The assessment of metazoan parasite diversity of native and extralimital *Clarias gariepinus* (Burchell, 1822) populations: a holistic approach

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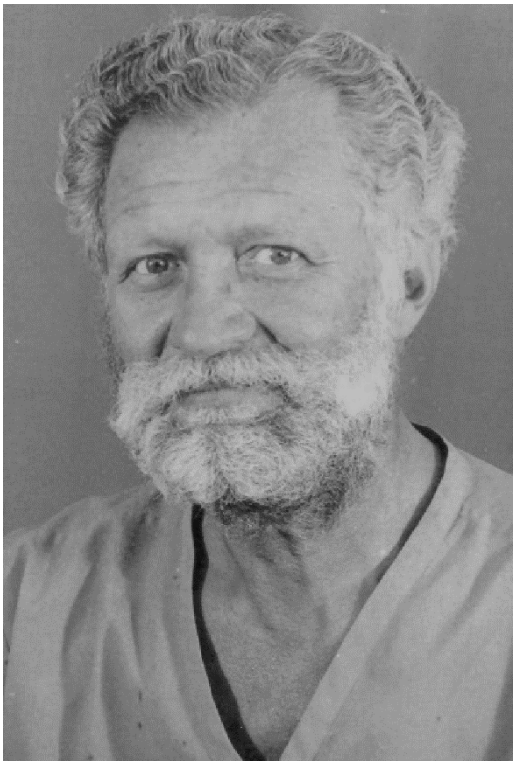
In liefdevolle herinnering aan Oom Pieter

~ met die blou bakkie, tarentale, perde hoewe, staaltjies, biltong en sterk koffie, adder-poetse bak ~

"…die ritsel in die gras, was toe al die tyd my sweep se punt."



Petrus Hendrikus Kemp

27 Augustus 1929 – 09 Januarie 2007

Declaration

This thesis comprises seven chapters and were prepared following the NWU Harvard style and prescribed template provided by the North-West University.

Information from two chapters (**Chapter 3** and **Chapter 4**) have been published in peer reviewed journals and include molecular, distribution and presence/absence data. I declare that this thesis has been composed by myself and that the work submitted is my own, except where work that has been published partially, or in its entirety as part of jointly authored publications has been included. For Chapters 1, 2, 5, 6 and 7, I was the lead and only author. For each of the manuscripts produced (based on data in **Chapter 3** and **Chapter 4**), there was joint responsibility between co-authors in components for study design, and I was responsible for data collection, laboratory work, data analysis, writing, manuscript preparation and consolidation or revision by co-authors and response to reviewers. Relevant authorship and contributions are noted below.

Data from **Section 3.5.** in **Chapter 3** has been published in *International Journal for Parasitology*: *Parasites and Wildlife* (Svitin et al. 2019). Dr Roman Svitin was lead author for this manuscript, and Dr Olena Kudlai, Prof Nico J. Smit and Prof Louis du Preez were co-authors. **Chapter 4** has been published in *Folia Parasitologica* (Truter et al. 2021a) and was co-authored by Dr Aline A. Acosta, Prof Olaf L. F. Weyl and Prof Nico J. Smit. The data from one of the study sites (Ndumo Game Reserve) in **Chapter 3** and **Chapter 4** formed part of a book chapter published by *IntechOpen* (Acosta et al. 2021). Dr Aline A. Acosta was the lead author, and the chapter was co-authored by experts across several disciplines, Dr Edward C. Netherlands, Prof Francios Retief, Dr Lizaan de Necker, Prof Louis du Preez, L., Dr Reece Alberts, Dr Ruan Gerber, Prof Victor Wepener, Dr Wynand Malherbe and Prof Nico J. Smit. All authors contributed equally to the manuscript design and writing aspects.

I declare that this thesis has been composed by myself and that the work has not be submitted for any other degree or professional qualification.

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Abstract

Parasites contribute substantially to global biodiversity. Similarly, parasites of aquatic species have a vast unexplored diversity, and in recent years received increasing attention, contributing to the global aim of scientists to catalogue the entirety of the world's biodiversity. Simultaneously, it became apparent that the diversity of parasites, as well as their assemblages within ecosystems, represent a complex interconnectivity of various components of an ecosystem and is not solely a reflection of the diversity or presence and absence of species. These macro- and microscopic organisms contribute to the dynamics of interactions between humans, the environment, and the hosts within said environments. Thus, leading to the present study that investigated diversity of the pan-African distributed sharptooth catfish, *Clarias gariepinus* (Burchell) in several geographic regions within southern Africa that is greatly underrepresented in parasitological data.

This study provides an overview on the validity and biogeography of the more than 107 metazoan parasite species currently known from *C. gariepinus*, and the number of listed digenean species known from this host is amended from 34 to 36. Furthermore, 44 parasitic species across the major taxonomic groups (Monogenea, Digenea, Cestoda, Nematoda and Crustacea) representing 12 orders and 19 families were recorded from various localities in southern Africa during this study. Of these, eight species are novel to the parasite fauna of *C. gariepinus*, 33 taxa are already known, and three species utilised the sharptooth catfish as paratenic host. Community assemblages were distinct across all sampled populations, and diversity and abundance [species richness (S)] varied greatly, with highest diversity recorded in the Upper Zambezi River system: Barotse floodplain (S = 31) and Kabompo River (S = 15) in Zambia, while catfish from the Lower Phongolo River and floodplain (S = 17) harboured the most diverse parasitic community in South Africa compared to the lower diversity found from populations in the Boskop Dam (BS) (S = 14), Vaal River (VR) (S = 13), Sand River (SR) (S = 10), Riet River (RR) (S = 9) and Gariep Dam (GD) (S = 8) on the Orange-Vaal River system. The parasite diversity and abundances from two translocated populations, one in the Great Fish River (GFR) (S = 9) and Riviersonderend River (RSE) (S = 7) were similar, however community assemblages were distinct. The GFR populations shared a similar community assemblage compared to its source population from GD, while RSE had a distinct community assemblage. The resilience of 12 parasitic species is confirmed, in their persistence upon co-introduction into novel environments, and provided support for the enemy release hypothesis on loss of native parasites upon entering a new environment.

Two alien invasive species, *Argulus japonicus* Thiele, 1900 and *Schyzocotyle acheilognathi* (Yamaguti, 1934) were also found to parasitise *C. gariepinus*. The former in GD, while *S.*

acheilognathi most probably spilled back to the translocated *C. gariepinus* in RSE from another alien invasive, *Cyprinus carpio,* already present in the system. Furthermore, a novel parasitic copepod species, *Ergasilus* sp. A was found on the gills of both translocated populations and suggests a spillback event from an unknown native host to the introduced *C. gariepinus* in GFR and RSE. The complexity and tolerance of parasitic taxa were illustrated in the absence of complex life cycle species from some native distribution populations, but presence in translocated populations, indicating that the ecosystems where they occur support similar host (intermediate or definitive) assemblages or that the parasites can adapt to a new host-parasite system in a novel environment. Finally, the responses of parasitic taxa to functional host traits and their environment (unimpacted or impacted), as well as inverse responses to increased elements, or in the case of positive responses to Au, Fe, Co, Hg, Sn and Se by respective taxa, indicate that the effect of pollutants on different organisms in aquatic environments is more complex than linking a generalised concept of pollution to species (intermediate and definitive hosts) absence or presence, or increased morbidity.

Keywords: Ecological hypotheses; Enemy release; Environmental parasitology; Freshwater fish parasites, Invasion mechanisms, Latitudinal diversity gradient; Translocations

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Chapter 1

Introduction to freshwater fishes and research on their parasites

1.1. General introduction

The known, the unknown and importance of research

Fishes constitute approximately half of the world's living vertebrates and exhibit exceptional diversity in morphology, ecological niche, habitat utilisation, physiology, and an associate diversity in almost all aquatic environments. Freshwater fishes are of unquestionable value to mankind as a food staple, contributing to macro- and micro-economies, education and knowledge across the globe.

"*Today fishes form an important element in the economy of many nations while giving incalculable recreational and psychological value to the naturalist, sports enthusiast and home aquarist. They are also the subject of international and domestic agreements…and disagreements… Particular species lend themselves to studies in behaviour, ecology, evolution, genetics, and physiology. They are used as general indicators of pollution, partly to the direct benefit of humans and partly to protect what people consider a valuable and necessary part of their heritage and life.*" – Nelson et al. (2016).

In contrast to the small proportion of the world's freshwater habitats, a disproportionately large number of fish species are found within them, and a similar dichotomy exists for this greatly diverse host group (approximately 13 000 species), with a disproportionately lesser-known parasite diversity. Africa holds about 25% of the world's fish diversity in its rivers and lakes, with a high percentage of endemism (at species and family level) and established historical host-parasite relationships ideal for ecological and evolutionary studies (Lundberg et al. 2000). However, the recent review of knowledge and diversity of African freshwater fish parasites stressed the need for development in research and expertise of the poorly studied parasite diversity of such a diverse fish host assemblage (Scholz et al. 2018c). Similarly, research on freshwater fish parasitology in South Africa is also predisposed to regions where biologists, ecologists and parasitologists are stationed with easy access to resources. In the case of South Africa, four major freshwater fish parasitology research groups are in the north-eastern provinces of the country and drive the dynamic advancement and intensity of ichthyoparasitology in the southern African region (Van As 2015). No major parasitological research group is based in the

coastal provinces of southern Africa, and thus research along these regions, the rest of the country and neighbouring countries are primarily dependent on collaboration or research initiatives by local research stations along inland lakes (Smit and Hadfield 2015; Van As 2015). A similar trend can be seen for research on the African sharptooth catfish, *Clarias gariepinus* (Burchell), which is one of the most widely distributed freshwater species in Africa and the subject of many other research disciplines (i.e., ecology, ecotoxicology). *Clarias gariepinus* is primarily utilised as a bioindicator in the highly and continuously impacted freshwater systems of a water scarce country (South Africa) with intensive mining activities and industrial productivity in addition to a rapidly growing population and economic demand (Pheiffer et al. 2018; Erasmus et al. 2022). Simultaneously, *C. gariepinus* serves as an ideal species for utilisation in economic initiatives to alleviate hunger and poverty and ensure local food security (Britz et al. 2015). Moreover, its parasite community has been widely studied in North African countries where fish culture and dependence on local small scale subsistence fisheries from local rivers and impoundments are primary practise for local micro-economies and food sourcing, such as Nigeria, Ethiopia, and Egypt (Abdel-Gaber et al. 2015; Dauda et al. 2018). Similarly, parasitology research along the African Great Lakes is intensively focused, with highly productive in-lake fish culture supporting an increasing local and international fisheries market and, providing local income to surrounding communities (FAO 2020). The opposite is true for the majority of southern Africa, where *C. gariepinus* is, 1) present in highly diverse and naturally pristine environments; 2) utilised as an indicator species in impact studies; 3) is repeatedly considered as an ideal candidate, along with other fishes, for local capture fisheries in South Africa (Britz et al. 2015); and 4) an alien invasive, translocated beyond natural borders into provinces with high fish endemism and critically endangered species (i.e., Cape Floristic Region and Eastern Cape) in South Africa.

The “why” of freshwater fish parasitology in a country such as South Africa

As mentioned above, *C. gariepinus* is a species that has the capacity to be utilised as a way forward in potentially reaching one or more of the 2030 Sustainability Development Goals (SDGs) i.e., no poverty; zero hunger; sustainable cities and communities through the establishment of local fisheries to create employment and income opportunities and affordable food sources; good health and wellbeing by developing local economy and infrastructure through job creation and opportunity to increase local income; as well as identifying potential risks (such as pathogens and parasites with epizootic and zoonotic potential) that may affect stocks, their desired market value or human health. More recently, leading researchers in the field of ichthyology, policy making, ecologists and parasitologists identified “the impacts of pathogenic diseases on fish populations” as one of the ten important themes that should guide future policy development in inland fisheries (Weyl et al. 2020). Within this theme framework, these authors emphasised that

there is a lack of historical information on baseline epidemiology data to draw a parallel between the norm and a changing environment.

What is a changing environment in parasitology?

Often parasites are associated with illness, death, and gruesome imagery of zoonotic disease. However, parasites in general are defined as individual organisms living in a symbiotic or close relationship, in or on a host, and is dependent on this host to provide it with various resources to ensure its survival and successful reproduction (Poulin et al. 2011; Dougherty et al. 2015). Thus, from this we can conclude that parasites are in fact dependent on its host for ultimate survival, or in some cases death, to succeed in its own survival. For parasites to succeed, they have specialised life cycles, exceptional lifestyle strategies and are adapted to live in a certain environment on their host. Endoparasites live inside organs such as the eye, cranial cavity, intestine, liver, spleen, gallbladder and even inside muscle tissue; while ectoparasites are found attached to external surfaces of its host's body like the gills, fins or skin; and finally, mesoparasites embed their attachment organs inside the muscle tissue while the rest of their body is visible on the outside of the host. Each of these lifestyles has its benefits, but due to increasing human impacts on the natural environment, the lifestyle may be at a disadvantage for the parasite, or of benefit to the host. Similarly, strategies linked to life cycles, such as direct (monoxenous) or indirect (heteroxenous) links are affected differently when exposed to adverse changes in the environment.

To understand what a changing environment is for a parasite, and how a particular species might react to changes, it is imperative that its biology (i.e., reproduction, physiology) and ecology (i.e., life cycle, habitat preference) is established under natural conditions. However, in an everchanging world this is often not possible, as the study of natural and impacted environments often intertwine and are only scrutinised and assessed once an adverse event occurred. The importance of parasites, not just as agents of biodiversity, but also as contributors to food web dynamics and energy flow across various trophic levels, through their life cycle and effect on host behaviour, have been emphasised (Poulin 1992; Marcogliese and Cone 1997; Marcogliese 2001; Lafferty and Kuris 2009; Lafferty 2012). To better understand the relationship between parasitic communities, a changing environment and the effect on hosts, the discipline of environmental parasitology aims to define or quantify the effects of alterations, such as pollution and a multitude of anthropogenic influences in the natural environment on parasitic communities. The relationships between parasitic species, their hosts, and sources of pollution i.e., thermal pollution and elemental pollution, are quantified and defined in using parasites as accumulation and effect indicators. As a result, their role as interacting agents, along with selected indices and

environmental or ecosystem health assessments, is used to draw parallels between anthropogenic stressors and responses of hosts and parasites.

1.2. Problem statement and hypotheses

Studies on the parasite fauna of *C. gariepinus* is restricted to the northern regions of South Africa, where the main freshwater fish parasitology research groups were founded and are maintained. Although these research groups contribute significantly to the discipline at national and international level, few studies focus on comprehensive assessments of a single host species. Ample research has also been done on the ecology, feeding and single parasite taxa of *C. gariepinus* in the major river systems surrounding the core of each parasitology research group's hosting institution, but few to no investigations has been done on *C. gariepinus* populations beyond these sampling localities that are often frequented by parasitologists. Similarly, few studies report on the parasite communities of the sharptooth catfish from neighbouring countries i.e., Mozambique, Zimbabwe, Botswana, and Namibia where, as in South Africa, it is of economic and staple food importance to local communities. Additionally, where information is available it is mostly inaccessible or outdated. Notwithstanding the above, the natural southern distribution range of *C. gariepinus* falls within South Africa, with the Orange, Vaal and Umtamvuna rivers as natural limiting boundaries, which has been breached through the construction of inter-basin water transfer (IBWT) schemes, resulting in translocation of species, such as *C. gariepinus* beyond their natural range. Furthermore, introductions beyond the natural limit have also in the past been facilitated by governing bodies through stockings of fingerlings in research and hatchery facilities or moved by locals to improve angling or food sourcing in some regions in the Eastern and Western Cape provinces in South Africa (Ellender and Weyl 2014; van Wilgen et al. 2018). Finally, to date not a single parasitological study has been done on translocated *C. gariepinus* in the Eastern and Western Cape provinces to determine whether any of its parasites were co-introduced into these novel environments and have the potential for spill-over or whether it has accumulated non-native parasites. Thus, this study will focus on the parasitic diversity and communities of *C. gariepinus* from: 1) two subtropical rivers in southern Africa; 2) the Orange-Vaal River system and associated tributaries; and 3) two translocated populations, one in the Eastern Cape and the other in the Western Cape with the aim to test the following hypotheses:

- **Hypothesis 1:** New specialist parasite species will be uncovered in the native region of *C. gariepinus* in southern Africa.

- **Hypothesis 2:** Expanded species distributions will be uncovered in the regions where parasitic communities of *C. gariepinus* were not previously investigated.

- **Hypothesis 3:** Co-introduction of specialist parasite species occurred during the translocation of *C. gariepinus* into novel environments in South Africa.

- **Hypothesis 4:** Parasite spillback occurred from native fishes to *C. gariepinus* in the Great Fish River and Riviersonderend River, where this host is an invasive species.

- **Hypothesis 5:** Enemy release occurred upon the introduction of *C. gariepinus* into novel environments.

- **Hypothesis 6:** There will be a decreased ecto-parasite richness and higher endo-parasite richness in more stressed environments.

1.3. Aims and objectives

To test the above hypotheses, the following aims and objectives were set:

Aim 1: Determine the complete known parasite diversity of *C. gariepinus* in Africa.

Objective: Perform a complete literature review of all known parasitic species of *C. gariepinus* in Africa for comparative purposes of species collected in the present study.

Aim 2: Determine the parasite community composition, diversity, and richness at all chosen localities in the native and extralimital distribution range of *C. gariepinus*.

Objective: Apply classical morphological and molecular approaches to identify species to the lowest taxonomic level, calculate descriptive statistics, along with diversity indices to describe parasite presence, absence and burden on the host, and determine parasitic assemblages at infra- and component community level.

Aim 3: To determine if there was co-introduction, enemy release and parasite spillback in the parasite communities of *C. gariepinus* from the two rivers where it is a translocated species.

Objective: Comparison of translocated communities to that of native populations in South Africa.

Aim 4: Determine if parasite effect indicators can be identified by quantifying the environmental quality.

Objective: Measure organic and metal pollution at the time of parasitic collection and subsequent comparison to the parasite diversity.

1.4. Thesis layout

Chapter 1 serves as a general introduction to the selected host species, its parasite communities and rationale for this study.

Chapter 2 presents a comprehensive summation of the distribution and translocation of *C. gariepinus* in South Africa. Detailed information on occurrence, potential impacts, and presence of alien and invasive fishes in the localities studied are provided, in conjunction with the methods used to identify and quantify data collected during the present study.

A comprehensive review of literature on, and validity of parasite species known from *C. gariepinus* in Africa is presented in **Chapter 3** and includes the comprehensive findings of the five native populations investigated during the present study.

Chapter 4 provides a complete discussion of the genus *Macrogyrodactylus* Malmberg, 1957, as a case study on one of the parasitic groups found during the present study (this chapter has been published in *Folia Parasitologica*).

The parasitic communities of the two translocated populations investigated is presented in **Chapter 5**, along with a thorough discussion on the alien and invasive parasite species found from *C. gariepinus* in the present study and a summary on the known species listed in literature.

Chapter 6 illustrates the association between environmental quality and parasitic community assemblage composition.

An extensive discussion on the findings, and conclusions drawn from the present study, as well as recommendations to consider in future studies are presented in **Chapter 7.**

1.5. Dissemination of results to date

1.5.1. Publications

Sections of this thesis have been published in peer reviewed journals, details listed below, and the relevant details to authorship and contribution are stipulated in the “Declaration” section.

- A section of data obtained during the present study on the Camallanidae (**Chapter 3**, **Section 3.5.)** infecting *C. gariepinus* from the Ndumo Game Reserve were included as part of novel information on nematodes of freshwater hosts in South Africa. Data was published in the *International Journal for Parasitology: Parasites and Wildlife* and is included as **Appendix A6** in this thesis, reference:

  Svitin, R., Truter, M., Kudlai, O., Smit, N. J., du Preez, L. 2019. Novel information on the morphology, phylogeny and distribution of camallanid nematodes from marine and freshwater hosts in South Africa, including the description of *Camallanus sodwanaensis* n. sp. I*nternational Journal for Parasitology: Parasites and Wildlife*, 10:263–273, https://doi.org/10.1016/j.ijppaw.2019.09.007

- Presence and absence data on the Monogenea from *C. gariepinus* in the Ndumo Game Reserve were published with *IntechOpen* (Acosta et al. 2021), the relevant book chapter is included as **Appendix A7** in this thesis, reference:

  Acosta, A. A., Netherlands, E. C., Retief, F., de Necker, L., du Preez, L., Truter, M., Alberts, R., Gerber, R., Wepener, V., Malherbe, W., Smit, N. J. 2020. Conserving freshwater biodiversity of an African subtropical wetland: South Africa's Lower Phongolo River and floodplain. *IntechOpen*, https://doi.org/10.5772/intechopen.93752

- **Chapter 4** has been published in *Folia Parasitologica* (Truter et al. 2021a) and was prepared to a derivative of the NWU Harvard style in this thesis, reference:

  Truter, M., Acosta, A. A., Weyl, O. L. F., Smit, N. J. 2021. Novel distribution records and molecular data for species of *Macrogyrodactylus* Malmberg, 1957 (Monogenea: Gyrodactylidae) from *Clarias gariepinus* (Burchell) (Siluriformes: Clariidae) in southern Africa. *Folia Parasitologica*, 68:027, https://doi.org/10.14411/fp.2021.027

1.5.2. Conference contributions

Findings on sections of the present study were presented as oral or poster presentations at national and international conferences. Details for each are listed below. Underlined bold text denotes that the author of this thesis presented the work, bold text indicates the author of this thesis as a co-author and an underlined author denotes the presenter:

International conferences

- **Truter, M.**, Hadfield, K.A., Weyl, O. L. F., Smit, N. J. 2021. The metazoan parasite community of invasive *Clarias gariepinus* (Burchell, 1822) in South Africa: testing the co-invasive, spillback and enemy release hypotheses. *49th Conference of the Parasitological Society of Southern Africa*, 14–16 September 2021, Online meeting (Oral, presented)

- **Truter, M.,** Acosta, A. A., Weyl, O. L. F., Smit, N. J. 2021. New distribution records and molecular data for species of *Macrogyrodactylus* Malmberg, 1957 (Monogenea: Gyrodactylidae) from *Clarias gariepinus* (Burchell) (Siluriformes: Clariidae) in southern Africa. *British Society for Parasitology: Parasites Online*, 21–25 June 2021, Online Meeting (Poster, presented)

- **Truter, M.**, Hadfield, K.A., Weyl, O. L. F., Smit, N. J. 2019. Metazoan parasitic infections for *Clarias gariepinus* (Burchell, 1822) in native and extralimital distribution ranges within southern Africa. *27th Conference of the World Association for the Advancements of Veterinary*

Parasitology, 7–11 July 2019, Madison, Wisconsin, United States of America (Poster, presented)

- **Truter, M.**, Acosta, A. A., Weyl, O. L. F., Smit, N. J. 2021. New distribution records and molecular data for species of *Macrogyrodactylus* Malmberg, 1957 (Monogenea: Gyrodactylidae) from Clarias gariepinus (Burchell) (Siluriformes: Clariidae) in southern Africa. *British Society for Parasitology: Parasites Online*, 21–25 June 2021, Online Meeting (Poster, presented)

National conferences

- **Truter, M.**, Hadfield, K. A., Weyl, O. L. F., Smit, N. J. 2021. The metazoan parasite community of invasive *Clarias gariepinus* (Burchell, 1822) in South Africa: Testing the co-invasive, spillback and enemy release hypotheses. *49th Virtual PARSA Conference*, 14–16 September 2021, Potchefstroom, South Africa (Oral, presented)

- Svitin, R., Schoeman, A., **Truter, M.**, Smit, N., Du Preez, L. 2018. Phylogenetic relationships of Camallanidae (Nematoda) parasitising freshwater vertebrates from South Africa. *Southern African Society of Aquatic Scientists Congress: Aquatic Ecology in the Anthropocene*, 24–28 June 2018, Cape St Francis, South Africa (Oral presentation, presented by Svitin, R.)

Annual institutional progress symposia

- **Truter, M.,** Hadfield, K. A., Weyl, O. L. F., Smit, N. J. 2020. Metazoan parasite diversity of native and extra-limital Clarias gariepinus (Burchell, 1822) populations in southern Africa. *2nd SAIAB Student Symposium*, 8 December 2020, Makhanda, South Africa (Oral, presented)

- **Truter, M.**, Hadfield, K. A., Weyl, O. L. F., Smit, N. J. 2019. Metazoan parasite diversity of native and extralimital *Clarias gariepinus* (Burchell, 1822) populations. *1st SAIAB Student Symposium*, 21–22 November 2020, Makhanda, South Africa (Oral, presented)

- **Truter, M.**, Hadfield, K. A., Weyl, O. L. F., Smit, N. J. 2019. The assessment of metazoan parasite diversity of native and extralimital *Clarias gariepinus* (Burchell, 1822) populations: a holistic approach. *2nd UESM Prestigious PhD Conference,* 28–29 August 2019, Potchefstroom, South Africa (Oral, presented)

- **Truter, M.**, Weyl, O. L. F., Smit, N. J. 2018. The assessment of metazoan parasite diversity of native and extralimital *Clarias gariepinus* (Burchell, 1822) populations: a holistic approach. *1st UESM Prestige Conference*, August 2018, Potchefstroom, South Africa (Poster, presented)

1.5.3. Community engagement

The concept of parasitism, presence in aquatic ecosystems and their interactions with hosts were presented as a five-minute video presentation as part of the “Freshwater 5” series hosted by the freshwater research group of the South African Institute of Aquatic Biodiversity. Material and photomicrographs obtained during the present study were included, reference:

Truter, M. 2021. FW5 Episode 7 - Waar woon parasiete? / Where do parasites live? Aired on 10 September 2021, access: FW5 Episode 7 - Where do parasites live? - YouTube

1.6. Awards

Awardee of the Best Senior Oral Presentation at the 49th Annual Conference of the Parasitological Society of Southern Africa, 14–16 September 2021 (Virtual Conference) for oral presentation entitled “The metazoan parasite community of invasive *Clarias gariepinus* (Burchell, 1822) in South Africa: Testing the co-invasive, spillback and enemy release hypotheses”.

Chapter 2

Materials, methods, and site descriptions

2.1. *Clarias gariepinus* (Burchell) as a model species

The African sharptooth catfish *Clarias gariepinus* (Burchell) is one of the most widely distributed freshwater fish in the world (Skelton and Teugels 1992; Skelton 2001). Its natural distribution includes regions of Asia (Israel, Syria and the south of Turkey) and its pan-African distribution reaches from the Nile River in Egypt to the Orange River in the Free State, the Vaal River in North West and the Umtamvuna River on the east coast of South Africa (**Fig. 2.1.**) (Skelton 1994, 2001; Weyl et al. 2016b). The distribution of *C. gariepinus* beyond it natural limits has been extended through various pathways for aquaculture, food sourcing, subsistence fisheries, recreational activities, and inter-basin water transfer (IBWT) schemes (Bruton and Merron 1985; Cambray 2003a, 2005; Vitule et al. 2006). The extralimital distribution of the catfish is also extended to Argentina, Brazil, China, Cote d'Ivoire, Gabon, Ghana, India, Japan, Saudi Arabia and to various European countries (Vitule et al. 2009; Weyl et al. 2016b; Froese and Pauly 2022).

In South Africa translocation of *C. gariepinus* to extralimital regions resulted from illegal stocking, escapes from hatcheries, recreational angling and through IBWT schemes in the Eastern Cape (Orange-Fish River tunnel, Orange-River Project) and Western Cape (Riviersonderend-Berg River Project) (Cambray and Jubb 1977; Bruton and Van As 1986; Hecht et al. 1988; Cambray 2003a, b; Slabbert 2007; Ellender and Weyl 2014; Weyl et al. 2016b). Establishment of the sharptooth catfish in the Eastern Cape is confirmed in the Buffalo, Gonubie, Great Fish, Keiskamma, Kouga, Kwelera, Mtata, Nahoon, Sundays, and Swartkops rivers. While rivers in the Western Cape including the Berg, Breede, Eerste, Kuils and Riviersonderend rivers did not escape the invasion of this hardy species. Additionally, dams in the Cape Flats and Sandvlei, Zeekoeivlei rivers and in tributaries in the Olifants River below its confluence with the Doring and Gourits rivers are also confirmed to harbour *C. gariepinus* (De Moor and Bruton 1988; Cambray 2003b; Booth et al. 2010; Kadye and Booth 2012 a, b, c, d; Ellender et al. 2011, 2015, 2017; Wartenberg et al. 2013; Ellender and Weyl 2014; Shelton et al. 2019). As both an ecologically and economically important food fish species of the world, the African sharptooth catfish is known to be an abundant species in almost all ecosystems where it occurs, it is easy to culture and naturally adapts to various environmental extremes. *Clarias gariepinus* also represent several trophic levels within aquatic food webs and can be a ferocious predator, an opportunistic omnivorous bottom-feeder and in some cases an intentional detritus feeder (Bruton 1979a, b,

1996; Teugels 1986; Kidd et al. 2001; Skelton 2001; Okeyo 2003; Van Steenberge et al. 2019). Food items found in the diet of *C. gariepinus* include other fishes, crustaceans, insects and macro-invertebrates, molluscs and oligochaetes, all of which can be potential intermediate hosts to larval stages of several parasitic taxa (Bruton 1979a). Furthermore, *C. gariepinus* is a resilient species, easily adapting to and tolerating some of the harshest conditions, including high turbidity waters, low oxygen concentrations, low temperatures, and desiccation (Bruton 1979b; De Moor and Bruton 1988; Skelton 2001; Picker and Griffiths 2011). This host species is an exceptional specimen for research in particular for ecological, evolutionary, ecotoxicology and parasitological studies. *Clarias gariepinus* has a rich biogeographical history and is widely studied in association with its parasitic communities throughout several African regions (see **Chapter 3**). In other fields of study such as ecological impacts, predation, competition, habitat destruction and food web disruption the sharptooth catfish is also a well-studied species (Cambray 2003a; Cucherousset and Olden 2011; Ellender and Weyl 2014). Its position in the food web and preference for bottom-dwelling in aquatic systems also render it an ideal model for studies in the aquatic environment as a bio-indicator and host (Fausch et al. 1990; Paller et al. 1996; Kidd et al. 2001). Based on the wide distribution range, hardiness, position across various trophic levels in aquatic food webs and being a well-studied species across various disciplines, *C. gariepinus* was selected as the ideal species for this multi-disciplinary study encompassing parasitological, diversity and pollutant exposure components.

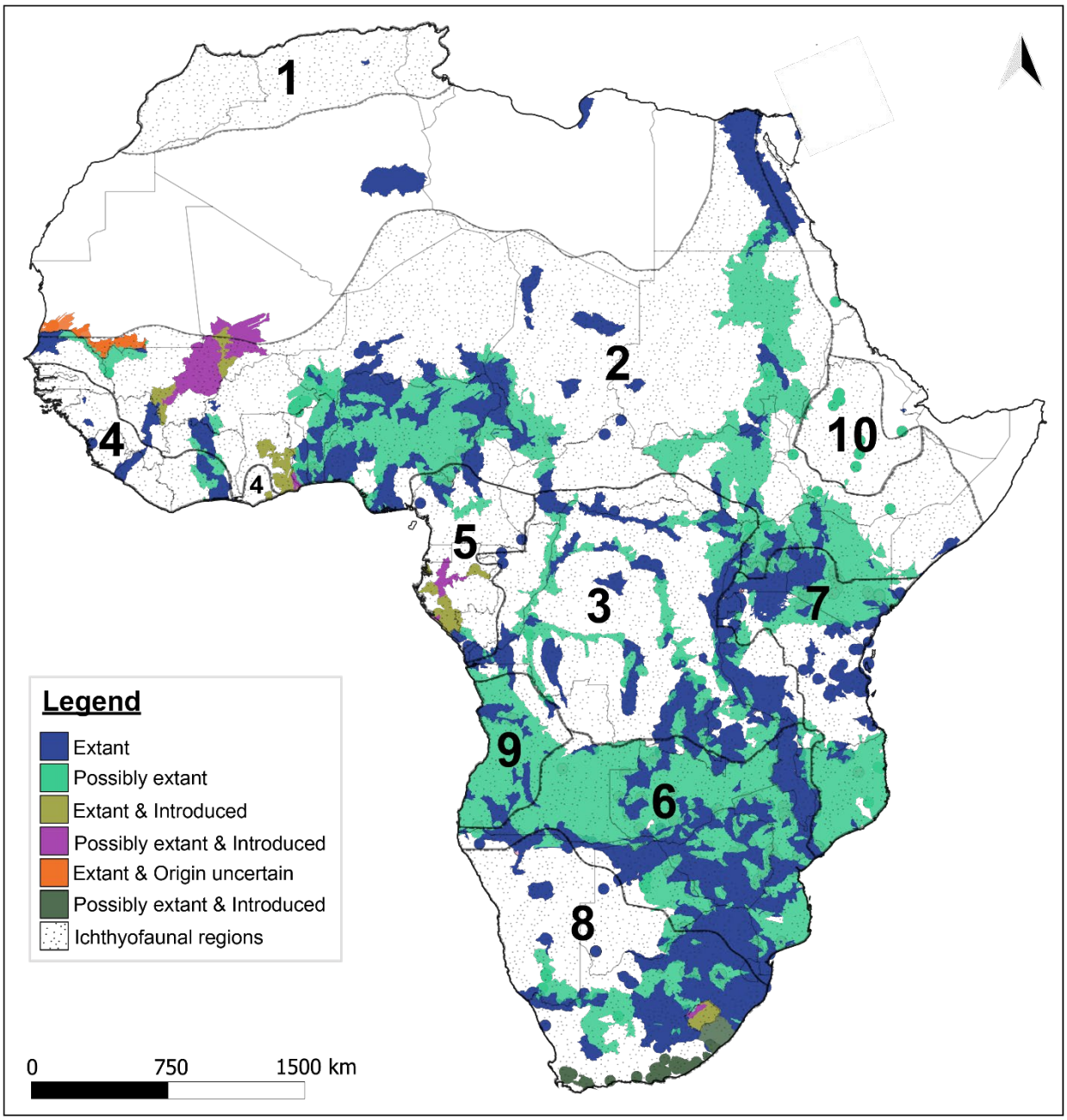

Figure 2.1. Map illustrating the natural and extra-limital distribution of *Clarias gariepinus* (Burchell) in Africa. The main ichthyofaunal regions in Africa: **1** – Maghreb; **2** – Nilo-Sudanian; **3** – Congo; **4** – Upper Guinea; **5** – Lower Guinea; **6** – Zambezi; **7** – East Coast; **8** – Southern Cape; **9** – Quanza and **10** – Abyssian Highland provinces. Data sources: CABI (2021), GBIF (2021) and IUCN (2021).

2.2. Site descriptions

This study was carried out in freshwater systems of assumed high biodiversity, where a series of stressors such as elemental pollution is known to impact the aquatic ecosystem as well as two rivers where *C. gariepinus* is translocated (see **Table 2.1.** and **Fig. 2.1.**). Coordinates of selected sampling localities, introduction status of the host and overall parasitic infection from each locality is summarised in **Table 2.1**. A summary of each sampled locality, fish fauna and potential anthropogenic influences follows below.

Table 2.1. Selected sampling localities in South Africa and Zambia for the present study. Abbreviations: N – native; T – translocated; n = number of hosts and parasitic infection, P – prevalence (%); mean intensity (MI) and range for all host individuals per locality.

Locality	Code	Coordinates	Status	n	P (%)	Overall MI	
Zambia							
Kabompo River	KR	13°06'10.42" S 24°41'38.93" E	N	2	100	3517	(814–6289)
Barotse floodplain	ZR	15°12'01.59" S 22°58'09.27" E	N	17	100	532	(21–3397)
South Africa							
Phongolo River and floodplain	NGR	26°55'47.00" S 32°19'25.80" E	N	37	100	248	(3–1102)
Vaal River	VR	26°51'59.63" S 27°17'40.03" E	N	18	100	55	(3–154)
Boskop Dam	BS	26°33'43.81" S 27°07'05.35" E	N	15	100	324	(29–1048)
Sand River	SR	28°19'23.59" S 27°21'35.06" E	N	15	93	243	(7–1046)
Riet River	RR	29°34'12.21" S 25°18'15.25" E	N	12	100	104	(11–374)
Gariep Dam	GD	30°34'05.77" S 25°33'53.03" E	N	15	100	22	(2–62)
Great Fish River	GFR	33°09'15.72" S 26°49'55.40" E	T	11	100	146	(23–316)
Riviersonderend	RSE	34°05'59.53" S 19°39'56.29" E	T	15	100	165	(49–311)

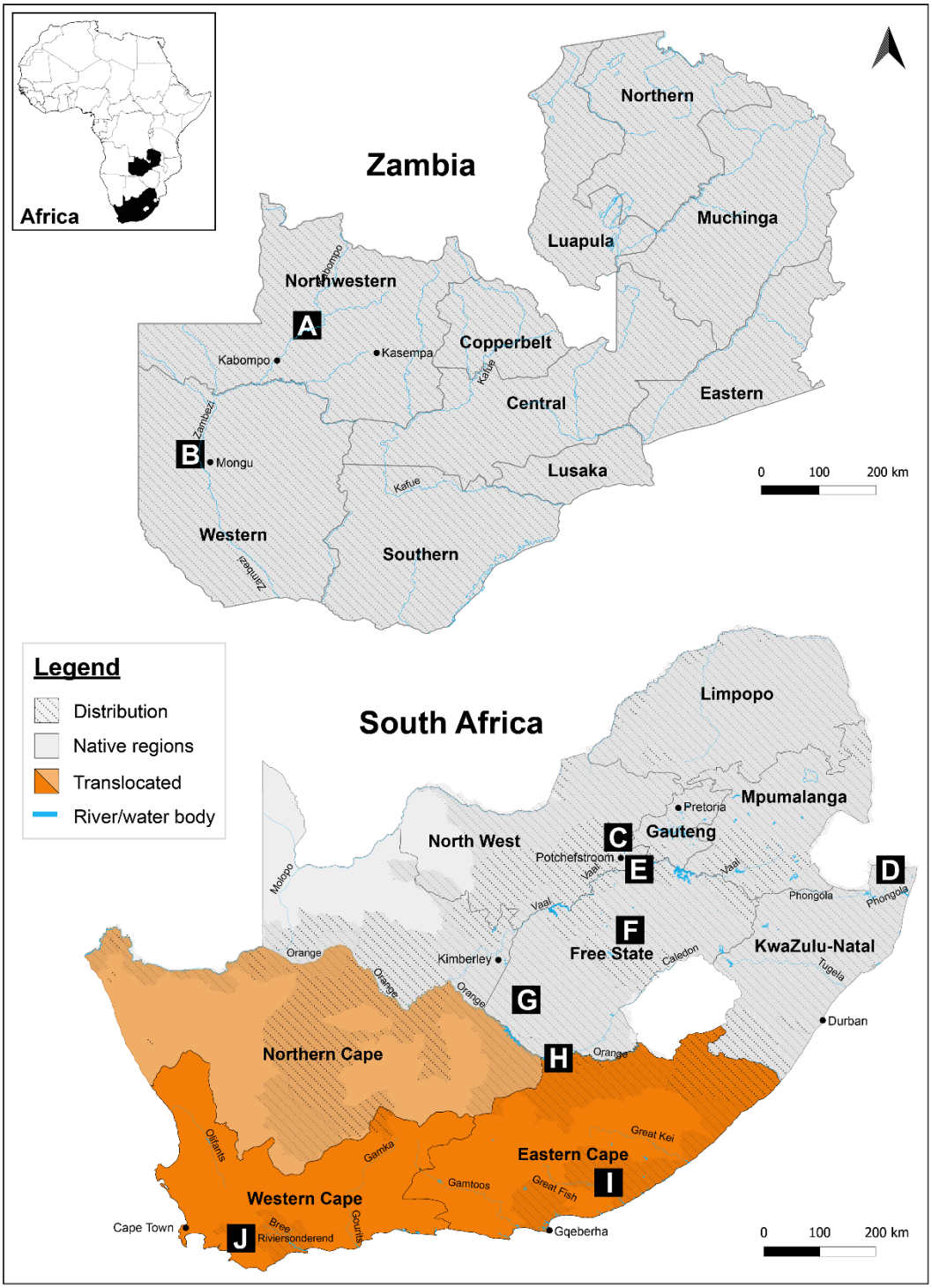

Figure 2.2. Sampling localities in the natural distribution range (A–I) and extra-limital regions (J–K) of *Clarias gariepinus* (Burchell). **A** – Kabompo River (KR) and **B** – Barotse floodplain (ZR); **C** – Boskop Dam; **D** – Phongolo floodplain (NGR); **E** – Vaal River (VR), **F** – Sand River (SR) and **G** – Riet River (RR); **H** – Gariep Dam (GD); **I** – Great Fish River (GFR) and **J** – Riviersonderend River (RSE).

2.2.1. Upper Zambezi basin, Zambia

The Zambezi River is the fourth longest river in Africa, having its origin near Mwinilunga in the Northwestern Province of Zambia. This east flowing river is divided into the Upper Zambezi basin that runs through the Northwestern Province of Zambia, enters Angola for a stretch of ±240km flowing south-west and enters Zambia again at the small village of Chingi, Northwestern Province. From here it flows south until it enters the Barotse floodplain (ZR) in the Western Province, and the river partially forms a border between Namibia and Zambia at Katima Mulilo. The Middle Zambezi basin follows where the Zambezi River briefly flows along the border between Botswana and Zambia continuing west as the border between Zambia and Zimbabwe, through Mozambique into the Cahora Bassa Dam (Lower Zambezi basin) and discharges into the Indian Ocean at the Zambezi Delta (Davies 1986; Tweddle 2010). In the present study sampling of *C. gariepinus* was carried out at two localities in the Upper Zambezi basin: from the Kabompo River (KR), the largest tributary of the Zambezi River, near the town of Jivundu in the Northwestern Province, and from ponds and stretches of the Zambezi River in the Barotse floodplain (ZR), near Mongu in the Western Province (**Fig. 2.3. A**–**C**).

Kabompo River, Northwestern Province

The origin of the Kabompo River is approximately 90km east of Mwinilunga in a watershed shared with the Democratic Republic of the Congo. The river flows southwest from its point of origin forming the eastern and southern borders of the West Lunga National Park, whereafter it joins the Zambezi River and the northern section of the Barotse floodplain north of the town Lukulu. Water from the Kabompo River is primarily used for irrigation purposes and supports a suspected high diversity of flora and fauna. Local communities, despite low population densities, are heavily dependent on the river, and surrounding forests are used for logging or converted to informal agricultural land (WorldBank Report 2010). More recently attention has been given to the effects of copper-nickel, gold and other mining activities in the headwaters of the Kabompo River as well as the flow regime of a river that supports a fairly understudied aquatic diversity. The construction of dams for small hydropower plants further contribute to direct impacts on this mostly undisturbed ecosystem (Ashton et al. 2001; HydroReview 2009; World Bank Report 2010; Michelo and Lange 2021; Ndhlovu and Woyessa 2021). Fishes in the system represent 16 families including the Alestidae, Amphilidae, Anabantidae, Characidae, Cichlidae, Clariidae, Claroteidae, Cyprinidae, Danionidae, Distichondontidae, Hepsetidae, Kneriidae, Mastacembelidae, Mochokidae, Mormyridae, Procatopodidae and Schilbidae (Winemiller 1991; Winemiller and Kelso-Winemiller 1996; Skelton 2001). During a survey in 2019 on the diversity, habitat use and preference of fish species in the Kabompo River, the fish diversity was confirmed at 88 species represented by 16 families. Only one introduced species, the Nile tilapia *Oreochromis niloticus* Linnaeus is present in the Kabompo River basin (Rennie 2021).

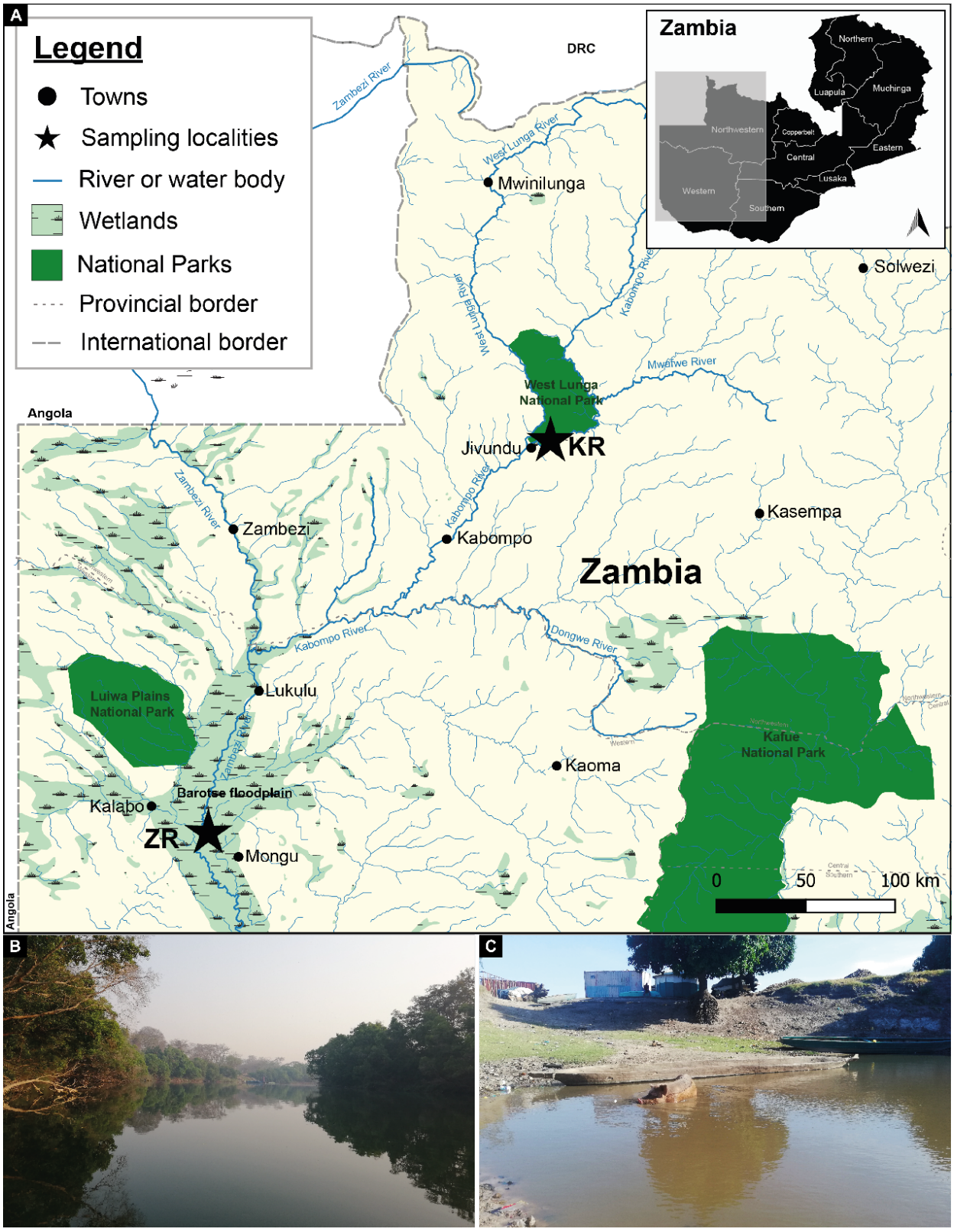

Figure 2.3. **A** – Map illustrating sampling localities in Zambia; **B** – Kabompo River and **C** – one of the ponds in the Barotse floodplain where *Clarias gariepinus* (Burchell) were collected.

Barotse floodplain, Western Province

Designated as a Ramsar site in 2007, the Barotse floodplain is the second largest wetland in Zambia. The floodplain is situated between Lukulu and Senanga in the Western Province, which also has one of the highest poverty rates of all sub-Saharan African countries. The province lacks mineral deposits that can generate income or provide employment, thus the local Lozi tribe utilise the floodplain as a primary source to sustain their livelihoods (Rajaratnam et al. 2015). During the wet season the Barotse floodplain is inundated for 3–5 months and provides fishing opportunities to locals. During the dry season, tribe members fish from the ponds remaining in the floodplain and extract water for their crops (maize, cassava and a variety of vegetables). Livestock (cattle, goats and pigs) also drink water from these ponds (Davies 1986; Turpie et al. 1999; World Bank report 2010; Zimba et al. 2018). This dynamic ecosystem supports the livelihoods of locals, fish trade and a diverse faunal community of birds, reptiles, and more than 80 fish species (Winemiller and Kelso-Winemiller 1996; Turpie et al. 1999; Pittock et al. 2009; Tweddle 2010; Baidu-Forson et al. 2014; Rajaratnam et al. 2015).

In addition to local human impacts, mining activities, deforestation along the riverbanks for logging and charcoal production and dam construction, the introduction of aquatic alien invasive species further threatens the preservation of native biota. The Australian red claw crayfish *Cherax quadricarinatus* (von Martens) (so far only present in the Barotse floodplain) and *O. niloticus* pose a direct threat to the native fish fauna i.e., *Oreochromis andersoni* (Castelnau) and may be a potential pathway for disease introduction (Darwall et al. 2009; Tweddle 2010; WorldBank Report 2010; Zengeya et al. 2015; FAO 2020; Jere et al. 2021a, b). Two examples include epizootic ulcerative syndrome (EUS) that was first reported in Africa from fish in the Zambezi River in 2006 and co-introduced temnocephalans of *C. quadricarinatus* (Huchzermeyer and Van der Waal 2012; Madzivanzira et al. 2021a, b; personal observations, M Truter). Other than the records of parasitic Crustacea from the sharptooth catfish in the Mulungushi Dam, the Kashizki River (Zambezi River system) and the Bangweulu Swamps (Congo River system) no studies on the parasitic fauna of the freshwater fishes in both these two river systems have been conducted (see **Chapter 3** and **Appendix A3** for further detail).

2.2.2. Lower Phongolo River and floodplain, KwaZulu-Natal, South Africa

The Lower Phongolo River and floodplain (PRF) is one of the largest and biodiverse floodplain systems in South Africa. The Phongolo River (PR) originates in the Mpumalanga Province flowing north through the Ubombo mountain range situated in the KwaZulu-Natal Province and the lower PRF stretches from where the river exits the gorge in the Ubombo mountains (also known as the Pongolapoort Dam). Here the PRF stretches for ±80 km to where the PR and Usuthu (US) rivers converge at the border between South Africa and Mozambique. The only formally protected

section of the PRF lies within the Ndumo Game Reserve (NGR) that is bordered in the north by the US and holds several unique pans i.e., natural saline pan, Lake Nyamithi that drains from its own catchment or is fed by the PR or US rivers during floods. The floodplain is characterised by temporary floodplain depressions (pans) that have permanent, intermittent or at times no connectivity with the river. Additionally, five distinct wetland types, all essential for breeding and foraging for migratory species as well as the exchange of organic matter and nutrients, can be found within the floodplain. Since the NGR was proclaimed a Ramsar wetland in 1997, an increase of research on this ecosystem followed (Smit et al. 2016 and references therein). The PRF is home to diverse amphibian, invertebrate as well as fish (46 species) communities, despite the major alteration in its physical and chemical profile brought on by the interference of the Pongolapoort Dam on natural flood regimes of the PFR (Acosta et al. 2021 and references therein).

Despite the diverse fauna and flora of the NGR the presence of alien species threatens local and endemic biodiversity. Local communities are also heavily dependent on the PRF for food (fish as protein) and resources (i.e., reeds, thatch, firewood and cultural practises), while water is mainly used for household activities and livestock. Although described as a resilient ecosystem, the PRF has been under immense stress in recent years as water abstraction increased due to an increase in subsistence farming practises serving as an alternative income to a fast-growing local population. In addition to historical remnants of intense spraying of insecticides, such as dichlorodiphentrichloroethane (DDT) and pesticides for malaria control, the increased agricultural activity in turn has created additional stressors that influence the PRF. Furthermore, an increase in use of chemicals such as pesticides and fertilisers on crops, as well as fuel and oil that leak from informally installed pumps, accompany the destruction of natural vegetation and riparian sections along riverbanks and increase the demand for resources from an already altered ecosystem.

Ample records exist for parasitic communities on several freshwater fishes of the NGR, however they only represent a fraction of the micro-diversity of the NGR and its macrofauna (du Preez and Smit 2013; Welicky et al. 2017; Svitin et al. 2019; Acosta et al. 2021; Truter et al. 2021a, b). During the present study, *C. gariepinus* was collected from the PR and UR rivers as well as from Lake Nyamithi (NYP) and KuShokwe pan (KSP) within the NGR (**Fig. 2.4. A**–**E**).

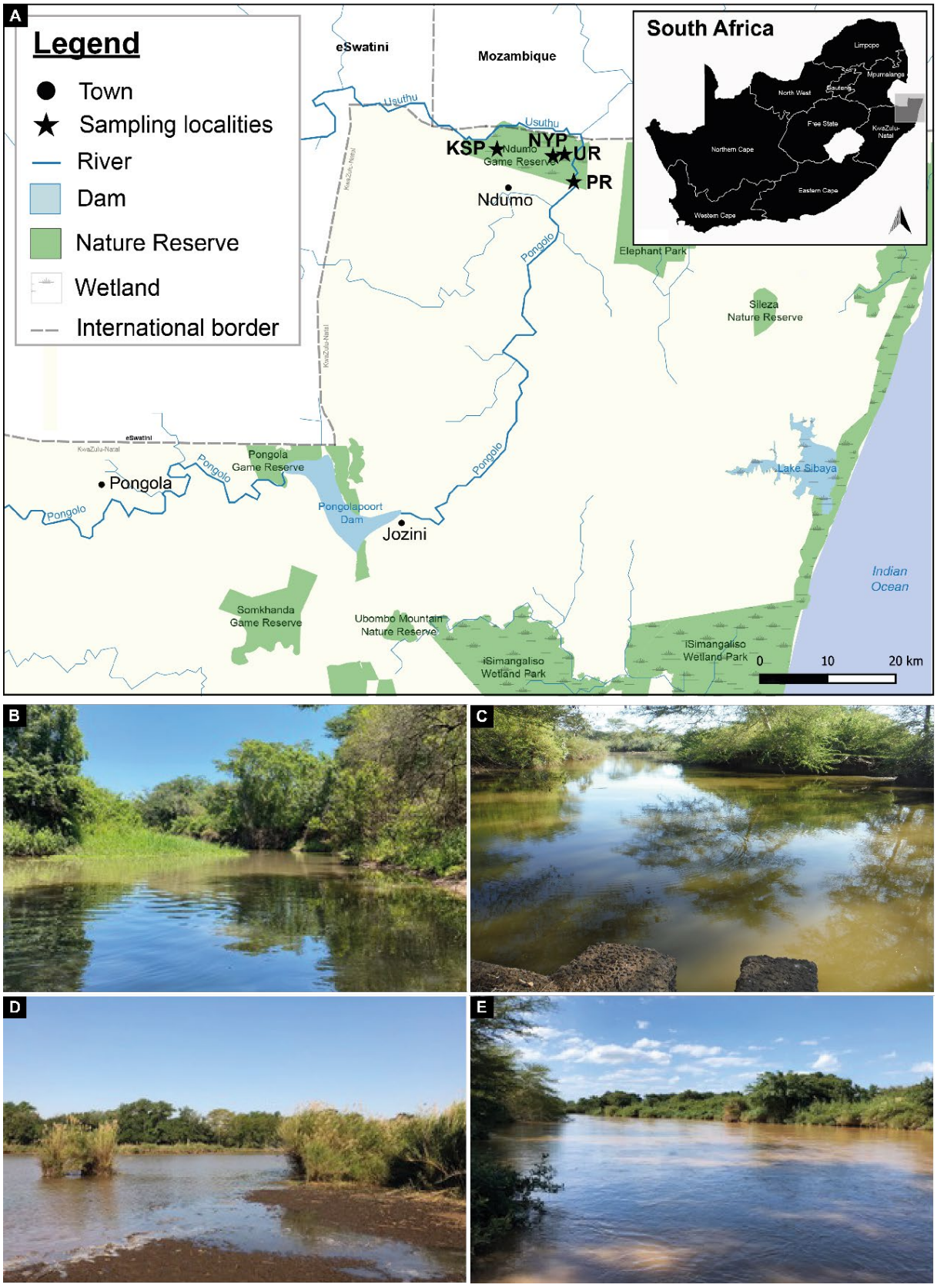

Figure 2.4. **A** – Localities sampled within the Lower Phongolo River and floodplain. **B** – Phongolo River; **C** – Nyamithi Pan; **D** – KuShokwe Pan and **E** – Usuthu River.

2.2.3. Orange-Vaal River system, South Africa

Vaal River, North West Province

The Vaal River is a tributary of the Orange River and the second largest river in South Africa, often referred to as the "water workhorse" and one of the most anthropogenically impacted rivers in the country. Situated in the industrial heartland of South Africa, water supply from the Vaal River (VR) sustains nearly 50% of the country's wealth, approximately 80% of the industries that produce electricity and several large gold and platinum mines and coal reserves (Brand et al. 2009; Barkhuizen 2015). Municipal wastewater treatment plants, runoff from mines, stormwater drainage from urban areas, industries and agriculture primarily impact the river's biodiversity and water quality. These impacts contribute to degradation and pollution of the other main tributaries of the river. In addition to habitat alteration, flow-regime modifications, migration barriers and impact on wildlife, the alterations to the VR result in increased mass fish mortalities and degraded water quality threatening ichthyofaunal health in the system. The presence of non-endemic or introduced fishes also pose a risk to native fish fauna of the VR (Winde and Van der Walt 2004; De Villiers 2007; O'Brien and De Villiers 2011; Wepener et al. 2011; Mahasa et al. 2015). Native fish fauna consists of cichlids, small barbs, large cyprinids, and catfishes. Several species such as the common carp *Cyprinus carpio* Linnaeus, grass carp *Ctenopharyngodon Idella* (Valenciennes), mosquito fish *Gambusia affinis* (Baird et Girard) and largemouth bass *Micropterus salmoides* (Lacépède) are alien to the river fauna (Barkhuizen 2015). Ample research is available on the parasitic communities of fishes from the Vaal River and barrage (see **Appendix A3**).

Sampling from the VR took place in a river stretch near the Takwasa Youth Camp approximately 30km downstream of Parys (**Fig. 2.5. A**, **B**).

Mooi River, North West Province

Boskop Dam (BS) (**Fig. 2.5. A**, **C**) is an impoundment on the Mooi River in the Lower Mooi River (Upper Vaal catchment) area that form part of three major sub-catchments i.e., Wonderfonteinspruit, Loopspruit and Mooi River proper (Labuschagne 2017). The dam is fed by dolomitic runoff from these sub-catchments. Upstream mining activities (gold and diamonds), urban and industrial run-off, informal settlements with insufficient waste and water infrastructure, stock grazing, informal slime dams, and agricultural activities such as maize crops and sunflower peat mining in the surrounding sub-catchments result in seepage into the underlying aquifer, with increased salt loads and deposition of trace metals (i.e., uranium) (Van der Walt et al. 2002; Annandale and Nealer 2011; Barnard et al. 2013; Pelser 2015; Manyatshe et al. 2017). The water from the Mooi River and BS is also used for irrigation and stock watering purposes, supplies water to the town of Potchefstroom, and serves as a popular destination for recreational activities such

as fishing, boating, canoeing, and camping. A diverse fish community with ten native species (including *C. gariepinus*), one translocated species, and five invasive species can be found in the waters of BS (pers. comm. Michael Kriel, Department of Water and Sanitation).

Sand River, Free State Province

The Sand River (SR) originates near the border of Lesotho and is a tributary of the Vet River which in turn is a tributary of the Vaal River. The SR flows in a western direction into the Allemanskraal Dam (Aldam) situated in the Willem Pretorius Nature Reserve near the town of Virginia in the goldmining capital of the Free State Province. Water from the river and dam is mainly allocated for irrigation of crops such as wheat, fodder crops, potatoes, maize, a variety of vegetables, and ground nuts. Water allocation to the mines in the region is supplied from the Vaal River (Sedibeng Water). Both the dam and river are characterised by salinisation due to mine discharges from gold mining activities, seepage from adjacent rivers such as the Wolwepan/Rietpan systems and irrigation return-flows (Herold et al. 1996). Municipal pollution, urbanisation and occasional spillage events from the surrounding mines are not uncommon with poor infrastructure to manage the capacities demanded by the surrounding communities and mines (Herold et al. 1996; RHP 2003; Mahasa et al. 2015).

During the River Health Programme, the overall health state of the SR was assessed and was classified as being in a “fair” to “poor” condition. Almost no natural flow is present in the river, with no water releases from the dams, and the river water is under great demand for irrigation and domestic use. It is also noted that during dry seasons the entire flow of the river consists of treated sewage effluent, and at times with irrigation return flows, industrial effluent (RHP 2003). Downstream aquatic habitats are sustained through seepage of water through the Allemanskraal Dam wall while upstream habitats are sustained with rainfall during summer months and several pools serve as refuge for fish and other aquatic species. The ichthyofaunal diversity of the SR is relatively low with few native species present and include large cyprinids [*Labeo capensis* (Smith), *L. umbratus* (Smith), *Labeobarbus aeneus* (Burchell) and *Labeobarbus kimberleyensis* (Gilchrist et Thompson)], small cyprinids [*Enteromius paludinosus* (Peters)], catfish (*C. gariepinus*). Reports of hybrids of *L. umbratus* x *L. capensis*, and invasives such as *G. affinis* and *M. salmoides* that displace indigenous species is also a concern (RHP 2003; Barkhuizen 2015; Erasmus et al. 2019). During the present study sampling of *C. gariepinus* took place from the river downstream of Allemanskraal Dam within the Willem Pretorius Nature Reserve (**Fig. 2.5. A**, **D**).

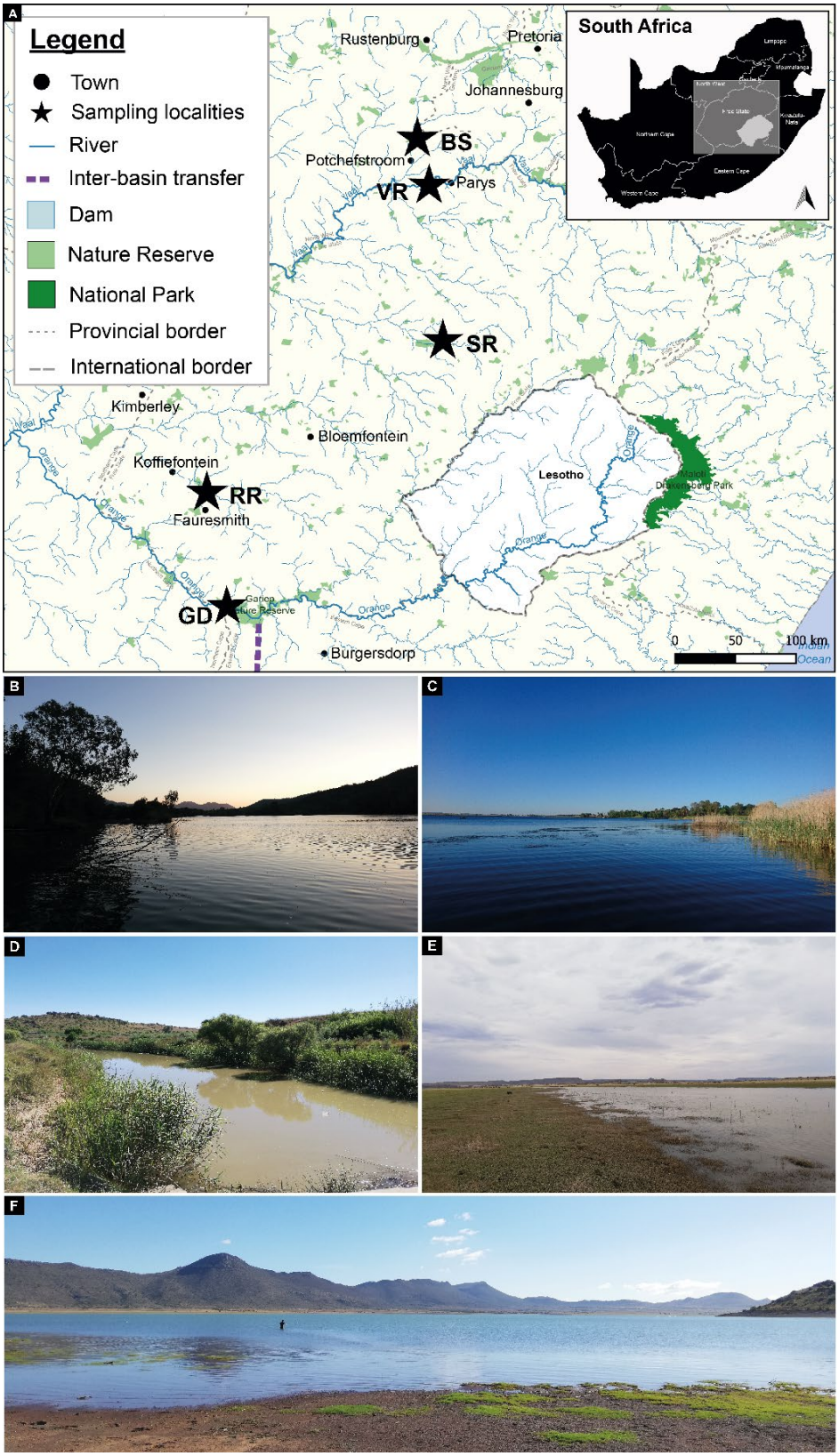

Figure 2.5. **A** – Localities on the Orange-Vaal system where sampling of *Clarias gariepinus* (Burchell) took place. **B** – Vaal River at Takwasa Youth Camp; **C** – Boskop Dam on the Mooi River; **D** – Sand River; **E** – Riet River and **F** – Gariep Dam on the Orange River.

Riet River, Free State Province

The Riet River (RR) is westward flowing draining through the arid south-western Free State Province and originates near Smithfield in southeast Free State. The Modder River, one of the tributaries of the RR, joins the RR near the town of Ritchie before the RR joins the Vaal River north of Douglas in the Northern Cape Province. Both rivers are highly regulated and impacted with numerous weirs resulting in the absence of natural flow. Structural, as well as species composition along the river is also altered because of over-abstraction for irrigation, industrial, municipal, domestic and hydropower usage, and droughts contribute to the overall poor health state of the RR prior to the confluence with the Modder River and downstream of the confluence of the two rivers (RHP 2003). One of the major impoundments on the RR is the Kalkfontein Dam, water from the dam is used for irrigation and domestic households in rural towns in the vicinity of the dam. Due to the alterations brought on by the constructed weirs and artificial water releases the habitat composition and freshwater fauna diversity remain low.

Fish species in the system had to adapt to lentic conditions, with some disappearing from river sections upstream and downstream from the dams constructed. In 2015, three indigenous species [*E. paludinosus*, *Pseudocrenilabrus philander* (Weber) and *Tilapia sparrmanii* Smith] and one invasive species, *M. salmoides*, were recorded for the first time from sections of the river (Barkhuizen 2015, 2019). Currently the fish reference list for the dam comprises of endemic and native species as including *C. gariepinus*, *Enteromius anoplus* (Weber), *L. capensis*, *L. umbratus, L. aeneus*, *L. kimberleyensis*, possible hybrids of *L. umbratus* x *L. capensis* and the invasive *C. carpio*, *G. affinis* and *M. salmoides* (Barkhuizen 1995, 1996, 2019).

Sampling of *C. gariepinus* during the present study was done in a stretch of the RR downstream of the Kalkfontein Dam in the Kalkfontein Nature Reserve between Koffiefontein and Fauresmith. The immediate surrounding area consist of mainly sheep and crop farms, and the Koffiefontein Mine is situated approximately 10km downstream of the present sampling locality (**Fig. 2.5. A**, **E**).

Gariep Dam, Orange River, Free State Province

The Gariep Dam (GD) in the Orange River, also known as Lake Gariep (**Fig. 2.5. A**, **F**), is the largest manmade impoundment in South Africa. It is situated in central South Africa, bordering the Eastern Cape, Northern Cape and Free State provinces and forms part of a highly regulated system and network of large transboundary water transfer schemes that feed other water management areas. Some of these, relevant to the current study, include the Orange-Fish tunnel from Oviston into the Teebus River and the Great Fish River (Snaddon et al. 1998, 1999; RHP 2003). The water of GD is mainly used for irrigation, tourism, hydro-electric power generation and its fish fauna supports nearby communities as a source for recreational or subsistence fishing

(Ellender et al. 2009; Barkhuizen et al. 2015, 2017). Situated in a region with natural low diversity in fish fauna, the fish community of the dam is represented by large cyprinids, particularly endemic species of *Labeo* Cuvier and some indigenous species of *Enteromius* Cope and the catfish *C. gariepinus*. Invasive species present include *C. carpio* and alien salmonids and centrarchids (Ellender et al. 2010; Barkhuizen et al. 2017). For this study sampling was carried out in the Gariep Dam Nature Reserve on the Free State side of the dam (**Fig. 2.5. F**).

2.2.4. Great Fish River, Eastern Cape Province, South Africa

The Great Fish River (GFR) (**Fig. 2.6. A**–**B**) is 650km long, rising at the Tandjiesberg mountains west of Cradock, drains the valley between Cradock and Queenstown (Fish River valley) in the Eastern Cape Province and enters the Indian Ocean approximately 25km East of Port Alfred (Watling and Watling 1983; O'Keeffe and De Moor 1988; Ter Morshuizen et al. 1996). After the construction of the Orange-Fish River IBWT, connecting the GD on the Orange River system with headwaters of the GFR, breaching the natural boundary between the two systems, the flow-regime of the river changed from seasonal to perennial (Cambray and Jubb 1977; O'Keeffe and De Moor 1988; Weyl et al. 2009). This flow-regime change supports the increased agricultural activities in the region, which otherwise would not have been possible. The surrounding valley demands high volumes of river water supporting livelihoods of many settlements and small livestock including angora goats, sheep, and bees, as well as for irrigation of lucerne, maize and wheat for stockfeed for the surrounding dairy industry (WUA 2004). Approximately 26 fish species inhabit the river, of which 10 are alien species (Kadye and Booth 2012d). Introduction of five of the 10 alien species, including *C. gariepinus* was a result of the transboundary Orange-Fish tunnel water scheme (Cambray and Jubb 1977; Laurenson and Hocutt 1986; Kadye and Booth 2012d; Weyl et al. 2016b).

Sampling was done in the river stretch near the Committees Drift Police station, situated north-west of the town Peddie (**Fig. 2.6. A**–**B**). Despite the remoteness of the immediate area small industrial and informal settlements exist on the eastern side of the river and police station, and local residents make use of the river to fish for large species including *C. gariepinus* and *C. carpio*, both of which are introduced species in the system. Upstream from the present sampling locality, the river winds through remote mountainous areas, mainly surrounded by nature reserves or private game farms. Hereafter, agricultural activity begins near the town of Middleton continuing upstream to Cookhouse and Cradock where the river has its origin.

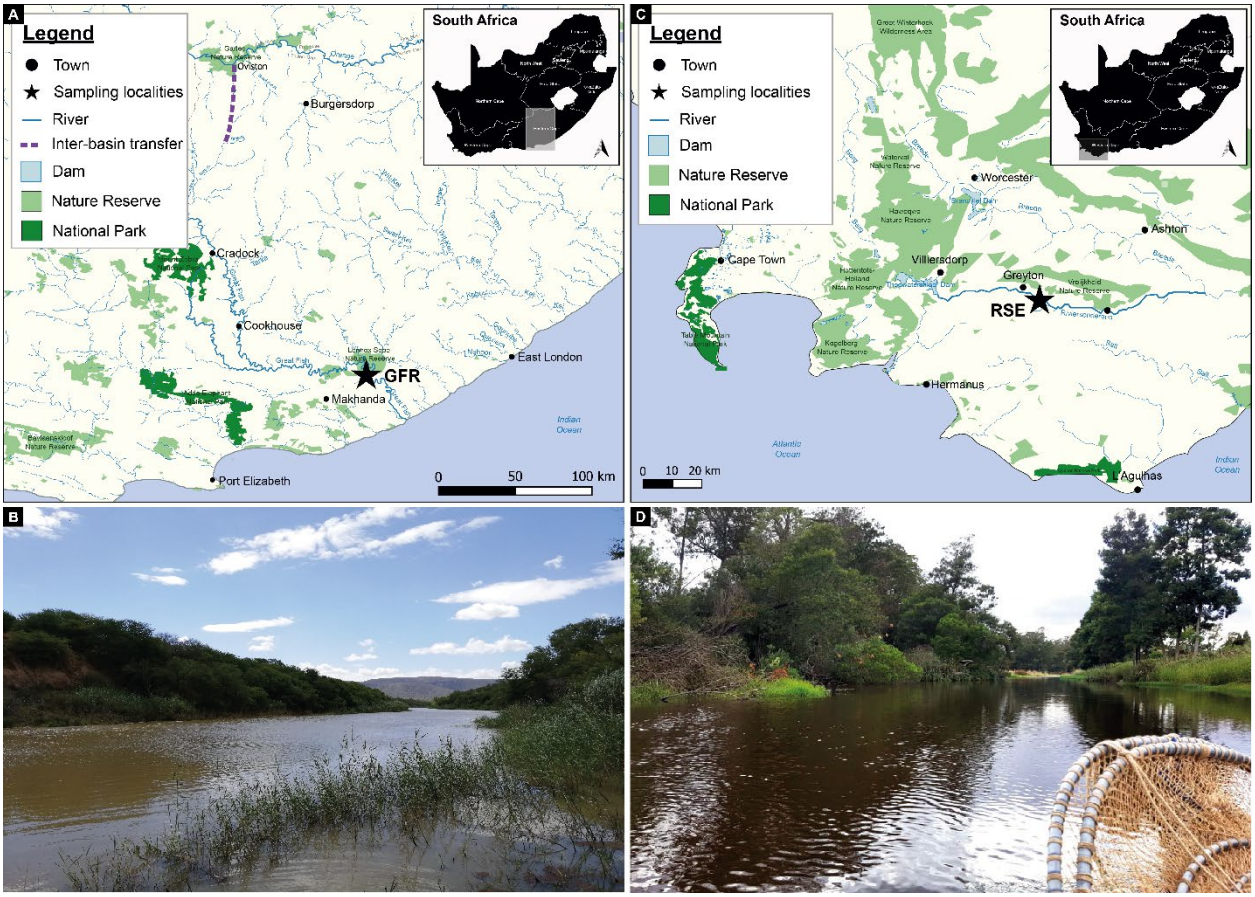

Figure 2.6. Localities where sampling of *Clarias gariepinus* (Burchell) took place in translocated systems. **A**, **B** – Great Fish River and **C**, **D** – Riviersonderend River.

2.2.5. Riviersonderend River, Western Cape Province, South Africa

The Riviersonderend River (RSE) (**Fig. 2.6. C**–**D**) fall within the Breede River Basin and is part of the Riviersonderend-Berg River IBWT in the Cape Floristic Region which is renowned for high plant diversity and high endemism in its ichthyofauna. . Surrounding the river course is one of the largest single food and wine producing regions in South Africa, with intense agricultural activity, cultivation of high value crops and is under pressure by increased water abstraction for irrigation of these commercial crops and urban demand (DWAF 2003; Aurecon 2011; Pegram and Baleta 2014; Shelton et al. 2019).

Aquatic diversity is also in the crossfire with degradation through a decline in indigenous riparian vegetation along riverbanks. Included in the hotspot of high endemism of freshwater fishes, critically endangered fish species [the Clanwilliam rock catlet *Austroglanis gilli* Barnard; Cape galaxias *Galaxias zebratus* (Castelnau); *Pseudobarbus burchelli* (Smith); the Fiery redfin *Pseudobarbus phlegothon* (Barnard); the Giant redfin *Pseudobarbus skeltoni* Chakona et Swartzandl and theCape kurper *Sandelia capensis* (Cuvier)] are also on the brink of extinction through physical threats such as loss of riparian vegetation as a result of agricultural and residential development, destruction of riverbeds, over-abstraction of water, dam construction, water pollution, as well as biological threats which include alien plants and presence of alien fish species (Chakona and Swartz 2013; Chakona et al. 2013; Weyl et al. 2014; Kadye et al. 2016; Paxton 2018). In the RSE the introduction of *C. gariepinus* occurred through the tunnels of the Riviersonderend-Berg River Project where the RSE supplies water to the Breede and Eerste rivers in the Western Cape (*C. gariepinus* is also a translocated species in these systems). Uncontrolled movement of catfish by farmers and anglers into farm dams or nearby rivers for “improved fishing” have contributed to the spread of the species into the river (Clark et al. 2009; Ellender and Weyl 2014; Sheasby 2016).

Riviersonderend River is the southernmost locality, with the highest latitude sampled during this project. Sampling was done ±2km south-east of the town Greyton on the mainstem of the RSE, downstream of the Theewaterkloof Dam and is surrounded by several agricultural activities including, dairy, vineyards and lucerne fields.

2.3. Host collection and processing

Clarias gariepinus (**Fig. 2.7. A–C**) individuals were collected using various sampling methods including rod and reel, baited longlines, gill nets and fyke nets (**Fig. 2.8. A**–**D**). Collection of the host species took place in November 2017 and May–June 2018 at localities in NGR; October 2018 from BS; November 2018 from the RR and SR rivers; December 2018 from RSE; February–March 2019 from GD; March–April 2019 from the VR in South Africa and September–October 2019 sampling was done from the ZR and KR in Zambia.

Live fish were kept in aerated containers at a field station until humanely killed by means of percussive stunning and cervical dislocation (Fouché 2016). Each fish was externally screened for macroscopic ectoparasites (see Dávidová and Smit 2018) on the body surface prior to being weighed (total) with a digital lip-grip scale and measured to the nearest millimeter (total and standard length). After the dissection of each fish, organs were removed and kept in separate petri dishes. The fins and gills were removed and placed in river or tap water and the eyes, brain matter and cerebrospinal fluid from the cranial cavity, and internal organs were removed and placed in a saline solution for screening of ecto- and endoparasites using a Zeiss Stemi 305 stereomicroscope with camera attachment. The muscle tissue of each individual was also sectioned off and screened for cysts of intermediate life stages of parasitic groups such as Digenea (Trematoda) and Nematoda. Following parasitic screening a muscle tissue sample was collected, placed in a labelled Ziplock bag and frozen for metal analyses (see **Section 2.7.**).

Figure 2.7. **A**–**C.** *Clarias gariepinus* (Burchell) collected during the present study.

Figure 2.8. Methods used to collect host species: **A** – Rod and reel; **B** – Colleagues deploying and baiting longlines in the Great Fish River; **C** – Locals gill netting in the Barotse floodplain; and **D** – Setting a fyke net in Boskop Dam.

Permits and permissions for sample transport were obtained from the relevant authorities: Ezemvelo KZN Wildlife (KwaZulu-Natal, permit Nos. OP 1075/2017, OP 1582/2018); the Department of Rural, Environmental and Agricultural Development (North West, permit no. HO 20/02/18-057 NW); the Department of Economic, Small Business Development, Tourism and Environmental Affairs (DESTEA, Free State, permit no. JM 4066/2018); the Department of Economic Development, Environmental Affairs and Tourism (Eastern Cape, permit no. CRO 20/18CR, CRO 22/18CR) and CapeNature (Western Cape, permit no. CN44-31-6790) for approving permits for collection of fish in the respective provinces. Ethics approval for this study involving the use and handling of host species was obtained from the AnimCare Ethics Committee of North-West University, Project Ethics number: NWU-00159-18-A5.

2.4. Parasite processing: collection and fixation

2.4.1. Collection of parasites

Ectoparasites present on the skin, fins and gills were counted, removed with a dissection needle or super fine paint brush and placed in water. Internal organs were individually screened and endoparasites present were counted and removed with dissection needles (or glass pipette) and placed in a 0.9% saline solution. Both ecto-and endoparasites were properly cleaned and fixed according to accepted protocols for morphological identification or molecular analyses applicable to each taxon (see below). When possible, images of live specimens were captured and identification of parasites was done up to the highest taxonomic level in the field using a Zeiss Primo Star compound microscope with camera attachment and associated software.

2.4.2. Parasite fixation for morphological identification and molecular analyses

Monogenea

To study morphological features individual monogenean specimens were removed from the gill filaments and placed in a drop of water on a microscope slide, specimens were flattened with a coverslip and cleared in glycerine-ammonium picrate (GAP) (Malmberg 1970). Fixation for molecular analyses was either by fixing whole specimens in 96% molecular grade ethanol or a partial section following the protocol of Řehulková (2018). Identification of *Quadriacanthus* spp. and *Gyrodactylus* spp. found in this study was done to the lowest taxonomic level.

Digenea, Cestoda and Nematoda

Trematode specimens were relaxed with hot saline and subsequently placed in 96% ethanol for both morphological and molecular analyses. Following the protocols of Justine et al. (2012) and Scholz et al. (2018a), specimens belonging to Cestoda and Nematoda were fixed with hot formalin or saline and preserved in 70% ethanol for morphological identification. For molecular analyses, specimens were fixed as described for the Digenea and stored in 96% molecular grade ethanol.

Crustacea

Specimens were gently removed from the host using a fine paint brush, placed in a petri dish with clean tap water, cleaned of any debris (such as mucus) and subsequently fixed in 70–96% ethanol following Dávidová and Smit (2018).

2.4.3. Morphological and molecular characterisation of parasitic taxa

Representatives of the Monogenea, Digenea, Cestoda and Nematoda were all studied using a Nikon N*i* compound microscope (Nikon, Tokyo, Japan) with differential interference contrast (DIC) and camera attachment. Measurements of specimens were taken using NIS-Elements BR 4.60© software and line drawings of taxonomic relevant structures were made with the aid of a camera lucida attached to the microscope and digitalised using a Wacom Intuos® Pro drawing tablet and Adobe Illustrator™ software package (Adobe, Inc., California, United States of America). Further, all sequences obtained during the course of this study was subjected to a Basic Local Alignment Tool (BLAST, https://blast.ncbi.nlm.nih.gov/Blast.cgi) search to identify congeners or closest relatives for inclusion in alignments implemented under the default parameter of MUSCLE for the respective alignments for each taxon in Geneious® v.7.1.3. (Kearse et al. 2012). Genetic divergences were calculated using uncorrected p-distance model in MEGA 7.0.26 (Kumar et al. 2016) for all alignments.

Monogenea

The general morphology of the sclerotised haptoral and reproductive structures of all specimens cleared in GAP (as described previously) were studied. Morphometrics and structures considered of taxonomic relevance for Dactylogyridea (*Quadriacanthus*) followed Francová et al. (2017). For the Gyrodactylidea (*Gyrodactylus* and *Macrogyrodactylus*), Christison et al. (2005) (for species of *Gyrodactylus*) and a combination of features from Khalil and Mashego (1998), N'Douba and Lambert (1999) and Přikrylová and Gelnar (2008) were studied for *Macrogyrodactylus*. Morphometric data for all three genera are presented as mean, followed by range in parentheses in **Appendix A2**. All measurements are in micrometres. No molecular analyses were performed for *Quadriacanthus* and *Gyrodactylus*. See **Chapter 4** for detail on morphological and molecular characterisation of *Macrogyrodactylus* found during this study.

Digenea

To examine metacercariae, specimens were removed from the 96% molecular grade ethanol, rehydrated in a drop of distilled water, and a temporary wet mount on a microscope slide with a cover slip was made. A morphological analysis was completed for each representative through photomicrography of taxonomically relevant structures (i.e., whole specimens, oral sucker, pseudosuckers, pharynx, ventral sucker, holdfast organ, excretory pore) and other observations, such as spines on the body surface. One specimen of each morphotype or genus was selected

from each locality as a molecular representative. Deoxyribonucleic acid (DNA) was extracted using the PCRBIO Rapid Extract PCR Kit (PCRBiosystems available from Analytical Solutions, Randburg, South Africa) following the manufacturer's protocol. The final reaction was diluted with 450 µl of molecular grade water instead of 900 µl to obtain DNA at a higher concentration. The entire ITS1-5.8S- ITS2 region, the large subunit nuclear ribosomal RNA (*lsrDNA* or 28S rRNA), region and cytochrome *c* oxidase subunit I (*cox*1) were amplified in polymerase chain reactions (PCR) using primer set combinations provided in **Table 2.2.**

Adult specimens were grouped into morphotypes. A representative specimen from each morphotype and locality was selected, and a small section was cut for molecular analysis, the remainder of these specimens with other congeners were stained with Mayer's Haematoxylin and mounted in dammar gum, or alternatively stained with acetocarmine, as described in Vermaak et al. (2021). DNA extraction for these specimens were done using PCRBIO Rapid Extract PCR Kit with modified volumes of lysis buffer (10 µl instead of 20 µl), protease containing buffer (5 µl instead of 10 µl) and the final reaction was diluted with 450 µl of molecular grade water instead of 900 µl to obtain DNA at a higher concentration. The manufacturers' recommended procedures and incubation durations were followed. The small subunit nuclear ribosomal RNA (ssrDNA or 18S rRNA), ITS2 region and 28S rRNA gene region were amplified using primer sets provided in **Table 2.2.**

Table 2.2. Primers used for the polymerase chain reactions for various loci of metacercariae and adult Digenea found in the present study. (F) – forward primer, (R) – reverse primer.

Locus	Primer		Sequence	Reference
Metacercariae				
ITS1-5.8S-ITS2	D1	(F)	5' – AGG AAT TCC TGG TAA GTG CAA G – 3'	Galazzo et al. (2002)
	D2	(R)	5' – CGT TAC TGA GGG AAT CCT GGT – 3'	Galazzo et al. (2002)
28S[*]	Digl2	(F)	5' – AAG CAT ATC ACT AAG CGG – 3'	Tkach et al. (2001)
	1500R	(R)	5' – GCT ATC CTG AGG GAA ACT TCG – 3'	Snyder and Tkach (2001)
	ECD2[a]	(F)	5' – CTT GGT CCG TGT TTC AAG ACG GG – 3'	Tkach et al. (2003)
	300F[a]	(R)	5' – CAA GTA CCG TGA GGG AAA GTT G – 3'	Littlewood et al. (2000)
***cox*1**	MplatCOX1dF[b]	(F)	5' – TGT AAA ACG ACG GCC AGT TTW CIT TRG ATC ATA AG – 3'	Moszczynska et al. (2009)
	MplatCOX1dR[b]	(R)	5' – CAG GAA ACA GCT ATG ACT GAA AYA AYA IIG GAT CIC CAC C – 3'	Moszczynska et al. (2009)
	Dice1F[c]	(F)	5' – ATT AAC CCT CAC TAA ATT WCN TTR GAT CAT AAG – 3'	Van Steenkiste et al. (2015)
	Dice14R[c]	(R)	5' – TAA TAC GAC TCA CTA TAC CHA CMR TAA ACA TAT GAT G – 3'	Van Steenkiste et al. (2015)
Adults				
18S	JLR24	(F)	5' – CGG AAT TCG CTA GAG GTG AAA TTC TTG G – 3'	Campos et al. (1998)
	JLR25	(R)	5' – CCG AAT TCC GCA GGT TCA CCT ACG G – 3'	Campos et al. (1998)
ITS2	3S	(F)	5' – GGT ACC GGT GGA TCA CGT GGC TAG TG – 3'	Morgan and Blair (1995)
	ITS2.2	(R)	5' – CCT GGT TAG TTT CTT TTC CTC CGC – 3'	Cribb et al. (1998)

[a] Internal primers used only for sequencing.
[b] Primers used specifically in amplification and sequencing for the *cox*1 mitochondrial region of *Clinostomum*.
[c] Primers specifically used for amplification and sequencing the *cox*1 mitochondrial region of Tetracotyle-type, *Tylodelphys* and Xiphidiata metacercariae.
[*] Same primers, internal primers and PCR thermal cycling protocol was used for amplification of the 28S rDNA region of adult Digenea.

Cestoda

Representative specimens of all morphotypes were sent to Prof Tomáš Scholz from the Institute of Parasitology Biology Centre, České Budějovice, Czech Republic and Prof Roman Kuchta from the Czech Academy of Sciences, Prague, Czech Republic, for morphological identification. These specimens were stained with Mayer's Carmine and permanent mounts in Canada balsam were prepared. Voucher material was deposited into the Parasite Collection of the National Museum (NMB), Bloemfontein, South Africa, and the Helminthological Collection of the Institute of Parasitology, Czech Academy of Sciences, České Budějovice, Czech Republic (IPCAS). DNA was extracted from representative individuals using 200 µl of a 5% suspension of Chelex in deionised water and 2 µl of proteinase K, followed by incubation for 3 hours at 56 °C and at an increased temperature of 90 °C for 8 min, whereafter the reaction mixture was centrifuged at 15 000 rpm for 10 min. PCR amplification of the 28S rRNA gene and *cox*1 were made in 25 µl final volume consisting of 12.5 µl of DreamTaq DNA Polymerase (ThermoFisher Scientific), 9.5 µl of double distilled water, 1 µl of each primer listed in **Table 2.3.** and 1 µl of DNA extraction supernatant.

Nematoda

Representative individuals of all morphotypes were cleared in lactophenol and temporary mounts were made to study the relevant cephalic and reproductive structures to identify specimens to the lowest taxonomic level (Magath 1916; Scholz et al. 2018a; Moravec 2019). Photomicrographs were taken of taxonomically relevant structures and specimens were transferred back into 70% ethanol for storage. Dr. Roman Svitin from the National Academy of Sciences of Ukraine, Kyiv, Ukraine aided in the assignment of all the nematode specimens to genus or species level and provided insights on further processing of these specimens.

Molecular characterisation was done on conspecific individuals for each morphotype where a small section was excised from the medial region of the body so as to not destroy any taxonomically relevant structures at the anterior or posterior ends of the specimens. The excised section was stored in 96% molecular grade ethanol until DNA was extracted. Genomic DNA extraction for specimens of the Diochotophymatidae was done using the Zymo Research *Quick*-DNA Microprep Plus Kit (Inqaba Biotechnical Industries, Pretoria, South Africa) following the recommended protocol. The final DNA was diluted with 50 µl of DNA Elution Buffer instead of 10 µl. Alternatively, DNA was extracted using the Chelex Buffer methods as described in the previous section for the Cestoda. PCR amplification of the 18S rRNA gene were performed with a 25 µl final volume consisting of 12.5 µl of DreamTaq DNA Polymerase (ThermoFisher Scientific), 9.5 µl of double distilled water, 1 µl of each primer listed in **Table 2.4.** and 1 µl of DNA extraction supernatant. DNA extraction from specimens of the Acuariidae, Anisakidae, Camallanidae,

Cystidicolidae, Gnathostomatidae and Quimperiidae was done using the PCRBIO Rapid Extract PCR Kit following the manufacturer's protocol. The final reaction was diluted with 450 µl of molecular grade water instead of 900 µl to obtain DNA at a higher concentration. The entire 18S rRNA region, 28S rRNA region and mitochondrial cytochrome *c* oxidase subunit I (*cox*1) were amplified in polymerase chain reactions (PCR) using primer set combinations provided in **Table 2.4.**

Table 2.3. Primers used for the polymerase chain reactions for loci of Cestoda collected during the present study. (F) – forward primer, (R) – reverse primer.

Locus	Primer		Sequence	Reference
28S*	LSU5[a]	(F)	5′ – TAG GTC GAC CCG CTG AAY TTA AGC A – 3′	Littlewood et al. (2000)
	1500R	(R)	5’ – GCT ATC CTG AGG GAA ACT TCG – 3’	Olson et al. (2003)
	ZX-1[b]	(F)	5’ – ACC CGC TGA ATT TAA GCA TAT – 3’	Lockyer et al. (2003)
	L1200R[b]	(R)	5’ – GCATAGTTCACCATCTTTCGG – 3′′	Van der Auwera et al. (1994)
***cox*1**	PBI_cox1F_PCR	(F)	5’ – CAT TTT GCT GCC GGT CAR CAY ATG TTY TGR TTT TTT GG – 3’	Scholz et al. (2013)
	PBI_cox1R_PCR	(R)	5’ – CCT TTG TCG ATA CTG CCA AAR TAA TGC ATD GGR AA – 3’	Scholz et al. (2013)
	PBI_cox1F_seq[b]	(F)	5’ – CAT TTT GCT GCC GGT CA – 3’	Scholz et al. (2013)
	PBI_cox1R_seq[b]	(R)	5’ – TAA TGC ATD GGR AAA AAA C – 3’	Scholz et al. (2013)

[a] Primer only used for PCR reaction and not for sequencing of the 28S rDNA region.
[b] Internal primers used only for sequencing.
* Thermal cycling conditions follow Brabec et al. (2012).

Table 2.4. Primers used for the polymerase chain reactions for loci of Nematoda collected during the present study. (F) – forward primer, (R) – reverse primer.

Locus	Primer		Sequence	Reference
18S	18SU467F[a]	(F)	5' – ATC CAA GGA AGG CAG CAG GC – 3'	Suzuki et al. (2006)
	18SL1310R[a]	(R)	5' – CTC CAC CAA CTA AGA ACG GC – 3'	Suzuki et al. (2006)
	NSF4/18[*]	(F)	5' – CTG GTT GAT CCT GCC AGT – 3'	Sakaguchi et al. (2020)
	NSR1787/18[*]	(R)	5' – CGA CGG GCG GTG TGT ACA – 3'	Sakaguchi et al. (2020)
	18S-1192R/20[b]	(F)	5' – CAG GTG AGT TTT CCC GTG TT – 3'	Sakaguchi et al. (2020)
	NSF573/19[b]	(R)	5' – CGC GGT AAT TCC AGC TCC A – 3'	Sakaguchi et al. (2020)
	S1[***]	(F)	5' – ATT CCG ATA ACG AAC GAG ACT – 3'	Sinnappah et al. (2001)
	IR8[***]	(R)	5' – GCT AGC TGC GTT CTT CAT CGA – 3'	Kaci-Chaouch et al. (2008)
28S	LSU5[**]	(F)	5' – TAG GTC GAC CCG CTG AAY TTA AGC A –3'	Littelwood et al. (1997)
	LSU3[**]	(R)	5' – TAG AAG CTT CCT GAG GGA AAC TTC GG – 3'	Littelwood et al. (1997)
***cox*1**	LCO1490	(F)	5' – GGT CAA CAA ATC ATA AAG ATA TTG G – 3'	Svitin et al. (2018)
	HCO2198	(R)	5' –TAA ACT TCA GGG TGA CCA AAA AAT CA – 3'	Svitin et al. (2018)

[a] Used in the PCR amplification reaction and sequencing of the *Eustrongylides* p. from Zambia.
[b] Internal primers used for sequencing only.
[*] Thermal cycling conditions follow Svitin et al. (2021). [**] Thermal cycling conditions follow Verneau et al. (2009). [***] Used only for *Gendria* sp., thermal cycling conditions follow Sinsch et al. (2020).

Crustacea

Individual specimens were removed from 70% or molecular grade ethanol and their morphology were studied using light microscopy as well as scanning electron microscopy (SEM) to identify individuals to the lowest taxonomic level. Branchiuran specimens (*Argulus* Müller, 1785) were processed according to protocols adapted from Rushton-Mellor (1994), Lutsch and Avenant (1995) and Ider et al. (2014), while copepod specimens (*Ergasilus* von Nordmann, 1832 and *Lamproglena* Nordmann, 1832) were processed and identified morphologically following adapted protocols of Marx and Avenant-Oldewage (1996), Ider et al. (2014) and Waicheim et al. (2021). Where possible, the morphological identity of the Branchiura and Copepoda morphotypes were confirmed using molecular markers including the small (18S) and large (28S) ribosomal RNA regions and the *cox*1 mitochondrial gene region (Møller et al. 2008; Song et al. 2008; Neethling and Avenant-Oldewage 2020; Van As et al. 2021).

2.5. Construction of parasite distribution maps

Distribution maps of parasitic taxa of *C. gariepinus* in Africa was generated using the open-source Geographic Information System (GIS) QGIS 3.10.14. Desktop software package. Data points for parasitic taxa were compiled from coordinates of sampling localities provided in each publication. In the case where coordinates were not provided, a coordinate was assigned based on the site description or nearest town. Taxa not identified to species level but included in recent checklists were included in the records tables and distribution maps i.e., for metacercariae of *Diplostomum* sp. However, where authors did not provide sufficient data to justify the mention of a species and provided identification only up to genus level (i.e., use of *Genus* sp. or *Genus* spp.) for taxa, the record was not included in the record tables or distribution map. Similarly, records with “Unidentified nematode sp.” were also excluded and are only discussed in the text. Example: In Hassan et al. (2010) the authors do not provide morphological nor molecular proof of their identification of specimens and only refer collectively to worms found as *Procamallanus* spp.

2.6. Determination of metal concentrations

2.6.1. Sample collection

Muscle tissue samples were collected from the left side of each fish. The skin was removed from the sample and placed in a plastic container. All tissue samples were labelled appropriately and stored at -4°C during transportation and at -20°C in the laboratory until processed.

2.6.2. Digestion of tissues samples

Tissue samples were analysed for a standard set of metals. For quality control purposes, triplicate blanks were included in each digestion cycle and certified reference material DORM-4 Fish Protein for tissue (certified reference material, National Research Council, Canada), were prepared. A Labconco ® FreeZone 6 freeze dryer and Ethos Easy MAXI-44 Advanced Microwave

Digestion System were used to process and prepare samples. All weights were noted with a 0.001g accuracy.

Preparation of samples were as follows:
Tissue samples were defrosted at room temperature, wet weight was noted, followed by freeze drying and a dry weight was noted (to calculate water content). Each sample was weighed up to 0.2g and a volume of 7.5 mL of 65% Nitric acid (HNO_3) (Rochelle Chemical) and 2.5 mL of 37% hydrochloric acid (HCl) (Rochelle Chemical) were added to each vessel. Digestion was performed on a pre-programmed method consisting of three steps: 1) ramping period of 20 min. to reach 180°C; 2) a holding period of 15 min at 180°C with pressure ranging between 80–100 bar; and 3) a cool down period of 15 min. After the cool down, samples were decanted and diluted to 50 mL with 1% HNO_3.

2.6.3. Metal analysis

Diluted tissue samples were analysed by Eco-Analytica Laboratory for the following elements: aluminium (Al), arsenic (As), cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), gold (Au), lead (Pb), manganese (Mn), mercury (Hg), nickel (Ni), silver (Ag), Selenium (Se), Tin (Sn), vanadium (V), zinc (Zn) and Uranium (U).

2.7. Statistical analyses

Parasitic community structure

Descriptive statistics for parasitic infection for overall parasitic population and community composition were defined using prevalence (P), mean abundance (MA) mean intensity (MI), and intensity of infection (IF), and were determined according to Bush et al. (1997) where:

Prevalence is defined as the proportion of hosts infected with one or more taxa (or species), calculated as:

$$\text{Prevalence (P\%)} = \frac{Number\ of\ infected\ fish}{Total\ number\ of\ hosts\ examined} \times 100$$

Mean abundance is defined as the proportion of hosts infected by a specific parasite taxon. i.e., the total number of individuals of a parasite species divided by the total number of host individuals examined, calculated as follows:

$$\text{Mean abundance (MA)} = \frac{Number\ of\ parasites}{Total\ number\ of\ hosts\ examined}$$

The mean intensity is the average intensity of a specific parasite taxon or species among the infected host individuals, thus the number of parasites of a particular parasite's species divided by the number of infected host individuals, calculated as:

$$\text{Mean intensity (MI)} = \frac{Number\ of\ parasites}{Total\ number\ of\ infected\ \ hosts}$$

Further, parasitic communities were defined at the infra- and component community hierarchical levels, where the infracommunity is defined by all the parasitic taxa within an individual host, and the component community represent all taxa in a host species at a given locality at a given point in time (see Bush et al. 1997; Scholz et al. 2018c and references therein).

Infracommunity assemblages

Parasite diversity indices were calculated using the statistical software package PRIMER v6 (Clarke and Gorley 2006), based on the infracommunity abundance data, where the mean ± 95% confidence interval was scatter plotted for each locality using GraphPad Prism v8.4.2. (GraphPad Software, 2022). Infracommunity composition was determined using taxa richness (S) and abundance, and the diversity of finite parasite assemblages were defined using the Brillouin's diversity index (D_B).

$$D_B = \frac{1}{N}\left(\ln N \sum_{i=1}^{S_o} \ln n_i\right)$$

N – total number of individuals in the assemblage

n_i – number of individuals belonging to species i

Taxa richness was used to determine the total number of species present in a community, thus at infracommunity level the mean number parasite taxa per host individual and dominant species were identified using the Berger-Parker Index (d) (see Poulin 2015; Scholz et al. 2018c).

$$d = \frac{N_{max}}{N}$$

N_{max} – abundance of the most abundance species

N – total number of species

Component community assemblages

The component community composition was defined using several indices. The Shannon-Wiener Diversity Index (H') to characterise species diversity in terms of abundance and richness (S) (Heip

1998; Poulin 2015). Greater values of H' indicate a greater biodiversity in a given locality and was calculated as follows:

$$H' = \sum_{i=1}^{S} p_i \ln p_i$$

S – number of species
n – one particular species
N – total number of individuals
p_i – proportion (*n*/*N*) of individuals with one particular species

The Simpson's Diversity Index (D) was used to determine the probability of any individuals randomly drawn from the community belonging to the same species, calculated as follows (Poulin 2015):

$$D = \frac{\sum n\,(n-1)}{N\,(N-1)}$$

n – number of individuals displaying a single trait
N – total number of all individuals

Pielou's Evenness Index (J') described the even distribution between species at a given sampling point. In a healthy ecosystem it is accepted that an even distribution between species in a given community will occur and community stability can be related. Values of J' vary between 0 and 1, with 1 indicating an even distribution and 0 an uneven distribution. Evenness is defined as the Shannon-Wiener Index divided by the natural logarithm of species richness:

$$J' = \frac{H'}{\ln SR}$$

H' – Shannon-Wiener Index value
SR – number of species (richness)

Comparisons of parasitic community, biodiversity and abiotic factors

Statistical significance of the spatial and temporal variation of biodiversity indices and selected abiotic factors were determined at $p < 0.05$. Data sets were subjected to normality and homogeneity tests to determine the distribution of data. The D'Agostino & Pearson (omnibus K2) normality test was used for all indices, following a One-way analysis of variance (ANOVA) with Tukey's multiple comparison test as a post test for parametrically distributed data sets. In the case of non-parametric data sets, the Kruskal-Wallis tests with Dunn's multiple comparison test were used (Pyrxzak 2016). Factors included in multivariate analyses were the life cycle (monoxenous or heteroxenous) of parasitic taxa, and heteroxenous/monoxenous ratio were determined at

infracommunity level (S_H/S_M) (Sures et al 2017b). Explanatory factors included were condition factor of host (CF), host size (mm) and weight (g) and parasite life cycle (heteroxenous or monoxenous), while overlaying categorical factors were host sex (male, female or juvenile) and habitat type (dam or river). The community similarity analysis was performed using standardised (square root transformed) taxa abundance data, based on the Bray-Curtis similarity coefficients at infra- and component community level (Primer Version 6). Taxa contributing to similarity (SIMPER) and dissimilarity (ANOSIM) between localities were also determined, where distinct community compositions are represented by a Global R-value of > 0.49 and high similarity communities characterised with Global R-values of < 0.51. Additionally, data were subjected to a non-metric multidimensional scaling (nMDS), cluster analysis with Pearson's correlation matrix (> 0.1) to determine ordination of infracommunity based on similarities in community composition, and taxa at each of the localities. An unconstrained principal coordinate analysis (PCoA) was performed to visualise an ordination of selected explanatory factors (condition factor, host size and sex, life cycle and habitat) to determine patterns of similarity between the infracommunities. A species accumulation curve (UGE) was also plotted to determine if the true total number of parasite taxa for *C. gariepinus* were represented by the sampling effort of the present study (Ugland et al. 2003; Deng et al. 2015). Further, a redundancy analysis (RDA) bi-plot was constructed using the computational software Canoco v5.12 (Ter Braak and Šmilauer 2018) to summarise variation in taxon composition and diversity with an overlay of environmental variables (host metal concentrations). Generalised linear models (GLM) were used to visualise the correlation between response curves of taxa explained by environmental variables, significant differences were regarded as $p < 0.05$. Abundance data of taxa were transformed (square root), whereas environmental variable data was log transformed [$y = log\ (x + 1)$], centred and standardised.

Chapter 3

Metazoan parasites of the African sharptooth catfish, *Clarias gariepinus* (Burchell) in Africa

3.1. Introduction

One of the most widely distributed freshwater fish on the African continent is the African sharptooth catfish *Clarias gariepinus* (Burchell). It is naturally present in a variety of freshwater habitats in almost all ten ichthyofaunal regions on the continent, except for the Maghreb, Upper Guinea, Lower Guinea and southern region of the Southern Cape ichthyofaunal provinces (see **Chapter 2**, **Fig. 2.1.**) (Skelton and Teugels 1992; Lim et al. 2001; Skelton 2001; Turan and Turan 2016). Apart from pure intellectual curiosity and conservation, biodiversity and taxonomic interest in the parasite-fauna of *C. gariepinus*, the fact that this fish inhabits a wide range of habitats and tolerate extreme conditions renders it a sought-after fish in several countries in the northern and tropical regions of Africa. Throughout the continent the sharptooth catfish is a highly valued and relatively cheap staple to local communities and an ideal aquaculture species (Van der Waal 1972; Weyl et al. 2016b). Consequently, interest in the parasitic communities of *C. gariepinus* has become eminent as parasites can accidentally be ingested by humans when eating uncooked fish or introduced into culture systems through fish stocks supplied from local rivers affecting yield, growth, and marketability.

From available and accessible literature, it seems that the first report on parasitic species from *C. gariepinus* was in the early 20th century. The parasitic branchiuran *Argulus rhipidiophorus* Monod, 1931 (reported as *Argulus africanus* Thiele, 1900) and *Argulus striatus* Cunnington, 1913 were found on the head, gill arches and the roof of the mouth from host individuals reported as *Clarias lazera* Valenciennes and *Clarias robecchii* Vinciguerra (both synonyms of *C. gariepinus*) in Lake Tanganyika (Cunnington 191; Fryer 1965b). The first inclusive study on parasitic communities (not single parasite records) of *C. gariepinus* only appeared in the late 1900s, as in the earlier years parasitological reports and descriptions were mostly the results of exploratory expeditions or observations of extremely high infections, noticeable pathology, or fish mortalities. However, these expeditions laid the foundation for what defines fish parasitology today.

In 1971, Khalil published what would be one of the first checklists on the helminth parasites of several freshwater fishes in Africa. This included the Monogenea, Cestoda, Nematoda and Acanthocephala (Khalil 1971). Approximately 30 years later, in the late 1990s, Khalil and Polling

(1997) published the second, updated edition of the checklist, providing the most inclusive checklist of helminth parasite from African freshwater fishes (see below) up to that point. These early compilations on the parasitic fauna found from freshwater fishes in Africa included 45 species from *C. gariepinus* (despite it being a widely distributed species) and included 18 monogeneans, 11 digeneans, seven cestodes, eight nematodes and one acanthocephalan species. It is evident that dedicated research efforts were absent, and researchers were unable to exploit the undiscovered parasite diversity that the diverse freshwater fish community of the continent holds (Smit and Hadfield 2015; Van As 2015; Scholz et al. 2016). It was only recently that a more comprehensive checklist including helminths, crustaceans, and leeches from African freshwater fishes saw the light (Scholz et al. 2018c).

Several works needed completion and taxa called for scrutiny to contribute and realise such comprehensive and informative collections such as checklists and monographs. Contributions of indispensable value to past and present freshwater parasitologists and to this thesis where the parasite-fauna of *C. gariepinus* is under scrutiny, include the works of several pioneers, their mentors, mentees and colleagues and is briefly summarised below.

Regional checklists, such as the one on the Sudanese freshwater fishes by Khalil (1969), highlighted the lack of information on parasitic species which can be of economic importance as well as their ecology, pathogenicity, control of infections, or the influence of man-made structures on community structures. Kibet et al. (2019) provided a checklist of parasitic species of importance to freshwater fisheries and aquaculture, however the authors did not provide host-specific data or if any species have shown cause for concern. Nonetheless, the information simply of presence and absence of parasites provide valuable information on the distribution and biology of parasitic species. In South Africa, Van As and Basson (1984) compiled a checklist to summarise known parasitic taxa in our freshwater systems as a result of the increasing interest in recreational angling, aquaculture and the introduction of species for bio-control measures. The authors listed 20 parasite species from *C. gariepinus*, which was at the time also considered as an aquaculture species (Van der Waal 1972; Mashego 1977; Hecht et al. 1988).

Lim et al. (2001) provided a complete discussion and catalogue of the Dactylogyridea (Monogenea) of Old World Siluriformes, and Přikrylová et al. (2012, 2013) provided the first overview and novel molecular data of the Gyrodactylidea (Monogenea) of African catfishes. The trematode (Digenea) diversity, or rather lack thereof in African fishes was intensively discussed by Scholz et al. (2016), while Kuchta et al. (2012) did a re-evaluation of the Bothriocephalidea (Cestoda) parasitising African freshwater fishes. The monographs and several works of Frantisek Moravec (summarised in Moravec 2019) included conclusions after the scrutiny of the parasitic nematodes of the African freshwater fishes. Cunnington (1913, 1914) provided species

descriptions and notes on parasitic Crustacea (the Branchiura and Copepoda) from museum specimens collected during the Third Tanganyika Expedition (1904–1905).

Geoffrey Fryer published a series of monographic notes, reports, and species descriptions of parasitic crustaceans from his work on fishes from the Nile and Niger rivers in northern Africa, the African Great Lakes and specimens in museum collections or acquired from acquaintances and fellow parasitologists of that time (see Fryer 1956, 1959a, b, 1961, 1964, 1965a, b). He also published a collection on the biology and distribution of African parasitic Crustacea that included *C. gariepinus* as host (Fryer 1968). Oldewage and Avenant-Oldewage (1993) produced a checklist on parasitic Copepoda (Crustacea) of African fishes. Avenant-Oldewage and Knight (1994) presented a checklist on the genus *Chonopeltis* Thiele (Branchiura: Crustacea), Marx and Avenant-Oldewage (1996) redescribed the copepod *Lamproglena clariae* Fryer, 1956 (Copepoda: Crustacea) and accounted for its distribution throughout Africa. In their compendium, Neethling and Avenant-Oldewage (2016) complimented the current knowledge on the African branchiurans by condensing all literature available on their taxonomic validity, distribution, ecology, and biology into a single publication. Van As and Van As (2015) accounted for branchiurans in two major systems, the Okavango (Botswana) and Zambezi (Namibia), and more recently Smit et al. (2019) complimented global knowledge on parasitic Crustacea with a comprehensive book on the origin and role of these organisms in freshwater systems, with numerous references to African species. As for the Acanthocephala, few records are included in the checklists of Khalil (1971) and Khalil and Polling (1997), while the comprehensive checklist on acanthocephalans of vertebrate hosts from South Africa of Halajian et al. (2018) did not include *C. gariepinus* as a host to acanthocephalan species. Due to the lack of knowledge and research, not much can be said for the Pentastomida (Crustacea) and leeches (Hirudinea) of African freshwater fishes. These two parasitic groups are greatly understudied, the former rather well-known from their definitive vertebrate hosts and the latter are included in sporadic reports on their role as vector to blood parasites such as *Trypanosoma* Gruby, 1843 (Kinetoplastea: Protozoa) (Oosthuizen 1989, 1991; Oosthuizen and Curtis 1990; Oosthuizen and Siddall 2003; Junker et al. 2016; Smit et al. 2020). Smit et al. (2017) provided a first insight into all alien and invasive parasitic taxa from freshwater fishes in South Africa, that included spill-over of a co-invasive alien species, the fish lice *Argulus japonicus* Thiele, 1900 to *C. gariepinus* in various freshwater system in the country (see Smit et al. 2017 and references therein).

It is evident that by the turn of the 21st century efforts to explore and document parasitic diversity of African freshwater fishes increased tremendously, however, we are still in a deficit as there are no reports on any parasitic fauna from several African countries (Van As 2015; Scholz et al. 2018c) (see **Fig. 3.1.**).

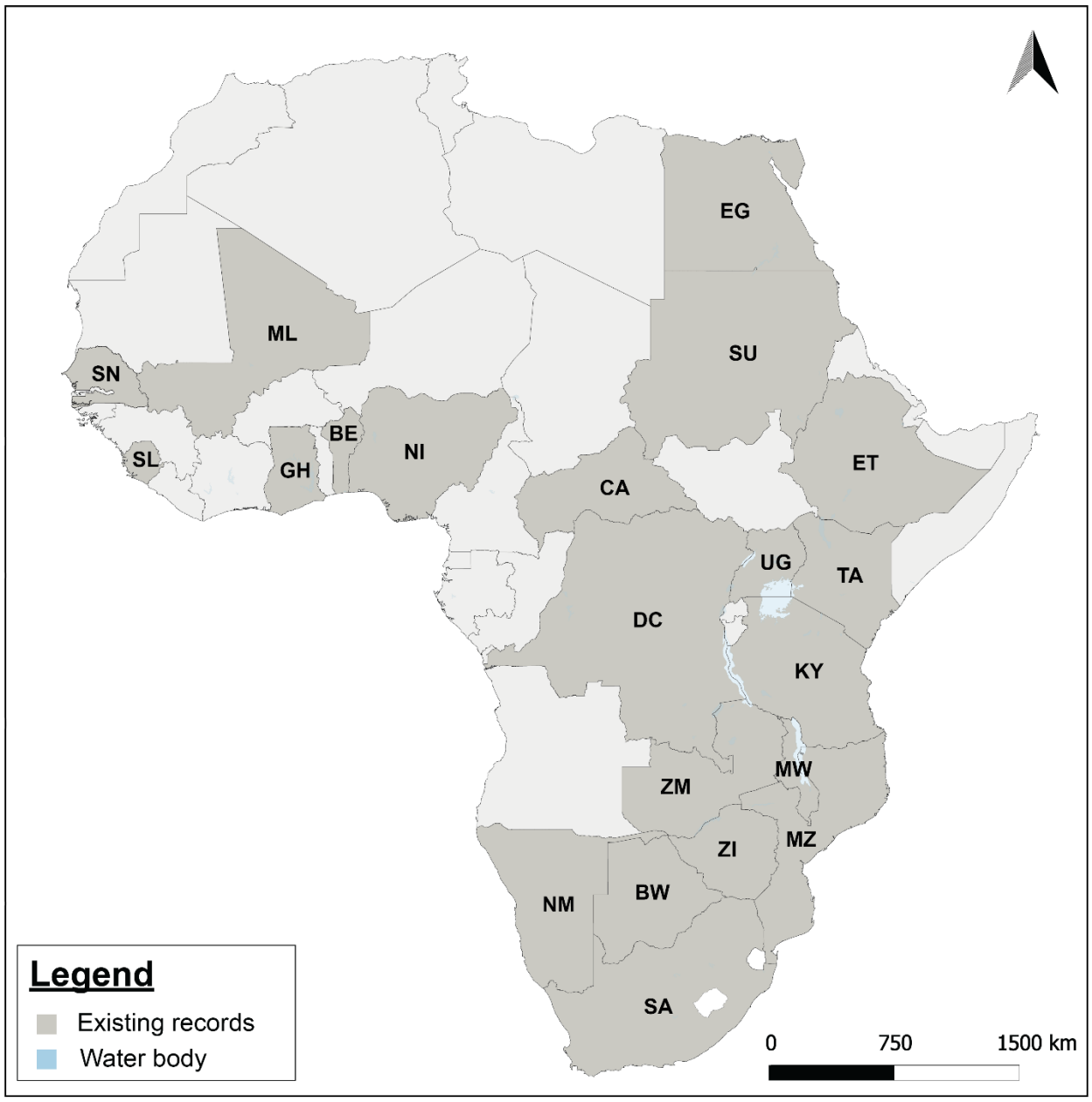

Figure 3.1. Map illustrating African countries (shaded) showing records of *Clarias gariepinus* (Burchell) with reported parasitic infections.

The most recent checklist on African freshwater fishes and their parasites, proves that exceptional effort has been made to produce an extensive overview and account on the known parasites of African freshwater fishes (Scholz et al. 2018c). In this document, *C. gariepinus* was identified as the species that hosts the most diverse parasitic communities on the continent, with ~107 metazoan parasitic species represented by 23 monogenean, 34 digenean, eight cestode, 20 nematode, four acanthocephalan, 17 crustacean and one hirudinid species (Scholz et al. 2018c; Carvalho Schaeffner 2018).

Below follows an account on the ~107 metazoan parasites infecting *C. gariepinus* in Africa, with a focus on their distribution throughout Africa, providing the first visual representation and literature verification for the checklist records. Data on infections found from native populations of *C. gariepinus* during the present study is also presented. Findings on the parasite communities extra-limital populations of *C. gariepinus* in the Riviersonderend (RSE), Western Cape and Great Fish River (GRF), Eastern Cape is presented in **Chapter 5**. Due to the diverse species contained in the genus *Clarias* Scopoli, all records from studied literature with records from *Clarias* sp. or *Clarias* spp. has been disregarded and are not included.

3.2. Class Monogenea

The Monogenea is divided into two subclasses, the Monopisthocotylea and Polyopisthocotylea that are primarily ectoparasites of fishes in freshwater, brackish and marine environments, with some species found associated with crustaceans, cephalopods amphibians, reptiles and even mammals (Rubio-Godoy 2007; Smit and Hadfield 2015). Several Monopisthocotylea have been found as endoparasites in the foregut, stomach and urinary bladder of their fish host (Madanire-Moyo and Avenant-Oldewage 2014; Řehulková et al. 2018), however here we will focus on ectoparasitic taxa of the Monopisthocotylea predominantly found on the skin, fins, gills, and rarely in the nostrils and mouth cavity of fishes. The life cycle of all monogenean species is simple (direct) where no intermediate host is required and transmission to new hosts is primarily dependent on direct contact and density of the host population within a freshwater system (Bakke et al. 2007; Řehulková et al. 2018). Individuals in this diverse group are hermaphroditic, where cross-fertilisation takes place, and oviparous or viviparous reproduction strategies are used. The keys to identification and classification of taxa within this group is highly dependent on the configuration of sclerotised structures in the opistohaptor (anterior end used for attachment to the host) and the reproductive system that may consist of soft tissue and/or sclerotised elements. Genera and species of the Monogenea usually exhibit a high affinity or specificity towards its host or a microhabitat on its preferred host (Bush et al. 2001; Arafa et al. 2009; Řehulková et al. 2018).

Species in two of the largest orders Dactylogyridea and Gyrodactylidea (Monogenea), are found to parasitise African freshwater fishes, with more than 5500 species allocated to ~750 genera

(Řehulková et al. 2018). From the most widely distributed clariid host in Africa, *C. gariepinus*, 23 monogenetic species from the Ancylodiscoididae (Dactylogyridea) (two genera, 12 species) and Gyrodactylidae (Gyrodactylidea) (two genera, 11 species) have been found, representing a fraction of the potential diversity that this host is able to contribute towards the helminth community on the continent (Přikrylová et al. 2012, 2013; Dos Santos et al. 2019). From the first checklists published by Khalil (1971) and Paperna (1979) a mere four and seven monogenetic species, respectively, from four African countries were known from *C. gariepinus*. By the time Khalil and Polling (1997) produced their comprehensive helminth checklist for African freshwater fishes, 16 monogenetic species from six African countries, an almost three-fold increase in monogenean species numbers, were recorded within a period of 18 years. In their review and validation of dactylogyridean species of Siluriformes, Lim (2001) included a total of eight species from the genera *Paraquadriacanthus* Ergens, 1988 and *Quadriacanthus* Paperna, 1961 (Ancylodiscoididae) for *C. gariepinus*, respectively, while Přikrylová et al. (2012, 2013) included gyrodactylids from all siluriform fishes in Africa in their review that included 3 species known from *C. gariepinus*. More recently, the checklist on all helminth parasites in African freshwaters listed 23 species from *C. gariepinus* (Řehulková et al. 2018) (see **Appendix A3**, **Table A3.1.** and **Table A3.2.**).

3.2.1. Order Dactylogyridea

Family Ancylodiscoididae

Paraquadriacanthus Ergens, 1988
(syn. *Quadriacanthoides* Kritsky et Kulo, 1988)

The monotypic genus *Paraquadriacanthus* was established by Ergens in 1988 based on two specimens found in the nasal cavities of *C. gariepinus* collected from the Nile River, Egypt and consists of a single species *Paraquadriacanthus nasalis* Ergens, 1988 (type species for the genus) (Ergens 1988). The species is characterised by the presence of seven pairs of marginal hooks, a pair of robust dorsal anchors with a well-developed inner and outer root, a pair of delicate ventral anchors with no differentiated roots, a complex dorsal bar and V-shaped ventral bar (**Fig. A1.1. A**–**C**). Inadvertently, Kritsky and Kulo (1988) proposed a new genus *Quadriacanthoides* Kritsky et Kulo, 1988 with type species, *Quadriacanthoides andersoni* Kritsky et Kulo, 1988 from a single specimen also collected from *C. gariepinus* in the Nile River. After re-examination of the type specimens of both species, it was confirmed they resemble each other and based on the principle of priority *Q. andersoni* was designated as a junior synonym of *P. nasalis* (see Kritsky 1990). The distribution of *P. nasalis* is restricted to the northern regions of the Nilo-Sudanian ichthyofaunal region, only ever recorded from the Nile River, Egypt (see **Fig. 3.2. A.**).

Present study: Host specimens were not subjected to a nasal cavity search for metazoan parasites, and none collected conformed to the characteristics of this genus.

Quadriacanthus Paperna, 1961
(syn. *Anacornautus* Dubey, Gupta et Argawal, 2004)

Paperna (1961) proposed the genus *Quadriacanthus* based on specimens from the gills of *C. gariepinus* collected in Israel; the type species is *Quadriacanthus clariadis* Paperna, 1961. Representative species can be distinguished from other dactylogyrideans in having a dorsal and ventral pair of anchors with accessory sclerites, a dorsal bar (V-shaped or Y-shaped) and a medially articulating ventral bar, a basally articulating copulatory tube (unsclerotised) with a sclerotised accessory piece in the male copulatory complex (MCO) and has seven pairs of marginal hooks following the ancyrocephaline distribution (Kritsky and Kulo 1988; Tripathi et al. 2007; Francová et al. 2017). Species from the genus have been reported from the gills of siluriform hosts in the Clariidae, Bagridae and phylogenetically distant Notopteridae (Osteoglaniformes) (see Nack et al. 2016; Francová et al. 2017). The genus diagnosis and its species have been amended on several occasions regarding generic characters. Kritsky and Kulo (1988) noted the medially articulating components of the ventral bar, not reported by Paperna (1961). Dubey et al. (1991), evidently unaware of the work of Kritsky and Kulo (1988), established *Anacornautus* Dubey, Gupta et Argawal, 1992 for species that possess the articulating ventral bar and Lim et al. (2001) synonymised *Anacornautus* with *Quadriacanthus* and placed its two species (then) as "*species inquirenda*".

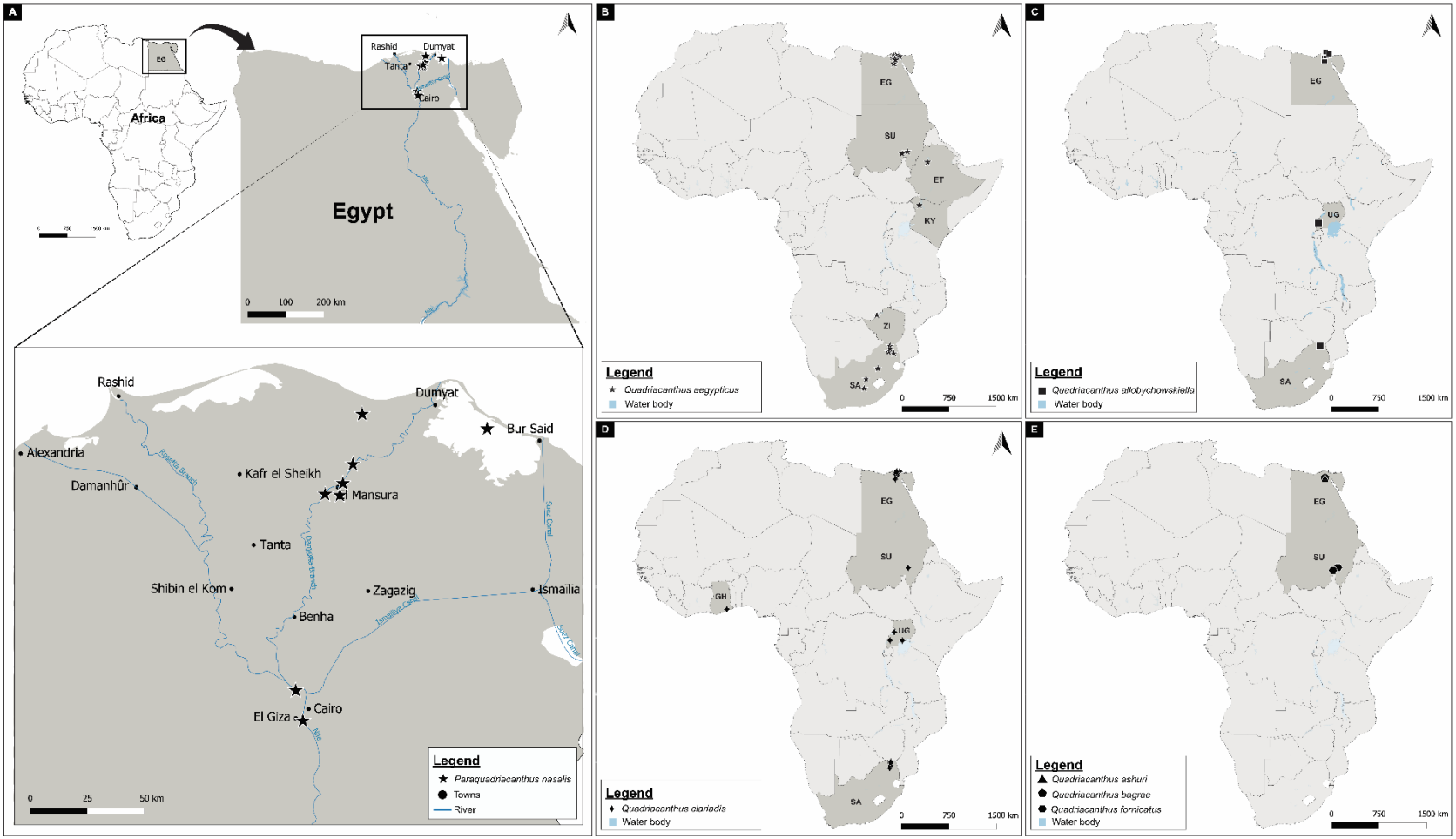

Figure 3.2. Distribution of **A** – *Paraquadriacanthus nasalis* Ergens, 1988; **B** – *Quadriacanthus aegypticus* El-Naggar et Serag, 1985; **C** – *Quadriacanthus allobychowskiella* Paperna, 1979; **D** – *Quadriacanthus clariadis* Paperna, 1961; and **E** – *Quadriacanthus ashuri* Kritsky et Kulo, 1988; *Quadriacanthus bagrae* Paperna, 1979 and *Quadriacanthus fornicatus* Francová et Řehulková, 2017 from *Clarias gariepinus* (Burchell) in Africa.

Currently, the genus *Quadriacanthus* contains 36 recognised species from African fishes, of which 11 are known from *C. gariepinus*, and are widely distributed throughout the continent (see **Appendix A3**, **Table A3.1.** and **Table A3.2.**). Paperna's (1979) checklist proposed three sub-species for *Q. clariadis* (**Fig. A1.1. D**–**E**) from the sharptooth catfish: *Quadriacanthus clariadis bagrae* Paperna, 1979; *Quadriacanthus clariadis clariadis* Paperna, 1979 and *Quadriacanthus clariadis allobychowskiella* Paperna, 1979. Continued confusion of *Q. clariadis* with the three sub-species and *Quadriacanthus aegypticus* El-Naggar et Serag, 1985 (**Fig. A1.2. A**–**B**) described from *C. gariepinus* in Egypt prompted the re-evaluation of the type-material. The three sub-species were elevated to species rank and supplemented with resolution on species specific features or redescriptions to differentiate them from *Q. clariadis* (Kritsky and Kulo 1988). Elevation of *Q. c. allobychowskiella* to species rank as *Quadriacanthus allobychowskiella* is based on its notable large and complex dorsal anchor accessory piece morphology (see **Fig. A1.2. C**–**E**). The proposed species *Quadriacanthus kearni* El-Naggar et Serag, 1985 was synonymised with *Q. allobychowskiella*, however several authors still recognise the former as a separate species. This can be understood when looking at the representation of the male copulatory organ by El-Naggar and Serag (1985) that differs markedly from *Q. allobychowskiella* (Mashalay et al. 2019). *Quadriacanthus bagrae* (syn. *Q. c. bagrae*) possess a dorsal anchor with an elongate point and the vagina is unsclerotised, both features easily separate this species from *Q. clariadis* (see **Fig. A1.1. D**–**E** and **Fig. A1.3. A**–**B**).

The species *Quadriacanthus ashuri* Kritsky et Kulo, 1988 (**Fig. A1.3. C**), *Quadriacanthus numidus* Kritsky et Kulo, 1988 (**Fig. A1.3. D**), *Quadriacanthus papernai* Kritsky et Kulo, 1988 (**Fig. A1.3. E**) *Quadriacanthus voltaensis* Paperna, 1965 (**Fig. A1.3. F**), except *Q. voltaensis*, possess a double curved point of the ventral anchor, differentiated from each other in the 1) anchor point size of ventral anchor; 2) size of the anchor pairs; and 3) MCO complex morphology (Kritsky and Kulo 1988). The most recent species described from *C. gariepinus* are *Quadriacanthus fornicatus* Francová et Řehulková, 2017 (**Fig. A1.4. A**); *Quadriacanthus pravus* Francová et Řehulková, 2017 (**Fig. A1.4. B**) and *Quadriacanthus zuheiri* Francová et Řehulková, 2017 (**Fig. A1.4. C**) from waters of the Nile River basin in Sudan (see **Fig. 3.2. E** and **Fig. 3.3. B**, **C**). All three species have been noted as being similar to known species infecting siluriform hosts but exhibit distinct morphological features in either one of the following or a combination thereof: 1) the size and morphology of the anchors and its accessory piece (i.e., double curved tip of ventral anchor or triangular shaped dorsal anchor accessory piece); 2) the size of the entire MCO complex; and 3) the complexity of the MCO accessory piece (Francová et al. 2017).

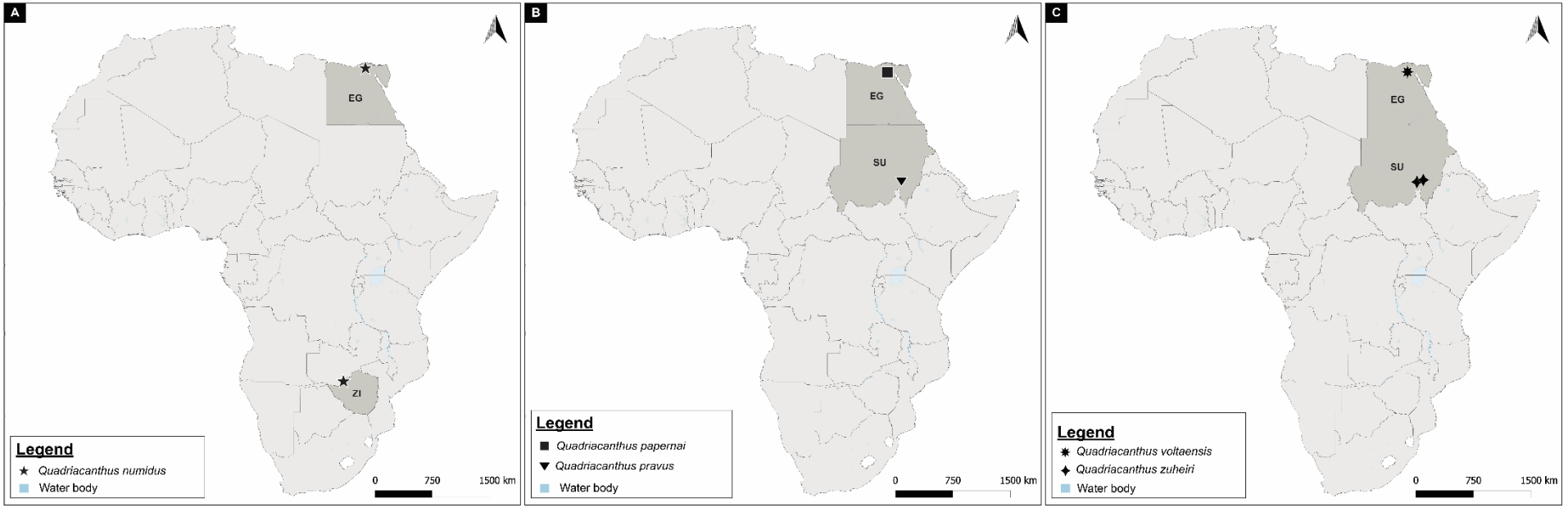

Figure 3.3. Distribution of **A** – *Quadriacanthus numidus* Kritsky et Kulo, 1988; **B** – *Quadriacanthus papernai* Kritsky et Kulo, 1988 and *Quadriacanthus pravus* Francová et Řehulková, 2017 and **C** – *Quadriacanthus voltaensis* Paperna, 1965 and *Quadriacanthus zuheiri* Francová et Řehulková, 2017 from *Clarias gariepinus* (Burchell) in Africa.

Paperna and Thurston (1968) reported on *Q. clariadis* from *C. gariepinus* in Uganda but was most likely dealing with *Q. aegypticus* based on the drawing provided of the accessory piece of the MCO (Kritsky and Kulo 1988). Photographs and drawings provided by Molnár and Mossalam (1985) indicate that they erroneously reported on a single species (*Q. clariadis*) or did not distinguish between the two species as *Q. aegypticus* (evident from the MCO in the drawings) and *Q. clariadis* (haptoral sclerite morphology in figures provided differ) (Kritsky and Kulo 1988). Douëllou and Chishawa (1995) erred in their line drawings of *Q. aegypticus* and *Q. bagrae,* most probably providing the illustrations of *Q. clariadis* (captioned as *Q. aegypticus* and *Q. bagrae*, respectively). This was later corrected (see Francová et al. 2017). A redescription of *Q. clariadis* (syn. *Q. c. clariadis*) highlight all discrepancies that distinguish *Q. clariadis* from all species mentioned thus far in that *C. clariadis*, 1) possesses a short, curved ventral anchor shaft; 2) a delicate sinistral vagina; 3) the dorsal anchors have a small accessory sclerite with unequal wings; 4) the terminal hook of the accessory piece is poorly developed and 5) the dorsal anchor has a short point (Kritsky and Kulo 1988; Francová et al. 2017).

Quadriacanthus aegypticus is currently the most widely distributed species of *Quadriacanthus* in Africa, followed by *Q. clariadis* (see **Fig. 3.2. B**, **D**). Both species have been found on *C. gariepinus* from various localities in the Nile River basin in Egypt and from the freshwaters of Sudan and various records exist from freshwater systems in South Africa. Single records for the two aforementioned species have also been reported from Ethiopia, Ghana, Kenya, Uganda and Zimbabwe. The Gariep Dam and Middle Letaba Dam (South Africa) are the southernmost distribution records for *Q. aegypticus* and *Q. clariadis* from *C. gariepinus*, respectively. The distribution of *Q. allobychowskiella* is limited to records from Egypt, Uganda and South Africa and *Q. bagrae* has only been recorded from *C. gariepinus* in Egypt as the record from Zimbabwe is deemed erroneous (see **Fig. 3.2. E**). The distribution of *Q. ashuri*, *Q. fornicatus* and *Q. numidus* (**Fig. 3.2. E** and **Fig. 3.3. A**) and *Q. papernai*, *Q. pravus*, *Q. voltaensis* and *Q. zuheiri* (**Fig. 3.3. B**–**C**) throughout the continent is rather scant. *Quadriacanthus ashuri*, *Q. papernai* and *Q. voltaensis* have only been recorded from the Nile River in Egypt, and *Q. fornicatus*, *Q. pravus* and *Q. zuheiri* are only known from the Blue Nile in Sudan.

Furthermore, a suspected accidental infection of *Oreochromis esculentus* [syn. *Tilapia esculenta* (Graham)] with *Quadriacanthus tilapiae* Paperna, 1973 is reported and the deficiency of diversity in the genus, and their distribution is evident from literature and the 16 different morphotypes reported in Africa not assigned to a species (see **Table 3.1.**). Two specimens, designated as *Quadriacanthus* sp. 1 and *Quadriacanthus* sp. 2 are reported from a closely related host species of *C. gariepinus*, *Heterobranchus isopterus* Bleeker, from Ghana and are presented in the work of Kritsky and Kulo (1988). In his unpublished thesis, Christison (2002) reported on two species

of *Quadriacanthus* from *C. gariepinus* and *Clarias theodorae* Weber, respectively, collected from various localities in the Okavango Delta, Botswana. The author indicated these morphotypes resemble *Q. aegypticus* and *Q. clariadis,* respectively when haptoral sclerite morphology is considered. However, differentiation from *Q. aegypticus*, Q. *agnebiensis* N'Douba, Lambert et Euzet, 1999 (ex. *H. isopterus*) and *Q. longifilisi* N'Douba, Lambert et Euzet, 1999 (ex. *H. longifilis*) is based on discrepancies in the size and morphology of the male copulatory complex, thus a re-examination is needed to confirm their identity (Christison 2002). Ten morphotypes of *Quadriacanthus* were reported from *C. gariepinus* collected in the Middle Letaba Dam, South Africa (Olivier et al. 2009). The authors did not provide a diagnosis indicating if the specimens represent new species or resemble known species of *Quadriacanthus* (see Olivier et al. 2009). More recently, Beletew et al. (2016) reported two undescribed species collected from *C. gariepinus* in Lake Tana, Ethiopia. The authors provided a short diagnosis of the specimens, designated them as *Quadriacanthus* sp. 1, closely resembling *Quadriacanthus simplex* N'Douba, Lambert et Euzet, 1999 (ex. *H. isopterus*) and *Quadriacanthus* sp. 2 that possess similar haptoral and reproductive sclerite morphology to *Q. clariadis* and *Q. longifilisi,* but are larger in overall size with a more slender and curved copulatory tube. All the above-mentioned specimens need re-examination to confirm if they indeed represent known or new species.

Table 3.1. List of undescribed specimens of *Quadriacanthus* Paperna, 1961 from siluriform hosts in Africa.

		Host	Country	Type material	Reference
1.	*Quadriacanthus* sp. 1	*Heterobranchus isopterus*	Ghana	MRAC 25.521	Kritsky and Kulo (1988)
2.	*Quadriacanthus* sp. 2	*Heterobranchus isopterus*	Ghana	MRAC 25.521	Kritsky and Kulo (1988)
3.	*Quadriacanthus katalensis*	*Clarias gariepinus*	Botswana	2001/10/04/09-07 and 2001/10/04/09-08 collection of Aquatic Parasitology, University of the Free State	Christison (2002)
4.	*Quadriacanthus theodorae*	*Clarias theodorae*	Botswana	99/07/20-08 and 99/07/20-09 collection of Aquatic Parasitology, University of the Free State	Christison (2002)
5.	*Quadriacanthus* sp. 1	*Clarias gariepinus*	South Africa	–	Olivier et al. (2009)
6.	*Quadriacanthus* sp. 2	*Clarias gariepinus*	South Africa	–	Olivier et al. (2009)
7.	*Quadriacanthus* sp. 3	*Clarias gariepinus*	South Africa	–	Olivier et al. (2009)
8.	*Quadriacanthus* sp. 4	*Clarias gariepinus*	South Africa	–	Olivier et al. (2009)
9.	*Quadriacanthus* sp. 5	*Clarias gariepinus*	South Africa	–	Olivier et al. (2009)
10.	*Quadriacanthus* sp. 6	*Clarias gariepinus*	South Africa	–	Olivier et al. (2009)
11.	*Quadriacanthus* sp. 7	*Clarias gariepinus*	South Africa	–	Olivier et al. (2009)
12.	*Quadriacanthus* sp. 8	*Clarias gariepinus*	South Africa	–	Olivier et al. (2009)
13.	*Quadriacanthus* sp. 9	*Clarias gariepinus*	South Africa	–	Olivier et al. (2009)
14.	*Quadriacanthus* sp. 10	*Clarias gariepinus*	South Africa	–	Olivier et al. (2009)
15.	*Quadriacanthus* sp. 1	*Clarias gariepinus*	Ethiopia	MRAC MT 37810, MRAC MT 37826	Beletew et al. (2016)
16.	*Quadriacanthus* sp. 2	*Clarias gariepinus*	Ethiopia	MRAC MT 37811	Beletew et al. (2016)

Present study: A total of eight species of *Quadriacanthus* were found to infect the gills of *C. gariepinus*. Presence and absence of species at the localities investigated in the native distribution range of *C. gariepinus* are presented in **Table A5.1.** and **Fig. 3.4**. Below follows a summary on the species found and a detailed account of the species. A morphological comparison to congeners is presented in **Appendix A2.**

Six species, *Quadriacanthus* sp. A–F, found from the gills of *C. gariepinus* in the present study resemble known species from clariid and bagrid hosts, while two, *Quadriacanthus* sp. G–H, are possibly new to the parasite fauna of *C. gariepinus* in Africa. *Quadriacanthus* sp. A (**Fig. 3.5. A**–**E** and **Table A2.1.**) was present on the gills of *C. gariepinus* at all native range localities, and *Quadriacanthus* sp. B (**Fig. 3.5. F**–**I** and **Table A2.3.**) was present at all localities sampled in the host's native distribution range, except the Kabompo River (KR). These two species are similar in the haptoral sclerite and MCO morphology of *Q. aegypticus* and *Q. fornicatus*, respectively (see **Table A2.2.** and **Table A2.4.**). *Quadriacanthus* sp. C (**Fig. 3.5. J**–**M** and **Table A2.5.**) was found at all native range localities, except sites within the Lower Phongolo River and floodplain (NGR). Morphologically *Quadriacanthus* sp. C resembles *Q. clariadis* (see **Table A2.6.**). *Quadriacanthus* sp. D (**Fig. 3.6. A**–**I** and **Table A2.7.**) is markedly similar to *Q. allobychowskiella* in having a complex accessory piece for the dorsal anchors. The dorsal anchor accessory piece resembles the morphology type presented by El-Naggar and Serag (1985) (see **Fig. A1.2. C**) and Kritsky and Kulo (1988) (**Fig. A1.2. D**), while the MCO morphology is similar to the type presented for *Q. kearni sensu* El-Naggar and Serag (1985) (see **Fig. A1.2. E** and **Table A2.7.** and **Table A2.8.** for morphometric comparisons). This species was absent from the KR and localities in NGR. *Quadriacanthus* sp. E (**Fig. 3.7. A**–**D**, **Table A2.9.**) was present on the gills of *C. gariepinus* from the Barotse floodplain (ZR) and localities in the NGR. The specimens studied share similarity in the haptoral sclerite morphology with four known species, *Q. ashuri*, *Q. numidus*, *Q. papernai* and *Q. pravus* in having a double curved tip of the ventral anchor (see **Fig. A1.3. C**, **D** and **Fig. A1.4. B**; **Table A2.10.**). However, based on similarities in both haptor sclerite as well as MCO morphology and size it is assigned to *Q. pravus. Quadriacanthus* sp. F (**Fig. 3.8. A**–**D** and **Table A2.11.**) also retain the same haptoral morphology as *Q. ashuri*, *Q. numidus*, *Q. papernai* and *Q. pravus* in having a double curved tip of the ventral anchor. However, *Quadriacanthus* sp. F can easily be distinguished from these species in having a tapering dorsal bar mid-region. This species can also be easily separated from the aforementioned four species in having a large MCO with a heavily sclerotised accessory piece, very similar to that of *Quadriacanthus gourenei* N'Douba, Lambert et Euzet, 1999 from *H. isopterus* (see **Table A2.10.** and N'Douba et al. 1999). This species was only present on the gills of *C. gariepinus* in the ZR.

Identification to species level was not possible for the following two morphotypes: *Quadriacanthus* sp. G (**Fig. 3.8. E**–**F** and **Table A2.11.**) retains the haptoral sclerite type of *Q. aegypticus* and has a MCO similar to *Quadriacanthus mandibulatus* Francová et Řehulková, 2017 described from *Heterobranchus bidorsalis* Geoffrey Saint-Hilaire. Dissimilarities can be observed in the overall size of *Quadriacanthus* sp. G if compared to *Q. aegypticus* and *Q. mandibulatus* (see **Table A2.2.** and **Table A2.12.**). Present on *C. gariepinus* from ZR only. *Quadriacanthus* sp. H (**Fig. 3.9. A**–**C** and **Table A2.13.**) shares haptoral sclerite morphology with *Q. aegypticus* and *Q. clariadis*, however it possesses a distinct MCO, not previously noted. This is a widely distributed species, present on *C. gariepinus* in the KR, ZR, Boskop Dam (BS), localities in NGR, the Sand River (SR) and Gariep Dam (GD).

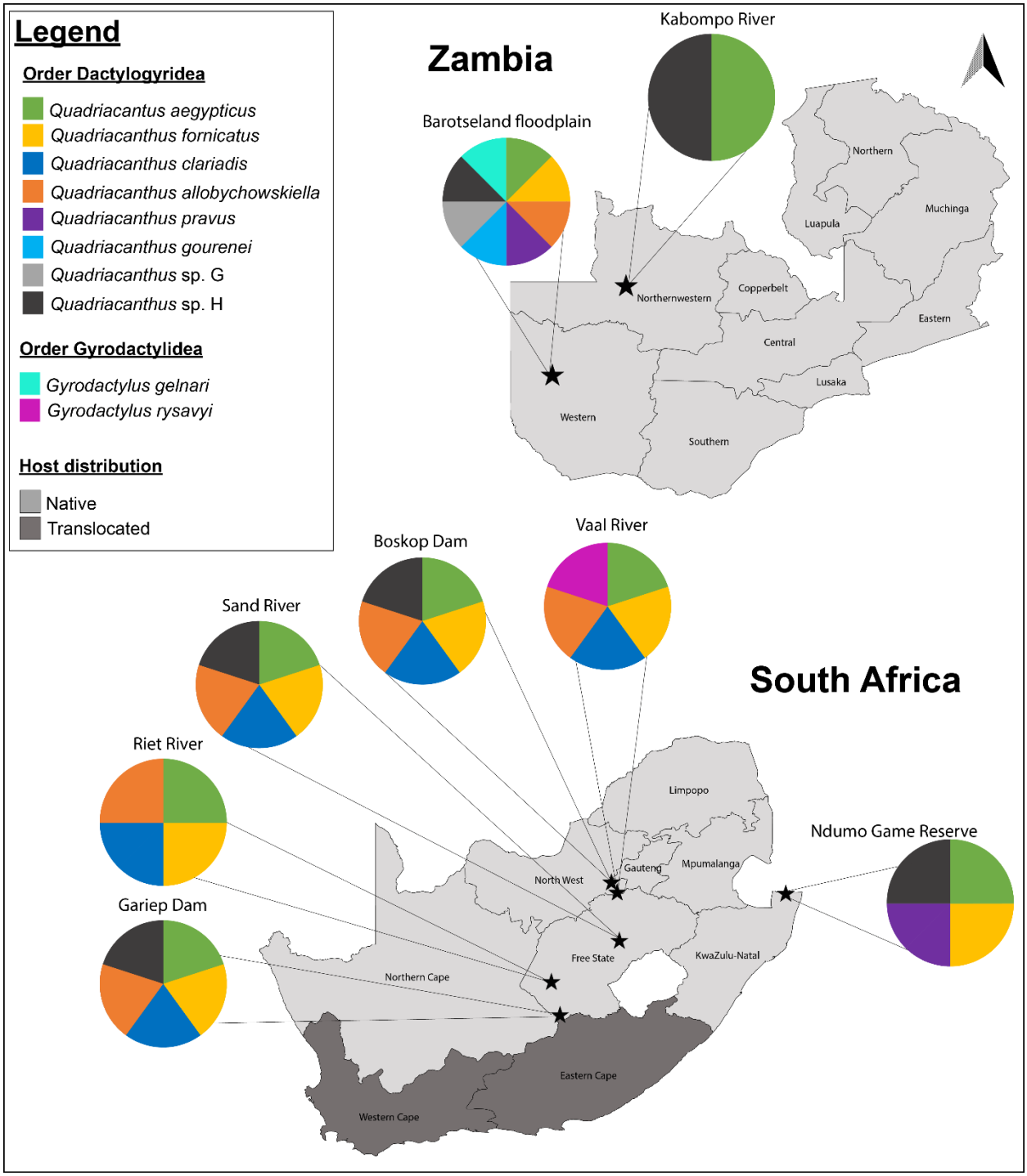

Figure 3.4. Map depicting presence and absence of species of *Quadriacanthus* Paperna, 1961 and *Gyrodactylus* von Nordmann, 1832 found on the gills of *Clarias gariepinus* (Burchell) during the present study. Note that the sectors of the pie-chart do not depict any numerical or statistical value regarding prevalence, intensity of infection or other infection statistic.

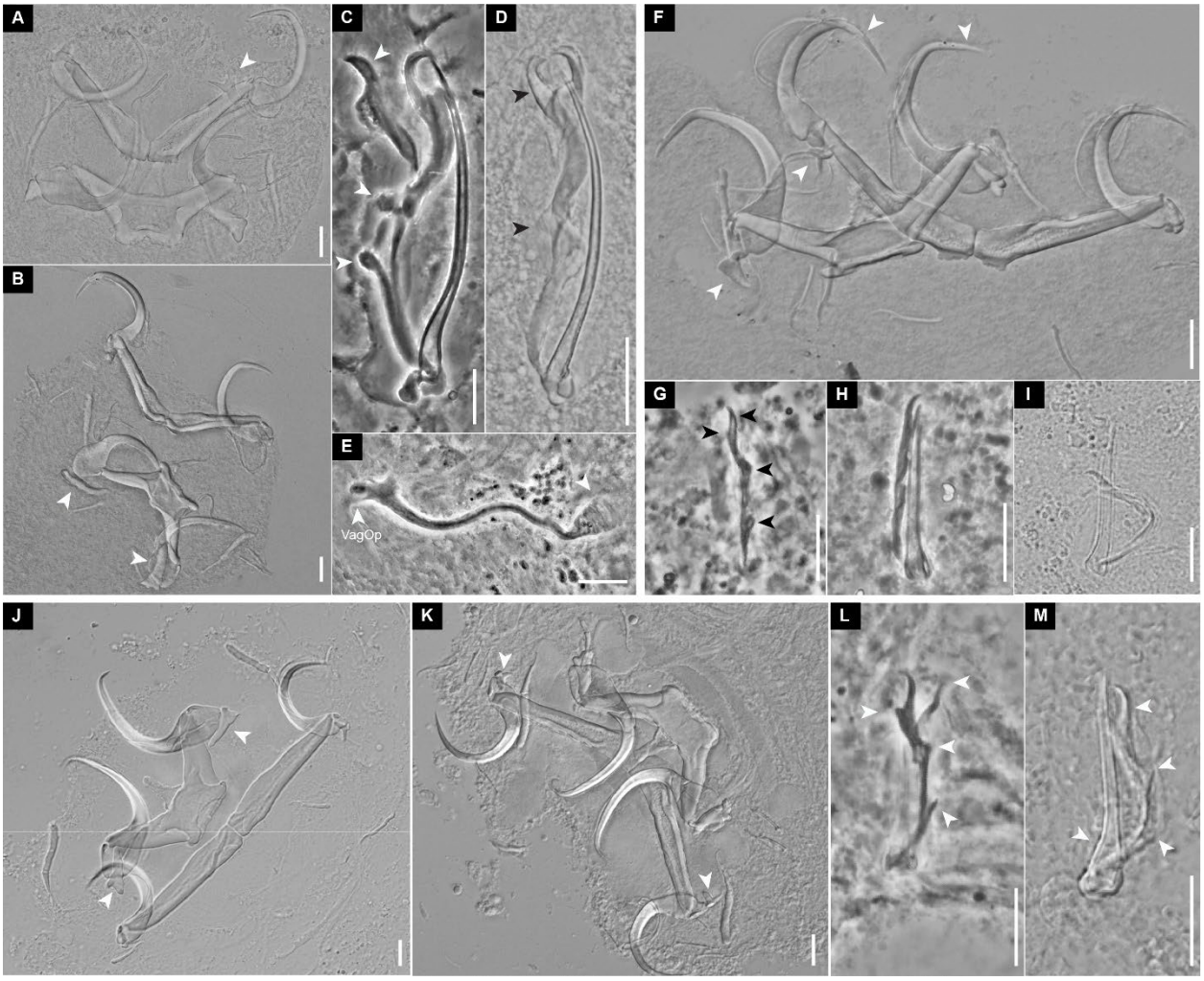

Figure 3.5. Micrographs of *Quadriacanthus aegypticus* El-Naggar et Serag, 1985 (A–E), *Quadriacanthus fornicatus* Francová et Řehulková, 2017 (F–I) and *Quadriacanthus clariadis* Paperna, 1961 (J–M) infecting the gills of *Clarias gariepinus* (Burchell) in the present study. **A**, **B**, **F**, **J**, **K** – Haptoral sclerite morphology. Arrows indicate variation in morphology of dorsal (B, F, J) and ventral anchor (A, F, K) accessory piece; **C**, **D**, **G**, **H**, **L**, **M** – Variation in male copulatory complex for each of the three species. Arrows indicate characters of interest and taxonomic significance in the accessory piece and **E** – Vagina, sclerotised (Vo – Opening of vagina). Scale bars: 10μm.

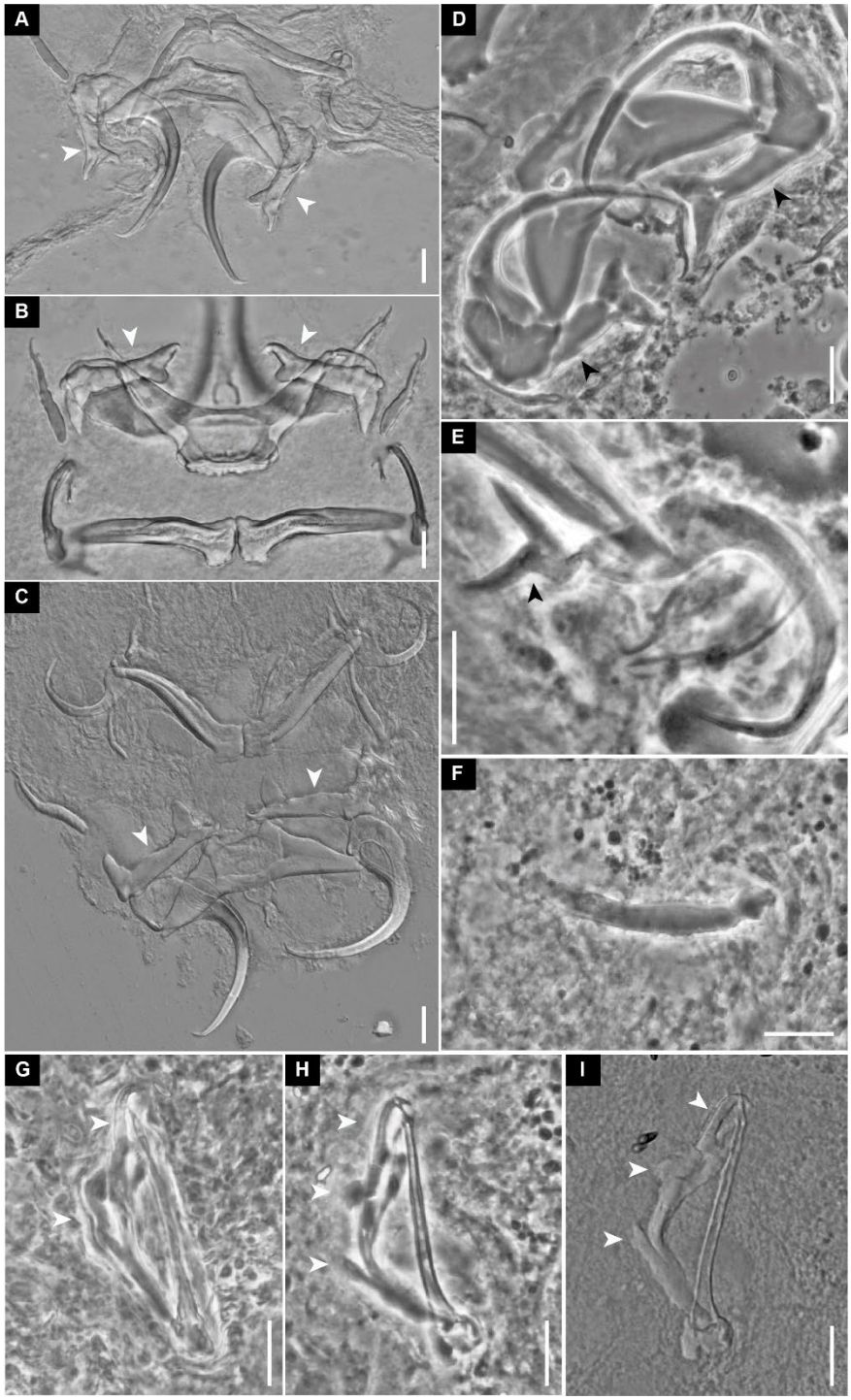

Figure 3.6. Micrographs of *Quadriacanthus allobychowskiella* Paperna, 1979 found from the gills of *Clarias gariepinus* (Burchell) in the present study. **A**–**D.** Haptoral sclerite morphology, with arrows indicating complex accessory piece of the dorsal anchor; **E** – Accessory piece morphology of the ventral anchors (black arrow); **F** – partially sclerotised vagina and **G**, **I** – Male copulatory complex with arrows indicating characters of taxonomic interest in the accessory piece. Scale bars: 10µm.

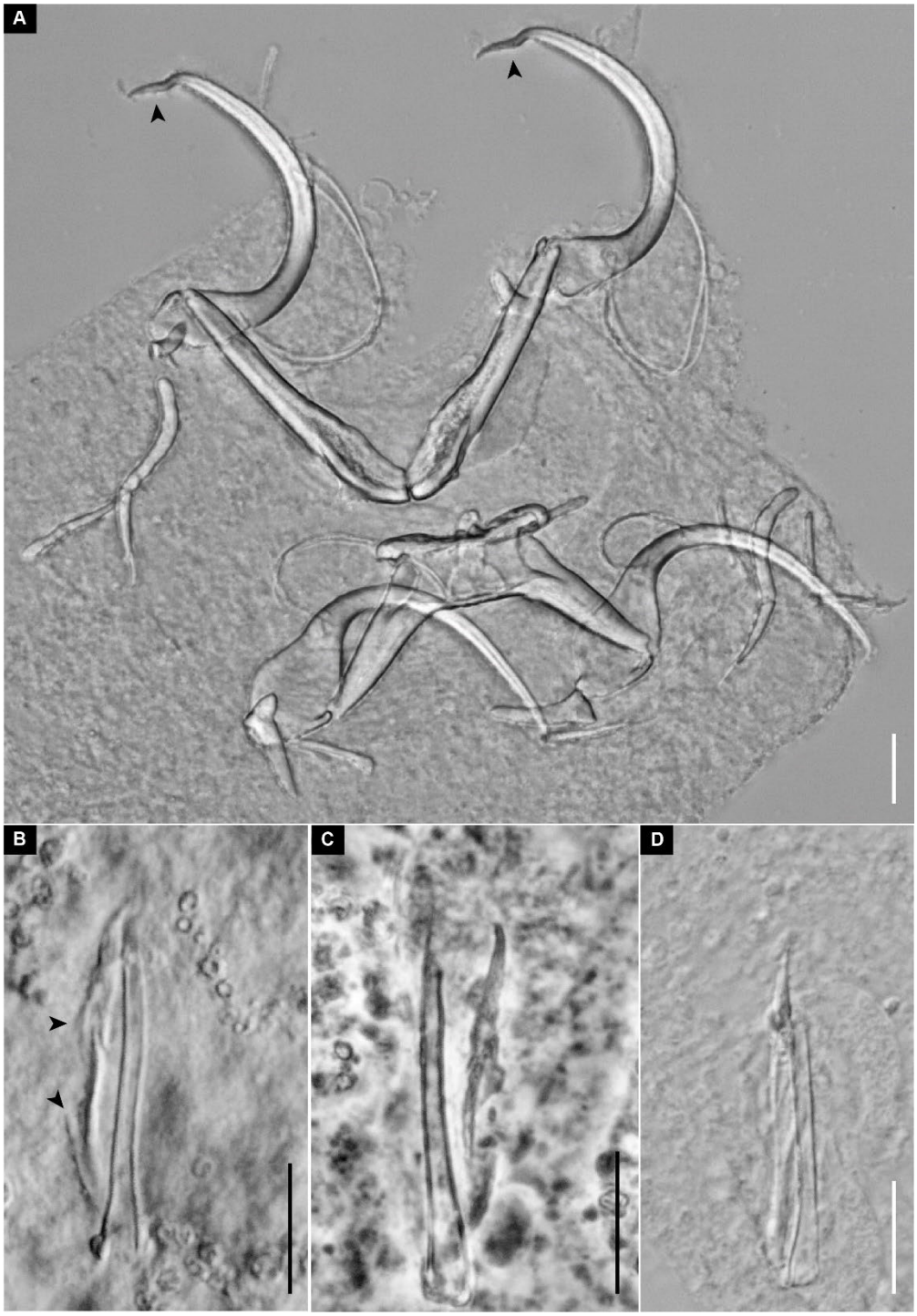

Figure 3.7. Micrographs of *Quadriacanthus pravus* Francová et Řehulková, 2017 infecting the gills of *Clarias gariepinus* (Burchell). **A** – Haptoral sclerite morphology (arrows indicate curved point of the ventral anchors; and **B**, **C**, **D** – Male copulatory organ (arrows indicate characters of taxonomic interest in the accessory piece). Scale bars: 10μm.

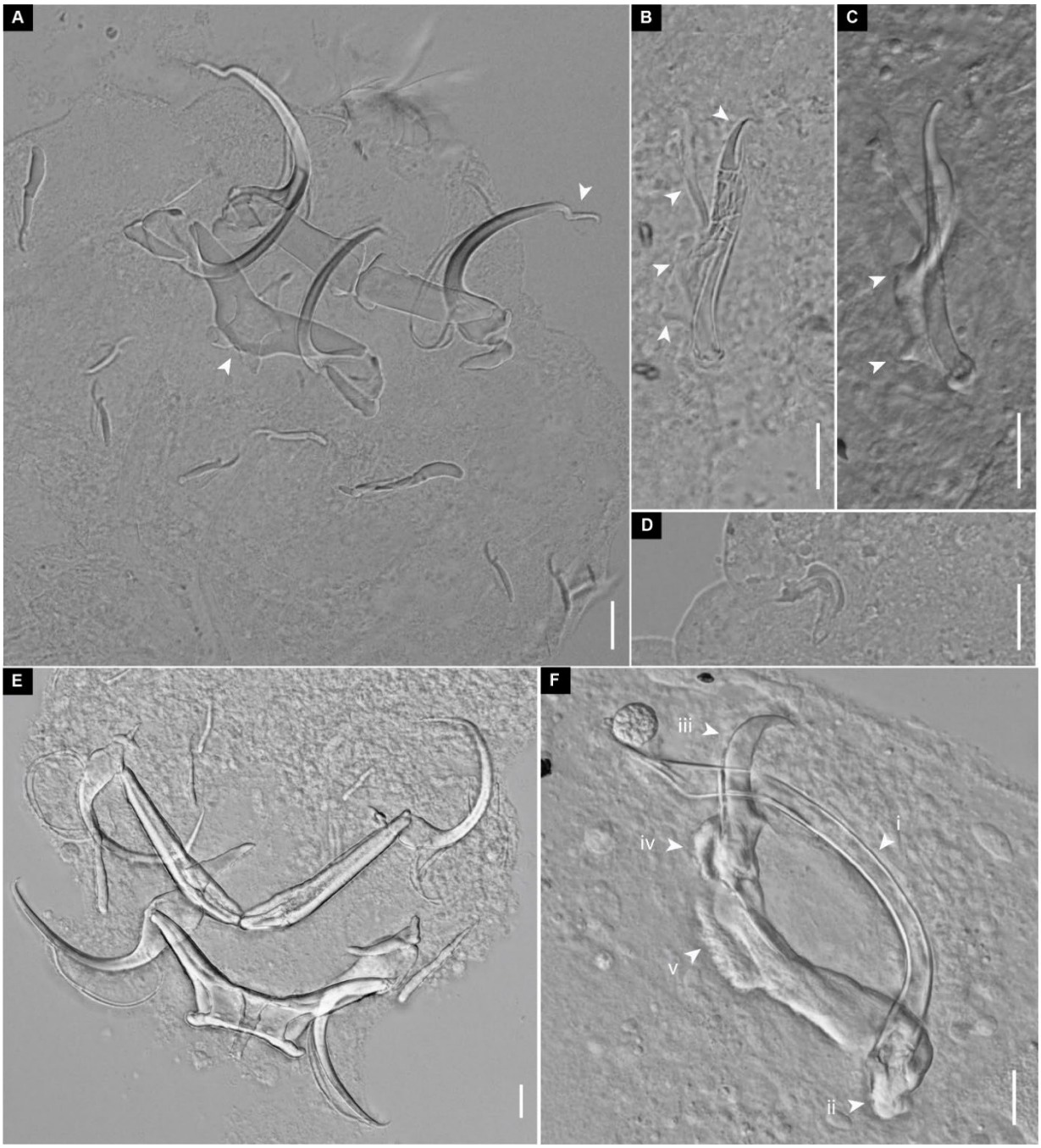

Figure 3.8. Micrographs of *Quadriacanthus gourenei* N'Douba, Lambert et Euzet, 1999and *Quadriacanthus* sp. G from the gills of *Clarias gariepinus* (Burchell) screened in the present study. **A**, **E** – Haptoral sclerite morphology. Arrows indicate curved point of the ventral anchors (A) and tapering of mid-region of the dorsal bar (A); **B**, **C**, **F** – Male copulatory organ. Arrows indicate characters of taxonomic interest in the accessory piece; and **D** – Unsclerotised vagina. Numbering in F: (i) bow-shaped copulatory tube; (ii) depicts basal protrusion of copulatory tube; (iii) sickle-shaped distal hook; (iv) and (v) depict the diverticulum on the posterior side of the distal hook and elongate lamellate thickening in the mid-region of the heavily sclerotised accessory piece. Scale bars: 10µm.

Figure 3.9. Micrographs of *Quadriacanthus* sp. H on the gills of *Clarias gariepinus* (Burchell) in this study. **A** – Haptoral sclerite morphology; **B**, **C** – Male copulatory organ. Arrows indicate characters of taxonomic interest in the accessory piece. Diverticula in mid-region of accessory piece – bottom arrow (B); middle and bottom arrows (C). Chelae-like morphology of the distal hook – top arrow (B, C). Scale bars: 10µm.

Concluding remarks

The eight species reported here from the various localities in the present study all represent new locality records and extend the distribution ranges of *Quadriacanthus* spp. known from *C. gariepinus*.

The distribution range of *Q. aegypticus* is extended in the native range of *C. gariepinus* to the Orange River system [Riet River (RR) and Gariep Dam (GD) in present study]. Previous records only exist from a tributary of the Orange River, the Modder River (Krugersdrift Dam); the Vaal River system [BS, SR and Vaal River (VR)] where previous records exist only from the Vaal Dam, upstream from our sampling locality; the NGR; and the Upper Zambezi River system (ZR and KR). *Quadriacanthus fornicatus* has only ever been recorded from *C. gariepinus* in the Blue and White Nile rivers in Sudan (see **Fig. 3.2. E**). The similarities in the haptoral and MCO sclerite complexes may prove that the species has a much wider distribution range than previously thought. The only species similar to *Q. fornicatus* is *Quadriacanthus bagrae* that was erroneously recorded from *C. gariepinus* in Zimbabwe (Douëllou and Chishawa 1995; Francová et al. 2017). In the present study, the distribution range for *Q. fornicatus* is extended in the native range of *C. gariepinus* in southern Africa to the Orange River system (RR and GD); the Vaal River system (BS, SR and VR); to the NGR and the Upper Zambezi River system (ZR). Despite being the second most widely distributed species in Africa, *Quadriacanthus clariadis* have only been noted from the northern regions of South Africa (see **Fig. 3.2. D**). Here we report on the extended distribution in southern Africa in the native range of *C. gariepinus* in the Orange River system (RR and GD) and the Vaal River system (BS, SS and VR). *Quadriacanthus allobychowskiella* has primarily been recorded from Egypt (see **Fig. 3.2. C**). Its distribution range is extended in the native range of *C. gariepinus* in southern Africa to the Orange River system (RR and GD); the Vaal River system (BS, SR and VR) and the Upper Zambezi system (ZR). The distribution of *Quadriacanthus pravus* is limited to its type locality in Sudan. Its distribution is extended to the NGR and ZR in the native range of its type host *C. gariepinus* in South Africa and Zambia, respectively. *Quadriacanthus gourenei* is only known from its type host *H. isopterus* from its type locality in the Ivory Coast (see N'Douba et al. 1999). We report here on the first record of this species from *C. gariepinus* from the ZR in Zambia. *Quadriacanthus* sp. G is only recorded from ZR. *Quadriacanthus* sp. H is recorded from the gills of *C. gariepinus* in the Orange River system (GD), the Vaal River system (BS and SR), the NGR and the Upper Zambezi River system (ZR and KR), all in the native range of the host.

3.2.2. Order Gyrodactylidea

Family Gyrodactylidae

The Gyrodactylidae is the second largest family of monogeneans, after the Dactylogyridae, that parasitise a wide range of freshwater fishes and representative species can be either viviparous or oviparous. In Africa, the Gyrodactylidae comprise six genera endemic to the continent: *Afrogyrodactylus* Paperna, 1968; *Citharodactylus* Přikrylová, Shinn et Paladini, 2017; *Diplogyrodactlyus* Přikrylová, Matějusová, Musilová, Gelnar et Harris, 2009; *Mormyrogyrodactylus* Luus-Powell, Mashego et Khalil, 2003 and *Tresuncinidactylus* Přikrylová, Barson et Shinn, 2021 parasitising fishes of the Alestidae, Citharinidae, Polypteridae and Mormyridae, respectively. Clariid hosts are, however, parasitised on the skin and gills by species from the genera *Gyrodactylus* von Nordmann, 1832 (cosmopolitan) and *Macrogyrodactylus* Malmberg, 1957 (endemic to Africa) on the skin and gills. *Gyrodactylus* species are generally smaller and possess an opistohaptor armed with a single row of 14 marginal hooks, one pair of hamuli, a dorsal bar and a ventral bar (with or without a membrane). In contrast *Macrogyrodactylus* is represented by individuals much larger in overall size as well as the sclerotised hamuli complex (Řehulková et al. 2018). Both genera are characterised by a bulbous male copulatory organ with one principal spine and a row/s of small spines and utilise parthenogenesis and hyper-viviparity (viviparous) as reproductive strategies (Bakke et al. 2007; Zahradníčková et al. 2016; Řehulková et al. 2018).

Gyrodactylus von Nordmann, 1832

Members of *Gyrodactylus* infect the body surface, fins and gills of its fish host and some species have also been found to infect amphibians, tetrapods and cephalopods (Paetow et al. 2009). Despite their small size, individuals in this group are economically significant as pests with mortalities mainly reported from cichlid and salmonids in aquaculture (Kabata 1985; Obiekezie and Taege 1991; Paperna 1996). Transmission occurs through direct contact between hosts and is thought to be dependent on host densities in a water body (see Bakke et al. 2007). Globally there are ~495 nominal species, however African representatives are still scanty and are predominantly found from cichlid and clariid hosts (see Harris et al. 2004; Zahradníčková et al. 2016; Dos Santos et al. 2019). Currently, 39 species are known from African fish hosts of the Alestidae (1), Anabantiidae (2), Amphilidae (1), Cichlidae (18), Cyprinidae (2), Mugilidae (1), Notobranchidae (1), Procatopodidae (1), Syngnathidae (1), Mochokidae (2), and the remaining nine have been described from the clariids, *C. gariepinus* (8) (see **Table A3.2.**) and *Clarias camerunensis* Lönnberg (see Paperna 1973; Price and Gery 1968; Ergens 1973; Prudhoe and Hussey 1977; El-Naggar and El-Abbassy 2003; Christison et al. 2005; Nack et al. 2005; El-Naggar

et al. 2007; García-Vásquez et al. 2007, 2010; Přikrylová et al. 2009, 2012; Vaughan et al. 2010; Vanhove et al. 2011; Zahradníčková et al. 2016; Dos Santos et al. 2019; Christison et al. 2021).

The first gyrodactylids from *C. gariepinus* were described in the work of Ergens (1973) and Paperna (1973), the latter author contributing greatly to early monogenetic research of African freshwater fishes. These authors provided descriptions of four species: *Gyrodactylus alberti* Paperna, 1973 (**Fig. A1.5. A**) and *Gyrodactylus clarii* Paperna, 1973 from Lake Albert in Uganda; and *Gyrodactylus groschafti* Ergens, 1973 (**Fig. A1.5. D**) and *Gyrodactylus rysavyi* Ergens, 1973 (**Fig. A1.5. E**–**F**) from the Nile River in Egypt (Ergens 1973; Paperna 1973). In his checklist of monogeneans from African freshwater fishes, Paperna denoted *G. clarii* as the junior synonym of *G. rysavyi* with no remarks on the re-assignment of the species. Upon further investigation in the literature, it seems the two species are regarded as separate, and a re-examination of the material is needed to confirm the validity of *G. clarii* (see Paperna 1979; Harris et al. 2004; Scholz et al. 2018c). *Gyrodactylus transvaalensis* Prudhoe et Hussey, 1977 (**Fig. A1.5. G**–**H**) was described from material collected from the skin and lower lip of the sharptooth catfish at the confluence of the Elands and Olifants rivers in the Limpopo Province, South Africa. Thirty-five years later, Přikrylová et al. (2012) discovered three new species from *C. gariepinus* and described the specimens in their review on *Gyrodactylus* from African catfishes. The three species are *Gyrodactylus alekosi* Přikrylová, Blažek et Vanhove, 2012 (**Fig. A1.5. B**) from temporary pools in Mozambique; *Gyrodactylus gelnari* Přikrylová, Blažek et Vanhove, 2012 (**Fig. A1.5. C**) (type host *Clarias anguillaris* Linnaeus) in Senegal and *Gyrodactylus turkanaensis* Přikrylová, Blažek et Vanhove, 2012 (**Fig. A1.5. I**) from Lake Turkana in Kenya.

Several authors indicate the presence of *Gyrodactylus* sp. on the gills of *C. gariepinus* from Lake Victoria, Tanzania and from whédos (artificial holes made in riverbanks to keep hosts for aquaculture purposes) in Benin. No photomicrographs are presented of specimens studied and material was not deposited in museum collections, rendering it difficult to determine if these specimens belong to known taxa, resemble one another, or are possible new species to the genus (Mwita and Nkwengulila 2004, 2008; Tossavi et al. 2014; Sèdogbo et al. 2019). To date, a single new species of *Gyrodactylus* from *C. gariepinus* that is morphologically similar to *G. turkanaensis* in haptoral sclerite morphology and marginal hooks with distinct sickle morphology is known from the White Nile, Sudan. The authors were able to obtain molecular evidence to confirm its separate species status, but morphological evidence to provide a complete description of the species was not possible from the two excised haptors collected (see Přikrylová et al. 2012).

Currently, the eight species known from *C. gariepinus* are distributed across seven countries in Africa. *Gyrodactylus groschafti* is confined to the Nile River, Egypt (see **Fig. 3.10. A**), and *Gyrodactylus rysavyi* is the most widely distributed, as far north as the Nile River in Egypt, West

in Senegal (West Africa) and in the southern hemisphere several records are known from South Africa, Mozambique and Zimbabwe (see **Fig. 3.10. A**). The remaining six species have only been recorded on single occasions from Egypt, Ethiopia, Kenya, Mozambique, South Africa, and Uganda (see **Fig. 3.10. B**). The low species richness of *Gyrodactylus* known from the widely distributed *C. gariepinus* is surprising, as the genus is one of the most diverse taxa to parasitise freshwater fishes. Moreover, *C. gariepinus* co-occur with several other siluriform hosts (*C. anguillaris*, *B. docmak*, *H. isopterus*) in East and West Africa, or has historically co-occurred with these hosts (see Van Steenberge 2019). The few species of gyrodactylids reported from the sharptooth catfish and disproportionate availability of data and reporting on infection highlight the unfaltering need for parasitological studies that include environmental, seasonal and host associations and the taxonomy and systematics of freshwater parasites of all African freshwater fishes.

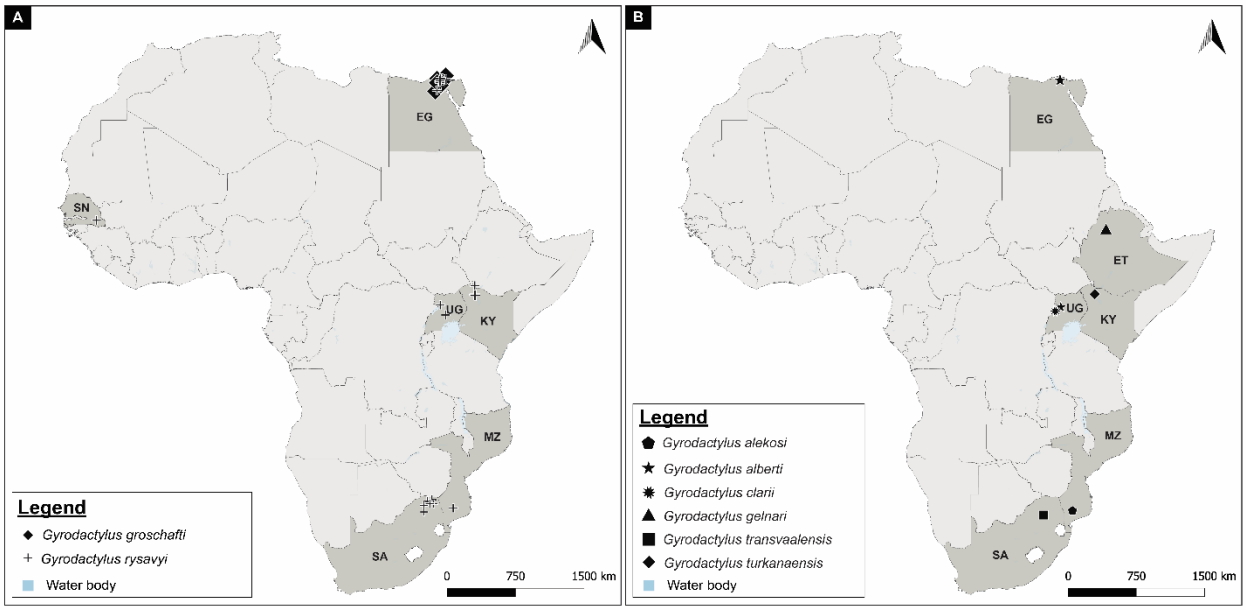

Figure 3.10. Distribution records of *Gyrodactylus* spp. known from *Clarias gariepinus* (Burchell) in Africa. **A** – *Gyrodactylus groschafti* Ergens, 1973 and *Gyrodactylus rysavyi* Ergens, 1973. **B.** – *Gyrodactylus alekosi* Přikrylová, Blažek et Vanhove, 2012, *Gyrodactylus alberti* Paperna, 1973; *Gyrodactylus clarii* Paperna, 1973; *Gyrodactylus gelnari* Přikrylová, Blažek et Vanhove, 2012; *Gyrodactylus transvaalensis* Prudhoe et Hussey, 1977 and *Gyrodactylus turkanaensis* Přikrylová, Blažek et Vanhove, 2012.

Present study: Two species of *Gyrodactylus* were found to parasitise the gills of *C. gariepinus* from the VR and the ZR (see **Fig. 3.4.** and **Table A5.1.**). *Gyrodactylus* sp. A **(Fig. 3.11. A**–**C**) was found from the gills of the sharptooth catfish in ZR and closely resemble *G. gelnari* (described from *C. anguillaris*). *Gyrodactylus* sp. B **(Fig. 3.11. D**–**F**) from the VR closely resemble *G. rysavyi*. The specimen from the VR conforms to the sizes of the sclerotised structures obtained from Kenya, Mozambique, and Senegal, but is slightly smaller in the size of the hamuli (see **Table A2.15.** in **Appendix A2** for comparative measurements of taxonomic relevant structures for both species).

Both records represent new locality records and extend the distribution range within the natural distribution range of *C. gariepinus*. This is only the second record of *G. gelnari* from *C. gariepinus* and the southernmost distribution of *G. rysavyi* is now from the Vaal River system, where it was previously restricted to the Limpopo River basin.

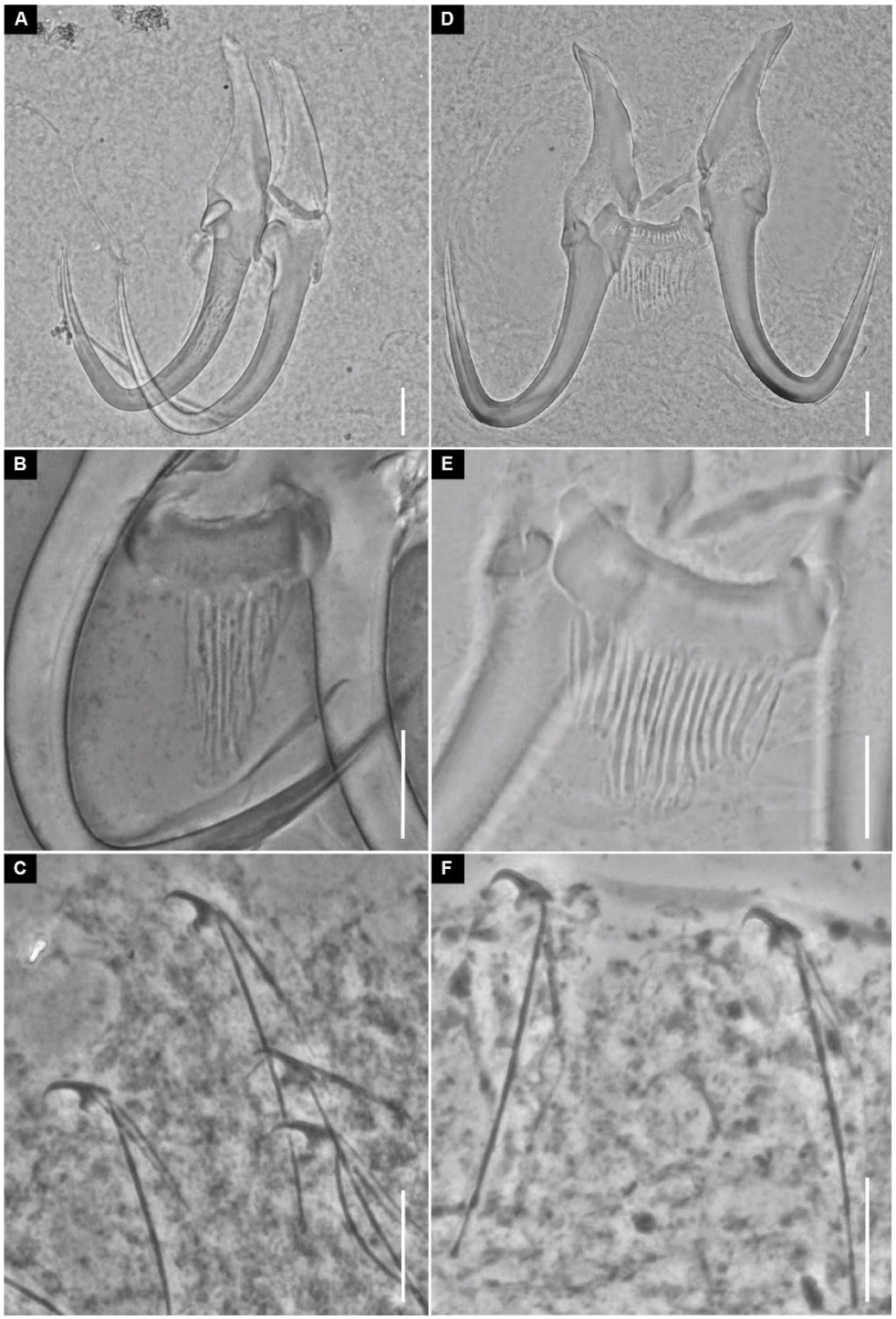

Figure 3.11. Microphotographs of *Gyrodactylus gelnari* Přikrylová, Blažek et Vanhove, 2012 (A–C) and *Gyrodactylus rysavyi* Ergens, 1973 (D–E) found on the gills of *Clarias gariepinus* (Burchell) during the present study. **A**, **D** – Haptoral sclerite morphology; **B**, **E** – Ventral bar and **C**, **F** – Marginal hooks. Scale bars: 10μm.

3.3. Subclass Digenea

The Digenea is one of the two subclasses of the class Trematoda, commonly referred to as flukes. This very large group of parasites has close to 20 000 nominal species primarily defined at superfamily level. Twenty-five superfamilies, 148 families and approximately 60 families that infect fishes are accommodated within the Digenea, of which ~38 families are exclusive parasites of fishes (Bray et al. 2008; Kostadinova and Pérez-del-Olmo 2014). Digenea are dorsally flattened obligate parasites almost exclusively endoparasites of bony fishes (Osteichthyes) and members represent the largest group of internal metazoan parasites of fishes. There is no specific set of unique morphological traits that differentiate families from one another and "individual families are primarily characterised by a unique combination of non-unique morphological characters" (Kudlai et al. 2018). Some species are causative agents of human fish-borne diseases in the liver and intestine; however, these are not common in Africa (Scholz 2008; Fried and Abruzzi 2010; Otranto and Eberhard 2011; Blasco-Costa and Locke 2017; Kudlai et al. 2017, 2018; Dumbo et al. 2019b). Other taxa, such as species of the Diplostomidae, have been found to alter host's behaviour to increase their transmission success (see Lozano 1991; Lafferty and Morris 1996; Seppälä et al. 2006; Poulin 2010; Dianne et al. 2011; Gopko et al. 2017; Sacco et al. 2021). Two types of life cycles are commonly recognised for all the members of digenetic worms that parasitise freshwater fishes in Africa: (i) fish serve as a 2nd intermediate host to metacercariae, and individuals can be encysted in tissues and organs or be pathogenic to the host when unencysted in the brain or eyes (**Fig. 3.12. A**); or (ii) fish serve as the definitive host and the adult digenean is found in various host organs such as in the intestine, gallbladder, urinary bladder, or even encapsulated in some tissues (**Fig. 3.12. B**). However, it should be noted that a wide spectrum of other second intermediate hosts can be utilised that include molluscs, insects and oligochaetes and fish may serve as the definitive host or in some instances as a paratenic host (Blasco-Costa and Locke 2017; Kudlai et al. 2018).

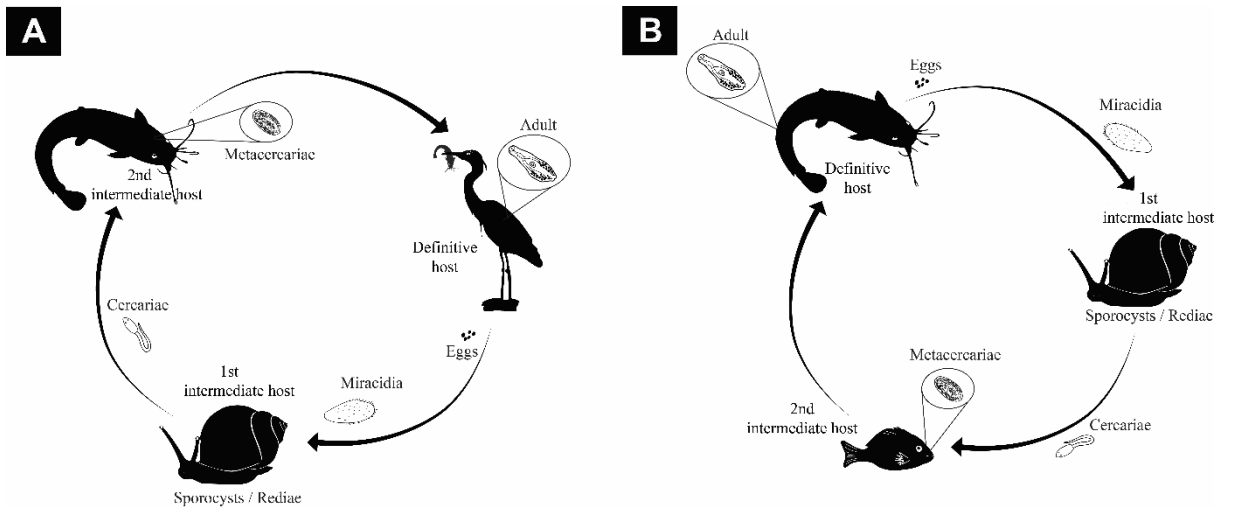

Figure 3.12. Schematic representation of the three-host life cycle of members of the Digenea. **A** – Fish serve as the 2nd intermediate host to metacercariae and **B** – fish as the definitive host to adult digenetic worms.

In attempts to resolve the classification and position of taxa within the Digenea, basic morphotypes were initially assigned to adult forms based on a general body scheme with the primary focus on the position of the suckers and the reproductive and digestive systems i.e., 'distome', 'amphistome', 'monostome', 'gasterostome', 'holostome', 'schinostome' and 'schistosome'(Blasco-Costa and Locke 2017). This approach was, however, unsatisfactory as most adults belong to the 'distomes', and additional efforts to include adult, cercarial, and asexual adult morphology resulted in conflicting opinions and placement of taxa within the Digenea (see Gibson 2002). Currently, the life cycle together with adult and cercarial morphology of all Digenea play a crucial role in defining individuals at family level, rendering it very difficult to present simple keys to the families of fish trematodes in Africa (Bray et al. 2008; Kudlai et al. 2018). Gibson (1996) proposed the division of the Digenea into three orders namely, the Strigeida (with forked cercarial tails), Echinostomida (cercariae encyst in the open) and the Plagiorchiida (cercariae without a forked tail), and this was applied in the keys by Jones et al. (2005). Olson et al. (2003) however did not recognise the Echinostomida (junior synonym of Digenea) but did acknowledge the Plagiorchiida and the Diplostomida as a subset of the Strigeida. Due to the controversy in the classification of the Digenea, information included on taxa in this section is selective and divided according to the orders Plagiorchiida and Diplostomida (*sensu* Olson et al. 2003). For the consistency purposes, the grouping of taxa at superfamily and family level within the two orders follow Jones et al. (2005) and Bray et al. (2008).

African freshwater fishes are hosts to Digenea in the orders Diplostomidae and Plagiorchiida. These parasites primarily belong to the distomous-type with an oral sucker at the anterior end of

the body and a ventral sucker in the mid-region of the body. They are also characterised by a well-developed digestive tract and attention is given to the relative position, shape, and extent of the genital organs; the position of the genital pore, cirrus sac structure and the size of eggs are all regarded as essential in identification. Some species are also known to occasionally have spines on the surface of their tegument while others have a smooth tegument, and at times the absence (or loss) of spines is regarded as an artefact of the fixation process (Gibson et al. 2002; Jones et al. 2005; Bray et al. 2008). In Africa, *C. gariepinus* hosts one of the most diverse digenean communities known from any freshwater fish on the continent (Scholz et al. 2018c). Infections with adult digenetic worms have been reported from various organs including the intestine, gallbladder and urinary bladder, whereas metacercariae have been found free in the branchial chambers, cranial cavity, eyes, coelom, and encysted or encapsulated in several tissues. In the most recent checklist on digeneans from African freshwater fishes, 34 species were reported to infect *C. gariepinus* (**Table A3.3.** and **Table A3.4.**) (Kudlai 2018); adult forms of 19 species from the families Allocreadiidae Stossich, 1903; Aporocotylidae Odhner, 1912; Cephalogonimidae Looss, 1899; Cladorchiidae Fischoeder, 1901; Gonocercidae Skrjabin et Guschanskaja, 1955; Gorgoderidae Looss, 1899; Heterophyidae, Leiper, 1900; Macroderoididae McMullen, 1937; Opisthorchiidae Looss, 1899 and Orientocreadiidae Yamaguti, 1958; and metacercariae of 15 species belonging to the Cladorchiidae; Clinostomidae Lühe, 1901; Cryptogonimidae Ward, 1917 and Diplostomidae Poirier, 1886 have been reported from *C. gariepinus* (Boomker 1984; Abdel-Gaber et al. 2015, 2016b; Scholz et al. 2016; Zhokhov et al. 2017; Kudlai et al. 2018).

In the present study, adult forms of species in the Gorgoderidae and Opisthorchiidae were found from the gallbladder; and a species of the Orientocreadiidae from the intestine of *C. gariepinus*. Metacercariae of the Clinostomidae were found unencysted (free) in the branchial chambers, while those belonging to the Diplostomidae were found encysted in the muscle tissue, liver and kidney or unencysted in the eyes, cranial cavity and visceral cavity (**Fig. 3.23.**). Taxa are listed alphabetically at the level of superfamily followed by the family and genera as in Gibson et al. (2002), Jones et al. (2005), Bray et al. (2008) and Kudlai et al. (2018). The aim of this section was not to resolve the taxonomic relationships within the Plagiorchiida and Diplostomida, thus the taxa is grouped and listed as mentioned above with a short introduction to each order and where relevant, for superfamilies or families. Information deemed appropriate on genera, their distribution, taxonomy, synonyms, and host range are included only for species known to parasitise *C. gariepinus*. Cited literature should be consulted for detail and further reading on the familial and taxonomic history and relations between taxa. Sequences used for comparison of alignments of the respective genes amplified are presented in **Table A4.1.** and **Table A4.2.**

3.3.1. Order Plagiorchiida

3.3.1.1. Superfamily Gorgoderoidea Looss, 1899

Family Allocreadiidae Stossich, 1903

Allocreadium Looss, 1900
(syn. *Allocreadoides* Yamaguti, 1971)

Representatives of the genus *Allocreadium* Looss, 1900 are relatively small cosmopolitan parasites found as adults in the digestive system of teleost hosts, and occasional infections are known from salamanders, snakes and frogs. Their three-host life cycle appears to be restricted to freshwater systems, with clams or snails as 1st intermediate hosts, while the 2nd intermediate hosts are aquatic arthropods, insects and crustaceans (Caira and Bogéa 2005). The taxonomy of the cercariae is complex and several species have erroneously been assigned to the genus after it was erected by Looss in 1899. Several species have also been removed from the genus based on morphological discrepancies (Peters 1957; Wootten 1957).

Seven species are known to parasitise African fishes of the Alestidae, Cichlidae, Cyprinidae, Danionidae, Mochokidae and a single species, *Allocreadium mazoensis* Beverley-Burton, 1962 is known from the intestine of the clariid host *C. gariepinus* originally described from Zimbabwe (**Fig. 3.13. A**) (Beverley-Burton 1962). The validity of *A. mazoensis* infecting *C. gariepinus* from Lake Victoria and Lake Malimbe in Tanzania is doubtful with no morphological evidence, and no comparative data for the 18S rRNA gene region they sequenced (see Mwita and Nkwengulila 2004, 2008, 2010; Mwita 2014a, b). Similarly, the mention of *Allocreadium* spp. found in the intestine of *C. gariepinus* in Nigeria is doubtful with no morphological or molecular data presented (Kawe et al. 2016).

Present study: No individuals of *Allocreadium* were found in the intestine of *C. gariepinus* during the present study.

Family Gorgoderidae Looss, 1901

Phyllodistomum Braun, 1899

The species of *Phyllodistomum* Braun, 1899 are cosmopolitan, and unlike other digeneans they have a two-host life cycle requiring no second intermediate host. Adults are usually found in the urinary bladder or ureters of the definitive fish host, while larvae emerge from clam intermediate hosts (Campbell 2008). Globally there are ~110 species, while only nine species from seven fish families [Anabantidae (1), Bagridae (1), Clariidae (3), Claroteidae (1), Gymnarchidae (1), Malapteruridae (1) and Mastacembelidae (1)] have been reported from Africa (Zhokhov 2010;

Kudlai et al. 2018). *Phyllodistomum ghanense* Thomas, 1985, *Phyllodistomum lingaule* Odhner, 1902, *Phyllodistomum spatula* (Odhner 1902), and *Phyllodistomum spatulaeforme* (Odhner, 1902) are all found in the urinary bladder of their respective hosts, whereas *Phyllodistomum symmetrorchis* Thomas, 1958 is the only species known to inhabit the coelom of its claroteid host (Thomas, 1958; Boomker 1984). The remaining three species, *Phyllodistomum bavuri* Boomker, 1984, *Phyllodistomum tana* Zhokhov, 2010 (syn. *Pseudolichorchis clarii* Bayoumy, El-Monem et El-Atti, 2013) and *Phyllodistomum vanderwaali* Prudhoe et Hussey, 1977, have been described from the urinary bladder (*P. bavuri* and *P. vanderwaali*) and ureters (*P. tana*) of *C. gariepinus* (Prudhoe and Hussey, 1977; Boomker 1984; Zhokhov 2010).

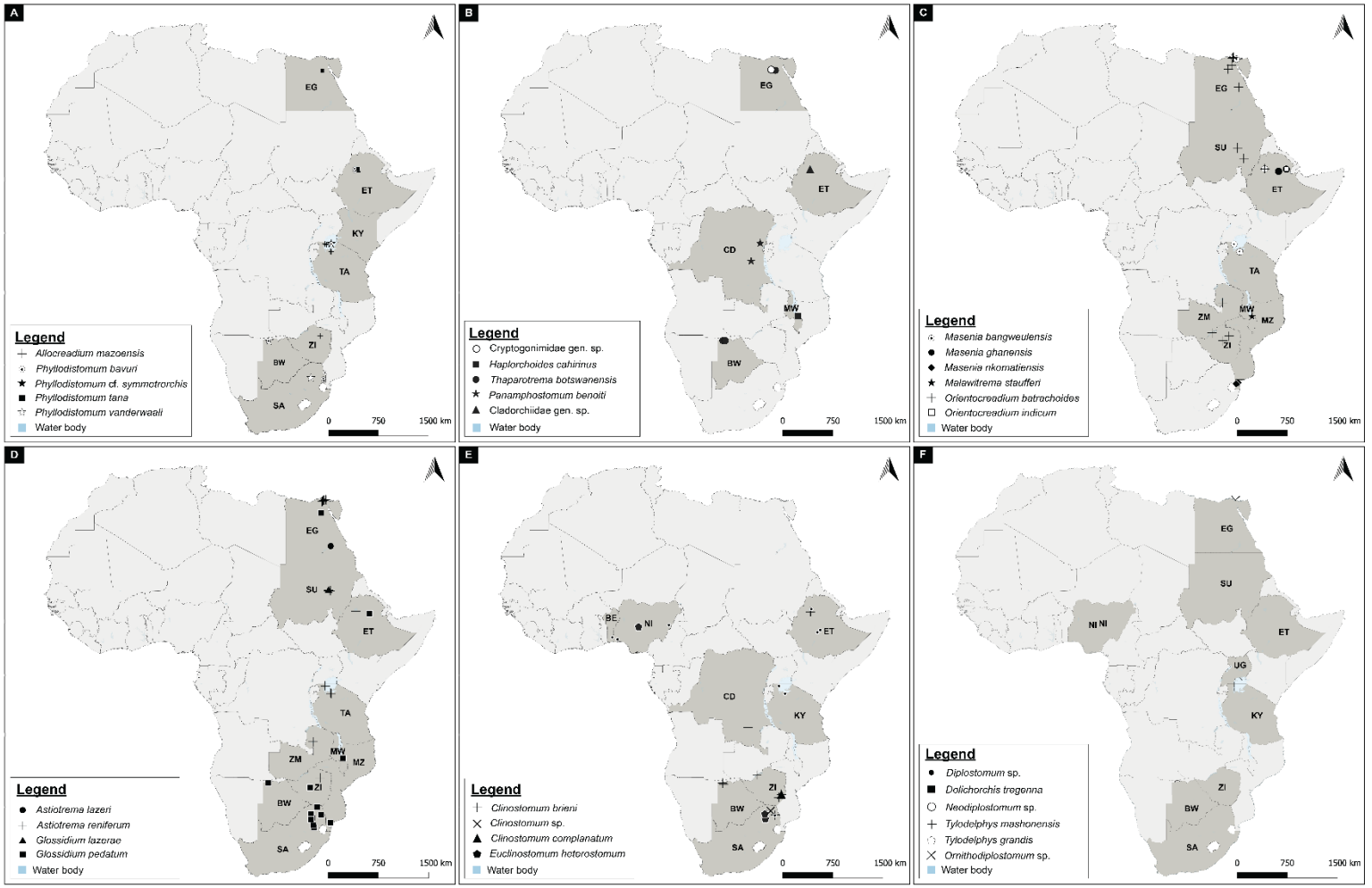

Figure 3.13. Distribution of the **A** – Gorgoderoidea Looss, 1899; **B** – Opisthorchioidea Looss, 1899 and Paramphistomoidea Fischoeder, 1901; **C** – Plagiorchidioidea Lühe, 1901; **D** – Plagiorchioidea Lühe, 1901 (*insertae sedis*); **E** – Clinostomoidea Lühe, 1901; and **F** – Diplostomoidea Poirier, 1886 infection *Clarias gariepinus* (Burchell) in Africa.

Boomker (1984) re-examined syntype material of *P. vanderwaali* and provided a redescription with notes on seasonal variation and a complete differential diagnosis on all the species known from African waters at the time. Species in the genus are mainly distinguished from each other in the position of the ovaries relative to the vitellarium, i.e., anterior or posterior (Prudhoe and Hussey 1977; Boomker 1984; Zhokhov 2010). *Phyllodistomum tana* found in the ureters of *C. gariepinus* in Ethiopia is the largest representative of the genus and on occasion co-occur with *P. bavuri* in individual hosts, each occupying a different organ (niche?) (Zhokhov 2010; Cutmore et al. 2013; Zhokhov et al. 2017). The most widely distributed species is *P. bavuri* known from *C. gariepinus* in various rivers in South Africa, the Okavango Delta (Botswana), and Lake Victoria (Kenya), and more recently it was found in Lake Tana (Ethiopia) (**Fig. 3.13. A**) (Boomker 1984; Zhokhov 2010; Cutmore et al. 2013; Jansen van Rensburg et al. 2013; Zhokhov et al. 2017). In their study on gorgoderiids from various hosts in Lake Victoria (Kenya), Cutmore et al. (2013) found specimens similar to *P. bavuri* from *C. gariepinus*. The authors also found another species from the urinary bladder of *C. gariepinus* and assigned it to *Phyllodistomum* cf. *symmetrorchis*. This assignment was based on the position of the ovary that is anterior to the vitellaria, closely resembling *P. symmetrorchis*, previously described from the coelom of the armoured catfish *Auchenoglanis occidentalis* (Valenciennes) (Claroteidae: Siluriformes) in Ghana (see Boomker 1984; Cutmore et al. 2013). Lastly, Gado et al. (2014) reported on *Phyllodistomum* sp. from the kidney of *C. gariepinus* in Egypt, providing images of stained specimens and indicating that their specimens are not representative of any species of the genus reported from a range of fish hosts to date.

Present study: A comprehensive morphological study was not performed on specimens found infecting the gallbladder of *C. gariepinus* from the Upper Zambezi system, ZR [P = 12%, IF =1–2], Zambia. Based on the characters listed below, the specimens in this study are assigned to *Phyllodistomum* cf. *vanderwaali* (**Fig. 3.14. A**–**D**): the size and shape (pear-shaped) body; short oesophagus and intestinal bifurcation immediately after the oral sucker; the opening of the genital pore halfway between the oral and ventral suckers; the shallowly indented, irregular lobed testes on either side (one slightly in front of the other) of the mid-line, posterior to the oral sucker and ovary; and the compact vitellaria.

Identification: Sequences of the 18S rRNA (817 bp), ITS1-2 (514 bp) and 28S rRNA (1233 bp) regions for one isolate of *Phyllodistomum* cf. *vanderwaali* collected from *C. gariepinus* in ZR (isolate MT14), were successfully amplified. The BLAST search yielded similarities to published sequences of *Phyllodistomum* from Africa (see **Table 3.2.**). No sequences of the 18S rRNA gene region for *Phyllodistomum* produced a significant alignment with isolate MT14, however the ITS1-2 region of MT14 showed a 16% (p-distance) divergence and 121 bp difference from

Phyllostomum cf. *symmetrorchis* collected from *C. gariepinus* from Lake Victoria, Kenya (see **Table 3.2.**) (Cutmore et al. 2013). There was a 14% divergence and 163 bp difference in the 28S rRNA region to the *P.* cf. *symmetrorchis* isolate from Kenya (see **Table 3.2.**)

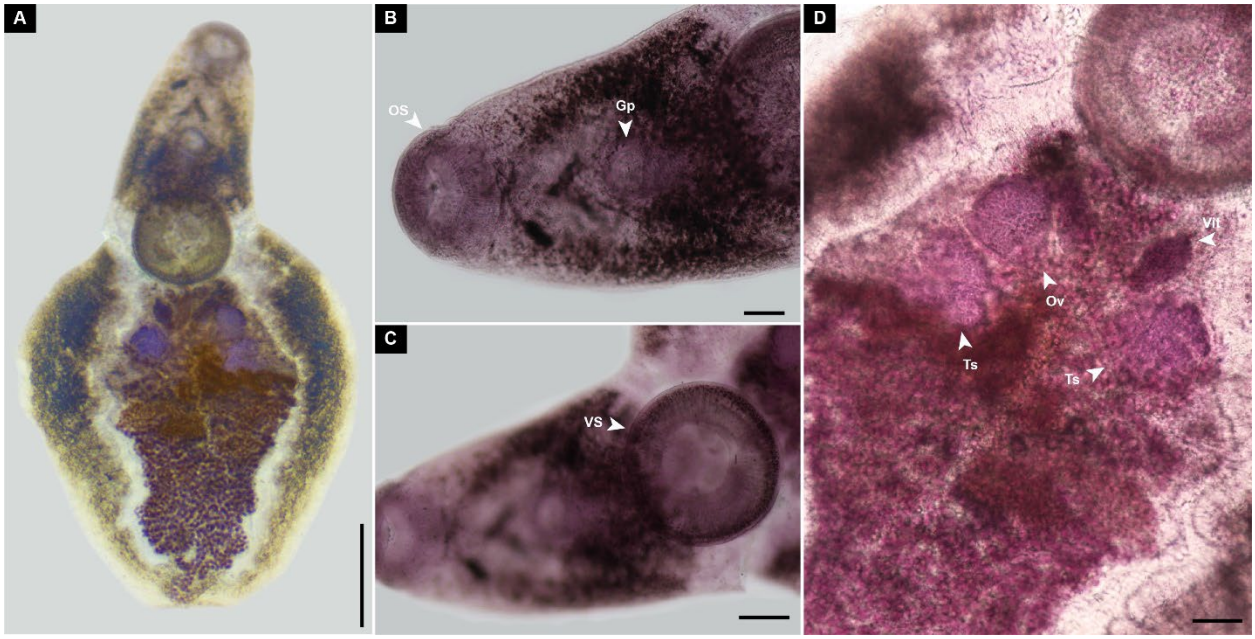

Figure 3.14. Micrographs of *Phyllodistomum* cf. *vanderwaali* Prudhoe et Hussey, 1977 found in the gallbladder of *Clarias gariepinus* (Burchell) collected in the Barotseland floodplain, Northwestern Province, Zambia. **A** – Whole specimen, **B** – oral sucker (OS) genital pore (Gp); **C** – ventral sucker (VS) and **D** – Vitellarium (Vit), testes (Ts) and ovary (Ov). Scale bars: 100µm (B, C, D) and 500µm (A).

Table 3.2. Divergence of species of *Phyllodistomum* Braun, 1899 based on ITS1-2 (529 bp) and 28S rRNA (1236 bp) regions. Values for p-distance (bottom diagonal) are expressed in percentage, while the top diagonal represent the number of differences in nucleotides. Species in bold were obtained from material collected during the present study.

ITS1-2		1	2	3
1	KF013152 *Phyllodistomum* cf. *symmetrorchis* KY	–	0	121
2	KF013162 *Phyllodistomum* cf. *symmetrorchis* KY	0.0	–	121
3	**MT14 *Phyllodistomum* cf. *vanderwaali* ZR**	16.2	16.0	–
28S rRNA		1	2	3
1	KF013171 *Phyllodistomum* cf. *symmetrorchis* KY	–	0	163
2	KF013174 *Phyllodistomum* cf. *symmetrorchis* KY	0.0	–	163
3	**MT14 *Phyllodistomum* cf. *vanderwaali* ZR**	13.7	13.7	–

3.3.1.2 Suborder Xiphidiata (*sensu* Olson, Cribb, Tkach, Bray et Littlewood, 2003

Present study: Two adult individuals belonging to the Xiphidiata **(Fig. 3.15. A–C)** were found in the eye viscera of *C. gariepinus* from the Barotse floodplain, Northwestern Province, Zambia [P = 12%, IF = 1]. The specimens from the present study are assigned to Xiphidiata gen. sp. 1.

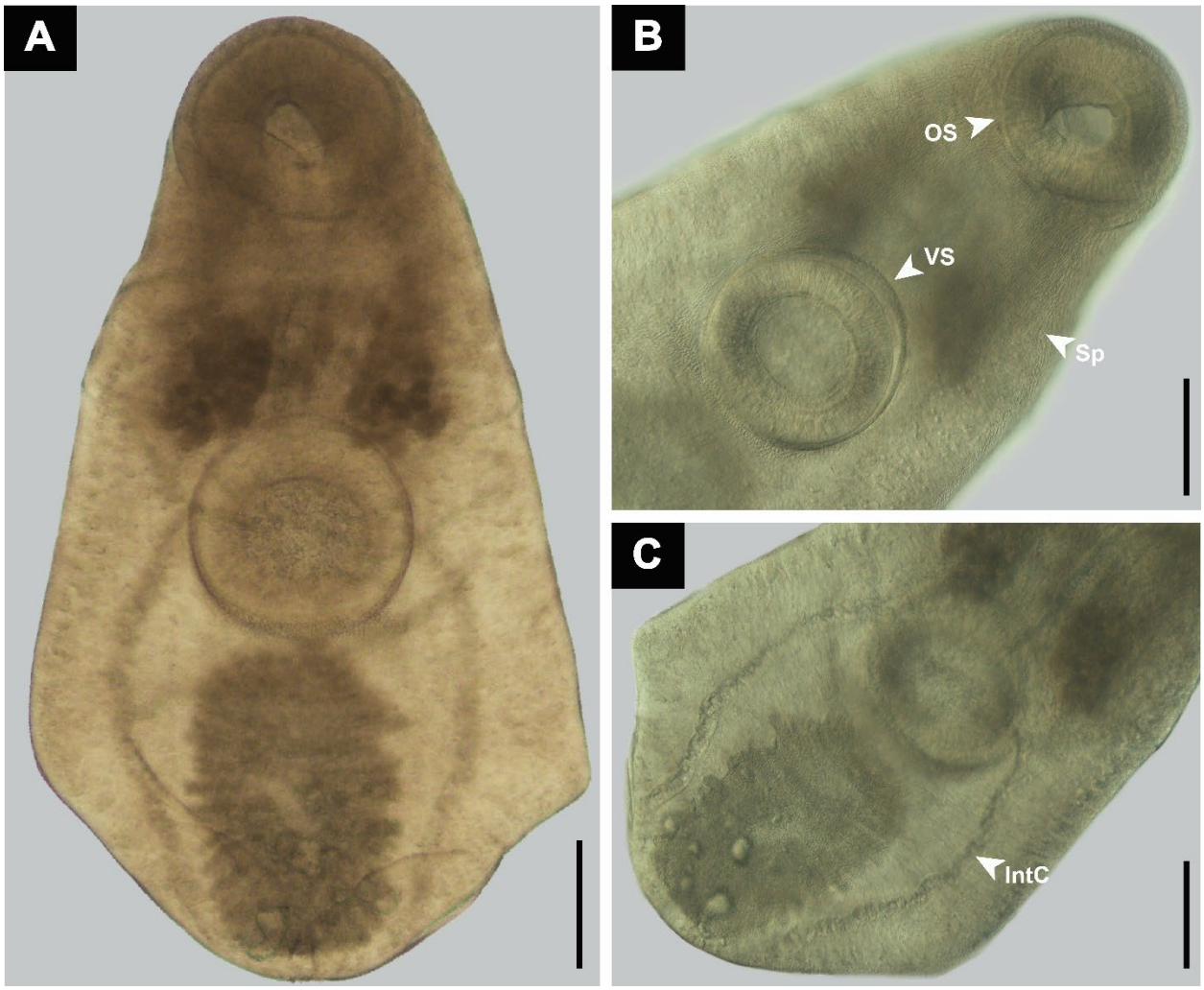

Figure 3.15. Micrographs Xiphidiata gen. sp. 1 found in the eye viscera of *Clarias gariepinus* (Burchell) from the Barotseland floodplain, Western Province, Zambia. **A** – whole specimen; **B** – oral sucker (OS), ventral sucker (VS) and spines (Sp) on body surface; **C** – bifurcate intestinal caeca. Scale bars: 100µm.

Identification: The 28S rRNA gene region was successfully amplified for one isolate from ZR (MT42). The closest alignments from the BLAST search indicated that MT42 had similarities with isolates of unidentified cercariae (reported as Macrophaloidea sp., Xiphidiata) found in freshwater snails, *Pilo ovata* (Olivier) in Nigeria and adults of *Travtrema stenocotyle* Cohn, 1902 (Xiphidiata)

from the intestine of the Lancehead snake, *Bothrops moojeni* Hoge in Brazil (see **Table 3.3.**) (Krupenko et al. 2020; Müller et al. 2021).

Table 3.3. Divergence of species of Xiphidiata gen. sp. 1 based on 28S rRNA (1274 bp) regions. Values for p-distance (bottom diagonal) are expressed in percentage, while the top diagonal represent the number of differences in nucleotides. Species in bold were obtained from material collected during the present study.

28S rRNA		1	2	3
1	KX022509 Microphalloidea sp. NI	–	88	99
2	MW317229 *Travtrema stenocotyle* BR	7.2	–	102
3	**MT42 Xiphidiata gen. sp. 1 ZR**	8.9	8.9	–

3.3.1.3. Superfamily Hemiuroidea

Family Gonocercidae Skrjabin et Guschanskaja, 1955

Gonocerca Manter, 1925

Members of the Gonocercidae are exclusively associated with marine environments and *Gonocerca* Manter, 1925 was erected for *Gonocerca phycidis* Manter, 1925 found from the gills, branchial cavity, and stomach of the marine squirrel hake *Urophycis chuss* (Walbaum) off the coast of Maine, United States of America (Manter 1925; Sokolov et al. 2017). To date, thirteen species have been reported as members of the genus, however controversy exists on the validity and placement of some, especially since they are generalists and there is no knowledge on their life history. A single record of *G. phycidis* is reported from *C. gariepinus* in Egypt, however this record is doubtful as the publication for this record is inaccessible and no species in the genus has been associated with freshwater systems (see Khalil and Polling 1997; Sokolov et al. 2017). Re-evaluation of these specimens from *C. gariepinus* is necessary to confirm its identity and placement within the Digenea.

Present study: No individuals of *Gonocerca* were found from *C. gariepinus* during the present study.

3.3.1.4. Superfamily Opisthorchioidea Looss, 1899

Family Cryptogonimidae Ward, 1917

Cryptogonimidae gen. sp.

Members of the Cryptogonimidae are parasitic to the intestine and pyloric caeca of marine and freshwater teleosts, reptiles and amphibians. Seven species from four genera are known to parasitise fishes from the freshwaters of Africa, however none of these species have been recorded from *C. gariepinus* (Kudlai et al. 2018; Kmentová et al. 2021 and references therein). Currently, the only recognised record of a cryptogonimiid is Cryptogonimidae gen. sp. that was collected from *C. gariepinus* in the Democratic Republic of the Congo (**Fig. 3.13. B**). This species was listed as *Metacercariae alestesi* Fain, 1953 in Khalil (1971) and Khalil and Polling (1997) in the family Acanthostomidae Poche, 1926 that is now considered a synonym of the Cryptogonimidae (see Bray et al. 2008; Kudlai et al. 2018).

Present study: Specimens resembling members of the Cryptogonimidae were not found to parasitise *C. gariepinus* in this study.

Family Heterophyidae Leiper, 1909

Haplorchoides Chen, 1949

Adult heterophyids are known from the intestine of their definitive fish hosts, primarily infecting freshwater siluriform fishes. Twenty-six species are known from siluriform catfishes in Australia, Egypt, India, Laos, Pakistan, Thailand and Uganda. Two species were described from perciform fishes in India and a single species (suspected accidental infection) is known from the Black kite *Milvus migrans* (Boddaert) in India (see Hostettler et al. 2018). The genus is diagnosed in having a complex ventral sucker structure, a single testis and for infecting siluriform fish. The type species for the genus, *Haplorchoides cahirinus* (Looss, 1896) is known from fishes of the Bagridae, close relatives of the siluriform family Clariidae. Several records from these bagrid hosts from Egypt and Uganda have been published (Khalil and Thurston 1973; Khalil and Polling 1997). The only record of this species from *C. gariepinus* is from the intestine of hosts individuals collected in Lake Malawi (**Fig. 3.13. B**) (Bray and Hendrix 2007).

Present study: No individuals of *Haplorchoides* were found to infect the intestine of *C. gariepinus* during the present study.

Family Opisthorchiidae Looss, 1899

Thaparotrema Gupta, 1955
(syn. *Agrawalotrema* Sahay et Sahay, 1988; *Opisthorchis* Blanchard, 1895)

Thaparotrema Gupta, 1955 was erected for *Thaparotrema vittalani* Gupta, 1955 (syn. *Opisthorchis vittalani*) described from the intestine of bagrid hosts in India (Gupta 1953). The genus is currently placed within the subfamily Opisthorchiinae Looss, 1899. These individuals are characterised in having a similar body plan to *Opisthorchis* Blanchard, 1895; the distribution of the uterine loops (intercaecal); the vitelline follicles presented as two bands along the lateral margins of body with different extensus (anterior or posterior); the position of the testes (tandem or near posterior extremity); the relative position of the ovary, seminal receptacle and testes and the shape of the body (lanceolate or elongate). Some species of the genus *Opisthorchis*, known from the gallbladder and intestine of teleost freshwater fishes in Africa and Asia, have been transferred to *Thaparotrema* based on the spined, elongate body opposed to the smooth, unspined lanceolate body of species of *Opistorchis* found in mammals and birds. Placement in *Thaparotrema* is also justified in the absence of a prepharynx, vitelline follicles that reach the ventral sucker or anterior to it, and testes are slightly diagonal (see Scholz 2008).

Currently, seven species are recognised in the genus, with *Thaparotrema botswanensis* Jansen van Rensburg, Van As et King, 2013 as the only species known from the gallbladder of *C.*

gariepinus in Africa (**Fig. 3.13. B**). The remaining species are primarily known from the teleost families Bagridae, Siluridae, Sisoridae (Silurifomres) and the Gymnarchidae (Osteoglaniformes).

Present study: Adult and developmental forms of *Thaparotrema* were found in the gallbladder of *C. gariepinus* from the Upper Zambezi system, KR [P = 50%, IF = 14] and ZR [P = 18%, IF = 2–4] in Zambia. Specimens collected from KR and ZR are assigned to *Thaparotrema* sp. A (**Fig. 3.16. A**–**H**). A full morphological study was not within the scope of the present study; thus comparison of specimens was not done to type material of *T. botswanensis* from Botswana. No comparative sequences are available for *T. botswanensis* (see below).

Co-occurrence: Jansen van Rensburg et al. (2013) reported on the co-occurrence of *Phyllodistomum* spp. in the urinary bladder of *C. gariepinus* from Botswana with *T. botswanensis* in the gallbladder of the same host individual. In the present study, *Thaparotrema* sp. A and *Phyllodistomum* cf. *vanderwaali* did not co-occur in any infected host individual from KR or ZR.

Identification: The 18S rRNA and ITS1-2 gene regions were successfully amplified for one isolate from KR (MT17; 18S: 843 bp, ITS1-2: 513 bp, 28S: 1180 bp); all three gene regions were successfully amplified for one isolate from ZR (MT13; 18S: 766 bp, ITS1-2: 396 bp, 28S: 1283 bp), and only the 18S and ITS1-2 regions for a second isolate from ZR (MT10; 18S: 804 bp; ITS1-1: 481 bp). The 18S rRNA and ITS1-2 regions were identical for all three isolates (see **Table 3.4.**).

Table 3.4. Divergence of species of *Thaparotrema* Gupta, 1955 based on the 18S rRNA (843 bp) and ITS1-2 (513 bp) regions. Values for p-distance (bottom diagonal) are expressed in percentage, while the top diagonal represent the number of differences in nucleotides. Species in bold were obtained from material collected during the present study.

18S rRNA		1	2	3
1	**MT10 *Thaparotrema* sp. A ZR**	–	0	0.0
2	**MT13 *Thaparotrema* sp. A ZR**	0.0	–	0.0
3	**MT17 *Thaparotrema* sp. A KR**	0.0	0.0	–
ITS1-2		1	2	3
1	**MT10 *Thaparotrema* sp. A ZR**	–	2	4
2	**MT13 *Thaparotrema* sp. A ZR**	0.0	–	4
3	**MT17 *Thaparotrema* sp. A KR**	0.0	0.0	–

Figure 3.16. Micrographs of *Thaparotrema* sp. A found from the gallbladder of *Clarias gariepinus* (Burchell) collected from the Barotseland floodplain, Western Province and Kabompo River, Northwestern Province in Zambia. **A**, **D** – whole specimens; **B, E** – oral sucker (OS) and pharynx (Ph); **F** – ventral sucker (VS) and gonopore (Go); **C**, **G**, **H** – anterior and posterior testes (Ts), ovaries (Ov), seminal receptacle (SR), uterus (Ut) and vitellaria (Vit). Scale bars: 50µm (E, F), 100µm (B, C, G, H) and 500µm (A, D).

3.3.1.5. Superfamily Paramphistomoidea Fischoeder, 1901

Family Cladorchiidae Fischoeder, 1901

Panamphistomum Manter et Pritchard, 1964

This genus is one of four paramphistome genera that occur in freshwater fishes. Their systematic placement is still disputed with no molecular or cladistic studies that attempt to resolve the genera within the superfamily other than a recent study by Pantoja et al. (2019) on paramphistomes from neotropical fishes. The lack of agreement on placement of taxa and insufficient data led to the use of host groups as basis for recognition at subfamily level, even though it is unsatisfactory "it is a logistical necessity" (Jones and Khalil 1990; Jones 2005). Presently, a single species *Panamphistomum benoiti* Manter et Pritchard, 1964 is known from *C. gariepinus* collected in the Democratic Republic of the Congo (**Fig. 3.13 B**) (Manter and Pritchard 1969).

More recently, a single specimen of a mature male that closely resembles *P. benoiti* was found from the intestine of *C. gariepinus* collected from Lake Tana, Ethiopia. The specimen, Cladorchiidae gen. sp. could not be placed in any of the known genera within the Cladorchiidae and is markedly different from *P. benoiti* in having a short oesophagus, long intestine reaching the ventral sucker, has a sub-medial ovary, compact vitelline glands and a uterus that spread outwards into the limits of the intestine (Zhokhov et al. 2017).

Present study: During the present study no infection with any cladorchiids were found from *C. gariepinus*.

3.3.1.6. Superfamily Plagiorchioidea Lühe, 1901

Family Cephalogonimidae Looss, 1899

Masenia Chatterji, 1933
(syn. *Eumasenia* Srivastava, 1951; *Pseudomasenia* Pan, 1984)

The genus *Masenia* Chatterji, 1933 is a small group of cephalogonimids primarily parasitising the intestine of marine and freshwater fishes of the Actinopterygii in Africa and Asia (Jones and Bray 2008a). Species delineation for *Masenia* is mainly based on body size, shape and arrangement of internal organs, number of circumoral spines, shape of excretory vesicle, size of eggs and the extension of vitelline follicles, caeca and cirrus sack (see Dumbo et al. 2019a and references therein). Representatives (adults) can be distinguished from species in the other four cephalogonimid genera in having two rows of enlarged circumoral spines and vitelline follicles located mainly in the hind body (Beverley-Burton 1962; Jones and Bray 2008a).

In Africa, five species are known from freshwater fishes of the Clariidae (4) and Mochokidae (1). The clariid, *C. gariepinus*, is a confirmed host for three species (**Fig. 3.13. C**). *Masenia bangweulensis* Beverley-Burton, 1962 (syn. *Eumasenia bangweulensis*) (ex. *Clarias ngamensis* Castelnau) was redescribed from the intestine of *C. gariepinus* in Egypt (El-Naggar et al. 1992). Records are also known from an unknown location in Egypt (most probably from the Nile River), from Lake Victoria in Tanzania and specimens from Lake Tana are reported to differ slightly from the type specimens in body size, the oral sucker and the cirrus sac and position (Mwita and Nkwengulila 2004, 2008, 2010; Radwan et al. 2009; Mwita 2011a, b; 2014a, b; Zhokhov et al. 2017). *Masenia ghanensis* (Fischthal et Thomas, 1968) was originally described from *C. anguillaris* in the Volta River (Ghana) and was later found to parasitise *C. gariepinus* from two rivers in Ethiopia (Fischthal 1973; Fischthal and Thomas 1972). Almost half a century later the description of *Masenia nkomatiensis* Dumbo, Dos Santos et Avenant-Oldewage, 2019 from *C. gariepinus* in the Incomati River in Mozambique was accompanied with the first key to the African maseniid species (see El-Naggar et al. 1992; Mwita and Nkwengulila 2004, 2008; Mwita 2014; Zhokhov et al. 2017; Dumbo et al. 2019a) (**Fig. 3.13. C**). A fourth possible species, *Eumasenia aegypticus* Imam, El-Askalany et Rashat, 1991, was reported by Gado et al. (2014) from the stomach of *C. gariepinus* in Egypt. This genus is synonymised with *Masenia* but *E. aegypticus* has a doubtful identity and has been placed into *species inquirenda*.

Present study: No infection with *Masenia* spp. was found in the intestine of *C. gariepinus* during the present study.

Family Macroderoididae McMullen, 1937

Malawitrema Bray et Hendrix, 2007

This genus was erected for specimens collected from the intestine of *C. gariepinus* in Lake Malawi (**Fig. 3.13. C**). The type species, *Malawitrema staufferi* Bray et Hendrix, 2007 is the only representative of the genus and was temporarily placed within the Macroderoididae until more material and data are available to clarify its position within the family. The authors noted that the placement of the species was rather difficult as it was not possible to accommodate it in any recognised family (see Bray and Hendrix 2007 and references therein).

Present study: No infection with *Malawitrema* was found from the intestine of *C. gariepinus* during the present study.

Family Orientocreadidae

Orientocreadium Tubangui, 1931

Species of *Orientocreadium* Tubangui, 1931 are often confused with *Astiotrema reniferum* (Looss, 1898) and *Masenia bangweulensis*; and several genera has been synonymised with *Orientocreadium* (see El-Naggar et al. 1993a; Jones and Bray 2008b). Two species are known from the intestine of *C. gariepinus*, namely *Orientocreadium batrachoides* Tubangui, 1931 (syn. *Orientocreadium lazeri* Khalil, 1961) and *Orientocreadium indicum* Pande, 1934. A single record of the latter is known from Ethiopia whereas *O. batrachoides* is widely distributed throughout the continent (**Fig. 3.13. C**). Numerous species assigned to the genus have been synonymised with the widespread species, *O. batrachoides* (see Beverly-Burton 1962). This species occurs as far north as the Nile River in Egypt and tributaries of the Nile in Sudan, and freshwater systems in Ethiopia. Its distributions extends southward to Zambia and Zimbabwe. Detailed redescriptions and studies of the tegument of *O. batrachoides* have also been published from *C. gariepinus* bought at a local fish market (fish were presumably collected in the Nile River and Delta in Egypt (Fischthal and Kuntz 1963; El-Naggar et al. 1993a, f). Dumbo et al. (2019b) provided novel morphological and molecular data from specimens collected in Mozambique in the southern hemisphere. Saoud et al. (1990), Mwita (2011b) and Awadin et al. (2012) reported on *Orientocreadium* sp. from the intestine of *C. gariepinus* from Egypt and Tanzania. These specimens most probably represent *O. batrachoides*, but morphological and molecular characterisation of these specimens are needed to confirm their identity.

Present study: *Orientocreadium batrachoides* (**Fig. 3.17. A**–**F**) was found in the intestine of native *C. gariepinus* from BS [P = 73%, IF = 1–617], Vaal River system, South Africa and ZR [P = 29%, IF = 1–4], Upper Zambezi system, Zambia.

Identification: The 18S rRNA, ITS1-2 and 28S rRNA gene regions were successfully amplified for one isolate from BS (MT4; 18S: 842 bp, ITS1-2: bp, ITS1-2: 468 bp, 28S: 1219 bp) and one isolate from ZR (MT8; 18S: 815bp, ITS1-2: 469 bp, 28S: 1143 bp). The BLAST search yielded significant alignments to published sequences of species of *Orientocreadium*. The 18S and 28S rRNA regions for both isolates were identical, while there was a 1-2% divergence in the 18S rRNA region to *O. batrachoides* from *C. gariepinus* in Egypt and MZ. A divergence of 2% was observed between MT4 and MT8 when these isolates were compared to *O. batrachoides* from Egypt and Mozambique. Interestingly, MT4 and MT8 also matched with *Orientocreadium* sp. from the freshwater snail, *Radix natalensis* (Krauss) from Zimbabwe, that may act as the 1st intermediate host in the life cycle of this species (see **Table 3.5.**).

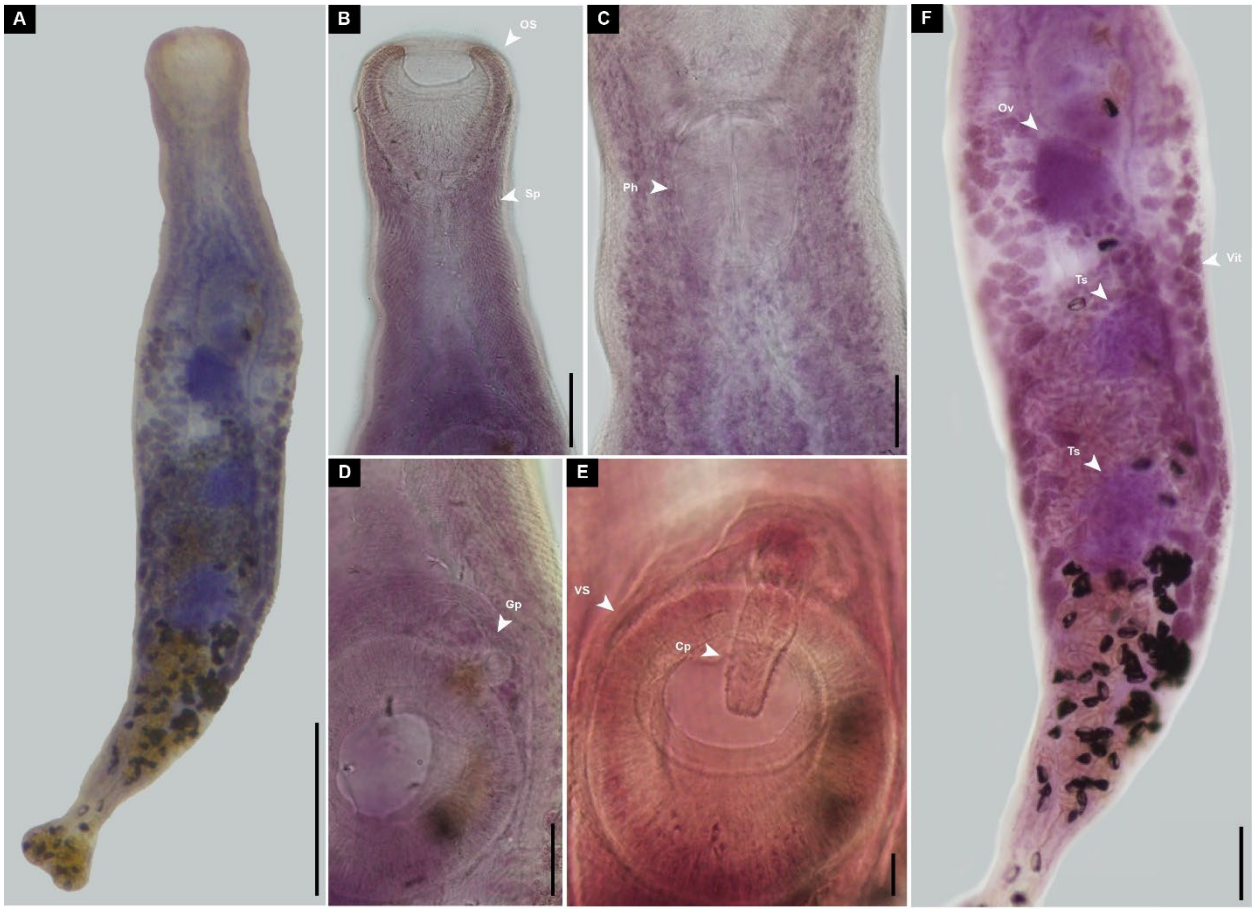

Figure 3.17. Micrographs of *Orientocreadium batrachoides* Tubangui, 1931 infecting the intestine of *Clarias gariepinus* (Burchell) from Boskop Dam, North West and the Great Fish River, Eastern Cape in South Africa and the Barotseland floodplain, Western Province, Zambia. **A** – whole specimens; **B** – oral sucker (OS) and tegument covered with spines (Sp); **C** – pharynx (Ph); **D** – genital pore (Gp); **E** – cirrus (cp) and ventral sucker (VS); and **F** – ovary (Ov), vitellaria (Vit) and the anterior and posterior testes (Ts). Scale bars: 50µm (C, D), 100µm (B, E, F) and 500µm (A).

Table 3.5. Divergence of species of *Orientocreadium* Tubangui, 1931 based on the 18S rRNA (919 bp), ITS1-2 (536 bp) and 28S rRNA (1312 bp) regions. Values for p-distance (bottom diagonal) are expressed in percentage, while the top diagonal represent the number of differences in nucleotides. Species in bold were obtained from material collected during the present study.

18S rRNA		1	2	3	4
1	MT797949 *Orientocreadium batrachoides* EG	–	5	11	9
2	MK496889 *Orientocreadium batrachoides* MZ	0.6	–		13
3	**MT4 *Orientocreadium batrachoides* BS**	1.3	1.8	–	1
4	**MT8 *Orientocreadium batrachoides* ZR**	1.1	1.6	0.1	–
ITS1-2		1	2	3	4
1	MZ600136 *Orientocreadium* sp. ZI	–	10	10	9
2	MZ600135 *Orientocreadium* sp. ZI	2.3	–		2
3	**MT4 *Orientocreadium batrachoides* BS**	2.9	0.0	–	2
4	**MT8 *Orientocreadium batrachoides* ZR**	2.5	1.0	0.4	–
28S rRNA		1	2	3	4
1	MT797948 *Orientocreadium batrachoides* EG	–	6	26	25
2	MK496882 *Orientocreadium batrachoides* MZ	0.2	–		25
3	**MT4 *Orientocreadium batrachoides* BS**	1.9	2.1	–	9
4	**MT8 *Orientocreadium batrachoides* ZR**	2.0	2.1	0.8	–

***Insertae sedis sensu lato* in the Superfamily Plagiorchioidea**

Four genera, *Astiotrema* Looss, 1900 (syn. *Astia* Looss, 1899), *Afromacroderoides* Khalil, 1972, *Alloglossidium* Simer, 1929 and *Glossidium* Looss, 1899 have been reported in the superfamily Plagiorchioidea from several freshwater fishes in Africa. There is however considerable confusion and disagreement on the placement of these genera within families, their validity and a possibility of a species complex (see Bray et al. 2006 and references therein).

Astiotrema Looss, 1900
(syn. *Astia* Looss, 1899)

Although the genus *Astiotrema* (syn. *Astia* Looss, 1899) was considered a member of the Plagiorchiidae, it was removed from the family (see Tkach 2008). The placement of the genus as *incertae sedis* is based on the fact that species within the genus have been found from several hosts including snakes, fish and turtles and there is limited knowledge on the life cycle and development of representative members. In conjunction with molecular and morphological data, the genus is currently placed within the Opisthorchiidae as *Astiotrema* clearly differs from plagiorchiids in having an undivided, sac-like seminal vesicle and the cercariae develop in rediae (see Olson et al. 2003; Pojamańska et al. 2008; Tkach 2008 and references therein).

Two species, *Astiotrema lazeri* El-Naffar, Saoud et Hassan, 1984 and *Astiotrema reniferum* (Looss, 1898) have been reported from *C. gariepinus* in Africa. A single record of *A. lazeri* is known from Lake Nasser in Egypt, which is also its type locality, while several records exist for *A. reniferum* from *C. gariepinus* in Egypt, Ethiopia, Sudan, Tanzania, Zambia and Zimbabwe (see **Fig. 3.13. D** and **Table A3.4**). Bray et al. (2006) consider the records of *A. reniferum* by Beverley-Burton (1962), Moravec (1977), El-Naggar et al. (1991) and those of *Glossidium lazerae* (syn. *Afromacroderoides lazera* Khalil, 1972) by Khalil (1972) and *A. lazeri* by El-Naffar et al. (1984) as the same or closely related species. Mwita and Nkwengulila (2008) reported an *Astiotrema* sp. from Lake Victoria, most probably conspecific with *A. reniferum* (see Mwita 2011a). In addition, Bray et al. (2006) is also of the opinion that *A. reniferum* is not a common parasite of African freshwater fishes as it was described from a freshwater turtle in India, and that African specimens are probably mistaken for *G. pedatum*. This may be the case as *Glossidium* and *Astiotrema* are rarely directly compared but are currently accepted as two genera (both *insertae sedis*) within the superfamily (see above and below).

Glossidium Looss, 1899
(syn. *Afromacroderoides* Khalil, 1972)

Glossidium was previously placed within the families, Allocreadiidae, Macroderoididae and Plagiorchiidae (Tkach 2008; Dumbo et al. 2019b and references therein). The genus was erected for the type species *Glossidium pedatum* Looss, 1899 collected from the intestine of *Bagrus bayad* (Forsskål) and *Bagrus docmak* (Forsskål) in the Nile River near Cairo in Egypt. In his diagnosis of the genus, Looss (1899) noted that *Glossidium* is reminiscent of *Astiotrema* but differs in the overall body shape and digestive system. Fischthal (1973) placed *G. pedatum* within the Macroderoididae, provided a redescription based on the smaller specimens he collected from *C. gariepinus* in Ethiopia, and noted its similarities with *Afromacroderoides lazerae* Khalil, 1972, also known from *C. gariepinus* (Khalil 1973). Mashego and Saayman (1989) revised the type material of *A. lazerae* and compared it with specimens of *G. pedatum* parasitising *C. gariepinus* from various localities in South Africa and assigned *A. lazerae* as a synonym of *G. pedatum*. Bray et al. (2006) and Bray and Hendrix (2007) provided insights on the taxonomic position of *G. pedatum* and concurred with Mashego and Saayman (1989) on the placement of the genus in the Plagiorchiidae. Jansen van Rensburg (2013) and Abdel-Gader et al. (2016b) however assigned *G. pedatum* collected from *C. gariepinus* in the Okavango Delta (Botswana) and the Nile River (Egypt), to the Macroderoididae. Despite synonymising *Afromacroderoides* with *Glossidium*, Tkach (2008) did not consider *A. lazerae* as a synonym of *G. pedatum* and this resulted in the new combination of *Glossidium lazerae*. This new combination was also accepted by Dumbo et al. (2019b) in their multidisciplinary approach to clarify the taxonomic position of *G. pedatum* and *O. batrachoides* within the Plagiochioidea using type material and specimens they collected from *C. gariepinus* in Mozambique.

From this summary, it is clear that *Glossidium* spp. is widely known from catfishes (see **Fig. 3.13. D**) in Africa and that the taxonomic reassignment and synonymy for species of *Glossidium* contribute to the inability to reach a consensus on the placement of the genus and its species to date. Although the genus is still considered *insertae sedis* (*sensu* Tkach 2008) the molecular data accompanied by a morphological re-evaluation and comparison to previously described material provided support for the uncertain status of *Glossidium* in the Plagiorchioidea as well as provided clarity on the validity of both *G. lazerae* and *G. pedatum*, presumed endemics to bagrid and clariid hosts in Africa, with additional morphological features highlighted (see Dumbo et al. 2019b).

Present study: No infection with representatives of *Glossidium* or *Astiotrema* were found from the intestine of *C. gariepinus*.

3.3.1.7. Superfamily Schistosomatoidea Stiles et Hassal, 1898

The superfamily contains three families that are parasitic flukes of the blood or circulatory system of their hosts. The Sanguinicolidae von Graff, 1907 are parasitic in fish, while the Spirorchiidae Stunkard, 1921 are parasitic in reptiles and turtles, and the Schistosomatidae Stiles et Hassall, 18998 are parasitic in reptiles, birds and mammals. At superfamily level unusual variety and diversity is found and the sanguinicolids are of economic, veterinary and medical significance (see Smith 2002a, b and references therein). Species of the sanguinicolids utilise aquatic gastropods and molluscs as intermediate hosts and terrestrial vertebrates as definitive hosts. The Aporocotylidae Odhner, 1912 is considered a junior synonym of the Sanguinicola however, in current literature the Aporocotylidae and Sanguinicolidae are treated as a separate families and are treated here as such (Smith 2002a; Olson et al. 2003).

Family Aporocotylidae Odhner, 1912

Sanguinicola Plehn, 1905 (*nomem dubium*, *incertae sedis*)
(syn. *Janickia* Rašín, 1929; *Phleniella* Szidat, 1951)

Recent efforts to confirm the validity of *Sanguinicola clarias* Imam, Marzouk, Hassan et Itman, 1984, the only aporocotylid known from *C. gariepinus* in Africa, was unsuccessful. No type material could be located for the blood flukes collected from the mesenteries and heart of *C. gariepinus* in Egypt (see Imam et al. 1984; Truong and Bullard 2013). *Sanguinicola clarias* remain a species with questionable validity and the proposed clarification of Truong and Bullard (2013) of *S. clarias*, together with *Plehniella dentata* Paperna, 1964 (both *insertae sedis*) is accepted where the authors are of opinion that they represent a single species or are two closely related species to *Nomasanguinicola* Truong et Bullard, 2013. Saoud et al. (1990) recorded *Sanguinicola* sp. occurring with *Orientocreadium* sp. in some *C. gariepinus* from a man-made lake in Egypt, but these specimens would need re-examination to confirm their validity.

Present study: No infection with specimens resembling blood flukes of *Sanguinicola* was found.

3.3.1.8. Superfamily Clinostomoidea Lühe, 1901

Members of the Clinostomoidea are commonly found unencysted in the buccal cavity, oesophagus and intestines of amphibians, reptiles, birds and on occasion from mammals. Controversy exists on the systematic position and validity of many subfamilies and genera within the superfamily. Two families are recognised within the superfamily, namely the Clinostomidae Luhem 1901 (syn. Nephrocephalidae Travassos, 1929) and Liolopidae Odhner, 1912 (syn. Harmotrematidae Yamaguti, 1933); the latter will not be discussed as there are no parasites belonging to the family recorded from *C. gariepinus* (Kanev et al. 2002a). Kanev et al. (2002b)

noted other recognised families and subfamilies within the Clinostomidae, but only recognised four subfamilies of which two, the Clinostominae Luhe, 1901 (containing *Clinostomoides* Dollfuss, 1950 and *Clinostomum*, Leidy, 1856) and the genus *Euclinostomum* Travassoss, 1928 are accommodated in the Euclinostominae Yamaguti, 1958. Species from these three genera are known from *C. gariepinus* in Africa.

Family Clinostomidae Lühe, 1901

Clinostomoides Dollfus, 1950 and *Clinostomum* Leidy, 1856

Clinostomoides and *Clinostomum* were established based on adult specimens found in the oesophagus of birds and metacercariae that are found unencysted in the buccal cavity, branchial chamber, and intestine or can be encysted in the muscle tissues, on the gills, or in the viscera from the 2nd intermediate fish hosts (see Manter and Pritchard 1969). These genera are distinguished from one another based on the body size, position of the genital complex and position of the genital pore (Dollfus 1950; Kanev et al. 2002b; Caffara et al. 2019). Despite these characteristics, the validity of *Clinostomum* has been in question, and is treated as such in Kanev et al. (2002a, b). Disagreement on the validity of the species contained in *Clinostomoides* also exists as metacercariae of *Clinostomoides dollfusi* Agarwal, 1959 (ex. *Clarias* sp.) were compared with the adult form of *Clinostomoides brieni* Dollfus, 1950 (ex. *Ardea goliath*) and not the metacercariae when initially described (Agarwal 1959). Subsequently, *Cl. dollfusi* was synonymised with *Cl. brieni* (see Dollfus 1950; Agarwal 1958; Fischthal and Thomas 1970; Caffara et al. 2019). In addition to the already existing confusion, several authors mistakenly reported *Cl. brieni* as *Clinostomum brieni* (see Prudhoe 1957; Douëllou 1992). More recently, Caffara et al. (2019) regarded all species of *Clinostomoides* (including *C. dollfusi*) as *species inquirenda*, transferred *Cl. brieni* to the genus *Clinostomum*, and amended the diagnosis of the genus based on morphological and molecular data.

Two clinostomids are known to infect *C. gariepinus*. The metacercariae of *C. brieni* has been widely reported as unencysted in the branchial chambers, unencysted or encysted in the gills, and encysted in muscle tissue from the sharptooth catfish in Botswana, the Democratic Republic of the Congo, Ethiopia, South Africa and Zimbabwe (**Fig. 3.13. E**). *Clinostomum complanatum* (Rudolphi, 1814) has only been found encysted in the muscle tissue of *C. gariepinus* collected in temporary pans and pools in the Save-Runde River floodplain in Zimbabwe (Barson et al. 2008). However, this record is not regarded as valid, as molecular confirmation needs to be done for all reports of this species from the Afrotropical region (see Caffara et al. 2019, 2020). From literature, the only record found of *Clinostomum macrosomum* Jaiswal, 1957 is from Nile tilapia *Oreochromis niloticus* (Linnaeus) collected in Lake Mugesera in Rwanda (Manter and Pritchard

1969). In addition to these species, several authors reported on infection with *Clinostomum* sp. from *C. gariepinus* from freshwater systems in Benin, Ethiopia, Nigeria, South Africa and Tanzania (Yimer 2000; Yimer and Enyew 2003; Mwita and Nkwengulila 2004, 2008; Gulelat et al. 2013; Salawu et al. 2013; Mahlatji 2014; Mwita 2014a; Domo and Ester 2015; Sèdogbo et al. 2019).

Present study: Unencysted metacercariae of *Clinostomum brieni* (**Fig. 3.18. A**–**D**) were found in the branchial cavity of *C. gariepinus* collected from ZR [P = 18%, IF = 2].

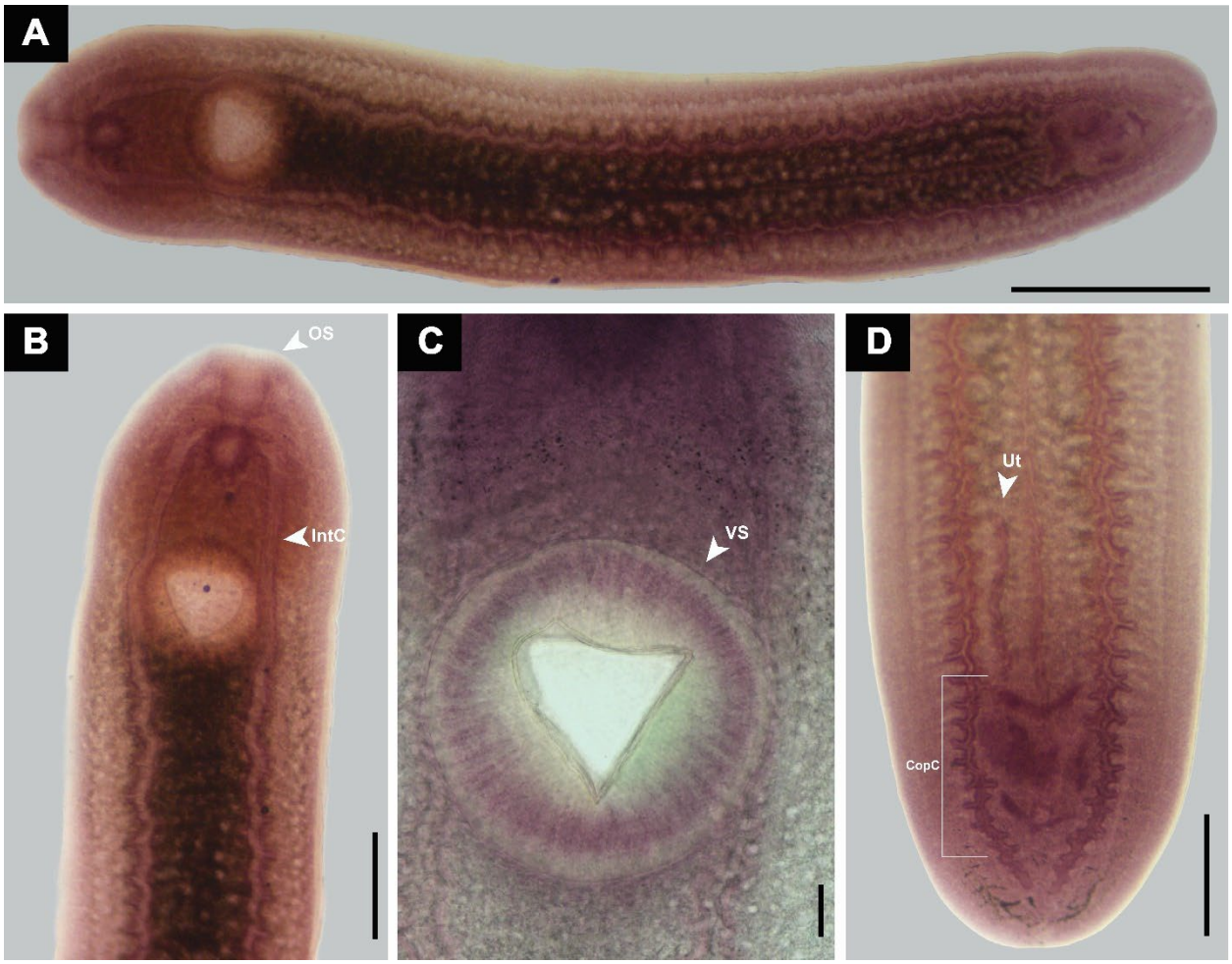

Figure 3.18. Micrographs of *Clinostomum brieni* (Dollfus, 1950) metacercariae found unencysted in the branchial chambers of *Clarias gariepinus* (Burchell) from the Barotseland floodplain, Western Province, Zambia. **A** – whole specimen; **B** – anterior region with oral sucker (OS) and bifurcate intestinal caeca (IntC); **C** – ventral sucker; and **D** – uterus and copulatory complex containing the testes and ovary. Scale bars: 100µm (C), 500µm (B, D) and 1000µm (A).

Identification: Sequences of the ITS1-2 (1236 bp), 28S rRNA region (1228 bp) and the *cox*1 mitochondrial gene region (610 bp) were successfully amplified for a single isolate from ZR (MT29). The BLAST search indicated that this isolate was similar to *C. brieni* collected from *C. gariepinus* in South Africa and *C. ngamensis* from the Democratic Republic of the Congo (Caffara et al. 2019). The 28S rRNA sequence of MT29 was most similar to *C. brieni* from *Heteropneustes fossilis* (Bloch) (Heteropneustidae: Siluriformes) collected in India (see **Table A4.2.)** (Tandon et al. 2015). The alignments of the three gene regions indicated that MT29 was conspecific with *C. brieni* (see **Table 3.6.**).

Table 3.6. Divergence of species of *Clinostomum brieni* (Dollfus, 1950) based on the partial 18S ITS1-2 (1236 bp), 28S rRNA (1250 bp) and *cox*1 mitochondrial (630 bp) regions. Values for p-distance (bottom diagonal) are expressed in percentage, while the top diagonal represent the number of differences in nucleotides. Species in bold were obtained from material collected during the present study.

ITS1-2		1	2	3	4	5	6
1	MH238412 *Clinostomum brieni* SA	–	0	0	0	0	0
2	MH238413 *Clinostomum brieni* SA	0.0	–	0	0	0	0
3	MH238414 *Clinostomum brieni* DC	0.0	0.0	–	0	0	0
4	MH238415 *Clinostomum brieni* DC	0.0	0.0	0.0	–	0	0
5	MH238416 *Clinostomum brieni* DC	0.0	0.0	0.0	0.0	–	1
6	**MT29 *Clinostomum brieni* ZR**	0.0	0.0	0.0	0.0	0.0	–

28S rRNA		1	2	3
1	KF811008 *Clinostomum brieni* IN	–	7	5
2	KF781299 *Clinostomum brieni* IN	0.6	–	29
3	**MT29 *Clinostomum brieni* ZR**	0.4	2.1	–

*cox*1		1	2	3	4	5	6
1	MH253044 *Clinostomum brieni* SA	–	69	1	0	0	1
2	MH253045 *Clinostomum brieni* SA	0.2	–	70	69	69	67
3	MH253046 *Clinostomum brieni* CD	0.0	11.3	–	1	1	2
4	MH253047 *Clinostomum brieni* CD	0.0	11.1	0.2	–	0	1
5	MH253048 *Clinostomum brieni* CD	0.2	11.1	0.2	0.0	–	1
6	**MT29 *Clinostomum brieni* ZR**	0.2	11.1	0.3	0.2	0.2	–

Euclinostomum Travassoss, 1928
(syn. *Distoma* Retzius, 1786)

Adult *Euclinostomum* are typically found in the mouth, oesophagus, pharynx and larynx of piscivorous bird definitive hosts, whereas metacercaria, commonly referred to as “yellow grub”, can be found embedded in the liver or encapsulated on the surface of the kidneys, mesenteries, small intestine, or in the muscles and the coelomic wall of various intermediate freshwater fish hosts (Lombard 1968; Kanev et al. 2002a, b; Caffara et al. 2016). The genus was erected to accommodate its type species *Euclinostomum heterostomum* (Rudolphi, 1809) (syn. *Distoma heterostomum* Rudolphi, 1809) described from specimens found in the oesophagus of the purple heron *Ardea purpurea* L. (Fischthal and Kuntz 1963a; Kanev et al. 2002a, b). Fischthal and Kuntz (1963a) provided a detailed history on the status of species contained in *Euclinostomum* and supplemented their work with a key to the (then) recognised adult and metacercarial forms. Since then, several species have been assigned to the genus based on generic characters and the validity of some of the existing species are questionable (see Mashego and Saayman 1989; Caffara et al. 2016 and references therein).

With regard to species in Africa, *Euclinostomum clarias* Dubois, 1930 (syn. *Clinostomum clarias* Dubois, 1930), *Euclinostomum dollfusi* Fischthal et Kuntz, 1963 and *E. heterostomum* are known from *C. gariepinus*, however only the latter two species are included in the most recent checklist on parasites of African freshwater fishes (Dollfuss 1950; Fischthal and Kuntz 1963a; Onuoha 2010; Kudlai et al. 2018). It is not surprising that metacercariae of *E. heterostomum* were found encysted in tissues of the intermediate host *C. gariepinus* from two dams in the Limpopo River basin as adults of *E. heterostomum* have previously been found from the grey heron *Ardea cinerea* L. and museum material from hammerhead *Scopus umbretta* Gmelin collected in South Africa and Zimbabwe (Prudhoe and Hussey 1977). More recently *E. clarias* and *E. heterostomum* were recorded from the intestine and liver of *C. gariepinus* in Nigeria (**Fig. 3.15. E**) (Mashego and Saayman 1989; Onuoha 2010; Dan-Kishiya et al. 2013). *Euclinostomum dollfusi* was proposed for adult specimens, previously identified as *E. heterostomum*, collected from the oesophagus of the giant heron, *Ardea goliath* in the Democratic Republic of Congo (see Fischthal and Kuntz 1963a). The record of *E. dollfusi* that was included in the checklist of Khalil and Polling (1997) is based on metacercariae found from *C. gariepinus* in South Africa, rendering the identification erroneous, as the authors compared it to adult specimens of *E. dollfusi* (see Mashego 1977; Mashego and Saayman 1989).

Furthermore, Feizullaev and Mirzoeva (1983) and Mashego and Saayman (1989) considered *E. dollfusi* a synonym of *E. heterostomum*. Although *E. clarias* is accepted as a valid species, it was not included in any checklist of African freshwater fishes and is morphologically considered a possible synonym of *E. heterostomum.* This synonymy was also supported by a recent study that

performed a comprehensive morphological and phylogenetic assessment of *Euclinostomum* spp. from Israel that suggests several *Euclinostomum* spp. possibly represent a single species, *E. heterostomum* (see Caffara et al. 2016).

Two records of unidentified metacercariae of *Euclinostomum* are known from *C. gariepinus* in South Africa and Ethiopia. Prudhoe and Hussey (1977) noted that their specimens, collected from the body cavity of *C. gariepinus* in South Africa, closely resemble the immature specimen of *E. dollfusi* collected from *A. ardea* in the Democratic Republic of the Congo. Similar metacercariae were found free in the orbital cavity of *C. gariepinus* in Ethiopia and possibly represent a new species. The new species was compared to metacercariae of *E. heterostomum* and can be differentiated based on its site of infection, overall large size, position of the testes, presence of a muscular bulb in the oesophagus, ratio of the ventral and oral sucker width and that this unidentified species was found unencysted, opposed to *E. heterostomum* metacercariae that are almost exclusively found encysted (Zhokhov and Morozova 2020). To confirm the identity of these metacercariae, as well as the known species of *Euclinostomum* in Africa, it is crucial that known and new material obtained be subjected to comparative morphological and complimentary molecular analyses from all hosts.

Present study: *Clarias gariepinus* collected during the present study was not parasitised with species of *Euclinostomum*.

3.3.2. Order Diplostomida Olson, Cribb, Tkach, Bray et Littlewood, 2003

As previously mentioned, various cercarial types have been identified and included in the identification for members of the Digenea (see **Section 3.4.**). The metacercarial forms of the Diplostomida parasitising African freshwater fishes are characterised by the ‘diplostomulum’, ‘neascus’, ‘prohemistomulum’ and ‘tetracotyle’ types (see **Fig. 3.14. J**–**O**). These diplostomid forms are distinguished on various features including (i) if encapsulated or free in the host; (ii) the shape and size of the forebody and hind body; (iii) the presence or absence of pseudosuckers and (iv) the extent or morphology of the reserve bladder (Niewiadomska 2002a, b).

3.3.2.1. Superfamily Diplostomoidea Poirier, 1886

Members of the superfamily Diplostomoidea Poirier, 1886 are characterised with the presence of a sucker-like or bilobed holdfast organ situated on the ventral side of the body, posterior to the ventral sucker. The function of the holdfast organ is primarily associated with adhesion and digestion (Niewadomska 2002a). Adult forms within the superfamily typically occur in mammals, birds and reptiles, with the metacercarial stages in intermediate hosts such as fish and cercarial stages in molluscs. The taxonomic position and life cycles of many species within the group have

not been resolved due to the taxonomic confusion created by: (i) disagreement between taxonomists (clarifying validity of taxa); (ii) the description of species from different life stages (phenotypic plasticity of adults and metacercariae); (iii) simple larval morphology; and (iv) difficulty in linking of life stages, due to species description solely based on a single life stage using only morphological evidence, or not providing morphological data when molecular identification is performed. The aforementioned resulted in misidentification and placement of specimens in a single species (based on different life stages) that belong to different species, or different species are assigned to a single species name (see Locke et al. 2010a, b; Georgieva et al. 2013a, b; Locke et al. 2015). In the latest revision of the Diplostomoidea, Niewiadomska (2002a) confirms that six families are enclosed within the superfamily, of which two are monotypic and the remaining families, including the Diplostomidae Poirier, 1886 are species rich. Four subfamilies based on the host-specificity of the metacercariae are also recognised within the Diplostomidae: Alarniinae Hall et Widgor, 1918, Crassiphialinae Sudarikov, 1960, Codonecephalinae Sudarikov, 1959 and Diplostominae Poirier, 1886. It is crucial that descriptions of species not be done from metacercariae found in the second intermediate host as it has proven to create too much confusion from a taxonomic perspective. Best practise is that all forms in a species' life cycle be known and when not possible, molecular characterisation of the ribosomal 28S rRNA, ITS, and the mitochondrial *cox*1 gene regions should be used alongside morphology to produce a comprehensive account of the identification of specimens.

Family Diplostomidae Poirier, 1886
(syn. Alariidae Tubangui, 1922)

The Diplostomidae have a typical three host life cycle (see **Fig. 3.12. A**) that includes a freshwater snail (1st intermediate host), a fish (2nd intermediate host) and piscivorous birds, mammals and occasionally amphibians, as definitive hosts (Kennedy and Burrough 1977; Niewiadomska 2002b; Chibwana et al. 2013). Metacercarial forms in the 2nd intermediate host (fish) are also used to distinguish between related genera within the family and are assigned the 'metacercaria type' as discussed earlier. The 'diplostomulum' metacercarial type is primarily retained within the Diplostomidae and is typically found encysted or encapsulated in muscles, mesenteries, and skin, or unencysted in the tissue of eye lenses, vitreous humour and retina of the eye, in the cranial cavity and central nervous system (see Gibson 1996; Chibwana et al. 2013).

Members of the Diplostomidae are also of significant economic and medical importance, where metacercarial forms play a role in altering the physiology or behaviour of the 2nd intermediate host, or in some cases shows pathology in humans (see Georgieva et. al. 2013, Blasco-Costa et al. 2014; Blasco-Costa and Locke 2017; Kudlai et al. 2017; Hoogendoorn 2020 and literature therein). Several species have been reported from the muscle, eyes and cranial cavity of *C.*

gariepinus and were placed in the genera of the subfamilies: Diplostominae (*Diplostomum* von Nordmann, 1932, *Dolichorchis* Dubois, 1961, *Neodiplostomum* Railliet, 1919, and *Tylodephys* Diesing, 1850) and the Crassiphialinae (*Ornithodisplostomum* Dubois, 1936).

Present study: Several underdeveloped metacercariae with no distinct morphological features were found encysted in the eyes, kidneys and connective tissues of *C. gariepinus* from KR [P = 100%, IF (6)] and ZR [P = 41%, IF (1–14)]. *Clarias gariepinus* collected from the RR and VR Rivers in South Africa during the present study were infected with a tetracotyle-type metacercariae found encysted in white cysts. Host individuals from RR were infected in the muscle tissue [P = 8%, IF = 4] while those from VR were infected in the liver [P = 17%, IF = 1]. Morphologically the metacercariae (assigned to Diplostomidae gen. sp. 1) (**Fig. 3.19. A–E**) resemble the tetracotyle species found encysted in the muscle tissue of *C. gariepinus* from the Save-Runde river system in Zimbabwe; the Nwanendi-Luphephe dams and Olifants- and Elands Rivers, Limpopo Province, South Africa (see Prudhoe and Hussey 1977; Barson et al. 2008; Madanire-Moyo 2011).

Identification: Alignments for the ITS1-ITS2 (1135 bp), 28S rRNA (1300 bp) and mitochondrial *cox*1 gene (639 bp) regions were successfully obtained for one isolate from RR (MT31). The GenBank search yielded similarities of MT31 to several genera of the Diplostomidae. From the three alignments for each gene regions, along with the morphological features retained in the tetracotyle metacercarial type, it is evident that MT31 belong within the Diplostomidae (see **Table 3.10.**).

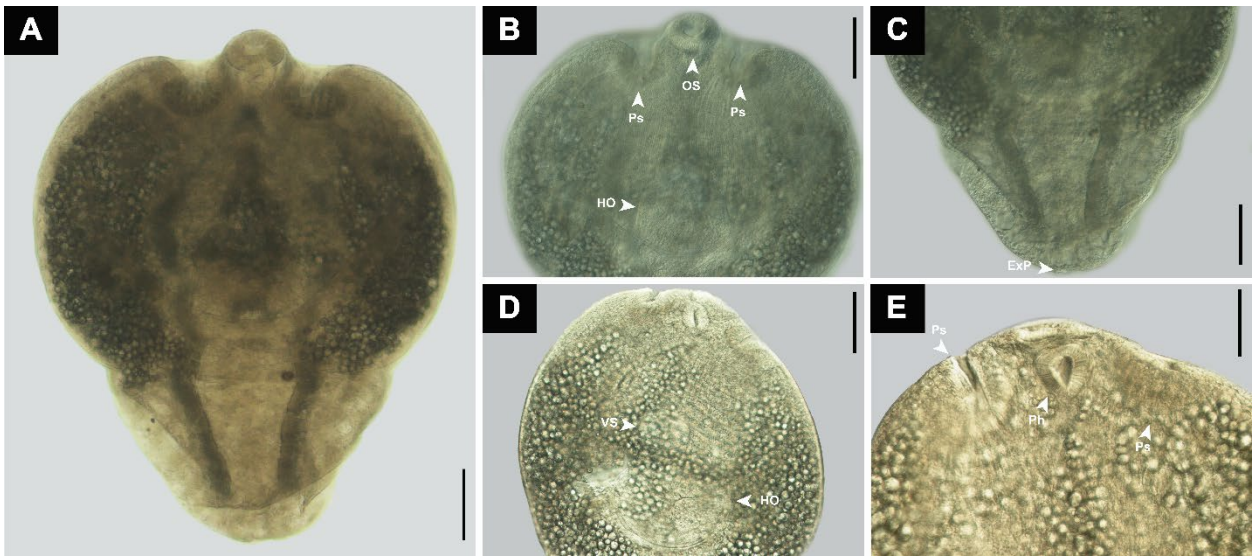

Figure 3.19. Photomicrographs of the tetracotyle-type metacercariae, Diplostomidae gen. sp. 1 found in encysted in the muscle tissue of *Clarias gariepinus* (Burchell) from the Riet and Vaal Rivers in South Africa. **A** – whole specimen; **B** – oral sucker (OS) with two sunken-type suckers and spines on tegument; **C** – posterior end of specimen and excretory pore (exp); **D** – ventral sucker (VS) and holdfast organ (Hf); and **E** – pharynx (Ph). Scale bars: 50µm (E) and 100µm (A, B, C, D).

Table 3.6. Divergence of species of Diplostomidae gen. sp. 1 based on ITS1-2 (1299 bp), 28S rRNA (1376) and cox1 (1002 bp) regions. Values for p-distance (bottom diagonal) are expressed in percentage, while the top diagonal represent the number of differences in nucleotides. Species in bold were obtained from material collected during the present study.

ITS1-2		1	2	3	4	5
1	MK604879 *Bolbophorus* sp. 3 SA	–	134	166	196	173
2	MK604885 Diplostomidae gen. sp. SA	10.4	–	138	167	155
3	MF568598 *Uvulifer spinatus* MX	13.5	12.4	–	67	139
4	MK604882 *Uvulifer* sp. 4 SA	11.9	10.2	3.5	–	122
5	**MT31 Diplostomidae gen. sp. 1 RR**	11.2	9.8	9.8	9.1	–

28S rRNA		1	2	3	4	5	6
1	MK604820 *Bolbophorus* sp. 3 SA	–	47	62	59	34	41
2	MK604826 Diplostomidae gen. sp. SA	3.5	–	55	44	27	35
3	MK604824 *Uvulifer* sp. 4 SA	4.9	4.1	–	61	23	46
4	OL799108 *Neodiplostomum vaucheri* EC	4.5	3.3	4.5	–	22	44
5	OL799087 *Neodiplosotmum banghami* USA	5.6	4.1	3.5	3.4	–	23
6	**MT31 Diplostomidae gen. sp. 1 RR**	3.2	2.5	3.4	3.2	3.6	–

*cox*1		1	2	3	4	5	6
1	MK605688 *Bolbophorus* sp. 3 SA	–	81	77	95	117	119
2	MK605692 Diplostomidae gen. sp. SA	19.2	–	72	79	71	78
3	MK605691 *Uvulifer* sp. 4 SA	18.0	20.4	–	74	75	90
4	OL770067 *Neodiplostomum vaucheri* EC	18.0	18.7	18.1	–	64	92
5	OL770054 *Neodiplosotmum banghami* USA	17.2	16.8	17.6	11.6	–	100
6	**MT31 Diplostomidae gen. sp. 1 RR**	18.6	18.5	21.1	18.0	15.6	–

Diplostomum von Nordmann, 1932

The genus *Diplostomum* represents the most specious group of the family Diplostomidae and was initially discovered during parasitological studies conducted near Berlin and Hamburg in Europe, andit was only decades later that the metacercarial characters were resolved (Braun 1894). The complete life cycle for *Diplostomum* is not fully understood in Africa, but several piscivorous birds, snails (lymnaeid and pulinoid) and fish were found infected with adult and larval stages, respectively, or identified as possible hosts for metacercaria (see Chibwana et al. 2013; Zhokhov 2014).

In Africa infection with diplostomid metacercariae occurs in fishes of nine families, namely the Centrarchidae, Characidae, Cichlidae, Clariidae, Cyprinidae, Hepsetidae, Mochokidae, Salmonidae and Schilbeidae. The records of *Diplostomum* spp. from various countries on the continent (**Fig. 3.13. F**) prove that *C. gariepinus* is a suitable intermediate host to metacercarial forms of the Diplostomidae, however the placement of these specimens needs critical revision to resolve their true identity and placement within the genus. The species *Diplostomum ardae* Dubois, 1969 and *Diplostomum magnicidum* El-Naffar, 1979 are known from Africa, however these records are from definitive piscivorous birds (see Zhokhov et al. 2010).

Prudhoe and Hussey (1977) reported on metacercaria of *Diplostomum* Type I (encysted in the mesenteries) and *Diplostomum* Type II (free in the cranial cavity) from *C. gariepinus* in South Africa. Hoogendoorn et al. (2020) contested the validity of these two species based on the state (encysted in mesenteries) and metacercarial morphology of the illustrations provided in the original description. The authors consider *Diplostomum* Type I as erroneous within the Diplostomidae and *Diplostomum* Type II as belonging the Diplostomidae, but not the genus *Diplostomum*.

Diplostomum Type 3, retaining the 'diplostomulum' metacercarial type is reported from the eyes and cranial cavity from *C. gariepinus* in South Africa and Tanzania (Madanire-Moyo 2011; Madanire-Moyo et. al. 2010). Several authors reported on *Diplostomum* sp. from the eyes and cranial cavity of the same hosts in Ethiopia, South Africa and Zimbabwe, however no morphological or molecular data is provided to support their placement of specimens within the genus (Madanire-Moyo and Barson 2010; Madanire-Moyo et. al. 2012; Gulelat et al. 2013; Mahlatji 2014). Similarly, *Diplostomum spathaceum* reported from the intestine of *C. gariepinus* in Nigeria (see Goselle et al. 2008) is most probably an erroneous identification, as the site of infection is not known for this species and no morphological or molecular support was provided.

Dolichorchis Dubois, 1961

Two species are included in this genus: *Dolichorchis marahoueense* Baer, 1957 and *Dolichorchis tregenna* (syn. *Diplostomum tregenna*) Nazmi, 1932. The status of *D. marahoueense* is uncertain, while *D. tregenna* has been reported from intermediate (fish) and definitive hosts (birds). The latter species was first described from adults found in the intestine of the definitive piscivorous host, the Egyptian Kite *Milvus migrans aegypticus* (Gmelin) in Egypt (Nazmi 1932). Three decades later, during parasitological surveys in Sudan, Khalil (1963) found *C. gariepinus* from the Blue and White Nile Rivers to be infected in the brain with 'diplostomulum' metacercaria. Identification was possible when the author succeeded in obtaining adults by feeding the metacercaria to chicks. Reports of *D. tregenna* metacercariae from the cranial cavity of *C. gariepinus* are known from Egypt and Ethiopia (see **Fig. 3.13. F**) (Khalil 1969; Ibraheem 2000; Zhokhov et al. 2010). The reports of *D. tregenna* from wild and cultured catfish in Nigeria are most probably erroneous as the specimens in the study of Anosike et al. (1992) provide no morphological or molecular support for their identification and Yakubu et al. (2002) note that they found the metacercariae from the intestine of *C. gariepinus*, a rather unusual find since metacercaria are known to occur in the cranial cavity of the intermediate host, and adults are known from the intestine of definitive bird hosts.

Neodiplostomum Railliet, 1919

The genus is distinguished from other genera based on its host-specificity and distribution, has 'diplostomulum' or "neodiplostomulum" metacercariae types in the intermediate fish host, and is primarily known from falconiform and strigiform birds, and the Nile crocodile in Africa (Bisseru 1956, 1957; Prudhoe and Hussey 1977; Niewiadomska 2002b). Metacercaria of species in the genus can only be assigned to species if the life cycle is known. Prudhoe and Hussey (1977) reported a *Neodiplostomum*-type from the mesenteric tissue of *C. gariepinus* in South Africa. Jansen van Rensburg et. al. (2013) found similar specimens encysted in the muscle of the same host in the Okavango Delta, Botswana (**Fig. 3.13. F**), however they differ from the *Neodiplostomum*-type from South Africa in the position of the gonads and holdfast organ and were assigned to *Neodiplostomum* Type 1.

Present study: During the present study two individuals that retain the neodiplostomulum-type metacercarial morphology was found from *C. gariepinus*. Metacercariae with similar morphology have been reported by Prudhoe and Hussey (1977) and Jansen van Rensburg et al. (2013) from *C. gariepinus*. The *Neodiplostomum*-type metacercariae from the present study (**Fig. 3.20. A**–**E**) were found encysted in white cysts in the muscle tissue of a single host specimen collected in ZR [P = 6%, IF = 2]. A conspecific individual was found from the eye viscera of a single host individual collected in the NGR [P = 5%, IF = 1]. Both specimens from this study resemble those found from catfish in the Okavango Delta, thus we assign our specimens to *Neodiplostomum* Type 1.

Identification: Sequences for the 28S rRNA (1226 bp) and *cox*1 mitochondrial gene (738 bp) regions for one isolate from NGR (isolate 42KS) were generated. The closest congeners to 42KS were species representing several genera of the Diplostomidae (see **Table A4.2.** and **Table 3.7.**).

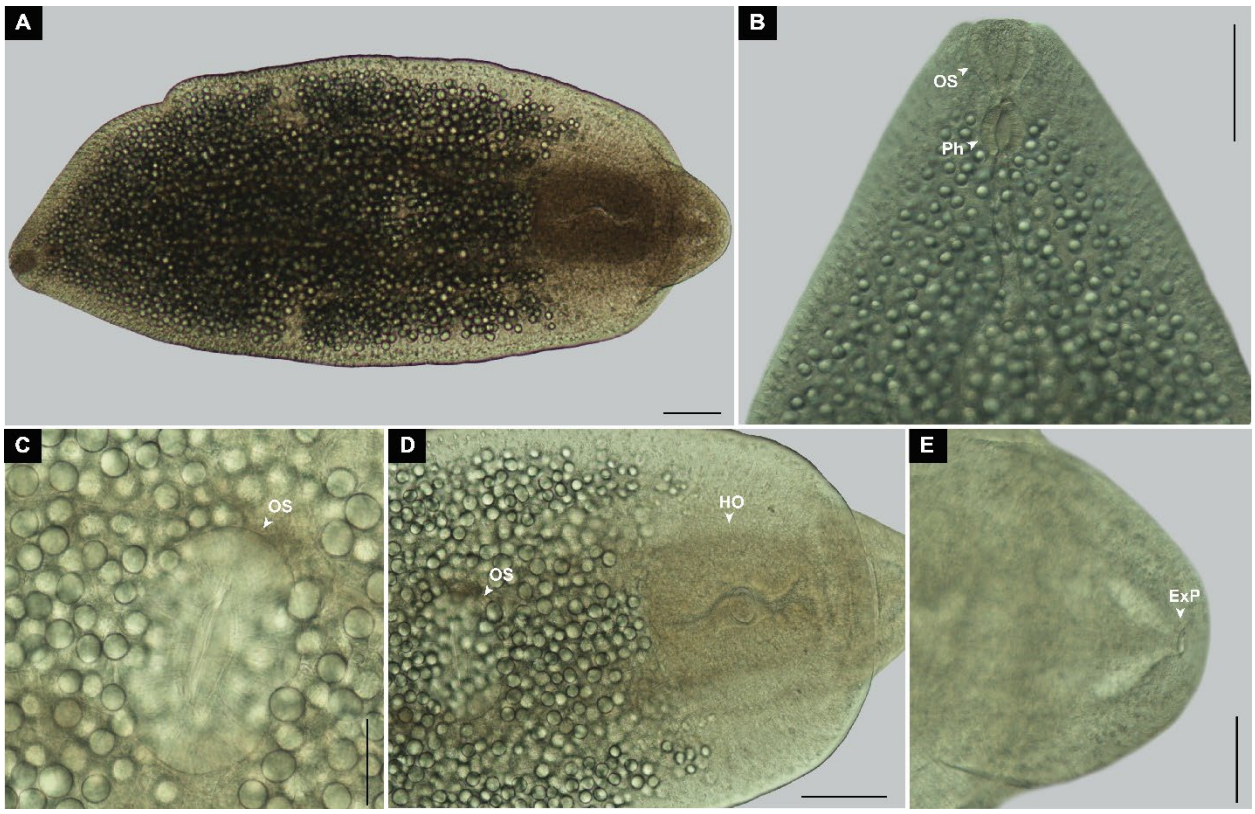

Figure 3.20. Photomicrographs of the *Neodiplostomum* Railliet, 1919 Type I metacercariae found from *Clarias gariepinus* (Burchell), encysted in muscle tissue of the host collected in the Barotseland floodplain, Northwestern Province, Zambia and unencysted in the eye viscera from a host specimen collected in the Ndumo Game Reserve, KwaZulu-Natal, South Africa. Scale bars: 10µm (A), 50µm (C, E) and 100µm (B, D).

Table 3.7. Divergence of species of *Neodiplostomum* Railliet, 1919 type based on 28S rRNA (1346 bp) and *cox*1 (1006 bp) regions. Values for p-distance (bottom diagonal) are expressed in percentage, while the top diagonal represent the number of differences in nucleotides. Species in bold were obtained from material collected during the present study.

28S rRNA		1	2	3	4	5	6	7	8
1	MK604824 *Uvulifer* sp. 4 SA	–	62	56	62	58	23	61	93
2	MK604820 *Bolbophorus* sp. 3 SA	4.7	–	47	59	48	34	59	93
3	MK604826 Diplostomidae gen. sp. SA	4.2	3.5	–	45	44	28	41	70
4	OL799108 *Neodiplostomum vaucheri* EC	4.4	4.3	3.3	–	42	23	14	71
6	MW411445 *Sphincterodiplostomum musculosum* BR	4.4	3.6	3.3	3.2	–	16	32	61
7	OL799087 *Neodiplostomum banghami* USA	3.5	5.2	4.3	3.2	2.5	–	20	32
8	OL799102 *Neodiplostomum* cf. *lucidum* USA	4.5	4.4	3.1	0.9	2.5	3.0	–	64
9	**42KS *Neodiplostomum* Type 1 NGR**	7.2	6.3	5.3	5.5	4.9	5.4	5.1	–

*cox*1		1	2	3	4	5	6	7	8
1	MK605691 *Uvulifer* sp. 4 SA	–	77	72	74	90	75	68	102
2	MK605688 *Bolbophorus* sp. 3 SA	18.0	–	81	95	123	117	92	144
3	MK605692 Diplostomidae gen. sp. SA	20.4	19.2	–	79	89	71	81	107
4	OL770067 *N. vaucheri* EC	18.1	18.0	18.7	–	92	64	49	120
5	MW410856 *S. musculosum* BR	22.3	23.5	21.1	16.9	–	99	85	139
6	OL770054 *N. banghami* USA	17.6	17.2	16.8	11.6	18.2	–	64	148
7	OL770043 *N.* cf. *lucidum USA*	18.2	19.2	19.2	10.2	17.7	13.4	–	112
8	**42KS *Neodiplostomum* Type 1 NGR**	23.9	21.2	25.4	21.7	25.5	20.2	23.4	–

Tylodelphys Diesing, 1850

Tylodelphys is a cosmopolitan species in freshwater systems, and the representatives retain the diplostomulum-type metacercariae. Metacercariae are most frequently encountered unencysted (free) in the cranial cavity and eyes of their second intermediate fish host. Definitive hosts in Africa have been identified as piscivorous birds i.e., the grey heron *Ardea cinerea* L. and great egret *Egretta alba* L., and the amphibian, the African clawed frog *Xenopus laevis* Daudin (King and Van As 1997; Chibwana 2015). Knowledge on the metacercaria of *Tylodelphys* is also of importance in the aquaculture sector as heavy infections can result in mass mortalities, behavioural changes that can increase susceptibility to predation, increase chances of secondary bacterial infection, and affect productivity in aquaculture (see Grobbelaar 2011; Chibwana et al. 2015) (**Fig. 3.13. F**).

Two species, *Tylodelphys mashonensis* (syn. *Diplostomum* (*Tylodelphys*) *mashonense*) Beverly-Burton, 1963 and *Tylodelphys grandis* Zhokhov, Morozova et Tessema, 2010 are known to infect *C. gariepinus* in Africa. *Tylodelphys mashonensis* was described from adult forms found in the intestine of the avian definitive host, *A. cinerea* in Zimbabwe (Beverley-Burton 1963). Metacercariae recovered from *C. gariepinus* during the same study were fed to chicks to obtain adults that were identical to those found in *A. cinerea*. The authors were able to experimentally obtain miracidia, as well as sporocysts from laboratory reared lymnaeids, however cercariae were

not obtained and studied (Beverley-Burton 1963). Nkwengulila and Kigadye (2005) reported that *Biomphilaria pfeifferi* (Krauss) from Tanzania is a suitable host to *Tylodelphys*-like metacercariae, while Chibwana et al. (2015) successfully obtained all life stages i.e., sporocysts, furcocercariae, metacercariae, and adults from the respective snail (*Bulinus* sp.), fish (*C. gariepinus*) and bird (*A. cinerea* and *E. alba*) hosts and could delineate them through morphological and molecular techniques. Mashego and Saayman (1989) and Moema et al. (2013) all reported on *T. mashonensis* from the cranial cavity of *C. gariepinus* from various freshwater impoundments in South Africa, while Barson et al. (2008) reported on unencysted *T. mashonensis* (reported as *Diplostomum mashonense*) metacercariae from the intestine of *C. gariepinus* from the Save-Runde system and noted that their species might be similar to the *T. mashonensis* [reported as *Diplostomum* sp. 4 - *Diplostomum* (*Tylodelphys mashonense*)] from Lake Kariba (Zimbabwe) by Douëllou (1992). *Tylodelphys* sp. X and *Tylodelphys* sp. Y reported from Lake Victoria in Tanzania by Mwita and Nkwengulila (2004) are synonymous with *Tylodelphys* sp. 1 and *Tylodelphys* sp. 2 reported from the same locality by Musiba and Nkwengulila (2006), Mwita and Nkwengulila (2008, 2010) and Mwita (2011b; 2014a, b). These authors identified three distinct diplostomid morphotypes from the cranial cavity of *C. gariepinus* viz., *Diplostomum mashonense* (Type 3), *Tylodelphys* sp. 1 and *Tylodelphys* sp. 2, however until recently it was uncertain if they belong to the same species. Chibwana and Nkwengulila (2010) performed an in-depth morphological analysis of the three morphotypes from the same host collected from Lake Victoria and the Msimbazi, Ruvu and Kilembero rivers in Tanzania. They observed a high degree of morphometric variation among the diplostmid morphotypes and concluded that the assignment of diplostomid metacercariae to a species based solely on morphological features should be done with caution. Based on an in-depth morphological study complimented with characterisation of the ITS and *cox*1 regions for the three morphotypes from Lake Victoria and the three previously mentioned rivers, Chibwana et al. (2013) confirmed that there is a high degree of morphological variation between diplostmid metacercariae from the cranial cavity of *C. gariepinus*. They suggested that the differentiation of *Tylodelphys* spp. from another diplsotmid, *D. tregenna* (see above) can be made on the size of the body and smaller suckers, pharynx and holdfast organ. Additionally, they re-assigned *D. mashonense* to *Tylodelphys*; confirmed that *Tylodelphys* sp. 1 and *Tylodelphys* sp. 2 are two distinct species and that the structure of the terminal genitalia, rather than symmetry of the testes, is relevant in species delineation.

Tylodelphys grandis was recently described based on metacercariae found in the cranial cavity and adipose tissue surrounding the brain of *C. gariepinus* from Lake Tana in Ethiopia. During the same study *T. grandis* specimens were also found to co-occur in low intensities with *D. tregenna* in some host individuals. Differentiation between *T. mashonensis* and *T. grandis* was not discussed when the latter species was described, however the authors mentioned the opaque

appearance of specimens (most probably to the high density of granules in the body) and confirmed that it is the largest metacercariae of the genus known to date.

Present study: Two morphotypes of *Tylodelphys* were found from *C. gariepinus* in the present study and are assigned to *T. mashonensis* and *T.* cf. *grandis* (sensu Zhokhov et al. 2010) based on the size difference in whole specimens, the, smaller ventral sucker size, and the opaque and highly granulated appearance of the latter morphotype. *Tylodelphys mashonensis* (**Fig. 3.21. A**–**D**) was recorded from the KR [P = 100%, IF = 753–4208] and ZR [P = 100%, IF = 1–3377] in Zambia; from sites within the NGR [P = 73%, IF = 1–1070], BS [P = 67%, IF = 1–443], the VR [P = 17%, IF = 1–4] and the SR [P = 73%, IF = 5–1001]. *Tylodelphys* cf. *grandis* (sensu Zhokhov et al. 2010) (**Fig. 3.22. A**–**D**) was only recorded from the KR [P = 100%, IF = 18–955] and ZR [P = 47%, IF = 79]. Infection with both species of *Tylodelphys* was primarily in the cranial cavity of the host at all localities. *Tylodelphys mashonensis* specimens from BS (n = 1) and the VR (n = 1) were found from the eye of *C. gariepinus* while infections from KR and ZR were found in the cranial cavity. When the latter infections were present in high intensities [IF = 37–4208], several hundreds were found unencysted in the body cavity and even on the gills of the host individuals.

Co-occurrence: *Tylodelphys mashonensis* and *T.* cf. *grandis* in *C. gariepinus* from KR and ZR.

Identification: Sequences for the ITS1-2 region, 28S rRNA region and *cox*1 mitochondrial gene were successfully amplified for five isolates: 3BB1 (NGR; ITS1-2: 1288 bp, 28S: 1220 bp, *cox*1: 762 bp) and isolates MT19-22 (ITS1-2: 1260–1285 bp, 28S: 949–1243 bp, *cox*1: 693–838 bp) from ZR. From an additional four, isolates 1US1, 1US2, 3US2, 32SR1 only the ITS1-2 (1237-1281 bp) and 28S rRNA region (1217–1244 bp) were successfully amplified. The BLAST search indicated similarities to congeners from *C. gariepinus* in Tanzania (see **Table A4.2.**). From the similarity table for the three alignments with congeners it is evident that the isolates from the present study belong to *Tylodelphys.* All three gene regions for the isolates from NGR were all conspecific (identical) to *T. mashonensis* from *C. gariepinus* in Tanzania and to *T. mashonensis* (reported as Diplostomidae spp.) from *T. sparrmanii* in South Africa (see **Table 3.8.**). All isolates from ZR were initially assigned to *T.* cf. *grandis*, however, divergence in the ITS1-2 and *cox*1 regions indicate that at least three species, *T. mashonensis*, *Tylodelphys* sp. 1 and *Tylodelphys* sp. 2, are present, similar to the reports of Chibwana et al. (2013) from Lake Victoria, Tanzania.

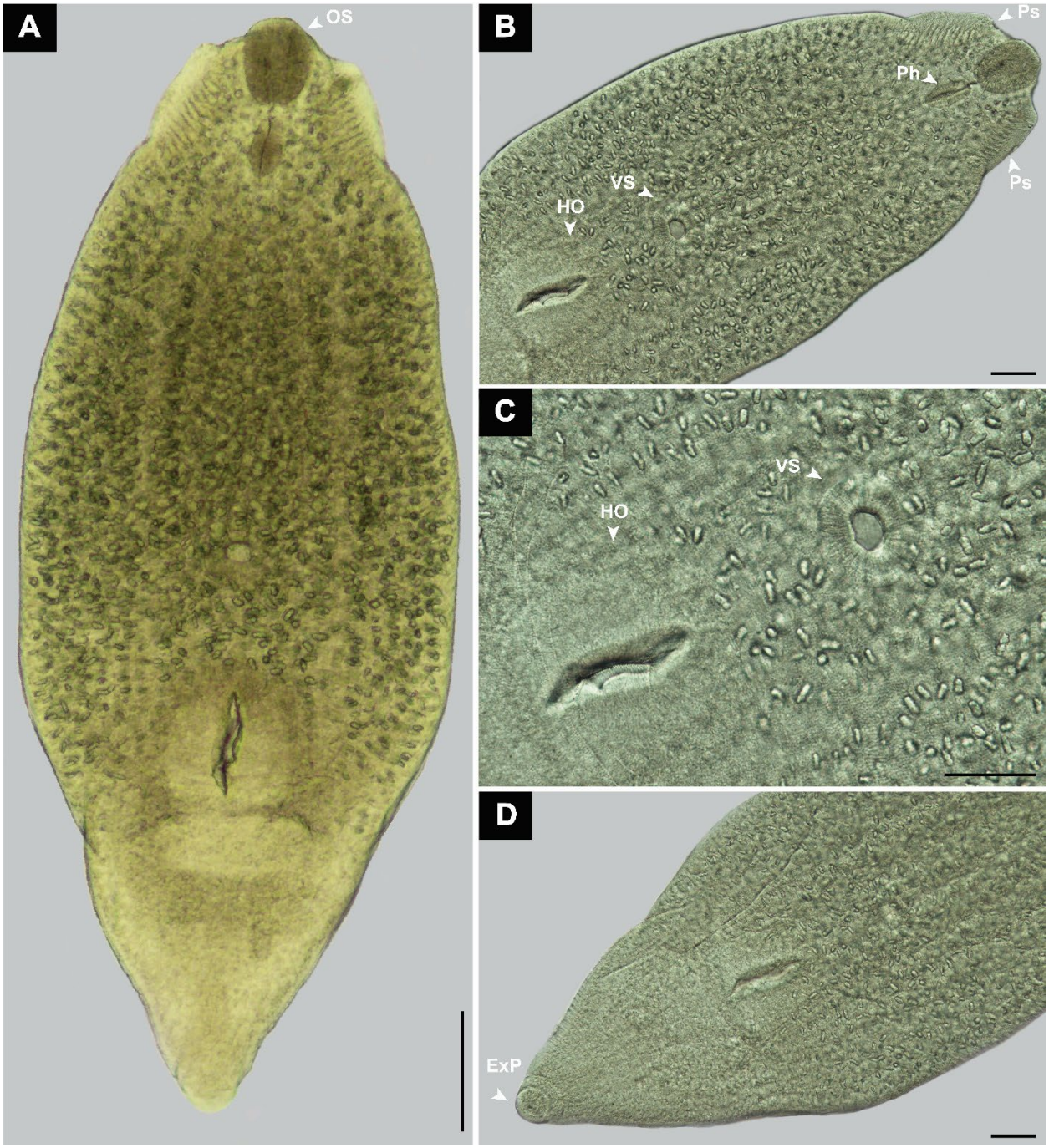

Figure 3.21. Photomicrographs of *Tylodelphys mashonensis* Beverley-Burton, 1963 found in the cranial cavity, eye viscera and body cavity of *Clarias gariepinus* (Burchell) collected from the Sand and Vaal rivers, Boskop Dam, the Ndumo Game Reserve, the Barotseland floodplain and Kabompo River in the host's native distribution range. Infected *C. gariepinus* from the Great Fish River (translocated host range). **A** – whole organism; **B** – oral sucker (OS) holdfast organ (HO), pharynx (Ph), pseudosuckers (Ps) and ventral sucker (VS); **C** – detail of HO and VS; and **D** – excretory pore (ExP). Scale bars: 50µm (B, C, D) and 100µm (A).

Figure 3.22. Photomicrographs of *Tylodelphys* cf. *grandis* Zhokhov, Morozova et Tessema, 2010 found in the cranial cavity of *Clarias gariepinus* (Burchell) collected from Barotseland floodplain, Western Province and Kabompo River, Northwestern Province, Zambia. **A** – whole organism; **B** – oral sucker (OS); **C** – ventral sucker (VS); **D** – ventral sucker (VS). Scale bars: 50µm (C, D) and 100µm (A, B).

Table 3.8. Divergence of species of *Tylodelphys* Diesing, 1850 based on the partial ITS1-2 (1293bp), 28S rRNA (1276 bp) and *cox*1 mitochondrial (838 bp) regions. Values for p-distance (bottom diagonal) are expressed in percentage, while the top diagonal represent the number of differences in nucleotides. Species in bold were obtained from material collected during the present study.

ITS1-2		1	2	3	4	5	6	7	8	9	10	11	12
1	KC685365 *Tylodelphys* sp. 1 TA	**–**	20	35	1	21	35	21	36	35	35	35	35
2	FJ470401 *Tylodelphys* sp. 2 TA	2.2	**–**	47	37	13	49	36	51	50	51	50	43
3	FJ470402 *Tylodelphys mashonensis* TA	4.0	4.1	**–**	48	48	5	48	6	5	5	4	3
4	**MT19 *Tylodelphys* cf. *grandis* ZR**	0.1	3.0	3.7	**–**	33	45	31	49	47	49	46	43
5	**MT20 *Tylodelphys* cf. *grandis* ZR**	2.1	0.9	3.9	2.1	**–**	43	31	47	45	47	44	43
6	**MT21 *Tylodelphys* cf. *grandis* ZR**	4.0	4.3	0.3	3.1	3.1	**–**	45	4	2	4	2	0
7	**MT22 *Tylodelphys* cf. *grandis* ZR**	2.1	3.1	3.9	2.0	2.1	3.3	**–**	49	47	49	46	42
8	**1US1 *Tylodelphys mashonensis* NGR**	4.0	4.3	0.2	3.1	3.1	0.0	3.3	**–**	4	7	5	4
9	**1US2 *Tylodelphys mashonensis* NGR**	4.0	4.3	0.2	3.1	3.1	0.0	3.3	0.0	**–**	3	2	2
10	**3US2 *Tylodelphys mashonensis* NGR**	4.0	4.4	0.2	3.2	3.2	0.1	3.3	0.0	0.0	**–**	4	4
11	**3BB1 *Tylodelphys mashonensis* NGR**	4.0	4.4	0.2	3.2	3.2	0.1	3.3	0.0	0.0	0.0	**–**	1
12	**32SR1 *Tylodelphys mashonensis* NGR**	4.0	3.8	0.2	3.0	3.2	0.0	3.1	0.0	0.0	0.1	0.1	**–**

28S rRNA		1	2	3	4	5	6	7	8	9	10	11
1	KF189071 *Tylodelphys* cf. *mashonensis* SA	–	14	11	12	0	15	1	5	0	0	1
2	KF189072 *Diplostomum* sp. SA	1.0	–	8	13	14	15	15	19	13	13	14
3	**MT19 *Tylodelphys* cf. *grandis* ZR**	0.9	0.7	–	9	11	11	12	12	11	11	12
4	**MT20 *Tylodelphys* cf. *grandis* ZR**	0.9	1.1	0.8	–	12	8	13	16	12	12	13
5	**MT21 *Tylodelphys* cf. *grandis* ZR**	0.0	1.0	0.9	0.9	–	15	1	5	0	0	1
6	**MT22 *Tylodelphys* cf. *grandis* ZR**	1.0	1.1	0.9	0.6	1.0	–	16	20	14	14	15
7	**1US1 *Tylodelphys mashonensis* NGR**	0.1	1.1	1.1	1.0	0.1	1.1	–	6	1	1	2
8	**1US2 *Tylodelphys mashonensis* NGR**	0.0	1.0	1.0	0.9	0.0	1.0	0.1	–	4	4	5
9	**3US2 *Tylodelphys mashonensis* NGR**	0.0	1.0	0.9	0.9	0.0	1.0	0.1	0.0	–	0	1
10	**3BB1 *Tylodelphys mashonensis* NGR**	0.0	1.0	0.9	0.9	0.0	1.0	0.1	0.0	0.0	–	1
11	**32SR1 *Tylodelphys mashonensis* NGR**	0.0	1.0	1.0	0.9	0.0	1.0	0.1	0.0	0.0	0.0	–

*cox*1 (838 bp)		1	2	3	4	5	6	8	9
1	KC685327 *Tylodelphys mashonensis* TA	–	63	61	65	60	2	63	2
2	KC685346 *Tylodelphys* sp. 1 TA	14.4	–	54	4	52	61	50	61
3	KC685356 *Tylodelphys* sp. 2 TA	13.9	12.3	–	53	6	61	46	61
4	**MT19 *Tylodelphys* cf. *grandis* ZR**	14.8	0.9	12.1	–	82	86	80	88
5	**MT20 *Tylodelphys* cf. *grandis* ZR**	13.7	11.9	1.4	12.1	–	94	70	93
6	**MT21 *Tylodelphys* cf. *grandis* ZR**	0.5	13.9	13.9	12.4	12.9	–	96	1
7	**MT22 *Tylodelphys* cf. *grandis* ZR**	14.4	11.4	10.5	11.5	9.4	12.9	–	98
8	**3BB1 *Tylodelphys mashonensis* NGR**	0.5	13.9	13.9	12.7	12.5	0.1	12.9	–

3.3.2.2. Subfamily Crassiphilinae Sudarikov, 1960

Ornithodiplostomum Dubois, 1936

Representatives of the genus are primarily known from the Holartic and northern African regions. They have a three-host life cycle where piscivorous birds are the definitive host, and gastropods and fishes are utilised as the 1st and 2nd intermediate hosts (Zhokhov and Pugacheva 2020). Members of *Ornithodiplostomum* are well-known for disease caused in their fish hosts as metacercariae encyst in the brain (Matisz et al. 2010). However, reports of metacercariae of *Ornithodiplostomum* spp. from the sharptooth catfish in Africa do not concur. Barson et al. (2006b) reported on the first infection of any morphotypes of *Ornithodiplostomum* from the southern hemisphere in South Africa when they found metacercariae encysted in white cysts in the muscle tissue of *C. gariepinus*. Their specimens had no pseudosuckers, although a large holdfast organ and developing immature testes was present. Rashied et al. (2016) found metacercariae encysted in different tissues of the sharptooth catfish from the Nile River in Egypt, while Akoll et al. (2012) collected their specimens of *Ornithodisplostomum* sp. in the viscera of the host. The latter authors did not indicate if the specimens were encysted or not. Achatz et al. (2021) recently re-evaluated the diversity of the genera in the Crassiphilinae and synonymised *Ornithodiplostomum* and another genus *Mesoophorodiplostomum* Dubois, 1936 with *Posthodiplosotmum* Dubois, 1936. Members of the latter genus have been found encysted in various organs in their fish hosts, including the vitreous humour of the eye and the liver, body cavity, mesenteries and hepatopancreas (Stoyanov et al. 2017). Although the taxonomic position of the genus *Ornithodiplostomum* is not debated nor clarified in this section, the synonymy of genera by Achatz et al. (2021) may explain the finding of the African species in the various organs, except for the brain.

Present study: No infection with specimens of *Ornithodiplostomum* were found to infect *C. gariepinus*.

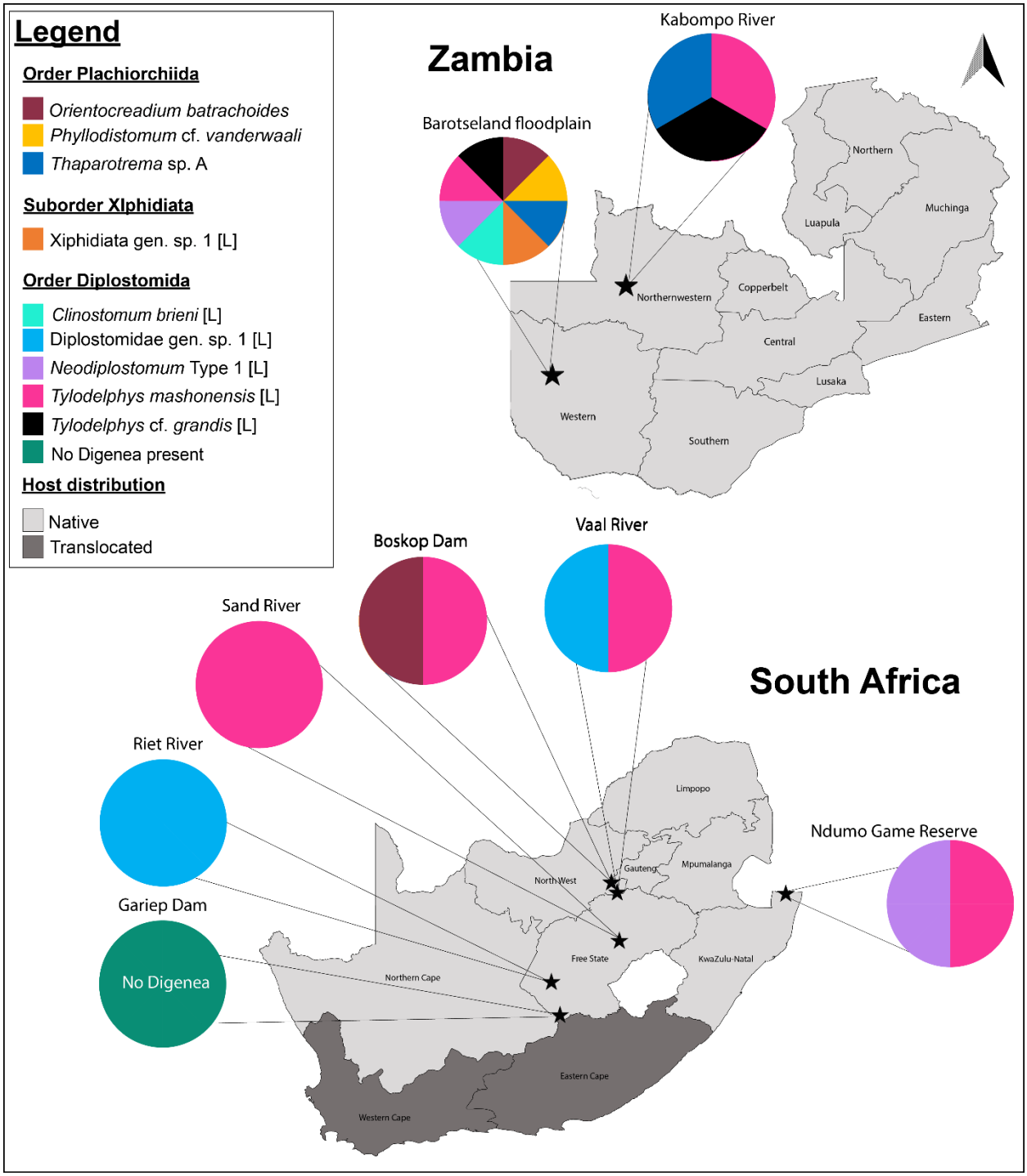

Figure 3.23. Visual representation of the presence and absence of Digenea infecting different organs of *Clarias gariepinus* (Burchell) in the present study. Note that the sectors of the pie-chart do not depict any numerical or statistical value regarding prevalence, intensity of infection or other infection statistic.

Concluding remarks

In addition to all the digenean species discussed above, several records of unidentified or unconfirmed species or morphotypes are found in the literature available on the parasite fauna of *C. gariepinus* in Africa. These records include the report of a *Prohemistomum* sp., most probably referring to the metacercarial type of the specimens and undifferentiated metacercariae and haplorchid metacercariae from the gill filaments, muscles, liver, spleen and kidneys from catfish in Egypt (Shaneen et al. 2014; Rashied et al. 2016); a *Neascus* sp., most probably referring to the metacercarial form of the specimens, from the skin of catfish in Nigeria (Abba et al. 2018a); an unidentified species from the same host in Lake Victoria, Tanzania (Mwita and Nkwengulila 2008); *Zoognoides* sp. from the intestines and *Gymnachitrema* sp. from *C. gariepinus* in Uganda and Benin, respectively (Akoll et al. 2012; Sèdogbo et al. 2019); and an *Echinostomum* sp. and several digenean cysts from the gills of catfish and *Acanthostomum* sp. encysted in the gills of *C. gariepinus* from the Phalaborwa barrage on the Olifants River and Nwanedi-Luphephe dams in South Africa, respectively (Madanire-Moyo et al. 2010, 2012; Mahlatji 2014). To date species of *Acanthostomum* have only been recorded from bagrid, claroteid and gymnarchid hosts, thus if this species from South Africa is found to belong to this cryptogonimid genus, it will be a new host record. Unidentified trematode cysts were also found from the sharptooth catfish in the Kwando River in Namibia (Avenant-Oldewage and Oldewage 1993). The report of *Paramacroderoides echinus* Venard, 1941 and *Emoleptalea* sp. from the intestine of sharptooth catfish in Egypt needs confirmation. The former species is known from shortnose gar, *Lepisosterus platostomus* Rafniesque in the United States of America, while species of *Emoleptalea* are known from bagrid, mochokid,notobranchiid and schilbeiid fishes and *C. gariepinus* may be a potential host (Gado et al. 2014; King et al. 2018; Kudlai et al. 2018; Curran et al. 2021). Lastly, the infection of *C. gariepinus* with *Paraclecithodendrium chilostomum* (Mehlis, 1831) (Lecithodendriidae: Paramphistomoidea) is deemed an accidental infection as this paramhpistomid is in fact a parasite of bats (see Morozova and Zhokhov 2016; Zhokhov et al. 2017).

Regarding digenetic worms found from *C. gariepinus* during this study (**Fig. 3.23.**), there is still a void in documenting its parasite community, although previous studies have already shown aspects of the biodiverse community. There is also a lot to learn on the role the sharptooth catfish plays in different parasite's life cycles, especially since it has a very unrestricted diet and would most probably be a suitable definitive or intermediate host for many parasitic taxa. However, despite this an incredible effort has already been done by past and present parasitologists to document the range of parasites for this host. Similar to the findings of Jansen Van Rensburg et al. (2013) who noted the very high diversity for digeneans in a single host individual in the Okavango Delta (up to four digeneans in a host individual each in a different niche), the present study highlighted the opportunity and role that *C. gariepinus* can play in any ecosystem. In this

study the adult digeneans *Orientocreadium batrachoides*, *Phyllodistomum* cf. *vanderwaali* and *Thaparotrema* sp. A utilised the sharptooth catfish as a definitive host. Metacercariae of *Clinostomum brieni*, *Neodiplostomum* Type 1, *T. mashonensis*, *T.* cf. *grandis*, Xiphidiata gen. sp. 1 and unidentified encysted diplostomids utilised *C. gariepinus* as a 2nd intermediate host. Up to two adults and four metacercarial forms were found from a single host individual that included *O. batrachoides* and *Thaparotrema* sp. A infecting the intestine and gallbladder, respectively. While unencysted metacercaria of *C. brieni* and Xiphidiata gen. sp. 1 infected the branchial cavity and eye, respectively. The cranial cavity was infected with two co-occurring unencysted metacercariae of *T. mashonensis* and *T.* cf. *grandis* and unidentified diplostomid metacercariae were encysted in the kidneys and connective tissues. *Clarias gariepinus* populations from all the localities sampled, except GD, that were within the natural distribution of the host were infected with digeneans. The ten digenean species found from *C. gariepinus* in this study all represent new distribution records in South Africa and Zambia. Infection of *C. gariepinus* with the adult *O. batrachoides* and metacercariae of Xiphidiata gen. sp. 1 are first records for South Africa. While infection of hosts individuals with adults of *P.* cf. *vanderwaali*, *Thaparotrema* sp. A, and metacercariae of *C. brieni*, *Neodiplostomum* Type 1, *T. mashonensis* and *T.* cf. *grandis* are all novel records from the sharptooth catfish in Zambia. The presence and absence of digeneans found from *C. gariepinus* are presented in **Fig. 3.23**. The record of *P.* cf. *vanderwaali* represents a new site of infection and extends it distribution range in southern Africa as it was previously only known from *C. gariepinus* from its type locality in the Olifants River and from the Okavango Delta in South Africa and Botswana, respectively (Jansen van Rensburg 2013). *Thaparotrema* sp. A from ZR is the second record of this opistorchiid infecting *C. gariepinus* and its distribution is expanded northward into the Upper Zambezi system. *Orientocreadium batrachoides* is one of the three most widely distributed digeneans known from *C. gariepinus*, the others being *A. reniferum* and *G. pedatum* (both *insertae sedis*). The southernmost distribution range of *O. batrachoides* was previously from Mozambique. The current distribution range of *O. batrachoides* is extended southward with the novel record in the natural distribution range of the host to BS in the Vaal River system South Africa (Dumbo et al. 2019b). The northernmost distribution for *C. brieni* in the native range of *C. gariepinus* is from Ethiopia, and the southernmost the Phalaborwa barrage (Olifants River) in South Africa. The novel record from ZR extends its known range northward in southern Africa. There are only two records for infection of *C. gariepinus* with *Neodiplostomum* Type 1 in southern Africa, viz., at the confluence of the Olifants and Elands rivers in the Limpopo Province (South Africa) and two sites in the Okavango Delta (Botswana). The distribution for this metacercariae is now extended to the NGR, KwaZulu-Natal Province in South Africa and the ZR in the Northwestern Province of Zambia as the southern- and northernmost records. *Tylodelphys grandis* is only known from its type locality in Ethiopia, but specimens of *T.* cf. *grandis* expands the distribution of this species southward to the KR and ZR, Zambia. *Tylodelphys mashonensis*

has been widely reported from *C. gariepinus* in the Limpopo River basin (Mpumalanga Province) in South Africa, its distribution is now extended southward to the NGR (Lower Phongolo River) and SR and VR in the Vaal River system.

The tetracotyle-type metacercariae (assigned to Diplostomidae gen. sp. 1 in this study) found from the muscle tissue of *C. gariepinus* in the VR and RR also extend its distribution southward in southern Africa, it has only been reported from the Save-Runde River system in Zimbabwe and the Limpopo River system in northern South Africa (see Barson et al. 2008; Madanire-Moyo 2011). The underdeveloped and unidentified diplostomid metacercariae found in this study are not regarded as a range extension for *Diplostomum* sp. as no specimens with notable features were successfully excysted. As indicated previously, the diplostomid metacercariae found the encysted in the muscle tissue of the sharptooth catfish (Diplostomidae gen. sp. 1) from the RR and VR retain the tetracotyle-type, similar to those found for *Tetracotyle* sp. (Barson et al. 2008; Madanire-Moyo et al. 2010, 2012) and *Diplostomum* type 1 (Prudhoe and Hussey 1977). The *Neodiplostomum*-type metacercariae from the eye and encysted in the muscle tissue for fish from ZR and NGR in our study morphologically represent the illustrations provided of the *Neodiplostomum* type 1 metacercariae found encysted in the mesenteries by Prudhoe and Hussey (1977), but not the *Diplostomum* gen. of Moravec (1977). From literature and the summary on each taxon in this section, it is apparent that all specimens, morphotypes or material obtained from any host should be subjected to both molecular and morphological characterisation if possible. It is imperative that specimens are characterised from the onset of a study to delineate morphotypes and taxonomic placement. A good example is the works of Dumbo et al. (2019a, b) who immediately shed light on the complex relationships of specimens of *G. pedatum* and *O. batrachoides*. The description of the adult *Masenia nkomatiensis* (Cephalogonimidae: Plagiorchidea) and report of Cladorchiidae gen. sp. (Paramphistomoidea) brings the total number of Digenea known from *C. gariepinus* in Africa from 34 to 36.

3.4. Class Cestoda

Helminths that belong to the Cestoda are commonly referred to as tapeworms. These worms are obligate endoparasites primarily found in the digestive tract of their vertebrate hosts and are characterised by a dorsoventrally flattened body (strobila) that constitute segments (proglottids) and a unique attachment complex referred to as the scolex (Margulis and Chapman 2009). The scolex features a combination of attachment structures such as hooks, suckers and bothria with a diverse morphological variation (see Scholz and Kuchta 2018 for examples). Their body surface is covered with hair-like structures (microtriches) for attachment and has an absorption function for nutrients (Scholz and Kuchta 2018). Globally cestodes are the second most specious group of the Platyhelminthes (approximately 32% parasitise freshwater fishes) and are well-defined into 19 orders based on 1) the morphology and complexity of the scolex, composed of bothria, bothridia, suckers and hooks; 2) the reproductive organs that include the vitelline follicles, position of the gonads, egg morphology (polyecithal or oligoecithal) and 3) the structure of the uterus which is situated in the strobila (Alves et al. 2017; Caira and Jensen 2017; Kuchta and Scholz 2017a, b; Yenova et al. 2020). Cestodes are hermaphroditic with well-developed vitellaria and their life cycle is indirect, where copepods, amphipods, oligochaetes and fishes are utilised as 1st and 2nd intermediate hosts. Some species of cestodes are associated with human fish-borne diseases, however, these are not common in Africa (Waeschenbach et al. 2017). Larval forms (metacestodes) are known as plerocercoids (or plerocerci) and occur outside of the intestinal lumen and are commonly found in the mesenteries, intestinal wall, gallbladder, spleen, and the liver of the host (Alves et al. 2017; Kuchta and Scholz 2017a, b).

Previously, the resolution of the taxonomy for cestodes was unsatisfactory (globally) and the use of invalid species names by several authors made records unreliable in addition to creating confusion on the true distribution and host range of several species. These issues were addressed or resolved just prior to the inception of, or during the Planetary Biodiversity Inventory (PBI) program with outputs that aimed to mobilise future biodiversity studies (see Caira and Jensen 2017 and references therein). The PBI program encompassed a comprehensive review on the biodiversity, classification, morphology, host associations, geographic distribution and inter-relationships of cestodes, and most certainly reached its goal in providing a solid foundation for the future of research on cestodes.

With regard to the higher taxa relevant to Africa and the present study: prior to the PBI program Kuchta and Scholz (2007) critically reviewed the Bothriocephalidea Kuchta, Scholz, Brabec et Bray, 2008 and subsequently circumscribed the order and highlighted that past radiations probably occurred in Africa and North America (see Kuchta et al. 2008a; Caira and Jensen 2017; Kuchta and Scholz 2017a; Scholz and Oros 2017). During the course of the PBI program, host

and geographic distribution of bothriocephalideans were scrutinised, the Bothriocephalidae (Blanchard, 1894) were confirmed to comprise a monophyletic group and the genus *Schyzocotyle* Akhmerov, 1960 was resurrected (Brabec et al. 2015; Kuchta and Scholz 2017a). Genera in the Caryophyllidea van Beneden in Carus, 1863 from the Afrotropic realm i.e., *Wenyonia* Woodland, 1923 have been revised and amended, the genus is confirmed as exclusive to mochokid hosts and an interim consensus has been reached on the order's status as the earliest diverging group of the eucestodes (Schaeffner et al. 2011). The Onchoproteocephalidea was erected and consists of the Proteocephalidea (in part) that parasitise freshwater vertebrates, amphibians, reptiles, snakes (as crown group of the new order) and Tetraphyllidea (in part) known from elasmobranchs (Caira et al. 2014; de Chambrier et al. 2017).

Nine adult cestode species from three orders, the Bothriocephalidea (1), Caryophyllidea (2) and Onchoproteocephalidea (6) are known to infect *C. gariepinus* in Africa and are discussed below (see **Table A3.5.** and **Table A3.6.**). The taxonomic classification in this section follows Caira and Jensen (2017) and Scholz and Kuchta (2018). A tenth species, the alien and co-invasive Asian tapeworm, *Schyzocotyle acheilognathi* (Yamaguti, 1934) (syn. *Bothriocephalus acheilognathi* Yamaguti, 1934) is also known to infect *C. gariepinus* in Africa and is discussed in detail in **Chapter 5.** Data on congeners used for comparative purposes with the sequence data obtained in the present study are in **Table A4.3.**

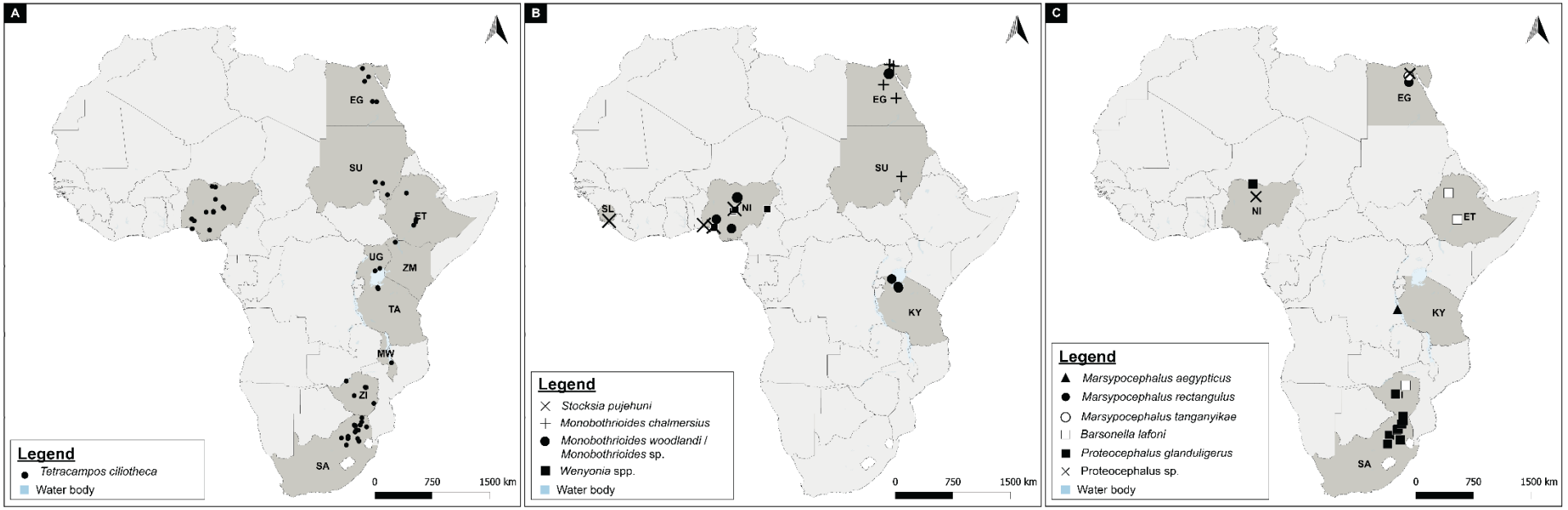

Figure 3.24. Distribution records of the **A** – Bothriocephalidea Kuchta, Scholz, Brabec et Bray, 2008; **B** – Caryophyllidea van Beneden in Carus, 1863 and **C** – Onchoproteocephalidea Caira, Jensen, Waeschenbach, Olson et Littlewood, 2014 parasitising the digestive system of *Clarias gariepinus* (Burchell) in Africa.

3.4.1. Order Bothriocephalidea Kuchta, Scholz, Brabec et Bray, 2008

(syn. Pseudophyllidea Beneden in Carus, 1863 in part)

The establishment of the Bothriocephalidea was a result of the division of the paraphyletic Pseudophyllidea van Beneden in Carus, 1963 into the Bothriocephalidea and Diphyllobothriidea Kuchta, Scholz, Brabec et Bray, 2008. This division is justified in representatives having unique morphological and life cycle features and molecular characters (see Kuchta et al. 2008b). The Bothriocephalidea is the most derived clade of the orders and is primarily characterised in having two dorsoventrally situated bothria. Representatives are common parasites of bony fishes in both marine and freshwater environments, and they have a cosmopolitan distribution with the freshwater species primarily known from Eurasia and North America and a disproportionately low diversity is known from other continents. Freshwater fishes from Africa host one polytypic and five monotypic genera, including the well-known invasive and pathogenic species *S. acheilognathi* (see **Chapter 5.**) (Kuchta and Scholz 2007; Kuchta et al. 2012; Brabec et al. 2015; Kuchta and Scholz 2017a).

Currently, four families are recognised within the order, but only two species, *Tetracampos ciliotheca* Wedl, 1861 and *S. acheilognathi* of the Bothriocephalidae (Blanchard, 1894) are known to infect *C. gariepinus*. These are also the only two species of the order that are found in fishes on other continents (Kuchta et al. 2012).

Family Bothriocephalidae (Blanchard, 1894)

Tetracampos Wedl, 1861

Tetracampos Wedl, 1861 was erected for the species *Tetracampos ciliotheca* [syn. *Clestobothrium clarias* Woodland, 1925; *Polyonchobothrium clarias* (Woodland, 1925); *Polyonchobothrium ciliohteca* (Wedl, 1861)] first found from *C. anguillaris* in Egypt. However, the original description was considered incomplete and the genus with its single species was designated as *nomen nudum* or placed within the Proteocephalidea or Tetraphyllidea van Beneden, 1850 (see Wedl 1861; Kuchta et al. 2008a and references therein). Kuchta et al. (2008a) resurrected the previously monotypic genus following a critical re-evaluation of the literature on its single widely distributed species infecting *Clarias* spp. Kuchta et al. (2012) examined new and type material of *Tetracampos* and did a comparison with type specimens from the type-host, *C. gariepinus* and other hosts. As a result, they discovered the medullary position of the vitelline follicles that contributed to the resurrection of the genus. Additionally, the genus diagnosis was amended and a second species, *Tetracampos martinae* Kuchta, 2012 was described from *Bagrus meridionalis* (L.) (Bagridae: Siluriformes) collected in Lake Malawi (Kuchta

et al. 2012). The latter authors also discussed the synonymy of *Polyonchobothrium* spp. with *T. ciliotheca* from all clariid host in Africa and commented on the reports of *T. ciliotheca* in Israel [as *Polyonchobothrium clarias* (Woodland 1925)] and Turkey [as *Polyonchobothrium magnum* (Zmeev, 1936)] that is most likely a result of importation of *Clarias* spp. for culture purposes (see Meggit 1930; Tadros 1968; Kuchta et al. 2008a, 2012 and references therein). Specimens of *T. ciliotheca* parasitising *C. gariepinus* are reported from several countries in Africa as *P. clarias* (junior synonym) or variations thereof[1] i.e., *P. clariae*, *P. fulgidum* or *P. ciliotheca*. Specimens of *Polyonchobothrium polypteri* (Leydig, 1853) reported from *C. gariepinus* in Nigeria are suspected to be a misidentification of *T. ciliotheca*, as *P. polypteri* is only known from hosts unrelated to the Clariidae i.e., the Claroteidae and Polypteridae (see Kuchta et al. 2012).

Adult forms of *T. ciliotheca* are also known from other commercially valued clariid species and can infect paratenic hosts. This was proven through experimental infection of cichlids, and immature individuals have been found from mochokid hosts in natural environments [see Kuchta et al. (2012) and references therein]. The species (*T. ciliotheca*) is also of commercial significance as it penetrates deeply into the mucosa of the intestinal wall resulting in injury. Penetration of the intestinal wall and attachment to organs including the liver, spleen and ovaries have been reported on a single occasion (Wakube-Bunoti 1980; Akinsanya and Otubanjo 2006; Kuchta et al. 2012 and references therein; Madanire-Moyo and Avenant-Oldewage 2014b). *Tetracampos ciliotheca* have also been found to infect the gall bladder, stomach, and bile duct in addition to the intestine of its clariid hosts (see Aderounmu and Adeniyi 1972; Amin 1978; Shotter 1980; Faisal et al. 1989; Adikwu and Ibrahim 2004; Barson et al. 2008; Sinaré et al. 2015; Ali et al. 2020).

Currently, *T. ciliotheca* is the most widely distributed cestode species on the continent, parasitising *C. gariepinus* from Egypt, Ethiopia, Kenya, Malawi, Nigeria, South Africa, Sudan, Tanzania, Uganda, and Zimbabwe (see **Fig. 3.24. A** and **Table A3.6.**).

Present study: *Tetracampos ciliotheca* was found embedded in the pyloric to mid-region of the intestinal wall of *C. gariepinus* from the GD [P = 13%, IF =1–7]; RR [P = 17%, IF =17–21]; SR [P

[1] See Woodland (1925c); Meggit (1930); Aderounmu and Adeniyi (1972); Khalil and Thurston (1973); Wakube-Bunoti (1980); Anosike et al. (1992); Douëllou (1992); Al-Bassel (2003); Nkwengulila and Mwita (2004); Oniye et al. (2004); Akinsanya and Otubanjo (2006); Barson and Avenant-Oldewage (2006b); Olofintoye (2006); Ayanda (2008, 2009a, b); Moyo et al. (2009); Barson et al. (2008); Ibrahim et al. (2008); Mwita and Nkwengulila (2008); Madanire-Moyo and Barson (2010); Aliyu and Solomon (2012); Mwita (2011b, 2014a); Awadin et al. (2012); Madanire-Moyo et al. (2012); Matla (2012); Madanire-Moyo and Avenant-Oldewage (2013a, 2014); Ajala and Fawole (2014a, b, 2021); Ugbor et al. (2014); Abdel-Gaber et al. (2015); Rashied et al. (2016); El-Shahawy et al. (2017); Abba et al. (2018a, b).

= 20%, IF =6–20]; BS [P = 20%, IF =1–4]; NGR [P = 30%, IF = 1–6]; ZR [P = 6% , IF = 1] and KR [P = 50%, IF = 1].

Co-occurrence: This bothriocephlaid co-occurred[2] with the proteocephalid *Barsonella lafoni* in host individuals from RR [2/12; (21, 17:4, 3)]; SR [(2/15) 6, 13:10, 1)]; BS [3/15 (4, 1, 1:12, 4, 1)] and NGR (1/37; 1:1).

Identification: The identity of specimens from this study was confirmed by Prof Roman Kuchta and the voucher material was deposited in the Helminthological collection of the Institute of Parasitology, Czech Academy of Sciences, České Budějovice, Czech Republic (IPCAS), collection number IPCAS C-466. Molecular analyses[3] of the partial 28S rRNA confirmed the morphological assignment of specimens to *T. ciliotheca*. One isolate (MT52) from BS was successfully amplified and aligned to species of the Bothriocephalidea when subjected to a blast BLAST search (see **Table A4.3.**). MT52 had a 1% divergence from *T. ciliotheca* from *C. gariepinus* in Ethiopia (see **Table 3.9.**) (Kuchta et al. 2012).

Table 3.9. Divergence between species of the Bothriocephalidea (Cestoda) based on 28S rRNA (1506 bp) alignments. Values for p-distance (bottom diagonal) are expressed in percentage, while the top diagonal represent the number of differences in nucleotides. Species in bold were obtained from material collected during the present study.

28S rRNA		1	2	3
1	JQ811836 *Polyonchobothrium polypteri* SU	–	52	49
2	JQ811835 *Tetracampos ciliotheca* ET	3.0	–	14
3	**MT52 28S *T. ciliotheca* BS**	3.7	0.8	–

3.4.2. Order Caryophyllidea van Beneden in Carus, 1863

Species of the Caryophyllidea are intestinal parasites of freshwater teleosts and are commonly associated with cypriniform and siluriform fishes. The latter order appears to be the dominant definitive hosts in the Afrotropical region. Intermediate hosts associated with caryophyllidean cestodes include annelids, and almost exclusively those from the Tubificinae Eisen. Caryophellidean cestodes are quite unique in having a monopleuroid or monozoic body plan, meaning there is a lack of segmentation, they possess a single set of reproductive organs, and no proglottisation is present in the strobila. These features make them similar to other orders i.e., the Amphilinidea Poche, 1922 and Gyrocotylidea Poche, 1926, however caryophyllideans do

[2] Co-occurrence of species presented for each site as; (number of hosts infected/number of hosts screened; abundance of *T. ciliotheca*: abundance of *B. lafoni* for each infected individual).
[3] see **Chapter 2** for details on methods used.

possess a scolex, and are absent from the amphilinideans and gyrocotylideans. This composite of morphological features creates some degree of consensus in that the Caryophyllidea represent the earliest diverging cestodes of the Eucestoda, often referred to as “true cestodes” (Mackiewicz 1994; Oros et al. 2008; Scholz and Oros 2017). Furthermore, cestodes of the Caryophyllidea are characterised by the arrangement of their longitudinal musculature (a non-adaptive trait), very diverse scolex morphology, vitelline follicle arrangement, ovarian morphology, the position and condition of the gonopore, presence of external seminal vesicle, the anterior extensus of the uterus, position of the post-ovarian vitelline follicles, and the ovarian morphology (Mackiewicz 1994).

Taxonomic inconsistency continues to be problematic for the caryophellideans, as several species or genera have been erected based on examination of immature specimens or the general lack of high-quality preserved material of representative material. Mackiewicz (1994) and Scholz and Kuchta (2017b) highlight the importance of describing species from a suitable sample size, fresh specimens, and properly fixed material as neglecting to do so can result in erroneous species descriptions based on generic or fictitious features or deformities that are artefacts of poor fixation or flattening of specimens prior to fixation. The aforementioned, in addition to the challenges already imposed by the monozoic body plan, make it almost impossible to discern intraspecific variation or generic complexes and retaining of proper specimens for both morphological and molecular vouchers (i.e., hologenophores).

Four families are recognised in the order and include the Balanotaeniidae Mackiewicz et Blair, 1978; Capingentidae, Hunter, 1930; Caryophylaeidae Leuckart, 1878 and Lytocestidae Hunter, 1927. Only two genera, the monotypic *Stocksia* Woodland, 1937 and one species of *Monobothrioides* Fuhrmann et Baer, 1925 (Lytocestidae) are associated with *C. gariepinus* in Africa. Members of the Lytocestidae are characterised by inner longitudinal muscles that separate the cortical vitellarium from the medullary testes. Furthermore, they lack diverse scolex morphology in not having loculi, bothria or acetubula, and all genera appear to lack an external seminal vesicle, pre-ovarian vitelline follicles in annular or lateral and media position predominate (see Mackiewicz 1994). The family tends to have more frequent generic synonymies than any other family as a result of descriptions made from poorly preserved material and misinterpretation of several anatomical features.

Family Lytocestidae Hunter, 1927

Stocksia Woodland, 1937

This is one of the numerous monotypic genera of the Caryophyllidea and was erected for a single specimen infecting *C. gariepinus* collected from the Waanje River in Sierra Leone (Woodland 1937). Other records from the sharptooth catfish are known from Nigeria, and Tossavi et al. (2014) reported this species as *Stoeksia pujehani* from sharptooth catfish in Benin (**Fig. 3.24. B.**) (Akinsanya and Otubanjo 2006).

Present study: No infection with representatives of *Stocksia* was found in the intestine of *C. gariepinus* during the present study.

Monobothrioides Fuhrmann et Baer, 1925

The genus consists of five species that infect clariid and claroteid hosts in Africa (Scholz and Kuchta 2018; Scholz et al. 2021). *Monobothrioides* Fuhrmann et Baer, 1925 seems to be confused with the monotypic genus *Monobothrium* Diesing, 1863 that is exclusively known from cyprinids and suckers in the Holartic Region (Scholz et al. 2015, 2021). The African records of *Monobothrioides wageneri* Nybelin, 1922 [reported as *Monobothrium wageneri* in Rashied et al. (2016)] are from infections of the intestine and stomach of *C. gariepinus* in Egypt and Nigeria (see Oniye et al. 2004; Rashied et al. 2016). These records are most probably excluded from recent checklists due to the lack of morphological or molecular data available for these specimens.

Similarly, species of *Wenyonia* Woodland, 1923 have incorrectly been reported to infect cultured and wild *C. gariepinus* in Nigeria (see Akinsanya and Otubanja 2006; Dan-Kishiya et al. 2013; Domo and Ester 2015). Cestodes of this genus are in fact parasites of the Mochokidae (squeakers or upside-down catfish) from several regions in Africa and have never been reported from *C. gariepinus*. Schaeffner et al. (2011) is of the opinion that these records are possibly the misidentification of *Monobothrioides chalmersius* (Woodland, 1924) (syn. *Caryophyllaeus chalmersius*) that is a common parasite of *C. gariepinus* in African waters. In addition to *M. chalmersius*, *Monobothrioides woodlandi* Mackiewicz et Beverley-Burton, 1967 have also been reported from *C. gariepinus* in Nigeria, Tanzania and Uganda (see **Fig. 3.24. B**) (Mwita and Nkwengulila 2004, 2008; Mwita 2014a, b; Ugbor et al. 2014). These records may be valid, as *M. woodlandi* is known from a closely related species, *Clarias ngamensis* Castelnau that occur sympatrically with *C. gariepinus* in certain water bodies of these countries. Nonetheless, it is very difficult or even impossible to confirm the identity of these species (*Monobothrium* spp., *Monobothrioides* spp. and *Wenyonia* spp.) as there is no morphological or molecular data presented in the publications that claim to have recorded these species from *C. gariepinus*, nor is there any mention of material deposited to recognised museum collections, or any museum for that matter.

Present study: *Clarias gariepinus* from the ZR [P = 53%, IF = 1–11)] were infected in the intestine with *Monobothrioides chalmersius*.

Co-occurrence: *Monobothrioides chalmersius* co-occurred with two proteocephalid species, *Barsonella lafoni* [1/17 (1)][4] and *Proteocephalus glanduligerus* [1/17 (1:3)][5] in the intestine of *C. gariepinus* collected in ZR.

Identification: *Monobothrioides chalmersius* was morphologically identified based on the strongly dorsoventrally flattened body, tapering slightly at the anterior end, the numerous longitudinal furrows apparent on the scolex, and a terminal introvert, this placement was confirmed by Prof Tomaš Scholz. Specimens stained with Mayer's Carmine and mounted as permanent slides were deposited in the Helminthological collection of the Institute of Parasitology, Czech Academy of Sciences, České Budějovice, Czech Republic, collection number: IPCAS C-504/1; and the Parasite Collection of the National Museum (NMB), Bloemfontein, South Africa, collection numbers: NMB P 696, 697, 698. The 28S rRNA region for MT53 had a 1% divergence when compared to *M. chalmersius* from *Clarias* spp. (including *C. gariepinus*) collected throughout Africa (see **Table 3.10.**) (Scholz et al. 2021).

[4] (number of hosts infected/number of hosts screened; abundance *M. chalmersius*: abundance *B. lafoni*).

[5] (number of hosts infected/number of hosts screened; abundance *M. chalmersius*: abundance *P. glanduligerus*).

Table 3.10. Divergence between species of the Caryophyllidea (Cestoda) based on 28S rDNA (1275 bp) alignment. Values for p-distance (bottom diagonal) are expressed in percentage, while the top diagonal represent the number of differences in nucleotides. Species in bold were obtained from material collected during the present study.

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	MW762944 *Monobothrioides chalmersius* SU		1	1	1	1	1	161	126	69	69	69	144	145	151	151	31
2	MW762950 *M. chalmersius* SU	0.0	–	1	0	0	0	161	126	69	69	69	144	145	151	151	31
3	EF095253 *M. chalmersius* EG	0.0	0.1	–	1	1	1	160	125	68	68	68	143	146	151	150	30
4	MW762954 *M. chalmersius* SU	0.0	0.0	0.1	–	0	0	161	126	69	69	69	144	145	151	151	31
5	MW027475 *M. chalmersius* SU	0.0	0.0	0.1	0.0	–	0	161	126	69	69	69	144	145	151	151	31
6	MW762948 *M. chalmersius* SU	0.0	0.0	0.1	0.0	0.0	–	161	126	69	69	69	144	145	151	151	31
7	MW027476 *M. tchadnesis* CD	5.9	6.0	5.9	6.0	6.0	6.0	–	118	159	159	159	113	114	142	142	157
8	MW762938 *M. cunningtoni* CA	6.0	6.0	5.9	6.0	6.0	6.0	4.8	–	128	128	128	104	105	109	110	124
9	MW762941 *M. longicollis* CA	0.9	1.0	0.9	1.0	1.0	1.0	4.9	4.7	–	0	0	151	152	141	141	81
10	MW762942 *M. longicollis* CD	0.9	1.0	0.9	1.0	1.0	1.0	4.9	4.7	0.0	–	0	151	152	141	141	81
11	MW762952 *M. longicollis* SU	1.0	1.1	1.0	1.1	1.1	1.1	5.1	4.8	0.0	0.0	–	151	152	140	141	81
12	MW762945 *M. zuheiri* SU	5.7	5.8	5.7	5.8	5.8	5.8	4.4	4.7	4.4	4.4	4.6	–	1	120	120	144
13	JQ034129 *M. zuheiri* SU	5.7	5.8	5.7	5.8	5.8	5.8	4.4	4.7	4.4	4.4	4.6	0.0	–	123	122	145
14	HQ848489 *Wenyonia acuminata* CD	6.3	6.4	6.2	6.4	6.4	6.4	5.1	5.3	5.6	5.6	5.8	4.5	4.4	–	3	155
15	HQ848519 *W. acuminata* CD	6.3	6.4	6.2	6.4	6.4	6.4	5.1	5.4	5.6	5.6	5.8	4.5	4.5	0.1	–	156
16	**MT53 28S *M. chalmersius* ZR**	1.3	1.3	1.3	1.3	1.3	1.3	5.5	5.5	1.2	1.2	1.2	5.8	5.9	6.5	6.6	–

3.4.3. Order Onchoproteocephalidea Caira, Jensen, Waeschenbach, Olson et Littlewood, 2014

(syn. Tetraphyllidea Carus, 1863 in part, Proteocephalidea Mola, 1928)

The Onchoproteocephalidea Caira, Jensen, Waeschenbach, Olson et Littlewood, 2014 constitutes all genera previously placed within the Proteocephalidea that are found in the intestines of freshwater fishes, amphibians, snakes and lizards and additionally include taxa of the Onchobrothiidae (Tetraphyllydea) known from sharks and stingrays (Ciara et al. 2014, de Chambrier et al. 2017). Proteocephalideans may also have a rostellum-like organ that is armed with hooklets and represent early diverging lineages with the freshwater fishes, snake and lizard taxa composing a crown group to those of marine hosts. They possess a scolex with four spherical or elongate, uniloculate suckers (acetabula) and some taxa have bi- or triloculate suckers. The apical region of the scolex may bear a rostellum-like muscular organ with hooks, an apical sucker (functional or vestigial) or simply a concentration of gland cells. Stobila bears numerous testes, bilobed ovary near the posterior margin of the proglittid (see Woodland 1925a; de Chambrier et al. 2017 and references therein for more detail on morphological characters of the order). More recently de Chambrier et al. (2004), de Chambrier and Vaucher (1999) and de Chambrier and Scholz (2008) identified more suitable morphological characters to distinguish between taxa within the order.

Among the freshwater fishes, siluriform catfishes represent the most extensive host group amid a very broad spectrum of hosts that are utilised by onchoproteocephalideans. Zoogeographic analysis suggests a Gondwana association and origin in Africa.

Currently the Onchoproteocephalidea constitute a single family, Proteocephalidae housing 13 subfamilies of which two contain parasites of *C. gariepinus* (de Chambrier et al. 2009b).

Family Proteocephalidae

Subfamily Marsypocephalinae Woodland, 1933

Marsypocephalus Wedl, 1861

Species of this genus are morphologically similar to *Barsonella* de Chambrier, Scholz, Beletew et Mariaux, 2009 and possess sphincter-like musculature on the anterior margin of the suckers and may occur sympatrically in African catfishes of genera *Clarias* and *Heterobranchus*. Species of *Marsypocephalus* can however easily be differentiated from *Barsonella* in the lack of additional sucker opening, cortical position of the testes, possession of well-developed osmoregulatory canals, and markedly larger cirrus sack in the former genus.

Of the five species known from African clariid hosts, three species; *Marsypocephalus aegypticus* El-Naffar, Saoud et Hassan, 1984; *Marsypocephalus rectangulus* Wedl, 1861 and *Marsypocephalus tanganyikae* (Fuhrmann et Baer, 1925) [reported as *Loennberg tanganyikae* in Fuhrmann (1925)] parasitise *C. gariepinus* from the Nile River system in Egypt, Sudan and Ethiopia (see **Fig. 3.24. C** and **Table A3.6.**) (Woodland 1925a). Additionally, *M. rectangulus* have on several occasions been recorded from *Heterobranchus anguillaris*, considered a synonym of *C. gariepinus* (see Khalil and Polling 1997).

Present study: No infection with species of *Marsypocephalus* was found in the intestine of *C. gariepinus* during the present study.

Subfamily Proteocephalinae Mola, 1929

Barsonella de Chambrier, Scholz, Beletew et Mariaux, 2009

This genus is monotypic, consisting of a single species, *Barsonella lafoni* de Chambrier, Scholz, Beletew et Mariaux, 2009, found in the intestine of *C. gariepinus* in Ethiopia. Material of *B. lafoni* collected from the same host in Tanzania and Zimbabwe prior to its description suggests that several reports of other proteocephalid species such as *Marsypocephalus* (Marsypocephalinae) and *Proteocephalus* (Proteocephalinae) may have been incorrectly identified from *C. gariepinus* and are in fact *B. lafoni* (de Chambrier et al. 2009b). To confirm the true distribution of *B. lafoni* in Africa it would be necessary to do a complete revision of all available material (museum collections) and where possible fresh material as *B. lafoni* do not seem to co-occur with the other two species with which it might be confused. It is however understandable that *B. lafoni* has been overlooked, even for such a large cestode as it is at first glance almost identical to the other two large proteocephalid species that have widely been reported from catfishes in Africa (see Khalil 1963, 1971; Khalil and Polling 1997).

Microscopically *B. lafoni* can easily be distinguished from species of *Marsypocephalus* and *Proteocephalus*. Similar to all Proteocephalinae its genital organs are all situated in the medulla (smaller in *Proteocephalus*). *Barsonella* has: 1) an additional slit-like transversely oval orifice on the suckers and a short distance posterior to the main opening of the sucker cavity (absent in *Marsypocephalus*); 2) circular musculature on the anterior margin of the sucker that serve as a sphincter; 3) the absence of well-developed osmoregulatory canals; 4) the presence of a small, elongate thin-walled glandular apical organ and 5) a massive strobila [see de Chambrier et al. (2009b) for more detail on generic characters].

Present study: *Barsonella lafoni* was found from the intestine of *C. gariepinus* from the GD [P = 40% , IF (1 – 3)]; RR [P = 75%, IF = 2–34)]; SR [P = 53%, IF = 1–57)]; BS [P = 73%, IF = 1–12)]; NGR [P = 8%, IF = 1]; ZR [P = 6%, IF = 3] and the KR [P = 50%, IF = 2].

Co-occurrence: For co-occurrence data, refer to the co-occurrence sections above for *M. chalmersius* and *T. ciliotheca*.

Identification: The species identity of *B. lafoni* was morphologically confirmed by Prof Tomaš Scholz and specimens stained with Mayer's Carmine were deposited in the Helminthological collection of the Institute of Parasitology, Czech Academy of Sciences, České Budějovice, Czech Republic, collection numbers: IPCAS C-590/1, IPCAS C-485/1; and the Parasite Collection of the National Museum (NMB), Bloemfontein, South Africa, collection numbers: NMB P 702, 703, 704, 705, 724, 728, 729. Molecular analyses and a BLAST search of the 28S rRNA region indicated that isolate MT54 from GD were similar to isolates of *B. lafoni* from *C. gariepinus* in Ethiopia, while the *cox*1 mitochondrial region matched with two onchoproteocephaliids, *Acanthostotaenia shipleyi* (Linstow, 1903) [ex. *Varanus salvator* (Shaw)] and *Monticalla magna* (Rego, Santos et Silva, 1974) from *Pimelodus fur* (Lütken) in Vietnam and Brazil, respectively (see **Table 3.10.**) (de Chambrier et al. 2019; Alves et al. 2021).

Table 3.10. Divergence between species of the Onchoproteocehpallidea (Cestoda) based on 28S rRNA (1690 bp) and cox1 mitochondrial (1641 bp) region alignments. Values for p-distance (bottom diagonal) are expressed in percentage, while the top diagonal represent the number of differences in nucleotides. Species in bold were obtained from material collected during the present study.

28S rRNA		1	2	3	4	5	6	7	8	9
1	**MT54 28S *B. lafoni* GD**	–	210	272	250	181	182	184	181	187
2	KP729392 *Proteocephalus glanduligerus* SU	3.4	–	144	105	33	34	36	32	59
3	KP729396 *Proteocephalus sulcatus* SU	5.6	5.5	–	107	92	93	84	90	125
4	KP729408 *M. heterobranchus* SU	5.3	4.2	4.3	–	77	78	69	74	104
5	FM955143 *B. lafoni* ET	1.8	2.2	5.7	5.7	–	1	10	2	2
6	FM955144 *B. lafoni* ET	1.9	2.3	5.8	5.8	0.1	–	11	3	3
7	FM955142 *B. lafoni* SU	1.7	2.2	5.8	5.7	0.0	0.1	–	12	12
8	FM955145 *B. lafoni* ET	1.8	2.0	5.6	5.5	0.0	0.1	0.0	–	2
9	KC786015 *B. lafoni* ET	1.9	3.1	4.6	4.5	0.2	0.3	0.2	0.0	–

*cox*1		1	2	3
1	MW244033 *Monticella magna*	–	348	86
2	MK328928 *Acanthostotaenia shipleyi*	20.6	–	85
3	**MT54 cox1 *Barsonella lafoni* GD**	16.7	16.5	–

Proteocephalus Weinland, 1858

The genus *Proteocephalus* Weinland, 1858 is non-monophyletic and the most diverse genus of the Onchoproteocephalidea (previously Proteocephalidea). Representatives also contain the genital organs in the medulla; however, they are much smaller than observed in *Barsonella*. The suckers are not accompanied with the additional opening and circular sphincter-like musculature as seen in *Barsonella*. Species of *Proteocephalus* also possess a large glandular organ, much larger than the suckers and have well-developed osmoregulatory canals throughout the strobila. In the recent checklist of Scholz and Kuchta (2018) ten species from African fishes of the Cichlidae, Clariidae, Claroteidae, Mochokidae and Polypteridae were reported. Only one of these, *Proteocephalus glanduligerus* (Janicki, 1928) (syn. *Ichthyotaenia glanduligera* Janicki, 1928) is known from *C. gariepinus*. This species (*P. glanduligerus*) is markedly different from all other species of *Proteocephalus* (sensu lato de Chambrier et al. 2004) and other proteocephallideans known from freshwater fishes in Africa and was originally described from *C. anguillaris* in Egypt as *Ichthyotaenia glanduligera*. This description was incomplete only including information on the scolex structures and lacking detailed illustrations of the strobila (see Scholz et al. 2009). Subsequently, redescriptions were done on two occasions; first by Mashego (2001) using material collected from the intestine (embedded in the epithelial lining of the intestine) of *C. gariepinus* from four impoundments in the Limpopo River system in South Africa; and later Scholz et al. (2009) amended the description and highlighted the unique glandular apical organ from a re-examination of museum material from *C. anguillaris* and *C. gariepinus* from Sudan, Ethiopia and South Africa (see Scholz et al. 2009 for details). On several occasions *P. glanduligerus* has been reported as *Proteocephalus glanduliger*, *Proteocephalus glanduligera*, and *P. glanduligeria* from *C. gariepinus* (see Janicki 1928; Mashego and Saayman 1989; Barson and Avenant-Oldewage 2006b; Moyo et al. 2009; Matla 2012; Abba et al. 2018a, b).

Proteocephalus sulcatus (Klaptocz, 1906) is primarily known from its type-host *Clarotes laticeps* (Claroteidae: Siluriformes) and accidental infections have been reported from several hosts of the Clatoreidae, Polypteridae and Clariidae (*C. anguillaris* and *C. gariepinus*) (de Chambrier et al. 2007; Scholz and Kuchta 2018). Material, if available, for the specimens reported as Proteocephalid sp., *Proteocephalus* sp., *Proteocephalus* sp. 1 in Nigeria and Zimbabwe, needs re-examination to determine if they belong to *P. glanduligerus* (Yakubu et al. 2002; Mwita and Nkwengulila 2004, 2008, 2010; Madanire-Moyo and Barson 2010; Mwita 2011a, 2014a, b).

Present study: *Proteocephalus glanduligerus* were recovered from the intestine of *Clarias gariepinus* from the ZR [(P = 12%, IF = 1–3].

Co-occurrence: For information on co-occurrence please refer to the section on co-occurrence for *M. chalmersius* above.

Identification: The identity of the specimens was morphologically confirmed by Prof Tomaš Scholz, based on the undeniable presence of the apical organ in the scolex. Voucher material stained with Mayer's Carmine is deposited in parasitological collections under numbers: IPCAS C-590/1 and NMB P 699. No molecular data was generated for representatives of *P. glanduligerus*.

Concluding remarks

In addition to the above species, the identity or validity of a number of records could not be confirmed due to the lack of morphological or genetic data and no mention was made of existing voucher material for these specimens in museum collections:

Akoll et al. (2012) reported on a "Amphinidae cestode" from *C. gariepinus* in Lake Victoria, Uganda. The only amphilinid cestode known from African waters is *Nesolecithus africanus* Dönges et Harder, 1966 from the Aba, *Gymnarchus niloticus* Cuvier (Osteoglaniformes: Gymnarchidae) in Nigeria (Scholz and Kuchta 2018). Records are also published for *Anomotaenia* sp. (Dilepididae: Cyclophyllidea) from the sharptooth catfish in reservoirs in Nigeria and Caryophyllidae sp. (Egypt) and *Caryophyllaeus* sp. (Zimbabwe) which seems to only parasitise cichlid and cyprinind hosts in Africa, respectively (see Oniye et al. 2004; Ayanda 2008, 2009a, b; Radwan et al. 2009; Madanire-Moyo and Barson 2010; Barson et al. 2014; Scholz and Kuchta 2018). Sèdogbo et al. (2019) listed Gryporhynchidae in their publication, but no further detail is given, nor any photomicrographs. Gryporhynchid cestodes are primarily known from various organs of cichlid, and cyprinid hosts in Africa (Khalil and Polling 1997; Scholz and Kuchta 2018; Scholz et al. 2018b). Waeschenbach et al. (2017) resurrected the genus *Dibothriocephalus* Lühe, 1899, amended its diagnosis to species utilising freshwater fish in temperate zones and the northern hemisphere. This resurrection, resulted in the assignment of *Diphyllobothrium* Cobbold, 1958 (in part) as a junior synonym of *Dibothriocephalus*. The considerable number of records for *Diphyllobothrium latus* (syn. *Taenia lata* and erroneously reported as *D. latum*) from *C. gariepinus* now belong in the newly resurrected genus. From the latest checklist available on African freshwater fishes, the only diphyllobothriid cestodes known from African freshwater fishes is the larvae (plerocercoids) of *Ligula intestinalis* (L., 1785) which have to date only been found from small and large cyprinids, cichlids and a single daniodid (Danionidae) (Scholz and Kuchta 2018). Thus, material, including the whole worms and plerocercoids of *D. latus* (all name variations) reported from the sharptooth catfish, in Nigeria needs re-evaluation (Yakubu et al. 2002; Goselle et al. 2008; Biu and Akorede 2013; Edeh and Solomon 2016; Uruku and Adikwa 2017). Currently, four species of *Lytocestus* Cohn, 1908 (Lytocetsidae: Caryophyllidea) are listed to infect alestid, claroteid, mormyrid and one clariid host in Africa. The records of *Lytocestus* sp. from Benin and Zimbabwe will need re-examination to confirm if the specimens represent any of the four species

of the genus known from African freshwater fishes, a new host record and expansion in distribution range or alternatively, represent a new species. The reports of "unidentified spp." from the intestine and mesenteries of *C. gariepinus* in Benin and Ethiopia cannot be considered valid until further investigation is done or more material is available (Gulelat et al. 2013; Tossavi et al. 2014). Saoud et al. (1984) also indicate that 5% of the *C. gariepinus* they screened were infected with cestodes, however no species are mentioned.

During the present study four of the nine cestode species known from *C. gariepinus* in Africa were found (see **Table 3.1.** and **Fig. 3.25.**). The bothriocephalid *Tetracampos ciliotheca* was found at all localities sampled in the native distribution range of *C. gariepinus*, except from VR and represent new locality records. The southernmost distribution of *T. ciliotheca* was previously reported from the Vaal Dam, a large impoundment in the Vaal River, upstream from the VR sampling locality in this study. The occurrence of *T. ciliotheca* in *C. gariepinus* is now extended to the lower Vaal River system, including the RR, SR and on the Orange River system, with GD being the southernmost occurrence for this cestode. *Monobothrioides chalmersius* has to date only been reported from the Nile River in Egypt, and the records of the suspected misidentification of *M. chalmersius* (as *M. woodlandi*, *Monobothrioides* sp. and *Wenyonia* spp.) is known from countries in the northern hemisphere, and Kenya in the southern hemisphere (see **Fig. 2.24. B**). Here the first record of *M. chalmersius* from *C. gariepinus* from ZR in Zambia is reported, now the only official record in the southern hemisphere for this species. *Barsonella lafoni* was identified as a separate species in 2009, and it is thought that its occurrence is underestimated, as the species was previously identified as *Marsypocephalus* spp. and *Proteocephalus* spp. (de Chambrier et al. 2009b). Few records exist for this species, from Lake Tana and Langano in Ethiopia and from Lake Chivero in Zimbabwe. The distribution for *B. lafoni* is now expanded to the Upper Zambezi River system (localities KR and ZR in the present study) and the southern distribution of this species is here reported from the Lower Phongolo River (locality NGR), the Vaal River system (localities RR and SR) and its southernmost distribution is from GD on the Orange River system. *Proteocephalus glanduligerus* has been repeatedly reported from *C. gariepinus* in South Africa (see **Fig. 3.25. C**). Interestingly, in the present study no infection of this species was found from any of the sampling localities in South Africa, however the record reported here from ZR in Zambia constitutes a new distribution record.

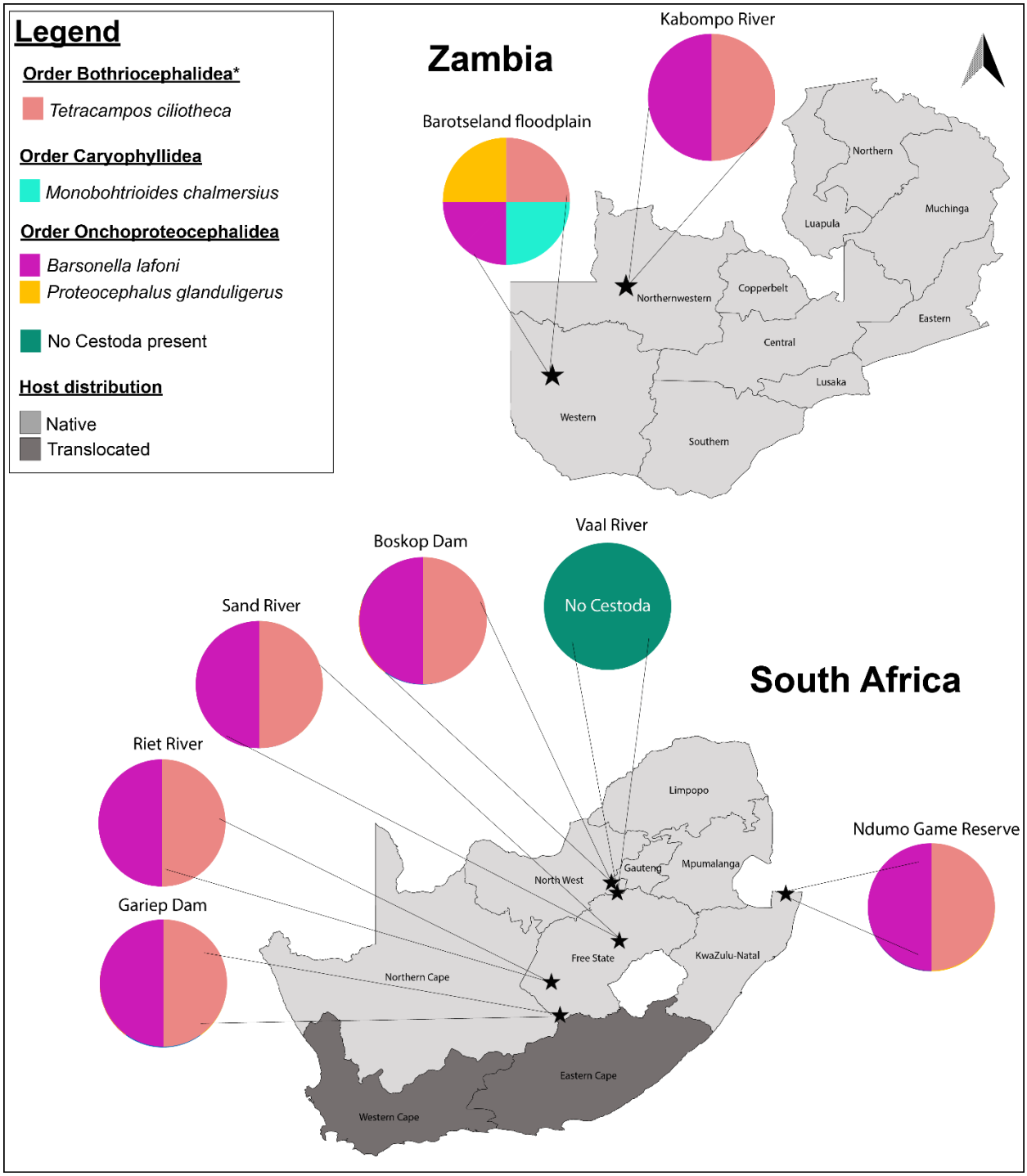

Figure 3.25. Visual representation of the presence and absence of Cestoda infecting the intestine of *Clarias gariepinus* (Burchell) in the present study. Note that the sectors of the pie-chart do not depict any numerical or statistical value regarding prevalence, intensity of infection or other infection statistics. * Please see Chapter 5.

3.5. Phylum Nematoda

Worms that belong to the Nematoda are commonly known as roundworms and are endoparasitic in several organs and tissues of some vertebrates. Roundworms are easily distinguished from the taxa dealt with up until now in this chapter, as they possess a secondary body cavity (pseudocoel) and complete or incomplete digestive system in all stages of development. Furthermore, their body is elongate, tapering to both ends (vermiform), cylindrical, and have a cuticle without cilia, protonephridia, respiratory organs or blood system (Mašová and Kuchta 2018). Nematodes also have a wide range of ecological adaptations, from free-living, semi-parasitic to parasitic. Individuals of the phylum are gonochoristic, have four larval stages with moulting of the cuticle between each life stage (there are some exceptions, see Moravec 2019) and can have a direct or indirect life cycle (Mašová and Kuchta 2018; Moravec 2019). An obligatory change of host is required to develop into the next life stage, thus intermediate hosts primarily utilised include aquatic insects, crustaceans and oligochaetes, while definitive hosts can be birds, mammals, reptiles or fish. Infective juvenile stages such as stage 3 larvae (L3) enter fish definitive hosts through ingestion, penetrate the skin and migrate through the tissues and mature in the intestines.

Research on nematodes of African freshwater fishes was initiated in the early 1800s. Nematodes have been used as indicators of man-made influence and changes in aquatic environments, in studies on fish population biology, as biological control agents, and for teaching models and research. Nonetheless, there is still an enormous knowledge deficit on their biology, ecology, taxonomy, and their geographical distribution on the continent. The apparent shortfall in knowledge of African fish nematodes is mainly due to the lack of suitable literary guides to aid in reputable identifications and descriptions, the absence, or inaccessibility of voucher material for comparisons. Additionally, very little is known on the life cycles and interactions between the host, the parasites, and their environment, especially in aquaculture and a continuously human-impacted natural environment. Currently, it is imperative that valuable sources, such as the monograph of Moravec (2019), be optimally exploited, complementary to morphometric and molecular approaches, in future taxonomic work for nematodes found from Africa's diverse freshwater fauna.

In the recent checklist of Mašová and Kuchta (2018) larval stages of six species and 14 adult nematode species were listed as parasites of *C. gariepinus*. However, in his monograph Moravec listed the larval stages and adults of nine and 15 species, respectively as suspected incidental infections and uncertain findings were included (see **Table A3.7.** and **Table A3.8.** for detail) (Moravec 2019). The 24 historic records of Nematoda parasitic to *C. gariepinus* are listed in and the following section provides a brief overview on all these. Taxa are listed alphabetically at the

class level and follows through to genus level. The classification systems used follows Mašová and Kuchta (2018) and Moravec (2019). Taxa (family or genus) denoted with a single asterisk (*) implies that larval stages are known from *C. gariepinus*, a double asterisk (**) indicates that both larvae and adults are known from this host, (★) denotes incidental infection, and unmarked taxa indicate that only adults are found in the fish host. A summary on existing records of all parasitic taxa discussed in this section is provided in **Appendix A3**.

During the course of this study, a total of eleven different nematode taxa were found to infect various organs of *C. gariepinus.* Third-stage larvae of *Contracaecum* spp. (Anisakidae: Ascaridida) were encysted in the body cavity, mesenteries and kidneys. Infection with adult nematodes were represented by spirurids (Camallanidae) infecting the stomach and intestine, while one species of the Cystidicolidae (*Spinitectus* sp. A) was found from the stomach of *C. gariepinus*. Additionally, incidental infections with larval stages of three species; *Eustrongylides* sp. (Dioctophymatidae: Enoplida), *Gendria* sp. (Quimperiidae: Ascaridida) and *Tanqua* sp. (Gnathostomatidae: Spirurida) were found from various organs and are discussed in the relevant sections below.

3.5.1. Class Adenophora

3.5.1.1. Order Enoplida

Family Capillariidae Railliet, 1915

Capillaria Zeder, 1800

Species of *Capillaria* Zeder, 1800 form part of trichinelloid nematodes that are considered highly pathogenic to their fish hosts. These nematodes are thin, thread-like and inhabit several internal organs such as the liver and intestinal tract of its fish host and directly lay their eggs in the tissues of these organs. There are numerous literature reports on the pathogenicity of species within this genus to wild fish populations and aquarium reared fishes. Debilitated conditions of fish hosts, inflammatory changes in the intestinal mucosa, formation of carcinomas and malignant neoplasms have been reported, moreover several capillariid nematodes have been identified as the causative agent of enteritis of the caeca and intestine in fish hosts (Moravec and Gut 1982; Kent et al. 2002; Moravec 2019 and references therein).

In the latest checklist for nematodes of African freshwater fishes, Mašová and Kuchta (2018) note that species of *Capillaria* are not known from fishes in Africa, and those reported are likely misidentification of other species. In contrast, Moravec (2019) indicates that one species, *Capillostrongyloides* (Travassos, 1914) is the only representative of capillarid nematodes in

African fishes. As such, the records of adult *Capillaria* spp. from *C. gariepinus* in Egypt, South Africa and pond reared or cultured populations of this host in Nigeria (see **Fig. 3.26. A** and **Table A3.8.**) are all doubtful and material should be re-examined where possible to confirm their placement within the Nematoda (Boomker 1994; Adeyemo and Falaye 2007; Hussen et al. 2012; Rufai and Adetona 2017; Abidemi-Iromini and Adelegan 2019). It should be noted that Moravec (2019) included Capillariidae gen. sp. from Moravec and Scholz (2017) as a parasite of *C. gariepinus* in his parasite-host list. This listing refers to the specimens of *Capillaria* sp. Boomker (1994) reported from this host in South Africa [see Moravec and Scholz (2017) for more detail]. The absence of these species in African waters may be a possible explanation for the lack of reporting on pathology from infections found in African fishes.

Present study: No infection with representatives of *Capillaria* were found from *C. gariepinus* during the present study.

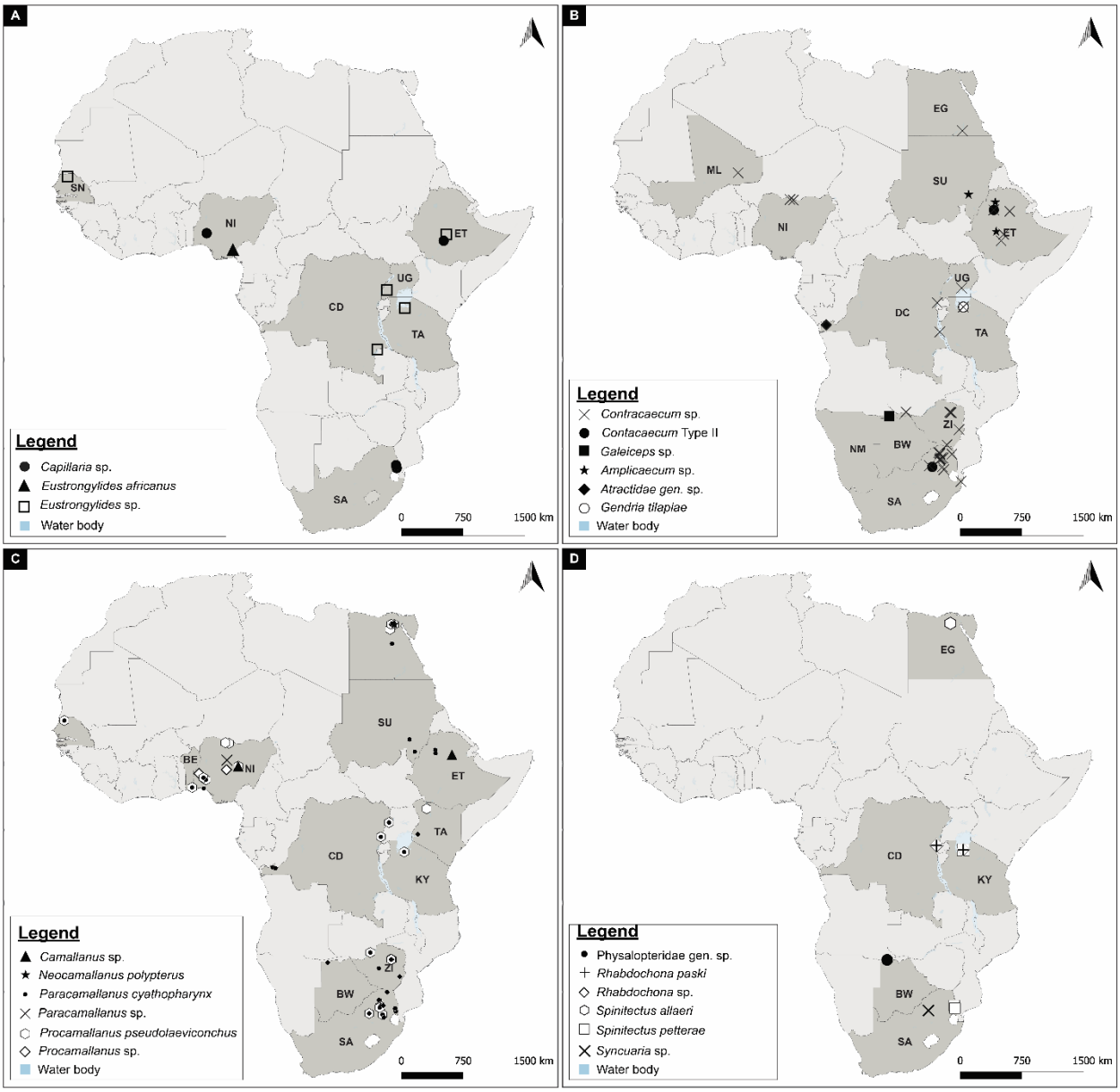

Figure 3.26. Maps depicting the known records of **A** – Enoplida; **B** – Ascaridida and the **C**, **D** – Spirurida infecting *Clarias gariepinus* (Burchell) in Africa.

Family Dioctophymatidae Rialliet, 1915

Eustrongylides Jägersköld, 1909*

Larval forms of *Eustrongylides* Jägersköld, 1909 are known from intermediate fish hosts, and those that comprise the genus are difficult to distinguish from those contained in the genus *Dioctophyme* Collet-Meygret, 1802, also parasitic in the abdominal cavity and viscera of freshwater fishes (Moravec 2019; Williams et al. 2022). Infection with larval *Eustrongylides* spp. is associated with disease, and in high intensities can cause destruction of the gonads (parasitic castration), fatty changes in the spleen or cause fibroblast formation surrounding unencapsulated parasites (Paperna 1974; Kaur et al. 2013). Larval stages of *Eustrongylides* spp. are capable of establishing themselves within paratenic hosts such as amphibians and aquatic reptiles, and on few occasions fourth-stage larvae have been found in the peritoneal cavity of humans who consumed raw fish (Kuperman et al. 2004; Eberhard and Ruiz-Tiben 2014; Menconi et al. 2020).

Eustrongylides africanus Jägersköld, 1909 is the only nominal species of the genus known from its definitive aquatic bird hosts in Africa and larval forms are known from *C. gariepinus* in the Bida floodplain in Nigeria (**Fig. 3.27. A**) (Paperna 1974; Ibiwoye et al. 2004, 2005a, b). These records of *E. africanus* are considered erroneous, as morphological identification of the species is only possible from adults obtained from the definitive bird host. Reports of larval *Eustrongylides* sp. from *C. gariepinus* in Lake Ziway (Ethiopia), from earthen and concrete ponds in Nigeria, from Lake George in Uganda, the Democratic Republic of the Congo, Lake Guiers in Senegal and the Insukamini Dam in Zimbabwe emphasise the dire need to obtain adult forms from the definitive piscivorous bird hosts as the current assignment of larval forms from freshwater fish may represent several species (see Baylis 1940; Paperna 1974; Vassiliades 1975; Moyo et al. 2009; Omeji et al. 2013; Bekele and Hussein 2015; Moravec 2019).

Present study: Individuals of *Eustrongyli*des sp. (**Fig. 3.27. B**–**D**) were found encysted in membranes filled with bright orange fluid in the muscle tissue of *C. gariepinus* from ZR [P = 59%, IF = 1–4].

Identification: Sequence data of the 18S rDNA region[6] was successfully generated for one isolate (MT27, 782 bp) from ZR. Significant alignments yielded from the BLAST search indicated species of *Dioctophyme renale* [ex. *Rattus norvegicus* (Berkenhout)] and *Eustrongylides* sp. [ex. *Chana punctata* Bloch] as the closest related taxa (Tokiwa et al. 2011; Williams et al. 2022). Congeners used for comparison are summarised in **Table A4.4.** and the alignment of the 28S rRNA region in **Table 3.11.** confirmed that the morphological assignment of these specimens was

[6] See **Section 2.4.3.** for protocol followed.

appropriate with a 1% divergence from *Eustrongylides* sp. from *C. punctata* as opposed to the 3.8% difference to *D. renale*.

Table 3.11. Similarity between species of the Dioctophymatidae Railliet, 1915 based on 18S rRNA gene region alignments (1548 bp). Values for p-distance (bottom diagonal) are expressed in percentage, while the top diagonal represent the number of differences in nucleotides. Species in bold were obtained from material collected during the present study.

18S rRNA		1	2	3	4	5
1	AB595139 *Dioctophyme renale* JA	–	0	0	14	31
2	LC389871 *D. renale* JA	0.0	–	0	14	31
3	LC389872 *D. renale* JA	0.0	0.0	–	14	31
4	OK104110 *Eustrongylides* sp. [L] BA	1.6	1.6	1.6	–	5
5	**MT27 *Eustrongylides* sp. [L] ZR**	3.8	3.8	3.8	1.0	–

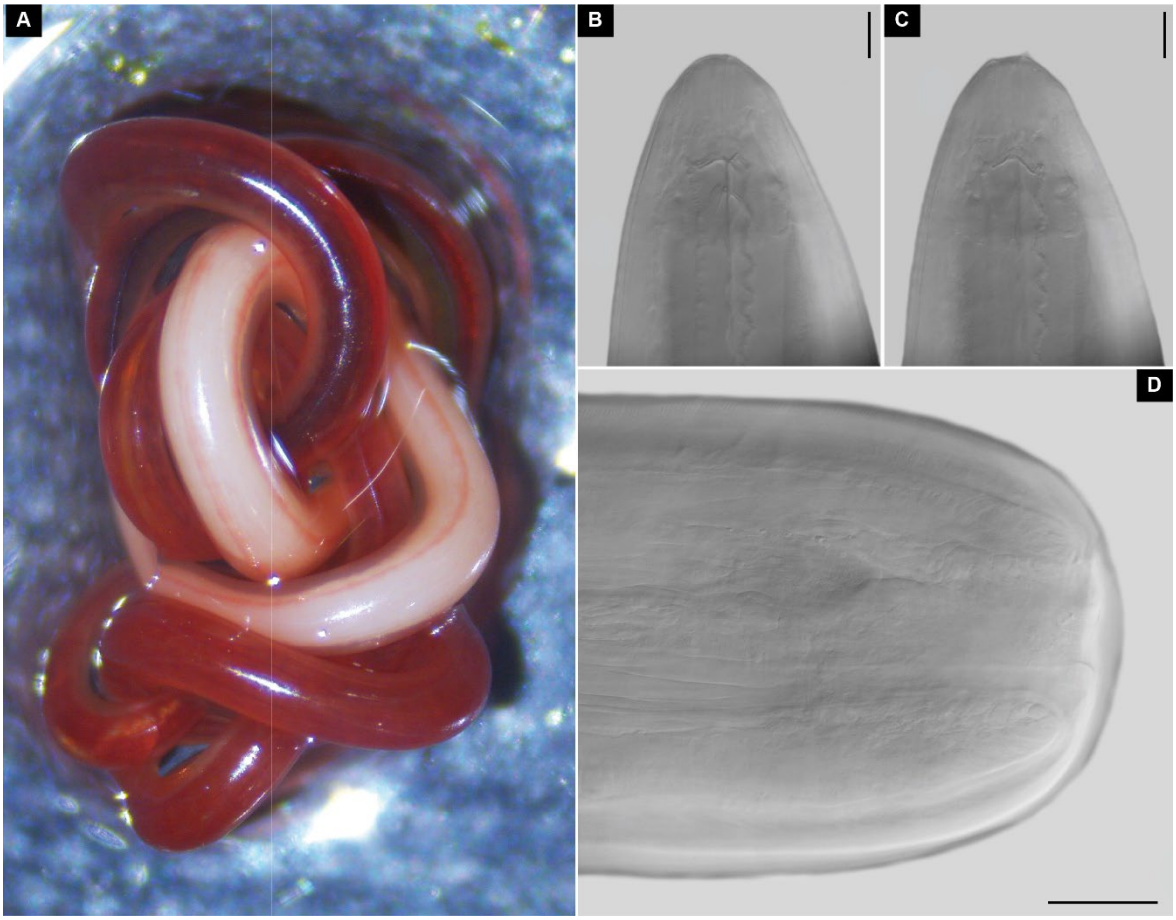

Figure 3.27. **A** – Live *Eustrongylides* sp. following removal from sac-like capsule in muscle tissue of *Clarias gariepinus* (Burchell) from the Barotse floodplain in Zambia. **B**, **C** – anterior end of body, and **D** – posterior end of specimen. Scale bars: 100µm.

3.5.2. Class Secernentea von Linstow, 1905

3.5.2.1. Order Ascaridida Skrjabin et Schulz, 1940

Family Anisakidae Railliet et Henry, 1912

Contracaecum Railliet et Henry, 1912*

Fish serve as the second intermediate host or in some cases as a paratenic host to larval stages of the anisakiid genus *Contracaecum* Railliet et Henry, 1912. Adult forms are found in the gut of the definitive piscivorous bird hosts or marine mammals. It is estimated that approximately 70 freshwater fish species from Africa are known to host larvae of *Contracaecum* (Tavakol et al. 2015; Moravec 2019). However, the close resemblance of *Contracaecum* larvae to those of *Galeiceps* Railliet, 1916 should be noted, as these larval forms are almost impossible to distinguish from one another based on morphology and may represent more than a single species (Moravec et al. 1993; Moravec 2019). The taxonomy of the genus is incomplete due to the paucity

in knowledge on its true diversity since species identification is based on the morphology of adult specimens obtained from the definitive host.

Infections with larval *Contracaecum* are known from the waters of eight African countries (**Fig. 3.26. B**) and infection is primarily of encysted larvae in the visceral cavity, with occasional infection of mesenteries, kidneys or even liver when the larvae were present in high intensities in the 2nd intermediate fish host. From literature three larval types; Type I, Type II and *Contracaecum* sp. are primarily reported on from the visceral cavity of fish hosts. *Contracaecum* sp. collected from the Rietvlei Dam in South Africa was assigned to Type II larvae and is characterised in having a conspicuously long intestinal caecum and short ventricular appendix (Moravec et al. 1993; Barson and Avenant-Oldewage 2006a; Moravec 2019). Larval specimens of *Contracaecum* sp. (Type II) have also been reported from *C. gariepinus* in Ethiopia (see Van As and Moravec 2015d; Moravec and Scholz 2017). Type I larvae are specimens with a very long ventricular appendix that exceed the length of the intestinal caecum. No morphotypes from *C. gariepinus* has been assigned to this form, despite the indication in the parasite-host list of Moravec (2019) that these forms are known from this host. Furthermore, Moravec (2019) did not assign a morphotype to the *Contracaecum* sp. reported by Tavakol et al. (2015), most probably due to the lack of a morphological account of their specimens. Here Type II larvae is reported on as indicated in Moravec (2019) (above) and all other records from the Democratic Republic of the Congo, Egypt, Ethiopia, Nigeria, Mali, South Africa, Tanzania, Uganda, and Zimbabwe will be listed under *Contracaecum* sp. in **Table A3.8.** Assignment of specimens from *C. gariepinus* to Type I larvae from the aforementioned countries needs confirmation.

Present study: All second and third-stage larvae collected in this study are assigned to the genus *Contracaecum* (**Fig. 3.28. A**–**H**). Molecular characterisation suggests that at least three species are present (see below). Infection with *Contracaecum* sp. A (**Fig. 3.28. B**, **F**) was found encysted in the body cavity, mesenteries and in the kidneys of *C. gariepinus* from KR [P = 50%, IF = 3]; ZR [P = 35%, IF = 1–3]. *Contracaecum* sp. B (**Fig. 3.28. A**, **D**, **G**) was encysted in the body cavity and mesenteries of sharptooth catfish from NGR [P = 89%, IF = 1–380], while third-stage larvae of *Contracaecum* sp. C (**Fig. 3.28. E**, **H**) from BS [P = 13%, IF = 2–12]; VR [P = 28%, IF = 1–2]; SR [P = 7%, IF = 1] and RR [P = 17%, IF = 1–2] were encysted in the kidneys, liver, muscle tissue and body cavity of *C. gariepinus*. Dark brown worm-like matter was encysted in the mesenteries of *C. gariepinus* from ZR and is presumably a result of the host's reaction to the nematode infections.

Identification: Sequence data of the 28S rDNA gene region was generated for one isolate from VR (MT44, 794 bp). The *cox*1 mitochondrial region of three isolates from ZR (MT39, 575 bp), NGR (MT45, 540 bp) and VR (MT44, 558 bp) were also successfully obtained. The BLAST search

yielded similarities of a third-stage larva of *Contracaecum* sp. from *Xenopus laevis* (Daudin) in South Africa for the 28S rDNA region for isolate MT44 as well as the *cox*1 regions of MT44 and MT39 (Schoeman et al. 2020). MT45 yielded no significant alignment with any anisakiid submission from fish hosts during the BLAST search (see **Table 3.12.**). An alignment of the 28S rDNA and *cox*1 regions with closest congeners indicated that MT44 from *C. gariepinus* in VR is indeed conspecific to the *Contracaecum* sp. from *X. laevis* in South Africa, while MT39 and MT45 differ from MT44 and *Contracaecum* sp. (**Table 3.12.**). The assignment of the three isolates to three morphotypes is based on the *cox*1 region for the three isolates; (MT39, MT44 and MT45) that are not identical.

Table 3.12. Similarity between the Ascaridida based on 28S rRNA (1414 bp) and cox1 mitochondrial (686 bp) gene region alignments. Values for p-distance (bottom diagonal) are expressed in percentage, while the top diagonal represent the number of differences in nucleotides. Species in bold were obtained from material collected during the present study.

28S rRNA		1	2	3	4
1	**MT44 *Contracaecum* sp. C VR**	**–**	5	214	215
2	MN526256 *Contracaecum* sp. SA	0.3	**–**	226	233
3	MN526257 *Paraquimperia* sp. SA	23.4	19.8	**–**	44
4	KY476350 *Icthyobronema hamulatum* RU	23.8	20.2	4.2	**–**
***cox*1**		1	2	3	4
1	**MT44 *Contracaecum* sp. C VR**	**–**	0	15	80
2	MN526248 *Contracaecum* sp. [L] SA	0.0	**–**	15	81
3	**MT39 *Contracaecum* sp. A ZR**	2.7	2.6	**–**	82
4	**MT45 *Contracaecum* sp. B NGR**	15.2	15.0	15.3	**–**

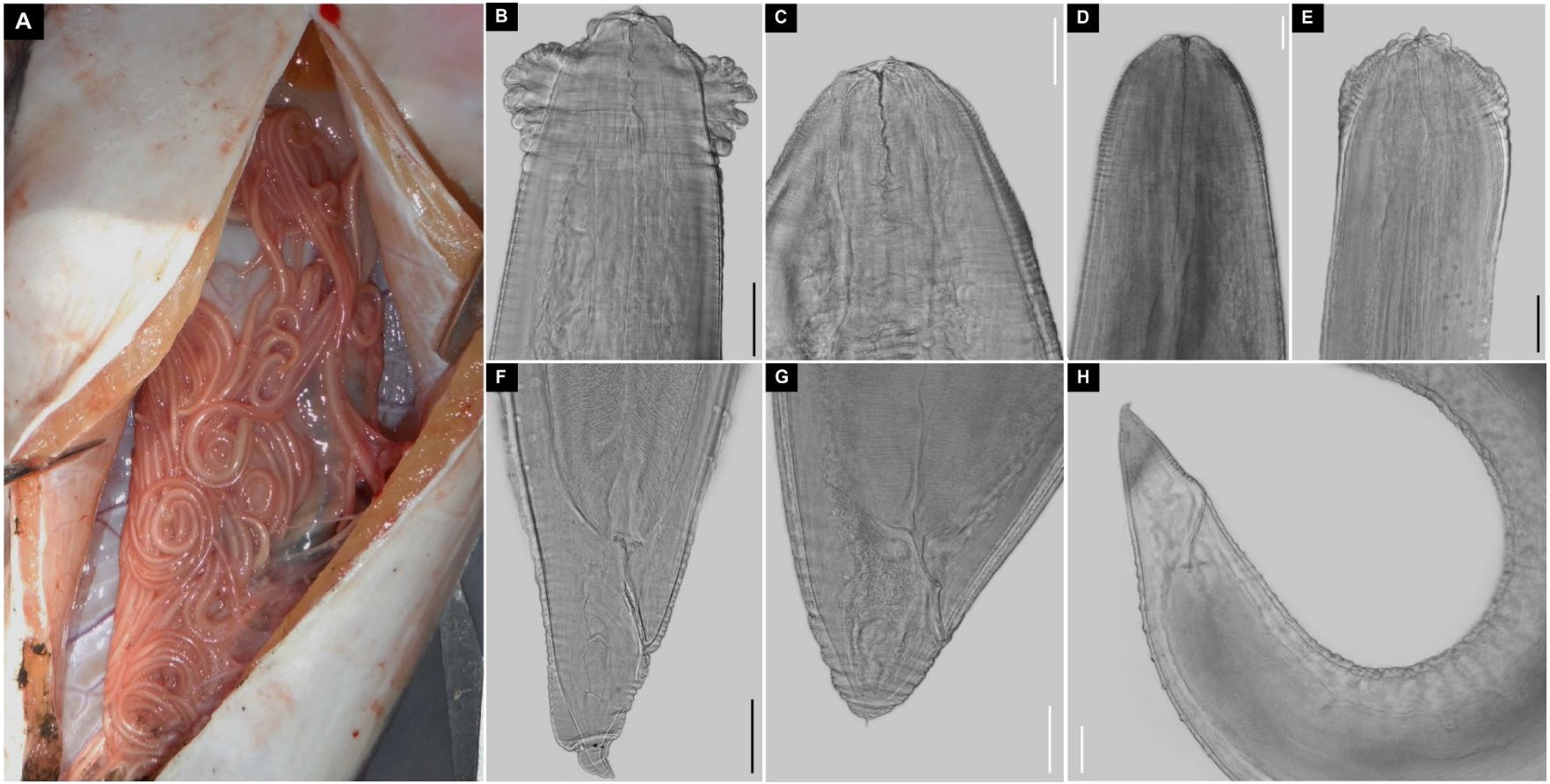

Figure 3.28. **A** – Larval *Contracaecum* sp. encysted in the mesenteries of *Clarias gariepinus* (Burchell) from the Lower Phongolo River and floodplain (NGR), South Africa. Anterior (B–E) and posterior (F–G) ends, lateral view of *Contracaecum* spp. infecting *C. gariepinus* from various localities in southern Africa. **B**, **F** – *Contracaecum* sp. A from the Zambezi River; **C**, **D**, **G** – *Contracaecum* sp. B from NGR and **E**, **H** – *Contracaecum* sp. C from the Riet River. Scale bars: 100µm.

Galeiceps Railliet, 1916*

As previously mentioned, larvae of *Galeiceps* are similar to that of *Contracaecum*, but can be distinguished from the former in having a cuticular collar with numerous small protruding cuticular plates and presence of cuticular bosses ("bumps" or nodule like structures on the cuticle), which are absent in *Contracaecum* but interestingly present in all other ascaridoid nematodes (larval or adult) (Moravec et al. 1993). Moreover, despite species of the two genera having similar life cycles, adults of *Galeiceps* are found in semi-aquatic mammals (i.e., giant otter shrew, marsh mongoose and monkeys), whereas piscivorous birds and marine mammals seem to be the definitive hosts for *Contracaecum*. Despite the lack of knowledge on the life cycle of *Galeiceps,* it seems apparent that the only similarities species of these genera share are the development of larval stages and mode of transmission from intermediate hosts to definitive hosts through ingestion of fish with infective third-stage larvae (Moravec and Van As 2015d).

A single record is known from *C. gariepinus* collected from the Okavango River, Botswana (Moravec and Van As 2015d) (**Fig. 3.26. B**).

Present study: No infection with specimens of *Galeiceps* were found from *C. gariepinus* during this study.

Family Ascarididae Baird, 1853

Amplicaecum Baylis, 1920*

Adults of *Amplicaecum* Baylis, 1920 nematodes are common among birds, amphibians, reptiles and it is thought that larvae are transmitted through ingestion of fish infected with larval stages (Yorke and Maplestone 1926; Khalil 1969; Moravec 2019). From available literature it seems that two types of larvae are recognised: Type I is characterised as large, sheathed and present in the body cavity of at least ten unrelated fish species, and Type II is a smaller unsheathed larva with host specificity for species of *Tilapia* Smith (Khalil 1969). It is possible that Type I larvae represent more than one species and possibly represent the anisakiid genera *Dujardinascaris* Baylis, 1947 or *Multicaecum* Baylis, 1923 that have ventriculi that is difficult to observe in congeneric larvae (Moravec 2019). Two records are known of larval *Amplicaecum* sp. from *C. gariepinus*, these are in Sudan from the Blue and White Nile and Lake Tana (Ethiopia) (Khalil 1969; Yimer 2000; Moravec 2019) (**Fig. 3.26. B**).

Present study: No infection with specimens of *Galeiceps* were found from *C. gariepinus* during the present study.

Family Atractidae Railliet, 1916*

Nematodes of the Atractidae Railliet, 1916 parasitise reptiles, amphibians and can be found in the intestine of their fish host. These nematodes are characterised in having sclerotised apparatus in the bulbous posterior portion of the oesophagus. Two genera, *Labeonema* Puylaert, 1970 and *Orientatractis* Petter, 1966 are known from freshwater fishes in Africa. Six *Labeonema* spp. are known from cypriniform, characiform and siluriform hosts and only a single species of *Orientatractis* is known from a siluriform (Mochokidae) host (see Moravec and Van As 2004; Moravec and Jirků 2017; Moravec 2019). Two morphotypes in the literature have been assigned to Atractidae gen. sp., one adult female from *Schilbe intermedius* Rüppel collected in the Okavango River in Botswana, while the other specimen, a fourth-stage female larva was found from *C. gariepinus* collected in the Lower Congo River in the Democratic Republic of the Congo (**Fig. 3.28. B**) (Moravec and Jirků 2017). The adult specimen from Botswana presented several morphological characters resembling *Labeonema* but differed from known species in this genus by the position of the vulva in the anterior part, which are usually located in the posterior section of the body. Similarly, the Atractidae gen. sp. from *C. gariepinus* presented with characters of atractiid nematodes but could not be compared to the specimen from Botswana as it harboured its vulva in the posterior section of the body (Moravec and Van As 2015; Moravec and Jirků 2017).

Present study: No specimens resembling the Atractidae were found from *C. gariepinus* during the present study.

Family Quimperiidae Gendre, 1928

Gendria Baylis, 1930[*, ★]

Three genera, namely *Gendria* Baylis, 1930 (syn. *Chabaudus* Inglis et Ogden, 1965); *Paraquimperia* Baylis, 1934 and *Quimperia* Gendre, 1926, are known to parasitise fishes in Africa. Species of the latter two genera are known from anguillid and anabantid hosts, respectively. Individuals of the genus *Gendria* are known from freshwater host, with adults of six species of *Gendria* known from the intestine of cichlid, clariid, pantodontid, schilbid and polypterid fishes in Africa (see Vassiliades 1975; Moravec and Jirků 2017; Moravec 2019 and references therein). One of the six species, *Gendria tilapiae* Baylis, 1930 formerly only known from *Sarotherodon galilaeus* L. in the Niger River in Mali, has been reported from *C. gariepinus* in Lake Victoria (Tanzania). This record from the sharptooth catfish needs confirmation with no morphological or molecular data presented by the authors (Baylis 1930; Mwita and Nkwengulila 2008; Mwita 2014a; Moravec 2019).

Present study: Adults of *Gendria* (**Fig. 3.29. A–E**) were found in the intestine of *C. gariepinus* from the KR [P = 50%, IF = 44] and ZR [P = 6%, IF = 6–22].

Identification: The18S rDNA, 28S rRNA regions and the *cox*1 mitochondrial region were successfully amplified and sequenced for one isolate, MT34 (18S rRNA:1353 bp; 28S rRNA: 723 bp; *cox*1: 558 bp) from ZR. The closest similarities were to species of the Kathalaniidae and Quimperiidae reported from fishes and frogs. Resolution at generic level is not depicted by the 18S rDNA (**Table 3.13.**), while the 28S rRNA alignment indicates MT34 is similar to other quimperiids, namely *Paraquimperia* sp. larva from the intestine of *X. laevis* in South Africa (see **Table 3.13.**).

Table 3.13. Similarity between species of the Quimperiidae Gendre, 1928 based on 18S rRNA (1812 bp) and 28SrRNA (1087 bp) gene region alignments. Values denote number of identical nucleotides as percentage (%). Species in bold obtained from material collected during the present study.

18S rRNA		1	2	3	4	5
1	JF803925 *Paraquimperia africana* SA	**–**	2	47	53	36
2	MN526251 *Paraquimperia* sp. SA	0.3	–	17	18	11
3	KR139827 *Spectatus spectatus* BR	2.7	2.5	–	46	23
4	KP275686 *Paraseuratum* sp. PM	3.3	2.6		**–**	30
5	**MT34 18S *Gendria* sp. ZR**	2.7	2.4	1.5	2.2	**–**

28S rRNA		1	2	3	4
1	KY476350 *Icthyobronema hamulatum* RU	**–**	43	132	223
2	MN526257 *Paraquimperia* sp. SA	4.1	–	123	213
3	MN526256 *Contracaecum* sp. SA	14.6	13.2	**–**	225
4	**MT34 *Gendria* sp. ZR**	18.5	17.8	25.4	–

Figure 3.29. **A** – Photomicrographs of *Gendria* sp. from the intestine of *Clarias gariepinus* (Burchell) in the Barotse floodplain, Zambia. **A**, **B** – Anterior end, lateral view; **C** – part of body at vulva region, lateral view. Posterior ends of D – female, lateral view; and E – male, lateral view. Abbreviations: Pa – papillae; Ph – bulbous pharynx; Sp – spicule; Vulv – vulva. Scale bars: 20µm (B) and 100µm (A, C, D, E).

3.5.3. Order Spirurida Chitwood, 1937

Family Acuariidae Railliet, Henry et Sisoff, 1912

Syncuaria Gilbert, 1927*, ⋆
Adult worms of *Syncuaria* Gilbert, 1927 (syn. *Chordocephalus* Alegret, 1941; *Decorataria* Soboleve 1949; *Skrjabinocara* Kurashvili, 1940) are primarily found in the gizzard lining of Pelecaniformes, Ciconiiformes and Podicipediformes (Digiani 1999). Ostracods and dragonfly larvae are utilised as intermediate hosts and, paratenic infections are reported in fish that are in some cases essential for transmission to the definitive piscivorous host (Moravec and Scholz 1994).

An accidental infection with *Syncuaria* sp. (initially reported as *Skrjabinocara* sp.) in the stomach of *C. gariepinus* from the Hartbeespoort Dam in South Africa is the only record known to date from South Africa (**Fig. 3.28. D**) (Boomker 1982; Moravec 2019). In the checklist of Khalil and Polling (1997) this same record is listed under *Chordocephalus* sp., and the authors considered it a synonym of *Skrjabinocara*. Mašová and Kuchta (2018) listed *Chordocephalus* sp. in their checklist, but not *Syncuaria* sp. We follow Moravec (2019) in considering all these to be records of species of *Syncuaria* sp.

Present study: No specimens representing *Syncuaria* were found from *C. gariepinus* during this study.

Family Camallanidae Railliet et Henry, 1915**

The Camallanidae Railliet et Henry, 1915 is a group of parasitic nematodes primarily infecting the digestive tract of marine and freshwater fish and less frequently amphibians, turtles, and snakes. Camallanids are globally distributed and are morphologically distinguished from other nematode groups in having a well-developed buccal capsule consisting of two lateral valves often supported by different structures (i.e., basal ring, longitudinal or spiral ridges, tridents). Species of two subfamilies, the Camallaninae Rialliet et Henry, 1915 (*Camallanus* Railliet et Henry, 1915; *Neocamallanus* Ali, 1957; *Paracamallanus* Yorke et Maplestone, 1926) and Procamallaninae Yeh, 1960 [*Procamallanus* (*Procamallanus*) Yeh, 1960 and *Procamallanus* (*Spirocamallanus*) Olsen, 1952] are known from African fishes. Morphological characters, such as the tridents present in the buccal capsule of camallaniniids and absence thereof in procamallaniniids, were previously used to distinguish members of the two subfamilies. It should be noted that these features of the buccal capsule and the presence or absence of a left spicule in the male caudal region are not sufficient for generic identification and more clarity is needed on the most suitable gene region (possibly the 28S rDNA region) to resolve relationships within the Camallanidae (see

Moravec 2019; Svitin et al. 2019 and references therein). Part of the work presented below was published in the paper by Svitin et al. (2019) titled: “Novel information on the morphology, phylogeny and distribution of camallanid nematodes from marine and freshwater hosts in South Africa, including the description of *Camallanus sodwanaensis* n. sp.”. The author of this thesis was second author on that paper and was responsible for the work done, results, description and discussion related to the camallanids from *C. gariepinus* and *Synodontis zambezensis* (Peters) presented in that paper.

Subfamily Camallaninae Riallier et Henry, 1915

Four species of *Camallanus* are known from anabantid, cyprinid, clariid, mochokid and polypterid hosts in Africa. However, none of these have been found to infect *C. gariepinus* in African waters. The reports of *Camallanus* sp. from the sharptooth catfish in the Uke River and various rivers in Nigeria, Lake Hugo in Ethiopia and Lake Chivero, Zimbabwe, need further investigation (Anosike et al. 1992; Yakubu et al. 2002; Amare et al. 2014). Abdel-Gaber et al. (2015) reported on the infection of *C. gariepinus* with *Neocamallanus polypteri* (syn. *Camallanus polypteri* Kabré et Petter, 1997) in Egypt. This record is considered a possible misidentification as *N. polypteri* is only known from polypterid hosts and no morphological account is given for the specimens found (see Moravec 2019).

Paracamallanus Yorke et Maplestone, 1926 seems to be a monotypic genus with *Paracamallanus cyathopharynx* (Baylis, 1923) (syns. *Camallanus cyathopharynx* Baylis, 1923; *Paracamallanus senegalensis* Vassiliadès, 1970) as the only species. *Paracamallanus cyathopharynx* was described from specimens infecting *C. gariepinus* from the Nile River. This species is amongst the most widely distributed camallanid reported from the sharptooth catfish, with records from 12 African countries (northern most country is Egypt and southernmost is South Africa) (**Fig. 3.25. C** and **Table A3.8.**). The life cycle and development of *Pa. cyathopharynx* was intensively studied, and although the development of larval stages in fish hosts were not conducted, copepods are confirmed as the intermediate host. Infection of the fish definitive host occurs through ingestion of infected copepods or through preying on small fishes that serve as paratenic hosts to infective larvae, which occur in the posterior end of the intestine (Moravec 1974a, b). *Paracamallanus* sp. reported from Nigeria by Ayanda (2009) has been assigned to *Pa. cyathopharynx* by Moravec (2019) and *Paracamallanus* sp. from Kaduna River, Nigeria needs re-examination to determine if it resembles *Pa. cyathopharynx* (Onwuliri and Mgbemena 1987; Auta et al. 2019).

Present study: Adults of potentially two species of *Paracamallanus* (**Fig. 3.30. A**–**F**) were found primarily from the medial to distal section of the intestine and on occasion from the stomach of *C. gariepinus*. Infection with *Pa. cyathopharynx* was found from KR [P = 50%, IF (1)], ZR [P = 71%,

IF = 1–12] and NGR [P = 70%, IF = 1–28]. *Paracamallanus* sp. A infected the intestine of *C. gariepinus* from BS [P = 40%, IF = 3–12], VR [P =89%, IF = 2–54], SR [P = 13%, IF = 2–17] and RR [P = 92%, IF = 1–38].

Identification: The 18S rDNA (MN514775) and *cox*1 (MN523683) gene regions with a morphological description of *Pa. cyathopharynx* from NGR, are presented in the publication of Svitin et al. (2019), see **Appendix A6**. In summary, from the alignment it is evident that generic resolution is not reached with the comparison of a conservative gene such as the 18S rDNA region (**Table 3.14.**), supporting the findings in Svitin et al. (2019). No new sequences of taxa from the present study were included in the 28S rDNA alignment, however generic differentiation is possible when using a less conservative gene region (**Table 3.14.**). The assignment of isolates to the two morphotypes are based on the alignment of *Pa. cyathopharynx* previously published in Svitin et al. (2019) and newly obtained sequences for isolates from BS (MT46, *cox*1: 544 bp) and RR (MT47, *cox*1: 508 bp). The alignment indicated that isolates MT46 had a 58 nucleotide difference to the *Pa. cyathopharynx* from NGR (**Table 3.14.**).

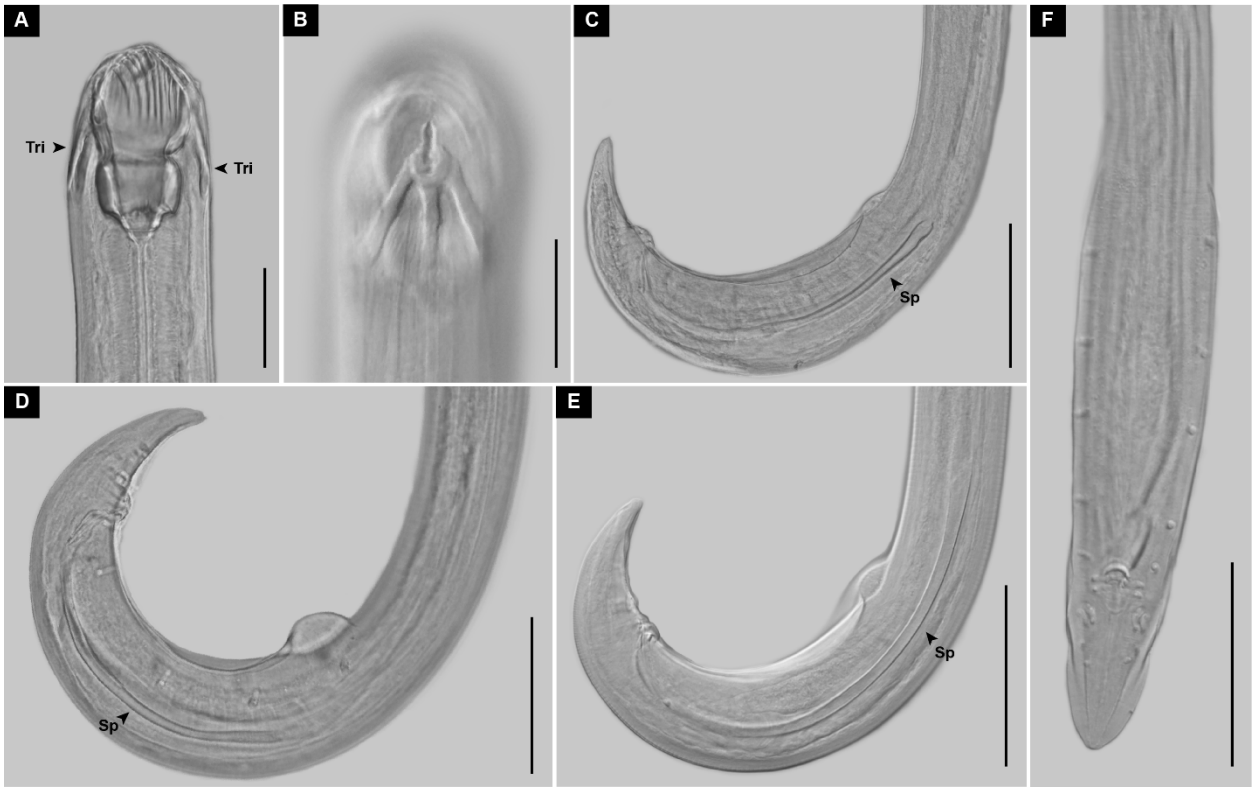

Figure 3.30. Photomicrographs showing general morphology of **A** – buccal capsule, lateral view and **B** – trident morphology, lateral view for male of *Paracamallanus cyathopharynx* (Baylis, 1923). Posterior end of males, lateral view: **C** – Boskop Dam; **D** – Riet River; **E** – Barotse floodplain (ZR); and **F** – ventral view of specimen from ZR. Abbreviations: Sp – spicule; Tri – trident. Scale bars: 50µm (A, B, C) and 100µm (D, E, F).

Table 3.14. Similarity between species of the Camallanidae Railliet et Henry, 1915 based on 18S rRNA (1790 bp), 28S rRNA (1525 bp) and cox1 mitochondrial gene (685 bp) region alignments. Values denote number of identical nucleotides as percentage (%). Species in bold obtained from material collected during the present study.

18S rRNA		1	2	3	4	5	6	7	8	9	10	11
1	MN514772 *Camallanus kaapstaadi* SA	–	3	8	11	13	8	13	13	13	16	17
2	MN514773 *Camallanus xenopodis* SA	0.4	–	8	15	16	10	12	15	15	18	15
3	MN514774 *Camallanus sodwanaensis* SA	1.1	1.1	–	16	16	7	10	11	9	14	22
4	**MN514775 *Paracamallanus cyathopharynx* NGR**	1.2	1.8	1.9	–	5	6	18	20	18	24	25
5	MN396556 *Paracamallanus cyathopharynx* KY	1.6	2.0	2.0	0.5	–	15	20	17	15	21	26
6	DQ813445 *Paracamallanus cyathopharynx* TA	1.6	2.1	1.4	0.9	0.6	–	6	403	402	405	10
7	**MN514770 *Procamallanus* (*P.*) *pseudolaeviconchus***	1.6	1.5	1.2	2.0	2.4	1.2	–	9	7	13	24
8	JF803914 *Procamallanus fulvidraconis* CH	1.7	1.9	1.4	2.4	1.8	1.1	1.3	–	33	26	22
9	JF803934 *Procamallanus laevichonchus* SU	1.7	1.9	1.1	2.1	1.6	1.0	1.0	2.0	–	42	20
10	DQ442665 *Procamallanus pacificus* NC	1.9	2.2	1.7	2.8	2.2	1.3	1.7	1.5	2.5	–	22
11	MN514771 *Spirocamallanus dalenae*	2.3	2.0	3.0	3.1	3.4	2.1	3.1	2.9	2.6	2.8	–

28S rRNA		1	2	3	4	5
1	MG947391 *Camallanus kaapstaadi* SA	–	108	240	302	367
2	MG947389 *Camallanus xenopodis* SA	6.3	–	252	315	375
3	MN525306 *Camallanus sodwanaensis* SA	18.2	17.0	–	338	372
4	**MN525307 *Procamallanus* (*P.*) *pseudolaeviconchus* NGR**	19.2	16.5	20.6	–	332
5	MN525304 *Spirocamallanus dalenae* SA	26.6	24.2	25.6	23.6	–

*cox*1		1	2	3	4	5	6	7	8	9
1	MG948462 *Camallanus xenopodis*	–	76	102	81	79	78	85	72	59
2	MG948461 *Camallanus kaapstadi*	12.0	–	91	85	81	80	96	66	60
3	**MN523683 *Paracamallanus cyathopharynx* NGR**	15.1	14.5	–	58	59	72	112	80	66
4	**MT46 *Paracamallanus* sp. A BS**	14.9	15.5	10.7	–	2	79	89	78	71
5	**MT47 *Paracamallanus* sp. A RR**	15.6	15.8	11.6	0.4	–	77	85	79	73
6	**MT49 *Procamallanus* sp. A ZR**	15.5	15.7	14.3	15.7	15.6	–	70	74	68
7	**MN523682 *Procamallanus* (*P.*) *pseudolaeviconchus* NGR**	12.6	15.2	16.5	16.4	16.7	13.9	–	80	75
8	EU598816 *Camallanus cotti*	16.8	15.2	18.7	18.2	18.5	17.8	18.7	–	54
9	EU598890 *Camallanus hypophthalmichthys*	13.8	13.8	15.4	16.6	17.1	16.4	17.5	12.6	–

Subfamily Procamallaninae Yeh, 1960

Members of a single genus occurs in freshwater fishes in Africa. As previously mentioned, there is still controversy on the validity and synonymy of several genera in the subfamily despite morphological and molecular scrutiny of taxa in the subfamily. Similar to species of *Paracamallanus*, it was experimentally proven that *Procamallanus* spp. utilise copepods as intermediate hosts (Moravec 1975). With regard to species of *Procamallanus* infecting *C. gariepinus*, two species of the genus have been found from the intestine.

The first is *Procamallanus* (*Procamallanus*) *pseudolaeviconchus* Moravec et Van As, 2015 that is the second most common camallanid (after *Pa. cyathopharynx*), known to infect the intestine and stomach of the sharptooth catfish in 10 African countries (see **Fig. 3.25. C** and **Table A3.8.**). There was controversy regarding the infection of *C. gariepinus* (and other fish hosts in Africa) with *Procamallanus* (*Procamallanus*) *laeviconchus* (Wedl, 1861) (syn. *Cucullanus laeviconchus* Wedl, 1861). Moravec and Van As (2015a) shed light on this, in proving that *P.* (*P.*) *laeviconchus* thought to infect *C. gariepinus* is in fact a separate species. *Procamallanus* (*P.*) *pseudolaeviconchus* possess an unlobed circumoral flange that is lobed in *P.* (*P.*) *laeviconchus*. Subsequently, all species previously recorded as *P.* (*P.*) *laeviconchus* or *Procamallanus* sp. from *C. gariepinus* and all *Clarias* spp. were regarded as erroneous (misidentifications) and were transferred to *P.* (*P.*) *pseudolaeviconchus* (see Moravec and Van As 2015a; Moravec 2019). *Procamallanus* (*Spirocamallanus*) *spiralis* Baylis, 1923 (syn. *Spirocamallanus mazabukae* Yeh, 1957) is the second species described from *C. gariepinus* (Egypt) and is easily distinguished from *P.* (*P.*) *pseudolaeviconchus* in having spiral ridges in the buccal capsule along with several other morphological features (see Moravec and Van As 2015b; Moravec 2019). The record of *Procamallanus* spp. from Lekki Lagoon in Nigeria most probably represents *P.* (*P.*) *pseudolaeviconchus* but this would need re-examination of the material for confirmation (Hassan et al. 2010).

Present study: Adult forms of *Procamallanus* (*P.*) *pseudolaeviconchus* (**Fig. 3.31. A**–**E**) were found from the medial to distal section of the intestine and on occasion from the stomach in *C. gariepinus* from the ZR [P = 71%, IF = 1–12], NGR [P = 35%, IF = 1–6] and BS [P = 40%, IF = 2–8].

Identification: A morphological description and molecular identification based on 18S rDNA (MN514770), 28S rDNA (MN525307) and mitochondrial *cox*1 (MN523682,) gene regions of *P.* (*P.*) *pseudolaeviconchus* from NGR is presented in the publication of Svitin et al. (2019), see **Appendix A4**. An additional isolate, MT49 from ZR was included in the *cox*1 alignment with a 13.9% divergence from *P.* (*P.*) *pseudolaeviconchus* from NGR and is thus assigned to *Procamallanus* sp. A (**Table 3.14.**).

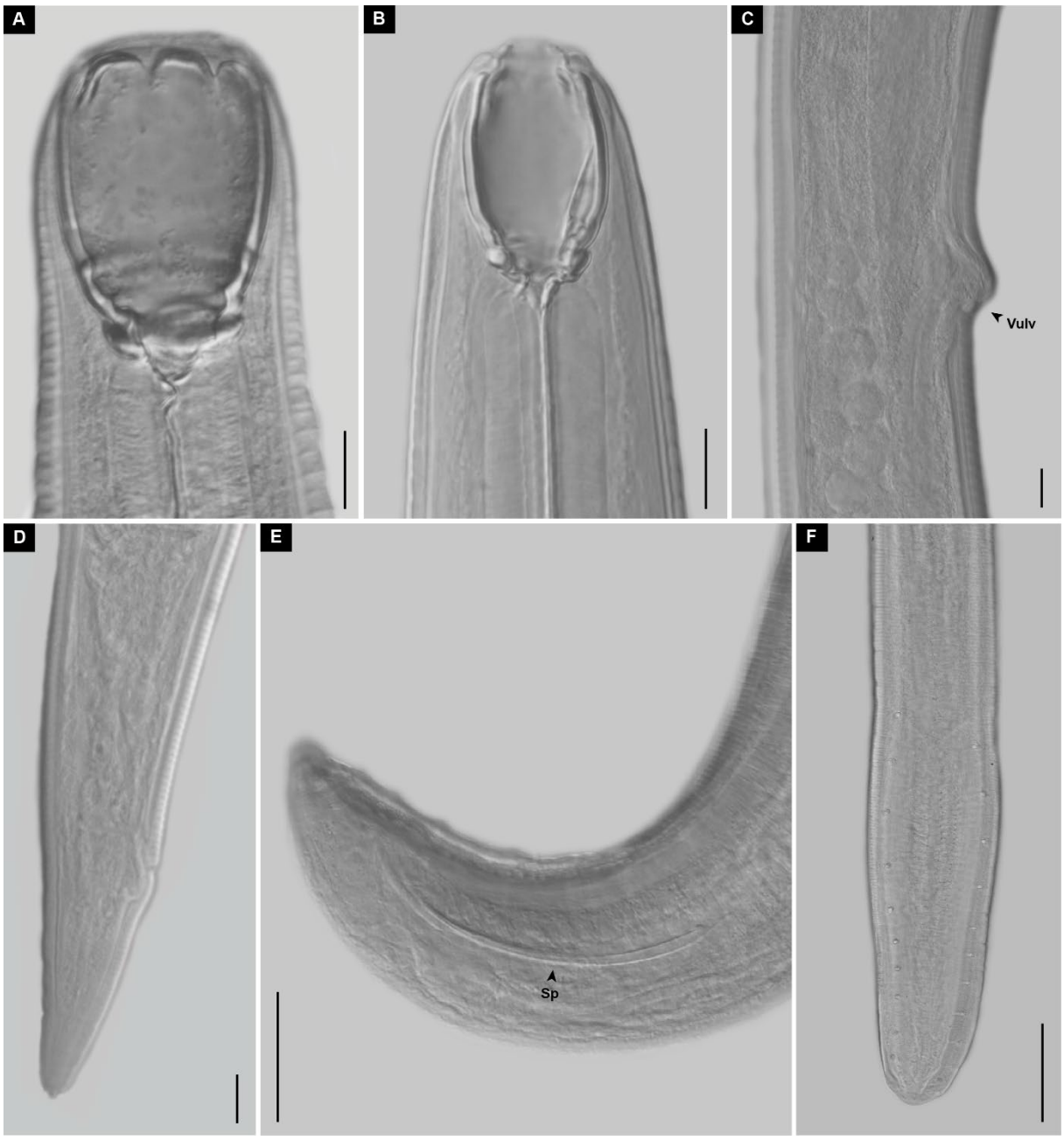

Figure 3.31. Photomicrographs of *Procamallanus* (*Procamallanus*) *pseudolaeviconchus* Moravec et Van As, 2015. **A** – buccal capsule, lateral view; **B** – optical section of buccal capsule; **C** – part of body at vulva region, female, lateral view; **D** – posterior end of body, female, lateral view; **E** – posterior end of body, male, lateral view and **F** – ventral view. Abbreviations: Sp – spicule; Vulv – vulva. Scale bars: 20μm (A, B, C, D) and 50μm (E).

Family Cystidicolidae Skryabin, 1946

Spinitectus Fourment, 1884

In one of the first checklists on the helminths of African freshwater fishes Khalil and Polling (1997) recognised 14 species of *Spinitectus* Fourment, 1884. However, only nine of these are currently recognised after a revision of nematodes of African freshwater fishes by Moravec (2019). It is worth mentioning that Moravec (2019) recommended an in-depth revision of material of the recognised species, as some may still be considered synonymous with other species or even to be invalid. To distinguish representatives of *Spinitectus* Fourment, 1884 from other nematodes, characters such as posteriorly directed cuticular spines that diminish posteriorly are prominent in adult specimens. Even though relatively nothing is known about the life cycle of several *Spinitectus* spp., it has been confirmed that aquatic insects such as mayflies and shrimps serve as intermediate hosts.

Two species, *Spinitectus allaeri* Campana-Rouget, 1961 (syn. *S. moraveci* Boomker et Puylaert, 1994) and *Spinitectus petterae* Boomker, 1993 are known from the stomach of *C. gariepinus* (Moravec et al. 1974a). *Spinitectus allaeri* is only known from the sharptooth catfish in Egypt, while the infection with *S. petterae* reported from Tanzania needs confirmation as this species is only known from *C. gariepinus* from rivers in South Africa.

Present study: Adult individuals of *Spinitectus* sp. were found in the stomach of *C. gariepinus* from the VR [P = 44%, IF = 1–6] (**Fig. 3.32. A–D**).

Identification: Assignment of specimens to *Spinitectus* sp. is based on the presence of the posteriorly directed cuticular spines of the cuticle of specimens from VR (see Fig. 34. A–B). Assignment to a species was not done as a full morphological account is not given and is not within the scope of this study. A good quality consensus sequence could not be obtained for isolates of *Spinitectus*. Two submissions of the 18S rDNA region for *S. petterae* from *C. gariepinus* in Egypt (KC291616) and Tanzania (DQ813447) are available in the GenBank database (Mwita and Nkwengulila 2010). It is evident that molecular characterisation, complimenting morphological identification will clarify species placement within the genus and provide insights into the true distribution of all the species currently contained in the genus.

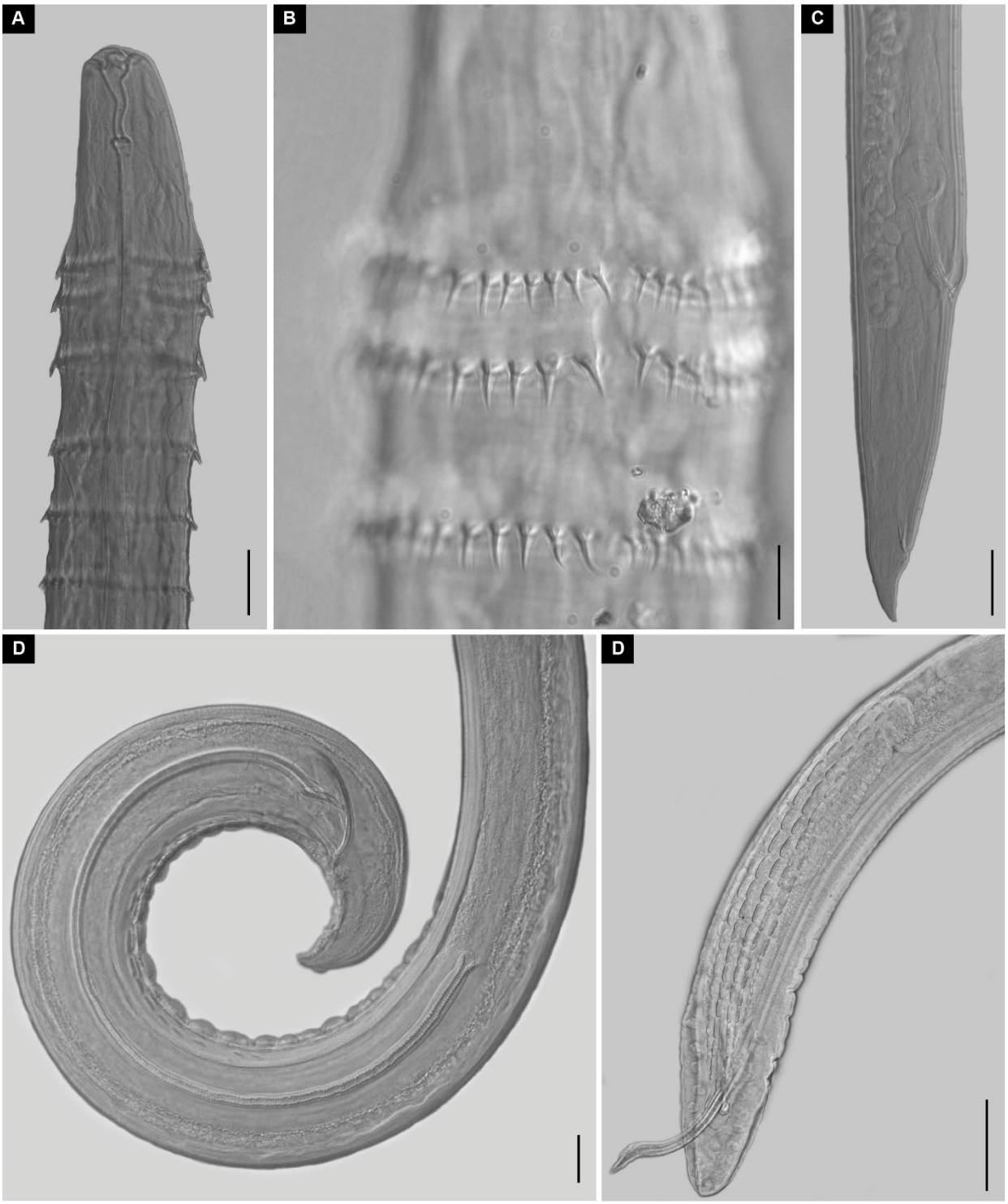

Figure 3.32. Photomicrographs of *Spinitectus* sp. from the stomach of *Clarias gariepinus* (Burchell) from the Vaal River. **A** – Anterior end of body, lateral view; **B** – spine detail, anterior end; **C** – posterior end, female, lateral view; **D**, **E** – posterior end, male, lateral and ventral view. Scale bars: 20µm (B, E); 50µm (A, C) and 100µm (D, F).

Family Gnathostomatidae

Tanqua Blanchard, 1904*, ⋆

Representatives of this genus are known from aquatic reptiles and have mostly been reported as parasites of *Varanus* lizards (Baylis 1940; Hering-Hagenbeck and Boomker 2000; Schoeman et al. 2020 and references therein). Adult forms are also parasites of amphibians and fish may serve as one of a range of paratenic hosts. Larvae of *Tanqua* have been reported but are yet to be described. Laetsch et al. (2012) reported on larvae encysted in the serosa of the swimbladder of African longfin eel, *Anguilla mossambica* (Peters) from South Africa (Laetsch et al. 2012; Moravec 2019). Similarly, Schoeman et al. (2019) found third-stage larvae encysted in the intestine of *X. laevis* also from South Africa. According to these author's comparison of 18S rRNA and 28S rRNA gene regions, these specimens are congeneric, and possibly belong to *Tanqua tiara* (von Linstow, 1879) reported from the definitive varanid host (see Schoeman et al. 2019 for detail). *Tanqua tiara* have also been reported from *Varanus* spp. in Africa, thus it is not impossible that *A. mossambica* and *X. laevis* connect pathways for transmission to definitive hosts that have diverse diets (Hering-Hagenbeck and Boomker 2000; Dalhuisjen et al. 2014; Williams et al. 2022). Formal assignment of the aforementioned larval stages to a species is pending a comparison of specimens as Laetsch et al. (2012) did not provide any morphological description of their specimens.

Present study: Larval individuals of *Tanqua* sp. (**Fig. 3.33. A–B**) were found encysted in the mesenteries and kidneys of *C. gariepinus* from KR [P = 50%, IF = 4] and ZR [(P = 76%, IF = 1–7)].

Identification: Sequence data was successfully obtained for the *cox*1 mitochondrial gene region for isolate MT33 from ZR. There were no significant alignments in the GenBank database with this specimen. Comparison to the isolates of Laetsch et al. (2012) and Schoeman et al. (2020) were not possible as PCR amplification of the 18S and 28S rDNA regions of isolates from the present study were not successful.

Figure 3.33. Photomicrographs of larval *Tanqua* sp. from the intestine of *Clarias gariepinus* (Burchell) from the Barotse floodplain, Zambia. **A** – Anterior end, lateral view; **B** – posterior end, lateral view. Scale bars: 50µm.

Family Physalopteridae Railliet, 1893*

Invertebrates harbouring third-stage larvae serve as intermediate host, while fish serve as a paratenic host to species of the Physalopteridae Railliet, 1839. Adult forms parasitise freshwater and marine fishes primarily belonging to the subfamily Proleptinae. A single species, *Heliconema africanum* (Linstow, 1899) (syn. *Spiropterina africana* Linstow, 1899) is known from eels in African waters while other species parasitise amphibians, reptiles, birds, and mammals (Anderson 2000). Moravec and Van As (2015c) reported a single specimen of Physalopteridae gen. sp. from *C. gariepinus* collected in the Okavango River in Botswana (**Fig. 3.26. D**).

Present study: No specimens representing Physalopteridae gen. sp. were found during the present study.

Family Rhabdochonidae Travassos, Artigas et Pereira, 1928

Rhabdochona Railliet, 1916*

Ten species are recognised from the intestine of freshwater fishes in Africa. Although the biology of the genus is largely unknown, aquatic arthropods are utilised as intermediate hosts and adults are found in the intestine of their fish host. A single species *Rhabdochona* (*Globochona*) *paski* Baylis, 1928 (syns. *Rhabdochona congolensis* Campana-Rouget, 1961; *Rhabdochona aegyptiaca* El-Naffar et Saoud, 1974; *Rhabdochona versterae* Boomker et Petter, 1993) is thought to parasitise characiform fishes (as definitive hosts) while other fish hosts harbour *Rhabdochona* spp. as paratenic hosts or postcyclic infections. A single record of *Rhabdochona* (*Gl.*) *paski* is known from the intestine of *C. gariepinus* from Lake Kivu in the Democratic Republic of the Congo, whereas several records are known of *Rhabdochona* spp. from the same host in the Olifants, Sabie and Crocodile rivers in South Africa (Campana-Rouget 1961; Boomker 1994) (see **Fig. 3.26. D**).

Present study: No infection with specimens resembling representatives of *Rhabdochona* were found.

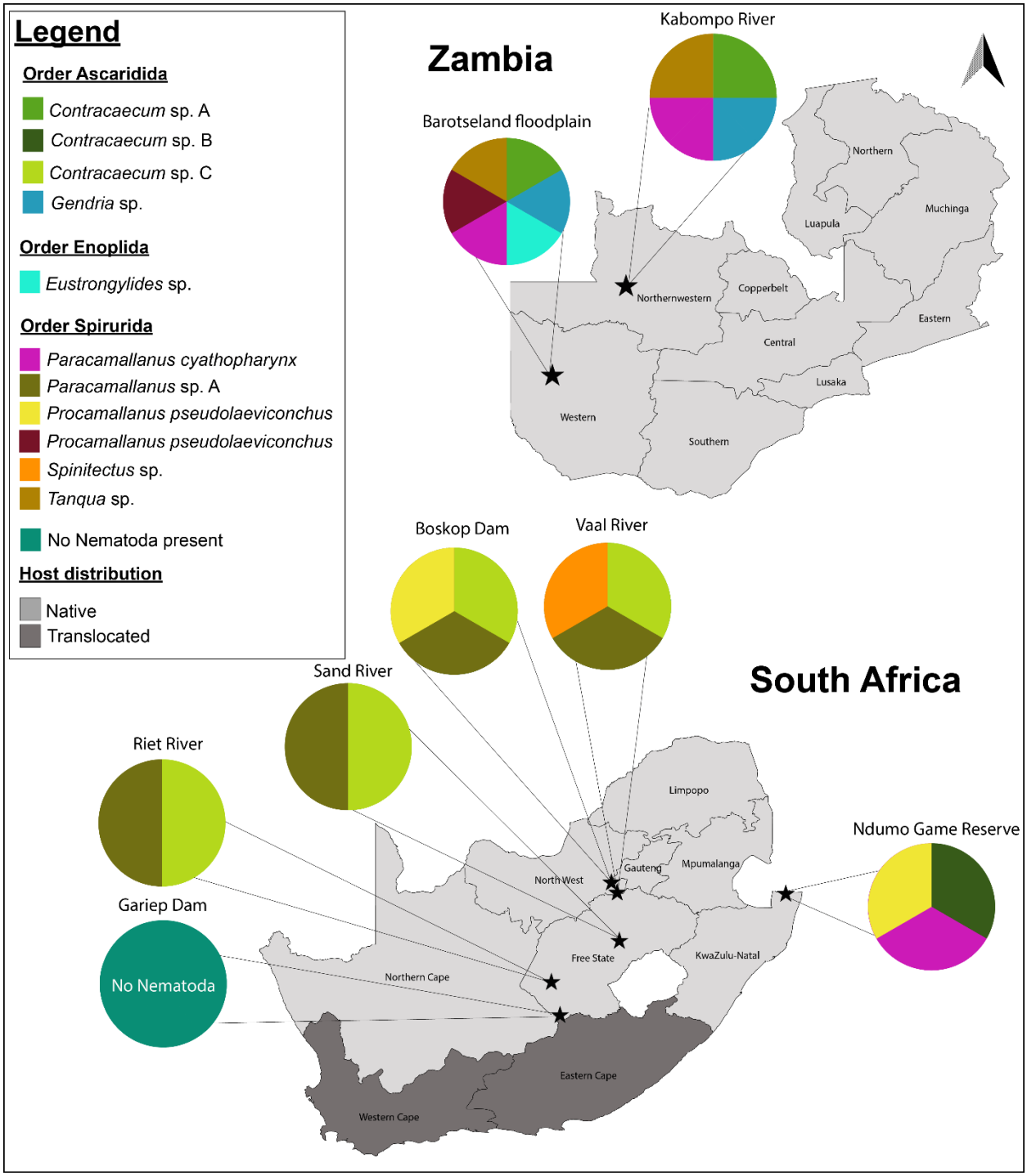

Figure 3.34. Map depicting presence and absence of nematodes of the Enoplida, Ascaridida and Spirurida infecting various organs of *Clarias gariepinus* (Burchell) in the present study. Note that the sectors of the pie-charts do not depict any numerical or statistical value regarding prevalence, intensity of infection or other infection statistics.

Concluding remarks

In contrast to the Digenea and Cestoda, few records of unidentified nematode infections exist. Boomker (1994a) investigated the nematode parasites of *C. gariepinus* from the Olifants, Sabie and Crocodile rivers in South Africa. The author listed all nematode larvae infecting *C. gariepinus* that did not correspond to the morphology species of *Contracaecum* as "unidentified nematode larvae". Similarly, Madanire-Moyo et al. (2012) found unidentified larvae in the sharptooth catfish from Flag Boshielo Dam and Barson et al. (2014) reported on unidentified nematodes from the Gwebi and Mukuvusi Rivers in Zimbabwe. In both these studies, no detail was provided on the morphology of the specimens they found.

Eleven species from the three nematode orders, Enoplida (1); Ascaridida (4) and Spirurida (5) were found to infect various organs of the *C. gariepinus* screened during this study (see **Fig. 3.34.**). Infections with adult forms are represented by the camallanids *Pa. cyathopharynx* (NGR, ZR and KR); *Paracamallanus* sp. A (RR, SR, VR, BS); and *P.* (*P.*) *pseudolaeviconchus* (BS, NGR) and *Paracamallanus* sp. A (ZR); and the cysticolid *Spinitectus* sp. of which all, except the morphotypes *Pa.* sp. A and *Paracamallanus.* sp. A are known from the intestine and stomach of *C. gariepinus*. Three morphotypes of larval *Contracaecum* were found encysted in various organs, while larval stages of *Eustrongylides* sp., *Gendria* sp. and *Tanqua* sp. utilised *C. gariepinus* as a paratenic host from localities in Zambia (KR and ZR).

Infection with all nematodes found in this study represent novel and extended geographic distribution records for all ten species in southern Africa. *Clarias gariepinus* as a paratenic host to *Tanqua* sp. is an expansion of the known host species for larvae of this genus. *Pa. cyathopharynx* were formerly only reported from the Limpopo and Komati River systems, but its occurrence is now extended southward to the NGR and *Paracamallanus* sp. A is reported for the first time from the Orange and Vaal rivers (RR, SR, VS and BS). Confirmation of *Paracamallanus* sp. A as a new species is pending a morphological study to complement the molecular data generated in this study. Similar to the previous camallanid, the distribution of *P.* (*P.*) *pseudolaeviconchus* is now extended southward to the NGR and Mooi River (part of the Vaal River system). The record here of a *Spinitectus* sp. extends the distribution of the genus to the Vaal River system, previously only known from *C. gariepinus* in the Nile River (Egypt) and the Crocodile and Sabie rivers in South Africa. Despite larvae of *Contracaecum* spp. being widely reported from nine African countries, their presence in sharptooth catfish in southern Africa is only known from the lower Zambezi and Save rivers (Zimbabwe), the Limpopo and Komati River systems and Lake Sibaya in South Africa. Here first records are reported from KR, ZR and NGR. Furthermore, the southward distribution of *Contracaecum* spp. is now extended to BS, VR, SR

and RR. An incidental infection with *Eustrongylides* sp. was found from ZR, representing the second reported infection from the southern hemisphere.

Additionally, from the molecular data generated in this study, it is clear that there is an urgent need for multi-disciplinary approaches to the identification of parasitic taxa from African freshwater fishes, including nematodes. It is apparent that further investigation will enlighten parasitologists, as well as ecologists, on the interaction between species within our ecosystems. Despite the reports of infection with *Eustrongylides* sp. and associated pathology from numerous fishes, including *C. gariepinus* no comparative sequence data is available to determine if this is a single, cosmopolitan non-host specific species, or several species. The African anisakiid fauna is represented by a single submission of a larval *Contracaecum* species from *X. laevis*. *Contracaecum* is a cosmopolitan generalist infecting several hosts and has an intricate taxonomic history. Moreover, larval stages are impossible to distinguish from other genera and may represent several species, prompting the need for multi-disciplinary identification of larval representatives to aid in future clarity on host preference, life cycles and biogeography within the diverse and unique ecosystems of Africa. Similarly, comparable sequence data for *Gendria* and *Tanqua* from African species are scant. Svitin et al. (2019) provided novel sequence data complimenting their morphological account of camallanids from *C. gariepinus* in NGR. The authors highlighted the need for complimentary molecular data, to the debated morphological assignment of species within the Camallanidae. The additional sequences generated for species of *Paracamallanus* and *Procamallanus* in this study, contribute to the very small molecular database currently available for camallanids in Africa and will certainly aid in the future understanding of relationships between species of the different genera, and should be complimented with further morphological characterisation. Another example of the importance of multidisciplinary approaches in species delineation is the genus *Spinitectus*. Despite the fact that no sequence data was obtained for *Spinitectus* isolates during this study, it is clear that the availability of such data would be of great value in initial species delineation. The two submissions of the 18S rDNA fragments for *S. petterae* in the GenBank database are currently the only available data for comparison. Both these submissions lack complimentary morphological and morphometric data and would need re-evaluation as *S. petterae* is only known from South Africa.

3.6. Phylum Acanthocephala

Acanthocephalans are a small group of helminths commonly known as spiny- or thorny-headed worms due to their armed proboscis found in the anterior end of individuals. Acanthocephalans have indirect life cycles, where adults utilise vertebrate definitive hosts, larvae (cystacanths) are found in intermediate arthropod hosts and, on occasion, vertebrates may act as paratenic hosts (Amin 1998; Sures et al. 2018). Globally 1300 species representing four classes (Archiacanthocephala; Eoacanthocephala; Palaeacanthocephala and the Polyacanthocephala) have been reported (Verweyen et al. 2011). Increasing attention has been given to these unique parasites with the emergence of environmental parasitology where acanthocephalans alongside nematodes have been identified as excellent indicators of environmental pollution (Nachev et al. 2013; Sures et al. 2017b). Few species are known from African freshwater fishes, where fishes of 15 families have been recorded as hosts of only 19 acanthocephalan species. The checklists on helminths of African freshwater fishes compiled by Khalil (1971) listed no infections with acanthocephalans for *C. gariepinus*. In the 2nd edition, only a single acanthocephalan was added to the parasitic community of the sharptooth catfish (Khalil and Polling 1997). Halajian et al. (2018) produced a checklist, the first of its kind on all acanthocephalans known from the diverse vertebrate fauna of South Africa, and unsurprisingly the sharptooth catfish from the southern tip of the continent were not listed as a host to any spiny-headed worm.

Only species from two families are known to infect *C. gariepinus*. These are *Neoechinorhynchus rutili* (Müller, 1780) and *Neoechnorhynchus* sp. (Neoechinorhynchidae: Neoechonorhynchida); and *Paragorgorhynchus aswanensis* Saoud et Wanas, 1990 and *Paragorgorhynchus* sp. (Rhabdinorhynchidae: Echinorhynchida). *Neoechinorhynchus rutili* was reported from *C. gariepinus* in Nigeria, as well as one record from Egypt, while a single record is known for *P. aswanensis* from the intestine of *C. gariepinus* in Egypt (**Fig. 3.35.**) (Saoud and Wannas 1984).

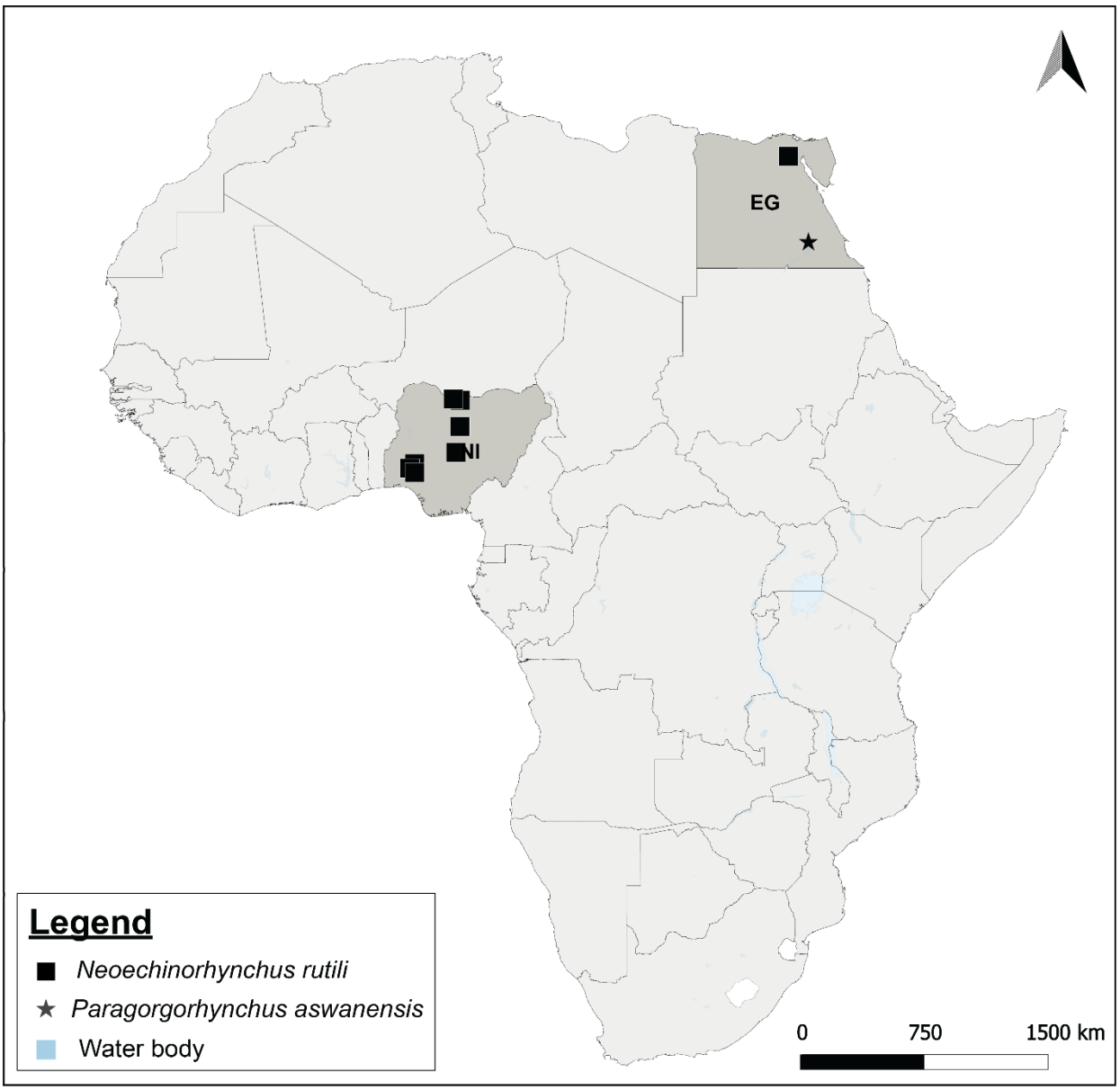

Figure 3.35. Distribution records for Acanthocephala infections from the intestine of *Clarias gariepinus* (Burchell) in Africa.

In addition to these known species, several unconfirmed records of acanthocephalans can be found in the literature reporting on general parasite communities of *C. gariepinus*. Mwita and Nkwengulila (2008) and Mwita (2014a, b) reported an "unidentified sp." found from *C. gariepinus* screened in Lake Victoria in Tanzania. Goselle et al. (2008) reported on a "Acanthocephalans sp." in their study on helminths of sharptooth catfish from Lamingo Dam in Nigeria, and Balogun and Solomon (2015) also found an "Acanthocephalan sp." during their study on the endohelminths of *C. gariepinus* that were fed with chicken droppings. Furthermore, Ibironke and Morenikeji (2018) reported on the infection of *C. gariepinus* with *Acanthogyrus* sp., a genus previously reported from at least 40 different fish species in Africa (see Sures et al. 2018), while Sèdogbo et al. (2019) claimed to have found a species of *Pallisentis* Van Cleave, 1928 from the Oueme River basin in Benin. These reports all need confirmation as none of the above authors provided any morphological description or molecular characterisation of their findings. Further, it should be noted that *N. rutili* is a Holarctic species reported from fishes and the designation of specimens from Nigeria to the species should be re-evaluated.

It is clear from available publications that there is a deficiency in research and understanding for the acanthocephalans, their life cycle, host range and interactions in African fish and specifically *C. gariepinus*.

Present study: No infection with species of acanthocephalans were found during the present study.

3.7. Subphylum Crustacea

Parasitic Crustacea (a subphylum within the Arthropoda) is globally considered the most successful and morphologically diverse parasitic group in aquatic environments. They occur on a range of vertebrate and invertebrate hosts and possess excellent life strategies adapted for a wide spectrum of parasitic lifestyles (Smit et al. 2019). One such example is the moving between host and substrate or permanent attachment for life cycle completion and in some cases only females become parasitic (see Smit and Hadfield 2018 for more detail). Features that discriminate these parasites from the parasitic groups discussed thus far is its chitinous exoskeleton that is shed between different life stages during moulting. Furthermore, representatives bear two antennae and three pairs of mouthparts, have two compound eyes on the head and have biramous appendages. More complex features of parasitic crustaceans include the absence of a heart (blood is not circulated in vessels) and their two-part nervous system consisting of a ventral nerve cord and ganglia system. Some species are hermaphroditic, but mostly sexual reproduction occurs between separate sexes (Smit and Hadfield 2018; Hadfield 2019). Taxa such as the copepods of the Lernaeidae Cobbold, 1879, are of economic and commercial importance as they can have devastating effects on fish populations in cases of high infestation rates. Several pathological conditions of the gill filaments and skin have been associated with infestations of copepods, and additionally they may facilitate disease transmission by acting as vectors for viruses (i.e., haematopoietic necrosis virus, spring viraemia of carp and carp pox) and larval nematodes (see Fryer 1968; Molnár and Székely 1995; Dezfuli et al 2003; Hadfield and Smit 2019; Johnson et al. 2019).

Species of three subclasses, the Branchiura, Copepoda and Pentastomida are known to parasitise *C. gariepinus* and are briefly discussed below (see **Table A3.11.** and **Table A3.12.**). During this study representatives of the Branchiura (*Argulus* Müller, 1785) and Copepoda (*Lamproglena* von Nordmann, 1832; *Ergasilus* von Nordmann, 1832) parasitised the gills, skin and fins of *C. gariepinus* (see **Table 3.1.**).

3.7.1. Subclass Branchiura

The taxonomic placement of the Branchiura have in earlier years been debated, however it is now widely accepted in the subclass rank. The confirmation and elevation of the subclass was based on a set of characters that include the morphology of the “tail”, cephalic appendages (in particular the second maxilla) and the absence of the first maxilla on the mouth cone. All members of the Branchiura are exclusively parasitic of marine and freshwater fishes and are commonly referred to as “fish lice”. These small dorsoventrally flattened ectoparasites have on occasion been found on alligators, salamanders and even tadpoles. All branchiurans belong to the order Arguloida Yamaguti, 1963 and the family Argulidae Leach, 1819 that holds four genera, *Argulus* Müller, 1785; *Chonopeltis* Thiele, 1900; *Dipteropeltis* Calman, 1912 and *Dolops* Audouin, 1837. All

genera, except *Argulus*, are confined to a parasitic lifestyle in freshwaters, while no species of *Dipteropeltis* are known to parasitise *C. gariepinus* (Møller 2009; Boxshall and Hayes 2019; Hadfield 2019).

3.7.1.1. Order Arguloida

Family Argulidae Leach, 1819

Argulus Müller, 1785

Argulus is the oldest and probably the most speciose and widespread branchiuran genus. Members of the genus are the only argulids that can be found in marine, brackish and freshwater environments. Species have separate sexes; individuals move between hosts and substrate to complete their life cycle and to date no host specificity has been reported for any species of *Argulus*. Since *Argulus* spp. are mobile and do not attach to a single site on the hosts, sites of infection vary from the head, branchial arches, roof of the mouth, ventral body surface, fins, gill filaments and sometimes the crevices of external appendages (i.e., fins) of hosts (Cunnington 1913; Kruger et al. 1983). In the recent checklist of parasitic Crustacea of African freshwater fishes, five *Argulus* spp., and the alien invasive *Argulus japonicus* Thiele, 1900 were listed to infect *C. gariepinus.* All species, except *A. japonicus* are discussed below (see **Chapter 5** for more detail on the alien invasive species).

The most widespread species known to parasitise *C. gariepinus* is *Argulus rhipidiophorus* Monod, 1931. This species has been recorded from catfish collected in tributaries of the Nile and Niger rivers in Sudan, Uganda, and the great lakes (Lake Albert, Lake Victoria) and the southern reaches of Lake Tanganyika in Zambia (**Fig. 3.36. A**) (Fryer 1959b, 1965b). *Argulus africanus* Thiele, 1900, another species known from *C. gariepinus* was previously confused with *A. rhipidiophorus* and is only known from this host in Lake Victoria and at the confluence of the Matetsi and Deka rivers with the Zambezi River in Zimbabwe (see Fryer 1960, 1961, 1964, 1959b, 1965b). *Argulus ambloplites* Wilson, 1920, known from *C. gariepinus* in the waters of Zambia and northern Botswana, is often confused with *Argulus jollymani* Fryer, 1956 formerly recorded from two cichlid hosts (see Fryer 1959b, 1960). *Argulus cunningtoni* Fryer, 1965 has only been recorded from *C. gariepinus* from its type locality, Lake Albert, until Van As and Van As (2015) found *A. cunningtoni* from *C. gariepinus* in the Zambezi River (**Fig. 3.36. A**). Infection of the sharptooth catfish with *Argulus striatus* Cunnington, 1913 from Lake Tanganyika, is the only record for this species to date. The alien invasive *A. japonicus* from *C. gariepinus* is discussed in **Chapter 5.**

Present study: Individuals morphologically resembling *Argulus cunningtoni* (**Fig. 3.37. A–D**) were found from the gills of *C. gariepinus* from KR [P = 50%, IF = 2].

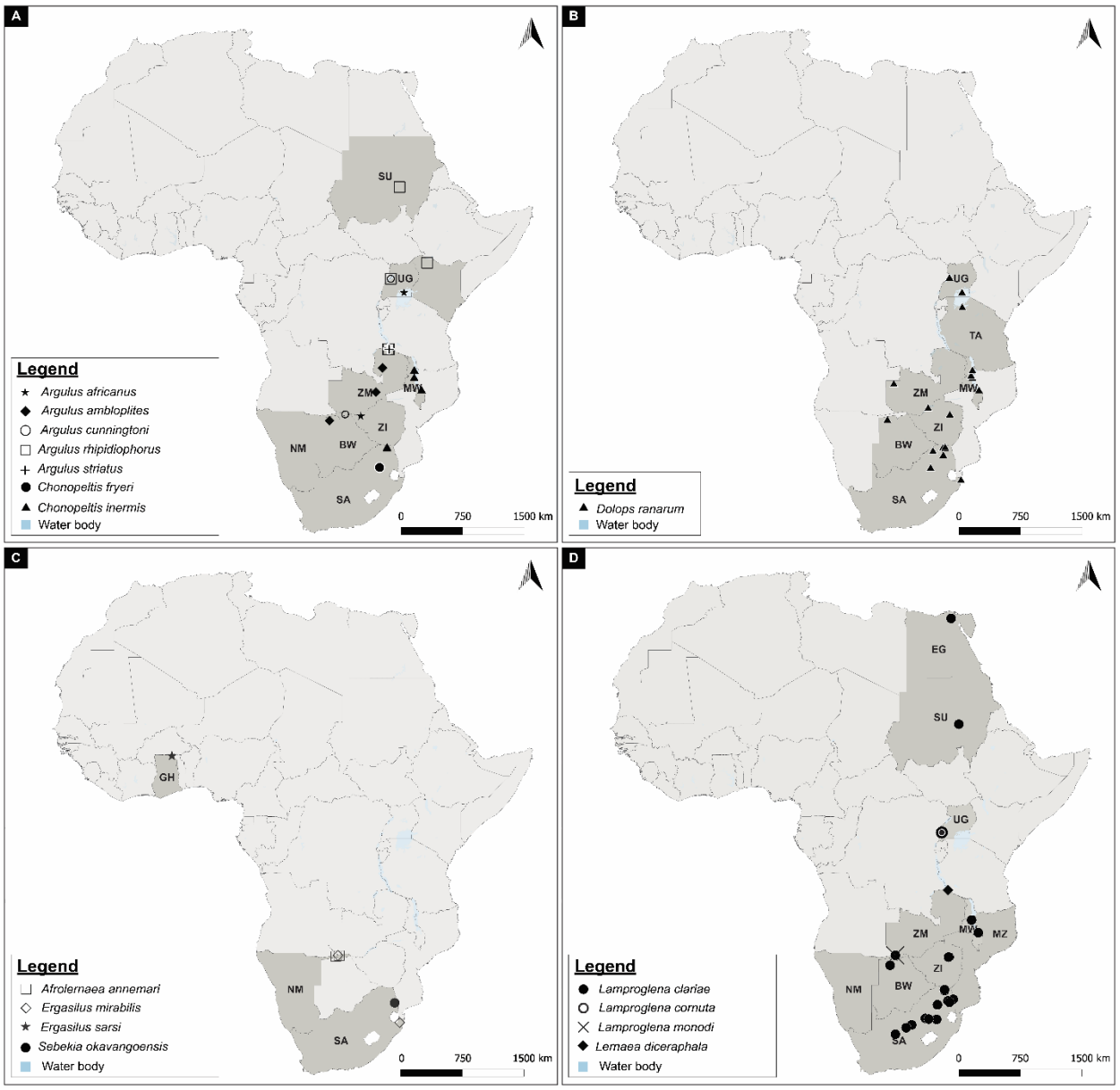

Figure 3.36. Current distribution of the Branchiura (A, B); Copepoda (C, D) and Pentastomida (C) infecting *Clarias gariepinus* (Burchell) in Africa.

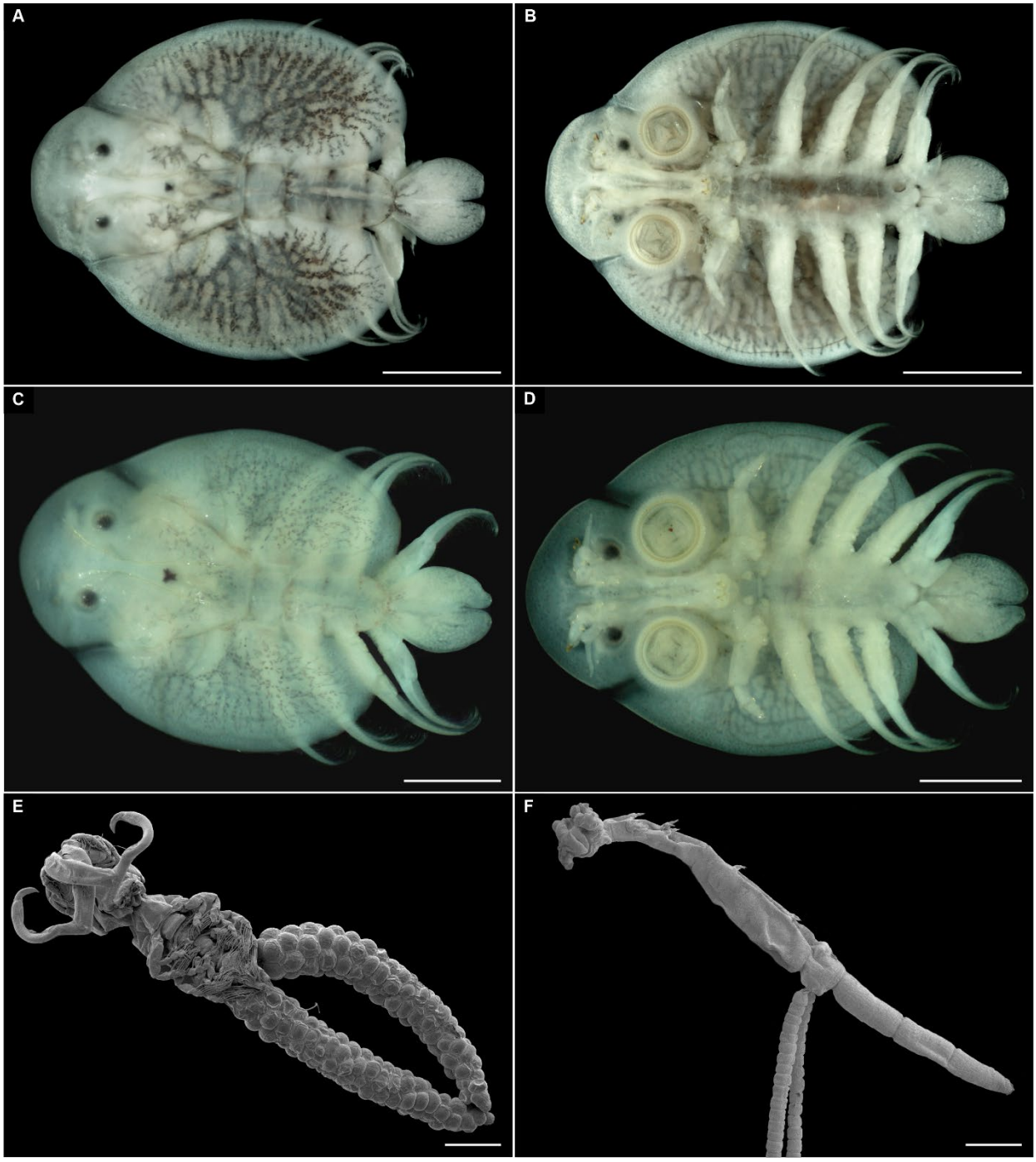

Figure 3.37. Photomicrographs of the Branchiura and Copepoda found from the gills of *Clarias gariepinus* (Burchell) during the present study. **A**, **C** – dorsal and **B**, **D** – ventral view of *Argulus* cf. *cunningtoni* Fryer, 1965 from the Kabompo River, Zambia; **E** – *Ergasilus* cf. *mirabilis* Oldewage et Van As, 1987; and **F** – *Lamproglena clariae* Fryer, 1956. Scale bars: 200µm (E), 500µm (F) and 1000µm (A–D).

Chonopeltis Thiele, 1900

This genus comprises 13 species all endemic to sub-Saharan Africa. Species of *Chonopeltis* are characterised by its sedentary lifestyle and possessing a cone- or funnel-shaped shield (Fryer 1956). From the host records noted in Smit and Hadfield (2018), a degree of host specificity is apparent for some species only parasitising cyprinid, mochokid or mormyrid hosts (also see Van As et al. 2017). The clariid host, *C. gariepinus* from Loskop Dam in South Africa is the type host for *Chonopeltis fryeri* Van As, 1986 (shared with *Clarias theodorae* Weber from the Mogalakwena River) (**Fig. 3.36. A**) (Van As 1986). A second species, *Chonopeltis inermis* Thiele, 1900 has also been recorded on the sharptooth catfish in South Africa and its type locality, Lake Malawi (Fryer 1956; Madanire-Moyo et al. 2010, 2012). Madanire-Moyo and Barson (2010) found *Chonopeltis* sp. on *C. gariepinus* from the Middle Zambezi River system (Nyatsime River) in Zimbabwe and indicated that based on morphological similarities, it is most probably *C. fryeri*. However, the identification of this *Chonopeltis* is not conclusive and it is possible that it might even be a different species, therefore further studies on the morphology and molecular aspects of the specimens found on sharptooth catfish from the Middle Zambezi River is recommended. Interestingly, Van As et al. (2021) provided morphological and molecular evidence that species of *Chonopeltis* are not restricted to drainage basins as previously thought.

Present study: During the present study no *Chonopeltis* specimens were found on *C. gariepinus* from the different sampling localities.

Dolops Audouin, 1837

Dolops is a genus presumed to be confined to the southern hemisphere, with representatives known to parasitise a wide range of fish hosts from Africa, South America and Australia (Tasmania). Species from this genus are generalist ectoparasites and can easily be distinguished from *Argulus* by the absence of suckers. Infestation with species of *Dolops* can be found on the skin of the host or in the mouth, branchial cavities, and gills. Species are active, non-sedentary and leave a distinct lesion where it was attached to the host, rendering it as another parasitic crustacean of importance (Avenant-Oldewage 1994).

A single species *Dolops ranarum* (Stuhlmann, 1892) (syn. *Gyropeltis ranarum* Stuhlmann, 1892), was originally described from tadpoles and is exclusively known from Africa. *Dolops ranarum* have been found in all major river drainage basins, except the Orange-Vaal system in South Africa and is absent from the southern extremity (southern Eastern and Western Cape provinces in South Africa) of the continent. Furthermore, the absences of *Dolops* in the southern extremes and the lack of radiation of the genus in Africa has been a topic of discussion in the past. Hypotheses for this include its intolerance to colder conditions, despite its tolerance to a wide range of

environmental conditions, or interspecific competition with other branchiurans i.e., aggressive behaviour exerted by female species of *Chonopeltis*, rather than the absence of suitable hosts (Fryer 1956; Van As and Van As 2019). Almost all research and information available on the genus emanated from the African species *D. ranarum* and is summarised in the compendium of Neethling and Avenant-Oldewage (2016) (also see Hadfield 2019 and references therein).

Records of *D. ranarum* parasitising *C. gariepinus* are well documented from as far north as the great lakes in Uganda and Tanzania and the southernmost record is from the natural freshwater Lake Sibaya on the east coast of South Africa (see **Fig. 3.36. B** and **Table A3.12.**). Several records from the sharptooth catfish have been reported from Lake Malawi, Lake Kariba, and single reports from the Okavango River and Upper and Middle Zambezi system in Zambia and Zimbabwe (Fryer 1956, 1959b; Bruton 1979a; Avenant and Van As 1985; Avenant et al. 1989b; Avenant-Oldewage and Van As 1990a, b; Avenant 1994; Douëllou and Erlwanger 1994; Møller et al. 2008; Tam and Avenant-Oldewage 2009; Madanire-Moyo et al. 2010, 2012; Madanire-Moyo and Barson 2010; Van As and Van As 2015). The Limpopo River drainage in northern South Africa represents the most well-documented region of occurrence of *D. ranarum* on *C. gariepinus* (and other fish hosts) and also represents the origin of the specimens used in the redescription of *D. ranarum* and several studies on its digestive system, reproductive system and life cycle (see Avenant et al. 1989a, b; Avenant-Oldewage and Van As 1990a, b; Avenant-Oldewage 1994; Tam and Avenant-Oldewage 2009)

Present study: During the present study no infection with specimens of *Dolops* were found on *C. gariepinus*.

3.7.2. Subclass Copepoda

The Copepoda is the most diverse group of the parasitic Crustacea in terms of morphology, host utilisation and species numbers. Representatives can be free-living, symbiotic or parasitic, some are endoparasites, but the majority are primarily found on the external surface of its host (i.e., in a microhabitat such as the gills, nostrils, mantle cavities, genital folds) (Hadfield 2019). Species of Copepoda that have been reported from *C. gariepinus* belong to the genera *Afrolernaea* Fryer, 1956; *Lamproglena* von Nordmann, 1832; *Lernaea* Linnaeus, 1758 and *Ergasilus* Nordmann, 1832. All genera, excluding *Ergasilus* that are free-swimming, are mesoparasitic, slender and embed their anchor into the cartilage of the gills or into the muscle tissue under scales of their hosts, while the remainder of their body and egg sacs are visible externally. Interestingly, only the females of these mesoparasites become parasitic after several moults and are referred to as copepodites (Smit and Hadfield 2018; Van As and Van As 2019).

3.7.2.1. Order Cyclopoida Burmeister, 1834

Family Ergasilidae Burmeister, 1835

Ergasilus von Nordmann, 1832

Ergasilus was the first of 29 genera described in the Ergasilidae and is unique in that the females, and not males, are parasitic on the fish host. Thirteen species of *Ergasilus* have been reported as parasites of fishes from marine, brackish and freshwater systems in Africa (Smit and Hadfield 2018). Representatives of the genus received much attention due to the tissue damage caused by their adapted second antennae, a robust claw, at the site of attachment on the gill filaments of their hosts. The attachment site to specific regions of the gills is species specific and may cause gill compression, compression of blood vessels and lead to hypoxia. Additionally, lesions caused as a result of infestation with *Ergasilus* may result in secondary infections and cause host mortalities (see Fryer 1968; Oldewage and Van As 1987; Hadfield 2019).

Ergasilus mirabilis Oldewage et Van As, 1987 has been reported from alestid, clariid, cyprinid, hepsetid, mormyrid and schilbidae hosts (Smit and Hadfield 2018). Infection on the gills of *C. gariepinus* is known from the Caprivi strip in Botswana and the Phongolo River and floodplain in South Africa (Oldewage and Van As 1988). From very mild infestations, Oldewage and Van As (1987) could determine that proliferation of the host tissues at the site of attachment was caused by this species as a result of its feeding activity rather than by the attachment of the parasite to the gills. However, the opposite is true for *Ergasilus sarsi* Capart, 1944 reported from the gills of *C. gariepinus* from Lake Volta in Ghana. Studies have not confirmed the pathology of *E. sarsi* infecting *C. gariepinus*, however a study on infested killifish of Lake Tanganyika confirmed that *E. sarsi* attaches to the tip of the gill filament, inserts its adapted claws (second antennae) into the gill filaments causing erosion of superficial epidermal tissues and eventually the loss of the tip of the filament [see Kilian and Avenant-Oldewage (2013) for a detailed account on pathology associated with infection of *E. sarsi*].

Present study: Ergasilids conforming to the general morphology of *Ergasilus mirabilis* were found **(Fig. 3.37. E**) on the gills of *C. gariepinus* in the VR [P = 50%, IF = 1–3], BS [P = 7%, IF = 1], NGR [P = 14%, IF = 1–14] and ZR [P = 24%, IF =1–160].

Family Lernaeidae Cobbold, 1879

Afrolernaea Fryer, 1956

Species of *Afrolernaea* are endemic to Africa and are thought to be the most primitive lernaeids based on a set of morphological features (see Oldewage 1994 and references therein). The six species described to date parasitise the gills and fins of Elephantfishes (Mormyridae) throughout

Africa, and only a single species, *Afrolernaea annemari* Oldewage, 1994 is known from a non-mormyrid host, *C. gariepinus* (Clariidae). The gills of *Clarias gariepinus* collected from a stretch of the Kwando River in the Caprivi strip in Namibia were infected with *A. annemari* (**Fig. 3.36. D**). Apart from the unusual infection on a clariid host, *A. annemari* is evidently different from the other five species in its cephalothorax comprising 2% of the total body length (Avenant-Oldewage and Oldewage 1993).

Present study: No specimens resembling *Afrolernaea* were found from the gills of *C. gariepinus* during the present study.

Lamproglena von Nordman, 1832

Lamproglena is a cosmopolitan parasitic genus comprising 28 species. Of these, 13 are known to parasitise a range of African freshwater fishes from the families Alestidae, Bargidae, Cichlidae, Citharinidae, Clariidae, Claroteidae, Cyprinidae, Hepsetidae, Latidae, and Malapteruridae (Smit and Hadfield 2018).

Lamproglena clariae Fryer, 1956 was described from the gills of *C. gariepinus* in Lake Malawi and is currently the most widely reported parasitic crustacean from the sharptooth catfish (**Fig. 3.38. D**). It has primarily been found to parasitise clariid hosts and is regarded as a pan-African species. In comparison, *Lamproglena monodi* Capart, 1944 has only been reported from *C. gariepinus* in Namibia (Avenant-Oldewage and Oldewage 1993), while the record of *Lamproglena cornuta* on *C. gariepinus* from Uganda needs confirmation. The references provided in the checklist of Oldewage and Avenant-Oldewage (1993), namely Thurston (1970) do not contain information on *L. cornuta*. However, Thurston (1970) does mention infestation of *C. gariepinus* from Lake George with *L. clariae* and Fryer (1965a) described *L. cornuta* from a closely related clariid, *H. bidorsalis* (see **Fig. 3.37. D** and **Table A3.12**). Although *Lamproglena* is not a genus of economic concern, lesions, hyperplasia and haemorrhaging around the site of attachment on the gill filaments have been observed. Hypertrophy of connective tissues and degeneration of blood capillaries within the gill filaments are also a concern (Fryer 1968; Marx and Avenant-Oldewage 1996; Tsotetsi et al. 2005).

Present study: *Lamproglena clariae* (**Fig. 3.37. F**) were found on the gills of *C. gariepinus* in the VR [P = 78%, IF = 1–31], BS [P = 20%, IF = 1], NGR [P = 8, IF =1–4] and the KR [P = 50%, IF = 2].

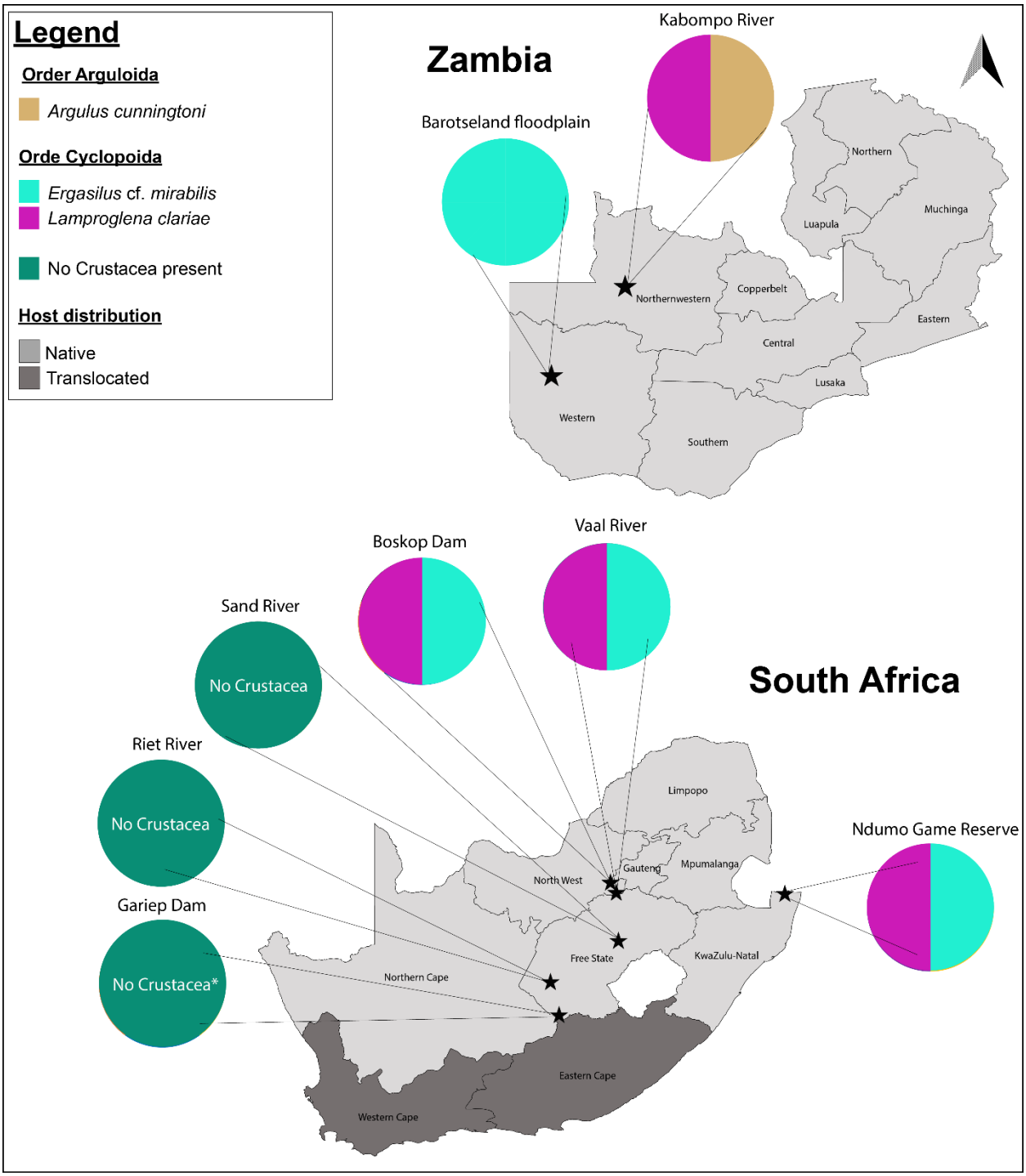

Figure 3.38. Map depicting presence and absence of parasitic Crustacea from *Clarias gariepinus* (Burchell) in the present study. Note that the sectors of the pie-charts do not depict any numerical or statistical value regarding prevalence, intensity of infection or other infection statistics. * Please see Chapter 5.

Lernaea Linnaeus, 1758

The genus *Lernaea* contains 57 species, with 16 of these listed in the recent checklist of parasitic copepods of African freshwater fishes (Smit and Hadfield 2018). Species of *Lernaea* are characterised by their head containing two to three, and rarely four pointed or swollen "horns" that are embedded in the host tissue. A single invasive species, *Lernaea cyprinacea* Linnaeus, 1758 is of high importance in natural as well as culture systems as even in low infestations it can be pathogenic to its host, whereas it seems that native *Lernaea* species are not prone to cause mortalities of hosts (Welicky et al. 2017, Van As and Van As 2019, Van As 2019).

Two species are known to parasitise *C. gariepinus*. *Lernaea composita* Wilson, 1924 [(syns. *Lernaea werneri* (Kurtz, 1922), Lernaeocera werneri Kurtz, 1922)] and *Lernaea diceracephala* (Cunington, 1914) (syn. *Lernaeocera diceracephala* Cunnington, 1914) has been recorded from sharptooth catfish collected in Lake Tanganyika (Cunnington 1914; Oldewage and Avenant-Oldewage 1993). The record of *L. composita* [reported as *L. werneri* in Oldewage and Avenant-Oldewage (1993)] on *C. gariepinus* (syn. *C. mossambicus*) from Lake Tanganyika needs confirmation, as no mention of *L. composita* or any of its synonyms could be found from the referenced literature in the checklist.

Present study: No individuals of the genus *Lernaea* were found to parasitise *C. gariepinus* during the present study.

3.7.3. Subclass Pentastomida Diesing, 1836

The Pentastomida is an ancient taxon previously placed in various groups including the annelids, mites, myriapods, onchyophorans and tardigrades. Finally, they were placed within the Arthropoda based on morphological and molecular studies that proved these specimens were more closely related to fish lice (*Argulus*) than any other group (Abele et al. 1989; de Oliveira Almeida and Christoffersen 1999; Hadfield 2019). Pentastomids or "tongue worms" are obligate vermiform parasites found in the respiratory passages of marine, freshwater and terrestrial vertebrates (Luus-Powell et al. 2008; Hadfield 2019; Smit et al. 2019). The adult forms lay their eggs in the respiratory passages of the definitive hosts, whereafter it is excreted through the digestive system and ingested by intermediate hosts such as fish. Larval forms of two families, the Sebekidae Sambon, 1922 and Subtriquetridae Fain, 1961 (Porocephalida) use freshwater fishes as intermediate hosts, while others utilise amphibians. Unfortunately, information on larval stages is limited, as adult forms are mostly found in reptiles, proving difficult to maintain or replicate in laboratory conditions. These challenges render the life cycles of these species relatively unexplored as well as limiting the investigating into the potential of fishes as hosts (Riley and Huchzermeyer 1995b; Junker 2016).

From Africa, infective larvae of six species in the genera *Alofia* Giglioli in Sambon, 1922 (1); *Leiperia* Sambon, 1922 (2); *Sebekia* Sambon, 1922 (2) and *Subtriquetra* Sambon, 1922 (1) have been found from alestid, cichlid, clariid, claroteid, lateid, mastacembalid, mormyrid and poecilid hosts in fresh- and brackish waters (see Luus-Powell et al. 2008; Smit and Hadfield 2018).

3.7.3.1. Order Porocephalida Heymons, 1935

Family Sebekidae Sambon, 1922

Sebekia Sambon, 1922

Luus-Powell et al. (2008) reported the first ever infection of *C. gariepinus* with a single encysted larva of *Sebekia okavangoensis* Riley et Huchzermeyer, 1995 in the mesenteries. This pentastomid was described from adult specimens found in the Nile crocodile *Crocodylus niloticus* Laurenti in the Okavango swamps in Botswana (Riley and Huchzermeyer 1995a). The hypothesis of Riley and Huchzermeyer (2000) that *C. gariepinus*, among several other fish hosts, is the true intermediate host for at least three sebekiid species that harboured final stage larvae is confirmed by this record of Luus-Powell et al. (2008). More investigation is needed on this parasite as the role of the sharptooth catfish in transmission of any pentastomids is still unknown.

Present study: No representatives of *Sebekia* or any other pentastomid were found from *C. gariepinus* during the present study.

Concluding remarks

In contrast to the major taxonomic groups treated thus far, no records of unconfirmed parasitic Crustacea are reported from *C. gariepinus* in Africa. Further, it will be advantageous to enocurage a complimentary (morphological and molecular) approach in future reporting and descriptions of parasitic crustaceans from African freshwater fishes. This data will contribute to global molecular databases and assist in easy access to species data for comparative purposes as well as diversity studies, determining host ranges of parasitic species and their biogeography.

3.8. Subclass Hirudinea

The Hirudinea is placed within the Phylum Annelida and is divided into two orders; the Arhynchobdellida and Rhynchobdellida (Clitellata – segmented worms) based on the morphology of their proboscis and vascular system (see Hadfield and Smit 2018 and references therein).

These organisms are commonly referred to as leeches and possess a cylindrical body with posterior and anterior disc-shaped suckers. The bodies of leeches are divided into segments that do not correspond to the rings or annuli observed on their external body surface and are of taxonomic importance. Sensillae (sensory structures) can also be found in a single transverse row in each segment of the body. Leeches have no internal skeletons, are hermaphroditic, and exchange gasses through their skin. It has also become clear that leeches are important organisms in ecosystems as they serve as intermediate hosts for some digeneans and in other cases as vectors for blood parasites. Leeches can be classified as temporary ectoparasites as they leave their host, that include frogs, turtles, fishes and birds after a blood meal, and when present in large numbers on a single host may cause the death of the host (Baker 1960; Oosthuizen and Siddall 2003; Smit et al. 2020 and references therein).

3.8.1. Order Rhynchobdellida

Family Glossiphoniidae

Batracobdelloides Oosthuizen in Sawyer, 1986

A single leech species *Batracobdelloides tricarinata* (Blanchard, 1897) [(syns. *Batrachobdella amnicola* Moore, 1958; *Clepsine nilotica* Johansson, 1909; *Dundjibdella dartevellei* Sciacchitano, 1939; *Dundjibdella plurilineata* Sciacchitano, 1939; *Dundjibdella rubra* Sciacchitano, 1939; *Dundjibdella trilineata* Sciacchitano, 1939; *Dundjibdella triserialis* Sciacchitano, 1939;*Helobdella tricarinata* Blanchard, 1897)] is known as a parasite of cichlid, clariid and cyprinid hosts in Africa, while several reports exist where *B. tricarinata* is identified as a vector for *Trypanosoma* Gruby, 1843 (Kinetoplastea: Protozoa) in the blood of freshwater fishes (Smit et al. 2004, 2020). From available literature, and as highlighted in Oosthuizen and Siddall (2003), research on leeches has to date received very little attention in most regions of Africa. With regard to infestation of *C. gariepinus*, no single publication provides explicit detail on location and host records for leeches parasitising *C. gariepinus*. Oosthuizen (1989) redescribed *B. tricarinata* and provides ample evidence on the synonymy of *Helobdella tricarinata* and *Clepsine nilotica* with *B. tricarinata*. The author only mentions that *B. tricarinata* was found from *C. gariepinus* in South Africa and Namibia. Furthermore, Oosthuizen (1989) provided an in-depth synopsis on the distribution of *B. tricarinata* at the time and confirmed its status as a non-specific haematophagous ectoparasite of fishes.

Oosthuizen and Curtis (1990) published a checklist of freshwater leeches of Namibia, where *B. tricarinata* was included, however no hosts were mentioned. Appleton et al. (2003) provided insights on the possible location of sampling of the latter authors in the wetlands of the eastern Caprivi strip in Namibia, however no mention was made of hosts. In 1991, Oosthuizen published a checklist of hirudinids of the Kruger National Park in South Africa that was accompanied with a key to known species, however no specifics were provided on hosts. This lack of host information may be due to the sampling being done across a wide range of habitats and the contributions of several individuals to this collection (Oosthuizen 1991).

Several reports of *C. gariepinus* being parasitised by leeches can be found from African literature; from the Nwanedi-Luphephe dams, South Africa (reported as “unidentified leech”), while several authors report on “leeches” or “piscicolid leech” from Nigeria and Tanzania (Mwita and Nkwengulila 2004; Madanire-Moyo et al. 2010; Mwita 2014a, b; Enyidi and Uwanna 2019). Tachia et al. (2012) and Edward et al. (2020) both report on *Piscicola geometra* (Linnaeus, 1961) from *C. gariepinus* from ponds in the agricultural research farm and the Digil dam in Nigeria, however these records will need confirmation as no description of these specimens are provided by the respective authors. Hadfield and Smit (2018) provide a short synopsis on other leech species that may parasitise fishes, however, no specific information is provided on their hosts and localities and the authors excluded these from the confirmed list of hirundinid parasites of African fishes.

Despite the lack of host information and specific localities mentioned by Oosthuizen (1989), he did report on the distribution range of the individuals of *B. tricarinata* in Africa. Material studied during his redescription of *B. tricarinata* included specimens from Angola, the Democratic Republic of the Congo, Egypt, Ghana, Kenya, Lesotho, Namibia, South Africa, Sudan, Uganda and Zambia. The presence of *B. tricarinata* from habitats from these countries, along with the above non-specific reports of leeches from *C. gariepinus*, indicate that interesting finds on host associations await discovery in these regions.

Present study: No infestation with any type of leech was found on *C. gariepinus* collected during the present study.

Chapter 4

Novel distribution records and molecular data for species of *Macrogyrodactylus* Malmberg, 1957 (Monogenea: Gyrodactylidae) from *Clarias gariepinus* (Burchell) (Siluriformes: Clariidae) in southern Africa

Abstract

The viviparous gyrodactylid genus *Macrogyrodactylus* Malmberg, 1957 is endemic to Africa, composed of nine species from hosts of four freshwater fish families, including catfishes (Siluriformes: Clariidae). Three species, *Macrogyrodactylus clarii* Gussev, 1961; *M. congolensis* (Prudhoe, 1957) and *M. karibae* Douëllou et Chishawa, 1995, are primarily known to parasitise the African sharptooth catfish *Clarias gariepinus* (Burchell) in various African countries. From November 2017 to September 2019, a total of 184 individuals of *C. gariepinus* were collected from selected localities in southern Africa and their skin, fins and gills were surveyed for monogeneans. Three species of *Macrogyrodactylus* (*M. clarii*, *M. congolensis* and *M. karibae*) were found parasitising *C. gariepinus* from five localities in South Africa and Zambia. Overall prevalence was 50% to 100% with intensities of up to 109 parasites per host individual. New locality records in southern Africa, morphological observations and additional molecular data on the complete Internal Transcriber Spacer (ITS-1-5.8S-ITS-2) regions of the rDNA gene for the three gyrodactylid species are presented in this study.

Keywords: fish parasites, gyrodactylids, endemic species, ITS region, phylogeny

4.1. Introduction

Macrogyrodactylus Malmberg, 1957 (Gyrodactylidae) is one of the six endemic viviparous monogenean genera parasitising African freshwater fishes (Barson et al. 2010; Přikrylová et al. 2017; Řehulková et al. 2018). The genus comprises nine species parasitising hosts from the Anabantidae, Clariidae, Latidae and Polypteridae (**Table 4.1.**). In addition to the nine species, a study on macrogyrodactylids from *Clarias gariepinus* (Burchell) in Kenya and Zimbabwe revealed a species possessing morphological features of *Macrogyrodactylus clarii* Gussev, 1961 and

Macrogyrodactylus heterobranchii N'Douba et Lambert, 1999. Molecular characterisation of these specimens suggested a hybrid origin partially corresponding to the ribosomal 18S, Internal Transcribed Spacer (ITS), and mitochondrial (*cox*1) gene regions of *M. clarii* and *M. heterobranchii* (see Barson et al. 2010). The majority of the literature on the species of *Macrogyrodactylus* originate from the Nilo-Sudanian, Abyssian Highlands and Zambezi ichthyofaunal provinces where *C. gariepinus* is highly valued in aquaculture and as food. Interest on this parasitic group was initially driven by its potential to exponentially increase in infection, first noted on grey bichir, *Polypterus senegalus* Cuvier, in the Sudan infected with *Macrogyrodactylus polypteri* Malmberg, 1957, and on *C. gariepinus* and *Heterobranchus longifilis* Valenciennes reared in earthen ponds in Nigeria. These infections were characterised by increased mucous secretion, skin damage resulting in the formation of white patches on the skin, increased turbidity of the water, and in some cases death of the host (Amirthalingam 1965; Khalil 1964, 1970; Saoud and Mageed 1969; Obiekezie and Ajah 1994). Later in southern Africa, similar infections on the clariid host *C. gariepinus* were observed in natural conditions and greyish-white patches were noted on the skin of the infected hosts (Khalil and Mashego 1998). Despite the small number of species in the genus, ample research has been published on the occurrence, morphology and ultrastructure, microhabitat and infection dynamics of some species in natural systems, earthen ponds and in aquaculture conditions (El-Naggar and Serag 1987; Cable et al. 1996; Khalil and Mashego 1998; Arafa 2000; El-Naggar et al. 2001a, b; 2004a, 2019; El-Naggar and Cable 2007; Madanire-Moyo et al. 2010; Arafa et al. 2012, 2013).

Increasing interest in the endemic species of gyrodactylids is the outcome of potential undiscovered high diversity, including morphological and molecular features that are suitable for advanced phylogenetic, evolutionary and parasite-host relationship studies (Bakke et al. 2002; Cribb et al. 2002; Poulin 2002; Boeger et al. 2003; Barson et al. 2010; Přikrylová et al. 2013). Although host specificity for all known species of *Macrogyrodactylus* has not been determined, specificity of *M. polypteri* towards bichirs has been confirmed, as only *Polypterus bichir* Lacépède and *P. senegalus* have been reported as hosts (Khalil 1964), and an affinity towards clariid hosts seems evident for three species of *Macrogyrodactylus*. Additionally, Barson et al. (2008) noted that *M. clarii*, *Macrogyrodactylus congolensis* (Prudhoe, 1957) and *Macrogyrodactylus karibae* Douëllou et Chishawa, 1995 exhibit a certain degree of specificity towards *C. gariepinus*.

In southern Africa, studies on macrogyrodactylids were initiated during the 1990's. Khalil and Mashego (1998) documented taxonomically significant features for the genus and provided a measurement scheme highlighting the sclerotised haptoral structures and cirrus spines. It is only in the last two decades that studies on the systematics of *Macrogyrodactylus* and other genera of endemic gyrodactylid species in Africa emerged (Matějusová et al. 2003; Barson et al. 2008;

2010; Přikrylová and Gelnar 2008; Vanhove et al. 2011, 2018; Přikrylová et al. 2013). The aforementioned authors emphasise the importance of studies of the morphological characters, distribution and genetics of these parasites. This study presents novel distribution records for *M. clarii*, *M. congolensis* and *M. karibae* from *C. gariepinus* in southern Africa, as well as additional morphological observations and molecular data (ITS-1-5.8S-ITS-2 regions of the rDNA gene).

Table 4.1. Species of *Macrogyrodactylus* Malmberg, 1957 from Africa with their host range.

	Type host	Type locality	Other hosts	Reference
Macrogyrodactylus clarii Gussev, 1961	*Clarias* sp.	Ghana	*C. anguillaris* (Linnaeus) *C. gariepinus*	Gussev (1961), Paperna (1969), Shotter (1980), El-Naggar and Serag (1987)
M. congolensis (Prudhoe, 1957)	*Clarias gariepinus* (Burchell)	Democratic Republic of the Congo	*C. anguillaris*	Prudhoe (1957), Thurston (1970), Paperna (1979), Přikrylová and Gelnar (2008)
M. heterobranchii N'Douba et Lambert, 1999	*Heterobranchus longifilis* Valenciennes	Senegal	*C. anguillaris*	N'Douba and Lambert (1999), Přikrylová and Gelnar (2008)
M. karibae Douëllou et Chishawa, 1995	*C. gariepinus*	Zimbabwe	-	Douëllou and Chishawa (1995), Khalil and Mashego (1998)
M. polypteri Malmberg, 1957	*Polypterus senegalus* Cuvier	Gambia	*P. bichir* Lacépède	Malmberg (1957)
M. simentiensis Přikrylová et Gelnar, 2008	*P. senegalus*	Senegal	-	Přikrylová and Gelnar (2008)
Species inquirenda				
M. anabantii Paperna, 1973	*Ctenopoma muriei* Boulenger	Uganda	-	Paperna (1973)
M. ctenopomii Paperna, 1973	*C. muriei* (Boulenger)	Uganda	-	Paperna (1973)
M. latesi Paperna, 1969	*Lates niloticus* (Linnaeus)	Ghana	-	Paperna (1969)

4.2. Materials and methods

Collection of hosts and parasites

A total of 184 individuals of *Clarias gariepinus* were collected from November 2017 to September 2019 at various localities in southern Africa (see **Table 4.2. and Fig. 4.1. A**, **B**). Individual hosts were euthanised by percussive stunning and severance of the spinal cord (ethics Nos. NWU-00156-18-A5, NWU-00264-15-A5) and subjected to a full parasitological screening for metazoan parasites (see detailed procedures in Řehulková 2018). Herein, we report only on the infection with species of *Macrogyrodactylus* found on the skin and gills of the host. Whole specimens of *Macrogyrodactylus* spp. from parasitised fish were removed from the skin or gills using fine dissecting needles and cleared on a microscope slide in glycerine-ammonium picrate (GAP – Malmberg 1970) for later morphological identification, which were subsequently remounted in Canada Balsam, following the procedure described in Ergens (1969). For molecular characterisation, specimens were fixed in 96% molecular-grade ethanol and processed as described below. The taxonomical relevant sclerites of individuals were measured using a Nikon Eclipse Ni compound microscope (Nikon Instruments, Tokyo, Japan) with DIC optics. Measurements were taken using NIS-Elements BR 4.60© software and line drawings of sclerotised structures were made with the aid of a camera lucida attached to the microscope and digitalised using a Wacom Intuos® Pro drawing tablet (Wacom, Saitana, Japan) and Adobe Illustrator™ software package. Measurements of sclerotised structures were performed ac cording to Khalil and Mashego (1998), N'Douba and Lambert (1999), and Přikrylová and Gelnar (2008). Morphometric data are presented as mean followed by range in parentheses. All measurements are in micrometres unless otherwise indicated. Whole-mounted voucher specimens were deposited in the parasite collection of the National Museum, Bloemfontein (NMB), South Africa. Ecological descriptors for the monogenean parasites (prevalence and mean intensity) followed Bush et al. (1997).

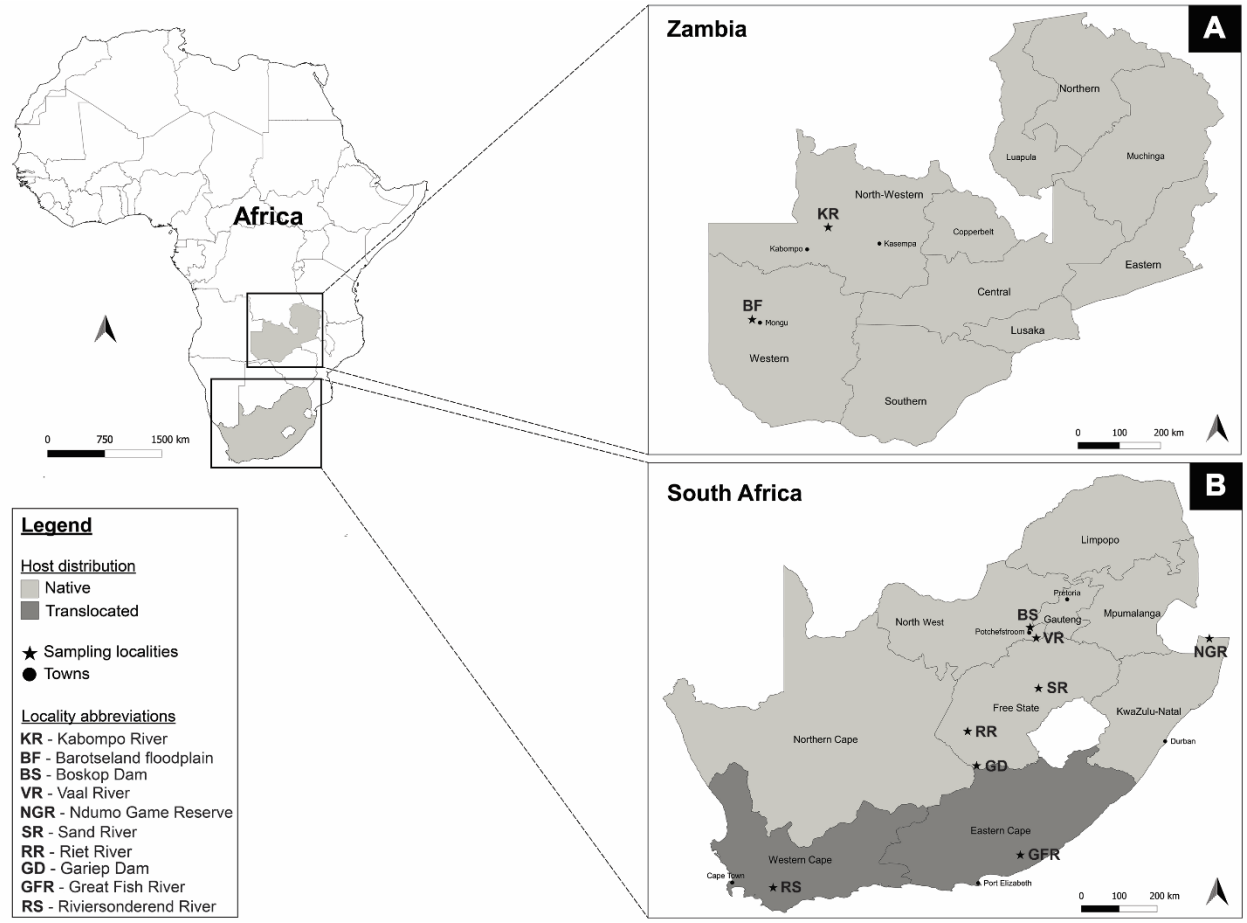

Figure 4.1. Map illustrating the sampling localities of *Clarias gariepinus* (Burchell) during the present study. **A** – Zambia; **B** – South Africa.

Table 4.2. Localities, number (N) of host individuals screened, host status and whether infected with *Macrogyrodactylus* spp. or not of 184 *Clarias gariepinus* (Burchell) collected from November 2017 to September 2019 in southern Africa.

Locality	Province	Host introduction status	Coordinates	N	Infected by one or more *Macrogyrodactylus* spp.
South Africa					
Riviersonderend River	Western Cape	Translocated	34.0998S, 19.6656E	15	No
Great Fish River	Eastern Cape	Translocated	33.1543S, 26.8320E	11	No
Gariep Dam (Orange River)	Free State	Native	30.5682S, 25.5647E	15	No
Riet River	Free State	Native	29.5700S, 25.3042E	12	No
Sand River	Free State	Native	28.3232S, 27.3597E	15	No
Vaal River	North West	Native	26.8665S, 27.2944E	18	Yes
Boskop Dam (Mooi River)	North West	Native	26.5621S, 27.1181E	15	Yes
Ndumo Game Reserve	KwaZulu-Natal				
KuShokwe Pan		Native	26.8717S, 32.2141E	15	Yes
Nyamithi Pan		Native	26.8829S, 32.3113E	15	Yes
Phongolo River		Native	26.9297S, 32.3238E	4	No
Usuthu River		Native	26.8824S, 32.3116E	3	Yes
Zambia					
Kabompo River	Northwestern	Native	13.1025S, 24.6920E	2	Yes
Barotse floodplain	Western	Native	15.1960S, 22.9647E	17	Yes

DNA extraction, PCR amplification and sequence analyses

The specimens used for molecular analyses had the haptor mounted on slides with GAP to confirm morphological identity (see Řehulková 2018) and deposited as e-vouchers and molecular vouchers (hologenophore, see Pleijel et al. 2008 for terminology). Thereafter, the anterior section of the parasite was used for DNA extraction using PCRBIO Rapid Extraction© (ThermoFischer Scientific, Waltham, Massachusetts, USA) kit following the manufacturer's protocol. DNA amplifications were performed on the Internal Transcriber Spacer ITS1-5.8S-ITS2 regions of the rRNA gene using the primers ITS1A (5'-GTAACAAGGTTTCCGTAGGTG-3') and ITS2 (5'-TCCTCCGCTTAGTGATA-3') (Matějusová et al. 2001). Each amplification reaction was performed with a total volume of 25 µl: 12.5 µl of DreamTaq PCR Master Mix (2X) (ThermoFischer Scientific, Waltham, Massachusetts, USA), 1 µl of 10 µM of each primer, 1 µl of DNA product and 9.5 µl of double distilled water. Thermocycling conditions followed Barson et al. (2010). PCR products were visually inspected on a 1% agarose gel electrophoresis and sent to Inqaba Biotechnical Industries (Pty) Ltd. (Pretoria, South Africa) for purification and sequencing. Basic Local Alignment Search Tool (BLAST) (https://blast.ncbi.nlm.nih.gov/) was used to compare and identify species closely related to the new sequences.

Seven newly generated sequences of *Macrogyrodactylus* spp. were aligned with those of ten other congeneric species available in GenBank, and one sequence of *Laminiscus gussevi* (Bychowsky et Polyansky, 1953) used as outgroup (see **Table 4.3.** for detailed information on the sequences). The alignment was generated in Geneious v. 7.1.3 using implemented default parameters of MUSCLE (Kearse et al. 2012). The alignment was trimmed, resulting in the length of 810 bp. Evolutionary model selection was performed in MEGA 7 (Kumar et al. 2016). Phylogenetic analyses were run under Maximum Likelihood (ML) and Bayesian Inference (BI) criteria, applying the model of nucleotide evolution GTR + G. ML trees were generated in RAxML (Guindon and Gascuel 2003). The model parameters and bootstrap support values (1,000 repetitions) were estimated using RAxML. BI trees were generated in MrBayes (Ronquist et al. 2012), running two independent MCMC runs of four chains for 10 million generations and sampling tree topologies every 1,000 generations. Burn-in periods were set to the first 25,000 generations. ML and BI analyses were performed on the computational resource CIPRES (Miller et al. 2010). Genetic divergences were calculated using uncorrected p-distances model in MEGA 7 and are expressed as percentage (p-distances) and differences in number of nucleotides. Trees were visualised in FigTree v1.4.4 (Rambaut 2009).

Table 4.3. Information on the sequences of *Macrogyrodactylus* spp. (Monogenea: Gyrodactylidae) used in the phylogenetic analyses of the ITS1-5.8S-ITS2 region of the rRNA gene. *Laminiscus gussevi* (Bychowsky et Polyansky, 1953) was used as outgroup. Species sequenced in this study are in bold.

Species	Host	Locality	GenBank ID	Reference
***Macrogyrodactylus clarii* (Isolate 1)**	***Clarias gariepinus***	**Barotse floodplain, Zambia**	**MZ869846**	**Present study**
***M. clarii* (Isolate 2)**	***Clarias gariepinus***	**Barotse floodplain, Zambia**	**MZ869850**	**Present study**
***M. clarii* (Isolate 3)**	***Clarias gariepinus***	**Barotse floodplain, Zambia**	**MZ869847**	**Present study**
***M. clarii* (Isolate 1)**	***Clarias gariepinus***	**Phongolo River floodplain, SA**	**MZ869849**	**Present study**
M. clarii	*Clarias gariepinus*	Save River, Zimbabwe	GU252711	Barson et al. (2010)
M. clarii × *heterobranchii*	*Clarias gariepinus*	Save River, Zimbabwe	GU252712	Barson et al. (2010)
M. clarii × *heterobranchii*	*Clarias gariepinus*	Lake Turkana, Kenya	GU252713	Barson et al. (2010)
M. clarii × *heterobranchii*	*Heterobranchus bidorsalis*	Lake Turkana, Kenya	HF548683	Přikrylová et al. (2013)
Macrogyrodactylus heterobranchii	*Clarias anguillaris*	Gambia River, Senegal	GU252714	Barson et al. (2010)
Macrogyrodactylus congolensis	*Clarias anguillaris*	Lake Turkana, Kenya	GU252716	Barson et al. (2010)
M. congolensis	*Clarias anguillaris*	Mare Simenti, Senegal	GU252717	Barson et al. (2010)
M. congolensis	***Clarias gariepinus***	**Vaal River, SA**	**MZ869848**	**Present study**
Macrogyrodactylus karibae	*Clarias gariepinus*	Save River, Zimbabwe	GU252715	Barson et al. (2010)
M. karibae	***Clarias gariepinus***	**Barotse floodplain, Zambia**	**MZ869845**	**Present study**
M. karibae	***Clarias gariepinus***	**Phongolo River floodplain, SA**	**MZ869851**	**Present study**
Macrogyrodactylus polypteri	*Polypterus senegalus*	–	AJ567672	Matějusová et al. (2003)
Macrogyrodactylus simentiensis	*Polypterus senegalus*	Mare Simenti, Senegal	HF548681	Přikrylová et al. (2013)
Laminiscus gussevi	*Mallotus villosus*	Iceland	HF548678	Přikrylová et al. (2013)

Abbreviations: SA – South Africa.

4.3. Results

In the present study, *Macrogyrodactylus clarii*, *M. congolensis* and *M. karibae* were found on the skin and gills of *Clarias gariepinus* from five localities in southern Africa (**Table 4.2.**). Infection of *C. gariepinus* with species of *Macrogyrodactylus* are recorded for the first time from the Vaal River (North West Province) and Ndumo Game Reserve (NGR) (KwaZulu-Natal Province), in South Africa (SA), and the Kabompo and Zambezi (Barotse floodplain) rivers in Zambia (ZM). The southernmost distribution records for *M. clarii* and *M. karibae* are recorded from NGR and for *M. congolensis* from the Vaal River (SA). All three species were present from sites within the NGR and Barotse floodplain, whereas no other species co-occurred with *M. congolensis* in the Vaal and Kabompo rivers. *Macrogyrodactylus congolensis* was the most widely distributed species occurring at four of the five localities, followed by *M. karibae* (three localities), and *M. clarii* (two localities). The least prevalent and abundant species was *M. karibae*, except in NGR where it was found to co-occur with *M. congolensis*. *Macrogyrodactylus clarii* was the most prevalent and abundant species even when in co-occurrence with the other two species found during this study in NGR and the Barotse floodplain. The highest intensity of infection was recorded from the Vaal River, with 109 individuals of *M. congolensis* found on a single host, and the same intensity of infection for an individual host was noted from the Barotse floodplain. However, no greyish-white mucous covering of the skin was observed. In the present study, the general morphology and morphometrics for *M. clarii*, *M. congolensis* and *M. karibae* corresponded with those from original descriptions and redescriptions for each species.

Gyrodactylidae Cobbold, 1864

Macrogyrodactylus Malmberg, 1957

Macrogyrodactylus clarii Gussev, 1961

Type host: *Clarias* sp. (Siluriformes: Clariidae).

Type locality: Lake Awasa, Ethiopia.

Other records: *Clarias gariepinus*: Egypt – El-Naggar and Serag (1987), El-Naggar (1993), Hagras et al. (1995, 1999),El-Naggar et al. (1997, 2001a, b, 2004a, b, 2016, 2017, 2019, 2020), El-Naggar and Cable (2007), Arafa et al. (2009, 2012), Radwan et al. (2009), El-Seify et al. (2011), Mashalay et al. (2019); Ethiopia – Gussev (1961), Beletew et al. (2016); Ghana – Paperna (1969, 1979); Kenya – Barson et al. (2010, 2014); Nigeria – Obiekezie and Ajah (1994), Eyo et al. (2015); South Africa – Khalil and Mashego (1998), Olivier et al. (2009), Madanire-Moyo et al. (2010, 2012), Matla (2012), Mahlatji (2014); Uganda – Paperna (1979); Zimbabwe – Barson et al. (2008,

2010), Madanire-Moyo and Barson (2010). *Clarias anguillaris* (Linnaeus); Nigeria – Shotter (1980).

Localities (present study): South Africa: KuShokwe Pan, Ndumo Game Reserve, KwaZulu-Natal Province; Zambia: Barotse floodplain, Zambezi River, Northwestern Province.

Site of infection: Skin and gills.

Voucher material: Twenty-three voucher specimens deposited in NMB (Coll. Nos. P 800–809, 820–822).

Representative DNA sequences: 710–753 bp long sequences of the ITS1-5.8S-ITS2 region of the rRNA gene of four isolates (GenBank accession numbers: MZ869846, MZ869850, MZ869847, MZ869849). Hologenophores: NMB (P 824–827).

Specimens studied: n = 48 (KuShokwe Pan, South Africa - SA); n = 25 (Barotse floodplain, Zambia - ZM).

Infection rate: KuShokwe Pan (SA): Prevalence (P) of 80% and intensity of infection (IF) 1–12; Barotse floodplain (ZM) P = 64%, IF = 1–4.

Morphological observations (measurements provided in **Table 4.4.**): Morphology of haptoral sclerites conform to description of Gussev (1961) and redescription of El-Naggar and Serag (1987). Individuals armed with 16 marginal hooks, two positioned in anterolateral lobes, 14 in posterior edge of haptor. Basal part of sickle has small indentation near anterior end of base, anterior end of sickle base thin, sharply rounded. Base of sickle rounded with slight curvature on distal side into slender sickle blade curving inward and tapering into a fine, sharp point that stops in line with anterior end of sickle base (**see Figs. 4.2. B, Fig. 4.3. D(i)**). Hamulus complex consisting of a pair of robust hamuli with curvature into point, sharp inward curved root, small dorsal bar consisting of two articulating sclerites, Y-shaped ventral bar with short anterolateral processes and long posterior central arm. Two narrow rod-like sclerites (R1 and R2) accompany ventral bar (**Figs. 4.2. A**–**C** and **Fig. 4.3.A**). Cirrus observed in 24 (SA) and 11 (ZM) specimens studied, each armed with 13–15 small spines and one large spine (**Fig. 4.2 C**).

Remarks. The dimensions of the hamulus (total length and length of the shaft, root and point) from specimens studied from NGR (SA) and Barotse floodplain (ZM) (**Table 4.4**.) are similar to those of specimens from the Middle Letaba Dam (SA) and localities in Kenya and Egypt (El-Naggar and Serag 1987; Khalil and Mashego 1998; Barson et al. 2010). Dimensions obtained for the dorsal bar in the present study do not deviate from the ranges found in other studies. Marginal hook size also conforms to previously recorded measurements. The marginal hook sickle morphology is identical to that illustrated for *M. clarii* specimens collected from Zimbabwe and *M. clarii* ×

Macrogyrodactylus heterobranchii from Kenya (see Barson et al. 2010). Gussev (1961) and El-Naggar and Serag (1987) reported 16 and 12 cirrus spines, with one large spine in their descriptions, respectively. Additionally, this study provides details on the morphology of the sickle, which was not described by Gussev (1961) and El-Naggar and Serag (1987). Comparative measurements are presented in **Table 4.4**.

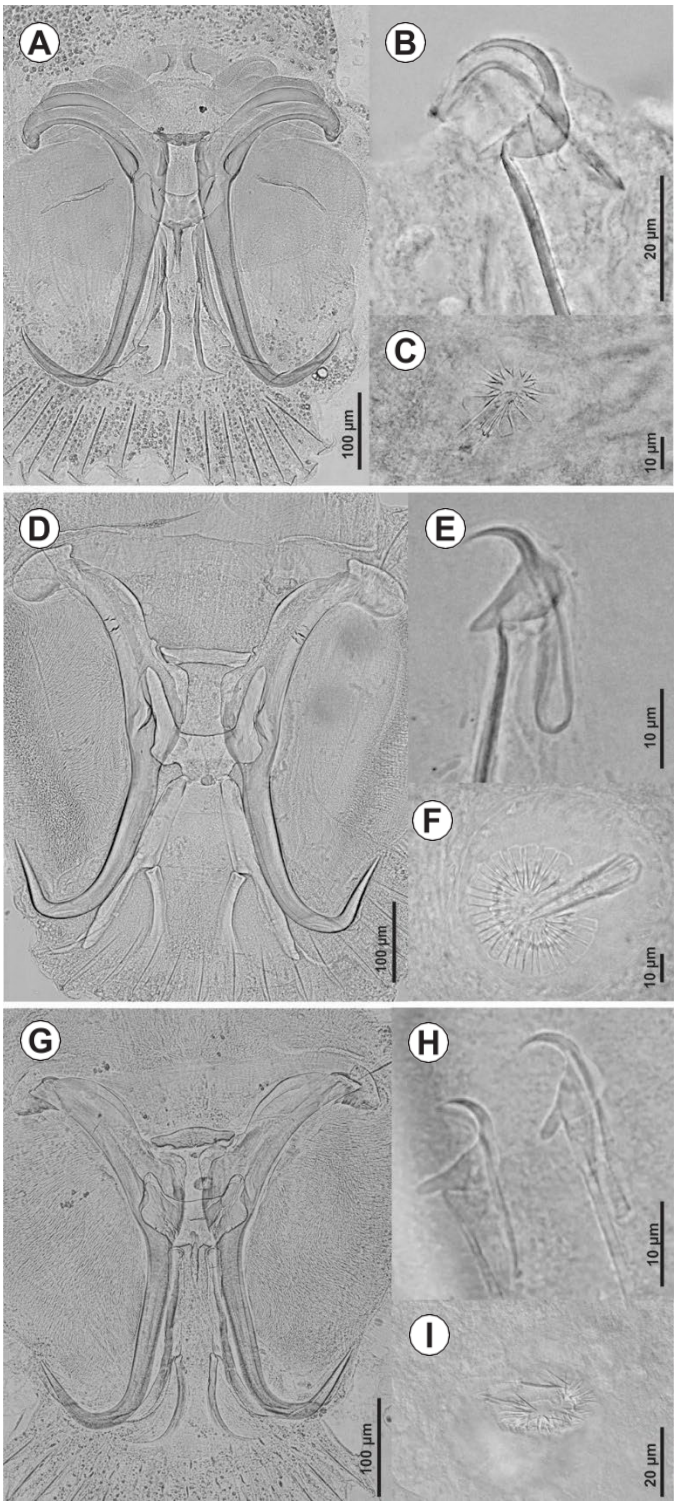

Figure 4.2. Micrographs showing the morphological features of *Macrogyrodactylus clarii* Gussev, 1961 (A–C), *Macrogyrodactylus. congolensis* (Prudhoe, 1957) (D–F), and *Macrogyrodactylus karibae* Douëllou et Chishawa, 1995 (G, H). **A**, **D**, **G** – hamuli complex; **B**, **E**, **H** – sickle of marginal hook; **C**, **F**, **I** – cirrus with spines.

Table 4.4. Morphometric comparison of *Macrogyrodactylus clarii* Gussev, 1961 parasitising *Clarias gariepinus* (Burchell) in Africa. Measurements are presented in micrometres as mean followed by range in parentheses. n = number of specimens studied.

	KuShokwe Pan	Barotseland Floodplain	Lake Turkana & Lake Chivero	Nile Delta	Middle Letaba Dam
	SA	ZM	KY / ZI	EG	SA
	n = 48	n = 25	n = 10	n = 10	–
	Present study	Present study	Barson et al. 2010	El-Naggar and Serag 1987	Khalil and Mashego 1998
Body					
Length	3389 (2190–5014)	3173 (2237–4351)	–	2330 (2000–2580)	1800–2600
Width	518 (221–788)	382 (278– 584)	–	400 (360–440)	320–440
Haptor					
Length	839 (544–1377)	680 (465–846)	–	460 (400–500)	–
Width	600 (420–1053)	352 (306–583)	–	440 (400–480)	–
Hamuli					
Total length	370 (193–465)	426 (378–497)	436 (374–475)	385 (376–392)	437–453
Point length	113 (98–134)	153 (110–143)	120 (108–136)	120 (112–128)	131–140
Shaft length	334 (281–411)	376 (340–440)	380 (350–457)	–	381–406
Root length	171 (139–218)	197 (156–222)	191 (135–238)	171 (176–184)	193–203
Ventral bar					
Anterior lateral arm	28 (17–38)	33 (23–43)	29 (17–38)	–	21–31
Posterior central arm	63 (44–80)	74 (59–90)	69 (61–85)	–	46–55
Total length	135 (110–166)	152 (122–175)	148 (137–172)	110 (104–116)	–
Total width	111 (89–135)	112 (106–133)	138 (122–157)	106 (96–112)	–
Ventral bar R1	250 (192–301)	272 (232–304)	191 (177–207)	–	–
Ventral bar R2	137 (111–185)	152 (129–170)	147 (141–157)	–	–
Dorsal bar	Divided	Divided	Divided	Divided	Divided
Total length	18 (12–23)	18 (14–22)	16.5 (13.5–20)	–	15–18
Total width	77 (61–93)	78 (68–92)	82.3 (72.5–92)	–	68–75
Marginal hooks					
Total length	110 (92–128)	117 (100–130)	114 (103–132)	96 (91–101)	109–125
Handle length	92 (72–108)	100 (84–112)	96 (87.5–112)	82	–
Sickle length	17 (13–21)	18 (15–20)	18.5 (17–21)	14	–
Sickle proximal width	12 (8–14)	13 (9–16)	13.8 (13–17)	–	–
Cirrus spines	13–15	13–15	–	12	12–13

Macrogyrodactylus congolensis (Prudhoe, 1957) Yamaguti, 1963

Synonym: *Neogyrodactylus congolensis* Prudhoe, 1957

Type host: *Clarias gariepinus* (Burchell) (Siluriformes: Clariidae).

Type locality: Lake Upemba (Lualaba River), Democratic Republic of the Congo.

Other records: *Clarias gariepinus*: Egypt – El-Naggar et al. (1999, 2001a, b, 2004b, 2007, 2017, 2019), Hagras et al. (1999), Arafa (2000, 2011), Arafa et al. (2003, 2013, 2014), Mashalay et al. (2019); Democratic Republic of the Congo – Prudhoe (1957), Vanhove et al. (2018); Kenya – Barson et al. (2010); South Africa – Khalil and Mashego (1998), Madanire-Moyo et al. (2010, 2012); Uganda – Thurston (1970), Akoll et al. (2012). *Clarias anguillaris*: Senegal – Přikrylová and Gelnar (2008), Barson et al. (2010).

Localities (present study): South Africa: Vaal River, North West Province and Usuthu River, Ndumo Game Reserve (NGR), KwaZulu-Natal Province; Zambia: Barotse floodplain, Zambezi River, Northwestern Province, Kabompo River, Northern Province.

Site of infection: Skin and gills.

Voucher material: Nineteen voucher specimens deposited in, NMB P 812–817, 823.

Representative DNA sequence: 696 bp long sequence of the ITS1-5.8S-ITS2 region of the rRNA gene of one isolate. GenBank accession number: MZ869848. Hologenophore, NMB P 828.

Specimens studied: n = 25 (Vaal River, SA), n = 2 (Usuthu River, SA), n = 1 (Barotse floodplain, ZM), n = 1 (Kabompo River, ZM).

Infection rate: Usuthu River (SA): P = 3%, IF = 1; Vaal River (SA): P = 33%, IF = 1–109; Barotse floodplain (ZM): P = 6%, IF = 1; Kabompo River (ZM): P = 50%, IF = 1.

Morphological observations (measurements provided in **Table 4.5.**): Morphology of haptoral sclerites similar to illustrations provided in description of Prudhoe (1957). Hamulus complex of *M. congolensis* large in size, has slender hamuli less sharply curved into point and root, armed with 16 marginal hooks. Marginal hook sickle robust, with noticeable curvature in base near anterior end of sickle. Anterior end of sickle base rounded and convex, with indentation on distal side as it joins sickle blade that is short, curved inward tapering into a fine point and stops before the proximal end of sickle base (**Fig. 4.2. E**, **Fig. 4.3. D(ii)**). Hamuli complex has small uniform dorsal bar, wider than long, Y-shaped ventral bar with long anterolateral processes, and short posterior central arm. Two narrow rod-like sclerites accompany ventral bar (**Fig. 4.2. D**–**F, Fig. 4.3. B**). Cirrus observed in one specimen from the Usuthu River (SA) and Barotse floodplain (ZM), respectively, and 17 individuals from the Vaal River (SA), each armed with 18–20 small spines and one large spine (**Fig. 4.2. F**).

Remarks. The dimensions of the hamulus (total length and the length of the shaft, root and point) from specimens studied from the Vaal and Usuthu rivers (SA) and the Kabompo River and Barotse floodplain (ZM) are similar in size to specimens from the Middle Letaba Dam and Lake Turkana in Kenya (Khalil and Mashego 1998; Barson et al. 2010). *Macrogyrodactylus congolensis* specimens from Senegal seems to have smaller hamuli compared to the present and other studies (see Přikrylová and Gelnar 2008). Dimensions obtained for the undivided dorsal bar in the present study do not deviate from the ranges found in other studies. Marginal hook measurements are also in accordance with previously recorded measurements (see **Table 4.5.**). The morphology of the marginal hook sickle conforms to those illustrated for *M. congolensis* from Kenya (see Barson et al. 2010). Prudhoe (1957) reported fewer cirrus spines (15), with one large spine in his description of *M. congolensis*. Details on the morphology of the marginal hooks were not provided in the description of Prudhoe (1957), which are added in the present study. Comparative measurements are presented in **Table 4.5.**

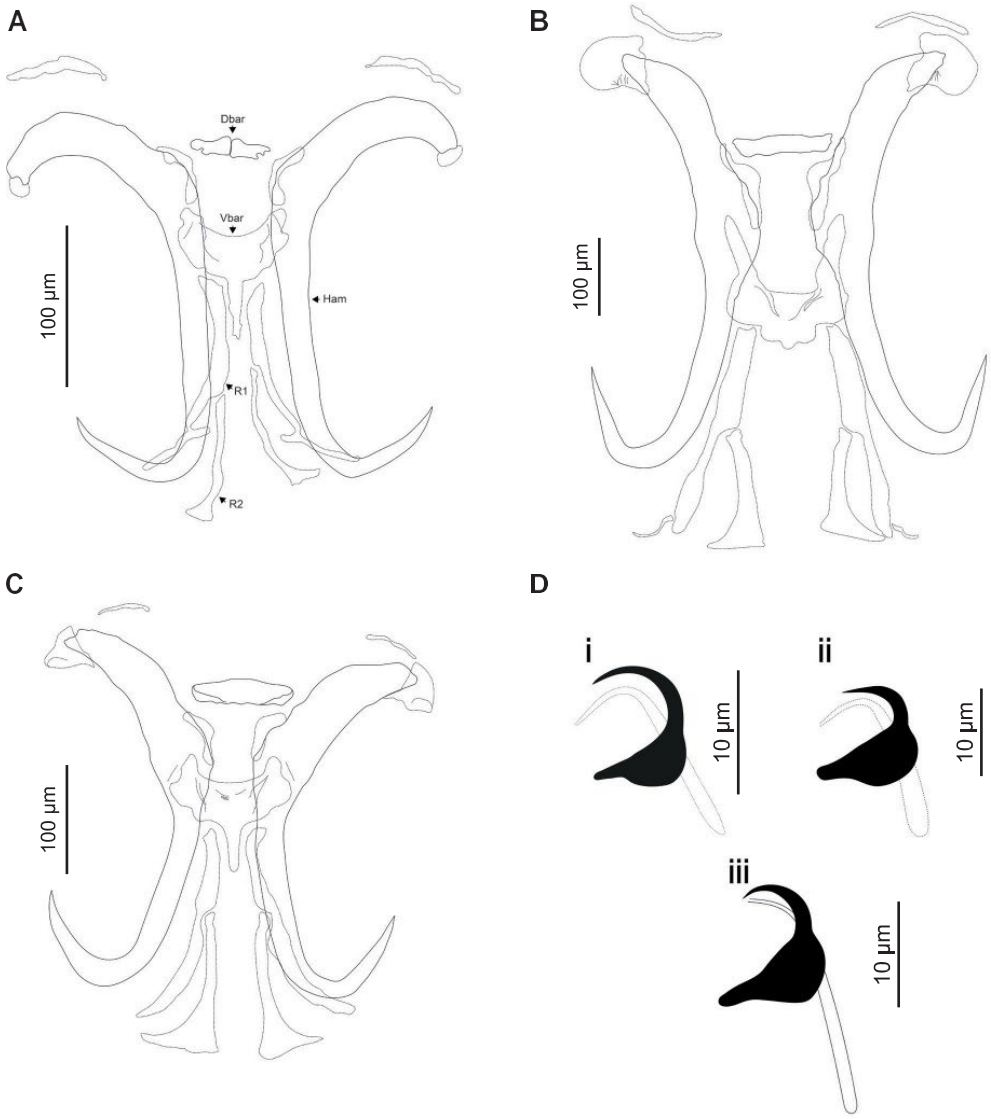

Figure 4.3. Illustrations of the hamuli of **A** – *Macrogyrodactylus clarii* Gussev, 1961; **B** – *M. congolensis* (Prudhoe, 1957); **C** – *M. karibae* Douëllou et Chishawa, 1995. **D** – sickle of marginal hooks of *M. clarii* (i), *M. congolensis* (ii), and *M. karibae* (iii). Abbreviations: Ham – hamuli, Vbar – ventral bar, Dbar – dorsal bar, R1 and R2 – ventral bar rod-like sclerites.

Table 4.5. Morphometric comparison of *Macrogyrodactylus congolensis* (Prudhoe, 1957) parasitising *Clarias gariepinus* (Burchell) in Africa. Measurements are presented in micrometres as mean followed by range in parentheses. n = number of specimens studied.

	Vaal River SA n = 25 Present study	Usuthu River SA n = 2 Present study	Kabompo River & Barotseland Floodplain ZM n = 2 Present study	Lake Turkana KY n = 7 Barson et al. 2010	Mare Simenti SN n = 3 Přikrylová and Gelnar 2008	Middle Letaba Dam SA n = Khalil and Mashego 1998
Body						
Length	3736 (2917–5998)	4751 (4569–4933)	3552 (2476–4629)	–	–	1700–2800
Width	464 (230–745)	643 (412–873)	374 (283–466)	–	–	220–580
Haptor						
Length	666 (513–827)	849 (798–901)	618 (508–728)	–	–	–
Width	552 (438–798)	760 (735–786)	546 (480–612)	–	–	–
Hamuli						
Total length	456 (390–537)	399 (391–407)	430 (419–440)	421 (385–481)	383 (381–385)	490–530
Point length	111 (94–131)	98 (95–102)	106 (105–107)	99 (93–103)	101 (99–103)	80–109
Shaft length	368 (310–431)	325 (318–332)	339 (339)	334 (310–361)	306 (299–313)	296–312
Root length	203 (166–272)	178 (177–179)	175 (165–186)	175 (153–203)	154 (145–165)	251–287
Ventral bar						
Anterior lateral arm	93 (84–101)	85 (84–85)	85 (82–88)	90 (81.5–118)	65 (58–71)	84–93
Posterior central arm	9 (5–13)	6 (5–7)	8 (7–10)	7 (5.5–8.5)	9 (8–10)	12–15
Total length	156 (134–170)	147 (146–148)	147 (145–150)	143 (131–150)	129 (124–134)	140–162
Total width	133 (110–153)	129 (126–133)	124 (122–125)	124 (118–129.5)	112 (109–114)	120–134
Ventral bar R1	254 (222–306)	239 (230–248)	238 (237–239)	236 (217–254)	217 (211–224)	–
Ventral bar R2	141 (118–179)	136 (134–137)	129 (118–140)	132 (126–141)	127 (120–137)	–
Dorsal bar	Undivided	Undivided	Undivided	Undivided	Undivided	Undivided
Total length	22 (16–25)	20	20 (19–22)	17.5 (15.5–19.5)	14 (13–15)	15–18
Total width	115 (97–140)	103	101	97.6 (93–102.5)	90 (88–94)	109–125
Marginal hooks						
Total length	104 (96–124)	95 (91–100)	96 (91–101)	96.6 (94.5–100.5)	95	81–87
Handle length	92 (83–114)	84 (80–87)	83 (77–89)	85.1 (83.5–89)	82.7 (78–86)	–
Sickle length	12 (10–14)	11	5 (11)	11.4 (10.8–11.7)	10.7 (10–12)	–
Sickle proximal width	11 (8–14)	9 (8–9)	5 (10–10)	11.2 (10.3–11.8)	10.3 (9–11)	–
Cirrus spines	18–20	–	19	–	–	14–15

Macrogyrodactylus karibae Douëllou et Chishawa, 1995

Type host: *Clarias gariepinus* (Burchell) (Siluriformes: Clariidae).

Type locality: Lake Kariba, Zimbabwe.

Other records: *Clarias gariepinus*. South Africa – Khalil and Mashego (1998), Olivier et al. (2009), Matla (2012); Zimbabwe – Douëllou and Chishawa (1995), Barson et al. (2008, 2010).

Localities (present study): South Africa: KuShokwe Pan and Usuthu River, Ndumo Game Reserve, KwaZulu-Natal Province; Zambia: Barotse floodplain, Zambezi River, Northwestern Province.

Site of infection: Skin and gills.

Voucher material: Four voucher specimens deposited in, NMB P 818–821.

Representative DNA sequences: 715–741 bp long sequence of the ITS1-5.8S-ITS2 region of the rRNA gene of two isolates. GenBank accession numbers: MZ869845, MZ869851. Hologenophore, NMB P 829.

Specimens studied: n = 8 (KuShokwe Pan, SA), n = 1 (Usuthu River, SA), n = 2 (Barotse floodplain, ZM).

Infection rate: KuShokwe Pan (SA): P = 20%, IF = 1–2; Usuthu River (SA): P = 66%, IF = 1; Barotse floodplain (ZM) P = 6%, IF = 1.

Morphological observations (measurements provided in **Table 4.6.**): General morphology of the haptoral sclerites is similar to the description of Douëllou and Chishawa (1995). Marginal hook sickle blade slender and inwardly curved point, distal side slightly convex as it joins sickle blade. Base of sickle presents noticeable curvature toward anterior end of sickle, has a convex distal side with an indentation as it joins sickle blade that sharply curve inward, tapering into a sharp point that stops before proximal side of sickle base; proximal side of sickle base curved. Cirrus observed in two specimens from KuShokwe Pan (SA) and Barotse floodplain (ZM), respectively, armed with 15 small spines and one large spine (**Fig. 4.2. I**).

Remarks. *Macrogyrodactylus karibae* can be easily distinguished from *M. congolensis* in having a ventral bar with short anterolateral processes and a short central arm. It can also be distinguished from *M. clarii* in having slender hamuli that is sharply curved into the point and root and in having a single uniform dorsal bar, wider than long (see **Fig. 4.2. G–H**, **Fig. 4.3. C**). Additionally, *M. karibae* presents a curved proximal side of sickle base, opposed to the absence of a curved appearance in *M. clarii* and *M. congolensis* (**Fig. 4.2. H** and **Fig. 4.3. D(iii)**). Dimensions for the hamuli (total length, point, shaft and root length) for specimens in the present study from NGR (SA) and Barotse floodplain (ZM) are within the ranges reported for the same

species from other localities in southern Africa (**Table 4.6.**) (Douëllou and Chishawa 1995; Khalil and Mashego 1998, Barson et al. 2010). The size of the dorsal and ventral bars is within the size ranges previously reported. The marginal hook size and morphology are comparable to previously reported data, specimens from NGR seem to have a slightly larger marginal hook sickle than previously reported. In the description and re-evaluation of *M. karibae*, Douëllou and Chishawa (1995) and Khalil and Mashego (1998) did not provide details on the morphology of the marginal hooks, which are provided in the present study. Comparative measurements are presented in **Table 4.6.**

Table 4.6. Morphometric comparison of *Macrogyrodactylus karibae* Douëllou et Chishawa, 1995 parasitising *Clarias gariepinus* (Burchell) in Africa. Measurements are presented in micrometres as mean followed by range in parentheses. n = number of specimens studied.

	KuShowke Pan & Usuthu River	Barotseland Floodplain	Lake Chivero and Save River	Lake Kariba	Middle Letaba dam
	SA	ZM	ZI	ZI	SA
	n = 9	n = 2	n = 6	n = 14	–
	Present study	Present study	Barson et al. 2010	Douëllou and Chishawa 1995	Khalil and Mashego 1998
Body					
Length	3309 (1791–3920)	2559 (2463–2656)	–	2300 (1300–3660)	1200–3950
Width	643 (496–806)	339 (282–395)	–	530 (390–720)	390–680
Haptor					
Length	622 (418–794)	420 (415–424)	–	–	–
Width	545 (379–652)	389 (355–423)	–	–	–
Hamuli					
Total length	298 (267–343)	306 (305–307)	312 (292–330)	289 (252–314)	296–375
Point length	81 (73–87)	84	83 (74–90)	84 (69–101)	68–93
Shaft length	249 (222–278)	262 (261–262)	259 (245–273)	–	251–296
Root length	141 (122–164)	153 (149–158)	144 (114–160)	115 (106–128)	125–156
Ventral bar					
Anterior lateral arm	23 (18–26)	22 (20–25)	18 (16.5–20)	20 (16–27)	21–31
Posterior central arm	37 (26–42)	41 (40–43)	42 (37–47)	41 (34–49)	24–40
Total length	98 (86–108)	101 (99–103)	104 (94–113)	94 (73–114)	84–111
Total width	103 (96–107)	104 (103–105)	109 (99–120)	100 (93–110)	78–111
Ventral bar R1	202 (182–231)	191 (190–192)	182 (177–189)	–	–
Ventral bar R2	125 (106–166)	126 (126–127)	114 (101–125)	–	–
Dorsal bar	Undivided	Undivided	Undivided	Undivided	Undivided
Total length	19 (14–24)	17 (17–18)	16.8 (14.5–19.5)	16 (14–18)	15–21
Total width	82 (76–90)	88 (87–90)	87.5 (80–92)	83 (78–93)	68–78
Marginal hooks					
Total length	77 (72–92)	81 (80–83)	80 (72–83.5)	78 (71–88)	68–78
Handle length	69 (65–97)	71 (70–73)	68.6 (61–73.5)	–	–
Sickle length	11 (10–15)	11	10.9 (10.5–11.5)	–	–
Sickle proximal width	10 (8–12)	9	9.3 (9–9.5)	–	–
Cirrus spines	15	11–13	–	–	14–15

Phylogenetic relationships

Seven sequences (696–753 bp long) of the ITS1-5.8S- ITS2 region of the rRNA gene of *Macrogyrodactylus* spp. from *C. gariepinus* were newly generated in the present study (see **Table 4.3.** for detailed information). Maximum likelihood and Bayesian inference analyses yielded trees with similar topology (**Fig. 4.4.**). The genetic distances presented as p-distance (in %) and number of differences in nucleotides among *Macrogyrodactylus* spp. and the out-group are given in **Table 4.7.** The tree depicted a separated branch with *Macrogyrodactylus simentiensis* Přikrylová et Gelnar, 2008 from a polypterid, one single branch with *Macrogyrodactylus polypteri* also from a polypterid, and a clade grouping *Macrogyrodactylus* spp. from clariids that divided into two subclades. The first subclade shows that *M. clarii* isolates 1, 2 and 3 from ZM that clustered together with *M. clarii* from Zimbabwe (ZI) (GU252711) are all identical, whereas *M. clarii* isolate 1 from SA is more related to *M. clarii* × *heterobranchii* from ZI (GU252713) showing slight interspecific differences (p-distance 0.1– 0.5%, number of differences in nucleotides 1–3); *M. clarii* × *heterobranchii* (GU252713 and HF548583) from Kenya (KY) and Senegal (SN) appeared closely related to *M. heterobranchii* from SN (p-distance 0.5–0.6%, number of differences in nucleotides 4). The second subclade showed that *M. congolensis* and *M. karibae* are closely related. *Macrogyrodactylus congolensis* isolate from KY (GU252716) and from SN (GU252717) appeared more related (p-distance 0.3%, number of differences in nucleotides 2), whereas the isolate from SA showed interspecific divergence (p-distance 0.6–0.8%, number of differences in nucleotides 4–5); *M. karibae* from ZM appeared more related to *M. karibae* from ZI (GU252715) (p-distance 0.1%, number of differences in nucleotides 1) whereas *M. karibae* from SA showed more interspecific divergence (p-distance 1.8–2%, number of differences in nucleotides 12–14).

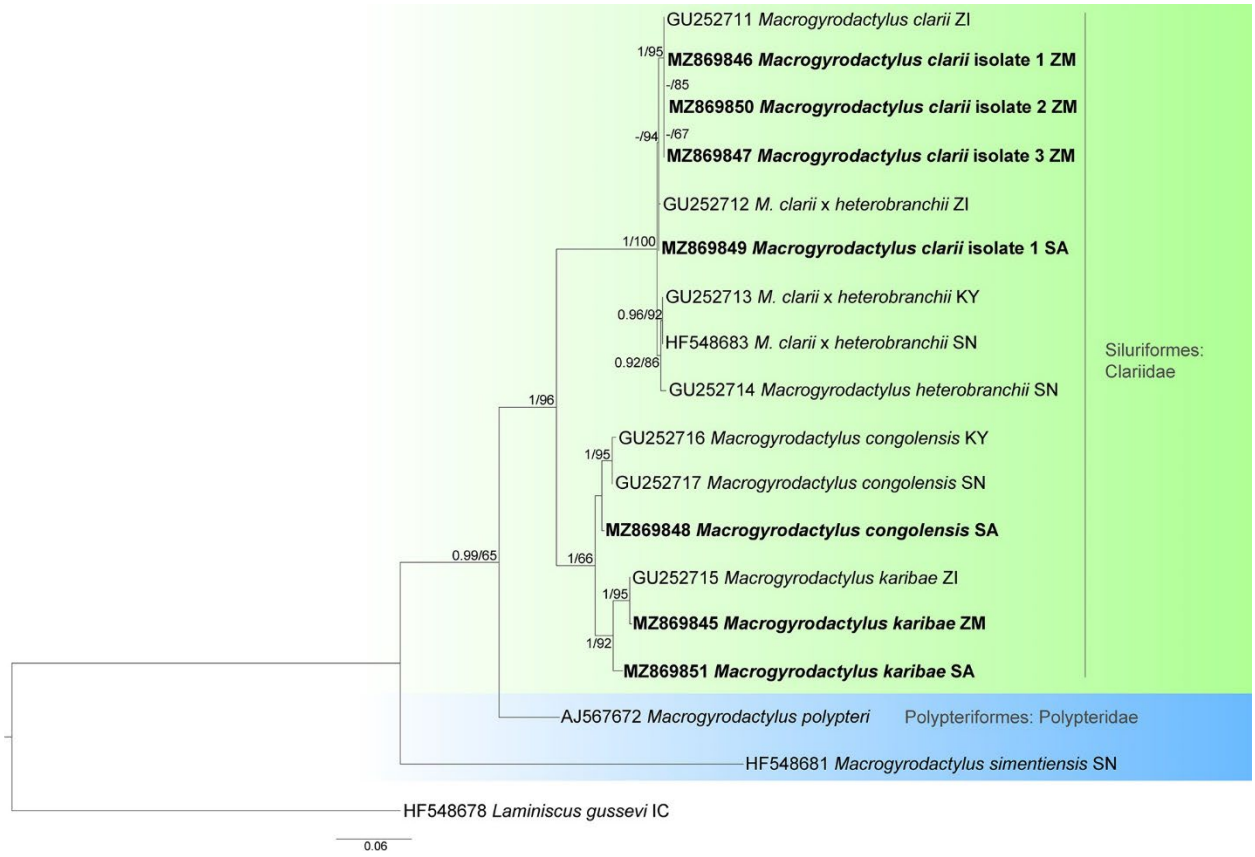

Figure 4.4. Maximum likelihood phylogram based on the ITS1-5.8S-ITS2 regions of the rDNA gene. GenBank accession number precedes species name. Species sequenced in the present study are in bold. Posterior probabilities and bootstrap support values are presented along branch nodes (posterior probability < 0.90 and bootstrap < 60 are not shown). *Laminiscus gussevi* (Bychowsky et Polyansky, 1953) was used as outgroup. Branch length scale bar indicates number of substitutions per site. *Abbreviations*: IC – Iceland; KY – Kenya; *M.* – *Macrogyrodactylus*; SA – South Africa; SN – Senegal; ZI – Zimbabwe; ZM – Zambia.

Table 4.7. Nucleotide genetic divergence values among sequences of the ITS1-5.8S-ITS-2 regions of the rDNA gene of *Macrogyrodactylus* spp. and *Laminiscus gussevi* (Bychowsky et Polyanky, 1953) from the 810 bp long alignment. Values below the diagonal are expressed in percentage (p-distance) while values above the diagonal represent number of differences in nucleotides. Newly sequenced taxa are in bold. Abbreviations: IC –Iceland, KY – Kenya, *M.* – *Macrogyrodactylus*, SN – Senegal, SA – South Africa, ZI – Zimbabwe, ZM – Zambia.

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1	GU252711 *Macrogyrodactylus clarii* ZI		0	0	0	4	3	7	7	9	76	75	54	72	69	69	91	159	196
2	**MZ869846 *M. clarii* isolate 1 ZM**	0.0		0	0	4	3	6	6	8	68	68	54	68	67	67	79	113	153
3	**MZ869850 *M. clarii* isolate 2 ZM**	0.0	0.0		0	4	3	7	7	9	75	74	54	71	69	74	87	125	160
4	**MZ869847 *M. clarii* isolate 3 ZM**	0.0	0.0	0.0		4	3	7	7	9	76	75	54	71	69	74	88	126	162
5	GU252712 *M. clarii* x *heterobranchii* ZI	0.5	0.6	0.6	0.6		1	5	5	7	74	73	52	72	69	75	89	156	198
6	**MZ869849 *M. clarii* isolate 1 SA**	0.4	0.5	0.4	0.4	0.1		4	4	6	68	67	51	69	68	71	79	116	156
7	GU252713 *M. clarii* x *heterobranchii* KY	0.9	0.9	1.0	1.0	0.7	0.6		0	4	75	74	51	71	68	76	88	160	197
8	HF548683 *M. clarii* x *heterobranchii* SN	1.0	0.9	1.0	1.0	0.7	0.6	0.0		4	70	69	51	69	68	73	79	119	156
9	GU252714 *M. heterobranchii* SN	1.2	1.2	1.3	1.3	0.9	0.9	0.5	0.6		74	73	50	70	67	75	87	158	199
10	GU252716 *M. congolensis* KY	10.3	10.3	11.0	11.1	10.1	10.1	10.2	10.4	10.1		2	5	30	27	23	69	147	194
11	GU252717 *M. congolensis* SN	10.2	10.3	10.9	11.0	9.9	10	10.1	10.3	9.9	0.3		4	28	26	23	68	145	194
12	**MZ869848 *M. congolensis* SA**	8.6	8.6	8.6	8.6	8.3	8.1	8.1	8.1	7.9	0.8	0.6		11	12	10	51	81	139
13	GU252715 *M. karibae* ZI	9.8	10.3	10.4	10.4	9.8	10.3	9.7	10.3	9.5	4.1	3.8	1.7		1	14	75	144	197
14	**MZ869845 *M. karibae* ZM**	10.3	10.2	10.3	10.3	10.3	10.1	10.1	10.1	10.0	4.0	3.9	1.9	0.1		12	75	102	157
15	**MZ869851 *M. karibae* SA**	10.7	10.2	10.9	10.9	10.9	10.6	11	10.9	10.9	3.3	3.3	1.6	2.0	1.8		72	119	168
16	AJ567672 *M. polypteri*	12.7	12.0	12.8	12.9	12.4	11.8	12.3	11.8	12.1	9.6	9.5	8.1	10.5	10.3	10.5		134	194
17	HF548681 *M. simentiensis* SN	23.2	18.5	19.7	19.8	22.7	18.6	23.3	19.1	23	21.6	21.3	21.3	21.2	18.8	18.8	20.0		199
18	HF548678 *Laminiscus gussevi* IC	27.5	23.8	24.3	24.5	27.8	24.0	27.7	24.0	27.9	27.4	27.4	27.4	27.9	25.4	25.4	27.9	29.8	

4.4. Discussion

Novel distribution records for *Macrogyrodactylus clarii*, *M. congolensis* and *M. karibae* are confirmed in southern Africa. The presence of the three *Macrogyrodactylus* spp. in the Vaal River and NGR (SA), and from the Barotse floodplain and Kabompo River (ZM) reflect similar community composition of species as in other localities in southern Africa and Africa (Khalil and Mashego 1998; Barson et al. 2008, 2010; Olivier et al. 2009; Madanire-Moyo et al. 2012). The absence of *Macrogyrodactylus* species in the Eastern and Western Cape in the South Cape ichthyofaunal province confirms that *Macrogyrodactylus* was not co-introduced with *Clarias gariepinus* from the Zambezi and northern regions of the Southern Cape ichthyofaunal provinces, the geographical origin of these translocated *C. gariepinus* populations (see Van Steenberge et al. 2019 and references therein). This implies that *C. gariepinus* in the translocated regions initially did not harbour any species of *Macrogyrodactylus* and is supported by the absence of *Macrogyrodactylus* spp. in the Free State, the southern-most limit of the host's natural distribution. These results suggest that the parasite is either naturally absent from these systems, seasonally present or absent, or restricted by environmental barriers such as physicochemical variables and organic pollution (see Khan and Thulin 1991; Madanire-Moyo and Barson 2010; Madanire-Moyo et al. 2012; Gilbert and Avenant-Oldewage 2017; Sures et al. 2017b).

The findings of the present study represent infection in- tensities in a single point in time and did not indicate high numbers or cause for concern as reported by Khalil and Mashego (1998). The host specimens from the Vaal River (SA) and Barotse floodplain (ZM) that had the highest worm burden (109 individuals of *Macrogyrodactylus* spp. each), did not present greyish-white patches on the skin, as previously recorded by the aforementioned authors in cases of severe infection in natural conditions. Infection of *C. gariepinus* with *M. clarii*, *M. congolensis* and *M. karibae* found in this study is low if compared to reports from the Middle Letaba Dam (SA) where intensities of *M. clarii* peaked from 1 to 700 during summer months, and *M. karibae* numbers increased up to 150 (while *M. clarii* decreased) during the cooler months of autumn. The co-occurrence of *M. clarii* and *M. karibae*, the latter in fewer numbers, reported from the Middle Letaba Dam coincides with findings from KuShokwe Pan, South Africa (Khalil and Mashego 1998). From available data, it seems that *M. clarii* is an ever-present species occurring at all, but one locality studied in the southern African region (Khalil and Mashego 1998; Barson et al. 2008; Barson 2009; Madanire-Moyo 2011).

The use of universal primers for the ITS region of the rDNA and its suitability as a diagnostic tool made it widely used within gyrodactylid systematics, resulting in the availability of many sequences for this group (Matějusová et al. 2003; Přikrylová et al. 2013). The first study to ever sequence the ITS region of a *Macrogyrodactylus* species (*Macrogyrodactylus polypteri*) was

conducted by Matějusová et al. (2003). These authors constructed a phylogeny including sequences of many species of *Gyrodactylus* von Nordmann, 1832, and a sequence of *Gyrdicotylus gallieni* (Vercammen Grandjean, 1960), and *Gyrodactyloides bychowsii* Albova, 1948. Their phylogenetic analyses recovered *M. polypteri* as the closest ancestor of *Gyrodactylus*, as suggested by Malmberg (1998) based on morphological characteristics, who also suggest that *Macrogyrodactylus* is supposed to be the earliest divergent gyrodactylid lineage. However, Malmberg's hypothesis has recently been refuted by Vanhove et al. (2018), who analysed the near-complete mitogenome of *M. karibae* and compared it with other gyrodactylid genera.

Barson et al. (2010) was the second study to include molecular analyses of the ITS region for *Macrogyrodactylus* spp., including for the first time *M. clarii, M. congolensis, M. heterobranchii* and *M. karibae* from African clariids. Sequences of the partial 18S rDNA, complete ITS and the mitochondrial gene *cox*1, together with morphology revealed a hybrid species that was intermediate between *M. clarii* and *M. heterobranchii*, which was confirmed by their phylogenetic analyses (Barson et al. 2010). Přikrylová et al. (2013) conducted a large-scale phylogenetic study on African gyrodactylids, including for the first time sequences of the 18S and ITS rDNA of all (but one) genera occurring in the continent. Both ITS and 18S phylogenetic analyses of Přikrylová et al. (2013) recovered, with strong support, *Macrogyrodactylus* as a monophyletic group. Boeger et al. (2021) in their phylogenetic study based on 18S rDNA sequences of several gyrodactylid genera also recovered *Macrogyrodactylus* as a monophyletic lineage related to *Diplogyrodactylus* Přikrylová, Matějusová, Musilová, Gelnar et Harris, 2009 and *Afrogyrodactylus* Paperna, 1968. In contrast, *Macrogyrodactylus* appeared related to *Paragyrodactylus* Gvozdev et Martechov, 1953 in their analyses of the mitochondrial gene *cox*2. The clustering of *Macrogyrodactylus* from clariid hosts as two distinctive lineages in the phylogenetic analyses in this study reflects their morphological synapomorphies. *Macrogyrodactylus clarii* and *M. heterobranchii* share the following morphological characteristics: 1) hamuli with a strong inward curvature in the root, 2) a divided dorsal bar with two articulating sections, 3) a ventral bar with two short anterolateral arms and a long central posterior arm, and, 4) slender marginal hook sickle and slender sickle blade tapering into a sharp, thin point ending in line with the anterior end of the sickle base.

Macrogyrodactylus congolensis and *M. karibae* share the following morphological characteristics: 1) hamuli with slight inward curvature of the root, 2) single undivided dorsal bar, and 3) robust marginal hook sickle and short sickle blade tapering into a fine point that end in line with the proximal end of the sickle base. However, the relationships of *Macrogyrodactylus* from polypterids remained inconclusive in the present study.

The phylogenetic analyses showed that the ITS-1-5.8S- ITS-2 sequences of *M. clarii* from South Africa differed slightly from those of *M. clarii* from Zambia and Zimbabwe; similarly, *M. congolensis*

from South Africa differed slightly from *M. congolensis* from Kenya and Senegal (see **Fig. 4.4.** and **Table 4.7**. for genetic distances). The ITS- 1-5.8S-ITS-2 sequences of *M. karibae* from South Africa also differed from those of *M. karibae* from Zambia and Zimbabwe (**Fig. 4.4.**, **Table 4.7.**). Such differences can be related to the geographical separation of the hosts among the African ichthyofaunal provinces of Zambezi and Southern (Cape) as proposed by Roberts (1975), Teugels (1986) and Van Steenberge et al. (2019) and references therein. *Clarias gariepinus* inhabiting the Southern Cape retains similar haplotypes as those from the central and eastern Zambezi Province, thus these catfish occurred sympatrically before a recent separation into the Southern Cape and brought along their parasites (Barson et al. 2010; Van Steenberge et al. 2019).

There is still disproportionate availability of data on freshwater fish parasite diversity from Africa. The continent holds ~25% of the world's freshwater fishes (with the second highest species richness on the globe), and some of the most widely distributed endemic freshwater species (Scholz et al. 2018c). It is also evident that there is disparity in distribution and diversity records throughout the continent, even from a widely distributed fish host such as *C. gariepinus*. No data on its parasitic communities exist from at least ¾ of African countries, where this fish is either native or translocated, and highly utilised for aquaculture. Therefore, more studies encompassing occurrence, environmental or seasonal variation, morphology and genetics of *Macrogyrodactylus* spp. are necessary to better understand their distribution and irradiation into the African continent.

Chapter 5

Co-introduction, parasite spillback and enemy release hypotheses in translocated *Clarias gariepinus* (Burchell) populations in South Africa

5.1. Introduction

Clarias gariepinus as an invasive in translocated regions

It is well-known thathe African sharptooth catfish *Clarias gariepinus* (Burchell) is a widely distributed species of economic importance on a local and global scale (see **Chapter 2**). This catfish has been introduced into several countries in Eurasia (i.e., China, Mongolia, Japan, India, Thailand) and South America (i.e., Brazil, Argentina), and in Africa the introduction into several freshwater systems is reported but the origin or introduced status is uncertain (Vitule 2006, 2009; Picker and Griffiths 2011; GISD 2022). Research on the distribution and species delineation of several representatives in the genus *Clarias* Scopoli, 1777, where *C. gariepinus* is concerned, has been thoroughly addressed (see Teugels 1986; Skelton and Teugels 1991, 1992; Tweddle et al. 2009). Recently, Van Steenberge et al. (2019) unravelled the historical landscape of African river basins using the biogeography (haplotype networks) of *C. gariepinus*. It was during this study that it became apparent that translocated and invasive populations of *C. gariepinus* in the Eastern and Western Cape provinces of South Africa are represented by the Southern-lineage (Southern ichthyofaunal province, **Fig. 2.1.**). They are however also present in the Orange-Vaal rivers, the limit of its natural distribution, while the populations present in the sub-tropical eastern systems of South Africa (Limpopo, Mpumalanga) form part of the Zambezi-lineage (**Fig. 2.1.**). The presence of *C. gariepinus* in the systems of the Eastern Cape is primarily a result of the construction of the Orange-Fish tunnel (Laurenson and Hocutt 1986, 1989; De Moor and Bruton 1988). Similarly, translocation into the Riviersonderend River in the Western Cape followed after the construction of another inter-basin water transfer (IBWT) scheme (Riviersonderend-Berg River Project) and movement of fish for angling (see **Chapter 2**, **Section 2.1.** for more detail).

Although *C. gariepinus* is considered an excellent aquaculture species and is successfully cultured in several countries in the northern regions of Africa. In South Africa, its diverse diet and tolerance to a wide range of environmental variables have rendered it a species of interest for developing inland commercial capture fisheries and local subsistence fisheries practices to alleviate poverty and hunger, as well as to increase local economy, employment opportunities

and food security in South Africa (see Van der Waal 1972; Mashego 1977; Bruton and Merron 1985; De Moor and Bruton 1988; Britz et al. 2015). From the comprehensive review in **Chapter 3** it is evident that the parasitic community of *C. gariepinus* in Africa is one of the most diverse and well-studied. However, despite all of the available literature, limited data is available for this host in southern Africa and no historical, nor present day, data is available for comparative purposes from the invaded regions to identify potential parasitic taxa of concern in highly crowded aquaculture conditions (if the host would be cultured). Furthermore, there is no data to evaluate the potential risks posed by parasitic taxa that are introduced into novel environments, where *C. gariepinus* is not part of the native fauna.

Invasion parasitology in South Africa

In his account of freshwater fish parasitology in South Africa, Van As (2015) highlighted the remarkable progress made by local research groups to document the parasitic communities (Protozoa and Metazoa) of several fishes in the country. The first extensive contribution summarising current (then) knowledge on freshwater fish parasites in South Africa was the checklist of Van As and Basson (1984). The authors provided a complete synopsis of South Africa's freshwater parasitic diversity mainly available from the northeastern provinces: the present Limpopo, Mpumalanga, Gauteng, North West, Free State, KwaZulu-Natal. No records for any parasite taxa from the freshwater fishes of the Northern Cape were included. The only records from the Eastern and Western Cape provinces comprised of the branchiuran *Chonopeltis minutus* Fryer, 1977 from the currently critically endangered Clanwilliam redfin, *Sedercypris calidus* (Barnard, 1938) and Twee River redfin, *Sedercypris erubescens* (Skelton, 1974) in the Groot Berg River (Van As and Basson 1984); and the branchiuran *Argulus capensis* Barnard, 1955 and the generalist *Contracaecum* sp. from the Cape kurper, *Sandelia capensis* (Cuvier) from Zoetendals Vlei and De Hoop Nature Reserve (Barnard 1955; Moravec et al. 2016). Further, *Lernaea barnimiana* (Hartman, 1865) and *Mugilicola smithae* Jones et Hine, 1978 were recorded from the moggel, *Labeo umbratus* (Smith) and *Anguilla mossambica* Peters, respectively, from rivers in the Eastern Cape. Invasive species included at the time were four co-invasive Protozoa and three co-invasive metazoans: *Schyzocotyle acheilognathi, Lernaea cyprinacea* and *Argulus japonicus*. Co-introduced parasites included the three protozoans and the metazoans *Acolpenteron uretorocetus* (as *Gyrodactylus* sp.) and most probably species of *Onchocleidus* (as *Dactylogyrus* sp.) from the largemouth bass, *Micropterus salmoides* Lacépède (Du Plessis 1948; Lombard 1968; Van As and Basson 1984 and references therein). All the invasive protozoans mentioned were reported from rivers in the Eastern Cape. Since then, the discipline of freshwater fish parasitology has advanced considerably with four main freshwater fish parasitology research groups across the Limpopo, North West, Gauteng, and Free State provinces. All these research groups contribute significantly to documenting diversity, distribution, host ranges, biology, and

more recently to environmental parasitology. However, very few focus on invasive fish and parasitic species in regions where one or both are introduced and established. It was only recently, almost three decades after initial concerns were raised, that Smit et al. (2017) and Weyl et al. (2018) reviewed current knowledge on the biogeography, introduction pathways, invasion status, and host ranges of several alien and invasive freshwater fish parasites. The need for research in the coastal provinces of South Africa, along with the focus on introduced and translocated hosts and their parasites, was emphasised in these extensive reviews of alien and invasive freshwater fish parasites in South Africa (Smit et al. 2017; Weyl et al. 2018).

Invasion mechanisms and alien freshwater fish parasites

The invasion of species in natural or introduced systems is primarily driven by human-mediated translocations of hosts, or so-called “vector species” (Welcomme 1992; Richardson et al. 2003). The book of Charles S. Elton “The Ecology of Invasions by Animals and Plants” prompted what would be the building blocks of invasion biology, concerning host-parasite interactions at individual, population, and community levels across all living taxa (Elton 1958; Heger et al. 2013; Van Wilgen et al. 2018). The main challenge scientists face within the discipline is to explain the disproportionate success and/or failure of species when introduced into novel environments. Although some light has been shed on the invasion success or failure of some species, several interactions and aspects of invasions remain a conundrum. Terminology and defining alien and invasive species in categories based on their success and extent has been addressed, and the interaction of invasives with novel environments are progressing at a gradual pace. The understanding of invasion success or lack thereof remains complex, while Jeschke et al. (2012) and Médoc et al. (2016) elaborate on the factors that possibly contribute to the advancement or collapse of species populations upon introduction (Laurenson and Hocutt 1986; Colautti et al. 2004; Blackburn et al. 2011; Lymbery et al. 2014; Médoc et al. 2016).

Briefly, parasites need to overcome similar barriers as their host when introduced into a novel environment and are either co-introduced with their alien host, meaning they successfully overcame barriers typically encountered during introduction, while co-invasives are successfully introduced and established with their alien host, however they spread to native hosts (now known as parasite spill-over) (Blackburn et al. 2011; Lymbery et al. 2014). Four major ecological theories in invasion biology define phenomena observed upon alien species introduction (see **Table 5.1.**) with a multitude of examples of natural aquatic experiments in the literature. Co-introduction of ancyrocephalid monogeneans has been widely reported from centrarchid hosts into Britain, Europe, Norway, South Africa and Ukraine (see Sterud and Jørgensen 2006; Ondračková et al. 2011; Truter et al. 2017); the co-invasion and subsequent spill-over of parasitic nematodes and three monogeneans of eels have also made headlines (Schabuss et al. 1997; Hayward et al.

2001a, b; Laetsch et al. 2012; Weyl et al. 2014; Hein et al. 2016; Truter et al. 2021b); while García-Vásquez et al. (2017, 2021) confirmed the spill-over of three previously co-introduced species of *Gyrodactylus* von Nordmann, 1832 (via their alien tilapia hosts) to native and invasive poecilids, as well as native cichlids in Mexico. Schoeman et al. (2019) illustrated that parasite loss is characteristic of the invasion process using the global invasive African clawed frog, *Xenopus laevis* Grendon in testing the enemy release hypothesis (also see Kvach and Stepien 2008); Paterson et al. (2011) found that introduction of brown trout *Salmo trutta* Linnaeus stimulated a reduced infection (dilution) in native roundhead galaxias, *Galaxias anomalus* Stokell with acanthocephalans in New Zealand (also see Mooring and Hart 1992; Kelly et al. 2009; Grendon and Marcogliese 2012); and lastly Hershberger et al. (2010) reported on the spill-over and resulting amplification of *Ichthyophonus* sp., an epizootic disease introduced with America shad *Alosa sapidissima* (Wilson) from the Atlantic to the Pacific coast of North America (also see Ortega et al. 2020). Furthermore, it should be noted that co-introduction, co-invasion, and associated invasion mechanisms (or hypotheses) are not limited to intercontinental translocation and introductions but can occur on a local scale of regional natives and endemic species.

Table 5.1. Ecological hypotheses (invasion mechanisms) on the success or failure of parasitic species in novel environments (as defined in Lymbery et al. 2014).

Mechanisms	Definition
Enemy release hypothesis	Alien hosts lose a proportion of its natural parasite fauna as a result in failure to overcome one or several barriers in the novel environment.
Spillback	Parasites of native fauna transfer to the introduced/alien host and may occur in increased numbers, also known as “parasite amplification”.
Dilution effect	An effect of spillback, where the presence of alien hosts dilutes the infection and transmission of native parasitic fauna on native hosts.
Spill-over (pathogen pollution)	Alien hosts introduce new parasite species that infect the native hosts in the novel environment.

The aims of this chapter were thus: 1) to present an overview of the alien invasive metazoan parasites infecting *C. gariepinus* throughout Africa; 2) to investigate the parasitic communities of two translocated populations of *C. gariepinus* in South Africa; 3) to determine if any of its native parasite species were co-introduced into the novel environments; and 4) to determine if enemy release or parasite spillback occurred upon introduction into the Great Fish River, Eastern Cape and Riviersonderend River, Western Cape in South Africa.

5.2. Materials and methods

A total of 26 *C. gariepinus* individuals were collected from the Great Fish River (GFR, n = 11) and the Riviersonderend River (RSE, n = 15). Infection statistics for *C. gariepinus* from Gariep Dam (GD, n = 15) were included to compare with GFR as it is the source population through the Great-Fish tunnel (IBWT). A detailed background on the introduction of *C. gariepinus* into the two rivers is provided above and in **Chapter 2**, **Section 2.2.3. (Subsection Gariep Dam, Orange River, Free State Province)**, **Section 2.2.4.** and **Section 2.2.5.**, respectively. Biometric information on host individuals and general parasitic infection (host size and mass, overall prevalence, intensity of infection) are summarised in **Table 5.2.** Data analysis of infra- and component community abundance and assemblages are provided in **Chapter 2**, **Section 2.8.** Collection and fixation methods, and morphological and molecular identification of parasitic taxa are described in detail in **Chapter 2**, **Section 2.4.**

Table 5.2. Biometric information of *Clarias gariepinus* (Burchell) collected from the two translocated populations in the Eastern and Western Cape Provinces of South Africa. Abbreviations: n – number of host individual; Mean M – mean mass (g); Mean TL – mean total length (mm); P – overall prevalence in % and MI mean – intensity of infection with minimum and maximum.

Locality	n	Mean Mass ±SD	Mean TL ±SD	P %	MI
Gariep Dam	15	3419 ± 3292	743.1 ± 214.6	100	5.1 (1 – 10)
Great Fish River	11	2666 ± 3368	624.1 ± 256.5	100	27.7 (13 – 53)
Riviersonderend River	15	684 ± 589.1	404.8 ± 168.1	100	26.0 (8 – 26)

5.3. Results

5.3.1. Invasive metazoan parasites of *Clarias gariepinus*

The co-introduction of 15, and co-invasion status of nine freshwater fish parasites, infecting native freshwater fishes of South Africa have been confirmed (see Smit et al. 2017; Weyl et al. 2018; Truter et al. 2021b). In their review of alien and invasive freshwater parasites in South Africa, Smit et al. (2017) found that two co-invasive species, *Schyzocotyle acheilognathi* (Yamaguti, 1934) and *Argulus japonicus* Thiele, 1900 are known to infect *C. gariepinus* in South Africa (see below).

The co-invasive Asian tapeworm, *Schyzocotyle acheilognathi* (Yamaguti, 1934) (syn. *Bothriocephalus acheilognathi* Yamaguti, 1934) (Cestoda: Bothriocephalidae) infects the intestines of more than 200 freshwater fish species (Kuchta and Scholz 2007). The Asian tapeworm has been widely reported from cyprinid hosts in South Africa since the mid-1970s and was most likely introduced with *Ctenopharyngodon idella* (Valenciennes) (Bruton and Merron 1985; De Moor and Bruton 1988). Currently, it is present in tributaries of the Incomati, Limpopo, Olifants, Vaal, Orange, Mthatha and Phongolo rivers infecting a wide range of native hosts (Smit

et al. 2017). It was also found to parasitise the endemic *Labeobarbus aeneus* (Burchell, 1822) that was translocated into the Great Fish River in the Eastern Cape (Stadtlander et al. 2011). A single record of *S. acheilognathi* infecting the intestine of *C. gariepinus* is reported from Gariep Dam (GD) in the Free State [unpublished thesis, Mogorosi (2019)], while numerous reports from African countries (as *Bothriocephalus aegypticus*) can be found from literature (see **Fig. 5.1.** and **Table A1.8.**) (Anosike et al. 1992; Yimer 2000; Moyo et al. 2009; Abba et al. 2018a, b). None of the latter studies, apart from Mogorosi (2019), provide morphological or molecular data to support their identification.

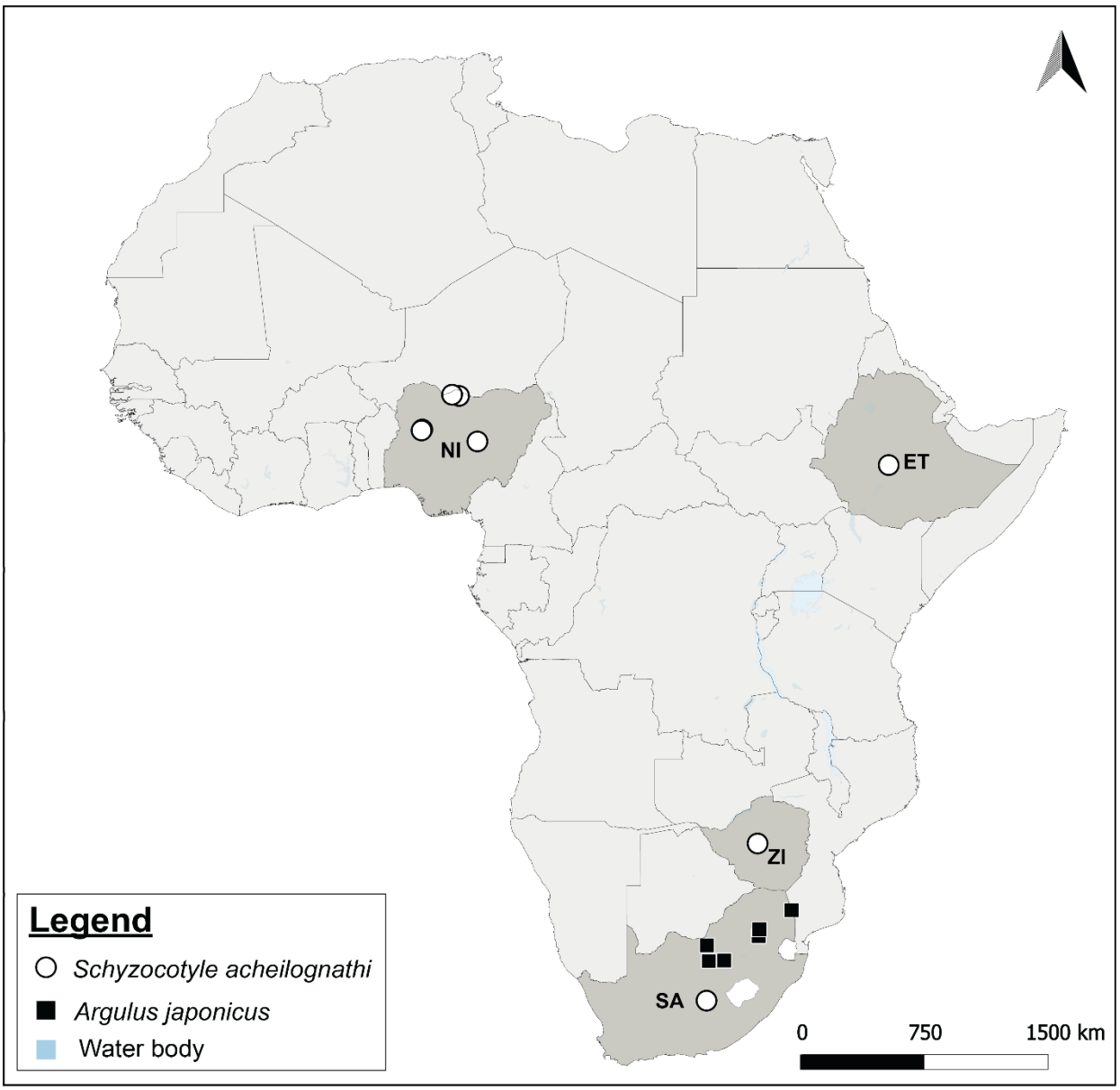

Figure 5.1. Distribution of the alien and invasive *Schyzocotyle acheilognathi* (Yamaguti, 1934) (Cestoda) *and Argulus japonicus* Thiele, 1900 (Crustacea) infecting *Clarias gariepinus (*Burchell) from various localities in Africa.

Argulus japonicus, commonly known as the Japanese fish louse, was first reported in South Africa from several fishes, including the alien *C. carpio*, from Bloemhof Dam and Barberspan on the Vaal River (Kruger et al. 1983). In 1984, the host range and distribution of *A. japonicus* were also expanded (see Smit et al. 2017). However, it would seem that the introduction into South Africa occurred much earlier since the suspected carrier hosts, the common carp (*C. carpio*) and goldfish *Carassius auratus* Linnaeus were already introduced into the country, in 1859 and 1726, respectively, and earlier records of an *Argulus* sp. from *C. gariepinus* in the Mpumalanga Province indicates that this global invasive was already present in the 1950s (Lombard 1968; Ellender and Weyl 2014). At present, this global invader is known to infect at least nine native fishes in South Africa, including *C. gariepinus* from the Vaal River system (Bloemhof Dam and Barberspan wetland) and the Olifants River (Loskop Dam, Witbank Dam and Olifants River mainstem) (see **Fig. 5.1.** and **Appendix A6**) (Kruger et al. 1983; Shafir and Oldewage 1992; Avenant-Oldewage 2001; Smit et al. 2017).

5.3.2. Native populations of *Clarias gariepinus*

During the present study a maximum of two specimens (per host individual) of *A. japonicus* (**Fig. 5.2. A–B)** were removed from the gills and fins of six *C. gariepinus* individuals [Prevalence (P) = 40%] collected from GD on the Orange River, Free State. There is a possibility that the infestation on *C. gariepinus*, might have been opportunistic, since the sharptooth catfish was held in containers with the smallmouth yellowfish, *Labeobarbus aeneus* (Burchell), also caught and transported by local authorities to the shore for another team of parasitologists. Nonetheless, it cannot be ignored that *C. gariepinus* is a suitable host since previous studies report on much higher infections of *A. japonicus* from *C. gariepinus*: Bloemhof Dam [P = 100%; highest intensity on an individual host (IF) = 17]; Barberspan [P = 78%; IF = 10]; Loskop Dam [P = 6.25%; IF = 1]; Witbank Dam [P = 100%; IF = 74] and Olifants River [P = 30.8%; IF = 5] (Kruger et al. 1983; Shafir and Oldewage 1992; Avenant-Oldewage 2001; Smit et al. 2017).

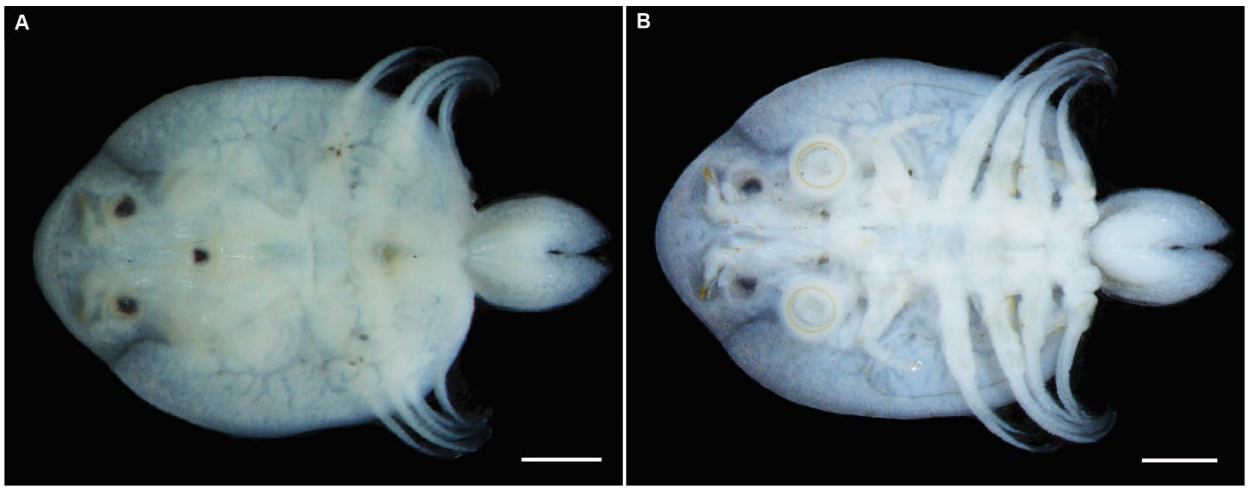

Figure 5.2. Photomicrograph of the alien invasive fish lice *Argulus japonicus* Thiele, 1900 found from the external body surface and gills of *Clarias gariepinus* (Burchell) collected at Gariep Dam. A – Dorsal view; B – ventral view. Scale bars: 1000µm.

5.3.3. Translocated populations of *Clarias gariepinus*

A single specimen, identified by the *cox*1 mitochondrial gene (**Table 5.3.**), of the co-invasive *Schyzocotyle acheilognathi* was found from the intestine of *C. gariepinus* collected from RSE.

Table 5.3. Divergence between species of the Bothriocephalidea (Cestoda) based on *cox*1 mitochondrial region (1641 bp) alignments. Values for p-distance (bottom diagonal) are expressed in percentage, while the top diagonal represents the number of differences in nucleotides. Species in bold were obtained from material collected during the present study. Abbreviations: BR – Brazil; ET – Ethiopia; MD – Madagascar; RSE – Riviersonderend; US – United States of America and VT – Vietnam.

*cox*1		1	2	3	4	5	6	7
1	**MT56 *Schyzocotyle acheilognathi* RSE**	–	0	0	91	98	80	79
2	MG968744 *S. acheilognathi* MD	0.0	–	4	416	437	327	339
3	MG968745 *S. acheilognathi* MD	0.0	0.3	–	415	434	326	338
4	MK328928 *Acanthostotaenia shipleyi* VT	19.9	24.4	24.4	–	338	361	372
5	MW244033 *Monticella magna* BR	21.4	25.1	24.9	20.8	–	364	381
6	KR780814 *Bothriocephalus* sp. US	17.5	17.0	16.8	22.7	23.2	–	106
7	KR780795 *T. ciliotheca* ET	17.3	19.0	18.8	24.5	26.0	17.7	–

5.3.4. Parasitic communities of translocated *Clarias gariepinus* in South Africa

Infra- and component community diversity

Overall individuals from GFR and RSE had similar overall infection statistics, while *C. gariepinus* from GD were less infected. A total of 13 metazoan parasite species were found from GD, GFR and RSE and were represented by five Monogenea [*Quadriacanthus aegypticus* (**Fig. 3.5. A-E**), *Q. clariadis* (**Fig. 3.5. J-M**), *Q. fornicatus* (**Fig. 3.7. A-D**), *Q. allobychowskiella* (**Fig. 3.6. A-I**), *Q. pravus* (**Fig. 3.5. F-I**) and *Quadriacanthus* sp. H (**Fig. 3.8. E-F**)] from the gills; two Digenea [*Orientocreadium batrachoides* (**Fig. 3.19. A-F**) and *Tylodelphys mashonensis* (**Fig. 3.23. A-E**)] from the intestine and cranial cavity, respectively; three Cestoda (*Barsonella lafoni*; *Tetracampos ciliotheca* and *Schyzocotyle acheilognathi*) from the intestine, two Nematoda [*Contracaecum* sp. C (**Fig. 3.30.**) and *Paracamallanus* sp. A (**Fig. 3.32.**)**]** from the mesenteries and intestine, respectively, and the copepod *Ergasilus* sp. A from the gills (see **Fig. 5.3.** and **Table 5.4.**). All translocated *C. gariepinus* from GFR (S = 9) were parasitised by any combination of the five monogeneans (*Q. aegypticus*; *Q. allobychowskiella*; *Q. clariadis*; *Q. fornicatus*; *Quadriacanthus* sp. H), two digeneans (*O. batrachoides*; *T. mashonensis*), the nematode larvae of *Contracaecum* sp. C, and the copepod, *Ergasilus* sp. A. While the 15 host individuals from RSE (S = 7) were only infected with any combination of three monogeneans (*Q. fornicatus; Q. clariadis; Q. fornicatus*), two cestodes (*T. ciliotheca* and *S. acheilognathi*), the nematode *Paracamallanus* sp. A, and the parasitic copepod, *Ergasilus* sp. A (see **Table 5.4.**).

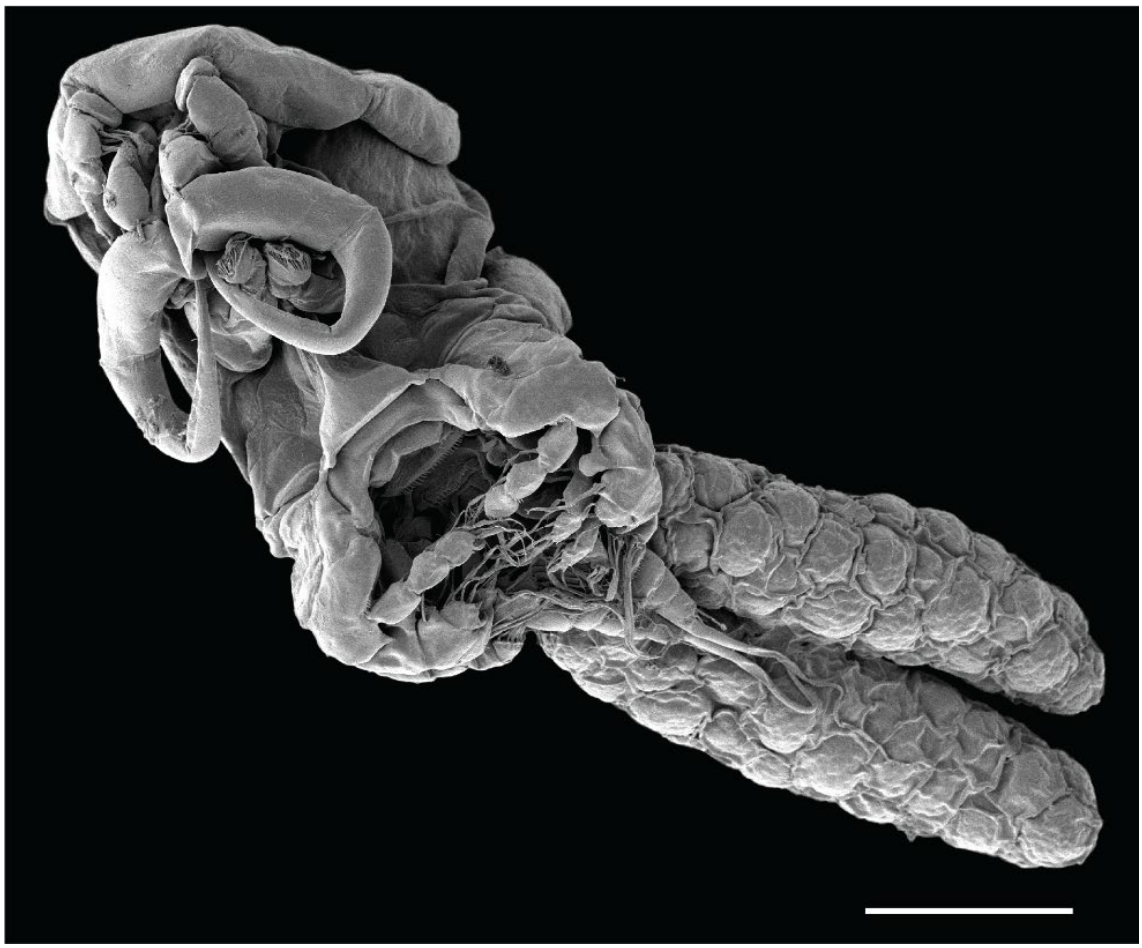

Figure 5.3. Scanning electron micrograph of *Ergasilus* sp. A found on the gills of *Clarias gariepinus* (Burchell) collected from the Great Fish River (Eastern Cape) in South Africa. Scale bar: 100µm.

Table 5.4. Descriptive statistics for individual metazoan parasitic communities of *Clarias gariepinus* (Burchell) from the Gariep Dam, Great Fish River and Riviersonderend River in South Africa. Abbreviations: HA – heteroxenous adult; HL – heteroxenous larvae; M – monoxenous; MA – mean abundance; MI –mean intensity; n – number of host and P – prevalence (%); individuals screened and infected per locality indicated in brackets after locality name; dominant species indicated in bold. Component community defined as taxa richness [S], life cycle ratio indicated as S_H:S_M below heading, , M – monoxenous; HA – heteroxenous adult and HL – heteroxenous larvae. ▲Alien invasive species.

Taxon	Species	Life cycle	P (%)	MA	MI
Gariep Dam (n = 15/15)	Component community [8]	1:3			
Monogenea	*Quadriacanthus aegypticus*	M	40.0	0.8	2.0
	Quadriacanthus fornicatus	M	40.0	0.8	2.0
	Quadriacanthus clariadis	M	26.7	0.4	1.5
	Quadriacanthus allobychowskiella	M	13.3	0.2	1.5
	***Quadriacanthus* sp. H[a]**	**M**	**33.3**	**1.2**	**3.6**
Cestoda	*Barsonella lafoni*	HA	40.0	0.7	1.7
	Tetracampos ciliotheca	HA	13.3	0.5	4.0
Crustacea	*Argulus japonicus*▲	M	40.0	0.5	1.2
Great Fish River (n = 11/11)	Component community [9]	1:2			
Monogenea	*Quadriacanthus aegypticus*	M	60.0	3.0	5.0
	Q uadriacanthusfornicatus	M	60.0	2.3	3.8
	Q uadriacanthusclariadis	M	33.3	1.1	3.2
	Q uadriacanthusallobychowskiella	M	20.0	0.3	1.3
	Quadriacanthus sp. H	M	26.7	0.6	2.3
Digenea	*Tylodelphys mashonensis*	HL	20.0	2.3	11.7
	***Orientocreadium batrachoides*[b]**	**HA**	**46.7**	**3.9**	**8.3**
Nematoda	*Contracaecum* sp. C [L]	HL	20.0	3.5	17.3
Crustacea	*Ergasilus* sp. A	M	60.0	3.5	5.8
Riviersonderend River (n = 15/15)	Component community [7]	1:1			
Monogenea	*Quadriacanthus fornicatus*	M	46.7	1.7	3.6
	Q uadriacanthusclariadis	M	60.0	1.8	3.0
	Q uadriacanthuspravus	M	13.3	0.1	1.0
Cestoda	*Tetracampos ciliotheca*	HA	86.7	8.2	9.5
	Schyzocotyle acheilognathi▲	HL	6.7	0.1	1.0
Nematoda	***Paracamallanus* sp. A[c]**	**HA**	93.3	14.1	15.1
Crustacea	*Ergasilus* sp. A	M	6.7	0.1	1.0
Berger-Parker Dominance index: [a] – 0.237; [b] – 0.190; [c] – 0.541.					

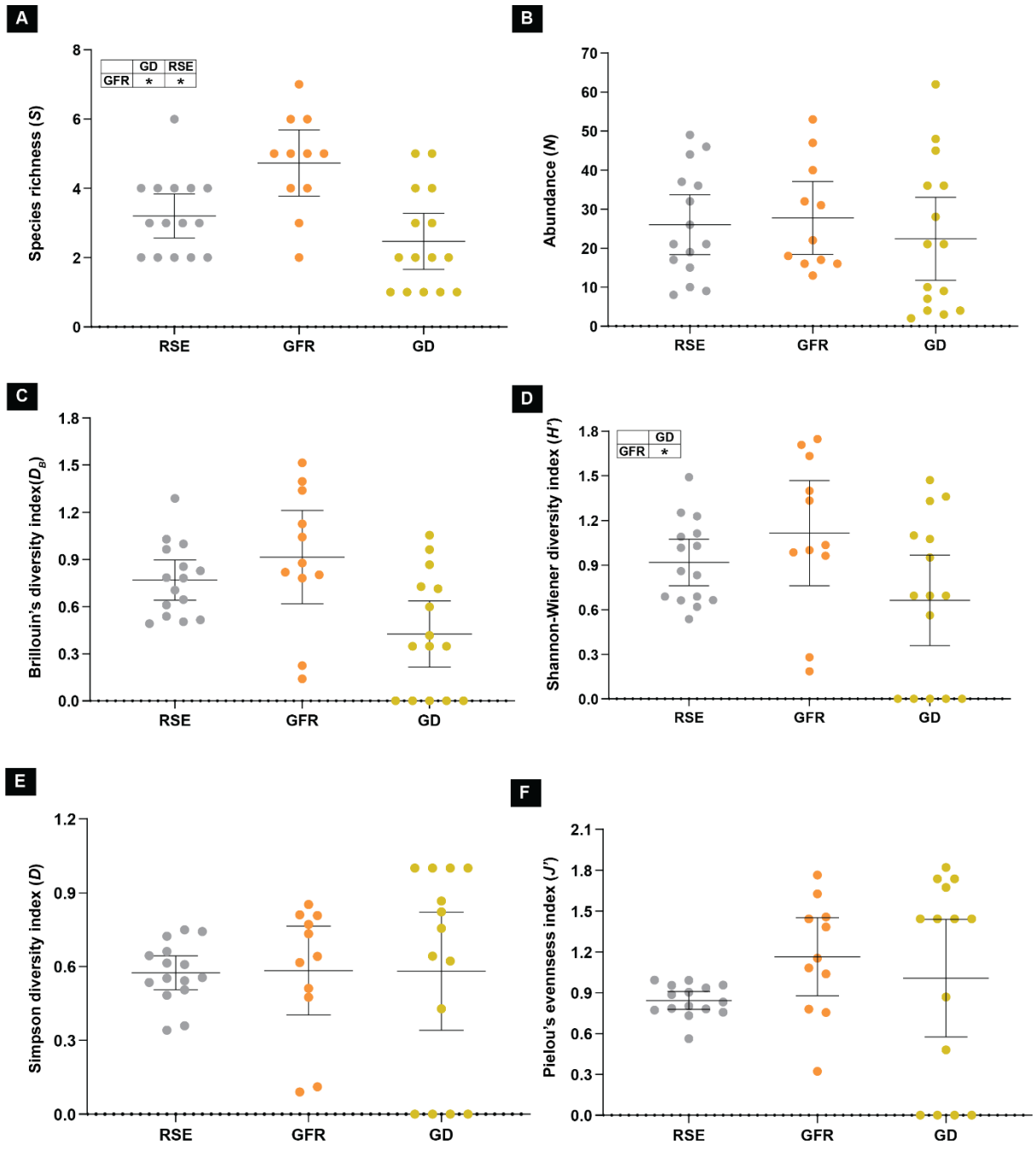

Figure 5.4. Infracommunity composition defined as **A** – taxa richness (S); **B** – abundance (N); **C** – Brillouin's diversity index (DB); **D** – Shannon-Wiener diversity index (H'); **E** – Simpson diversity index (D) and **F** – evenness [Pielou's evenness index (J')]. The mean and 95% confidence interval of each index is presented for *Clarias gariepinus* (Burchell) from Gariep Dam (GD), Great Fish River (GFR) and Riviersonderend River (RSE). Significant differences considered as $p < 0.05$ and denoted with an asterisk (*) in a table for each figure.

There was a significant difference between the mean infracommunity species richness (*p = 0.0179*) (**Fig. 5.4. A**) of GFR (mean S = 4.73) and RSE (mean S = 3.20), but not mean abundance (*p = 0.9603*) (**Fig. 5.4. B**). The mean species richness of GFR (mean S = 4.73) was significantly higher than that of GD (mean S = 2.47; *p = 0.0004*), the source population of the GFR *C. gariepinus*. The Brillouin's diversity index (D_B), that measures finite diversity at infracommunity level, and Shannon-Wiener diversity index (H'), which measures the component community diversity, both indicated that high diversity was present at GFR (D_B = 0.914; H' = 1.15) and RSE (D_{BD} = 0.769; H' = 0.917) (**Fig. 5.4. C**, **D**). The Simpson diversity index (D) indicated that a moderate diversity was present at all three localities: GD (D = 0.5813), GFR (D = 0.5839) and RSE (D = 0.5747) (**Fig. 5.4. E**). Mean parasitic species abundances were evenly spread among host individuals from GD (J' = 1.006), GFR (J' = 1.164) and RSE (0.8431) and did not significantly differ between localities (*p > 0.05*) (**Fig. 5.4. F**).

Based on the Bray Curtis similarity coefficient, and stress level of 0.12, the infracommunities of GFR and GD primarily overlapped with one another, while the infracommunities of GFR and RSE are distinct (**Fig. 5.5.**). The analysis of similarity (ANOSIM), or dissimilarity matrix, indicated that the community assemblages of RSE were distinct, compared to those of GFR and GD, while the latter two share highly similar community assemblages (see **Table 5.5.**). The Global R-statistic was defined at > 0.583 for distinct infracommunity assemblages.

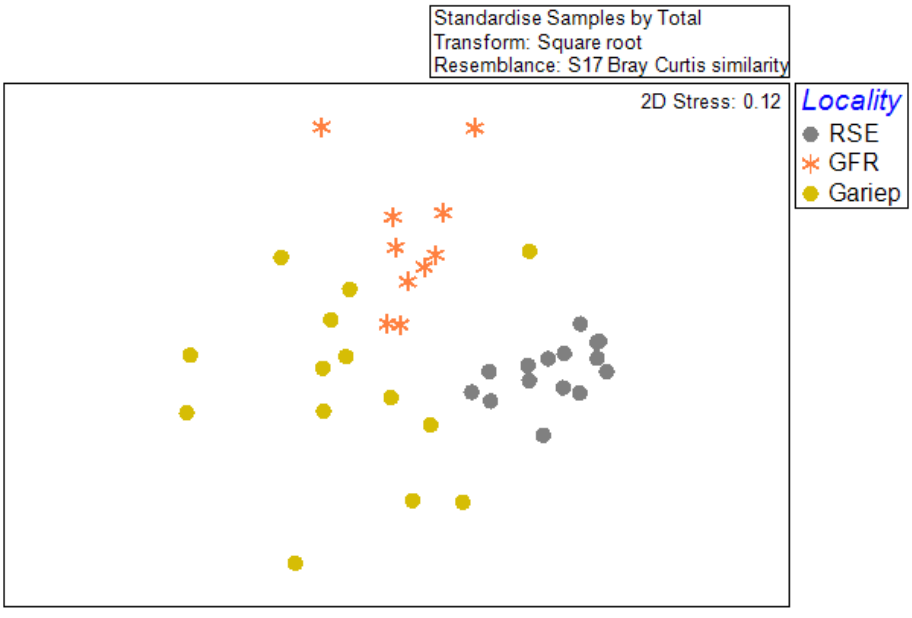

Figure 5.5. Non-metric Multi-dimensional Scaling (nMDS) scatter plot explaining the diversity and abundance of the parasite infracommunities of Clarias gariepinus (Burchell) from Gariep Dam, Great Fish River and Riviersonderend.

Table 5.5. ANOSIM analysis R-statistic (below diagonal) for each locality where 0.5 < R < 0.75 indicate distinct community assemblages; 0.25 < R < 0.5 indicate similar assemblages and highly similar assemblages is observed when, 0.1 < R < 0.25. Statistical significance (above diagonal) between community assemblages regarded as 0.1%.

		1	2	3
1	Riviersonderend River	–	0.1	0.1
2	Great Fish River	0.922	–	0.2
3	Gariep Dam	0.576	0.258	–

Molecular identification of parasite species

Identification of the following species from RSE and GFR were confirmed through comparison of sequence data obtained to congeneric sequences available from GenBank. Accession numbers of comparative sequences are listed in **Appendix 4**. In the present study, the following sequences were generated from parasite species found to parasitise *C. gariepinus* in the translocated regions: for *O. batrachoides* and *T. mashonensis,* the 18Sr RNA, ITS1-2 regions and 28S rRNA; for *T. ciliotheca* and *S. acheilognathi,* the 28S rRNA region and *cox*1 mitochondrial gene region and the *cox*1 mitochondrial gene region for *Paracamallanus* sp. A; and the 18S rRNA region for *Ergasilus* sp. A.

The assignment of specimens from the intestine of *C. gariepinus* from GFR (MT18) to *O. batrachoides* is confirmed in the low divergence observed between congeners, also found from the intestine of *C. gariepinus* in the native range localities investigated and to that of individuals found in the intestine of *C. gariepinus* in Egypt and Mozambique (**Table 5.6.**)

Sequence data generated for the ITS1-2 and 28S rRNA regions for the isolate of *T. mashonensis* (24FR1) is conspecific to the isolates infecting *C. gariepinus* from NGR in the native range, as well as to the isolate of *T. mashonensis* previously found from the cranial cavity of *C. gariepinus* in South Africa (**Table 5.7.**) (Moema et al. 2013).

Alignment of the 28S rRNA region for *T. ciliotheca* with congeners confirmed the assignment of specimens from RSE as *T. ciliotheca* (**Table 5.8.**)

Comparison of the *Paracamallanus* isolate from RSE (MT36) to the isolates previously published for *Pa. cyathopharynx* (MN523683) and the newly obtained sequences from the native range in the present study, indicate that MT36 is identical to isolates MT46 and MT47 infecting *C. gariepinus* collected from RR and BS (**Table 5.9.**). Supporting the assignment of the camallanids from RSE to *Paracamallanus* sp. A.

Table 5.6. Divergence of species of *Orientocreadium* based on the 18S rRNA (919 bp), ITS1-2 (536 bp) and 28S rRNA (1312 bp) regions. Values for p-distance (bottom diagonal) are expressed in percentage, while the top diagonal represent the number of differences in nucleotides. Species in bold were obtained from material collected during the present study. Abbreviations: BS – Boskop Dam; EG – Egypt; GFR – Great Fish River; MZ – Mozambique; ZR – Barotse floodplain.

18S rRNA		1	2	3	4	5
1	**MT18 *Orientocreadium batrachoides* GFR**	–	1	0	10	14
2	**MT8 *O. batrachoides* ZR**	0.1	–	1	9	13
3	**MT4 *O. batrachoides* BS**	0.0	0.1	–	11	15
4	MT797949 *O. batrachoides* EG	1.3	1.1	1.3	–	5
5	MK496889 *O. batrachoides* MZ	1.9	1.6	1.8	0.6	–
ITS1-2		1	2	3	4	5
1	**MT18 *Orientocreadium batrachoides* GFR**	–	2	0	1	10
2	**MT8 *O. batrachoides* ZR**	0.4	–	2	2	9
3	**MT4 *O. batrachoides* BS**	0.0	0.4	–	1	10
4	MZ600135 *Orientocreadium* sp. ZI	0.4	0.7	0.4	–	10
5	MZ600136 *Orientocreadium* sp. ZI	2.9	2.5	2.9	2.3	–
28S rRNA		1	2	3	4	5
1	**MT18 *Orientocreadium batrachoides* GFR**	–	7	2	25	24
2	**MT8 *O. batrachoides* ZR**	0.6	–	9	25	25
3	**MT4 *O. batrachoides* BS**	0.2	0.8	–	27	26
4	MT797948 *O. batrachoides* EG	1.9	2.1	2.1	–	6
5	MK496882 *O. batrachoides* MZ	1.8	2.0	1.9	0.2	–

Table 5.7. Divergence of species of *Tylodelphys* based on the partial ITS1-2 (1293bp), 28S rRNA (1276 bp) and *cox*1 mitochondrial (838 bp) regions. Values for p-distance (bottom diagonal) are expressed in percentage, while the top diagonal represent the number of differences in nucleotides. Species in bold were obtained from material collected during the present study. Abbreviations: GFR – Great Fish River; NGR – Ndumo Game Reserve; SA – South Africa; TA – Tanzania; ZR – Barotse floodplain.

ITS1-2		1	2	3	4	5	6	7	8	9	10	11	12	13
1	FJ470400 *Tylodelphys* sp. 1 TA	–	32	49	8	39	51	36	53	52	53	52	44	57
2	FJ470401 *Tylodelphys* sp. 2 TA	2.6	–	47	37	13	49	36	51	50	51	50	43	55
3	FJ470402 *Tylodelphys mashonensis* TA	3.9	4.1	–	48	48	5	48	6	5	5	4	3	12
4	**MT19 *Tylodelphys* cf. *grandis* ZR**	0.6	3.0	3.7	–	33	45	31	49	47	49	46	43	51
5	**MT20 *Tylodelphys* cf. *grandis* ZR**	3.0	0.9	3.9	2.1	–	43	31	47	45	47	44	43	49
6	**MT21 *Tylodelphys* cf. *grandis* ZR**	4.1	4.3	0.3	3.1	3.1	–	45	4	2	4	2	0	7
7	**MT22 *Tylodelphys* cf. *grandis* ZR**	2.7	3.1	3.9	2.0	2.1	3.3	–	49	47	49	46	42	49
8	**1US1 *T. mashonensis* NGR**	4.1	4.3	0.2	3.1	3.1	0.0	3.3	–	4	7	5	4	9
9	**1US2 *T. mashonensis* NGR**	4.1	4.3	0.2	3.1	3.1	0.0	3.3	0.0	–	3	2	2	9
10	**3US2 *T. mashonensis* NGR**	4.2	4.4	0.2	3.2	3.2	0.1	3.3	0.0	0.0	–	4	4	11
11	**3BB1 *T. mashonensis* NGR**	4.2	4.4	0.2	3.2	3.2	0.1	3.3	0.0	0.0	0.0	–	1	9
12	**32SR1 *T. mashonensis* NGR**	3.5	3.8	0.2	3.0	3.2	0.0	3.1	0.0	0.0	0.1	0.1	–	7
13	**24FR1 *T. mashonensis* GFR**	4.0	4.2	0.3	3.0	3.0	0.0	3.2	0.0	0.0	0.1	0.1	0.0	–

28S rRNA		1	2	3	4	5	6	7	8	9	10	11	12
1	KF189071 *T. mashonensis* SA	–	14	11	12	0	15	1	5	0	0	1	4
2	KF189072 *Diplostomum* sp. SA	1.0	–	8	13	14	15	15	19	13	13	14	15
3	**MT19 *Tylodelphys* cf. *grandis* ZR**	0.9	0.7	–	9	11	11	12	12	11	11	12	11
4	**MT20 *Tylodelphys* cf. *grandis* ZR**	0.9	1.1	0.8	–	12	8	13	16	12	12	13	14
5	**MT21 *Tylodelphys* cf. *grandis* ZR**	0.0	1.0	0.9	0.9	–	15	1	5	0	0	1	4
6	**MT22 *Tylodelphys* cf. *grandis* ZR**	1.0	1.1	0.9	0.6	1.0	–	16	20	14	14	15	16
7	**1US1 *Tylodelphys mashonensis* NGR**	0.1	1.1	1.1	1.0	0.1	1.1	–	6	1	1	2	5
8	**1US2 *Tylodelphys mashonensis* NGR**	0.0	1.0	1.0	0.9	0.0	1.0	0.1	–	4	4	5	6
9	**3US2 *Tylodelphys mashonensis* NGR**	0.0	1.0	0.9	0.9	0.0	1.0	0.1	0.0	–	0	1	4
10	**3BB1 *Tylodelphys mashonensis* NGR**	0.0	1.0	0.9	0.9	0.0	1.0	0.1	0.0	0.0	–	1	4
11	**32SR1 *Tylodelphys mashonensis* NGR**	0.0	1.0	1.0	0.9	0.0	1.0	0.1	0.0	0.0	0.0	–	5
12	**24FR1 *Tylodelphys mashonensis* GFR**	0.0	1.0	0.9	0.9	0.0	1.0	0.1	0.0	0.0	0.0	0.0	–

Table 5.8. Divergence between species of the Bothriocephalidea (Cestoda) based on 28S rDNA (1506 bp) alignments. Values for p-distance (bottom diagonal) are expressed in percentage, while the top diagonal represent the number of differences in nucleotides. Species in bold were obtained from material collected during the present study. Abbreviations: BR – Brazil; BS – Boskop Dam; ET – Ethiopia; GD – Gariep Dam; MD – Madagascar; RSE – Riviersonderend River; SU – Sudan; US – United States of America; VT – Vietnam.

28S rRNA		1	2	3	4
1	**MT52 28S *T. ciliotheca* BS**	–	4	14	49
2	**MT51 28S *T. ciliotheca* RSE**	0.3	–	10	47
3	JQ811835 *Tetracampos ciliotheca* ET	0.9	0.8	–	52
4	JQ811836 *Polyonchobothrium polypteri* SU	3.4	3.7	3.0	–

Comparison of the *Paracamallanus* isolate from RSE (MT36) to the isolates previously published for *Pa. cyathopharynx* (MN523683) and the newly obtained sequences from the native range in the present study, indicate that MT36 is identical to isolates MT46 and MT47 infecting *C. gariepinus* collected from RR and BS (**Table 5.9.**). Supporting the assignment of the camallanids from RSE to *Paracamallanus* sp. A.

Table 5.9. Similarity between species of the Camallanidae based on the *cox*1 mitochondrial gene (685 bp) region alignments. Values denote number of identical nucleotides as percentage (%). Species in bold obtained from material collected during the present study. Abbreviations: BS – Boskop Dam; NGR – Ndumo Game Reserve; RR – Riet River; RSE – Riviersonderend River.

*cox*1		1	2	3	4
1	**MT36 *Paracamallanus* sp. A RSE**	–	0	2	55
2	**MT47 *Paracamallanus* sp. A RR**	0.0	–	2	59
3	**MT46 *Paracamallanus* sp. A BS**	0.4	0.4	–	58
4	**MN523683 *Paracamallanus cyathopharynx* NGR**	11.5	11.6	10.7	–

Morphological assignment of ergasilid specimens found from the gills of *C. gariepinus* from GFR and RSE to *Ergasilus* sp. A is confirmed in the high divergence observed in the highly conservative 18S rRNA region. Isolates from ZR (P33) and VR (P38) were almost identical in having only a 0.1% divergence, while the isolate from GFR (P40) had a 2.6% divergence from its congeners.

Table 5.10. Divergence between species of the Ergasilidae (Copepoda) colecected during the present study based on the 18S rRNA region (1634 bp) alignment. Values for p-distance (bottom diagonal) are expressed in percentage, while the top diagonal represents the number of differences in nucleotides.

18S rRNA		1	2	3
1	**P38 *Ergasilus* cf. *mirabilis* VR**	–	17	43
2	**P33 *Ergasilus* cf. *mirabilis* ZR**	0.1	–	60
3	**P40 *Ergasilus* sp. A GFR**	2.6	2.7	–

5.4. Discussion

General observations

This is the first study to present parasitic community data of translocated populations of *C. gariepinus* in South Africa. A total of nine and eight species were found to parasitise various organs of the sharptooth catfish from in the GFR and RSE, respectively, where it is a translocated species. The parasitic community of RSE was represented by species of four major taxa, the Monogenea (3); Cestoda (2), Nematoda (1) and Copepoda (1), however no representatives of the Digenea were present. From GFR, a similar species richness was observed, however species composition was different. The same five species, *Q. aegypticus*, *Q. allobychowskiella*, *Q. clariadis*, *Q. fornicatus,* and *Quadriacanthus* sp. H were present at both GFR and GD, however, no Digenea, Nematoda or Copepoda infected *C. gariepinus* from GD, while two digeneans, *O. batrachoides* and *T. mashonensis*, one nematode *Contracaecum* sp. C and one copepod, *Ergasilus* sp. A were found from *C. gariepinus* from GFR. Fewer monogenean species (3) were present at RSE, of which one species, *Q. pravus*, did not occur at GFR, but have been found from *C. gariepinus* at two other localities (NGR and ZR) in the native range during the present study (**Chapter 3**).

The infestation of *C. gariepinus* with the invasive *A. japonicus* from GD is the second report of this co-invasion in the dam, previously also reported in the thesis of Mogorosi (2019). *Schyzocotyle acheilognathi* infecting *C. gariepinus* in RSE is a first report on the spill-over of this invasive into the Western Cape, second to the spill-over to *L. aeneus* in the Great Fish River. All other parasitic species found during this study represent specialist species of *C. gariepinus*, while the co-introduced *Quadriacanthus* sp. H is new to the parasite fauna of *C. gariepinus* (see **Appendix A2).** The infestation of *C. gariepinus* with *Ergasilus* sp. A is most likely parasite spillback from a native fish species that occurred in both GFR and RSE since it is not conspecific to *Ergasilus* cf. *mirabilis* found from *C. gariepinus* from the localities on the Vaal River (BS and VR) and in the Ndumo Game Reserve. The only species of *Ergasilus* von Nordmann, 1832 known from the coastal provinces of South Africa are *Ergasilus ilani* Oldewage et Van As, 1988; *Ergasilus lizae* Krøyer, 1863 and *Ergasilus sieboldi* von Nordmann, 1832, all known from marine hosts. The identity of *Ergasilus* sp. A need to be confirmed since it does not represent any species previously reported from *C. gariepinus* or from other hosts in the country.

Alien and invasive parasites on *Clarias gariepinus*

From the literature review on the alien and invasive species infecting *C. gariepinus* in South Africa, it is not surprising that the native GD population was infected with the generalist ectoparasite, *A. japonicus*. Although no previous records of *A. japonicus* have been published from the Orange River system, or from *C. gariepinus*, the thesis of Mogorosi (2019) provided evidence that *A.*

japonicus was already present in the system, infecting the large cyprinid, *L. aeneus* from GD. Moreover, during this study *A. japonicus* was also found from *L. aeneus* and *Labeo capensis* (Smith) from the Knellpoort Dam at the confluence of the Klip- and Rietspruit rivers with the Caledon River. This is a tributary of the Orange River and the alien invasive *C. carpio*, with which *A. japonicus* is thought to have been introduced with, is also present in GD. The absence of *A. japonicus* from all other localities investigated on the Vaal River should not be seen as the definite absence of this invasive, since several records have been published where *C. gariepinus,* along with other native or endemic host species from tributaries and impoundments the Vaal River (Bloemhof Dam, Barberspan wetland, Mooi River), have been infected by this opportunistic invasive (see above and Smit et al. 2017 and references therein).

The fact that the Asian tapeworm, *S. acheilognathi,* was not found from *C. gariepinus* collected at any of the localities in its native range, including GD during the present study, is not evident of its total absence from these systems. *Schyzocotyle acheilognathi* has been reported on one occasion from *C. gariepinus* from GD, and several native cyprinids have been parasitised by this invasive species along the Vaal River (Smit et al. 2017). The report of *S. acheilognathi* from *C. gariepinus* from GD in the thesis of Mogorosi (2019) is the only existing record in South Africa and it should be monitored to see if the spill-over to any other natives such *L. aeneus*, also previously infected, amplifies or causes mortality. Introduction of *S. acheilognathi* into GFR was most probably mediated with the introduction of the invasive *C. carpio* into GD. As for the presence of *S. acheilognathi* in the intestine of *C. gariepinus* from RSE, it is a first record of this alien invasive from any fish in the Western Cape, and was most likely co-introduced with *C. carpio*, an alien invasive species already present in several of the freshwater systems of the Western Cape, including the Breede and Riviersonderend Rivers (see Paxton 2018). Thus, spillback occurred to another invasive in the Western Cape, and should be closely monitored. Very little information is available on the impact of this generalist invasive on native fish populations; however, infection is associated with high pathogenicity in infected hosts, and is of specific concern to small fishes, in which it causes intestinal blockages leading to reduced growth and survival (see Scholz et al. 2012). Moreover, there is a very high endemicity of small fishes in the Western Cape (see **Chapter 2**, **Section 2.2.5.**) that are at high risk to an invasive that can grow extremely large within a small host, leading to increased morbidity and eventual mortality.

Furthermore, the complexity of the success in invasions is highlighted by the findings in this study. The communities of the translocated populations are rather diverse, with eight and nine parasite species present at GFR and RSE, respectively, similar in richness to the southernmost native communities of *C. gariepinus*. Five specialist monogeneans (*Q. aegypticus*; *Q. allobychowskiella*; *Q. clariadis*; *Q. fornicatus* and *Quadriacanthus* sp. H) were all co-introduced from GD into the

GFR, while the specialist cestodes *B. lafoni* and *T. ciliotheca* were absent, providing support for enemy release upon introduction into GFR. The translocation of *C. gariepinus* via the Breede tunnel further supports enemy release, with only three co-introduced monogeneans (*Q. clariadis*; *Q. fornicatus* and *Q. pravus*) present, while *T. ciliotheca* did not "shed" from *C. gariepinus* during the introduction event into RSE. A specialist nematode, *Paracamallanus* sp. A, only found in the Vaal River (from Boskop Dam and Riet River in this study) was also present in *C. gariepinus* from RSE, and *Ergasilus* sp. A was present on *C. gariepinus* from both GFR and RSE. From the community composition it is evident that there is great disparity in our knowledge of the true parasitic communities of *C. gariepinus*. The possible translocation of the parasitic community of *C. gariepinus* from GD into GFR can be seen with the communities having similar monoxenous specialist species present (Monogenea). The importance of intermediate hosts was also highlighted by the absence of specialist heteroxenous cestodes, but presence of digeneans (also heteroxenous) at GFR, while the inverse was true for GD. The parasitic community of the RSE population, however, paints a different picture and the source population of these *C. gariepinus* may be from systems other than the Vaal or Orange rivers, especially regarding the presence of *Q. pravus* in RSE. *Quadriacanthus pravus* has only been reported from its type locality in the Sudan, and during the present study it was found only from *C. gariepinus* in Zambia (Barotse floodplain) and the Ndumo Game Reserve (see **Chapter 3** and **Table A5.1**). Furthermore, *Q. pravus* was not detected on the gills of the sharptooth catfish collected from the Orange-Vaal system, further supporting a hypothesis that the introductions of *C. gariepinus* into the Western Cape might include populations from a wider geographic range. Although some specialist heteroxenous species such as *T. ciliotheca* are present, the absence of the monoxenous species, in particular *Q. pravus,* and the heteroxenous nematode *Paracamallanus* sp. A and *Contracaecum* sp. C may support a further two hypotheses; 1) the preceding hypothesis of a different source population within the Southern Cape ichthyofaunal region is correct; 2) suitable intermediate copepods are not present at GD and GFR for *Paracamallanus* sp. A to continue its life cycle to the fish intermediate host.

From the above findings it is clear that *C. gariepinus* is a fortress for parasites, housing a number of parasite specialists, and is potentially a hub for invasive parasites too. With regard to the four ecological hypotheses of invasions, the findings of the present study support the enemy release hypothesis, as well as spillback. However, in order to test the dilution effect and spill-over there is a dire need to perform comprehensive parasitological surveys of indigenous and endemic fishes of the Eastern and Western Cape.

Chapter 6

The role of environmental variables in the metazoan parasitic community composition of *Clarias gariepinus* (Burchell)

6.1. Introduction

The use of parasitic species as indicators of environmental impacts has been a topic of interest for three decades. The establishment of parasitic species in ecotoxicological studies has resulted in coining of the term “Environmental Parasitology” (EP), which is currently a widely accepted discipline (Goater et al. 2014 and references therein). Lafferty (1997) concluded that environmental change affects parasites, and they may be indicators of anthropogenic stress or impacts. The stress may be observed through,1) an increase in parasitic infection due to a decrease in host resistance because of environmental impact, or an increase in intermediate or definitive host density; and 2) a decrease in parasitic infection due to declines in intermediate or definitive hosts, or direct toxicity to parasites exposed to impacted environments and indirectly through differentially high mortality. At the time, few studies provided association data for these opposing scenarios, and Lafferty (1997) indicated that suspected drivers, i.e., pollutants, are measured, but physical or ecological impact is not quantified. Subsequently, several authors advocated for the inclusion of parasitic species as sentinels in assessment of ecosystem health or effect monitoring along with their hosts and suitable indices (see Poulin 1992; Lafferty and Kuris 1999; Macogliese 2004; Lafferty 2008, 2012; Blanar 2009; Nachev and Sures 2016; Sures et al. 2017a, b).

Following reviews on application and relevance of EP, as well as identifying suitable taxa as indicators or sentinels, Blanar et al. (2009) summarised the effect size of parasites to contaminants at the time. The authors found that the most common responses documented were to eutrophication and elemental contamination, and that these responses were highly variable among individual taxa i.e., metal pollution exerted significantly negative effects on Monogenea and Digenea at population level, and only slight impacts were found on community richness; while monoxenous species, ectoparasites, and free-living transmission stages of endoparasites were more susceptible to a wide range of pollutants compared to internal (endo) parasites.

Parasites have been used as accumulation indicators, effect indicators and as interacting agents with pollutants (Sures et al. 1999, 2017b). Acanthocephala and Cestoda species have been identified as the most suitable sentinels in ecosystems that have high accumulation capabilities and are tolerant to a wide array of pollutants (metals and organic compounds). Furthermore, these taxa are also suitable as bioavailability indicators and toxic-effect determinants due to their simple physiology (i.e., tegument, lack of digestive system), compared to Nematoda (probably suitable as an indicator for thermal pollution or eutrophication) that possess complex morphology and physiological barriers (i.e., cuticle, membranes of/encapsulated larvae, digestive system) (Sures et al. 1999, 2017a, b; Sures 2001, 2003, 2004, 2008; Nachev and Sures 2016).

However, the parasite fauna of South African freshwater fishes is naturally poor in acanthocephalans (see Halajian et al. 2018), and *C. gariepinus* only harbours representatives of this taxon in North Africa (see **Chapter 3.8**. and **Table A3.10**). Although present, cestodes do not necessarily contribute significantly to the parasite community of *C. gariepinus* in southern Africa where a general low abundance and prevalence is found or are prevalent, but low in abundance for the two species, *Tetracampos ciliotheca* Wedl, 1861 and *Proteocephalus glanduligerus* (Janicki, 1928) (Mashego and Saayman 1989; Barson and Avenant-Oldewage 2006b; Madanire-Moyo et al. 2010; Madanire-Moyo and Avenant-Oldewage 2013a; Sara et al. 2014). Thus, the search for sentinels and effect indicators in southern Africa focusses primarily on the other major parasitic taxa. Where *C. gariepinus* is concerned, a single study along the Vaal River system assessed the potential of the monoxenous copepod *Lamproglena clariae* Fryer, 1956 as effect indicator of nutrient levels in water, and metals in both water and sediment, but not in the host or parasite (Pretorius and Avenant-Oldewage 2021). Since the Vaal River has been the subject of recent studies relating aquatic biota community responses to different pollutants such as metals and organic pollutants (organochlorines and perfluorinated compounds) (Wepener et al. 2011; Groffen et al. 2018), this system was selected as the study area. There are limited studies that focus on parasitic community responses to pollutants (e.g., metal element) exposure as reflected by the metal concentrations in hosts tissue. Therefore, the aims of this chapter were to assess the diversity of parasitic communities in *C. gariepinus* and determine the drivers affecting parasitic community composition between localities associated with different physical (lotic or lentic systems), environmental conditions such as elemental pollution sources, using five native populations from the Orange-Vaal River system as case study.

6.2. Results

Infra-, component and supracommunities of the Orange and Vaal River system

A total of 11 466 metazoan parasite specimens were collected from 75 individuals of *C. gariepinus* from five localities on the Orange and Vaal rivers in South Africa. These represented 19 parasite species grouped into five major taxonomic groups (**Table 6.1.**). The 19 parasite species were represented by nine heteroxenous species (six adult and three larval stages) and ten monoxenous species. All genera have previously been reported from *C. gariepinus* (see **Chapter 3** and **Appendix A3** and references therein). Boskop Dam (BS) and Vaal River (VR) had a higher component community richness of 14 and 13 species, respectively, compared to the 10 (Sand River, SR), nine (Riet River, RR) and eight (Gariep Dam, GD) species present at the other localities (**Table 6.1.**). Infracommunity species richness was significantly higher at BS (S = 6.2) compared to VR (S = 4.6; $p = 0.0345$), SR (S = 2.9; $p < 0.0001$), RR (S = 3.5; $p = 0.0002$) and GD (S = 2.4; $p = 0.0016$). Similarly, the mean species richness was statistically higher at VR compared to that observed for SR ($p = 0.0254$) (**Fig. 6.1. A**). Infracommunity species abundance varied between the sampled communities, where GD had a significantly lower infracommunity mean abundance than BS, VR and SR ($p < 0.0001$) (**Fig. 6.1. B**). This variation can be explained by the presence of dominant taxa, *Orientocreadium batrachoides* (MA = 88.3; d = 0.503), *Macrogyrodactylus congolensis* (MA = 25.7; d = 0.518) and *Tylodelphys mashonensis* (MA = 230.2; d = 0.941) at the latter three localities, respectively (see **Table 6.1.** for detail on mean abundances). The mean finite diversity of the infracommunity, calculated with the Brillouin's diversity index (D_B), sampled from each locality was significantly higher at BS (D_B = 0.868) and VR (D_B = 0.847), compared to the lower mean index values for GD (D_B = 0.425; $p = 0.016$ and $p = 0.0111$) (**Fig. 6.1. C**). The mean index values for SR (D_B = 0.293) were also significantly lower than observed for BS (D_B = 0.868; $p = 0.0007$), VR (D_B = 0.847; $p = 0.0007$) and RR (D_B = 0.740; $p = 0.0227$).

Table 6.1. Descriptive statistics for individual metazoan parasitic communities of *Clarias gariepinus* (Burchell) from the Orange and Vaal River systems in South Africa. Abbreviations: P – prevalence (%); MA – mean abundance; MI –mean intensity; n – number of host individuals screened and infected per locality indicated in brackets after locality name; dominant species indicated in bold. Component community defined as taxa richness [S], life cycle ratio indicated as S_H:S_M below heading, M – monoxenous; HA – heteroxenous adult and HL – heteroxenous larvae.

Taxon	Species	Life cycle [S_H:S_M]	P %	MA	MI
Boskop Dam [n = 15/15]	Component community [14]	1:1			
Monogenea	*Quadriacanthus aegypticus*	M	80.0	3.7	4.6
	Quadriacanthus fornicatus	M	53.3	1.1	2.0
	Quadriacanthus clariadis	M	33.3	0.8	2.4
	Quadriacanthus allobychowskiella	M	6.7	0.1	1.0
	Quadriacanthus sp. H	M	93.3	9.0	9.6
Digenea	*Tylodelphys mashonensis*	HL	66.7	63.6	95.4
	Orientocreadium batrachoides [a]	**HA**	**73.3**	**88.3**	**120.4**
Cestoda	*Barsonella lafoni*	HA	73.3	2.9	4.0
	Tetracampos ciliotheca	HA	20.0	0.4	2.0
Nematoda	*Contracaecum* sp. C	HL	93.3	9.0	9.6
	Paracamallanus sp. A	HA	40.0	2.7	6.8
	Procamallanus (*P.*) *pseudolaeviconchus*	HA	40.0	1.9	4.7
Crustacea	*Ergasilus* cf. *mirabilis*	M	6.7	0.1	1.0
	Lamproglena clariae	M	20.0	0.2	1.0
Vaal River [n = 18/18]	Component community [13]	1:1.6			
Monogenea	*Quadriacanthus aegypticus*	M	16.7	0.2	1.3
	Quadriacanthus fornicatus	M	11.1	0.5	4.5
	Quadriacanthus clariadis	M	16.7	0.2	1.3
	Quadriacanthus allobychowskiella	M	5.6	0.1	1.0
	Gyrodactylus rysavyi	M	5.6	0.1	1.0
	Macrogyrodactylus congolensis [b]	**M**	**83.3**	**25.7**	**30.8**
Digenea	*Tylodelphys mashonensis*	HL	16.7	0.3	2.0
	Diplostomidae gen. sp. 1	HL	16.7	0.2	1.0
Nematoda	*Contracaecum* sp. C	HL	27.8	0.4	1.4
	Paracamallanus sp. A	HA	88.9	10.1	11.4
	Spinitectus sp.	HA	44.4	1.3	3.0
Crustacea	*Ergasilus* cf. *mirabilis*	M	50.0	0.9	1.9
	Lamproglena clariae	M	77.8	9.6	12.3
Sand River [n = 14/15]	Component community [10]	1:1			
Monogenea	*Quadriacanthus aegypticus*	M	64.3	2.3	3.6
	Quadriacanthus fornicatus	M	21.4	0.4	1.7
	Quadriacanthus clariadis	M	7.1	0.1	1.0
	Quadriacanthus allobychowskiella	M	14.3	0.2	1.5
Digenea	***Tylodelphys mashonensis*** [c]	**HL**	**78.6**	**230.2**	**293.0**
Cestoda	*Barsonella lafoni*	HA	57.1	7.1	12.5
	Tetracampos ciliotheca	HA	21.4	2.8	13.0
Nematoda	*Contracaecum* sp. C	HL	7.1	0.1	1.0
	Paracamallanus sp. A	HA	14.3	1.4	9.5
Crustacea	*Argulus japonicus*	M	7.1	0.1	1.0
Riet River [n = 12/12]	Component community [9]	1.25:1			
Monogenea	*Quadriacanthus aegypticus*	M	83.3	6.1	7.3
	Quadriacanthus fornicatus	M	8.3	0.1	1.0
	Quadriacanthus clariadis	M	33.3	0.9	2.8
	Quadriacanthus allobychowskiella	M	16.7	0.2	1.0
Digenea	Diplostomidae gen. sp. 1	HL	8.3	0.3	4.0
Cestoda	*Barsonella lafoni*	HA	75.0	5.7	7.6
	Tetracampos ciliotheca	HA	16.7	3.2	19.0
Nematoda	*Contracaecum* sp. C	HL	16.7	0.3	1.5
	***Paracamallanus* sp. A** [d]	**HA**	**91.7**	**13.8**	**15**

Table 6.1. (continued) Descriptive statistics for individual metazoan parasitic communities of ***Clarias gariepinus*** (Burchell) from the Orange and Vaal River systems in South Africa. Abbreviations: P – prevalence (%); MA – mean abundance; MI –mean intensity; n – number of host individuals screened and infected per locality indicated in brackets after locality name; dominant species indicated in bold. Component community defined as taxa richness [S], life cycle ratio indicated as S_H:S_M below heading, M – monoxenous; HA – heteroxenous adult and HL – heteroxenous larvae.

Taxon	Species	Life cycle [S_H:S_M]	P %	MA	MI
Gariep Dam [n = 15/15]	Component community [8]	1:3			
Monogenea	*Quadriacanthus aegypticus*	M	40.0	0.8	2.0
	Quadriacanthus fornicatus	M	40.0	0.8	2.0
	Quadriacanthus clariadis	M	26.7	0.4	1.5
	Quadriacanthus allobychowskiella	M	13.3	0.2	1.5
	***Quadriacanthus* sp. H[e]**	**M**	**33.3**	**1.2**	**3.6**
Cestoda	*Barsonella lafoni*	HA	40.0	0.7	1.7
	Tetracampos ciliotheca	HA	13.3	0.5	4.0
Crustacea	*Argulus japonicus*	M	40.0	0.5	1.2

Berger-Parker Dominance values: [a] – 0.503; [b] – 0.518; [c] – 0.941; [d] – 0.452; [e] – 0.237

Diversity of the component communities, expressed as richness and abundance through the Shannon-Wiener diversity index (H'), reflects similar diversity rankings as observed when using D_B, except that H' indicates no difference in diversity for GD when compared to those of BS and VR ($p > 0.05$) (**Fig. 6.1. D**). The Simpson diversity index (D) weakly corroborates ($r^2 = 0.214$) the significantly lower diversity of SR (D = 0.208) compared to that of VR (D = 0.580; $p = 0.0025$), RR (D = 0.516; $p = 0.0413$) and GD (D = 0.581; $p = 0.004$) (**Fig. 6.1. E**). The significantly lower mean diversity (H') of the component community of SR can be explained when the evenness of species distribution is considered, as explained by the Pielou's evenness index (J') that measures diversity as a function of the relative abundance of different taxa within an assemblage. The parasite species present in and on hosts from SR are not evenly distributed (J' = 0.349). The uneven distribution of parasite species at infracommunity level is evident from the scatter plots of species abundance and diversity indices, where the majority of host individuals in the sample have a low species richness, three to four species, and high abundances (**Fig. 6.1. A**, **B**) when compared to the other localities represented by higher species richness in relatively low abundances (**Fig. 6.1. C–F**). For ease of interpretation, **Fig. 6.2.** illustrates the species richness for each component community with an overlay of the mean diversity and evenness index values of the respective indices calculated. Furthermore, the dominant species, *Tylodelphys mashonensis* with a mean abundance of 230.2 ($d = 0.914$) contributes to 84.31% of the community diversity of SR, compared to the relatively low mean abundances (ranging between 0.1 and 19.1) and contributing weights of the other taxa from SR, as determined by the SIMPER analysis (see **Table 6.1.** and **Table 6.2.**).

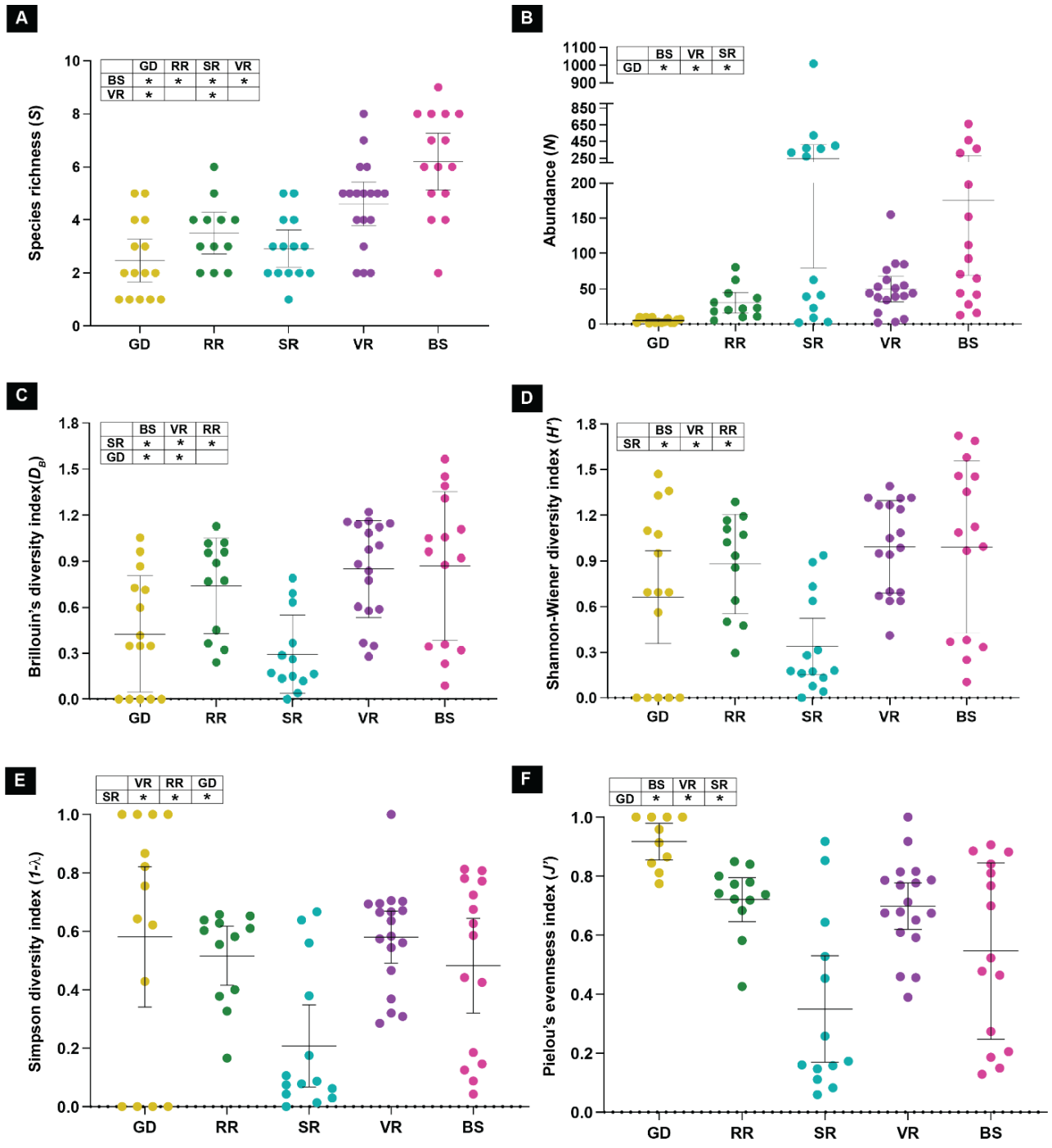

Figure 6.1. Infracommunity composition defined as **A** – taxa richness (S); **B** – abundance (N); **C** – Brillouin's diversity index (DB); **D** – Shannon-Wiener diversity index (H'); **E** – Simpson diversity index (D) and **F** – evenness [Pielou's evenness index (J')]. The mean and 95% confidence interval of each index is presented for Clarias gariepinus (Burchell) from Boskop Dam (BS), Vaal River (VR), Sand River (SR), Riet River (RR) and Gariep Dam (GD). Significant differences considered as $p < 0.05$ and denoted in with an asterisk (*) in the table for each figure.

Table 6.2. SIMPER analysis mean similarity for each locality, and mean abundance (MA), mean similarity ±SD, contribution (%) and cumulative contribution (%) for most abundant species per locality**.**

Locality	MA	Mean similarity ±SD	Contribution %	Cumulative %
Boskop Dam	Mean similarity = 41.52%			
Orientocreadium batrachoides	4.3	11.2 ± 0.68	27.05	27.05
Quadriacanthus sp. H	3.2	9.7 ± 1.69	23.45	50.5
Tylodelphys mashonensis	3.7	7.9 ± 0.59	18.95	69.45
Quadriacanthus aegypticus	1.9	4.2 ± 0.86	10.08	79.53
Barsonella lafoni	1.9	3.8 ± 0.80	9.24	88.78
Quadriacanthus fornicatus	0.9	1.5 ± 0.56	3.58	92.36
Vaal River	Mena similarity = 50.92%			
Macrogyrodactylus congolensis	5.7	20.6 ± 1.24	40.42	40.42
Paracamallanus sp. A	4.1	14.3 ± 1.5	28.1	68.51
Lamproglena clariae	3.6	11.7 ± 1.08	22.89	91.4
Sand River	Mean similarity = 44.96%			
Tylodelphys mashonensis	7.0	37.9 ± 1.08	84.31	84.31
Quadriacanthus aegypticus	1.7	3.2 ± 0.56	7.14	91.44
Riet River	Mean similarity = 50.81%			
Paracamallanus sp. A	5.8	25.0 ± 1.59	49.21	49.21
Quadriacanthus aegypticus	4.0	13.9 ± 1.2	27.29	76.5
Barsonella lafoni	3.5	10.1 ± 0.91	19.83	96.33
Gariep Dam	Mean similarity = 20.83%			
Barsonella lafoni	2.7	5.3 ± 0.35	25.32	25.32
Quadriacanthus fornicatus	2.2	3.6 ± 0.38	17.21	42.53
Argulus japonicus	2.2	3.3 ± 0.37	16.03	58.56
Quadriacanthus aegypticus	2.0	3.0 ± 0.39	14.17	72.73
Quadriacanthus sp. H	2.1	2.9 ± 0.32	13.8	86.53
Quadriacanthus clariadis	1.9	2.3 ± 0.23	11.23	97.76

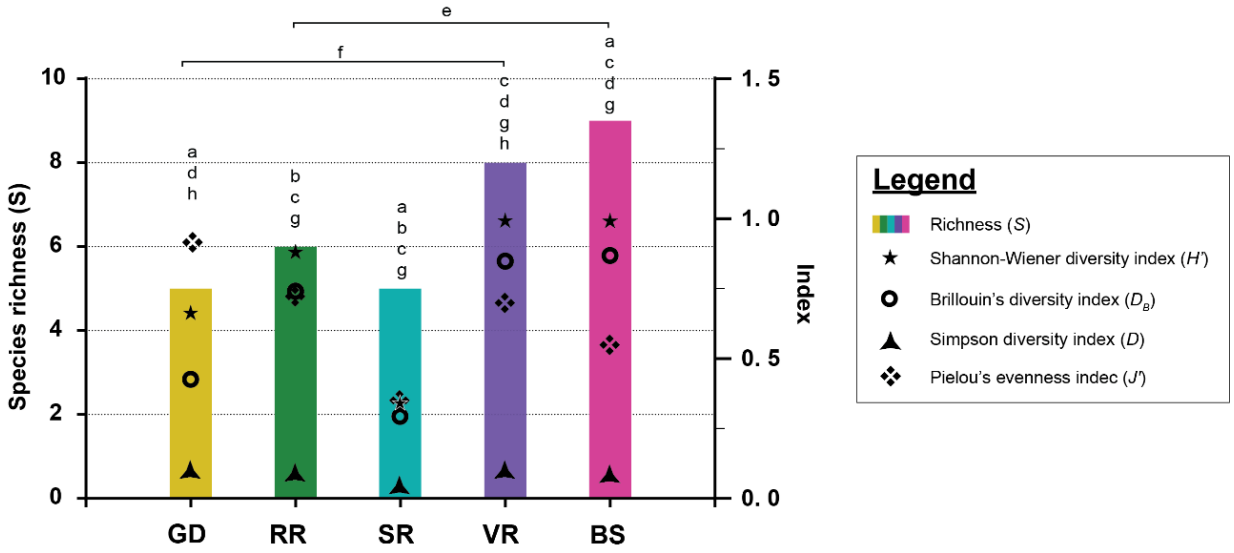

Figure 6.2. Taxa richness from each locality with an overlay of the mean index values for diversity and evenness. Significant differences for all indices were considered as $p < 0.05$ and are indicated between sites for: a, b – taxa richness (solid bars); c, d – Brillouin's diversity index (🅾); e – Shannon-Wiener diversity index (★); f – Simpson diversity index (▲) and g, h – Pielou's evenness index (❖).

A heterogenous distribution is indicated for infracommunity evenness from BS (J' = 0.546) when compared to the homogenous species distribution of VR (J' = 0.698; *p* = *0.2803*), RR (J' = 0.721; *p* = *0.2441*) and a significantly higher evenness observed for GD (J' = 0.916; *p* = *0.0009*) (**Fig. 6.1. F**). Based on the species accumulation curve (**Fig. 6.3.**), the total supracommunity (regional parasite fauna) of *C. gariepinus* in the Orange-Vaal River system is represented in the present study. The initial exponential climb and early plateau suggest that the complete parasitic diversity of *C. gariepinus* is optimally sampled at approximately 16 host individuals (S_{max} = 15.86), and that approximately four host individuals need to be sampled to detect 50% of its parasitic diversity (B = 3.79) in the Orange-Vaal River system.

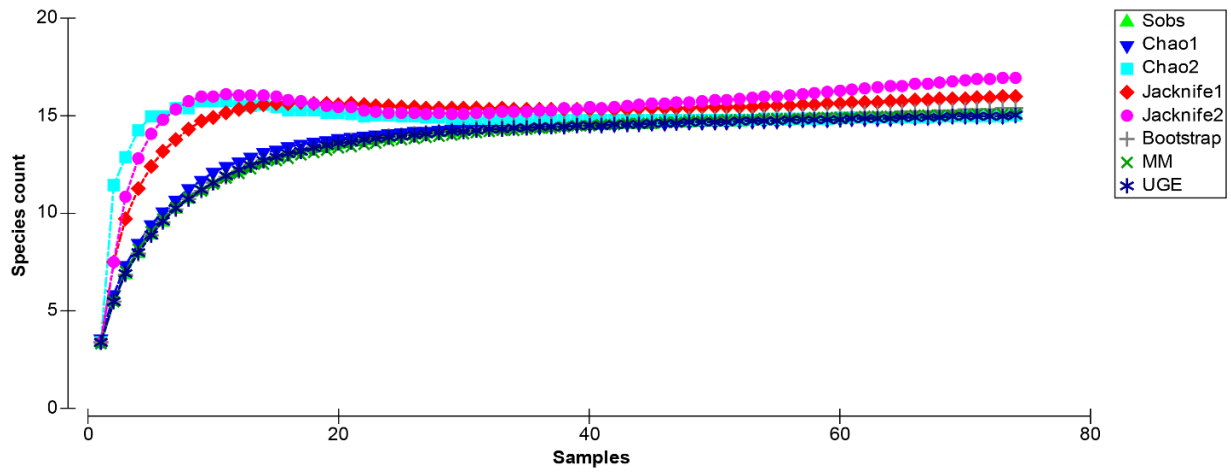

Figure 6.3. Species accumulation curve estimating total species richness as a function of sample size using the following estimators: Sobs – number of species in the sample; Chao1 – based on number of rare species; Chao2 – based on presence-absence data; Jack1 – species that occur in one sample; Jack2 – second order jack-knife for species estimate as a whole ; Bootstrap – proportion of quadrants containing each species; Michaelis-Menton curve (MM) – fitted to Sobs-curve, and UGE – species accumulation curve as estimation of species richness (S_{max} = 15.86; B = 3.79).

Table 6.3. SIMPER, average dissimilarity between localities.

		1	2	3	4	5
1	Boskop Dam					
2	Vaal River	91.27				
3	Sand River	73.17	93.25			
4	Riet River	80.05	78.21	81.57		
5	Gariep Dam	84.55	97.2	90.46	82.14	

Table 6.4. ANOSIM analysis R-statistic (below diagonal) for each locality where 0.5 < R < 0.75 indicate distinct community assemblages; 0.25 < R < 0.5 indicate similar assemblages and highly similar assemblages is observed when, 0.1 < R < 0.25. Statistical significance (above diagonal) between community assemblages regarded as 0.1%.

R > 0.592		1	2	3	4	5
1	Boskop Dam		0.1	0.1	0.1	0.1
2	Vaal River	0.745		0.1	0.1	0.1
3	Sand River	0.328	0.682		0.1	0.1
4	Riet River	0.669	0.628	0.515		0.3
5	Gariep Dam	0.356	0.629	0.348	0.303	

The Non-metric Multi-Dimensional Scaling (nMDS) ordination, based on the Bray-Curtis similarity coefficient, had a stress level of 0.12 (**Fig. 6.4.**). All infracommunities grouped together according to locality, with a 30% overlap in infracommunities of GD, RR and BS. The infracommunity of VR did not show significant overlap with any of the other localities; and is supported by the average dissimilarity between sites, as determined by the SIMPER analysis (see **Table 6.3.**). The ANOSIM analysis, used to test for similarity or distinct infracommunity assemblages, where Global R-values ≥ 0.592 defined distinct infracommunity assemblages. Classification was based on differences chosen for the factor, locality, where $p = 0.001$ (or 0.1%) was considered as significant for the Global R statistic. Distinct community assemblages were present at SR, RR and GD when compared to VR; VR and RR had distinct assemblages compared to BS, while SR and RR also each presented a unique community assemblage (R-statistic for all comparison > 0.5; $p = 0.001$). BS, SR and GD shared similar community assemblages (R-statistic < 0.05; $p = 0.001$), while the similarity in assemblage for RR and GD were not significant (R-statistic < 0.05; $p = 0.003$) (see **Table 6.4.**). A vector overlay of the Pearson's correlation of major taxa abundances, where vector correlations of > 0.1 were included, represent the correlation between taxa where the vector direction and length are defined as an increase in the vector abundance across the plot (**Fig. 6.4.**). Infracommunity assemblages for VR were positively associated with increased abundances of nematodes and parasitic crustaceans, while SR associated with an increasing gradient of Digenea and a negative (decreasing) abundance of Monogenea. Localities BS, RR

and GD all correlated with Monogenea, Cestoda and Digenea with respect to their increasing abundance.

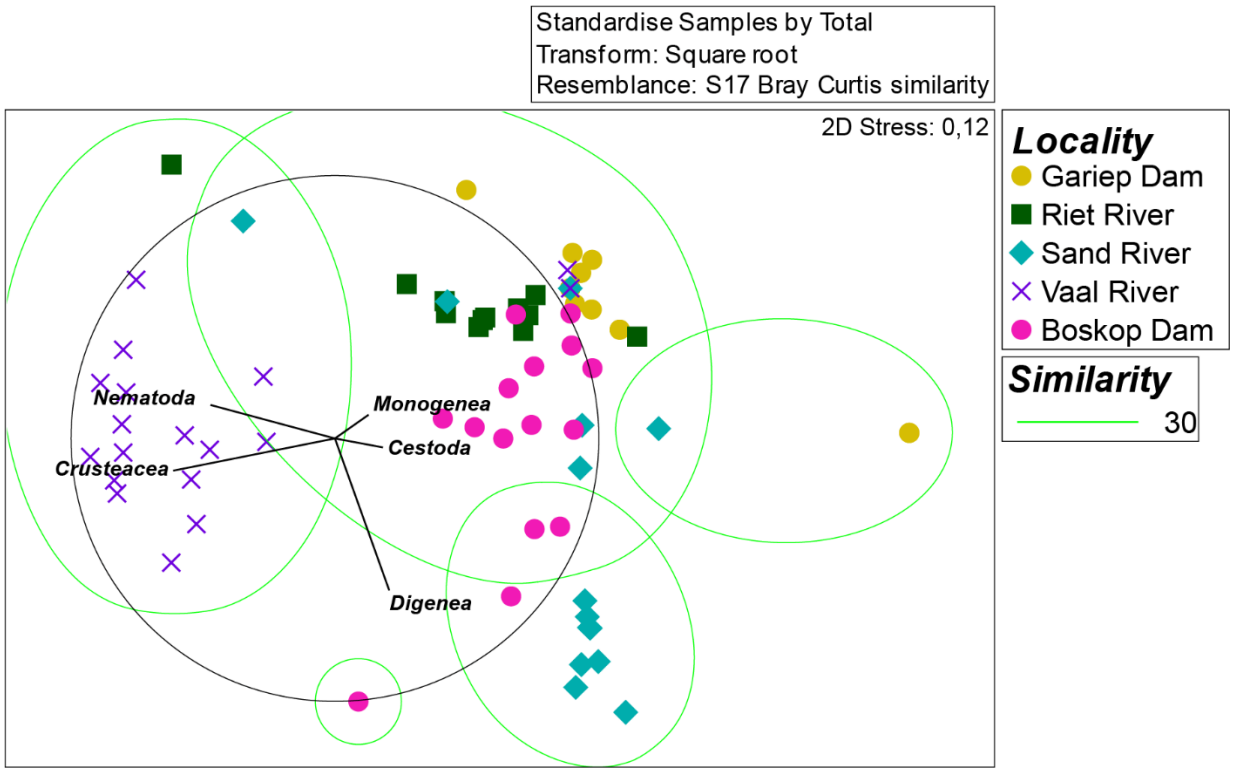

Figure 6.4. Non-metric Multi-dimensional Scaling (nMDS) scatter plot explaining the diversity and abundance of the parasite infracommunities of *Clarias gariepinus* (Burchell) from Boskop Dam, Vaal River, Sand River, Riet River and Gariep Dam. The ordination illustrates similarity between the parasite infracommunities, with a Pearson's correlation vector overlay of the major taxonomic groups (Monogenea, Digenea, Cestoda, Nematoda and Crustacea). Similarity levels (10, 20, 30) selected based on the hierarchical cluster analysis (Resemblance = 50) of Bray Curtis coefficients.

Functional traits

The PCoA analysis (**Fig. 6.5.**) illustrated similarity of the infracommunity abundance explained for all the localities with a Pearson's correlation vector overlay of functional traits selected (life cycle, host sex, size, weight and condition factor (CF); and habitat type). Similar to the nMDS, infracommunity assemblages for each locality were correlated by similar vectors for individuals within each population. Vector length and direction correlates with the increasing magnitude of the vector in relation to the similarities present at each locality. VR associated with monoxenous life cycle type, most probably a result of the presence of the dominant monogenean *M. congolensis* (MA = 25.7; MI = 30.8).

The response curves of communities across all localities sampled indicated that an overall negative association was observed for the Monogenea, Nematoda and Crustacea with respect to the functional traits of host weight, size, and CF (**Fig. 6.6. A**, **B**). The Cestoda had a significant positive response to host weight ($p = 0.02189$) and size ($p = 0.00789$), while the Digenea had a positive response to all three host functional traits, host weight, size, and CF, although not significant (**Fig. 6.6. A**–**C**). At component community level, parasitic abundance had positive and negative responses to functional traits. Here only significant responses ($p < 0.05$) are reported: the Monogenea from BS responded positively to host size ($p\ 0.0296$) and Nematoda negatively to CF ($p = 0.01993$), the Crustacea to weight ($p = 0.02837$) and size ($p = 0.02869$) and Monogenea from RR responded negatively to host weight ($p = 0.01908$) and length ($p = 0.04106$), while Digenea at SR responded negatively to CF ($p = 0.0142$).

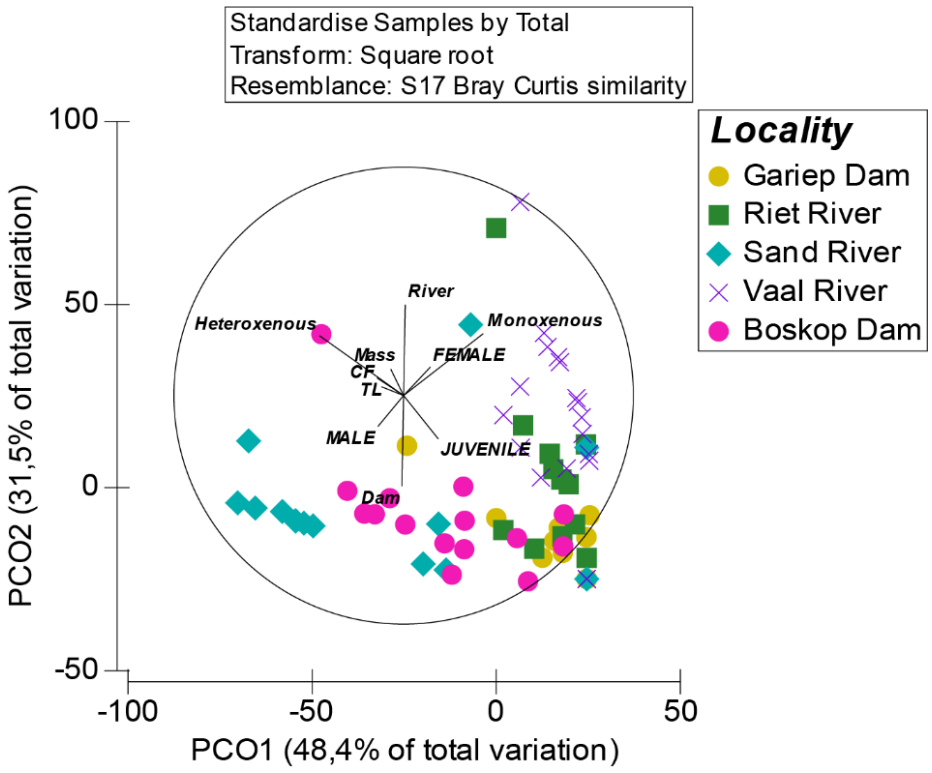

Figure 6.5. Principal Co-ordinate Analysis (PCoA) explaining diversity and abundance of the parasite infracommunities of *Clarias gariepinus* (Burchell) from Boskop Dam, Vaal River, Riet River, Sand River and Gariep Dam. The biplot illustrates similarity between parasite infracommunities for each locality, with a Pearson's correlation vector overlay of functional traits (life cycle, host sex, weight and size and habitat type), correlations of > 0.2 were included.

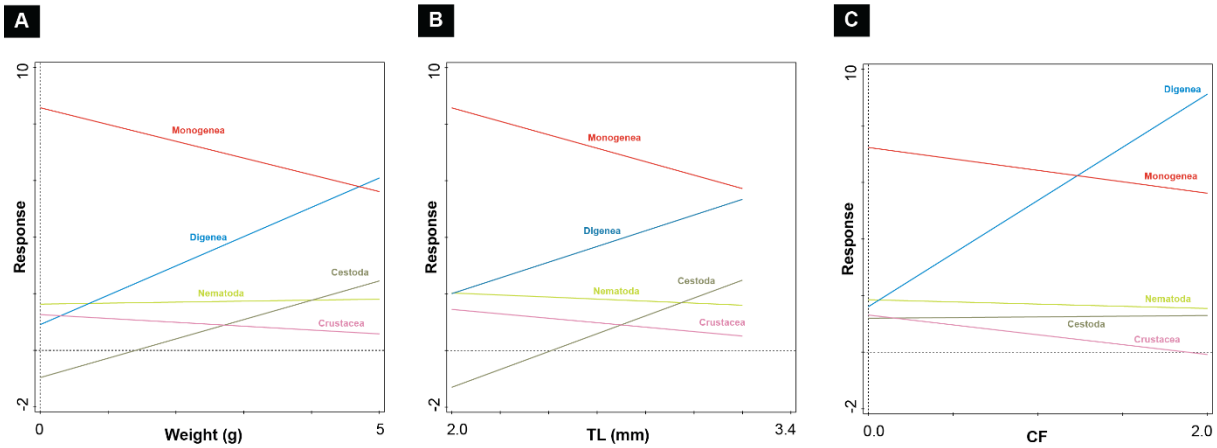

Figure 6.6. Response curves of selected functional traits: **A** – weight; **B** – total length and **C** – condition factor to the major taxonomic groups Monogenea, Digenea, Cestoda, Nematoda and Crustacea. Significant correlations regarded as $p < 0.05$.

Elemental data and parasite communities

There was variation in elemental concentrations and individuals from each locality grouped together, however a clear variation is observed between localities (**Fig. 6.7.**). Boskop Dam associated with Au, Co, Pb and V, and VR associated with higher concentrations of Au, Cd, Cr and Pb. SR associated with As, Co and Hg, while RR correlated with Hg. VR grouped together with the major influencing vectors Au, Al, Cr and Pb, while SR associated with Co and Hg. Localities that associated with increasing concentrations of As and V were RR and GD, while BS had a strong association with As, Co, V and Hg. The generalised linear models (GLM) based on species abundance data and metal concentrations indicated significant correlations between the abundance of major taxonomic groups, Monogenea ($p = 0.00068$); Digenea ($p = 0.02411$); Cestoda ($p = 0.00129$); Nematoda ($p = 0.00075$) and Crustacea ($p < 0.0001$) and some of the metals. The Monogenea had a significant positive response to increasing concentrations of Fe ($p = 0.0007$) and significant negative responses to Co ($p = 0.03686$), Cr ($p = 0.03442$), Hg ($p = 0.00467$), Mn ($p = 0.03324$) (**Fig. 6.8. A**–**E**). Significant positive responses were seen to Co ($p = 0.00446$) and Fe ($p = 0.02814$) for the Digenea (**Fig. 6.8. F**–**G**). The Cestoda correlated significantly positive with increasing concentrations of Hg ($p = 0.01026$) and significantly negative with Se ($p = 0.00627$) and U ($p = 0.02586$) (**Fig. 6.9. A**–**C**), while the Nematoda responded significantly negative to Co ($p = 0.02383$) and significantly positive to Sn ($p = 0.00484$) (**Fig. 6.9. E**, **F**). A significant negative response was seen between V ($p = 0.00743$), and a significant positive response to Au ($p = 0.00009$) and Se ($p = 0.04898$) for the Crustacea (**Fig. 6.9. G**, **H**).

Clustering of component community assemblages between localities associated greatly with different components (environmental variables), categorical factors (functional traits) and species composition (**Fig. 6.10.**). The primary factors contributing significantly to community composition were the life cycle type, host sex and condition factor and the following elements: Au, As, Cd, Cr, Co, Fe, Hg, Pb, Se, Zn, V ($p < 0.05$). Component community diversity at each locality was driven by a distinct combination of factors: for BS Monogenea, Digenea and Cestoda were the main drivers in community diversity, along with heteroxenous life cycle parasites (1:1), increasing concentrations of Fe; for VR the Crustacea strongly associated with river habitat, monoxenous life cycle (1:1.6) and Au, Cr and Se; while the Cestoda and Digenea associated with CF of the host and Hg and the Monogenea associated with Fe and contributed substantially to the community composition at RR. No single factor drove community composition at GD, although it associated with Cd, Co and Se.

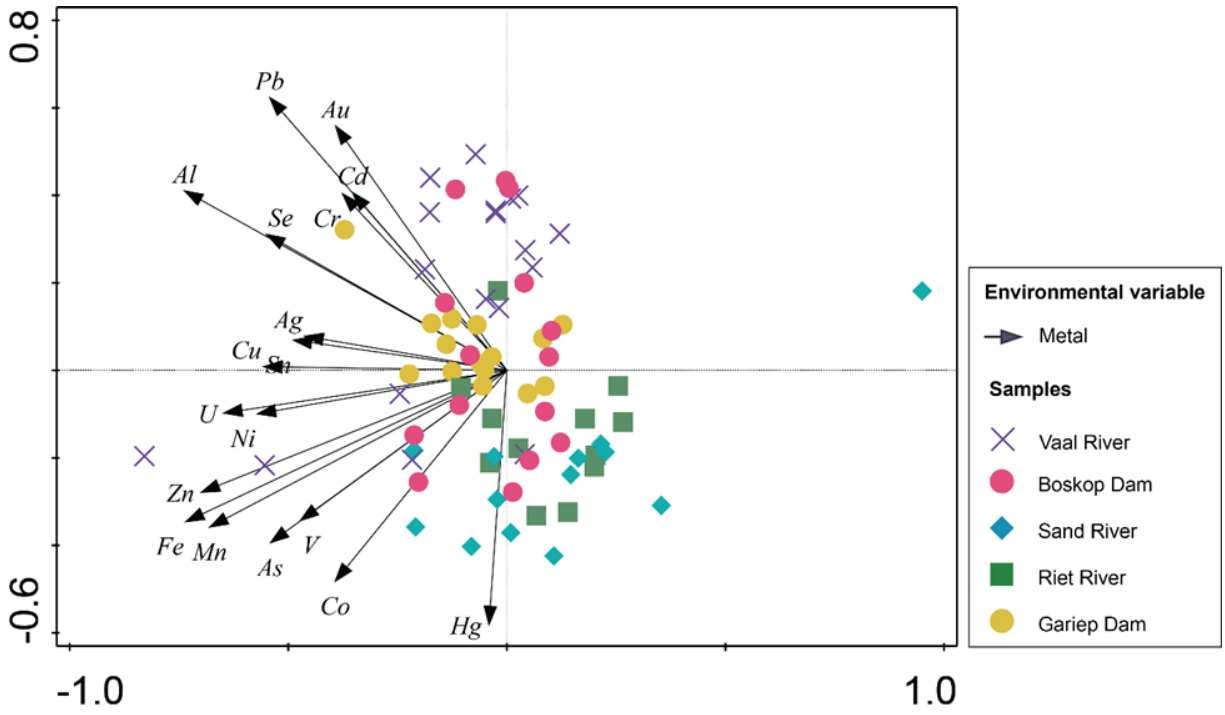

Figure 6.7. Principal Component Analysis (PCA) biplot illustrating the variation in metal concentrations (Al, Ag, As, Au, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Se, Sn, Pb, U, V, Zn) in the muscle tissue of *Clarias gariepinus* (Burchell) from different localities. The biplot explains 42.9% of the total variation, with 29.1% on the first axis and 13.8% on the second axis.

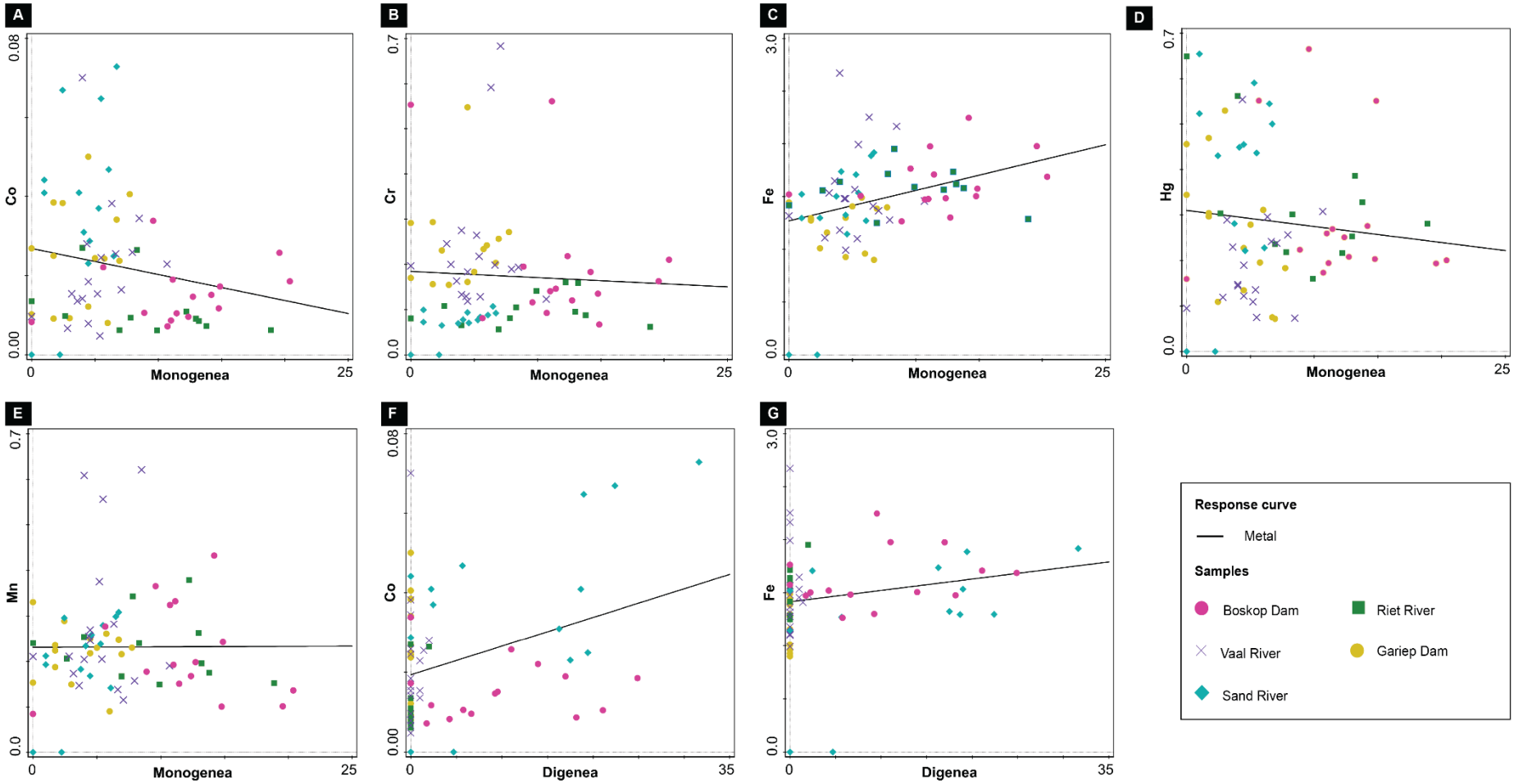

Figure 6.8. Generalised linear regressions of the abundance of major taxonomic groups to increasing metal concentration: **A**–**F**. Monogenea (Co, Cr, Fe, Hg, Mn and Ni) and **G**–**I**. Digenea (Co, Fe and Ni). Only significant correlations (p = <*0.05*) were presented.

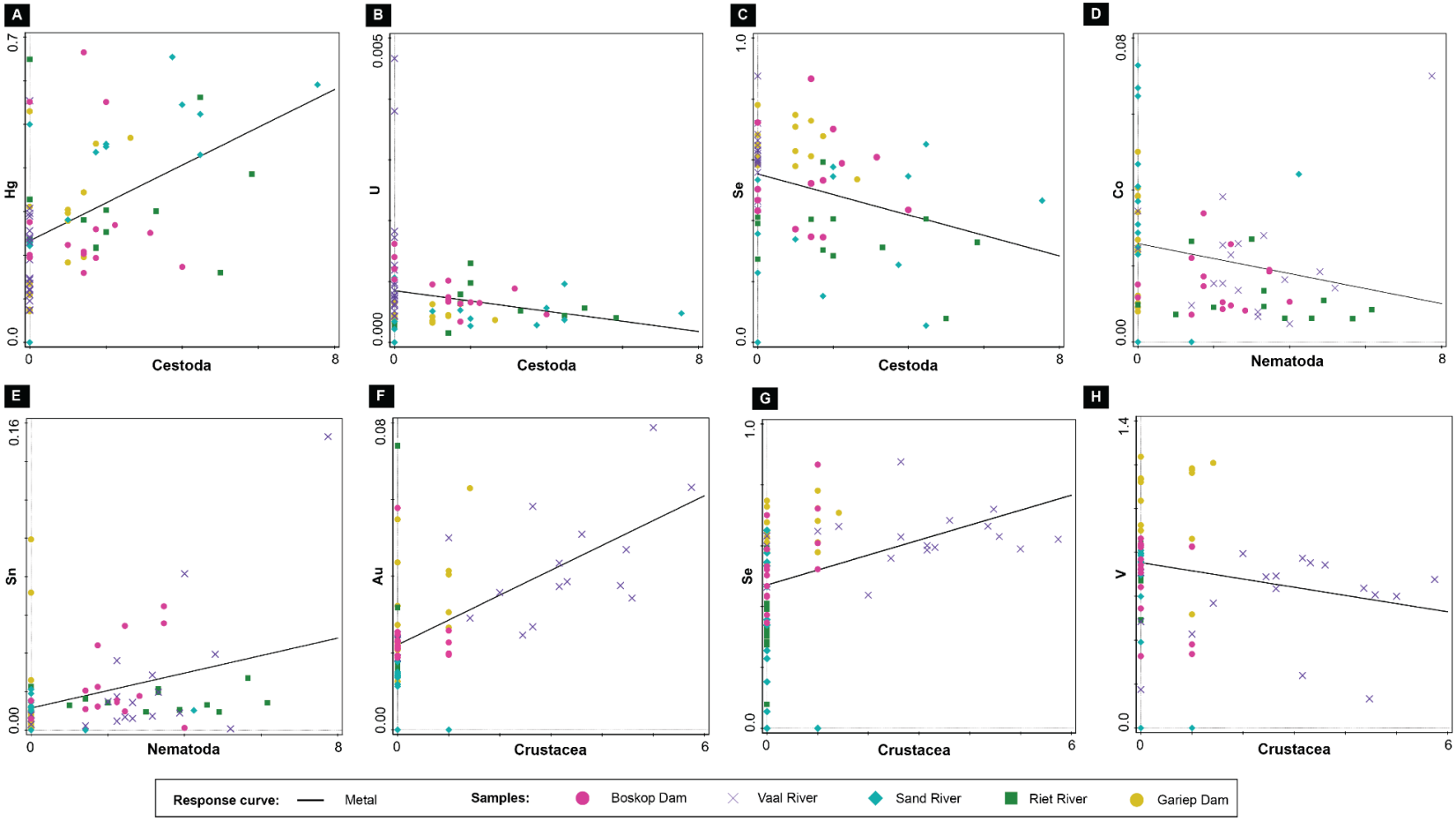

Figure 6.9. Generalised linear regressions of the abundance of major taxonomic groups to increasing metal concentrations: **A**–**C**. Cestoda (Hg, Se, U); **D**–**E**. Nematoda (Co, Sn) and **F**–**I**. Crustacea (As, Au, Se and V). Only significant correlations (p = < *0.05*) were presented.

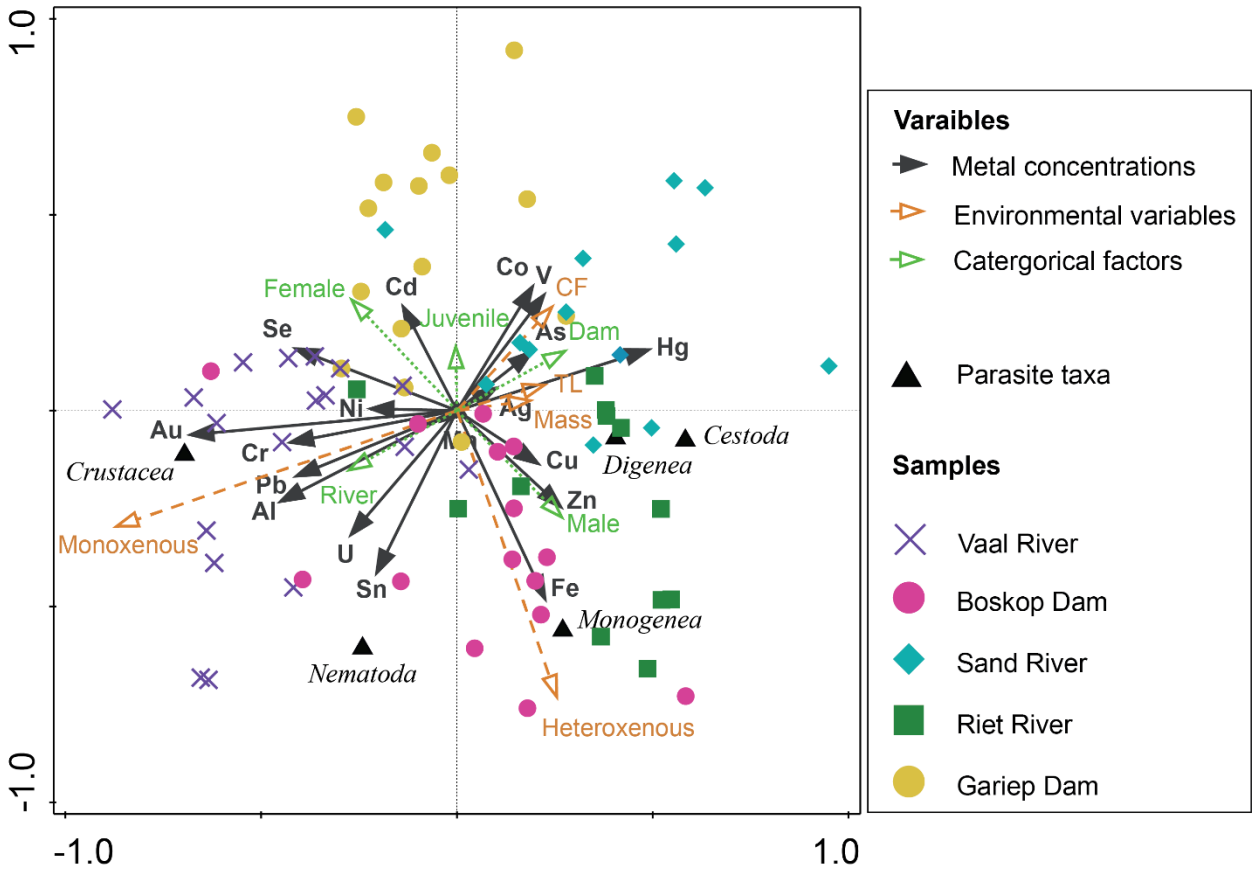

Figure 6.10. Redundancy analysis (RDA) based on major parasite taxa in *Clarias gariepinus* (Burchell) at the sampled localities with metal concentrations in host muscle tissue overlain as environmental variables and the functional traits (host weight, total length and sex, monoxenous and heteroxenous life cycle, dam and river habitat) as explanatory variables. The quad-plot explains 36.5% of the variation with 22.4% on the first axis and 14.1% on the second axis (pseudo-F = 3, $p = 0.002$).

6.3. Discussion

It has been established that there is a great number of factors that can shape communities in aquatic systems and hosts as environments. In **Chapter 5**, it was seen that the translocation of a host can lead to the ultimate change in a parasitic community diversity and assemblage when a host enters a novel environment, and parasites, along with their host are faced with a range of barriers to overcome before they can succeed. Similarly, natural and/or native populations encounter interactions with their environment that determine the success of species and their transmission across trophic levels to complete life cycles and eventually reproduce. Earlier in this chapter, the two opposing hypotheses of Lafferty (1997) were introduced, and it was highlighted that there is a need to quantify physical or ecological response of parasitic communities to drivers that potentially lead to increasing or decreasing community assemblage composition. Subsequently, community diversity and assemblages were investigated for five native *C. gariepinus* populations, and potential drivers i.e., environmental variables (elemental concentration in the host) and functional traits (host sex, weight, size and CF, habitat, and life cycle type) were quantified.

The overall high parasite diversity and abundances in *C. gariepinus* at all localities cannot directly be related to a decrease in host resistance, since the CF indicated that all individuals were in fair to good condition. This reflects that there are suitable food sources in the respective systems and active feeding occurs. It is assumed that when a host has a compromised health status, this would be reflected in the CF, that represents the volumetric relationship between the body weight and total size (length) of the individual (Barnham and Baxter 1998; Lückoff 2005). Additionally, the positive responses of the major taxonomic groups (Cestoda and Digenea) to the host traits indicate that these heteroxenous taxa are optimally exploiting the feeding behaviour of the host and are successfully transferred through trophic levels in each system. The inverse response of Monogenea, Nematoda and Crustacea is probably indicative of a change in behaviour, decreasing the chances of transmission through direct contact for the monoxenous taxa and diet by larger individuals, as infected copepods are mainly the intermediate hosts to larval nematodes and form part of smaller individual diets, and larger *C. gariepinus* eat more detritus than copepods or small fishes (Bruton 1979a).

The significantly lower parasitic community composition, assemblage, and diversity at the two lentic systems (GD lower compared to BS), and the lotic systems (RR, SR and VR) are probably more related to physical habitat conditions than to anthropogenic stressors (i.e., as reflected in element accumulation by the host). These substantial community diversity and assemblage differences can firstly be explained by the physical attributes of these two seemingly different lentic ecosystems: BS is a very small and dynamic waterbody with a surface area of ca. 3.73 km^2;

the upstream river is influenced by several agricultural and mining (uranium) activities; and habitat availability within the dam with a substantial number of aquatic plants and a diverse fish community. In comparison, GD is 100-fold larger in surface area (374 km^2), with a highly regulated flow-regime system and harbours a very low natural fish diversity. Even though it is situated downstream of a growing urban population, with increased agricultural and industrial activities in its tributaries, it is unlikely that contamination from these sources would currently have an influence on aquatic biota, mainly due to the sheer volume of the GD. The decrease in diversity at GD, compared to BS, may be explained by the hypothesis of decrease in parasite infection (diversity and abundance) due to a decline in availability of intermediate hosts (Lafferty 1997). This is also reflected in the dominance of monoxenous species present on the hosts in GD, with very few heteroxenous endoparasites. Similarly, varying parasitic diversity and community compositions at VR, SR and RR cannot be directly attributed to host resistance expressed by the CF. The lower diversity (richness and abundance) of RR compared to VR and SR may be attributed to a suite of factors, where the increase in diversity is a result of suitable intermediate and definitive hosts presence, as well as individual responses (sensitivity or tolerance) of parasite taxa to environmental factors, in this case elemental concentrations in the host.

The ratios of monoxenous and heteroxenous species at BS, VR, SR and RR were almost equal for all localities, suggesting that the host as habitat, as well as the environment, consist of a suitable composition of biotic (intermediate hosts and habitat) and abiotic constituents to support reproduction and transmission (trophically or contact and density based). Monoxenous species dominated at GD and suggest that: 1) there is an absence of suitable intermediate hosts in order for cercarial forms of complex life cycle species to transmit; 2) the diet of the sharptooth catfish from GD differs compared to other localities; or alternatively, 3) infection intensities are low and infect few host individuals. Similarly, the absence of the monoxenous species *Ergasilus* cf. *mirabilis* and *Lamproglena clariae* from RR and GD may simply be a result of barriers yet to be identified and may include limits of their natural distribution and driven by temperature, latitude, or water quality. The varying absence of *Spinitectus* sp. (from BS, SR, RR, GD); *Procamallanus* (*P.*) *pseudolaeviconchus* (from VR, SR, RR, GD); *Orientocreadium batrachoides* (from VR, SR, RR, GD) and *Contracaecum* sp. A (from GD) may be a result of the lack of suitable intermediate hosts or introductory definitive hosts, rather than the effect of environmental variables on these parasitic species and needs further investigation.

The Digenea and Monogenea as accumulation indicators are still debated due to their small size, proving difficult to effectively quantify accumulation and bioavailability of pollutants (Sures et al. 2017b), however their role as response indicators should not be disregarded (Gilbert and Avenant-Oldewage 2021). Identifying suitable response indicators is more difficult, since their

natural response may be stochastic, rather than related to environmental change or, in some instances, opposing correlations have been found to similar exposures for different species within a taxon (Lafferty 1997). The suitability of Digenea has been assessed on occasion and, although there are challenges in detection, due to low abundances and small size of cercarial stages a decline in cercarial survival has been linked to high elemental concentrations (Lafferty and Kuris 1999; Pietrock et al. 2002; Sures 2004).

The effect size approach of Blanar et al. (2009) which states that 1) Monogenea and Digenea are primarily negatively affected, with slight alterations were seen for community richness; and 2) there is an increased susceptibility of monoxenous and ectoparasites and free-living stages of endoparasites to pollutants can be confirmed. Furthermore, the results from the present study corroborate these findings in the significant inverse response observed for the major taxa toward several elements (Co, Cr, Fe, Hg, Mn, Se, Sn, U and V). However, it is also evident that when comparing the results from known element polluted systems such as the Vaal River (Wepener et al. 2011; Janse van Rensburg et al. 2019; Connell et al. 2020) with other systems that are not known to be contaminated with metals, elemental exposure as a whole is not an effective collective measure in characterising size effect on taxa. No single element is a concrete determinant of overall pollution or response, each contribute significantly to the effect exerted at individual taxa or species level, as was observed for their sensitivity or tolerance to a suite of elements. It can be reasoned that high abundance of Digenea may be indicative of high Co and Hg concentrations and low Fe; together with a diverse and abundant monogenean or cestode component indicate potential high Fe; similarly, Crustacea may be indicative of high Au and Se in the system of interest, and Nematoda may be an indicator for increased Sn as seen from the results obtained in the present study.

Overall, caution should be taken when assumptions are made regarding the effect of environmental alterations or pollution on parasites on individual, community, or population level. Generalisation of species, taxa and effect should be avoided until complete ecology and physiological processes of selected species is understood. Responses of individual taxa and functional groups to specific contaminants is yet to be assessed quantitively and proves to be challenging given the varying complexity and uncertainty of responses of parasitic taxa to any given combination of pollutants. The use of parasitic species should however not be disregarded in the face of challenges, as they may prove useful in predictions of effect across trophic levels, as pointed out in the present study (Riggs and Esch 1987; Sures 2004; Blanar et al. 2009). Several factors lead to variation in water quality or pollution, and it should be considered that high or low species abundance may reflect natural occurrence in a specific ecosystem. Presence or absence of a particular parasite is not causally related to impacted ecosystems and may well

reflect other ecological factors such as compromised hosts, missing linkages at various trophic levels (i.e., natural absence of suitable intermediate hosts) or represent investigation near or outside the natural distribution limit of parasitic taxa, which in turn may be more sensitive to subtle environmental changes (Möller 1978; MacKenzie 1987). Currently, defining parasite natural occurrence, as well as understanding the biology and ecology of potential sentinels, agents or effect indicators are important, and comparisons between functional groups (endoparasites vs. ectoparasites or heteroxenous vs. monoxenous) are essential. Predictions cannot be made on the basis of “belonging to a specific taxon” (i.e., Acanthocephala, Cestoda, Monogenea) alone, since quantitative limits for the “typical responses” of species at individual, population and community level to these pollutants do not yet exist (Lafferty 1997; Sures 2001, 2003, 2008; Blanar et al. 2009 and references therein).

Lastly, this study also provide useful information on the use of species accumulation curves. Dove and Cribb (2006) evaluated the application of species accumulation curves and confirmed its usefulness in understanding patterns of diversity in parasite assemblages. The accumulation curve derived from data obtained in this study indicated that the parasitic communities of the investigated *C. gariepinus* populations are uniform and that the community is highly interactive. Further, this is also a measure that can contribute to address ethical aspects of studying the biodiversity of sensitive and endemic species in estimating minimal impact through determining the minimum number of individuals and sampling effort needed to fully determine the biodiversity of an ecosystems.

Chapter 7

General discussion

Diversity, or rather biodiversity, within aquatic ecosystems is essential to all life forms, not just on species level but also on a community, regional and global scale. Aquatic organisms interact and resonate with terrestrial fauna and flora, and where fishes are concerned, also provide an important ecosystems service to mankind in a social, economic, educational, and nutritional capacity. The importance of fishes, their utilisation, and concurrent conservation is frequently highlighted in research and policy making regarding freshwater life across the globe. However, there is still a great need in advocating, and subsequent action, for smaller aquatic fauna such as parasites, which form an important component of all aquatic life and biological units (Windsor 1995, 2021; Carlson et al. 2017). It has been established across all ecosystems (freshwater, marine and terrestrial), that parasitic species and communities contribute substantially to the equilibrium (although not always) of any given system and have a key role in their ecological as well as evolutionary progression (Bordes and Morand 2009; Gomez and Nichols 2013). In an increasingly fast paced world, the importance of biodiversity conservation through investigation and empirical quantification is of importance. Scientists struggle to understand ecological and evolutionary processes underlying the origin, distribution, and maintenance of biodiversity and at times, diversity is much higher or lower than previously anticipated (Beheregaray and Caccone 2007; Vaughn 2010). Several factors, such as exploitation of water and its inhabitants, pollution, habitat destruction, and modifications in natural flow regimes all contribute to an accelerating decline in biodiversity (Poff et al. 2007; Vaughn 2010). These factors are also applicable to ecosystems on a smaller scale and should be assessed on local ground to understand and identify potential concerns in developing regions, such as South Africa. It is essential to understand impacts on ecosystems and their biotic (parasites and their hosts, species interactions) and abiotic (environment, pollution) link chains (food web structuring) and their ranges, and bear in mind that human impact has a deleterious effect in the ecological function of the very systems we are dependent on for survival.

The rationale behind the importance of parasites, their study and conservation are at times ambiguous since the initial negative association i.e., harmfulness to hosts, difficult to study, taxonomic impediments, zoonoses, economic effect, cost related morbidity, impacting food security, and problems posed to conservation of hosts, often overwhelm a misinformed global community and result in a lack of support toward knowledge gathering and conservation of

parasites, and indirectly to overall biodiversity (Bordes and Morand 2009; Gomez and Nichols 2013). Furthermore, the contribution and role of parasites in food webs and their robustness, energy flow and as indicators of host population history and/or structure are of immense importance (Marcogliese and Cone 1997; Lafferty and Kuris 2009; Buck 2019; Morris et al. 2019). The co-evolutionary relationship between hosts and their parasites has also recently been brought to the attention of parasitologists and ecologists, with the risk of co-extinction or resulting low parasite diversity host populations (Dallas and Cornelius 2015; Milotic et al. 2020). It is important to document biodiversity effectively and, where possible, before human impacts occurr to provide comparisons for the future's past (Beheregaray and Caccone 2007). It is clear that global changes and a true understanding on diversity and complexity of parasites in ecosystems, as well as their contribution to overall biodiversity (species, community and genetic), are at a crossroad. The present survival and conservation of important species that drive future ecosystems are already under immense pressure, because of human impact and an overall deficit in the extent of our knowledge in understanding parasitic species and the dynamic interaction with their environments.

With respect to the above, this thesis contributed to diversity knowledge and motivation for conservation through the evaluation of natural diversity in native and translocated populations of *C. gariepinus*, as well as potential biotic and abiotic impacts on native natural diversity. Specific aims were to: 1) summarise the complete known diversity of a host with a wide geographic range in Africa; 2) determine natural diversity in native populations in southern Africa; 3) compare parasitic diversity in non-natural geographic distribution ranges to native population diversity in a widely understudied region in South Africa; and 4) identify relationships of biotic and abiotic impacts on major parasitic groups in a natural environment.

The findings of this thesis contribute substantially to the parasitic diversity knowledge of an exceptional host species. Although widely studied in its natural range, it is apparent from results obtained here that there is still a gap in knowledge in the already known and extremely diverse parasitic community of *C. gariepinus* in its natural range. Forty-four parasitic species representing five major taxa (12 orders and 19 families) were identified. Distinct community assemblages and compositions were apparent in different geographic regions in southern Africa. The existing potential for discovery of novel specialist parasitic species, in addition to establishing true community composition and distribution within several natural geographic ranges of *C. gariepinus* in southern Africa, were also demonstrated through characterisation of species through morphological and molecular analyses (**Chapter 3** and C**hapter 4**). Furthermore, it has been established that the documenting of the natural distribution of known species is still far from complete in the discovery of several species parasitising *C. gariepinus* from novel geographic

regions (**Chapter 3** and **Chapter 4**). The reach and implications of alien invasive parasitic species, as well as the success of co-introduced parasite species in novel environments were also illustrated, in the presence of similar parasitic species (infra- and community) richness, but distinct parasite community assemblages in translocated populations (**Chapter 5**). Lastly, the value of environmental parasitology through continued investigation of parasitic species as effect indicators were highlighted in the positive responses observed for specific major taxonomic groups to a suite of elemental compounds present in one the of most impacted river systems, the Orange-Vaal River system in South Africa (**Chapter 6**).

In the following section, I will highlight the main findings of this thesis focusing on the themes of taxonomy and systematics, global to local parasite diversity, translocation beyond natural geographic barriers, ecosystem health and identify challenges and gaps in current knowledge as well as provide goals for future research.

Taxonomy and systematics

'*Before we can learn anything else about parasites, we must first discover them, characterise them carefully in ways that will allow us to distinguish them from other species, identify their main hosts, and determine where they fit in our growing, evolutionary-based classification of living organisms*' – Poulin et al. (2020).

In their study on the state of fish parasite discovery, taxonomy, and systematics, Poulin et al. (2020) found that the discovery and documenting of fish parasitic species is progressing at a steady pace, but that there is a disproportionate availability of data, for example molecular data, compared to physical observations and reports in some geographical regions. Similarly, a mismatch was observed for spatial distribution and species discoveries among higher taxa. Furthermore, taxonomists are dealing with parasitic groups that are quite simple in having direct life cycles, but an equally large number of complex life cycle parasites, if not more, are present in fish. This substantially complicates the basics of taxonomy in rendering morphological identification alone insufficient and calls for more sensitive assessment of species. Fortunately, in modern parasite taxonomy an excellent toolkit is at our disposal to discriminate between species with great advancement on the fronts of classical microscopy (light, scanning and fluorescence to name a few) as well as molecular (DNA barcoding) techniques, and a growing collaborative spirit among researchers across several continents and different disciplines to produce accurate species identification (Poulin et al. 2020; Heneberg and Sitko 2021). Although the main aims of this thesis were not to characterise all the parasite taxa morphologically and molecularly to species level, it was inevitable to consider the power of this technology and its value to accurately delineate species. Specifically, where community and population diversity are concerned and its relationship to environmental change. Not all studies and interests are in the

taxonomy and systematics of species or communities, however, correct and accurate identification is a crucial part in the understanding of interconnectivity between hosts and their parasites. This brings the concept of cryptic species to the forefront where morphological discrimination between species is not always possible, but their role in ecosystems is of substantial importance (Bray and Cribb 2015; Pinacho-Pinacho et al. 2018; Artim et al. 2020; Heneberg and Sitko 2021).

The parasitic community of *C. gariepinus* is exceptionally well studied throughout Africa and has been described as the most widely distributed host with the most diverse metazoan community (Scholz et al. 2018c). This may well be true, but there are ample studies reporting on the parasitic communities of *C. gariepinus* without providing morphological or molecular support for the findings (see **Chapter 3**, **Chapter 4** and **Chapter 5**). Firstly, the absence of complete descriptions provides challenges in determining the true host range and geographic reach of species that are of importance in the documenting of global biodiversity. Secondly, challenges are faced when these parasitic species are of epizootic (aquaculture or natural systems) or zoonotic (human disease) importance, but no identification and subsequent epidemiological information is available to identify, manage and potentially eradicate such individuals. Lastly, all major taxonomic group harbour cryptic species, they are ever-present and represent a world of hidden diversity and complexity. Cryptic speciation among known parasites of *C. gariepinus*, the Diplostomidae (*Diplostomum* and *Tylodelphys*) and genera within the Camallanidae have recently been identified and need further investigation for species from Africa (Georgieva et al. 2013a, b; Ailán-Choke et al. 2020; Ailán-Choke and Pereira 2021; Heneberg and Sitko 2021). In this thesis, the use of molecular tools were of great value to delineate parasitic species that were grouped into morphotypes. Novel sequences were generated for 29 of the 44 parasite species found in this study. These were novel sequences for eight known parasitic species of *C. gariepinus* and included the Digenea (adults *Phyllodistomum* cf. *vanderwaali*, *Thaparotrema* sp. A, and the metacercariae *Neodiplostomum* Type 1, *Tylodelphys* cf. *grandis*, Diplostomidae gen. sp. 1); Nematoda (*Paracamallanus cyathopharynx*, *Procamallanus* (*Procamallanus*) *pseudolaeviconchus*; and Crustacea (*Ergasilus mirabilis*). Sequences were obtained for six species, for which no conspecific comparisons from GenBank were available, and include the Digenea (Xiphidiata gen. sp. 1) and Nematoda (*Contracaecum* sp. A, *Contracaecum* sp. B, *Contracaecum* sp. C; *Paracamallanus* sp. A, *Procamallanus* (*P.*) sp. A). Additional molecular data were generated for eight species for which comparative sequence data were available from the GenBank database and included the Monogenea (*Macrogyrodactylus clarii*, *M. congolensis*, *M. karibae*); Digenea (*Tylodelphys mashonensis*, *Clinostomum brieni*, *O. batrachoides*) and Cestoda (*Tetracampos ciliotheca*, *Barsonella lafoni*). Two invasive species, *Argulus japonicus* and *Schyzocotyle acheilognathi,* were successfully identified through molecular comparison to

congeners and *Ergasilus* sp. A could be separated as a species from *E. mirabilis*. Finally, three nematodes that infected *C. gariepinus* as a paratenic host could be confirmed as larval forms of *Eustrongylides* sp., *Gendria* sp. and *Tanqua* sp.

Parasite biodiversity of *Clarias gariepinus* in Africa

Recently, a complete inventory on the freshwater parasites of all fishes in Africa was published and holds great value for the future of taxonomy and parasitological finds on the continent (Scholz et al. 2018c; Moravec 2019). With the review on the biogeography of all known parasites of *C. gariepinus* in Africa (**Chapter 3**), it became apparent that such an overview contributes to the identification of regions where communities are greatly understudied and subsequently this thesis presented a graphic presentation of the distribution of known parasitic species of *C. gariepinus* in Africa and a complete record list. From the literature search it became apparent that a call for confirmation and re-examination of several species reported in the literature was essential. This was based on the lack of morphological or molecular data to support the records. As mentioned in the previous section on taxonomy and systematics, a substantial contribution was made during the present study in providing molecular data for the species of Digenea, Cestoda and Nematoda found in this study, almost all representing novel data for species from South Africa and Zambia (**Chapter 3**, **Chapter 4**, and **Chapter 5**). Additionally, this thesis provided the taxonomic description of eight dactylogyrids; *Quadriacanthus aegypticus, Quadriacanthus allobychowskiella*, *Quadriacanthus clariadis*, *Quadriacanthus fornicatus*, *Quadriacanthus gourenei* and *Quadriacanthus pravus* from several localities in southern Africa, and two new species, *Quadriacanthus* sp. G and *Quadriacanthus* sp. H (**Chapter 3**). The finding of *Macrogyrodactylus clarii*, *Macrogyrodactylus congolensis* and *Macrogyrodactylus karibae* from two floodplain ecosystems, the Barotse floodplain (ZR) in Zambia and Ndumo Game Reserve (NGR) in South Africa; as well as *M. congolensis* from the Kabompo (KR) and Vaal Rivers (VR) in Zambia and South Africa, respectively, with complementary molecular data and morphological descriptions were published (see Truter et al. 2021a) and were presented in **Chapter 4** of this thesis.

Briefly, all infection records in the native distribution range reported in this thesis were novel geographic records. All species of *Quadriacanthus* and *Macrogyrodactylus* reported were novel to the respective localities, while *Q. fornicatus* and *Q. pravus* were reported from localities other than their type localities for the first time. Here *C. gariepinus* was reported as a new host for *Quadriacanthus gourenei* (ZR) and novel distribution records for *Gyrodactylus gelnari* (ZR) and *G. rysavyi* (VR) were provided. The digenean, *O. batrachoides,* was reported from South Africa (BS) and Zambia (ZR) for the first time; *P. cf. vanderwaali* from the gallbladder of *C. gariepinus* in ZR represent a new site of infection and there was an expanded distribution for metacercariae

of *Neodiplostomum* Type 1 to NGR and ZR, suggesting a migratory bird host or generalist nature of this parasite. The records here of the digenean, *T.* cf. *grandis* from KR and ZR were a first from localities other than their type localities and the metacercaria of *T. mashonensis* seem to be more widely occurring in South Africa than previously thought (found from NGR, SR, VR). The present study also provided the first morphological and molecular data for Diplostomidae gen. sp. 1 (RR and VR) (previously reported as *Tetracotyle* sp. or *Diplostomum* type 3); novel geographic records for the cestodes, *T. ciliotheca* from RR, SR and GD; for *M. chalmersius* and *Proteocephalus glanduligerus* from ZR; and *B. lafoni* from KR and ZR in Zambia, and NGR, RR, SR and GD in South Africa were also presented. The nematodes, *Pa. cyathopharynx* from NGR, KR and ZR; *P.* (*P.*) *pseudolaeviconchus* from BS and NGR (published in Svitin et al. 2019); *Argulus cunningtoni* from ZR; *E. mirabilis* from VR, BS and ZR; and *Lamproglena clariae* from BS and NGR were also all new geographic distribution records. A novel species, Xiphidiata gen. sp. 1 from the eye viscera of *C. gariepinus* in ZR was reported, as well as larval nematodes, *Contracaecum* sp. A (ZR and KR); *Contracaecum* sp. B (NGR); *Contracaecum* sp. C (RR, SR, VR and BS); *Pa.* sp. A (RR, SR, VR, BS); *P.* (*P.*) sp. A (ZR) and paratenic infections with larval *Eustrongylides* sp., *Gendria* sp. and *Tanqua* sp. Furthermore, the known digenetic parasitic community of *C. gariepinus* was increased from 34 to 36 species following the addition of Cladorchiidae gen. sp. and the description of *Masenia nkomatiensis* in recent years (Zhokhov et al. 2017; Dumbo et al. 2019a).

It was also found that a single host individual has the potential to harbour up to four monogeneans, six digeneans, one cestode and three nematodes, highlighting the suitability of *C. gariepinus* as habitat for a multitude of specialist and generalist parasite species, and also serve as a paratenic host in the life cycle of heterogeneous species.

Translocation beyond natural geographic limits

The presence of invasive species in novel environments globally, but also in South Africa, has resulted in several debates on their eradication or removal of hosts through piscicide treatments and subsequent rehabilitation of native species. In the Western Cape Province of South Africa, several such eradication plans have successfully been executed with desired results (Weyl et al. 2016a; Dalu et al. 2020). On the other hand, the benefit of potentially eradicating alien and co-invasive parasites, particularly those that have not yet spilled over to native fishes or established in newly acquired intermediate hosts as part of the eradication process, should also be assessed. However, it is important to monitor these systems closely before and after eradication and as such not to destroy a river's existing biota and productivity until policy guidelines have been assessed and published (see Weyl et al. 2016a; Marr et al. 2019; Davies et al. 2020). As a result, few host eradication strategies are executed, and it is of interest to rather attempt to monitor the effect and potential restructuring of the interactions between organisms within these altered systems. Britton

(2013) evaluated the potential of introduced parasite species to alter novel environment food webs through topological changes and shifts in trophic relationships between species in relation to the ecological hypotheses of parasite spillback, spill-over and enemy release, among other factors such as life cycle complexity. The author concluded that the presence of co-introduced, or co-invasive parasites and their hosts resulted in the addition of new nodes and links across trophic levels, increasing overall connectivity and complexity, while shifts are resultant of a cascade effect from the acquisition of parasite species (spill-over). However, in South Africa there is almost no data available on parasitic communities of native and translocated or invasive host species, even in aquatic systems where there are sensitive species and high endemicity (particularly the Western Cape and Eastern Cape Provinces). Thus, it is difficult to attempt to assess the effects of introduced species on the interconnectivity of hosts and parasites in these environments and identify potential ecosystem functionality loss or cascading effects in the food web where several other organisms such as macro-invertebrates, molluscs, and birds are also concerned and indirectly exposed to novel parasitic species.

Torchin and Mitchell (2004) estimated that introduced host species escape approximately 75% of their native parasite fauna upon entering a novel environment. The two parasite communities from the GFR population and RSE population of *C. gariepinus* were only infected with ~24% of the most species rich community discovered during this study (the Barotse floodplain), and only shared three (RSE) and seven (GFR) specialist parasitic species found from any of the native populations in the present study, thus supporting Torchin and Mitchell's (2004) estimate (Chapter 5). Despite having similar richness to the southernmost native populations in South Africa, RSE had a distinct community assemblage, while GFR mirrored the parasite community of its source population. Life cycle complexity and interconnectivity of species in different ecosystems were also illustrated in this thesis (Chapter 5), in the presence of two heteroxenous species, the digeneans *O. batrachoides* and *T. mashonensis* in hosts from GFR, but absent from their GD source population. Both these species utilise *C. gariepinus* as a different host link (i.e., intermediate and definitive host) in their life cycle and provide insights on the barriers these species may or may not encounter in the novel environment that affect their survival and establishment, and ability to adapt (or their resilience and specificity) and utilise available hosts to succeed within the recipient ecosystem. Evidence is provided for the co-introduction of 12 specialist species of *C. gariepinus* into the novel environments (GFR and RSE), with support for the enemy release hypothesis in translocated hosts in GFR and RSE harbouring substantially fewer native parasite species (richness) compared to native populations. Finally, parasite spillback from native as well as another alien invasive host is confirmed with *Ergasilus* sp. A (from an unknown native host) in GFR and RSE, and *S. acheilognathi* (from the common carp, *C. carpio*) parasitising the intestine of *C. gariepinus* in RSE.

Ecosystem health

Parasitology is one of the most successful modes of life displayed by organisms in nature and concomitantly they also represent one of the most diverse groups on the planet (Dobson et al. 2008; Timi and Poulin 2020). The premise of Lafferty (1997) on the potential of parasites to reflect anthropogenic impacts as defined by: 1) the increase in parasitism (abundance or richness) as a function of decline in host resistance, or increase in intermediate host density; and 2) decrease in parasitism as a function of an increased exposure to direct environmental toxicity (or indirect increase of host mortality or a decline in intermediate and definitive host), is a fine beginning when interrogating the role of parasitic species in freshwater environments. One cannot assess ecosystem health solely based on theories, including that of the inverted parasite index that ectoparasites are more directly exposed than endoparasites to the effect of water quality (or environmental quality) and will thus be sentinels of good water quality (Crafford and Avenant-Oldewage 2009). Nor can it be ecosystems be assessed by the niche breadth hypothesis, that postulates that specialists can only tolerate limited abiotic conditions or feed on a restricted range of resources, and only exist in a narrow subset of localities, while generalists have a broad tolerance and diets exist in a wider range (Poulin et al. 2011). From results in this thesis (Chapter 6), it is evident that the complexity of parasite interactions with their host as an environment is quite vast, with added factors as to where hosts occur naturally, where they came from, and if they are subjected to external factors such as pollution or the availability of suitable trophic linkages and barriers within a novel environment. There is a high interconnectivity of each parasite with its direct and indirect environment, and its presence or absence, and high infra- or component community richness or abundances may not be attributed to any single aspect of an ecosystem or be a causation of one single impact or factor (to which we often resolve). The comparison of the parasitic community assemblage composition from lentic (GD and BS) and lotic (VR, SR, RR) systems illustrated that it is extremely important to evaluate the ecological milieu of each system we assess, at all levels where possible i.e., all biotic contributors and their responses to the potential cascading effects imposed by various abiotic changes.

Recommendations for the future

Throughout this thesis it became apparent that: 1) diversity is defined by the time and effort of the parasitologist investigating communities of populations and often restricted to a taxon of interest or within a set of expertise and available technologies; 2) general assignment of taxa to species was previously based on assumptions that morphological grouping validates the reporting of species, not reflecting true host range and distribution, and diversity across a wide geographical range; 3) the natural diversity and occurrence for many parasitic species, as well as community assemblages are yet to be defined, even for the parasite community of a widely studied host; and

4) there is a hidden knowledgebase on the ecology of parasites with respect to defining natural and impacted diversity.

Based on the above, it would be desirable for continued research within the field of fish parasitology and future exploration to include comprehensive surveying and training across all parasitic taxa and geographic regions, not just for *C. gariepinus*, but for all hosts. Where taxa are of less interest to experts or researchers, the effort should be made toward outreach and collaboration with relevant stakeholders to ensure accurate assignment and reporting through accredited platforms. Before we can attempt to understand and decipher the ecology of a single species or community, we must first know its identity whereafter the preference and intrinsic responses to its environment can be documented. Often these will occur simultaneously, and thus the more important it becomes to accurately identify species, especially where several trophic links are involved and prompts the use of complimentary modern technologies, such as the application of molecular analyses (Poulin et al. 2020). Although not explicitly tested in this thesis, some assemblages of parasitic communities hinted at diversity gradients at latitudinal as well as longitudinal scales. These hypotheses suggest a diversity gradient (richness) at species, population, and genetic level (Conord et al. 2012; Lawrence and Fraser 2020). In the present study, infra- and community diversity (as richness) was high at low latitudes and low and high latitudes, some variation was observed for populations studied from east to west and subsequently linking historical geographic origin of each of the populations of *C. gariepinus* studied in this thesis. Based on the haplotype network in the work of Van Steenberge et al. (2019), the question is posed as to whether geographic origin defines parasitic community assemblages and warrants further research. Lastly, the potential of success in collective eradication of co-introduced and co-invasive parasites with their hosts during eradication exercises should be included in the monitoring of these programmes and form part of the pre- and post-eradication rehabilitation monitoring.

Conclusion

In conclusion, the parasite diversity of *C. gariepinus* has been intensively and thoroughly reviewed and novel data presented in this thesis includes the discovery of two monogeneans, one digenetic metacercaria, five nematodes and one parasitic crustacean from *C. gariepinus* in southern Africa. Moreover, expanded distributions are reported for all 44 species collected in the present study, highlighting the need for intensive parasitological studies of all fish hosts in the future, particularly in regions previously unexplored. The resilience of taxa was identified in the co-introduction of at least 12 parasite species into recipient ecosystems where the host does not naturally occur, as well as in the responses of specific taxa to specific elemental contamination in highly polluted ecosystems. Moreover, the suitability of *C. gariepinus* as paratenic host to three nematodes in its

native distribution range, and spillback of two alien invasive species (*A. japonicus* and *S. acheilognathi*) and a native copepod, highlight the urgent need for a critical review of the potential of *C. gariepinus* as an amplification vector in translocated regions.

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Appendix A1

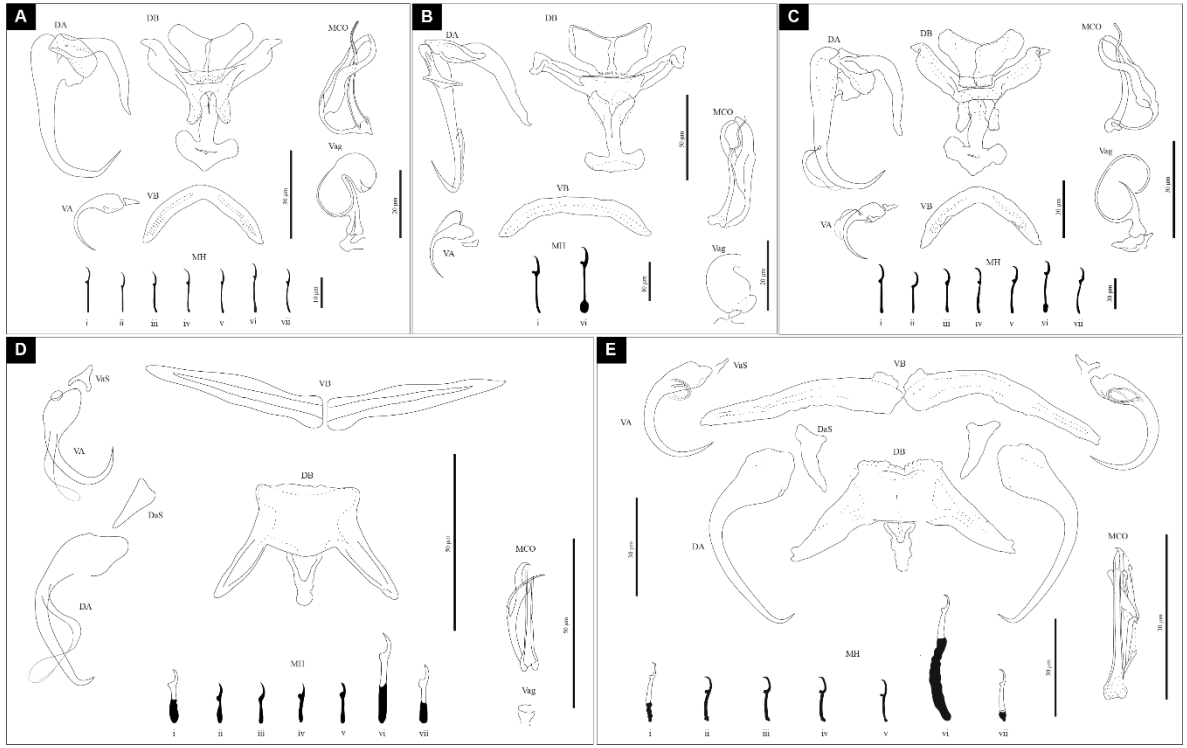

Figure A1.1. The haptoral sclerite complex and reproductive structures of *Paraquadriacanthus nasalis* Ergens, 1988 found in the nostril of *Clarias gariepinus* (Burchell, 1822) in Egypt. Illustrated and modified from holotype drawings presented in **A** – Ergens (1988), **B** – Kritsky and Kulo (1988) and voucher material in **C** – Řehulková (2018). *Quadriacanthus clariadis* Paperna, 1961: **D** – Egypt (El-Naggar and Serag 1985) and **E** – Sudan (Francová et al. 2017). Illustrations redrawn and modified from respective literature. Illustrations redrawn and modified from respective literature. Abbreviations: DA – dorsal anchor, DB – dorsal bar, VA – ventral anchor, VB – ventral bar, MH – marginal hook pairs, MCO – male copulatory complex, Vag – vagina.

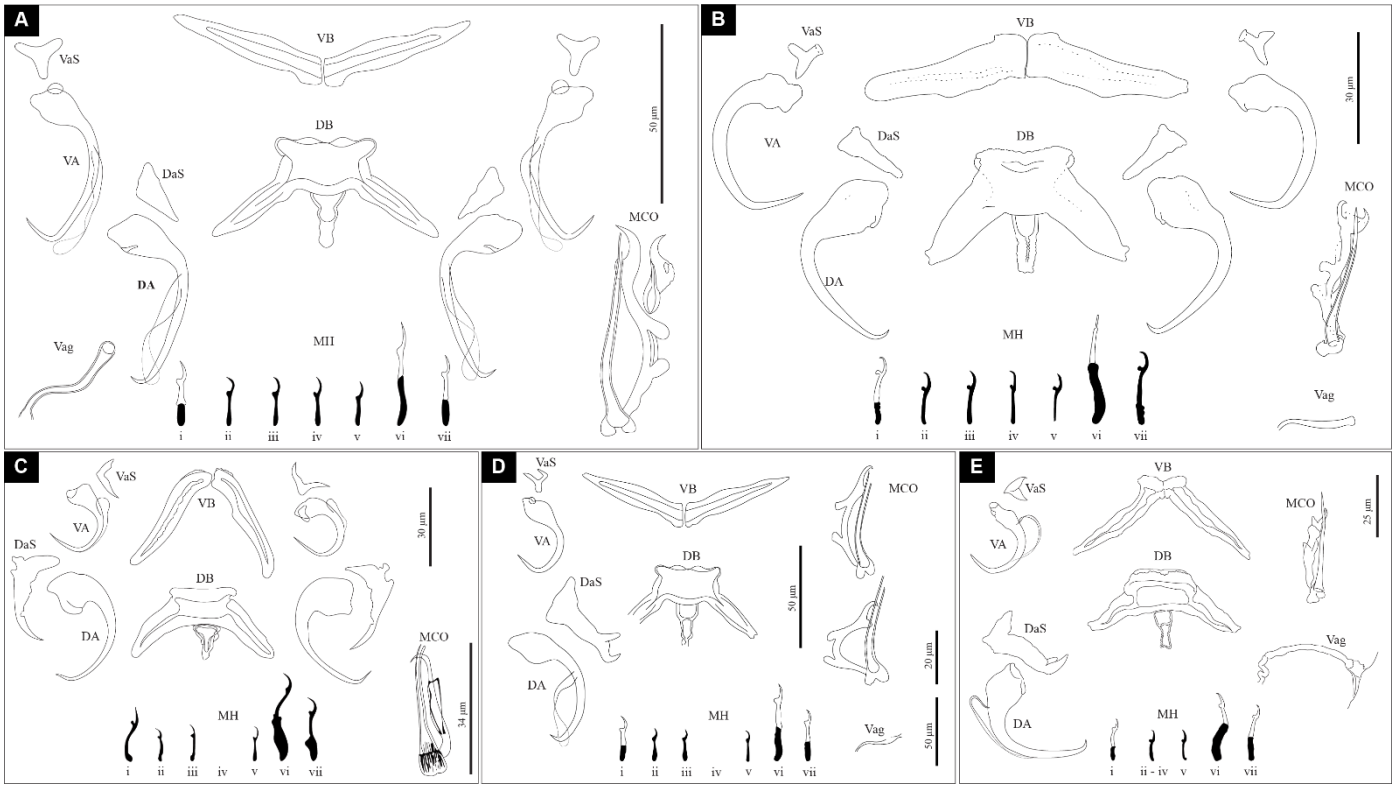

Figure A1.2. Haptoral sclerite morphology of *Quadriacanthus aegypticus* El-Naggar et Serag, 1985 from the gills of *Clarias gariepinus* (Burchell, 1822). Redrawn and modified from **A** – El-Naggar and Serag (1985) (Egypt) and **B** – Francová et al. (2017) (Kenya). *Quadriacanthus allobychowskiella* Paperna, 1979 from the gills of *C. gariepinus* in **C** – Uganda (Paperna 1979); **D** – Egypt (El-Naggar and Serag 1985) and **E** – Egypt (Kritsky and Kulo 1988). Illustrations redrawn and modified from respective literature. Abbreviations: VA – ventral anchor; VaS – ventral anchor supplementary piece; VB – ventral bar; DA – dorsal anchor; DaS – dorsal anchor supplementary piece; DB – dorsal bar; MH – marginal hooks; MCO – male copulatory organ and Vag – vagina.

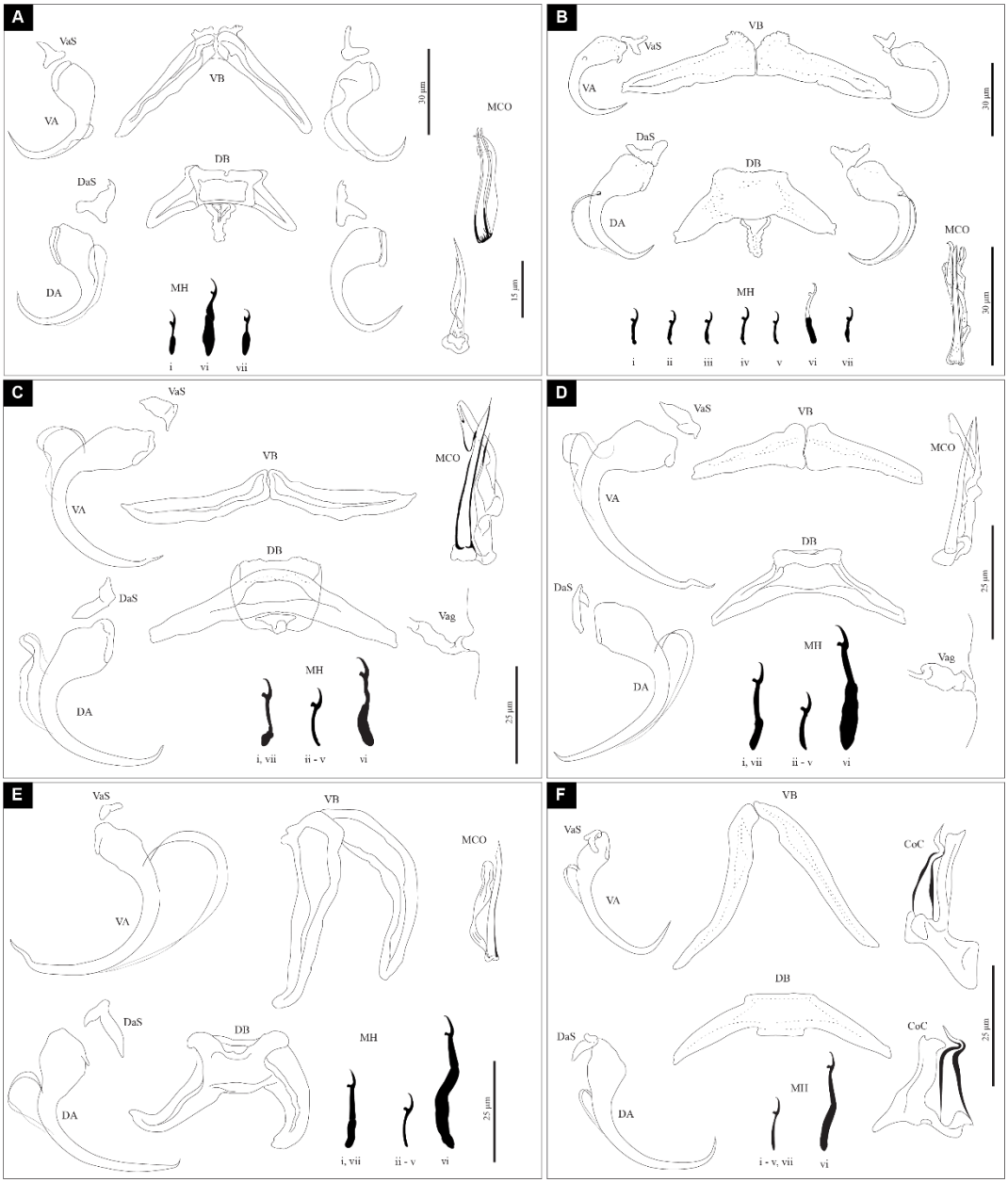

Figure A1.3. Haptoral sclerite morphology of *Quadriacanthus bagrae* Paperna, 1979 redrawn from **A** – Uganda (Paperna 1979) from gills of *Bagrus docmak* (Forsskål, 1775); **B** – Egypt (Kritsky and Kulo 1988) from the gills of *Clarias gariepinus* (Burchell, 1822); **C**– *Quadriacanthus ashuri* Kritsky et Kulo, 1988; **D** – *Quadriacanthus papernai* Kritsky et Kulo, 1988 **E** – *Quadriacanthus numidus* Kritsky et Kulo, 1988; and **F** – *Quadriacanthus voltaensis* Paperna, 1965 from the gills of *Clarias gariepinus* (Burchell, 1822) in Egypt (illustrations redrawn and modified from Kritsky and Kulo (1988)). Abbreviations: VA – ventral anchor; VaS – ventral anchor supplementary piece; VB – ventral bar; DA – dorsal anchor; DaS – dorsal anchor supplementary piece; DB – dorsal bar; MH – marginal hooks; MCO – male copulatory organ and Vag – vagina.

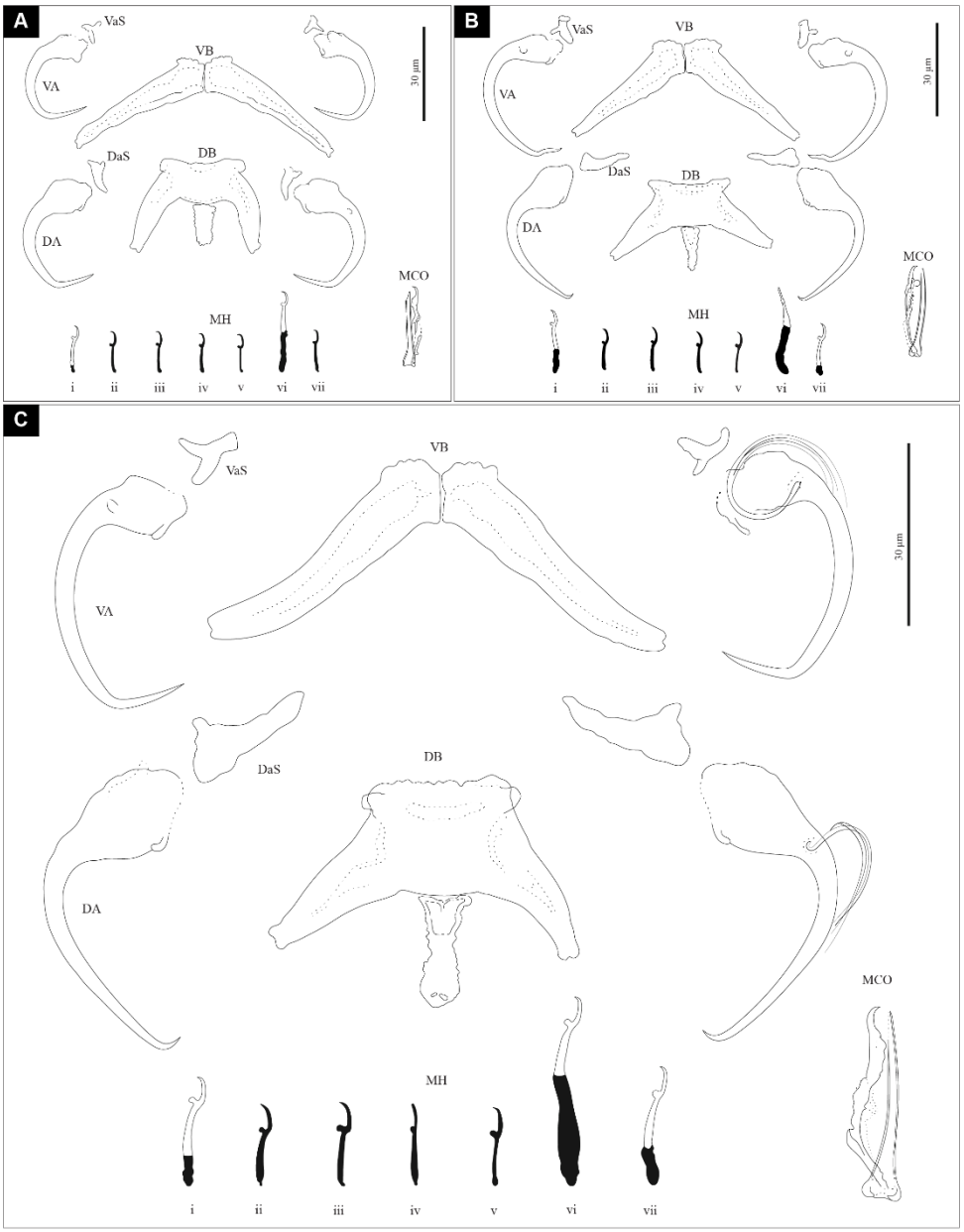

Figure A1.4. Haptoral sclerite morphology of **A** – *Quadriacanthus fornicatus* Francová et Řehulková, 2017; **B** – *Quadriacanthus pravus* Francová et Řehulková, 2017 and **C** – *Quadriacanthus zuheiri* Francová et Řehulková, 2017 from the gills of *Clarias gariepinus* (Burchell, 1822) in Sudan (illustrations redrawn and modified from Řehulková et al. (2017)). Abbreviations: VA – ventral anchor; VaS – ventral anchor supplementary piece; VB – ventral bar; DA – dorsal anchor; DaS – dorsal anchor supplementary piece; DB – dorsal bar; MH – marginal hooks; MCO – male copulatory organ and Vag – vagina.

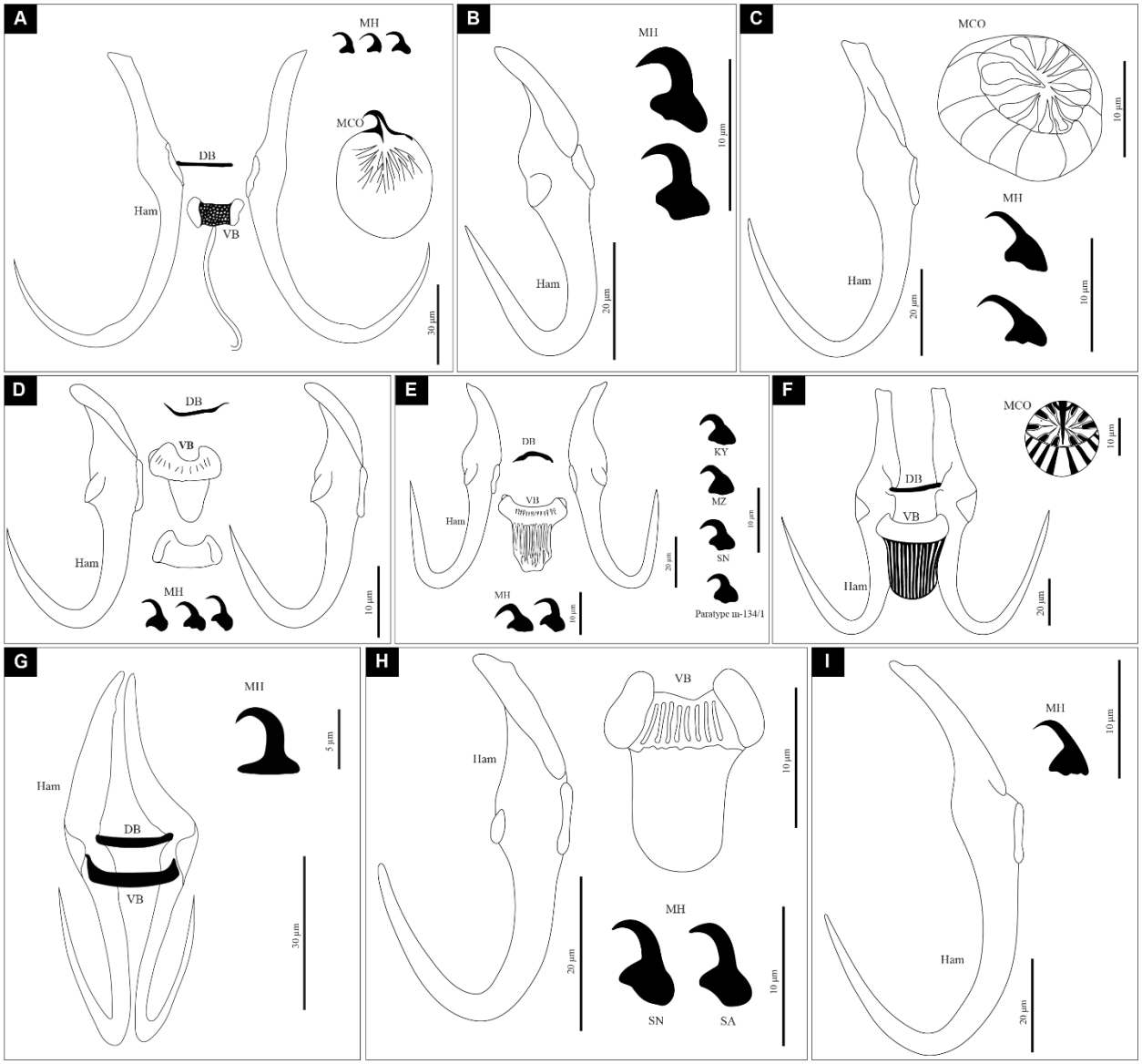

Figure A1.5. Haptoral sclerite morphology of **A** – *Gyrodactylus alberti* Paperna, 1973; **B** – *Gyrodactylus alekosi* Přikrylová, Blažek et Vanhove, 2012; **C** – *Gyrodactylus gelnari* Přikrylová, Blažek et Vanhove, 2012 from *Clarias anguillaris* (Linnaeus, 1758); **D** – *Gyrodactylus groschafti* Ergens, 1973; **E**, **F** – *Gyrodactylus rysavyi* Ergens, 1973; **G**, **H** – *Gyrodactylus transvaalensis* Prudhoe et Hussey, 1977 and **I** – *Gyrodactylus turkanaensis* Přikrylová, Blažek et Vanhove, 2012). Illustrations redrawn from literature. Abbreviations: Ham – hamuli; DB – dorsal bar; VB – ventral bar; MCO – male copulatory organ and MH – marginal hook sickle, morphological variations presented are redrawn from Přikrylová et al. (2012).

Appendix A2

During the present study several representatives of the Monogenea were recovered from the gill lamellae of *Clarias gariepinus* (Burchell). Here we present a detailed account on the morphological and morphometric comparisons of the species found in the present study (see **Chapter 3**, **Section 3.3.1.** and **Section 3.3.2.)**, with known species of *Quadriacanthus* (Ancylodiscoididae: Dactylogyridea) and *Gyrodactylus* (Gyrodactylidae: Gyrodactylidea). The following abbreviations appear in the tables: USNM – United Stated National Museum; HWML – University of Nebraska State Museum; INPA – Instituto Nacional de Pesquisas da Amazônia; MRAC – Musée Royal de l'Afrique Centrale.

Monogenea
Monopisthocotylea
Dactylogyridea
Ancylodiscoididae Gusev, 1961

Quadriacanthus Paperna, 1961

Quadriacanthus sp. A (**Fig. 3.5. A–E**; **Table A2.1.)**
Host: *Clarias gariepinus* (Burchell) (Siluriformes: Clariidae).
Localities (present study): Zambia – Kabompo River (KR), Northwestern Province and Barotse floodplain (ZR), Western Province. South Africa –the Ndumo Game Reserve (NGR, Phongolo and Usuthu rivers), KwaZulu-Natal Province; Vaal River (VR) and Boskop Dam (BS), North West Province; Gariep Dam (GD), Riet (RR) and Sand (SR) rivers, Free State Province and Great Fish River (GFR), Eastern Cape Province.
Site of infection: Gills .
Number of specimens studied: n = 4 (US), n = 15 (PH), n = 7 (BS), n = 2 (VR), n = 15 (GD).
Morphological observations: The general haptoral sclerite and male copulatory organ morphology of *Quadriacanthus* sp. A (**Fig. 3.5. A–E**) conform to the description for *Quadriacanthus aegypticus* El-Naggar et Serag, 1985 by El-Naggar and Serag (1985) and the redescription provided by Kritsky and Kulo (1988) (see **Fig. A1.2. A–B** and **Table A2.2.**). The haptoral sclerite complex consist of a dorsal bar, a medially articulating ventral bar, a pair of dorsal anchors with a long shaft and short, recurved points and a pair of ventral anchors with a long shaft and long recurved points, both pairs with accessory sclerites similar to that of the genus type species *Quadriacanthus clariadis* Paperna, 1961 (see **Fig. A1.1. D–E**). *Quadriacanthus aegypticus* can easily be distinguished from *Q. clariadis* in having larger ventral anchors in relation to the ventral bar, ventral anchors with more elongate shafts and a more robust and defined male copulatory organ (MCO). The MCO of our specimens are comparable to that of *Q. aegypticus*, in having a slender copulatory tube tapering into a fine tip and the accessory piece possess two protruding

diverticula in the medial section and terminates in a claw-like hook at the distal end. A smaller sclerite accompanies the distal hook of the accessory piece and can either be separate (**Fig. 3.5. C**) or attached (**Fig. 3.5. D**). Vagina is sclerotised (**Fig. 3.5. E**).

General morphometric comparisons fall within the ranges reported for *Q. aegypticus* by El-Naggar and Serag (1986), Kritsky and Kulo (1988), Douëllou and Chishawa (1995) and Francová et al. (2017) (see **Table A2.1.** and **Table A2.2.).** Based on comparative morphology and measurements, we assign *Quadriacantus* sp. A to *Quadriacanthus aegypticus*.

Table A2.1. Morphometrics of sclerotised structures of the haptor and male copulatory organ for ***Quadriacantus* sp. A** parasitising the gills of *Clarias gariepinus* (Burchell) in the present study. Measurements are presented in micrometres as mean followed by range in parentheses. Abbreviations: SA – South Africa, ZM – Zambia.

	Phongolo River KwaZulu-Natal SA n = 15		Usuthu River KwaZulu-Natal SA n = 4		Boskop Dam North West SA n = 7		Vaal River SA n = 2		Gariep Dam Free State SA n = 15	
Ventral anchor										
Total length	29.9	(24.3– 32.9) [14]	27.9	(26.2–29.8)	42.6	(38–45.2)	35	(34.6–35.4)	37.9	(35.6–44.3)
Point length	10.3	(4– 13.8) [14]	12.5	(10.8–13.6)	13.9	(12.4–14.9)	12.6	(12–13.3)	12.9	(11.8–14.1)
Accessory piece length	8.5	(5.1–12.6) [13]	6.6	(6.4–6.8)	11.8	(10.5–13.3)	10.1	(9.9–10.3)	10.4	(7.7–13.6)
Shaft curvature (x/y)	1.3	(1–1.2) [14]	1	(0.9–1)	1.4	(1.4–1.5)	1.4		1.4	(1.6–1.7)
Dorsal anchor										
Total length	38.7	(35.7–41.5) [13]	43.8	(42.9–45.5)	51.1	(45.1–60.1)	43.6[1]		47.7	(44.3–54.7) [14]
Point length	4.0	(2.5–6.1) [13]	5.4	(4.9–6.1)	5	(3.9–5.9)	3.6[1]		5	(3.6–6.3) [14]
Accessory piece length	16.8	(12.4–20.7) [13]	14.9	(14.3–15.8)	19.3	(14.9–23.5) [6]	21	(19.7–22.4)	20.4	17.8–23.8)
Shaft curvature (x/y)	1.1	(0.9–1.1) [13]	1.2	(1.3–1.4)	1.1	(1.0–1.2)	0.97[1]		1.2	(1.1–1.3) [14]
Ventral bar length									47.1	(42.5–49.6)
Dorsal bar	38.4	(29.9–49.2) [14]	49.7	(48.7–51.3)	47.5	(40.6–63.2)	41	(39.4–42.6)		
Total length									54.3	(50.5–62.9)
Total width	44.5	(30–56.1) [13]	56.3	(51.2–59.8)	58.2	(47.2–70.9)	49	(48.4–49.6)	33.8	(27.1–43.6)
Mid-region thickness	23.1	(16–29.2) [14]	26.6	(25.4–28.2)	34.1	(25.6–42.3)	25.9	(25.7–26.1)	18.8	(15.5–22.2)
Process length	12.5	(7.6–17.5) [14]	15.1	(11.4–18.3)	18.1	(14.6–20.4)	16.7		18.3	(16.6–22.3)
Marginal hooks	13.1	(9.4–17.7) [10]	11.9	(11.5–12.5)	18.8	(16.2–22.2)	15.5			
Pair I									19	(16.9–23.2) [14]
Pair II-V	16.3	(15.8–17.2)	16.1	(14.9–17.2)	20.7	(20.4–21.1) [5]	17.1	(16.5–17.6)	13.4	(11.4–19.7)
Pair VI	13.3	(12.6–13.9)	14.1	(13.7–14.5)	15.8	(13.6–17.3) [6]	14.1	(13.1–16.8)	31.5	(29.3–37.1) [12]
Pair VII	26.3	(18.6–31.4) [11]	32.0	(31.5–32.8)	30.6	(23.1–34.7) [6]	29.5		18.9	(10.4–23.1) [14]
Vagina total length	18.1	(17.2–18.7)	16.5	(15.2–18.3) [3]	20.7	(18.5–22.6) [6]	18.6	(18.3–18.8)	30.8	
	22.1	(15.5–26.3) [7]	–		34.9	(20.7–44.5) [5]	–			
Male copulatory organ										
Total length	39.0	(22–43.4) [13]	26.2	(24.8–27.6)[2]	53.1	(40.8–60.9)	42.9	(40.8–45)	47.4	(41.8–68.3)
Total trace length (copulatory tube)	36.4	(21.8–40.7) [14]	23.2	(22–24.3)[3]	50.2	(42.1–57.6)	42.2	(41.2–43.1)	47	(40.9–63)

Numeral value in superscript indicates the number of individuals measured.

Table A2.2. Comparative morphometrics of ***Quadriacanthus aegypticus*** El-Naggar et Serag, 1985 from type host *Clarias gariepinus* (Burchell) from various localities in Africa. Measurements are presented in micrometres as mean followed by range in parentheses. Abbreviations: EG – Egypt, KY – Kenya, SU – Sudan, ZI – Zimbabwe.

	Nile River[†] EG n = 10 El-Naggar & Serag (1986)		Voucher material[a] n = 26 Kritsky & Kulo (1988)		Lake Kariba ZI n = 14 Douëllou & Chishawa (1995)		Lake Turkana / Blue and White Nile KY / SU n = 13 Francová et al. (2017)	
Ventral anchor								
Total length	41	(35–43)	38	(60 –89)	31.9	(29.4– 33.5)	35	(33–37)[10]
Point length	11	(9–13)	–		12.6	(10.5– 14.1)	12	(11–13)[10]
Accessory piece length	–		–		8.8	(10.5– 14.1)	10	(9–11)[10]
Shaft curvature (x/y)	–		–		–		1.4	(1.2–1.7)[10]
Dorsal anchor								
Total length	46	(42–49)	47	(43 –51)	37.6	(34.3– 40.6)	42	(40–44)[10]
Point length	3	(3–4)	–		33.7	(3.3– 4.1	4	(3– 5)[10]
Accessory piece length	17	(14–19)	–		16.1	(14.5–17.8)	19	(6–21)[10]
Shaft curvature (x/y)	–		–		–		1.1	(1.1–1.2) [10]
Ventral bar length	44	(40–47)	47	(39–53)	34.9	(31.5–37.2)	43	(40–46)[10]
Dorsal bar								
Total length	–		57	(45–74)	38.8	(34.8–43.9)	49	(39–52) [10]
Total width	–		–		–		28	(21–31) [10]
Mid-region thickness	–		–		–		15	(10–17) [10]
Process length	15	(12–18)	–		15.8	(13.2–16.6)	15	(14–17) [10]
Marginal hooks								
Pair I	18	(16–19)	18	(16–22)	15	(13.2–16.6)	17	(16–19) [10]
Pair II-V	13	(11–14)	14	(13–16)	–		13	(11–15) [10]
Pair VI	30	(27–36)	33	(31–36)	25.2	(24–26.9)	29	(26–29) [10]
Pair VII	20	(19–22)		(20–21)	15.4	(13.2–17.3)	18	(18–19) [10]
Vagina total length	31	(18–38)	–		19.4	(14.5–21.5)	19	(17–21) [10]
Male copulatory organ								
Total length	–		39	(33–49)	40	(36.4–43.9)	41	(39–44) [10]
Total trace length (copulatory tube)	53	(42 – 56)	43	(40–52)	37.3	(34–40.1)	38	(36–40) [10]

Numeral value in superscript indicates number of individuals measured.

[†] Type locality.

[a] Voucher material collection numbers: HWML 20765; INPA PA315-1, 2; MRAC 37.163; USNM 79900.

Quadriacanthus sp. B (**Fig. 3.5. F–I**; **Table A2.3.)**

Host: *Clarias gariepinus* (Burchell) (Siluriformes: Clariidae).

Localities (present study): Zambia – Barotse floodplain (ZR), Western Province. South Africa – Phongolo and Usuthu rivers in the Ndumo Game Reserve (NGR), KwaZulu-Natal Province; Boskop Dam (BS) and Vaal River (VR), North West Province; Gariep Dam (GD), Sand River (SR), Free State Province; Great Fish River (GFR), Eastern Cape Province; Riviersonderend (RSE), Western Cape.

Site of infection: Gills .

Number of specimens studied: n = 3 (US), n = 4 (PH), n = 4 (BS), n = 6 (VR), n = 11 (GD).

Morphological observations: General sclerite morphology of *Quadriacanthus* sp. B (**Fig. 3.5. F**) is similar to that of *Quadriacanthus bagrae* Paperna, 1979*, Quadriacanthus fornicatus* Francová et Řehulková, 2017 and *Quadriacanthus simplex* N'Douba, Lambert et Euzet, 1999. The male copulatory organ components of *Quadriacanthus* sp. B resemble that of *Q. clariadis*, *Q. bagrae* and *Q. fornicatus* (see **Fig. 3.5. G–I**, **Fig. A1.1. D–E**, **Fig. A1.3. A–B** and **Fig. A1.4. A**) (Kritsky and Kulo, 1988; N'Douba et al. 1999; Francová et al. 2017). From specimens studied in the present study it is apparent that there is undeniable similarity in haptoral morphology of *Quadriacanthus* sp. B to those of *Q. bagrae*, *Q. fornicatus* and *Q. simplex* in sharing the same anchor types of the dorsal and ventral anchors pairs that both have short shafts and long recurving points (see **Fig. A1.3. C–F** and **Fig. 3.5. F**, and N'Douba et al. 1999) that easily distinguish these species from the genus type species *Q. clariadis* (**Fig. A1.1. D–E**) (dorsal anchors of *Q. clariadis* has a long shaft and short recurved point and ventral anchors with a short, smoothly curved shaft that taper into a recurved long point). These features are adequately characterised in the descriptions, redescriptions and reports of the respective species by Kritsky and Kulo (1988), N'Douba et al. (1999), Tripathi et al. (2007) and Francová et al. (2017). Morphological size comparison of *Quadriacanthus* sp. B to *Q. bagrae*, *Q. fornicatus* and *Q. simplex* (Table A.3. and Table A.4.) does not provide substantial support to distinguish between the species, as their morphometric ranges overlap. Based on morphological comparison of the dorsal and ventral anchors, *Quadriacanthus* sp. B retain more slender and longer shafts in both anchor pairs that taper into long recurved point, compared to the more robust and stout dorsal and ventral anchor appearance of *Q. bagrae* (**Fig. A1.3. A–B** and **Fig. 3.5. F**). Despite size similarities in the ventral bar lengths for *Q. bagrae* and *Q. fornicatus*, the latter seems to have more slender ventral bars than *Q. bagrae*, similar to that of *Quadriacanthus* sp. B. Both Tripathi et al. (2007) and Francová et al. (2017) support the redescription of *Q. bagrae* by Kritsky and Kulo (1988) and only report on the morphological findings in their respective surveys. Francová et al. (2017) described *Q. fornicatus* (Fig. 3.12. A) and provides a comparison to *Q. simplex*, but no differentiation from *Q. bagrae* is provided. Morphological comparison of the MCO eliminates *Q. simplex* as a possible identity, as *Quadriacanthus* sp. B has a more complex accessory piece terminating in a distal

hook with a broad base and slightly sclerotised medial part of the accessory piece, while the MCO of *Q. simplex* is rather simple with no distinct features (see **Fig. 3.5. G–I** and N'Douba et al. 1999). In their redescription of *Q. bagrae*, Kritsky and Kulo (1988) noted that *Q. bagrae* is similar to *Q. voltaensis*, however these two species can easily be distinguished from one another based on the MCO morphology (see **Fig. A1.3. A–B**, **F**). The MCO of our specimens has similar morphology as the MCO of *Q. bagrae* and *Q. fornicatus*, thus it would be necessary to do a proper comparison of our species to type material of both *Q. bagrae* and *Q. fornicatus* to confirm their identity. Additionally, as in these two species, the vagina was not observed in our specimens. Based on general haptoral sclerite, ventral anchor morphology and MCO morphology and size (see **Table A2.3.** and **Table A2.4.**), we temporarily assign *Quadriacanthus* sp. B from the present study as *Quadriacanthus fornicatus*.

Table A2.3. Morphometrics of sclerotised structures of the haptor and male copulatory organ for ***Quadriacantus* sp. B** parasitising the gills of *Clarias gariepinus* (Burchell, 1822) in the present study. Measurements are presented in micrometres as mean followed by range in parentheses. Abbreviations: SA – South Africa, ZM – Zambia.

	Phongolo River KwaZulu-Natal SA n = 4		Usuthu River KwaZulu-Natal SA n = 3		Boskop Dam North West SA n = 4		Vaal River North West SA n = 6		Gariep Dam Free State SA n = 11	
Ventral anchor										
Total length	24.9	(23–25.8)	28	(27.1–29.1)	31.3	(30.5–32.1)	25.6	(25.1–26.3)	29.6	(28.4–31)
Point length	11.2	(8–14.1)[2]	13.3	(12–15.5)	14.4	(13.1–15.1)	11.1	(10.5–11.6)	13	(11.9–15.1)
Accessory piece length	5.6	(5.3–5.9)[3]	6.5	(5.7–7)	10.1	(9.4–10.8)[2]	5.8	(4.7–6.5)[4]	7	(6.5–7.7)
Shaft curvature (x/y)	1.3	(1.2–1.3)[3]	1.1		1.2	(1.1–1.3)	1.1	(1.3–1.4)	1.3	(1.4–1.5)
Dorsal anchor										
Total length	30.6	(29.1–32)	33.7	(32.4–35.9)	38.9	(36.2–41.5)	33.1	(31.7–34.7)	36.2	(34.4–38.23)
Point length	10.5	(9.5–11.7)	11.5	(9.7–13)	13.9	(13.3–14.4)	10.6	(8.7–12.1)	13	(11.8–13.7)
Accessory piece length	8.8	(6.5–10.1)	8.8	(8–9.7)	10.1	(9.2–11.9)	9.3	(8.1–10.1)[4]	10.1	(7.1–11.3)
Shaft curvature (x/y)	1	(1–1.1)	1.2	(1.2–1.4)	1.2		1.1	(1.1–1.2)	1.2	
Ventral bar length	42.5	(39–44.9)	45.7	(41.9–49)	52.7	(49.2–55.3)	42.9	(40–46.3)	51.8	(48.1–54.7)
Dorsal bar										
Total length	51.6	(47.9–54.4)	56.5	(53.7–58.8)	67.4	(63.7–70.2)	48.4	(44.9–52.8)	61.3	(51.5–70.5)
Total width	22.4	(19.9–24.1)	24.6	(21.2–28.5)	29.2	(28.1–30.2)	24.6	(20.6–29.4)	28.2	(24.2–31.3)
Mid-region thickness	9.2	(8.3–10)	12	(10.5–13.7)	13.3	(12.6–13.9)	10.0	(8.8–11.3)	13	(10.7–16.3)
Process length	11.1	(10.5–12)[3]	11	(9.7–13.4)	14.2	(13.8–14.8)	10.1	(8.9–11.8)[4]	14.2	(11.4–18.1)
Marginal hooks										
Pair I	–		16.1	(14.6–17.4)	17.4	(15.6–19.1)[2]	14.6	(13.65–15)[4]	16.1	(15–17.1)
Pair II-V	–		13.5	(12.5–14.9)	15.6	(15–16.1)[2]	14.2	(13.9–14.8)[3]	13.7	(11.7–14.8)
Pair VI	21.4	(18.5–22.9)	24.1	(21.7–26.6)[2]	25.4	(25.2–3)	21.7	(18.5–24.3)	24.2	(22.8–25.7)
Pair VII	–		14.6	(14.1–14.9)	17.2	(16.8–17.6)[2]	14.3	(13.3–15)[4]	14.9	(14.4–15.7)
Vagina total length	–		–		–		–		–	
Male copulatory organ										
Total length	25.6	(24.6–26.9)[3]	25.9	(24.9–26.9)[2]	29.6	(28.2–30.4)	26.0	(23–27.9)	27.3	(25.6–28.9)
Total trace length (copulatory tube)	19.3	(14.5–22.8)[3]	25.5	(23.8–27.3)[2]	27.2	(25.3–29.3)	27.8	(26.5–32.1)	26.0	(24–28.4)

Numeral value in superscript indicates the number of individuals measured.

Table A2.4. Comparative morphometrics for *Quadriacanthus bagrae* Paperna, 1979, *Quadriacanthus fornicatus* Francová et Řehulková, 2017 and *Quadriacanthus simplex* N'Douba, Lambert et Euzet, 1999 from bagrid and clariid host in Africa. Measurements are presented in micrometres as mean followed by range in parentheses. Abbreviations: IC – Ivory Coast, ID – India, SU – Sudan.

	Quadriacanthus bagrae	*Q. bagrae*	*Q. bagrae*	*Q. fornicatus*	*Q. simplex*
	Voucher material[a]	Gomti River	Lake Turkana / Blue and White Nile	Blue and White Nile[†]	Bia River[†]
		ID	SU	SU	IC
	n = 31	n = 22	n = 13	n = 7	n = 12
	Various hosts	*Clarias gariepinus*	*B. docmak*[#]	*C. gariepinus*[#]	*Heterobranchus isopterus*[#]
	Kritsky & Kulo (1988)	Tripathi et al. (2007)	Francová et al. (2017)	Francová et al. (2017)	N'Douba et al. (1999)
Ventral anchor					
Total length	32 (30–34)	27 (24–29)[15]	30 (27–33)	28 (26–31)	27 (24–29)
Point length	–	–	12 (9–14)	12 (11–13)	15 (13–16)
Accessory piece length	–	–	9 (8–11)	7 (7–8)	–
Shaft curvature (x/y)	–	–	1.3 (1.1–1.6)	1.2 (1.0–1.3)	–
Dorsal anchor					
Total length	39 (33–42)	32 (29–35)[15]	40 (37–43)	35 (32–39)	35 (33–37)
Point length	–	–	9 (8–9)	12 (12–13)	11 (10–13)
Accessory piece length	–	–	17 (16–18)	12 (9–14)	6 (5–7)
Shaft curvature (x/y)			1.2 (0.9–1.3)	1.1 (0.9–1.2)	–
Ventral bar length	52 (42–64)	52 (48–54)[15]	52 (47–57)	49 (44–54)	46 (41–49)
Dorsal bar					
Total length	63 (51–78)	55 (40–58)[15]	64 (58–73)	54 (42–64)	–
Total width	–	–	29 (26–33)	27 (17–33)	–
Mid-region thickness	–	–	20 (17–23)	12 (8–14)	–
Process length	–	15 (13–19)[15]	13 (12–15)	12 (9–13)	10 (9–12)
Marginal hooks					
Pair I	18 (17–19)	15 (14–16)[15]	15 (14–16)[3]	15 (13–17)	15 (14–17)
Pair II-V	–	12 (10–12)[15]	14 (12–16)[3]	13 (12–14)	14 (13–17)
Pair VI	–	25 (22–27)[15]	23 (21–25)	27 (25–29)	25 (24–28)
Pair VII	–	–	15 (13–15)[3]	14 (13–14)[6]	16 (14–17)
Vagina total length	–	–	–	–	–
Male copulatory organ					
Total length	19 (17–21)	–	34 (32–35)	26 (23–29)	25 (24–28)
Total trace length (copulatory tube)	25 (23–27)	–	31 (29–33)	24 (22–26)	27 (26–28)

Numeral value in superscript indicates number of individuals measured.
[#] Type host.
[†] Type locality.
[a] Voucher material collection numbers: HWML 20759; INPA PAS 10-1-3; MRAC 34290; MRAC 37.157; USNM 79891.

Table A2.5. Morphometrics of sclerotised structures of the haptor and male copulatory organ for ***Quadriacantus* sp. C** parasitising the gills of *Clarias gariepinus* (Burchell) in during the present study. Measurements are presented in micrometres as mean followed by range in parentheses. Abbreviations: SA – South Africa, ZM – Zambia.

	Boskop Dam North West SA n = 3		Vaal River North West SA n = 3		Gariep Dam Free State SA n = 2	
Ventral anchor						
Total length	33.2	(31.8–34)	28.2	(26.8–29.5)	29.9	(29.6–30.1)
Point length	13.9	(12.4–14.7)	11.7	(9.7–13)	12.7	(12–13.4)
Accessory piece length	8.1	(6.8–9.2)	6.1	(5.5–6.5)	8	(7.9–8)
Shaft curvature (x/y)	1.6	(1.5–2)	1.4	(1.3–1.5)	1.1	(1.1–1.2)
Dorsal anchor						
Total length	51.7	(50.4–53.1)	43.9	(42.4–46.1)	47.8	(47.6–48)
Point length	5.9	(4.8–6.7)	6.1	(4.4–7)	5.8	(5.5–6.1)
Accessory piece length	17.6	(17.1–18)	16.2	(14.9–17.6)	16.5	(16.4–16.5)
Shaft curvature (x/y)	1	(0.9–1)	1	(1–1.1)	1	(0.9–1)
Ventral bar length	58.9	(55.7–61.1)	56.3	(45.7– 62.8)	53.5	(53.1–53.9)
Dorsal bar						
Total length	67.8	(63.6–72)	61.9	(52.9–66.8)	59.3	(56.5–62.1)
Total width	34.5	(32.2–36)	29.4	(22.2–36.5)	29.3	(25.7–33)
Mid-region thickness	19.0	(18.6–20)	16.9	(10.9–22.8)	16.4	(13.5–19.4)
Process length	16.2	(15.3–17.1)	15.2	(12.7–17.8)^{2}	14	(13.1–14.9)
Marginal hooks						
Pair I			18.7	(17.2–19.5)	19	(18–19.9)
Pair II - V			14.6	(14.2–15)^{2}	14.9	(14–15.9)
Pair VI	35.1	(32.2–37.5)	31.6	(31.1–32.2)	35	(34.3–35.7)
Pair VII			15.7	(15.5–15.9)^{2}	16	(15.8 –16.26)
Vagina total length	–		–		–	
Male copulatory organ						
Total length	28.4	(25.1–31)	24.9	(23.7–26)	26.4	(25.8–27.1)
Total trace length (copulatory tube)	25.4	(23.9–26.8)	25.9	(24.9–26.8)^{2}	28.0	(27.3–28.8)

Numeral value in superscript indicates the number of individuals measured.

Quadriacanthus sp. C (**Fig. 3.5. J–M**; **Table A2.5.**)

Host: *Clarias gariepinus* (Burchell) (Siluriformes: Clariidae).

Localities (present study): South Africa – Boskop Dam (BS) and Vaal River (VR), North West Province; Gariep Dam (GD) and Sand River (SR), Free State Province; Great Fish River (GFR), Eastern Cape Province and Riviersonderend (RSE), Western Cape Province.

Site of infection: Gills .

Number of specimens studied: n = 3 (BS), n = 3 (VR), n = 2 (GD).

Morphological observations: The general sclerite morphology of *Quadriacanthus* sp. C (**Fig. 3.5. J–K**) closely resemble to those of *Q. aegypticus*, *Quadriacanthus agnebiensis* N'Douba, Lambert et Euzet, 1999, *Quadriacanthus allobychowskiella* Paperna, 1979, *Q. clariadis*, *Quadriacanthus longifilisi* N'Douba, Lambert et Euzet, 1999 and *Quadriacanthus zuheiri* Francová et Řehulková, 2017 (see Paperna 1961; El-Naggar and Serag 1985; Kritsky and Kulo 1988; N'Douba et al. 1999). The haptoral sclerite complex of *Quadriacanthus* sp. C have a dorsal bar, a medially articulating ventral bar and a pair of dorsal anchors with a long shaft and short recurving point, and ventral anchors with a short shaft, smoothly curving into a tapering long point (**Fig. 3.5. J**). Based on comparative morphology, *Quadriacanthus* sp. C can easily be distinguished from *Q. aegypticus* in the latter having ventral anchors with a long shaft, sharply tapering into a recurved long point and large MCO with a highly differentiated accessory piece with two knob-like projections in the medial region (see **Fig. A1.2. A–B** and **Fig. 3.5. J–K**; **Table A2.2.** and **Table A2.5.**). *Quadriacantus allobychowskiella* differ markedly from *Q uadriacanthus* sp. C in having a large and differentiated accessory piece on the dorsal anchor pairs and a larger MCO with a robust accessory piece (see **Fig. A1.2. C–E**). *Quadriacanthus agnebiensis* and *Q. longifilisi*, described from *Heterobranchus isopterus* Bleeker and *H. longifilis* Valenciennes, respectively, possess a longer hook pair VII and the MCO size and morphology of both species is markedly different from that of *Q uadriacanthus* sp. C (see **Fig. 3.5. J–K**, **Table A2.5.** and **Table A2.12.**) (N'Douba et al. 1999). *Quadriacanthus* sp. C may be confused with *Q. zuheiri*, also known from *C. gariepinus*, as their MCO morphology is very similar, if not identical, in the hook with a wide base at the distal end of the accessory sclerite, however it can be separated as two distinct species in the size difference (*Q. zuheiri* has a markedly longer copulatory tube and accessory piece). The two species can also be easily distinguished in the larger

dorsal and ventral anchor size of *Q. zuheiri* (see **Fig. A1.4. C** and **Table A2.5.**, **Table A2.12.**). Similarities of *Quadriacanthus* sp. C to *Q. clariadis* can be seen in the conspecific dorsal and ventral anchor morphology and the size of haptoral sclerite components (see **Fig. A1.1. D–E**, **Table A2.5.**, **Table A2.6.** and Kritsky and Kulo 1988). Tripathi et al. (2007) and Francová et al. (2017) were the first to provide a detailed illustration of the MCO of *Q. clariadis* compared to the representation of the MCO in the description of *Q. clariadis* by Paperna (1979) and in the report of El-Naggar and Serag (1985). Comparative morphology indicates that the MCO of *Q uadriacanthus* sp. C (**Fig. 3.5. L–M**) is very similar to the representation provided by Tripathi et al. (2007) and Francová et al. (2017). The specimens studied in the present study conforms to the general haptoral sclerite morphology of *Q. clariadis* as well as in the size and morphology of the MCO. The only difference observed in our specimens when compared to *Q. clariadis* is the finger-like projection on the posterior end of the hook sickle of the distal part of the accessory piece (see **Fig. 3.5. L–M**). Vagina not observed. We assign *Quadriacanthus* sp. C to *Quadriacanthus clariadis*.

Table A2.6. Comparative morphometrics of *Quadriacanthus clariadis* Paperna, 1961 from bagrid and clariid hosts in Africa. Measurements are presented in micrometres as mean followed by range in parentheses. Abbreviations: EG – Egypt, ID – India, IL – Israel.

	Lake Galilee[#]	Nile River	Voucher specimens[a]	Gomti River	Lake Turkana / Blue and White Nile
	IL	EG		ID	KY/SU
	n =	n = 10	n = 7	n = 9	n = 13
	Clarias gariepinus[#]	*C. gariepinus*	*C. gariepinus*	*C. gariepinus*	*Bagrus docmak*
	Paperna (1961)	El-Naggar & Serag (1985)	Kritsky & Kulo (1988)	Tripathi et al. 2007	Francová et al. (2017)
Ventral anchor					
Total length	(27–38)	41 (35–43)	32 (29–34)	24 (22–26)	29 (28–31)
Point length		–	–	–	11 (10–13)
Accessory piece length		–	–	–	8 (7–10)
Shaft curvature (x/y)		–	–	–	1.4 (1.1–1.9)
Dorsal anchor					
Total length	(38–55)	46 (42–49)	49 (47–51)	40 (36–42)	48 (45–51)
Point length		–	–	–	6 (5–7)
Accessory piece length		17 (14–19)	–	–	17 (15–20)
Shaft curvature (x/y)		–	–	–	1.0 (0.9–1)
Ventral bar length		–	53 (42–65)	44 (40–46)	60 (53–64)
Dorsal bar					
Total length		–	63 (52–72)	50 (42–54)	60 (54–65)
Total width		–	–	–	32 (29–37)
Mid-region thickness		–	–	–	17 (13–19)
Process length		15 (13–17)	–	12 (9–14)	15 (13–16)
Marginal hooks					
Pair I		16 (15–18)	19 (18–20)	17 (15–18)	20 (18–22)
Pair II-V		–	14 (13–15)	12 (12–13)	14 (12–15)
Pair VI		–	35 (32–39)	30 (27–32)	35 (33–39)
Pair VII		16 (15–18)	(16–17)	15 (13–16)	16 (15–16)
Vagina total length		–	–	–	–
Male copulatory organ					
Total length		–	21 (17–26)	22 (21–24)	27 (26–28)
Total trace length (copulatory tube)	(22–35)	27 (23–30)	25 (22–31)	20 (18–22)	26 (24–27)

Numeral value in superscript indicates number of individuals measured.
[#] Type host.
[†] Type locality.
[a] Voucher material collection numbers: HWML 20762; INPA PA 313-1; MRAC 37.160; USNM 79896.

Quadriacanthus sp. D (**Fig. 3.6. A**–**I**; **Table A2.7**.)

Host: *Clarias gariepinus* (Burchell) (Siluriformes: Clariidae).

Localities: Zambia – Barotse floodplain (ZR), Western Province. South Africa – Boskop Dam (BS) and Vaal River (VR), North West Province; Gariep Dam (GD), Riet (RR) and Sand (SR) rivers, Free State Province; Great Fish River (GFR), Eastern Cape Province.

Site of infection: Gills .

Number of specimens studied: n = 2 (ZR), n = 1 (BS), n = 1 (VR), n = 3 (SR), n = 3 (SR), n = 1 (RR), n = 2 (GD), n = 3 (GFR).

Morphological observations: Morphological and size comparison render *Quadriacanthus* sp. D as identical to *Quadriacanthus allobychowskiella* Paperna, 1979 (see **Table A2.7.** and **Table A2.8.**) (Kritsky and Kulo 1988; El-Naggar and Serag 1985). Kritsky and Kulo (1988) redescribed *Q. allobychowskiella*, originally proposed as a sub-species of *Q. clariadis* by Paperna (1979), and synonymised *Q. kearni sensu* El-Naggar and Serag (1985) with *Q. allobychowskiella* (see Section 3.3.1.2. for detail). Specimens of *Quadriacanthus* sp. D studied during this study, retain a dorsal anchor with a long shaft, tapering into a short recurving point and a distinctly large accessory piece characterised by a terminal hook and bifid subterminal lobes not observed in any other species of *Quadriacanthus* (see **Fig. 3.6. A–D**). The ventral anchors have a short shaft, tapering into a long point with an accessory piece that is well-defined with to projections, almost perpendicular to each other, ending in a sharp point (see **Fig. 3.6. E**). The male copulatory organ of *Quadriacanthus* sp. D is similar to the MCO morphology of *Q. aegypticus* that has a long copulatory tube and accessory piece that is distinctly sclerotised, however it has a longer copulatory tube than *Q. aegypticus* and a smaller (in length) accessory piece that is attached to the base of copulatory tube and terminates in a single hook with a very broad base on the distal end. In the mid-region of the accessory piece two diverticula is present, also noted in the description of El-Naggar and Serag (1985) (see **Fig. 3.6. G–I** and **Fig. A1.2. C–E**). In some specimens it seems as if the diverticula are connected (see **Fig. 3.6. G**). Vagina observed, sclerotised (**Fig. 3.6. F**).

Table A2.7. Morphometrics of sclerotised structures of the haptor and male copulatory organ for ***Quadriacantus* sp. D** parasitising the gills of *Clarias gariepinus* (Burchell) in the present study. Measurements are presented in micrometres as mean followed by range in parentheses. Abbreviations: SA – South Africa, ZM – Zambia.

	Great Fish River		Gariep Dam		Riet River	Sand River		Vaal River	Boskop Dam	Barotseland floodplain	
	Eastern Cape		Free State		Free State	Free State		North West	North West	Northwestern Province	
	SA		SA		SA	SA		SA	SA	ZM	
	n = 3		n = 2		n = 1	n = 3		n = 1	n = 1	n = 2	
Ventral anchor											
Total length	27.4	(26.7–28.3)	28.2	(27.4–29.1)	27.9	25.6	(24–26.8)	23.2	26.3	25.1	(24.7–25.5)
Point length	10.5	(9.7–10.9)	9.5	(8.7–10.4)	12.2	10.1	(9.3–10.4)	9.8	8.5	9.3[1]	
Accessory piece length	12.9	(12.6–13.2)	12.2	(12.2–12.3)	10.1	11.3	(8.8–14.6)	–	11.7	9.7	(8.4–11)
Shaft curvature (x/y)	1.4	(1.3–1.4)	1.1	(1–1.1)	1.2	1.3	(1.1–1.3)	1.2	1.1	1.5[1]	
Dorsal anchor											
Total length	47.3	(44.3–49)	46.6		48.2	46.4	(44.2–47.7)	47.9	49.3	42.2	(40.5–43.8)
Point length	4.3	(4.3–4.4)	4.7	(3.8–5.6)	3.5	4.1	(3.5–4.6)	3.4	3.1	3.7	(3.5–4)
Accessory piece length	31.2	(28.5–34.8)	37.4	(32–42.9)	29.8	32.2	(30.6–33.5)	26.6	35.6	28.1	(27.6–28.7)
Shaft curvature (x/y)	1.5	(1.7–1.4)	1.1	(1–1.1)	1.3	1.3	(1–1.2)	1.1	1.4	1.4	(1.6–1.3)
Ventral bar length	46	(44.4–49.2)	47.9[1]		45.7	48.0	(46.3–49.6)	39.4	46.3	40.9	(40.6–41.2)
Dorsal bar											
Total length	54.6	(53.6–56.3)	56.6[1]		63.8	63.8	(61.7–67)	55.7	56.6	52.7	(52.2–53.1)
Total width	30.3	(26.6–36.6)	31.5[1]		30.6	30.3	(30–30.8)	22.2	29	27.4	(27–27.8)
Mid-region thickness	18.4	(17.5–19.2)	19.5[1]		21.2	21.3	(19.6–24)	13.5	19.5	15.5	(15.4–15.5)
Process length	15.8	(13.1–17.6)	16.5[1]		12.8	15.3	(14.5–16.1)	11.7	13	12.9	(12.9–12.9)
Marginal hooks											
Pair I	17.6	(17.2–18.3)	16.7[1]		17.6	17.6	(17–18.6)	18.0	16.9	17.2	(17.2–17.2)
Pair II-V	14.4	(13.8–14.8)	14.4[1]		11.8	14.3	(13.8–14.2)	14.4	14.4	14.6	(13.1–15.3)
Pair VI	28.6	(26.1–30)	27[1]		30.0	29.2	(28.6–29.6)	26.0	29.8	26.9	(26–27.8)
Pair VII	20.9	(20.4–21.5)	22.9[1]		16.7	21.5	(20.9–22.6)	18.4	20.5	21.0	(20.7–21.3)
Vagina total length	24.1	(35.6–36.7)[2]	31.8[1]		53.65	37.99	(34.47–41.51)[2]	29.88	38	29.61	(24.52–34.7)
Male copulatory organ											
Total length	43.0	(36–49.5)	45.3[1]		40.5	43.8	(42.5–46.3)	45.8	38.0	42.8	(41.6–44.1)
Total trace length (copulatory tube)	45.5	(43.9–46.9)	45.3[1]		44.6	46.7	(45.3–47.8)	40.9	45.2	41.0	(39.4–42.6)

Numeral value in superscript indicates the number of individuals measured.

Quadriacanthus sp. E (**Fig. 3.7. A–D**; **Table A2.9.)**

Host: *Clarias gariepinus* (Burchell) (Siluriformes: Clariidae).

South Africa – Riviersonderend (RSE), Western Cape; Phongolo River in Ndumo Game Reserve (NGR), KwaZulu-Natal; Zambia – Barotse floodplain (ZR), Western Province.

Site of infection: Gills.

Number of specimens studied: n = 3 (ZR), n = 1 (PH).

Morphological observations: *Quadriacanthus* sp. E (**Fig. 3.7. A–D**) share the same haptoral sclerite morphology, that retain a double curved point in the ventral anchor, as seen in *Quadriacanthus ashuri* Kritsky et Kulo, 1988*, Q. gourenei*, *Q. numidus* Kritsky et Kulo, 1988*, Q. papernai* Kritsky et Kulo, 1988 *and Q. pravus* Francová et Řehulková, 2017 (see Kritsky and Kulo 1988; N'Douba et al. 1999; Francová et al. 2017). The pair of dorsal anchors of *Quadriacanthus* sp. E have a long shaft with a short recurving point (as seen in *Q. clariadis*) and the ventral anchors have a long shaft tapering into the double curved point. Based on morphological comparison, *Quadriacanthus* sp. E can be distinguished from *Q. ashuri* in the ventral anchors that has a more slender and longer shaft with a prominent curvature into the double curved point (in *Q. ashuri* and *Q. papernai* the shaft is short and smoothly curves into the doubly curved point). Both *Q. ashuri* and *Q. papernai* have a small spine arising near the mid-region of the accessory piece (see **Fig. A1.3. C**, **E** and Kritsky and Kulo 1988), not present in *Quadriacanthus* sp. E. Our species can also be differentiated from *Q. gourenei* in the shape of the dorsal bar and ventral anchor that is similar to that of *Q. ashuri* and the heavily sclerotised MCO accessory piece, not observed in our species. Similarly, *Quadriacanthus* sp. E is different from *Q. numidus* that has a more smoothly curved shaft of the ventral anchor, and a small MCO with a lamellate accessory piece (see **Fig. A1.3. D**). *Quadriacanthus* sp. E is most similar in haptoral morphology to *Q. numidus* and *Q. pravus*, specifically the ventral anchor. Similarities between *Q. pravus* are clear in the triangular shape of the accessory sclerites of the dorsal anchors (wing-shaped in *Q. numidus*) and the accessory piece of the MCO (see **Fig. A1.4. B**). Francová et al. (2017) describe the morphology of the accessory piece as having a "terminal hook and subterminal pestle", and no vagina was observed, this is similar to the morphology observed in our specimens (**Fig. 3.7. B–D**). Based on the aforementioned diagnosis and morphometric comparisons (**Table A2.9.** and **Table A2.10.**) we assign *Quadriacanthus* sp. E to *Quadriacanthus pravus*.

Table A2.8. Comparative morphometrics of sclerotised structures of the haptor and male copulatory organ of *Quadriacanthus allobychowskiella* Paperna, 1979 parasitising the gills of *Clarias gariepinus* (Burchell) (type host) in Africa. Measurements are presented in micrometres as mean followed by range in parentheses. Abbreviations: EG – Egypt.

	Nile River EG n = 10 El-Naggar & Serag (1985)		Voucher material[a] n = 16 Kritsky & Kulo (1988)	
Ventral anchor				
Total length	26	(23–28)	29	(26–31)
Point length	–		–	
Accessory piece length	–		–	
Shaft curvature (x/y)	–		–	
Dorsal anchor				
Total length	42	(39–45)	48	(44–51)
Point length	–		–	
Accessory piece length	35	(31–39)	–	
Shaft curvature (x/y)				
Ventral bar length				
Dorsal bar	44	(42–47)	50	(44–56)
Total length				
Total width	–		63	(51–74)
Mid-region thickness	–		–	
Process length	–		–	
Marginal hooks	15	(13–17)	–	
Pair I	–			
Pair II-V	18	(15–20)	18	(17–20)
Pair VI	–		13.5	(12–15)
Pair VII	20	(18–23)	29	(28–31)
	–		21	(19–23)
Vagina total length	26	(23–29)	–	
Male copulatory organ				
Total length	–		36	(30–43)
Total trace length (copulatory tube)	42	(39–45)	45	(41–48)

Numeral value in superscript indicates number of individuals measured.
[a] USNM 79899; HWML 20764; INPA PA314-1, 2; MRAC 37.162.

Table A2.9. Morphometrics of sclerotised structures of the haptor and male copulatory organ for ***Quadriacantus* sp. E** parasitising the gills of *Clarias gariepinus* (Burchell, 1822) in the present study. Measurements are presented in micrometres as mean followed by range in parentheses. Abbreviations: SA – South Africa, ZM – Zambia.

	Riviersonderend Western Cape SA n = 1	Phongolo River KwaZulu-Natal SA n = 1	Barotseland floodplain Northwestern Province ZM n = 3	
Ventral anchor				
Total length		37.5	37.2	(31.76 – 40.33)
Point length		7.33	7.3	(6.45 – 8.52)
Accessory piece length		9	8.2	(7.2 – 9.38)
Shaft curvature (x/y)		1.3	1.3	(1.4– 1.2)
Dorsal anchor				
Total length		38.5	38.9	(31.84 – 42.62)
Point length		3.1	2.9	(2.58 – 3.11)
Accessory piece length		14.3	14.2	(12.17 – 15.28)
Shaft curvature (x/y)		2	0.9	(1.1 – 0.9)
Ventral bar length		36	37.7	(26.83 – 43.48)
Dorsal bar				
Total length		43.1	43.6	(36.83 – 47.32)
Total width		20.2	22.6	(19.61 – 24.25)
Mid-region thickness		9.9	10.2	(9.69 – 10.69)
Process length		–	13.8	(13.44 – 14.15)
Marginal hooks				
Pair I		–	21.9	(21.34 – 22.36)
Pair II-V		11.3	13.8	(11.37 – 15.36)
Pair VI		30	28.0	(23.42 – 31.07)
Pair VII		17.4	17.9	(17.75 – 18)
Vagina total length		–	–	
Male copulatory organ				
Total length		26.8	32.8	(27.78 – 39.72)
Total trace length (copulatory tube)		25.3	28.8	(26.89 – 32.41)

Numeral value in superscript indicates number of individuals measured.

Quadriacanthus sp. F (**Fig. 3.8. A–D**; **Table A2.11.)**

Host: *Clarias gariepinus* (Burchell) (Siluriformes: Clariidae)

Localities (present study): Zambia – Barotse floodplain (ZR), Western Province.

Site of infection: Gills

Number of specimens studied: n = 5 (ZR)

Morphological observations: *Quadriacanthus* sp. F (**Fig. 3.8. A–D**) share the same haptoral sclerite morphology, that retain a double curved point in the ventral anchor, as seen in *Quadriacanthus ashuri, Q. gourenei*, *Q. numidus, Q. papernai and Q. pravus* (see Kritsky and Kulo 1988; Francová et al. 2017). This species can easily be differentiated from *Q. ashuri*, *Q. numidus, Q. papernai* and *Q. pravus* (**Fig. A1.3. A–E** and **Fig. A1.4. B**) in the shape of the dorsal bar, that tapers into a rounded curve on the distal side of the mid-region and the morphology of the MCO accessory piece (**Fig. 3.8. A–C**). Distinction can also be made from *Q. ashuri* and *Q. numidus* in their ventral anchors that both have a shorter shaft with a smooth curvature into the double recurving point and a less distinct curvature into the double curved point, respectively (see **Table A2.11.**). *Quadriacanthus* sp. F has a large sclerotised accessory piece with two diverticula, one half-way between the base of articulation with the copulatory tube and the mid-region, and the other in the mid-region that also seems to be the point of attachment for a finger-like rod projecting to the posterior end of the single hooked termination at the distal end (**Fig. 3.8. B–C**). Based on the above morphological comparison and morphometrics presented in **Table A2.10.** and **Table A2.11.**, we assign *Quadriacanthus* sp. F to *Q. gourenei* described by N'Douba et al. (1999) from the gills of *Heterobranchus isopterus* in the Ivory Coast. Vagina unsclerotised (**Fig. 3.8. D**).

Table A2.10. Comparative morphometrics of sclerotised structures of the haptor and male copulatory organ for ***Quadriacanthus* spp.** parasitising the gills of *Clarias gariepinus* (Burchell) and *Heterobranchus isopterus* Bleeker in Africa. Measurements are presented in micrometres as mean followed by range in parentheses. Abbreviations: EG – Egypt, IC – Ivory Coast, SU – Sudan.

	Q. ashuri Nile River† EG n = 16 *C. gariepinus*# Kritsky & Kulo (1988)	*Q. gourenei* Agneby† and Bia River IC n = 4 *H. isopterus*# N'Douba et al. (1999)	*Q. numidus* Nile River† EG n = 4 *C. gariepinus*# Kritsky & Kulo (1988)	*Q. papernai* Nile River† EG n = 20 *C. gariepinus*# Kritsky & Kulo (1988)	*Q. pravus* Nile River basin SU n = 4 *C. gariepinus*# Francová et al. (2017)
Ventral anchor					
Total length	37 (35-38)	30 (27–32)	42 (40–44)	37 (33–41)	40 (39–40)
Point length	–	7 (6–8)	–	–	8 (8–9)
Accessory piece length	–	–	–	–	9 (8–10)[3]
Shaft curvature (x/y)	–	–	–	–	1.5 (1.4–1.6)
Dorsal anchor					43 (41–44)
Total length	40 (37-42)	31 (28–34)	45 (42–47)	36 (34-37)	2 (2–3)
Point length	–	3 (2–4)	–	–	16 (15–17)
Accessory piece length	–	4 (2–5)	–	–	1
Shaft curvature (x/y)	–	–	–	–	
					41 (38–44)
Ventral bar length	38 (33–45)	28 (24–32)	46 (44–48)	29 (24–33)	50 (47–53)
Dorsal bar					26 (25–26)
Total length	64 (56–75)	–	46 (44–47)	45 (40–49)	12 (11–14)
Total width	–	–	–		13 (11–15)
Mid-region thickness	–	10 (9–13)	–		
Process length	–	8 (7–9)	–	–	–
Marginal hooks					
Pair I	16 (15–17)	15 (14–16)	21 (20–22)	19 (17–21)	20 (19–21)
Pair II-V	14 (13–15)	–	14 (13–15)	14 (13–15)	13 (12–14)
Pair VI	26–27	21 (20–23)	32–33	25 (23–27)	28 (28–29)
Pair VII	16 (15–17)	15 (14–16)	18 (17–19)	16–17	17 (16–17)
Vagina total length	–	12 (10–14)	–	–	–
Male copulatory organ					
Total length	34 (28–39)	42 (40–45)	22 (20–24)	33 (29–37)	30 (29–32)
Total trace length (copulatory tube)	41 (38–43)	36 (34–39)	30 (28–32)	39 (35–43)	28 (27–30)

Numeral value in superscript indicates the number of individuals measured.
Type host.
† Type locality.

Table A2.11. Morphometrics of sclerotised structures of the haptor and male copulatory organ for ***Quadriacantus* sp. F** and ***Quadriacanthus* sp. G** parasitising the gills of *Clarias gariepinus* (Burchell) in Zambia. Measurements are presented in micrometres as mean followed by range in parentheses.

	Quadriacanthus sp. F		*Quadriacanthus* sp. G			
	Barotseland floodplain		Barotseland floodplain		Kabompo River	
	ZM		ZM		ZM	
	n = 5		n = 9		n = 3	
Ventral anchor						
Total length	33.9	(33.4–34.2)	43.3	(41.3–45.7)[8]	46.3	(46.2–46.4)[2]
Point length	6.8	(5.9–7.6)	15.5	(12.8–18.4)[8]	16.8	(15.6–17.9)[2]
Accessory piece length	11.5	(10.1–12)	9.2	(5.1–14)[7]	9.1	(7.6–10.1)
Shaft curvature:	1.6	(1.5–1.6)	1.6	(1.6–1.7)[8]	1.6	(1.5–1.8)[2]
Dorsal anchor						
Total length	34.6	(33.8–35.8)	58.1	(52.7–63.4)[8]	63.0	(60.8–65.2)[2]
Point length	2.1	(1.6–2.8)	4	(3.3–4.9)[8]	4.3	(3.6–4.9)[2]
Accessory piece length	13.6	(12.3–14.6)	23.5	(20–27.1)[8]	24.9	(23.9–26.1)
Shaft curvature:	1.4	(1.1–1.9)	1		0.9	(0.9–1)[2]
Ventral bar length	26.8	(24.5–28.8)	58.8	(52.1– 69.6)[8]	65.6	(58.8–69.5)
Dorsal bar						
Total length	40.7	(39.1–44.6)	72.9	(59.9–84.8)[8]	84.0	(79.8–86.8)
Total width	14.6	(12.8–16.7)	32.3	(30–38.4)[6]	36.0	(35.4–36.6)[2]
Mid-region thickness	9.2	(7.6–11)	15.3	(12.3–21.3)[7]	16.0	(15.2–16.5)
Process length	8.5	(7.4–9.9)	20.9	(16.4–26.6)[7]	21.5	(20.7–23)
Marginal hooks						
Pair I	14.1	(12.8–15.6)[4]	21.5	(15.8–25.7)[8]	24.0	(22.1–25.7)
Pair II - V	12.6	(10.4–14.6)	14.7	(12.5–16.4)[7]	15.8	(14.2–17.4)[2]
Pair VI	21.5	(20.9–22.2)	37.2	(31.6–39.8)[7]	41.1	(39.2–42.9)[2]
Pair VII	11.3	(13.2–16.8)[4]	22.5	(20.1–25.1)[8]	23.4	(22.1–25.1)
Vagina total length	16.4	(14.5–18.4)	–		–	
Male copulatory organ						
Total length	37.5	(36.7–38.4)	79.2	(57.8–93.4)	82.5	(81.1–84.4)
Total trace length (copulatory tube)	34.6	(33.8–35.8)	94.9	(86.9–104.7)	102.7	(93.4–111.1)

Numeral value in superscript indicates number of individuals measured.

Quadriacanthus sp. G (**Fig. 3.8. E–F**; **Table A2.11.**)

Host: *Clarias gariepinus* (Burchell) (Siluriformes: Clariidae).

Localities (present study): Zambia – Barotse floodplain (ZR), Western Province and Kabompo River (KR), Northwestern Province.

Site of infection: Gills

Number of specimens studied: n = 3 (KR), n = 9 (ZR).

Morphological observations: General sclerite morphology of *Quadriacanthus* sp. G is comparable to *Q. aegypticus,* and *Quadriacanthus mandibulatus* Francová et Řehulková, 2017 known from the gills of *Heterobranchus bidorsalis* Geoffrey Saint-Hilaire in Kenya and Sudan (Francová et al. 2017). The haptoral sclerite configuration of *Quadriacanthus* sp. G include a dorsal anchor with a long shaft tapering into a short, recurved point, and a ventral anchor with a long shaft tapering into a long, recurved point, as in *Q. aegypticus* (see **Fig. A1.2. A–B**). Our species, *Quadriacanthus* sp. G, do not share haptoral sclerite morphology with *Q. mandibulatus* (see **Fig. 3.8. E**, and Francová et al. 2017), however the MCO of *Quadriacanthus* sp. G retain a large, curved copulatory tube (bow-shaped) with a large accessory piece, similar to the MCO of *Q. mandibulatus.* The MCO of *Quadriacanthus* sp. G differs from *Q. aegypticus* that lack a sickle-shape distal hook and is larger in size to both that observed in *Q. aegypticus* and *Q. mandibulatus* (see **Table A2.2.**, **Table A2.11.** and **Table A2.12.**). The copulatory tube of *Quadriacanthus* sp. G is arched (bow-shaped) with a basal protrusion, has a heavily sclerotised accessory piece terminating in a single hook, a diverticulum is present on the posterior side of the distal hook-handle and an elongate lamellate thickening in the mid-region of the accessory piece (**Fig. 3.8. F**). No vagina observed.

Quadriacanthus sp. H (**Fig. 3.9. A–C**; **Table A2.13.**)

Host: *Clarias gariepinus* (Burchell) (Siluriformes: Clariidae).

Localities (present study): Zambia – Barotse floodplain (ZR), Northern Province. South Africa – Boskop Dam (BS), North West province; Gariep Dam (GD), Riet (RR) and Sand (SR) rivers, Free State Province and Great Fish River (GFR), Eastern Cape Province.

Site of infection: Gills .

Number of specimens studied: n = 7 (BS), n = 16 (GD).

Morphological observations: The haptoral sclerite morphology of *Quadriacanthus* sp. H (**Fig. 3.9. A–C**) is comparable to that of *Quadriacanthus clariadis* and *Q. aegypticus* in having a dorsal anchor with a long shaft tapering into a short, recurved point, a long triangular shaped accessory sclerite and a ventral anchor with a long shaft tapering into a long, recurved point (see **Fig. 3.9. A**, **Fig. A1.1. D–E** and **Fig. A1.2. A–B**). Based on morphological comparison *Quadriacanthus* sp. H has larger dorsal and ventral anchors than *Q. aegypticus* (see **Table A2.2.** and **Table A2.13.**), hook pair VI is also markedly longer than that of other species found from *C. gariepinus* during the present study. The MCO of *Quadriacanthus* sp. H is also substantially longer than seen in *Q. clariadis* and is equipped with a long copulatory tube tapering into an open end/tip and an accessory piece, similar to that of *Q. aegypticus* that have two diverticula in the mid-region and hook at the distal end. The accessory piece can firstly be distinguished from that of *Q. aegypticus* in the distal hook morphology resembling that of the chelae of a crab consisting of only the manus and dactyl, and secondly on the thin articulating (suspected) mid-region between the two diverticula (see **Fig. 3.9. B–C**). No vagina observed.

Table A2.12. Comparative morphometrics of *Quadriacanthus agnebiensis* N'Douba, Lambert et Euzet, 1999; *Quadriacanthus gourenei* N'Douba, Lambert et Euzet, 1999; *Quadriacanthus pravus* Francová et Řehulková, 2017 and *Quadriacanthus zuheiri* Francová et Řehulková, 2017 from various hosts. Measurements are presented in micrometres as mean followed by range in parentheses. Abbreviations: IC – Ivory Coast; SU – Sudan.

	Q. agnebiensis		*Q. longifilisi*		*Q. mandibulatus*		*Q. zuheiri*	
	Agneby and Bia River†		Agneby River†		Nile River basin† & Lake Turkana		Nile River basin†	
	IC		IC		SU		SU	
	n = 13		n = 26		n = 10		n = 8	
	H. isopterus#		*H. longifilis*#		*H. bidorsalis*#		*C. gariepinus*#	
	N'Douba et al. (1999)		N'Douba et al. (1999)		Francová et al. (2017)		Francová et al. (2017)	
Ventral anchor								
Total length	31	(28–33)	38	(33–41)	29	(28–30)	37	(37–38)
Point length	10	(8–11)	15	(13–17)	13	(12–14)	11	(10–11)
Accessory piece length	14	(11–16)	16	(13–20)	13	(9–15)	9	(8–11)
Shaft curvature (x/y)	–		–		1.1	(1.0–1.3)	1.4	(1.1–1.6)
Dorsal anchor								
Total length	42	(37–44)	48	(42–55)	79	(72–86)	45	(43–47)
Point length	5	(4–6)	7	(5–8)	8	(8–9)	3	(2–3)
Accessory piece length	8	(7–10)	11	(7–17)	26	(24–27)	20	(18–22)
Shaft curvature (x/y)	–		–		1.1	(0.9–1.4)	1.1	(1–1.3)
Ventral bar length	46	(43–49)	54	(50–60)	62	(54–68)	41	(39–44)[5]
Dorsal bar								
Total length	–		–		95	(84–102)	46	(42–51)[5]
Total width	–		–		34	(28–41)	29	(24–32)
Mid–region thickness	14	(12–16)	17	(14–20)	15	(12–18)	15	(15–18)
Process length	11	(7–15)	18	(15–21)	15	(13–17)[6]	14	(11–18)[5]
Marginal hooks								
Pair I	20	(19–21)	21	(18–23)	16	(14–18)	18	(17–18)
Pair II–V	–		–		14	(12–17)	13	(12–14)[5]
Pair VI	35	(31–37)	37	(33–41)	70	(67–74)	29	(27–31)
Pair VII	15	(13–16)	27	(24–29)	52	(49–56)	19	
Vagina total length	9	(8–12)	13	(10–15)	–		–	
Male copulatory organ								
Total length	31	(28–34)	40	(38–43)	74	(70–79)	32	(30–34)
Total trace length (copulatory tube)	29	(28–31)	39	(35–42)	69	(66–71)	30	(28–30)

Numeral value in superscript indicates the number of individuals measured.
Type host.
† Type locality.

Table A2.13. Morphometrics of sclerotised structures of the haptor and male copulatory organ for ***Quadriacantus* sp. H** parasitising the gills of *Clarias gariepinus* (Burchell) in the present study. Measurements are presented in micrometres as mean followed by range in parentheses. Abbreviations: SA – South Africa, ZM – Zambia.

	Boskop Dam North West SA n = 14		Gariep Dam Free State SA n = 16	
Ventral anchor				
Total length	42.7	(39.2–46.8)	41.0	(39.2–42.6)
Point length	15.0	(13.2–16.9)	13.8	(11.7–15.1)
Accessory piece length	10.3	(8.4–12.3)	10.4	(8.6–12.3)
Shaft curvature (x/y)	24.8	(21.2–28.4)	24.2	(22.5–25.8)
	17.4	(15.3–20.2)	16.7	(14.5–20.9)
Dorsal anchor				
Total length	56.6	(52.9– 56)	54.7	(51.81–57.09)[14]
Point length	4.9	(3.7–5.8)	4.5	(3.6–5.4)[14]
Accessory piece length	19.8	(15.5–22.6)	21.4	(19–23.2)
Shaft curvature (x/y)	26.3	(22.4–32.7)	26	(24.6–27.5)[14]
	24.9	(17.8–27.9)	24.8	(20.3–30.3)[14]
Ventral bar length	59.7	(50.8–66.1)	61.1	(55–65.9)
Dorsal bar				
Total length	67.5	(61.1–72.9)	69.6	(57.3–77.9)
Total width	33.7	(26.1–39.6)	35.4	(33.5–40.8)
Mid-region thickness	19.1	(13.1–21.1)	19.5	(17.2–22.1)
Process length	20.2	(10.8–24.3)	20.9	(16.8–23.3)
Marginal hooks				
Pair I	21.3	(16.7–24.5)	24.1	(21.4–25.2)
Pair II - V	15.8	(13.5–18.6)	14.5	(10.5–17)
Pair VI	36.1	(34.1–38.9)	38.5	(36.3–41.9)[14]
Pair VII	22.4	(20.4–24)	23.2	(20.9–24.5)[15]
Vagina total length	–		–	
Male copulatory organ				
Total length	41.2	(33.1–44.7)	40	(36.6–44.6)
Total trace length (copulatory tube)	41.7	(31.9–46.4)	40.8	(37.9–45.8)

Numeral value in superscript indicates number of individuals measured.

Gyrodactylidea

Gyrodactylidae Cobblod, 1864

Gyrodactylus Von Nordmann, 1832

Gyrodactylus gelnari Přikrylová, Blazek et Vanhove, 2012 (**Fig. 3.12. A–C**; **Table A2.14.**)
Type host and locality: *Clarias anguillaris* (L.) (Siluriformes: Clariidae), Mare Simenti, Niokolo Koba National Park (13°01.79′ N, 13°17.6′ W), Senegal.
Host (present study): *Clarias gariepinus* (Burchell) (Siluriformes: Clariidae).
Localities (present study): Zambia – Barotse floodplain (ZR), Western Province.
Site of infection: Gills .
Other records: *Clarias gariepinus*, Lake Tana, Ethiopia (Beletew et al. 2019).
Number of specimens studied: n = 10 (ZR).
Morphological observations: Hamuli slender, with flattened area on inner part of the root (see **Fig. 3.12. A**). Dorsal bar simple. Ventral bar proper and associated membrane ribbed (**Fig. 3.12. B**). Marginal hook sickle has a narrow shaft, distinctly points/angled forward from the foot and curves slightly tapering into a point that extend beyond the toe (**Fig. 3.12. C**). Male copulatory organ observed, but not able to determine presence of principal spine and number of smaller spines. Morphometric comparison with previous records of *G. gelnari* is presented in **Table A2.14**.

Gyrodactylus rysavyi Ergens, 1973 (**Fig. 3.12. D–E**; **Table A2.14.**)
Type host and locality: *Clarias gariepinus* (Burchell) (Siluriformes: Clariidae), Nile River, Cairo, Egypt.
Host (present study): *Clarias gariepinus*.
Localities (present study): South Africa – Vaal River (VR), North West Province.
Site of infection: Gills .
Other records: *Clarias gariepinus* – Egypt, Kenya, Mozambique, Senegal and Uganda (see **Table A1.6.** for details).
Number of specimens studied: n = 2 (VR).
Morphological observations: Hamuli slender, root narrow substantially into point after wide joint in shaft, at attachment of dorsal bar (**Fig. 3.12. D**). Dorsal bar simple. Ventral bar proper ribbed, associated membrane short, ribbed with irregular end (**Fig. 3.12. E**).

Marginal hook sickle has a broad shaft, slanted and curving forward, tapering into a thin point ending beyond the toe (**Fig. 3.12. F**). Morphometric comparison to other records of *G. rysavyi* is presented in **Table A2.14.**

Table A2.14. Morphometric comparison of *Gyrodactylus* Gussev, 1961 spp. parasitising the gills of *Clarias gariepinus* (Burchell) found during the present study. Measurements are presented in micrometres as mean followed by range in parentheses.

	Gyrodactylus sp. A Barotseland Floodplain ZM n = 2 Present study		*G. gelnari** Mare Simenti SN n = 22 Přikrylová et al. (2012)		*Gyrodactylus* sp. B Vaal River SA n = 1 Present study	*G. rysavyi* Paratypes n = 3 Přikrylová et al. (2012)		KY/MZ/SN n = 25		**EG**
Hamuli										
Total length	75.9	(74.6–77.3)	71.4	(69–75)	84.6	91.8	(90–93)	98.6	(89–106.5)	
Point length	37.4	(37.3–37.6)	35.7	(33.5–38)	43.8	46.4	(45–47.5)	48.9	(45.5–53)	
Shaft length	39.7	(39.7–39.7)	44.4	(42.5–47)	45.9	57.8	(57.5–58.5)	59.7	(54.5–64)	
Root length	30.8	(30–31.7)	34.4	(31.5–37.5)	36.2	42.5	(40–44)	49	(38–57)	
Ventral bar										
Median length	7.3	(7–7.7)	6.6	(5.5–8)	7.8	6	(5.5–6.5)	8.3	(6–10.5)	
Membrane length	18.1	(17–19.1)	17	(14.5–19)	16.4	19.7	(19–21.5)	21.8	(16–27)	
Width	24.3	(19.2–29.4)	19.5	(17.5–22)	27.6	29.9	(28–31.5)	33.4	(28.5–38.5)	
Dorsal bar										
Total length	1.9	(1.7–2.2)	1.9	(1.5–2.5)	2.3	2.3	(2–2.5)	2.8	(1.8–3.5)	
Total width	15.6	(13.8–17.4)	19	(17.5–20.5)	19.5	21.5	(19.5–22.5)	23.7	(19–28)	
Marginal hooks										
Total length	35.4	(34.6–36.3)	33.3	(32–34.5)	34.4	30.7		32.6	(30.5–36.5)	
Sickle length	5.2	(5–5.5)	4.3	(3.5 -5)	4.1	4.3	(4–4.5)	4.2	(4–5)	
Handle length	31.3	(30.8–31.7)	29	(27.5–31)	30.3	26.8	(26.5–27)	28.4	(26.2–32)	
Sickle distal width	1.8	(1.7–1.9)	4.3	(4–5)	2.8	–		3.5	(3–4)	
Sickle proximal width	2.9	(2.8–2.9)	3.5	(3–4)	3.3	3.4	(3–4)	3.6	(3–4)	
Sickle aperture distance	5.6	(5.5–5.7)	5.7	(5.5–6)	5.4	4.3	(4–5)	4.5	(4–5)	
Cirrus spines (small)	–		10-11			–		11		

* Species data, from type host *Clarias anguillaris* (Linnaeus, 1758).

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Appendix A3

Table A3.1. List **of Monogenea** included in checklists parasitising the African sharptooth catfish, *Clarias gariepinus (*Burchell) in Africa. Abbreviations: CD – Democratic Republic of Congo, EG – Egypt, GH – Ghana, SA – South Africa, UG – Uganda, ZI – Zimbabwe. Symbols: (+) Present, (–) no record.

		Khalil (1971)	Paperna (1979)	Van As and Basson (1984)	Khalil and Polling (1997)	Lim et al (2001)	Řehulková et al. (2018)
1.	*Gyrodactylus alberti*	–	UG	–	UG	–	+
2.	*Gyrodactylus alekosi*	–	–	–	–	–	+
3.	*Gyrodactylus clarii*	–	–	–	UG	–	+
4.	*Gyrodactylus gelnari*	–	–	–	–	–	+
5.	*Gyrodactylus groschafti*	–	EG	–	EG	–	+
6.	*Gyrodactylus rysavyi*	–	EG, UG	–	EG	–	+
7.	*Gyrodactylus transvaalensis*	–	–	SA	SA	–	+
8.	*Gyrodactylus turkanaensis*	–	–	–	–	–	+
9.	*Macrogyrodactylus clarii*	GH	GH, UG	–	GH	–	+
10.	*Macrogyrodactylus congolensis*	CD	CD, UG	–	CD, UG	–	+
11.	*Macrogyrodactylus karibae*	–	–	–	ZI	–	+
12.	*Paraquadriacanthus nasalis*	–	–	–	–	–	+
13.	*Quadriacanthus aegypticus*	–	–	–	EG, ZI	+	+
14.	*Quadriacanthus allobychowskiella*	–	UG	–	EG, UG	+	+
15.	*Quadriacanthus ashuri*	–	–	–	EG	+	+
16.	*Quadriacanthus bagrae*	–	–	–	EG, ZI	+	+
17.	*Quadriacanthus clariadis*	GH, UG	UG	–	EG, GH, UG, ZI	+	+
18.	*Quadriacanthus fornicatus*	–	–	–	–	–	+
19.	*Quadriacanthus numidus*	–	–	–	EG, ZI	+	+
20.	*Quadriacanthus papernai*	–	–	–	EG	+	+
21.	*Quadriacanthus pravus*	–	–	–	–	–	+
22.	*Quadriacanthus voltaensis*	GH	–	–	EG	+	+
23.	*Quadriacanthus zuheiri*	–	–	–	–	–	+

Table A3.2. Records of Monogenea parasitising the African sharptooth catfish, *Clarias gariepinus (*Burchell) in Africa. Abbreviations: CD – Democratic Republic of Congo, EG – Egypt, ET – Ethiopia, GH – Ghana, KY – Kenya, MZ – Mozambique, NI – Nigeria, SN – Senegal, SA – South Africa, SU – Sudan, UG – Uganda, ZI – Zimbabwe.

Classification	Localities	Country	Reference
Phylum Platyhelminthes			
Class Monogenea			
Subclass Monopisthocotylea			
Order Dactylogyridea			
Family Ancylodiscoididae			
Paraquadriacanthus nasalis[#] Ergens	Nile River[†]: Ammar drainage canal, Mansoura canal, Lake Manzala	EG	Ergens (1988)[†]; Kritsky and Kulo (1988); Hagras et al. (1995, 1999); Arafa (2004, 2012); El-Naggar et al. (2017)
Quadriacanthus aegypticus[#] El-Naggar et Serag	Nile River basin: Ammar drainage canal, Bahr Mouis, Damietta branch[†], Damroo drainage canal, Lake Manzala[†], Mansoura drainage canal, Nawasa El-Gheit drainage canal, Nile River barrage, Telbanah drainage canal	EG	Molnár and Mossalam (1985)▪; El-Naggar and Serag (1985, 1986)[†]; Kritsky and Kulo (1988); Hagras et al. (1995); Reda et al. (2003); El-Naggar et al. (1997, 2017, 2019b); El-Seify et al. (2011); Mashalay et al. (2019, 2020)
	Lake Tana	ET	Beletew et al. (2016)
	Lake Turkana	KY	Francová et al. (2017)
	Krugerdrift Dam, Gariep Dam, Lake Tzaneen, Middle Letaba Dam, Nwanedi-Luphephe Dams, Phalaborwa Barrage, Vaal Dam	SA	Venter (2002)[‡]; Olivier et al. (2009); Madanire-Moyo et al. (2010, 2012); Matla (2012)[‡]; Crafford et al. (2014); Mahlatji 2014[‡]; Mogorosi (2019)[‡]
	Nile River basin: Blue Nile, White Nile	SU	Francová et al. (2017)
	Lake Kariba	ZI	Douëllou and Chishawa (1995)
Quadriacanthus allobychowskiella[#] Paperna	Nile River basin: Ammar drainage canal, Damietta branch[†], Lake Manzala[†], Mansoura drainage canal, Nawasa El-Gheit drainage canal, Nile River barrage, Telbanah drainage canal	EG	El-Naggar and Serag (1985); Kritsky and Kulo (1988); Hagras et al. (1995); El-Naggar et al. (1997, 2017); Reda et al. (2003); Mashalay et al. (2019, 2020)
	Lake George[†]	UG	Paperna (1979)[†]
	Middle Letaba Dam	SA	Olivier et al. (2009)
Quadriacanthus ashuri[#] Kritsky et Kulo	Nile River barrage[†]	EG	Kritsky and Kulo (1988)[†]
Quadriacanthus bagrae Paperna	Nile River barrage	EG	Kritsky and Kulo 1988)

Table A3.2. (continued) Records of Monogenea parasitising the African sharptooth catfish, *Clarias gariepinus (*Burchell) in Africa. Abbreviations: CD – Democratic Republic of Congo, EG – Egypt, ET – Ethiopia, GH – Ghana, KY – Kenya, MZ – Mozambique, NI – Nigeria, SN – Senegal, SA – South Africa, SU – Sudan, UG – Uganda, ZI – Zimbabwe.

Classification	Localities	Country	Reference
Quadriacanthus clariadis# Paperna	Nile River basin: Ammar drainage canal, Bahr Mouis, Al-Manzala fish farm*, Demietta branch, Lake Manzala, Mansoura drainage canal, Nawasa El-Gheit drainage canal, Nile River barrage, Telbanah Drain	EG	Molnár and Mossalam (1985)▪; El-Naggar and Serag (1985); Kritsky and Kulo (1988); Hagras et al. (1995); Reda et al. (2003); Awadin et al. (2012); El-Naggar et al. (1997, 2017); Mashalay et al. (2019, 2020)
	Nsaki River	GH	Paperna 1969, 1979)
	Lake Turkana	KY	Francová et al. 2017)
	Nile River basin: Blue Nile	SU	Francová et al. 2017)
	Hout River, Lake Tzaneen, Middle Letaba Dam, Nwanedi-Luphephe Dams	SA	Madanire-Moyo et al. (2010, 2012); Olivier et al. (2009); Matla (2012)‡; Sara et al. (2014)
	Lake Albert, Lake George, Siba River, Lake Victoria	UG	Paperna and Thurston (1968); Thurston (1970); Paperna (1979)
Quadriacanthus fornicatus# Francová et Řehulková	Nile River basin: Blue Nile†, White Nile	SU	Francová et al. (2017)†
Quadriacanthus numidus# Kritsky et Kulo	Nile River barrage†	EG	Kritsky and Kulo 1988†
	Lake Kariba	ZI	Douëllou and Chishawa (1995)
Quadriacanthus papernai# Kritsky et Kulo	Nile River barrage†	EG	Kritsky and Kulo (1988)†
Quadriacanthus pravus# Francová et Řehulková	Nile River basin: Blue Nile†, White Nile	SU	Francová et al. (2017)†
Quadriacanthus voltaensis Paperna	Volta River†	GH	Paperna (1965)†; Kritsky and Kulo (1988)
Quadriacanthus zuheiri# Francová et Řehulková	Nile River basin: Blue Nile†, White Nile	SU	Francová et al. (2017)†
Order Gyrodactylidea			
Family Gyrodactylidae			
Gyrodactylus alberti# Paperna	Nile River: Demietta branch	EG	El-Naggar et al. (2001b)
	Siba River (Lake Albert)†	UG	Paperna (1973)†
Gyrodactylus alekosi# Prikrlyova, Blažek, Maarten et Vanhove	Temporal pans near Bala Bala Village†	MZ	Přikrylová et al. (2012)†

Table A3.2. (continued) Records of Monogenea parasitising the African sharptooth catfish, *Clarias gariepinus (*Burchell) in Africa. Abbreviations: CD – Democratic Republic of Congo, EG – Egypt, ET – Ethiopia, GH – Ghana, KY – Kenya, MZ – Mozambique, NI – Nigeria, SN – Senegal, SA – South Africa, SU – Sudan, UG – Uganda, ZI – Zimbabwe.

Classification	Localities	Country	Reference
Gyrodactylus clarii Paperna	Siba River (Lake Albert)†	UG	Paperna (1973)†
Gyrodactylus gelnari Prikrlyova, Blažek, Maarten et Vanhove	Lake Tana	ET	Beletew et al. (2016)
Gyrodactylus groschafti# Ergens	Nile River†, Abu Sarda irrigation canal†, Bahr Mouis, Demietta branch, Mansoura drainage canal, Lake Manzala	EG	Ergens (1973)†; Molnár and Mossalam (1985); El-Naggar (1992)
Gyrodactylus rysavyi# Ergens	Nile River†, Abu Sarda irrigation canal†, Ammar Drain, Bahr Mouis, Demietta branch, Lake Manzala, Telbanah Drain	EG	Ergens (1973)†; Molnár and Mossalam (1985); El-Naggar et al. (2004b, 2017); El-Naggar and El-Abbassy (2003); Mashalay et al. (2019)
	Lake Turkana	KY	Přikrylová et al. (2012)
	Temporal pans near Bala Bala Village	MZ	Přikrylová et al. (2012)
	Flag Boshielo Dam, Lake Tzaneen, Middle Letaba Dam, Nwanedi-Luphephe Dams, Return water dam	SA	Olivier et al. (2009); Madanire-Moyo et al. (2010, 2012)
	Mare Simenti: Kalokol, streams near Todonyang Village	SN	Přikrylová et al. (2012)
	Siba River (Lake Albert)	UG	Paperna (1979)
Gyrodactylus transvaalensis# Prudhoe et Hussey	Confluence of the Elands and Olifants rivers near Marble Hall Veld Fisheries Research Station†	SA	Prudhoe and Hussy (1977)†
Gyrodactylus turkanaensis# Přikrylová, Blažek, Maarten et Vanhove	Lake Turkana†	KY	Přikrylová et al. (2012)†

† Type locality and associated publication.
# Type host is *Clarias gariepinus*.
‡ Unpublished theses data.
* Fish farm/aquaculture/earthen ponds.
■ Species possibly misidentified based on images provided in the publication.

Table A3.3. Historic records of **Digenea** included in checklists of freshwater parasitic species in Africa for the African sharptooth catfish, *Clarias gariepinus (*Burchell). Abbreviations: Democratic CD – Republic of Congo, EG – Egypt, GH – Ghana, SA – South Africa, UG – Uganda, ZI – Zimbabwe. Symbols: (+) Present, (–) no record, Metacercariae – [L].

		Khalil (1971)	Van As and Basson (1984)	Khalil and Polling (1997)	Kudlai et al. (2018)
1.	*Allocreadium mazoensis*	ZI	–	ZI	+
2.	*Astiotrema lazeri*		–	EG	+
3.	*Astiotrema reniferum*[a]	SU, ZI, ZM	–	EG, SU, ZA, ZI	+
4.	*Clinostomoides brieni* [L]	CD	–	CD, ZI	+
5.	*Clinostomum macrosomum* [L]	–[b]	–	–[b]	+
6.	*Clinostomum* sp. [L]	–	SA	–	+
7.	*Diplostomum heterobranchi* [L][c]	EG,	–	EG	+
8.	*Diplostomum* sp. [L]	–	SA	–	+
9.	*Dolichorchis tregenna* [L]	–	–	–	+
10.	*Euclinostomum dollfusi* [L]	–	SA	SA	+
11.	*Euclinostomum heterostomum* [L]	–	–	SA	+
12.	*Glossidium lazerae*	–	–	–	+
13.	*Glossidium pedatum*[d]	–	SA	ET, SA, SU	+
14.	*Gonocerca phycidis*	–	–	EG	+
15.	*Haplorchoides cahirinus*	–	–	–	+
16.	*Malawitrema staufferi*	–	–	–	+
17.	*Masenia bangweulensis*	–	–	–	+
18.	*Masenia ghanensis*	–	–	ET	+
19.	*Neodiplostomum* sp. [L]	–	SA	SA	+
20.	*Orientocreadium batrachoides*[e]	EG, ZM	–	SU, ZA, ZI	+
21.	*Orientocreadium indicum*	–	–	ET	+
22.	*Ornithodiplostomum* sp. [L]	–	–	–	+
23.	*Panamphistomum benoiti*	CD	–	CD	+
24.	*Phyllodistomum bavuri*	–	–	–	+
25.	*Phyllodistomum tana*	–	–	–	+
26.	*Phyllodistomum vanderwaali*	–	SA	SA	+
27.	*Phyllodistomum* cf. *symmetrorchis*	–	–	–	+
28.	*Sanguinicola clarias*	–	–	–	+
29.	*Thaparotrema botswanensis*	–	–	–	+
30.	*Tylodelphys grandis* [L]	–	–	–	+
31.	*Tylodelphys mashonensis* [L]	ZI[f]	SA	SA, ZI	+
32.	*Tylodelphys* sp. [L]	–	–	–	+
33.	Cryptogonimidae gen. sp. [L][g]	CD	–	CD	+
34.	Diplostomidae gen. sp. [L]	–	–	–	+

[a] – syn. *Distoma reniferum, D. unicum*
[b] – Only reported from *Oreochromis nilotia* (Linnaeus) (syn. *Tilapia nilotica*) from Rwanda in Manter and Pritchard (1969).
[c] – syn. *Monocerca heterobranchi* Wedl, 1861 (see Khalil and Polling 1997).
[d] – syn. *Afromacroderoides lazerae* (see Mashego and Saayman 1989).
[e] – syn. *Orientocreaduim lazeri* (see Khalil 1961).
[f] – Reported as *Diplostomum* of *Diplostomum mashonense*.
[g] – Reported as *Metacercaria alestesi* Fain, 1953 in Khalil (1971) and Khalil and Polling (1997).

Table A3.4. Records of Digenea parasitising the African sharptooth catfish, *Clarias gariepinus* (Burchell) in Africa. Abbreviations: BE – Benin, BW – Botswana – BW, CD – Democratic Republic of the Congo, EG – Egypt, ET – Ethiopia, GH – Ghana, IC – Ivory Coast, KY – Kenya, MW – Malawi, MZ – Mozambique, NM – Namibia, NI – Nigeria, SN – Senegal, SA – South Africa, SU – Sudan, TA – Tanzania; UG – Uganda, ZM – Zambia, ZI – Zimbabwe. Metacercariae – [L].

Classification	Localities	Country	Reference
Phylum Platyhelminthes			
Class Trematoda			
Subclass Digenea			
Order Plagiorchiida			
Superfamily Gorgoderoidea			
Family Allocreadiidae			
Allocreadium mazoensis[#] Beverley-Burton, 1962	Lake Malimbe, Mwanza Gulf, Lake Victoria	TA	Mwita and Nkwengulila (2004, 2008, 2010); Mwita (2011a, b; 2014a, b)
	Mazowe Dam[†]	ZI	Beverley-Burton (1962)[†]
Family Gorgoderidae			
Phyllodistomum bavuri[#] Boomker, 1984	Okavango Delta: Shakawe mainstream	BW	Jansen van Rensburg et al. (2013)
	Lake Tana	ET	Zhokov (2010); Zhokhov et al. (2017)
	Lake Victoria	KY	Cutmore et al. (2013)
	Bangu River[†], Sabie River, Crocodile River	SA	Boomker (1984)[†]
Phyllodistomum cf *symmetrorchis* sensu Cutmore, Miller, Curran, Bennett et Cribb, 2013	Lake Victoria	KY	Cutmore et al. (2013)
Phyllodistomum tana[#] Zhokov, 2010	Lake Tana[†]	ET	Zhokhov (2010)[†]; Zhokhov et al. (2017)
	Nile River	EG	Abdel-Gaber et al. (2016b)
Phyllodistomum vanderwaali[#] Prudhoe et Hussey, 1977	Okavango Delta: Xaro mainstream and lagoon	BW	Jansen van Rensburg et al. (2013)
	Olifants River[†]	SA	Prudhoe and Hussey (1977)[†]
Superfamily Hemiuroidea			
Family Gonocercidae			
Gonocerca phycidis Manter, 1925	–	EG	Khalil and Polling (1997)[⁂]
Superfamily Opisthorchioidea			
Family Cryptogonimidae			
Cryptogonimidae gen. sp. [L]	–	CD	Fain (1953)[⁋] - see Khalil and Polling (1997)
Family Heterophyidae			
Haplorchoides cahirinus (Looss, 1896)	Lake Malawi	MW	Bray and Hendrix (2007)

Table A3.4. (continued) Records of Digenea parasitising the African sharptooth catfish, *Clarias gariepinus* (Burchell) in Africa. Abbreviations: BE – Benin, BW – Botswana – BW, CD – Democratic Republic of the Congo, EG – Egypt, ET – Ethiopia, GH – Ghana, IC – Ivory Coast, KY – Kenya, MW – Malawi, MZ – Mozambique, NM – Namibia, NI – Nigeria, SN – Senegal, SA – South Africa, SU – Sudan, TA – Tanzania; UG – Uganda, ZM – Zambia, ZI – Zimbabwe. Metacercariae – [L].

Classification	Localities	Country	Reference
Family Opisthorchiidae			
Thaparotrema botswanensis# Jansen van Rensburg, Van As et King, 2013	Okavango Delta: Kalatog channel, Shakawe mainstream†	BW	Jansen van Rensburg et al. (2013)†
	Nile River	EG	Abdel-Gaber et al. (2016b)
Superfamily Paramphistomoidea Family Cladorchiidae *Panamphistomum benoiti* Manter et Pritchard, 1964	Kasongo, Maniema	CD	Manter and Pritchard (1964)⁋ - see Jones and Khalil (1990)
Cladorchiidae gen. sp. [L]	Lake Tana	ET	Zhokov et al. (2017)
Superfamily Plagiorchioidea Family Cephalogonimidae *Masenia bangweulensis* (Beverley-Burton, 1962)	Nile River: Damietta branch, Lake Manzala	EG	El-Naggar et al. (1992); Radwan et al. (2009)
	Lake Tana	ET	Zhokov et al. (2017)
	Lake Malimbe, Mwanza Gulf, Lake Victoria	TA	Mwita and Nkwengulila (2004, 2010); Mwita (2011a, b; 2014a, b)
Masenia ghanensis (Fischthal et Thomas, 1968)	Awash River; Lake Haik	ET	Fischthal (1973)
Masenia nkomatiensis# Dumbo, Dos Santos et Avenant-Oldewage, 2019	Incomati River†	MZ	Dumbo et al. (2019a)†
Family Macroderoididae *Malawitrema staufferi*# Bray et Hendrix, 2007	Lake Malawi†	MW	Bray and Hendrix (2007)†
Family Orientocreadiidae *Orientocreadium batrachoides*	Nile River: Nile Delta, Lake Manzala, Damietta Branch; Bahr Youssef fish farm, Lake Qarun, Giza Fish Market*	EG	Fischthal and Kuntz (1963b)*; Moravec (1997); El-Naggar et al. (1993a, f); Al-Bassel (2003); Abdel-Gaber et al. (2015); El-Shahawy et al. (2017)
	Lake Tana	ET	Zhokov et al. (2017)

Table A3.4. (continued) Records of Digenea parasitising the African sharptooth catfish, *Clarias gariepinus* (Burchell) in Africa. Abbreviations: BE – Benin, BW – Botswana – BW, CD – Democratic Republic of the Congo, EG – Egypt, ET – Ethiopia, GH – Ghana, IC – Ivory Coast, KY – Kenya, MW – Malawi, MZ – Mozambique, NM – Namibia, NI – Nigeria, SN – Senegal, SA – South Africa, SU – Sudan, TA – Tanzania; UG – Uganda, ZM – Zambia, ZI – Zimbabwe. Metacercariae – [L].

Classification	Localities	Country	Reference
	Incomati River	MZ	Dumbo et al. (2019b)
	Blue Nile, White Nile	SU	Khalil (1961, 1969)
	Bangweulu Swamps: Lake Chali	ZA	Beverley-Burton (1962)
	Mazoe Dam, Gatooma, Lake Kariba	ZI	Beverley-Burton (1962); Douëllou (1992)
Orientocreadium indicum	Awash River	ET	Fischthal (1973)
Insertae sedis in the Superfamily Plagiorchiodea			
Astiotrema lazeri El-Naffar, Saoud et Hassan, 1984	Lake Nasser†	EG	El-Naffar et al. (1984)‼ - see Khalil and Polling (1997)
Astiotrema reniferum	Nile River: Damietta branch, Lake Manzala	EG	Moravec (1977); El-Naggar et al. (1991,1993b, c, d)
	Blue Nile, White Nile	SU	Khalil (1959, 1969)
	Lake Tana	ET	Zhokov et al. (2017)
	Lake Malimbe, Mwanza Gulf, Lake Victoria	TA	Mwita and Nkwengulila (2004, 2008, 2010); Mwita (2011a, b; 2014a, b)
	Bangweulu Swamps	ZA	Beverley-Burton (1962)
	Mazowe Dam	ZI	Beverley-Burton (1962)
Glossidium lazerae#	White Nile†	SU	Khalil (1972)†
Glossidium pedatum	Okavango Delta: Shakawe mainstream	BW	Jansen van Rensburg et al. (2013)
	Nile River	EG	Abdel-Gaber et al. (2016b)
	Lake Haik	ET	Fischthal (1973)
	Lake Malawi	MW	Bray and Hendrix (2007)
	Incomati River	MZ	Dumbo et al. (2019b)
	Makotswane Dam, Coetzeesdraai Dam, Krokodilsheuwel Dam, Olifants River, Piet Gouws Dam, Seshego Dam, Turfloop Dam; Nwanedi-Luphephe Dams, Flag Boshielo Dam, Phalaborwa barrage, Hout River	SA	Mashego and Saayman (1989); Madanire-Moyo et al. (2010, 2012); Mahlatji (2014); Sara et al. (2014)

Table A3.4. (continued) Records of Digenea parasitising the African sharptooth catfish, *Clarias gariepinus* (Burchell) in Africa. Abbreviations: BE – Benin, BW – Botswana – BW, CD – Democratic Republic of the Congo, EG – Egypt, ET – Ethiopia, GH – Ghana, IC – Ivory Coast, KY – Kenya, MW – Malawi, MZ – Mozambique, NM – Namibia, NI – Nigeria, SN – Senegal, SA – South Africa, SU – Sudan, TA – Tanzania; UG – Uganda, ZM – Zambia, ZI – Zimbabwe. Metacercariae – [L].

Classification	Localities	Country	Reference
	Insukamini Dam	ZI	Moyo et al. (2009)
Superfamily Schistosomatoidea Family Aporocotylidae *Sanguinicola clarias*# Imam, Marzouk, Hassan et Itman, 1984	–	EG	Imam et al. (1984)†, ‼ - see Truong and Bullard (2013)
Family Clinostomidae			
Clinostomum brieni [L]	Okavango Delta: Skakawe mainstream; Kalatog channel; Nxamaseri backwaters; Mokoro lagoon; Samochima lagoon	BW	Jansen van Rensburg et al. (2013)
	Lake Upemba (Lualabu River) Mabwe?	CD	Prudhoe (1957)
	Lake Tana	ET	Zhokov and Morozova (2020)
	Phalaborwa Barrage	SA	Caffara et al. (2019)
	Lake Kariba; Malilangwe Dam, Save-Runde River Floodplain	ZI	Douëllou (1992); Barson et al. (2008); Dalu et al. (2012)
Clinostomum complanatum [L]	Save-Runde River Floodplain	ZI	Barson et al. (2008) need ID confirmation in Afrotropical region
Clinostomum macrosomum [L]	–	–	–
Clinostomum sp. [L]	Koka Reservoir, Lake Tana, Lake Ziway	ET	Yimer (2000); Yimer and Enyew (2003); Guletat et al. (2013)
	Oueme upper delta	BE	Sedegbo et al. (2019)
	Ogun River, Lake Geriyo	NI	Salawu et al. (2013); Domo and Ester (2015)
	Phalaborwa barrage	SA	Mahlatji (2014)
	Lake Victoria	TA	Mwita and Nkwengulila (2004, 2008)
Euclinostomum dollfusi [L]		SA	Mashego (1977) misID of *E. heterostomum*?
Euclinostomum heterostomum [L]	Makotswane Dam; Seshego Dam	SA	Mashego and Saayman (1989)
	Gwagwadala region	NI	Dan-Kishiya et al. (2013)

Table A3.4. (continued) Records of Digenea parasitising the African sharptooth catfish, *Clarias gariepinus* (Burchell) in Africa. Abbreviations: BE – Benin, BW – Botswana – BW, CD – Democratic Republic of the Congo, EG – Egypt, ET – Ethiopia, GH – Ghana, IC – Ivory Coast, KY – Kenya, MW – Malawi, MZ – Mozambique, NM – Namibia, NI – Nigeria, SN – Senegal, SA – South Africa, SU – Sudan, TA – Tanzania; UG – Uganda, ZM – Zambia, ZI – Zimbabwe. Metacercariae – [L].

Classification	Localities	Country	Reference
Euclinostomum sp. [L]	Middle Letaba Dam	SA	Olivier et al. (2009)
	Lake Tana	ET	Zhokov and Morozova (2020)
Order Diplostomida			
Superfamily Diplostomoidea			
Family Diplostomidae			
Diplostomum heterobranchii (Wedl, 1861) [L]	–	EG	Wedl (1861)
Diplostomum sp. [L]	Koka Reservoir	ET	Gulelat et al. (2013)
	Nwanedi-Luphephe dams, Return Water dam, Phalaborwa barrage	SA	Madanire-Moyo et al. (2010, 2012); Mahlatji (2014)
	Manyame River, Nyatsime River, Mukuvusi River	ZI	Madanire and Barson (2010)
Diplostomidae gen. sp. [L]	Nile River	EG	Moravec (1977)[a]
Dolichorchis tregenna Nazmi Gohar, 1932 [L]	White Nile, Blue Nile	SU	Khalil (1963, 1969)
	Lake Tana	ET	Zhokhov et al. (2010)
	Rockwater and Panyam fish farms; Uke River	NI	Anoisike et al. (1992)⁂; Yakubu et al. (2002)⁂
	Nile River	EG	Ibraheem 2000• (see Zhokov et al. 2010)
Neodiplostomum sp. [L]	Confluence of the Elands and Olifants rivers	SA	Prudhoe and Hussey (1977)
	Shakawe and Zaro rivers	BW	Jansen van Rensburg (2013)
Tylodephys grandis# Zhokov, Morozova et Tessema, 2010 [L]	Lake Tana†	ET	Zhokhov et al. (2010)†
Tylodelphys mashonensis [L]	Lake Malimbe, Mwanza Gulf, Lake Victoria	TA	Mwita and Nkwengulila (2004, 2008, 2010); Musiba and Nkwengulila (2006); Mwita (2011a, b; 2014a, b)
	Boekenhoutskloof Dam; Buffelsdoorn Dam, Coetzeesdraai Dam, Krokodilsheuwel Dam, Lepellane Dam, Namakgale Dam, Olifants River, Piet Gouws Dam, Seshego Dam, Turfloop Dam	SA	Van As and Basson (1984); Mashego and Saayman (1989); Moema et al. (2013)
	Mazoe Dam†, Save-Runde River Floodplain	ZI	Beverley-Burton (1963)†; Barson et al. (2008)
Subfamily Crassiphialinae			

Table A3.4. (continued) Records of Digenea parasitising the African sharptooth catfish, *Clarias gariepinus* (Burchell) in Africa. Abbreviations: BE – Benin, BW – Botswana – BW, CD – Democratic Republic of the Congo, EG – Egypt, ET – Ethiopia, GH – Ghana, IC – Ivory Coast, KY – Kenya, MW – Malawi, MZ – Mozambique, NM – Namibia, NI – Nigeria, SN – Senegal, SA – South Africa, SU – Sudan, TA – Tanzania; UG – Uganda, ZM – Zambia, ZI – Zimbabwe. Metacercariae – [L].

Classification	Localities	Country	Reference
Ornithodiplostomum sp. [L]	Manzala Lake	EG	Rashied et al. (2016)
	Rietvlei Dam	SA	Barson and Avenant-Oldewage (2006b)
	Lake Victoria	UG	Akoll et al. (2012)[*]

[†] Type locality and associated publication; [#] Type host is *Clarias gariepinus*; [‡] Unpublished theses data; [*] Fish farm/aquaculture/whédos; [⁂] Doubtful validity; [•] Original literature not accessible; [a] Validity refuted by Hoogendoorn et al. (2019).

Table A3.5. Historic records of Cestoda parasitising *Clarias gariepinus* (Burchell) included in checklists of freshwater parasitic species in Africa. Abbreviations: EG – Egypt, GH – Ghana, SA – South Africa, UG – Uganda, ZI – Zimbabwe. Symbols: (+) Present, (–) no record, Metacercariae – [L].

		Khalil (1971)	Khalil and Polling (1997)	Scholz and Kuchta (2018)
1.	*Barsonella lafoni*	–	–	+
2.	*Marsypocephalus aegypticus*	–	EG	+
3.	*Marsypocephalus rectangulus*	EG	–	+
4.	*Marsypocephalus tanganyikae*[a]	–	–	+
5.	*Monobothrioides chalmersius*	–	–	+
6.	*Proteocephalus glanduligerus*	–	SA	+
7.	*Proteocephalus sulcatus*[b]	–	EG[*]	+
8.	*Stocksia pujehani*	SL	SL	+
9.	*Tetracampos ciliotheca*[c]	EG	EG, NI, SA, UG	+

[a] – syn. *Loennbergia tanganyikae* Fuhrmann et Baer, 1925 (see Khalil and Polling 1997).
[b] – accidental infection in several host species of the Clariidae, Claroteidae and Polypteridae (see Scholz and Kuchta 2018).

Table A3.6. Records of Cestoda parasitising the African sharptooth catfish, *Clarias gariepinus (*Burchell) in Africa. Abbreviations: BE – Benin, EG – Egypt, ET – Ethiopia, KY – Kenya, MW – Malawi, NI – Nigeria, SL – Sierra Leone, SA – South Africa, SU – Sudan, TA – Tanzania; UG – Uganda, ZI – Zimbabwe.

Classification	Localities	Country	Reference
Class Cestoda			
Order Bothriocephalidea			
Family Bothriocephalidae			
Tetracampos ciliotheca Wedl, 1861	Nile River[†]: Lake Manzala, Bahr Youssef	EG	Wedl (1861)[†]; Meggit (1930); Faisal et al. (1989); Al-Bassel (2003); Ibrahim et al. (2008)[★]; Shaneen et al. (2014)[◆]; El-Shahawy et al. (2017); Abdel-Gaber et al. (2015, 2016a); Rashied et al. (2016); Mohammed et al. (2018)[■]; Ali et al. (2020)[★]
	Lake Awasa, Lake Langano, Lake Tana, Lake Ziway	ET	Kuchta et al. (2012); Brabec et al. (2015)
	Lake Turkana	KY	Kuchta et al. (2012)
	Lake Malawi	MW	Kuchta et al. (2012)
	Ajiwa Reservoir, Anambra River, Asa Dam, Chanchaga River, Erinle Reservoir, Ife Ife pond, Jibia Reservoir, Lamingo Dam, Lekki Lagoon, Oba Reservoir, Usman Dam, Utako drainage canal, Wase Dam	NI	Aderounmu and Adeniyi (1972)[◆]; Anosike et al. (1992)[■, ◆]; Adikwu and Ibrahim (2004)[■]; Oniye et al. (2004)[★]; Akinsanya and Otubanjo (2006); Olofintoye (2006)[★]; Ayanda (2008[★], 2009a[★], b); Goselle et al. (2008); Bichi and Yelwa (2010)[■, f]; Aliyua and Solomon (2012); Ajala and Fawole (2014a, b); Ugbor et al. (2014); Edeh and Solomon (2016); Kawe et al. (2016)[★]; El-Shahawy et al. (2017); Ikechukwu et al. (2017)[★]; Abba et al. (2018a, b); Mgbemena et al. (2020)
	Coetzeesdraai Dam, Flag Boshielo Dam, Hout River, Krokodilsheuwel Dam, Lake Tzaneen, Lepellane Dam, Middle Letaba Dam, Mokotswane Dam, Nwanedi-Luphephe Dams, Olifants River, Olifantsnek Dam (Hex River), Phalaborwa Barrage, Piet Gouws Dam, Return water dam, Rietvlei Dam, Seshego Dam, Turfloop Dam, Vaal Dam	SA	Mashego and Saayman (1989); Saayman et al. (1991)[c]; Paperna (1996)[c]; Barson and Avenant-Oldewage (2006b); Madanire-Moyo et al. (2010, 2012); Matla (2012)[⁑]; Madanire-Moyo and Avenant-Oldewage (2013a, 2014); Mahlatji (2014)[⁑]; Sara et al. (2014); Yenova et al. (2020)
	Blue Nile (Sennar Dam and Er Roseires), Lake Victoria, While Nile (Al Kawah and Khartoum)	SU	Woodland (1925a); Kuchta et al. (2012)
	Lake Victoria	TA	Mwita (2014a, b); Mwita and Nkwengulila (2004, 2008)
	Lake Victoria	UG	Wakbuke-Bunoti (1980); Khalil and Thurston (1973)

Table A3.6. (continued) Records of Cestoda parasitising the African sharptooth catfish, *Clarias gariepinus (*Burchell) in Africa. Abbreviations: BE – Benin, EG – Egypt, ET – Ethiopia, KY – Kenya, MW – Malawi, NI – Nigeria, SL – Sierra Leone, SA – South Africa, SU – Sudan, TA – Tanzania; UG – Uganda, ZI – Zimbabwe.

Classification	Locality	Country	Reference
	Gwebi River, Insukamini Dam, Lake Kariba, Manyame River, Mukuvusi River, Nyatsime River, Save-Runde River floodplain	ZI	Douëllou (1992); Barson et al. (2008, 2014); Moyo et al (2009); Madanire-Moyo and Barson (2010)
Order Caryophyllidea			
Family Lytocestidae			
Stocksia pujehuni[#] Woodland, 1937	Angolin-Lowe in the Oueme River delta	BE	Tossavi et al. (2014)
	Lekki Lagoon	NI	Akinsanya and Otubanjo (2006); Ikechukwu et al. (2017)[★]
	Waanje River[†]	SL	Woodland (1937)[†]
Monobothrioides chalmersius (Woodland, 1924)	Nile River: Damietta Branch, Nile Delta	EG	Hamada and El-Naggar (2003); Hamada et al. (2004); Waeschenbach et al. (2007); El-Shahawy et al. (2017)
	Blue Nile (Sennar Dam)	SU	Scholz et al. (2021)
Order Onchoproteocephalidea			
Family Proteocephalidae			
Subfamily Marsypocephalinae			
Marsypocephalus aegypticus[#] El-Naffar, Saoud et Hassan, 1984	-	EG	El-Naffar et al. (1983, 1984)[◆, ●, b]
Marsypocephalus rectangulus[#] Wedl, 1861	Nile River[†]	EG	Wedl (1861)[†]
Marsypocephalus tanganyikae[#] (Fuhrmann et Baer, 1925)	Lake Tanganyika[†]	ET	Fuhrmann and Baer 1925[†, a]
Subfamily Proteocephalinae			
Barsonella lafoni[#] de Chambrier, Scholz, Beletew et Mariaux, 2009	Lake Langano, Lake Tana[†]	ET	de Chambrier et al. (2009b)[†]
	Lake Victoria	TA	de Chambrier et al. (2009b)
	Lake Chivero	ZI	de Chambrier et al. (2009b)
Proteocephalus glanduligerus Janicki, 1928	–	EG	Mohammed et al. (2018)[■]
	Jibia Reservoir	NI	Abba et al. (2018a, b)

Table A3.6. (continued) Records of Cestoda parasitising the African sharptooth catfish, *Clarias gariepinus (*Burchell) in Africa. Abbreviations: BE – Benin, EG – Egypt, ET – Ethiopia, KY – Kenya, MW – Malawi, NI – Nigeria, SL – Sierra Leone, SA – South Africa, SU – Sudan, TA – Tanzania; UG – Uganda, ZI – Zimbabwe.

Classification	Locality	Country	Reference
	Coetzeesdraai, Krokodilsheuwel, Lake Tzaneen, Middle Letaba Dam, Mokotswane Dam, Nwanedi-Luphephe Dams, Piet Gouws, Rietvlei Dam, Vaal Dam	SA	Janicki (1928) ◆, [e]; Mashego and Saayman (1989); Saayman et al. (1991)[c]; Barson and Avenant-Oldewage (2006b); Madanire-Moyo et al. (2010, 2012); Matla (2012)⁂; Madanire-Moyo and Avenant-Oldewage (2013a)
	Lake Tana	ET	Scholz et al. (2009)
	Insukamini Dam	ZI	Moyo et al. (2009)
Proteocephalus sulcatus (Klaptocz, 1906)	–	EG	El-Naffar et al. (1984)●, [b, d]

Refer to the following literature for more detail: [a] – Woodland (1925a); [b] – Khalil and Polling (1997); [c] – Barson and Avenant-Oldewage (2006b); [d] – de Chambrier et al. (2007); [e] – Scholz et al. (2009); [f] – Kuchta et al. (2012).

† Type locality and associated literature.
# Type host is *Clarias gariepinus* (Burchell).
■ Infection reported from fish obtained from local fish farms or cultured in fish farms or whédos.
◆ No specific locality or river system mentioned.
⋆ Fish bought from local fish markets, but suspected water body from where fish were caught is mentioned.
● Original literature not accessible.
‡ Unpublished theses data or conference contributions.
⁂ Doubtful validity of species.
▲ Introduced co-invasive parasitic species.

Table A3.7. Historic records of **Nematoda** parasitic to African sharptooth catfish, *Clarias gariepinus (*Burchell) previously included in checklists. Abbreviations: CD – Democratic Republic of Congo; EG – Egypt; ML – Mali; SA – South Africa; SU – Sudan. [L] – larval stages; ⁂ – considered incidental infection.

		Khalil (1971)	Khalil and Polling (1997)	Scholz and Kuchta (2018)	Moravec (2019)
1.	*Amplicaecum* sp. (Type I) [L]	SU	EG, SU	+	+
2.	*Camallanus* sp.	–	–	+	–
3.	*Capillaria* sp.	–	SA	+	+[a]
4.	*Contracaecum* sp.	CD, ML	CD, ML, SA	+	–
5.	*Contracaecum* sp. Type I [L]	–	–	–	+[b]
6.	*Contracaecum* sp. Type II [L]	–	–	–	+
7.	*Eustrongylides africanus* [L][c]	–	–	+	–
8.	*Eustrongylides* sp. [L]	CD	CD	+	+
9.	*Galeiceps* sp. [L]	–	–	+	+
10.	*Gendria tilapiae*⁂	–	–	–	+
11.	*Neocamallanus polypteri*⁂	–	–	–	+
12.	*Paracamallanus cyathopharynx*[d]	EG, CD, SU	EG, CD, SU, SA	+	+
13.	*Paracamallanus* sp.	–	–	+	–
14.	*Procamallanus* (*Procamallanus*) *pseudolaeviconchus*[e]	CD, SU	CD, SU, EG	+	+
15.	*Procamallanus* (*Spirocamallanus*) *spiralis*[f]	EG[g]	EG	+	+
16.	*Procamallanus* sp.	–	–	+	–
17.	*Rhabdochona* (*Globochona*) *paski*⁂, [h]	CD	CD	+	+
18.	*Rhabdochona* sp.	–	SA	+	+
19.	*Spinitectus allaeri*[i]	–	EG	+	+
20.	*Spinitectus petterae*	–	SA	+	+
21..	*Syncuaria* sp. [L][i]	–	SA	+	+
22.	Atractidae gen. sp. [L]	–	–	+	+
23.	Nematoda gen. sp. [L]	–	–	–	+[j]
24.	Physalopteridae gen. sp. 1	–	–	+	+

[a] – Reported as Capillaridae gen. sp.
[b] – No formal assignment of *Contracaecum* sp. Type I larvae to specimens found from *C. gariepinus* in literature.
[c] – Refer to Moravec (2019) for validity.
[d] – Synonyms: *Camallanus cyathopharynx* Baylis, 1923 and *Paracamallanus senegalensis* Vassiliades, 1970.
[e] – All species previously reported as *P. laeviconchus* from *C. gariepinus* is synonymised with *P.* (*P.*) *pseudolaeviconchus* (see Moravec and Van As 2015d; Moravec 2019).
[f] – Synonym: *Spirocamallanus mazabukae* Yeh, 1957.
[g] – Reported as *Spirocamallanus spiralis* (Baylis, 1923).
[h] – Synonym: *Rhabdochona congolensis* Campana-Rouget, 1961.
[i] – Synonyms: *Chordocephalus* sp. and *Skrjabinocara* sp.
[j] – Author do not refer to literature reporting infection with specific nematodes assigned to Nematoda gen. sp.

Table A3.8. Distribution records of the Nematoda known to parasitise the African sharptooth catfish, *Clarias gariepinus (*Burchell) in Africa. Abbreviations: BE – Benin; BW – Botswana; DC – Democratic Republic of the Congo; EG – Egypt; ET – Ethiopia; KY – Kenya; ML – Mali; NM – Namibia; NI – Nigeria; SN – Senegal; SA – South Africa; SU – Sudan; TA – Tanzania; UG – Uganda; ZI – Zimbabwe. [L] – larval stages.

Classification	Localities	Country	Reference
Phylum Nematoda			
Class Adenophorea			
Order Enoplia			
Family Capillaridae			
Capillaria sp.	Lake Awassa	NI	Adeyemo and Falaye (2007)▪; Hussen et al. (2012)⁂; Rufai and Adetona (2017) ◆, ⁂
	Crocodile River, Sabie River	SA	Boomker (1994a)
Family Dioctophymatidae			
Eustrongylides africanus [L]	Bida floodplain	NI	Ibiwoye et al. (2004, 2005a, b)⁂, c
Eustrongylides sp. [L]	–	DC	Baylis (1940)◆
	Lake Ziway,	ET	Bekele and Hussein (2015)
	earthen and concrete ponds▪	NI	Omeji et al. (2013)▪;
	–	SN	Vassiliades (1975)
	Lake Victoria (Mwanza Gulf)	TA	Mwita and Nkwengulila (2004, 2008)
	Lake George	UG	Paperna (1974)
Order Ascaridida			
Family Anisakidae			
Contracaecum sp. [L]	Lake Kivu, Lake Tanganyika	DC	Baylis (1940)◆; Campana-Rouget (1961)
	Lake Nasser	EG	See references in Younis et al. (2017)
	Lake Tana; Lake Ziway	ET	Yimer (2000); Amare et al. (2014); Bekele and Hussein (2015)
	Ajiwa Reservoir, Jibia Reservoir	NI	Hussen et al. (2012) ⁂; Abba et al. (2018a, b)
	Lake Liambezi	NM	McHugh et al. (2016)
	Niger River, Lake Déblo	ML	Baylis (1930)
	Hartbeespoort Dam, Flag Boshielo Dam, Hout River, Return Water Dam, Nwanedi-Luphephe Dams, Buffelsdoorn Dam, Seshego Dam, Piet Gouws Dam, Olifants River, Coetzeesdraai, Krokodilsheuwel, Lepellane, Namagkale, Turloop Dam, Sabie River, Crocodile River, Lake St Lucia, Rietvlei Dam	SA	Mashego and Saayman (1981); Boomker (1982, 1994a); Madanire-Moyo et al. (2010, 2012), Sara et al. (2014); Prudhoe and Hussey (1977); Whitfield and Heeg (1977)
	Lake Victoria (Mwanza Gulf)	TA	Mwita and Nkwengulila (2004, 2008)
	Lake Victoria	UG	Akoll et al. (2012)▪
	Lake Chivero, Save-Runde River Floodplain, Gwebi River, Manyame River, Nyatsime River, Mukuvusi River	ZI	Barson (2004); Barson et al. (2008, 2014); Madanire-Moyo and Barson (2010)
Contracaecum sp. [L] (Type II)	Lake Tana	ET	Moravec and Scholz (2017)
	Rietvlei Dam	SA	Barson and Avenant-Oldewage (2006a)
Galeiceps sp. [L]	Okavango River (Mokoro Lagoon)	BW	Moravec and van As (2015d)

Table A3.8. (continued) Distribution records of the Nematoda known to parasitise the African sharptooth catfish, *Clarias gariepinus (*Burchell) in Africa. Abbreviations: BE – Benin; BW – Botswana; DC – Democratic Republic of the Congo; EG – Egypt; ET – Ethiopia; KY – Kenya; ML – Mali; NM – Namibia; NI – Nigeria; SN – Senegal; SA – South Africa; SU – Sudan; TA – Tanzania; UG – Uganda; ZI – Zimbabwe. [L] – larval stages.

Classification	Localities	Country	Reference
Family Ascarididae			
Amplicaecum sp. (Type I) [L]	Lake Tana	ET	Yimer (2000)
	White and Blue Nile rivers	SU	Khalil (1969)
Family Atractidae			
Atractidae gen. sp. 1	Lower Congo River	DC	Moravec and Jirků (2017)
Family Quimperiidae			
Gendria tilapiae Baylis, 1930	Lake Victoria	TA	Mwita and Nkwengulila (2008)⁂, c
Order Spirurida			
Family Acuariidae			
Syncuaria sp.	Hartebeespoort Dam	SA	Boomker (1982)
Family Camallanidae			
Camallanus sp.	Lake Hugo	ET	Amare et al. (2014)
	Uke River, various streams and rivers in Plateu State (around Jos). earthen and concrete ponds▪	NI	Onwuliri and Mgbemena (1987); Anosike et al. (1992), Yakubu et al. (2002); Omeji et al. (2013)▪
Neocamallanus polypteri	Manzala Lake	EG	Abdel-Gaber et al. (2015) ⁂, c
Paracamallanus cyathopharynx (Baylis, 1923)#	Oueme River (Angolin-Lowe)	BE	Tossavi et al. (2014)
	Okavango River (Shakawe)	BW	Moravec and van As (2015c)
	Congo basin, Poikal Lower Congo River: Bas Congo, Poiko; Lake Albert, Lake Edward	DC	Campana-Rouget (1961); Khalil (1969); Moravec and Jirků (2017)
	Nile River†	EG	Baylis (1923b†); Myers (1962); Khalil (1969); Moravec (1974a, b)★; Kawe et al. (2016)◆; Shaneen et al. (2020)◆;
	Lake Tana	ET	Moravec and Scholz (2017)
	-	KY	Rindoria et al. (2020)▪
	Lekki Lagoon, Asa Dam, Erinle Reservoir, Oba Reservoir, Kaduna River	NI	Akinsanya and Otubanjo (2006); Ayanda (2008, 2009a, b)★; Ajala and Fawole (2014a, b); Olumuyiwa et al. (2014)
	Crocodile River basin, Hartebeespoort Dam, Hout River, Sabie River, Olifants River, Nwanedi-Luphephe Dams, Flag Boshielo Dam, Seshego Dam, Turfloop Dam, Buffelsdoorn Dam, Krokodilsheuwel Dam, Piet Gouws Dam, Coetzeesdraai Dam, Lepellane Dam	SA	Mashego and Saayman (1981); Boomker (1982, 1994a); Madanire-Moyo et al. (2010, 2012); Sara et al. (2014)
	Lake Guiers	SN	Vassiliades (1975)
	Blue Nile, White Nile	SU	Khalil (1969); Moravec and Scholz (2017)
	Lake Victoria (Mwanza Gulf)	TA	Mwita and Nkwengulila (2004, 2008); Mwita (2014a)
	Save-Runde River Floodplain, Gwebi River, Manyame River, Mukuvusi River, Nyatsime River, Insukamini Dam, Lake Kariba	ZI	Douëllou (1992); Barson et al. (2008, 2014); Moyo et al. (2009); Madanire-Moyo and Barson (2010);

Table A3.8. (continued) Distribution records of the Nematoda known to parasitise the African sharptooth catfish, *Clarias gariepinus (*Burchell) in Africa. Abbreviations: BE – Benin; BW – Botswana; DC – Democratic Republic of the Congo; EG – Egypt; ET – Ethiopia; KY – Kenya; ML – Mali; NM – Namibia; NI – Nigeria; SN – Senegal; SA – South Africa; SU – Sudan; TA – Tanzania; UG – Uganda; ZI – Zimbabwe. [L] – larval stages.

Classification	Localities	Country	Reference
Paracamallanus sp.	Kaduna River, rivers around Jos,	NI	Auta et al. (2019); Ali et al. (2020)◆, ❖
Procamallanus (Procamallanus) pseudolaeviconchus	Nile River: Bahr Youssef, Lake Manzala,	EG	Al-Bassel (2003); Moravec (1974a★, 1975a)[a]; Abdel-Gaber et al. (2015); Shaneen et al. (2020)◆
	Oueme River (Angolin-Lowe)	BE	Tossavi et al. (2014)
	Lake Albert, Lake Edward	DC	Campana-Rouget (1961)
	Uke River	ET	Yakubu et al. (2002, 2017)
	Lake Turkana	KY	Mašová et al. (2011)
	Ajiwa Reservoir, Asa Dam, Erinle Reservoir, Jibia Reservoir, Lekki Lagoon, Oba Reservoir, Uke River; Usman Dam, Erinle Reservoir, various streams and rivers in Plateu State	NI	Anosike et al. (1992); Yakubu et al. (2002); Bichi and Yelwa (2010)▪, Ayanda (2008, 2009a, b)★; Aliyu and Solomon (2012); Ajala and Fawole (2014a, b); Olumuyiwa et al. (2014); Kawe et al. (2016)◆; Abba et al. (2018a, b)
	Manyame River, Mukuvusi River, Nyatsime River, Lake Kariba	ZI	Douëllou (1992); Madanire-Moyo and Barson (2010)
	Flag Boshielo Dam; krokodilsheuwel Dam, Turfloop Dam, Olifants River, Hartebeespoort Dam, Olifants River, Nwanedi-Luphephe Dams	SA	Mashego and Saayman (1981); Boomker (1982, 1994a); Madanire-Moyo et al. (2012)
	Lake Guiers	SN	Vassiliadès (1975)
	Lake Victoria (Mwanza Gulf)	TA	Mwita and Nkwengulila (2004, 2008)
Procamallanus sp.	Ogun River, rivers around Jos	NI	Onwuliri and Mgbemena (1987); Salawu et al. (2013)
P. (Spirocamallanus) spiralis[#] Baylis, 1923	Nile River[†]	EG	Baylis (1923a, b)[†]
	-	SU	Khalil (1969)◆, [b]
Family Cystidicolidae			
Spinitectus allaeri Campana-Rouget, 1961	Nile River	EG	Moravec (1974b)★
Spinitectus petterae[#] Boomker, 1993	Crocodile River[†], Sabie River	SA	Boomker (1993[†], 1994a)
	Lake Victoria (Mwanza Gulf)	TA	Mwita and Nkwengulila (2004); Mwita (2014a)
Family Physalopteridae			
Physalopteridae gen. sp. 1	Okavango River: Shakawe Stream	BW	Moravec and van As (2015c)

Table A3.8. (continued) Distribution records of the Nematoda known to parasitise the African sharptooth catfish, *Clarias gariepinus (*Burchell) in Africa. Abbreviations: BE – Benin; BW – Botswana; DC – Democratic Republic of the Congo; EG – Egypt; ET – Ethiopia; KY – Kenya; ML – Mali; NM – Namibia; NI – Nigeria; SN – Senegal; SA – South Africa; SU – Sudan; TA – Tanzania; UG – Uganda; ZI – Zimbabwe. [L] – larval stages.

Classification	Localities	Country	Reference
Family Rhabdochonidae			
Rhabdochona (Globochona) paski	Lake Kivu	DC	Campana-Rouget (1961)
	Lake Victoria	NI	Mwiata and Nkwengulila (2008, 2010); Mwita (2014a)
Rhabdochona sp.	Olifants River, Sabie River, Crocodile River	SA	Boomker (1994a)

Refer to the following literature for more detail: [a] – Moravec and Van As (2015b); [b] – Moravec (2019).
The following literature could not be obtained to confirm records from *Clarias gariepinus,* or alternatively confirm parasitic species recorded from this host: Paperna (1964); Moravec (1975a, b); Fahmy et al. (1976); Shotter (1983); Ezenwaji and Ilozumba (1992); El Garhy (2003); Yimer and Enyew (2003).

† Type locality and associated literature.
# Type host is *Clarias gariepinus* (Burchell).
▪ Infection reported from fish obtained from local fish farms, aquaculture facilities or whédos.
◆ No specific locality or river system mentioned.
⋆ Fish bought from local fish markets, but suspected water body from where fish were caught is mentioned in the text.
⁂ Doubtful validity of species due to lack of provision of morphological or molecular data.
❖ Reported as *Procamallanus pseudolaeviconchus*, photomicrographs provided do not support this identification, specimens most probably belong to *Paracamallanus* based on buccal capsule morphology.

Table A3.9. Historic records of Acanthocephala included in checklists of freshwater parasitic species in Africa known to parasitise the African sharptooth catfish, *Clarias gariepinus (*Burchell). Abbreviations: EG – Egypt.

		Khalil (1971)	Khalil and Polling (1997)	Scholz and Kuchta (2018)
1.	*Neoechinorhynchus rutili*	–	–	+
2.	*Neoechinorhynchus* sp.	–	–	+
3.	*Paragorgorhynchus aswanensis*	–	EG	+
4.	*Paragogohynchus* sp.	–	–	+

Table A3.10. Records of Acanthocephala from the African sharptooth catfish, *Clarias gariepinus (*Burchell, 1822) in Africa. Abbreviations: EG – Egypt; NI – Nigeria.

Classification	Localities	Country	Reference
Phylum Acanthocephala Class Eoacanthocephala Order Echinorhynchida FamilyRhadinorhynchidae			
Paragorgorhynchus aswanensis Saoud et Wanas, 1990	Aswan High Dam Lake	EG	Saoud and Wannas (1990)
Paragorgorhynchus sp.			
Order Neoechinorhynchida Family Neoenchinorhynchidae			
Neoechinorhynchus rutili (Müller, 1789)			
	Lake Manzala	EG	Rashied et al. (2016)
	Ajiwia reservoir, Asa Dam, Jibia Reservoir, Oba Reservoir, Usman Dam	NI	Oniye et al. (2004); Ayanda (2008); Aliyu and Solomon (2012); Ajala and Fawole (2014a, b); Shaneen et al. (2014); Abba et al. (2018a, b)
Neoechinorhynchus sp.			

Table A3.11. Parasitic Crustacea from the African sharptooth catfish, *Clarias gariepinus* (Burchell) included in checklists.

		Oldewage and Avenant-Oldewage (1993)	Smit and Hadfield (2018)
1.	*Afrolernaea annemari*	–	+
2.	*Argulus africanus*	–	+
3.	*Argulus ambloplites*	–	+
4.	*Argulus cunningtoni*	–	+
5.	*Argulus japonicus*	–	+
6.	*Argulus rhipidiophorus*	–	+
7.	*Argulus striatus*	–	+
8.	*Chonopeltis fryeri*	–	+
9.	*Chonopeltis inermis*	–	+
10.	*Dolops ranarum*	–	+
11.	*Ergasilus mirabilis*	+	+
12.	*Ergasilus sarsi*	+	+
13.	*Lamproglena clariae*	–	+
14.	*Lamproglena cornuta*	+	+
15.	*Lernaea composita*[a]	+	+
16.	*Lernaea diceracephala*	–	+
17.	*Sebekia okavangoensis*	–	+

[a] – Synonyms: *Lernaea werneri* (Kurtz, 1992) and *Lernaeocera werneri* Kurtz, 1922.

Table A3.12. Records of Crustacea from the African sharptooth catfish, *Clarias gariepinus (*Burchell, 1822) in Africa. Abbreviations: BW – Botswana; EG – Egypt; GH – Ghana; KY – Kenya; MW – Malawi; NM – Namibia; SA – South Africa; SU – Sudan; TA – Tanzania; UG – Uganda; ZI – Zimbabwe; ZM - Zambia.

Classification	Localities	Country	Reference
Phylum Arthropoda Subphylum Crustacea Class Hexanauplia Subclass Copepoda Order Cyclopoida Family Ergasilidae			
Ergasilus mirabilis Oldewage et Van As, 1987	Kwando River (Caprivi strip)	NM	Avenant-Oldewage and Oldewage (1993)
	Pongolo River and floodplain	SA	Oldewage and Van As (1988)
Ergasilus sarsi Capart, 1944	Malwi River (Lake Volta)	GH	Paperna (1969b)
Family Lernaeidae			
Afrolernaea annemari Oldewage, 1994[#]	Kwando River (Caprivi strip)[†]	NM	Avenant-Oldewage and Oldewage (1993), Oldewage (1994)[†]
Lamproglena clariae Fryer, 1956[#]	Okavango River and Delta	BW	Unpublished data of APRG[*], see van As and van As (2007)
	Nile River, Abbassa fish farms	EG	Hassanin et al. (2010)
	Lake Malawi[†], Banga River	MW	Fryer (1956)[†]
	Kwando River (Caprivi strip)	NM	Marx and Avenant-Oldewage (1996)
	Olifants River, Vaal River, Grootdraai Dam, Vaalharts Dam, Douglaskeerwal, Vaal River Barrage, Vaal Dam, Nwanedi-Luphephe Dams, Flag Boshielo Dam Mamba, Balule Reservoir	SA	Euler and Avenant-Oldewage (1992)[b], Marx and Avenant-Oldewage (1996), Tsotetsi et al. (2004, 2005), Madanire-Moyo et al. (2010, 2012), Madanire-Moyo and Avenant-Oldewage (2013b), Moll and Avenant-Oldewage (2017), Esterhuyze and Avenant-Oldewage (2019)
	White Nile	SU	Fryer (1965b)
	Lake Kazinga channel between Lake Edward and Lake George	UG	Thurston (1970)*
	Manyame River, Nyatsime River	ZI	Madanire-Moyo and Barson (2010)
Lamproglena cornuta Fryer, 1965	Lake Kazinga channel between Lake Edward and Lake George	UG	Thurston (1970)[a]

Table A3.12. (continued) Records of Crustacea from the African sharptooth catfish, *Clarias gariepinus (*Burchell, 1822) in Africa. Abbreviations: BW – Botswana; EG – Egypt; GH – Ghana; KY – Kenya; MW – Malawi; NM – Namibia; SA – South Africa; SU – Sudan; TA – Tanzania; UG – Uganda; ZI – Zimbabwe; ZM - Zambia.

Classification	Localities	Country	Reference
Lamproglena monodi Capart, 1944	Kwando River (Caprivi strip)	NM	Avenant-Oldewage and Oldewage (1993)
Lernaea composita Wilson, 1924	Lake Tanganyika	–	Need confirmation see text.
Lernaea diceracephala (Cunnington, 1914) #	Lake Tanganyika†	TA	Cunnington (1914)†
Subclass Branchiura Order Arguloida Superfamily Arguloidea Family Argulidae			
Argulus africanus Thiele, 1900	Lake Victoria	UG	Fryer (1961)
	Confluence of Matetsi and Deka rivers with Zambezi River	ZI	Fryer (1964)
Argulus ambloplites Wilson, 1920	Okavango River and Delta	BW	Van As and Van As 2015
	Lake Malawi	MW	
	Mulungushi Dam, Lake Bangweulu	ZM	Fryer (1959a, b, 1960)
Argulus cunningtoni Fryer, 1965	Zambezi River	NM	Van As and Van As 2015
	Lake Albert†	UG	Fryer (1965b)
Argulus japonicus Thiele, 1900▲	Barberspan wetland; Bloemhof Dam; Loskop Dam; Olifants River, Witbank Dam	SA	Kruger et al. (1983), Shafir and Oldewage (1992), Avenant-Oldewage 2001
Argulus rhipidiophorus Monod, 1931	Lake Turkana	KY	Fryer (1959a, b)
	White Nile	SU	Fryer (1959b)
	Lake Albert†	UG	Fryer (1965b)
	Lake Tanganyika (Sumbu and Vua)	TA	Cunnington, (1913)
Argulus striatus Cunnington, 1913	Lake Tanganyika (Sumbu and Vua)	TA	Cunnington (1913)†
Chonopeltis fryeri Van As, 1986#	Loskop Dam†	SA	Van As (1986)†
Chonopeltis inermis Thiele, 1900	Nwanedi-Luphephe Dams	SA	Madanire-Moyo et al. (2010, 2012)
	Lake Malawi†, Banga River, Nkata Bay	MW	Fryer (1956)
Chonopeltis sp.	Nyatsime River	ZI	Madanire-Moyo and Barson (2010)

Table A3.12. (continued) Records of Crustacea from the African sharptooth catfish, *Clarias gariepinus (*Burchell, 1822) in Africa. Abbreviations: BW – Botswana; EG – Egypt; GH – Ghana; KY – Kenya; MW – Malawi; NM – Namibia; SA – South Africa; SU – Sudan; TA – Tanzania; UG – Uganda; ZI – Zimbabwe; ZM - Zambia.

Classification	Localities	Country	Reference
Dolops ranarum (Stuhlmann, 1892)	Okavango River	BW	Van As and Van As (2015)
	Lake Malawi, Banga River, Bana Lagoon, Nkata Bay	MW	Fryer (1956)
	Bloemhof Dam; Limpopo River drainage (Glen Alpine, Nwanedi-Luphephe, Nzhelele, Roodeplaat, Tzaneen dams); Lake Sibaya	SA	Bruton (1979a), Avenant and Van As (1985), Avenant et al. (1989b), Avenant-Oldewage and Van As (1990a, b), Avenant-Oldewage (1994), Møller et al. (2008), Tam and Avenant-Oldewage (2009), Madanire-Moyo et al. (2010, 2012)
	Mwanza Gulf; Lake Victoria	TA	Nkwengulila and Mwita (2004)
	Lake Albert, Lake Victoria	UG	Fryer (1961, 1965b), Thurston (1970)
	Lake Kariba, Manyame River	ZI	Douëllou and Erlwanger (1994), Madanire-Moyo and Barson (2010)
	Kashizki River	ZM	Fryer (1959a, b)
Subclass Pentastomida Order Porocephalida Superfamily Porocepgaloidea Family Sebekidae			
Sebekia okavangoensis Riley et Huchzermeyer, 1995 [L]	Incomati River system	SA	Luus-Powell et al. (2008)

Refer to the following literature for more detail: [a] – Oldewage and Avenant-Oldewage (1993); [b] – Marx and Avenant-Oldewage (1996) and [c] – Van As and Van As (2007).

† Type locality and associated publication.
# Type host is *Clarias gariepinus* (Burchell).
▲ Alien invasive species, classified as co-invasive in Smit et al. (2017).

Table A3.13. Records of Hirudinea parasitising the African sharptooth catfish, *Clarias gariepinus (*Burchell, 1822) in Africa. Abbreviations: NM – Namibia; SA – South Africa.

Classification	Localities	Country	Reference
Phylum Annelida Class Clitellata Subclass Hirudinea Order Rhynchobdellida Family Glossiphoniidae			
Batracobdelloides tricarinata (Blanchard, 1897)	Phongolo River, Kruger National Park	SA	Oosthuizen 1989, Smit et al. (2020)
	Eastern Caprivi strip	NM	Oosthuizen 1989, 1990, 1991

Appendix A4

Table A4.1. List of Digenea selected from the GenBank database for comparison to sequence data obtained for adults during the present study. Species in bold obtained from material collected during the present study. Abbreviations: EG – Egypt; KY – Kenya; MZ – Mozambique; ZI – Zimbabwe. Present study localities in South Africa (BS – Boskop Dam) and Zambia (KR – Kabompo River; ZR – Barotse floodplain).

	Host	Family	Locality	18S rDNA	ITS1-2	28S rDNA	Reference
Plagiorchiida							
Family Gorgoderidae							
Phyllosdistomum cf. *symmetrorchis*	*Clarias gariepinus* (Burchell)	Clariidae	KY	–	KF013152	KF013171	Cutmore et al. (2013)
			KY	–	KF013162	KF013174	Cutmore et al. (2013)
Phyllodistomum cf. *vanderwaali*	*C. gariepinus*	Clariidae	**ZR**	**MT14**	**MT14**	**MT14**	**Present study**
Family Opistorchiidae							
Thaparotrema sp.	*C. gariepinus*	Clariidae	**KR**	**MT17**	**MT17**	**–**	**Present study**
			ZR	**MT13**	**MT13**	**–**	**Present study**
			ZR	**MT10**	**MT10**	**–**	**Present study**
Family Orientocreadidae							
Orientocreadium batrachoides	*C. gariepinus*	Clariidae	**BS**	**MT4**	**MT4**	**MT4**	**Present study**
			ZR	**MT8**	**MT8**	**MT8**	**Present study**
			EG	MT797949	**–**	MT797948	Attia et al. (2021)
			MZ	MK496889	**–**	MK496882	Dumbo et al. (2019b)
			ZI	**–**	MZ600135	**–**	Hammoud et al. (2022)
Orientocreadium sp.	*Radix natalensis* (Krauss)[a]	Lymnaeidae	ZI	**–**	MZ600136	**–**	Hammoud et al. (2022)

[a] – Belong to the Gastropoda, possibly intermediate host.

Table A4.2. List of Digenea selected from the GenBank database for comparison to sequence data obtained for metacercariae during the present study. Species in bold obtained from material collected during the present study. Abbreviations: BR – Brazil; CD – Democratic Republic of the Congo; EC – Ecuador; EG – Egypt; IN – India; KY – Kenya; ME – Mexico; NI – Nigeria; SA – South Africa; TA – Tanzania; USA – United Stated of America. Present study localities in South Africa (BS – Boskop Dam; NGR – Ndumo Game Reserve; RR – Riet River; SR – Sand River) and Zambia (KR – Kabompo River; ZR – Barotse floodplain).

	Host	Family	Locality	ITS1-2	28S rDNA	*cox*1	Reference
Suborder Xiphidiata							
Macrophalloidea sp.	*Pila ovata*[a]	Ampullaridae	NI	–	KX022509	–	Krupenko et al. (2020)
Travtrema stenocotyle	*Bothrops moojeni*[b]	Viperidae	BR	–	MW317229	–	Muller et al. (2021)
Xiphidiata gen. sp. 1	*C. gariepinus*	Clariidae	**ZR**	**–**	**MT42**	**–**	**Present study**
Family Clinostomidae							
Clinostomum brieni	*C. gariepinus*	Clariidae	ZR	**MT29**	**MT29**	**MT29**	**Present study**
			SA	MH238412	–	–	Caffara et al. (2019)
			SA	MH238413	–	–	Caffara et al. (2019)
			SA	–	–	MH253044	Caffara et al. (2019)
			SA	–	–	MH253045	Caffara et al. (2019)
	Clarias ngamensis	Clariidae	CD	MH238414	–	MH253046	Caffara et al. (2019)
			CD	MH238415	–	MH253047	Caffara et al. (2019)
			CD	MH238416	–	MH253048	Caffara et al. (2019)
	Heteropneustes fossilis (Bloch)	Heterpneustidae	IN	–	KF811008	–	Tandon et al. (2015)
			IN	–	KF781299	–	Athokpam et al. (2014)
Family Diplostomidae							
Bolbophorus sp. 3	*T. sparrmanii*	Cichlidae	SA	MK604879	MK604820	MK605688	Hoogendoorn et al. (2019)
Diplostomidae gen. sp. 1	*C. gariepinus*	Clariidae	**RR**	**MT31**	**MT31**	**MT31**	**Present study**
Diplostomidae gen. sp.	*Tilapia sparrmanii*	Cichlidae	SA	MK604885	MK604826	MK605692	Hoogendoorn et al. (2019)
Neodiplostomum banghami	*Falco columbarius*[c]	Falconidae	USA	–	OL799087	OL770054	Achatz et al. (2022)
Neodiplostomum cf. *lucidum*	*Procyon lotor*[d]		USA	**–**	OL799102	OL770043	Achatz et al. (2022)
Neodiplostmum Type 1	*C. gariepinus*	Claridae	**NGR**	**–**	**42KS**	**42KS**	**Present study**
Neodiplostomum vaucheri	*Trachops cirrhosis*[d]	Phyllostomidae	EC	–	OL799108	OL770067	Achatz et al. (2022)
Sphincterodiplostomum musculosum	*Caiman yacare*		BR	**–**	MW411445	MW410856	Achatz et al. (2021)
Uvulifer sp. 4	*Tilapia sparrmanii*	Cichlidae	**SA**	MK604882	MK604824	MK605691	Hoogendoorn et al. (2019)
Uvulifer spinatus	*Alfaro huberi*	Poeciliidae	ME	MF568598	–	–	Lopez-Jimenez et al (2017)

Table A4.2. (continued) List of Digenea selected from the GenBank database for comparison to sequence data obtained for metacercariae during the present study. Species in bold obtained from material collected during the present study. Abbreviations: BR – Brazil; CD – Democratic Republic of the Congo; EC – Ecuador; EG – Egypt; IN – India; KY – Kenya; ME – Mexico; NI – Nigeria; SA – South Africa; TA – Tanzania; USA – United Stated of America. Present study localities in South Africa (BS – Boskop Dam; NGR – Ndumo Game Reserve; RR – Riet River; SR – Sand River) and Zambia (KR – Kabompo River; ZR – Barotse floodplain).

	Host	Family	Locality	ITS1-2	28S rDNA	*cox*1	Reference
Tylodelphys mashonensis	*C. gariepinus*	Claridae	**NGR**	**1US1**	**1US1**	–	**Present study**
			NGR	**1US2**	**1US2**	–	**Present study**
			NGR	**3BB1**	**3BB1**	**3BB1**	**Present study**
			NGR	**3US2**	**3US2**	–	**Present study**
			SR	**32SR1**	**32SR1**	–	**Present study**
			GFR	**24FR1**	**24FR1**	–	**Present study**
			TA	FJ470402	–	–	Chibwana et al. (2013)
			TA		–	KC685327	Chibwana et al. (2013)
			SA	–	KF189071	–	Moema et al. (2013)
			SA	–	KF189072	–	Moema et al. (2013)
Tylodelphys cf. *grandis*	*C. gariepinus*	Clariidae	**ZR**	**MT19**	**MT19**	**MT19**	**Present study**
			ZR	**MT20**	**MT20**	**MT20**	**Present study**
			ZR	**MT21**	**MT21**	**MT21**	**Present study**
			ZR	**MT22**	**MT22**	**MT22**	**Present study**
Tylodelphys sp. 1	*C. gariepinus*	Clariidae	TA	KC685365	**–**	KC685346	Chibwana et al. (2013)
Tylodelphys sp. 2	*C. gariepinus*	Clariidae	TA	FJ470401	**–**	**–**	Chibwana et al. (2013)

Non-fish hosts: [a] – Gastropoda; [b] – Reptilia; [c] – Aves; [d] – Mammalia.

Table A4.3. List of Cestoda selected from the GenBank database for comparison to sequence data obtained during the present study. Species in bold obtained from material collected during the present study. Abbreviations: BR – Brazil; CA – Central African Republic; CD – Democratic Republic of the Congo; CH – China; EG – Egypt; ET – Ethiopia; MD – Madagascar; SU – Sudan; US – United States of America; VT – Vietnam.

	Host	Family	Locality	28S rDNA	*cox*1	Reference
Bothriocephalidea						
Bothriocephalus sp.	*Lepomis gibbosus* (Linnaeus)	Centrarchidae	US	–	KR780814	Brabec et al. (2015)
Polyonchobothrium polypteri	*Polypterus senegalus* Cuvier	Polypteridae	SU	JQ811836	–	Kuchta et al. (2012)
Tetracampos ciliotheca	*Clarias gariepinus* (Burchell)	Clariidae	ET	–	KR780795	Brabec et al. (2015)
			ET	JQ811835	–	Kuchta et al. (2012)
			BS*	**MT52**	–	**Present study**
			GFR	**MT51**	–	**Present study**
Schyzocotyle acheilognathi	*Ptychochromis* cf. *inornatus*	Cichlidae	MD	–	MG968744	Kuchta et al. (2018)
	Saurogobio dabryi	Gobinidae	CH	–	MG968745	Kuchta et al. (2018)
	C. gariepinus	Clariidae	**RSE**	–	**MT51**	**Present study**
Caryophyllidea						
Monobothrioides chalmersius	*Clarias anguillaris* (Linnaeus)	Clariidae	SU	MW762944	–	Scholz et al. (2021)
	C. anguillaris	Clariidae	SU	MW762950	–	Scholz et al. (2021)
	C. gariepinus	Clariidae	EG	EF095253	–	Waeschenbach et al. (2007)
	C. gariepinus	Clariidae	SU	MW762954	–	Scholz et al. (2021)
	C. gariepinus	Clariidae	**ZR**	**MT53**	–	**Present study**
	Clarias sp.	Clariidae	SU	MW027475	–	Scholz et al. (2021)
			SU	MW762948	–	Scholz et al. (2021)
Monobothrioides tchadnesis	*Auchenoglanis occidentalis* (Valenciennes)	Claroteidae	CD	MW027476	–	Scholz et al. (2021)
Monobothrioides cunningtoni	*Parauchenoglanis panctatus* (Boulenger)	Claroteidae	CA	MW762938	–	Scholz et al. (2021)
Monobothrioiodes longicollis	*A. occidentalis*	Claroteidae	CA	MW762941	–	Scholz et al. (2021)
	A. occidentalis	Claroteidae	CD	MW762942	–	Scholz et al. (2021)
			CD	MW762943	–	Scholz et al. (2021)
			SU	MW762952	–	Scholz et al. (2021)
Monobothrioides zuheiri	*Auchenoglanis biscutatus* (Geoffrey Saint-Hilaire)	Claroteidae	SU	MW762945	–	Scholz et al. (2021)
	A. occidentalis	Claroteidae	SU	JQ034129	–	Brabec et al. (2012)
Wenyonia acuminata	*Synodontis acanthomias* Boulenger	Mochokidae	CD	HQ848489	–	Schaeffner et al. (2011)
			CD	HQ848519	–	Schaeffner et al. (2011)
Onchoproteocephalidea						
Acanthostotaenia shipleyi	*Varanus salvator*[a]	Varanidae	VT	–	MK328928	de Chambrier et al. (2019)
Barsonella lafoni	*Clarias* cf. *anguillaris*	Clariidae	SU	FM955142	–	de Chambrier et al. (2009b)
	C. gariepinus	Clariidae	ET	FM955143	–	de Chambrier et al. (2009b)
			ET	FM955144	–	de Chambrier et al. (2009b)
			ET	KC786015	–	Scholz et al. (2013)
			GD*	**MT54**	**MT54**	**Present study**

Table A4.3. (continued) List of Cestoda selected from the GenBank database for comparison to sequence data obtained during the present study. Species in bold obtained from material collected during the present study. Abbreviations: BR – Brazil; CA – Central African Republic; CD – Democratic Republic of the Congo; CH – China; EG – Egypt; ET – Ethiopia; MD – Madagascar; SU – Sudan; US – United States of America; VT – Vietnam.

	Host	Family	Locality	28S rDNA	*cox*1	Reference
	C. gariepinus	Clariidae	ET	FM955145	–	de Chambrier et al. (2009b)
Marsypocephalus heterobranchus	*Heterobranchus bidorsalis* Geoffrey Saint-Hilaire	Clariidae	SU	KP729408	–	de Chambrier et al. (2015)
Monticella magna	*Pimelodus fur* (Lütken)	Pomelodidae	BR	–	MW244033	Alves et al. (2021)
Proteocephalus glanduligerus	*Clarias* sp.	Clariidae	SU	KP729392	–	de Chambrier et al. (2015)
Proteocephalus sulcatus	*Clarotes laticeps* (Rüppell)	Claroteidae	SU	KP729396	–	de Chambrier et al. (2015)

[*] Abbreviations of sampling localities during present study: BS – Boskop Dam; GD – Gariep Dam; RSE – Riviersonderend River and ZR – Barotse floodplain.
[a] Reptilia.

Table A4.4. List of Nematoda selected from the GenBank database for comparison to sequence data obtained during the present study. Species in bold obtained from material collected during the present study. Abbreviations: BR – Brazil; CA – Central African Republic; CD – Democratic Republic of the Congo; CH – China; EG – Egypt; ET – Ethiopia; MD – Madagascar; SU – Sudan; US – United States of America; VT – Vietnam.

	Host	Family	Locality	18S rDNA	28S rDNA	*cox*1	Reference
Ascaridida							
Family Anisakidae							
Contracaecum sp. A [L]	*Clarias gariepinus* (Burchell)	Clariidae	**ZR**	–	–	**MT39**	**Present study**
Contracaecum sp. B [L]	*C. gariepinus*	Clariidae	**NGR**	–	–	**MT45**	**Present study**
Contracaecum sp. C [L]	*C. gariepinus*	Clariidae	**VR**	–	**MT44**	**MT44**	**Present study**
Contracaecum sp. [L]	*Xenopus laevis* (Duadin)[a]	Pipidae	SA	–	MN526256	MN526248	Schoeman et al. (2020)
Family Kathalaniidae							
Spectatus spectatus	*Piaractus mesopotamicus* (Holmberg)	Serrasalmidae	BR	KR139827	–	–	Pereira et al. (2015)
Family Quimperiidae							
Gendria sp.	*C. gariepinus*	Clariidae	**ZR**	**MT34**	**MT34**	–	**Present study**
Ichthyobronema hamulatum	*Lota lota* (L.)	Lotidae	RU	–	KY476350	–	Sokolov and Malysheva (2017)
Paraseuratum sp.	*Hoplias microlepis* (Günther)	Erythrinidae	PM	KP275686	–	–	Choudhury and Nadler (2015)
Paraquimperia africana	*Anguilla marmorata*	Anguillidae	SA	JF803925	–	–	Moravec (2007)
Paraquimperia sp.	*X. laevis*[a]	Pipidae	SA	MN526251	MN526257	–	Schoeman et al. (2020)
Enoplida							
Family Dioctophymatidae							
Dioctophyme renale	*Rattus norvegicus* (Berkenhout)*	Muridae	JA	AB595139	–	–	Tokiwa et al. (2011)
			JA	LC389871	–	–	Banzai et al. (2018)
			JA	LC389872	–	–	Banzai et al. (2018)
Eustrongylides sp.	*Channa punctata* (Bloch)	Channidae	BA	OK104110	–	–	Williams et al. (2021)
Eustrongylides sp. [L]	*C. gariepinus*	Clariidae	**ZR**	**MT27**	–	–	**Present study**
Spirurida							
Family Camallanidae							
Batrachocamallanus slomei	*X. laevis*[a]	Pipidae	SA	MN514769	MG947390	MG948463	Svitin et al. (2018, 2019)
Batrachocamallanus xenopodis	*Xenopus muelleri* (Peters)[a]	Pipidae	SA	MN514768	MN525305	MN523681	Svitin et al. (2019)
Camallanus kaapstaadi	*X. laevis*[a]	Pipidae	SA	MN514772	MG947391	MG948461	Svitin et al. (2018, 2019)
Camallanus sodwanaensis	*Pempheris adusta* Bleeker	Pempheridae	SA	MN514774	MN525306	–	Svitin et al. (2019)
Camallanus xenopodis	*X. laevis*[a]	Pipidae	SA	MN514773	MG947389	MG948462	Svitin et al. (2018, 2019)
Paracamallanus cyathopharynx	*C. gariepinus*	Clariidae	KY	MN396556	–	–	Rindoria et al. (2020)
			NGR	**MN514775**	–	**MN523683**	**Svitin et al. (2019)**
			BS	–	–	**MT46**	**Present study**
			RR	–	–	**MT47**	**Present study**
			RSE	–	–	**MT36**	**Present study**
	C. werneri	Clariidae	TA	DQ813445	–	–	Mwita and Nkwengulila (2010)
Procamallanus fulvidraconis	*Pelteobagrus fulvidraco* (Richardson)	Bagridae	CH	JF803914	–	–	Moravec (2007)
Procamallanus laeviconchus	*Synodontis schall* (Bloch et Schneider)	Mochokidae	SU	JF803934	–	–	Moravec (2007)
Procamallanus pacificus	*Anguilla obscura* Günther	Anguillidae	NC	DQ442665	–	–	Winjova et al. (2006)

Table A4.4. (continued) List of Nematoda selected from the GenBank database for comparison to sequence data obtained during the present study. Species in bold obtained from material collected during the present study. Abbreviations: BR – Brazil; CA – Central African Republic; CD – Democratic Republic of the Congo; CH – China; EG – Egypt; ET – Ethiopia; MD – Madagascar; SU – Sudan; US – United States of America; VT – Vietnam.

	Host	Family	Locality	18S rDNA	28S rDNA	*cox*1	Reference
Procamallanus (*P.*) *pseudolaeviconchus*	*C. gariepinus*	Clariidae	**NGR**	**MN514770**	**MN525307**	**MN523682**	**Svitin et al. (2019)**
			ZR	**–**	**–**	**MT49**	**Present study**
Spirocamallanus daleneae	*Synodontis zambezensis* (Peters)	Mochokidae	SA	MN514771	MN525304	**–**	Svitin et al. (2019)
Family Gnathostomidae							
Tanqua sp. [L]	*C. gariepinus*	Clariidae	**ZR**	**–**	**–**	**MT33**	**Present study**

Abbreviations of sampling localities during present study: BS – Boskop Dam; GD – Gariep Dam; RSE – Riviersonderend River and ZR – Barotse floodplain.
Non-fish hosts: [a] – Amphibia; [b] **–** Mammalia .

Appendix A5

Table A5.1. Species of the Monogenea, Digenea, Cestoda, Nematoda and Crustacea found to parasitise various organs of *Clarias gariepinus* (Burchell) during the present study. Symbols: Present (+); Absent (**–**); [L] – larval or metacercarial stages; ⁂ incidental infection; ▲ alien invasive species.

		Sol	RSE	GFR	GD	RR	SR	VR	BS	NGR	ZR	KR
Monogenea	**Oder Dactylogyridea**											
	Quadriacanthus aegypticus	G	+	+	+	+	+	+	+	+	+	+
	Quadriacanthus fornicatus	G	–	+	+	+	+	+	+	+	+	–
	Quadriacanthus clariadis	G	+	+	+	+	+	+	+	–	–	–
	Quadriacanthus allobychowskiella	G	–	+	+	+	+	+	+	–	+	–
	Quadriacanthus pravus	G	+	–	–	–	–	–	–	+	+	–
	Quadriacanthus gourenei	G	–	–	–	–	–	–	–	–	+	–
	Quadriacanthus sp. G	G	–	–	–	–	–	–	–	–	+	–
	Quadriacanthus sp. H	G	–	+	+	–	+	–	+	+	+	+
	Order Gyrodactylidea											
	Gyrodactylus gelnari	G	–	–	–	–	–	–	–	–	+	–
	Gyrodactylus rysavyi	G	–	–	–	–	–	+	–	–	–	–
	Macrogyrodactylus clarii	F, G	–	–	–	–	–	–	–	+	+	–
	Macrogyrodactylus congolensis	F, G	–	–	–	–	–	+	–	+	+	+
	Macrogyrodactylus karibae	F, G	–	–	–	–	–	–	–	+	+	–
Digenea	**Order Plagiorchiida**											
	Orientocreadium batrachoides	I	–	+	–	–	–	–	+	–	+	–
	Phyllodistomum cf. *vanderwaali*	GB	–	–	–	–	–	–	–	–	+	–
	Thaparotrema sp. A	GB	–	–	–	–	–	–	–	–	+	+
	Xiphidiata											
	Xiphidiata gen. sp. 1 [L]	E	–	–	–	–	–	–	–	–	+	–
	Order Diplostomida											
	Clinostomum brieni [L]	BrC	–	–	–	–	–	–	–	–	+	–
	Diplostomidae spp. [L]	VO	–	–	–	–	–	–	–	+	+	+
	Diplostomidae gen. sp. 1 [L]	MT	–	–	–	+	–	+	–	–	–	–
	Neodiplostomum Type 1 [L]	E, MT	–	–	–	–	–	–	–	+	+	–
	Tylodelphys mashonensis [L]	CC, BC, G	–	+	–	–	+	+	+	+	+	+
	Tylodelphys cf. *grandis*[L]	CC, BC, G	–	–	–	–	–	–	–	–	+	+
Cestoda	**Order Bothriocephalidea**											
	Tetracampos ciliotheca	I	+	–	+	+	+	–	+	+	+	+
	Schyzocotyle acheilognathi▲	I	+	–	–	–	–	–	–	–	–	–
	Order Caryophyllidea											
	Monobothrioides chalmersius	I	–	–	–	–	–	–	–	–	+	–
	Order Onchoproteocephalidea											
	Barsonella lafoni	I	–	–	+	+	+	–	+	+	+	+
	Proteocephalus glanduligerus	I	–	–	–	–	–	–	–	–	+	–

Table A5.1. (continued) Species of the Monogenea, Digenea, Cestoda, Nematoda and Crustacea found to parasitise various organs of *Clarias gariepinus* (Burchell) during the present study. Symbols: Present (+); Absent (**–**); [L] – larval or metacercarial stages; ⁂ incidental infection; ▲ alien invasive species.

	Sol		RSE	GFR	GD	RR	SR	VR	BS	NGR	ZR	KR
	Contracaecum sp. A [L]	BC, K, MS	–	–	–	–	–	–	–	–	+	+
	Contracaecum sp. B [L]	BC, MS	–	–	–	–	–	–	–	+	–	–
	Contracaecum sp. C [L]	BC, K, L, MT	–	+	–	+	+	+	+	–	–	–
	Gendria sp.⁂	I	–	–	–	–	–	–	–	–	+	+
Nematoda	**Order Enoplida**											
	Eustrongylides sp. ⁂	MT	–	–	–	–	–	–	–	–	+	–
	Order Spirurida											
	Paracamallanus cyathopharynx	I, S	–	–	–	–	–	–	–	+	+	+
	Paracamallanus sp. A	I, S	+	–	–	+	+	+	+	–	–	–
	Procamallanus (*P.*) *pseudolaeviconchus*	I, S	–	–	–	–	–	–	+	+	–	–
	Procamallanus sp. A	I	–	–	–	–	–	–	–	–	+	–
	Spinitectus sp.	S	–	–	–	–	–	+	–	–	–	–
	Tanqua sp. [L] ⁂	MS	–	–	–	–	–	–	–	–	+	+
	Order Arguloida											
Crustacea	*Argulus* cf. *cunningtoni*	G	–	–	–	–	–	–	–	–	–	+
	Argulus japonicus▲	G, F	–	–	+	–	–	–	–	–	–	–
	Order Cyclopoida											
	Ergasilus cf. *mirabilis*	G, F	–	–	–	–	–	+	+	+	+	–
	Ergasilus sp. A	G	+	+	–	–	–	–	–	–	–	–
	Lamproglena clariae	G	–	–	–	–	–	+	+	+	–	+

Localities: BS – Boskop Dam, GD – Gariep Dam, NGR – Ndumo Game Reserve, RR – Riet River, SR – Sand River (South Africa) and KR – Kabompo River, ZR – Barotse floodplain (Zambia).

Site of infection: BC – Body cavity; BrC – Branchial chamber; CC – Cranial cavity; E – Eyes; F – Fins; G – Gills; GB – Gallbladder; I – Intestine; K – Kidneys; L – Liver; MS – Mesenteries; MT – Muscle tissue; VO – various organs.

Appendix A6

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Novel information on the morphology, phylogeny and distribution of camallanid nematodes from marine and freshwater hosts in South Africa, including the description of *Camallanus sodwanaensis* n. sp.

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ABSTRACT

Four species of previously known nematodes from the family Camallanidae were found from different hosts in South Africa: *Batrachocamallanus xenopodis* from the frog *Xenopus muelleri*, *Paracamallanus cyathopharynx* and *Procamallanus pseudolaeviconchus* from the catfish *Clarias gariepinus* and *Spirocamallanus daleneae* from the catfish *Synodontis zambezensis*. In the material collected from various marine fishes, several specimens of nematodes from the genus *Camallanus* clearly differed from all previously known species. Based on morphological differences these specimens are assigned to a new species, *C. sodwanaensis*. Molecular data of 18S and 28S rDNA and COI sequences are provided for the collected species and a phylogenetic analyses based on 28S gene fragmets are presented.

1. Introduction

The Camallanidae is a globally distributed group of parasitic nematodes that primarily infects the digestive tract of marine and freshwater fish and less often amphibians, turtles and snakes (Stromberg and Crites, 1974; Rigby and Rigby, 2014). These nematodes can be morphologicaly distinguished from all other groups by the presence of a well-developed buccal capsule often supported by different structures (basal ring, longitudinal or spiral ridges, tridents, etc.) (Rigby and Rigby, 2014).

Hitherto, the camallanid fauna of African vertebrates is poorly studied. Although numerous genera from the subfamilies Procamallaninae and Camallaninae were erected, most of them consist of only a few species. Of the Procamallaninae, three species of the genus *Procamallanus* Baylis, 1923 were described from freshwater fishes: *P. laeviconchus* Wedl, 1861, *P. armatus* Campana-Rouget et Therezien, 1965 and *P. pseudolaeviconchus* Moravec et Van As, 2015a. Seven species of the genus *Spirocamallanus* Olsen, 1952 were described from African freshwater fishes: *S. daleneae* Boomker, 1993, *S. mazabukae* Yeh, 1957, *S. spiralis* (Baylis, 1923), *S. olseni* Campana-Rouget et Razarihelissoa, 1965, *S. serranochromis* Moravec et Van As, 2015b, *S. parachannae* Moravec et Jirků, 2015 and *S. pseudospiralis* Moravec et Scholtz, 2017. Jackson and Tinsley (1995) established a new genus *Batrachocamallanus* Jackson et Tinsley, 1995 to include two species from pipid frogs, *Batrachocamallanus slomei* Southwell et Kirschner, 1937 (described as *P. slomei*) and *B. xenopodis* Jackson et Tinsley, 1995 (described as *S. xenopodis*), and also described two new species *B. occidentalis* Jackson et Tinsley, 1995 and *B. siluranae* Jackson et Tinsley, 1995. Of these, *B. slomei* and *B. xenopodis* were subsequently found from *Xenopus* spp. in different regions of Africa (Jackson and Tinsley, 1995; Svitin et al., 2018).

Four genera of the Camallaninae were described and subsequently found in aquatic vertebrates from Africa. Representitives of three genera, namely *Paracamallanus* Yorke et Maplestone, 1926, *Zeylanema* Yeh, 1960 and *Neocamallanus* Ali, 1957 were found in freshwater fishes, each represented by a single species: *P. cyathopharynx* (Baylis, 1923), *Z. ctenopomae* (Vassiliadès et Petter, 1972) (described as *Camallanus ctenopomae*) and *N. polypteri* (Kabre et Petter, 1997) (described as

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Camallanus polypteri). The genus *Camallanus* Railliet et Henry, 1915 includes two species found in freshwater fishes: *C. longicaudatus* Moravec (1973) and *C. kirandensis* Baylis (1928); four species found in frogs: *C. kaapstaadi* Southwell et Kirshner, 1937, *C. dimitrovi* Durette-Desset et Batcharov, 1974, *C. xenopodis* Jackson et Tinsley, 1995 and *C. macrocephalus* Jackson et Tinsley, 1995; and one species found in a freshwater turtle, *C. chelonius* Baker, 1983. It should be noted that all previously described species in South Africa were reported from freshwater hosts while nematode parasites of marine organisms are still poorly studied and no camallanin has been reported from this host group (Smit and Hadfield, 2015).

Details of the morphology of the buccal capsule were traditionally used for generic differentiation within the family. Nevertheless, reliability of some characters and, as a result, number of genera within the Camallanidae, are still debated. Moravec and co-authors (1988, 2006, 2015a, 2015b) considered five taxa of the Procamallaninae (*Procamallanus*; *Spirocamallanus*; *Platicamallanus* Bilqees et Akram, 1982; *Punctocamallanus* Moravec et Scholz, 1991 and *Denticamallanus* Moravec et Thatcher, 1997) as subgenera of *Procamallanus*. Jackson and Tinsley (1995) followed the opinion of Moravec and colleagues in considering differences of the buccal capsule structure alone as not sufficient for the generic differentiation. At the same time these authors distinguished *Batrachocamallanus* mostly based on the presence of the large number of mucrons (more than five) on the female tail, relatively smaller body size and specificity to the amphibian hosts. Later on Moravec et al. (2006) considered the latter proposed differences as not reliable generic characters and advocated for the reduction of *Batrachocamallanus* to a junior synonym of *Procamallanus*. Rigby and Rigby (2014) supported the synonymy of *Batrachocamallanus*, although recognizing the genera that Moravec et al. (2006) considered as subgenera. Within the subfamily Camallaninae, Rigby and Rigby (2014) recognized three valid genera: *Camallanus* with *Zeylanema* and *Serpinema* Yeh, 1960 as junior synonyms, *Neocamallanus* Ali, 1957 with junior synonym *Neozeylanema* Sinha et Sahay, 1966 and *Oncophora* Diesing, 1851 with *Paracamallanus* as a synonym. At the same time, Moravec and colleagues (2015c, 2017) considered *Zeylanema* as subgenus of *Camallanus* and *Paracamallanus* as a valid genus separate from *Oncophora*. In numerous works on camallanid nematodes different authors considered different characters (details of buccal structure, female tail morphology, male genital system, etc.) as generic, subgeneric or species differentiators. As a result, based on different opinions, the number of genera within the family Camallanidae varies from two to twelve (Moravec and Thatcher, 1997; Moravec and Sey, 1988; Moravec and Van As, 2015a; Moravec and Van As, 2015b; Moravec and Jirků, 2017; Rigby and Adamson, 1998; Anderson et al., 2009; Rigby and Rigby, 2014). Therefore, it is clear that additional and detailed studies, including molecular analyses, are necessary to revise the status of the different taxa within the Camallanidae.

Several molecular studies which included camallanid nematodes were published recently. Černotíková et al. (2011) studied the phylogenetic relationships of spirurine nematodes including members of the families Philometridae, Dracunculidae, Cysticolidae, Quimperidae, Rhabdochonidae, Cucullanidae and Camallanidae based on 18S rDNA gene data. In the tree provided by the authors the subclades within the clade represented by members of the Camallanidae received overall low support with *C. carangis* Olsen, 1954 appearing in the Procamallaninae subclade and *P. rarus* Travassos et Artigas 1928 at the basal position to the subclades consisted of *Procamallanus* spp. and *Camallanus* spp., albeit without support. Later, Sardella et al. (2017) redescribed *S. macaensis* Vicente et Santos, 1972 and included this species in the phylogenetic analyses of the Camallanidae based on 18S rDNA gene data. Similarly, in studies of Černotíková et al. (2011), *Procamallanus*, *Spirocamallanus* and *Camallanus* formed weakly supported clades. Recently, Chaudhary et al. (2017) provided a phylogenetic tree based on the 18S rDNA gene with overall weakly supported clades and the members of the Procamallaninae and Camallaninae simultaneously appeared in different clades.

Three publications dealt with genes other than 18S rRNA. Wu et al. (2008) showed the variability between two species, namely *C. cotti* Fujita, 1927 and *C. hypophthalmichthys* Dogel and Akhmerov, 1959 from fish in China using sequences of the internal transcribed spacer (ITS) regions of rDNA, ITS1 and ITS2. Kuzmin et al. (2011) showed the phylogenetic relationships of five species of *Camallanus* from Australian turtles based on the partial 28S rDNA alignments. Svitin et al. (2018) showed the phylogenetic relationships of two *Camallanus* species from African frogs with two species from Chinese fish based on the mitochondrial cytochrome *c* oxidase 1 (COI) gene dataset and five species from Australian turtles based on 28S rDNA dataset.

To date, phylogenetic studies based on the 18S rRNA gene contained numerous controversies and studies based on other genetic markers included very few species, therefore questions on the evolutionary relationships amongst the Camallanidae and the status of different taxa within the family are still not resolved.

During parasitological surveys in the KwaZulu-Natal Province of South Africa several species of camallanid nematodes were found: *B. xenopodis* in the frog *Xenopus muelleri* (Peters, 1844); *Pa. cyathopharynx* and *P. pseodolaeviconchus* from catfish *Clarias gariepinus* Burchell, 1822; *S. daleneae* from catfish *Synodontis zambezensis* (Peters, 1852); and specimens of *Camallanus* clearly different from previously known species from five species of marine fishes (*Pempheris adusta* Bleeker, 1877, *Cirrhitus pinnulatus* (Förster, 1801), *Pomadasys furcatus* (Bloch et Schneider, 1801), *Terapon jarbua* (Forsskal, 1775) and *Trachinotus botla* (Shaw, 1803)). In present study we follow Anderson et al. (2009) for generic identification of the species, with some modifications (see below). Detailed descriptions and molecular characterisation based on three genes (18S and 28S rDNA and COI) of found species followed by molecular analyses based on 28S rRNA gene are presented.

2. Materials and methods

Material was collected from different localities in KwaZulu-Natal Province in South Africa during August, October and November 2017, and August 2018. In total, three frogs, *X. muelleri*, 15 African sharptooth catfish, *Clarias gariepinus*, 25 Brown squeakers, *Synodontis zambezensis*, 22 Dusky sweepers, *Pempheris adusta,* four Stocky hawkfish, four *Cirrhitus pinnulatus*, four Banded grunters, *Pomadasys furcatum*, four Largespotted darts, *Trachinotus botla* and three Jarbua terapons, *Terapon jarbua* were examined for the presence of parasites.

Amphibian hosts were anaesthetised in 6% ethyl-3-aminobenzoate methanesulfonate (MS222) (Sigma-Aldrich Co., St. Louis, Missouri, USA) and subsequently euthanised through severing the spine and destroying the brain according to internationally accepted standard operating procedures (ethics number: NWU-00492-16-S5). Fish hosts were euthanised by cranial pithing and spinal severance (ethics number: NWU-00159-18-S5).

During the total dissection, the digestive tract was removed and placed in 9% saline. Nematodes were gently removed, washed in saline and fixed in hot 70% ethanol and subsequently stored in 70% ethanol. Prior to microscopical examination, nematodes were placed in distilled water for about 20 min and then cleared in lactophenol. Apical sections were prepared manually using a thin razor and examined *en face* on temporary mounts. Morphology of the nematodes was studied using Nikon E800 and Nikon ECLIPSE *Ni* compound microscopes equipped with DIC optics.

In total, 77 nematodes were studied of which 46 were measured. All measurements in the text are given in micrometres unless otherwise indicated. Measurements are presented as ranges followed by mean values in parentheses and measurements of type specimens are in square brackets (if applicable).

For molecular analysis, the middle fragments of the nematodes were used while anterior and posterior parts were preserved for microscopic identification. DNA was extracted using PCRBIO Rapid Extract PCR Kit

following the standard protocol method recommended by the manufacturer. Polymerase chain reaction for COI was performed using the primer pair LCO1490 (5′-GGT CAA CAA ATC ATA AAG ATA TTG G-3′) and HCO2198 (5′-TAA ACT TCA GGG TGA CCA AAA AAT CA-3′). The thermocycling profile was as follows: 3 min denaturation at 94 °C, 10 cycles of 94 °C for 30 s, 45 °C for 30 s, 72 °C for 60 s and 40 cycles at 94 °C for 30 s, 51 °C for 60 s, 72 °C for 60 s for amplification, 72 °C for 10 min for extension (Folmer et al., 1994; Svitin et al., 2018). The 18S rRNA sequence fragments were amplified using the primer pair F18ScF1 (5′-ACC GCC CTA GTT CTG ACC GTA AA-3′) and F18ScR1 (5′-GGT TCA AGC CAC TGC GAT TAA AGC-3′). The thermocycling profile was as follows: 2 min denaturation at 95 °C for 30 s, 40 cycles of 95 °C for 30 s, 58 °C for 30 s and 72 °C for 90 s for amplification, 72 °C for 10 min for extinction (Lefoulon et al., 2015). The partial fragments of the 28S rRNA gene were amplified using a pair of newly designed primers: CTEf (5′-AGT GAA TGG GGA AAA GCC CA-3′) and CTEr (5′-GGA CCT CCA CCA GAG TTT CC-3′). The thermocycling profile was as follows: 3 min denaturation at 95 °C; 40 cycles of 30 s at 95 °C, 30 s at 54 °C, 2 min at 72 °C for amplification; 7 min for extension at 72 °C. Unpurified PCR products were sent to a commercial sequencing company (Inqaba Biotechnical Industries (Pty) Ltd, Pretoria, South Africa). DNA products were sequenced in both directions using the PCR primer pairs. Resulting sequences were assembled and chromatogram-based contigs were generated and trimmed using Geneious (V. 9.0) software and submitted to GenBank under the following accession numbers: COI [MN523681 – MN523683], 28S [MN525304 – MN525307], 18S [MN514768 – MN514775].

Novel partial 18S and 28S rDNA sequences and COI sequences obtained during this study were aligned with the sequences for the Camallanidae downloaded from GenBank using MUSCLE v3.7 implemented in Geneious ver. 9.1. Two alignments for the partial 28S rRNA gene were constructed. Alignment 1 was based on the longest sequences (867 nucleotide (nt)). Alignment 2 was much shorter (491 nt) in order to include the short sequences of five species of *Camallanus* spp. and a species of *Serpinema* published by Kuzmin et al. (2009) and Kuzmin et al. (2011). The final length of the alignment for 18S rDNA was 717 nt and the alignment for COI was 428 nt. The outgroup for each alignment was estimated using the basic local alignment searching tool (BLAST). The best-fitting model for each dataset was estimated prior to analyses using jModelTest (V. 2.1.2) (Guindon and Gascuel, 2003; Darriba et al., 2012). This was GTR + G for both the 28S rDNA, as well as for the 18S and COI datasets. Bayesian inference analyses were run using MrBayes (V. 3.2.2) software with the following nucleotide substitution model settings: lset nst = 6, rates = invgamma, ncat = 4, shape = estimate, inferrates = yes and basefreq = empirical. Further analyses were performed using the following parameters: mcmc ngen = 3 000 000 for 28S and COI fragments and 10 000 000 for 18S, samplefreq = 100, printfreq = 100 and diagnfreq = 1000. The maximum likelihood analyses were performed using PhyML version 3.0 (Guindon et al., 2010) run on the ATGC bioinformatics platform [http://www.atgc-montpellier.fr/ngs]. Nodal support in the maximum likelihood analyses was estimated from 100 bootstrap pseudoreplicates. Trees were visualised using the FigTree (V. 1.4.3) software (Rambaut, 2012). The p-distance and the number of difference matrix in Mega (V. 7.0) (Kumar et al., 2015) software were used for the pairwise analyses.

3. Results

In total, five species of camallanid nematodes were recovered: *Batrachocamallanus xenopodis* from Müller's platanna *Xenopus muelleri*; *Paracamallanus cyathopharynx* and *Procamallanus pseudolaeviconchus* from African sharptooth catfish *Clarias gariepinus*; *Spirocamallanus daleneae* from Brown squeaker *Synodontis zambezensis*; and *Camallanus sodwanaensis* n. sp. from five species of marine fish (*Pempheris adusta*, *Cirrhitus pinnulatus*, *Pomadasys furcatus*, *Terapon jarbua* and *Trachinotus botla*).

3.1. Species descriptions

Family Camallanidae Railliet et Henry, 1915.

Genus *Camallanus* Railliet et Henry, 1915.

Camallanus sodwanaensis n. sp.

Type host: Dusky sweeper *Pempheris adusta* Bleeker, 1877 (Perciformes: Pempheridae).

Other hosts: Stocky hawkfish *Cirrhitus pinnulatus* (Förster, 1801) (Perciformes: Cirrhitidae), Banded grunter *Pomadasys furcatus* (Bloch et Schneider, 1801) (Perciformes: Haemulidae), Jarbua terapon *Terapon jarbua* (Forsskål, 1775) (Perciformes: Terapontidae), and Largespotted dart *Trachinotus botla* (Shaw, 1803) (Perciformes: Carangidae).

Site of infection: Intestine.

Type locality: Sodwana Bay, KwaZulu-Natal Province, South Africa (32°40′46"E; 27°32′24"S).

Type material: Holotype (male, [**NMB P**509]), allotype (female, [**NMB P**510]), paratypes [**NMB P**511] deposited in the National Museum Parasite Collection (Bloemfontein, South Africa).

Intensity: *Pempheris adusta*: 1–15 (5.7); *Cirrhitus pinnulatus*: 1–5 (2.3); *Terapon jarbua*: 1–2 (1.5); *Pomadasys furcatum*: 1–1 (1); *Trachinotus botla*: 1; total: 1–15 (3.0).

Prevalence: *Pempheris adusta* – 14% (six of 22 specimens were infected); *Cirrhitus pinnulatus* – 75% (three of four specimens were infected); *Terapon jarbua* – 67%; *Pomadasys furcatum* – 50% (two of four specimens were infected); *Trachinotus botla* – 25% (one of four specimens were infected); total – 27%.

Abundance: *Pempheris adusta* – 0.8; *Cirrhitus pinnulatus* – 1.8; *Terapon jarbua* – 1; *Pomadasys furcatum* – 0.5; *Trachinotus botla* – 0.3; total – 0.8.

Representative DNA sequences: 28S [MN525306], 18S [MN514774].

ZooBank registration: To comply with the regulations set out in article 8.5 of the amended 2012 version of the International Code of Zoological Nomenclature (ICZN, 2012), details of the new species have been submitted to ZooBank. The Life Science Identifier (LSID) for *Camallanus sodwanaensis* n. sp. is urn:lsid:zoobank.org:act:BCEC589F-46B3-4645-9EC1-F6FA36B11213

Etymology: The species is named after its type locality.

Description (Figs. 1 and 2).

General. Body thin, elongated with maximum width at mid-length. Females generally larger than males. Cuticle with conspicuous transverse and fine longitudinal striations. Apical: oral opening narrow slit-like, surrounded by four conspicuous cephalic papillae (Fig. 1C; 2F). Four sclerotised plates situated on external surface of buccal capsule valves near their anterior margin. Buccal capsule with well developed valves supported by numerous ridges (Fig. 1A,B,C; 2C,D,F,G). Thick sclerotised basal ring present at base of buccal capsule. Oesophageal cup well developed. Two prominent tridents situated on ventral and dorsal sides of buccal capsule valves. Dorsal and ventral tridents equal in size and shape, each consisted of three posteriorly directed prongs. Central prong somewhat longer than sublateral ones, often reaching anterior margin of nerve ring (Fig. 1A,B,E; 2B,D,E). Muscular and glandular oesophagus almost cylindrical, slightly widening in posterior third. Nerve ring encircling oesophagus close to its anterior end. Excretory pore situated at level of nerve ring or slightly posterior to it (Fig. 1A; 2B). Deirids not observed. Intestine and rectum straight, narrow. Tail tapering with prominent phasmids situated at level of its anterior third.

Males. Measurements based on three specimens. Body 4.7–8.5 (6.6) [8.5] mm long, 112–173 (152) [173] maximum wide (Fig. 2A). Buccal capsule valves 91–101 (95) [91] long, 84–95 (92) [87] maximum wide, supported by 23–25 (25) [23] ridges, of which 9–9 (9) [9] incomplete. Basal ring 16–27 (22) [22] long, 61–69 (66) [69] wide. Oesophageal cup 13–27 (19) [16] long, 25–32 (30) [32] wide. Dorsal trident 134–160 (147) [160] long, 20–25 (23) [23] wide in lateral projection, ventral trident 131–169 (148) [160] long, 24–25 (25) [25] wide.

Muscular oesophagus 655–880 (794) [880] long, 10–14 (12) [10]% of body length; 62–79 (71) [62], 82–98 (90) [98] and 100–113 (107)

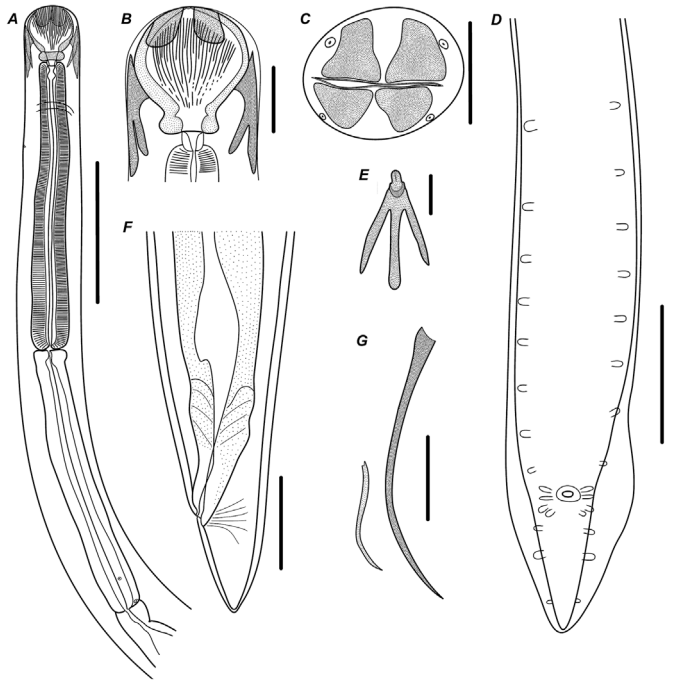

Fig. 1. *Camallanus sodwanaensis* n. sp., line-drawings. A – anterior part of body, female, lateral view; B – buccal capsule, female, lateral view; C – anterior part of body, female, apical view; D – posterior part of body, male, ventral view; E – dorsal trident, male, lateral view; F – posterior part of body, female, lateral view; G – spicules, lateral view. Scale bars: A – 500; B–D, F–G – 100; E – 50.

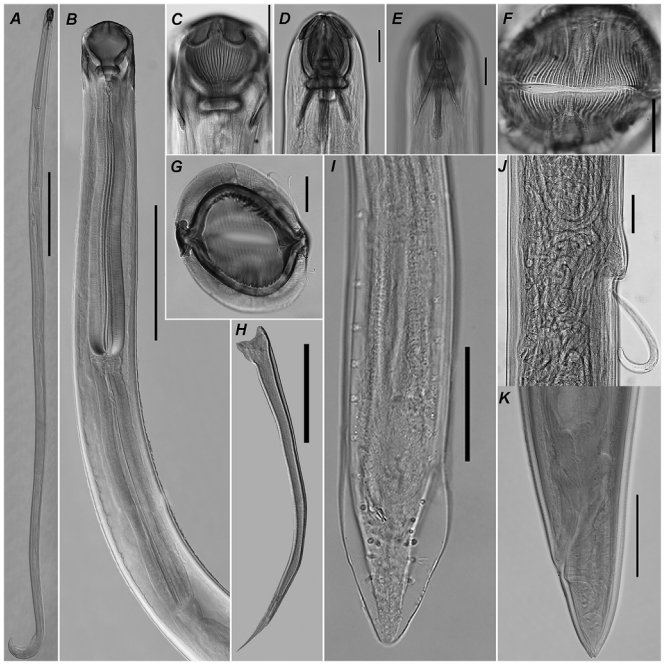

Fig. 2. *Camallanus sodwanaensis* n. sp., photomicrographs. A – male, general view; B – anterior part of body, female, lateral view; C – buccal capsule, female, lateral view; D – optical section at level of buccal capsule valves mid-width, male, dorsal view; E – dorsal trident, male, dorsal view; F – anterior part of body, female, apical view; G - optical section at level of buccal capsule valves mid-length, male, apical view; H – right spicule, lateral view; I – posterior end of body, male, ventral view; J – part of body at vulva region, lateral view; K – posterior end of body, female, lateral view. Scale bars: A – 1 mm, B – 500, C–K – 100.

[113] wide at anterior, mid-length and posterior levels, respectively. Glandular oesophagus 562–940 (767) [940] long, 11–12 (12) [12]% of body length; 67–89 (78) [89], 77–88 (83) [88] and 82–110 (96) [110] wide at anterior, mid-length and posterior levels, respectively. Nerve ring at 205–211 (207) [211], 24–31 (27) [24]% of muscular oesophagus length. Excretory pore at 210–242 (218) [242] from anterior end of body, 3–4 (4) [3]% of body length.

Caudal alae narrow, supported by papillae: eight pairs of pedunculated precloacal; two pairs of adcloacal (anterior and posterior to cloaca), five pairs of postcloacal (two pairs grouped slightly posterior to cloaca, one pair at level of tail mid-length and one close to tail end) (Fig. 1D; 2I). Spicules unequal, simple-shaped with sharpened tips (Fig. 1G; 2H). Right spicule prominent, 303–328 (313) [303] long, 4–7 (5) [4]% of body length; left one less sclerotised, poorly visible 162–205 (184) [205] long, 2–3 (3) [2]% of body length. Tail conical, tapering to rounded tip, 86–101 (94) [94] long, 1–2 (2) [2]% of body length.

Females. Measurements based on nine gravid (larvigerous) specimens. Body 6.0–11.8 (9.0) [10.9] mm long, 130–283 (222) [316] wide. Buccal capsule valves 106–173 (141) [115] long, 102–178 (141) [111] wide, supported by 19–33 (28) [28] ridges, of which 12–20 (16) [14] incomplete. Basal ring 20–30 (25) [22] long, 64–99 (83) [68] wide. Oesophageal cup 14–26 (21) [23] long, 30–40 (35) [35] wide. Dorsal trident 136–205 (171) [152] long, 20–36 (28) [20] wide in lateral projection, ventral one 134–206 (173) [151] long, 17–34 (28) [21] wide.

Muscular oesophagus 800–1173 (1053) [1087] long, 10–14 (12) [10]% of body length; 75–98 (88) [68], 89–124 (108) [112] and 104–154 (132) [113] wide at anterior, mid-length and posterior level, respectively. Glandular oesophagus 758–1152 (938) [1141] long, 9–14 (11) [10]% of body length; 86–126 (106) [63], 78–134 (111) [81] and 100–140 (123) [103] wide at anterior, mid-length and posterior level, respectively. Nerve ring at 202–287 (252) [246] from anterior end of body, 17–29 (24) [23]% of muscular oesophagus length. Excretory pore at 210–308 (263) [275], 2–4 (3) [3]% of body length. Viviparous. Vulva with distinct lips (Fig. 2J), opening posterior to small projection to body wall at 3.5–5.7 (4.8) [5.1] mm from anterior end of body, 46–58 (53) [46]% of body length. Tail 87–140 (104) [111] long, 1–2 (1) [1]% of body length. Tail tip rounded in mature females and bearing two small mucrons in immature ones (Fig. 1F; 2K).

Remarks. The species belongs to the genus *Camallanus* based on the presence of a well developed buccal capsule consisting of two valves, each supported by longitudinal ridges (not divided in dorsal and ventral group with a gap between), and presence of tridents on the dorsal and ventral sides of the buccal capsule valves (Anderson et al., 2009). *Camallanus sodwanaensis* n. sp. is the first species of the genus found in marine fish from Southern Africa. Only two species of *Camallanus* described from African freshwater fishes are still considered as valid and were subsequently found after the first description: *C. longicaudatus* and *C. kirandensis*. The new species can be distinguished from *C. longicaudatus* by the relatively smaller length of the female tail (1–2% of body length *vs* 12–14%) and number of postcloacal papillae in males (6 in *C. longicaudatus vs* 5 in *C. sodwanaensis* n. sp.) (Moravec, 1973). By the same characters, *C. sodwanaensis* n. sp. can be easily distinguised from *C. kirandensis* that has a comparatively long tail (868–1400 long in 8.4–20.0 mm long females, comprising approximately 7%). and only three pairs of postcloacal papillae (Baylis, 1928; Amin, 1978). Out of the species described from marine fish, *C. sodwanaensis* n. sp. is morphologically (size and shape of buccal capsule and tridents, morphology male spicules, general body measurements) and geographically the most closely related to *C. carangis*. The most reliable character to distinguish between the two species is the number of postcloacal papillae – five pairs in *C. sodwanaensis* n. sp. and six pairs in *C. carangis* (Rigby et al., 1998).

Genus ***Paracamallanus*** Yorke et Maplestone, 1926.

Paracamallanus cyathopharynx (Baylis, 1923).

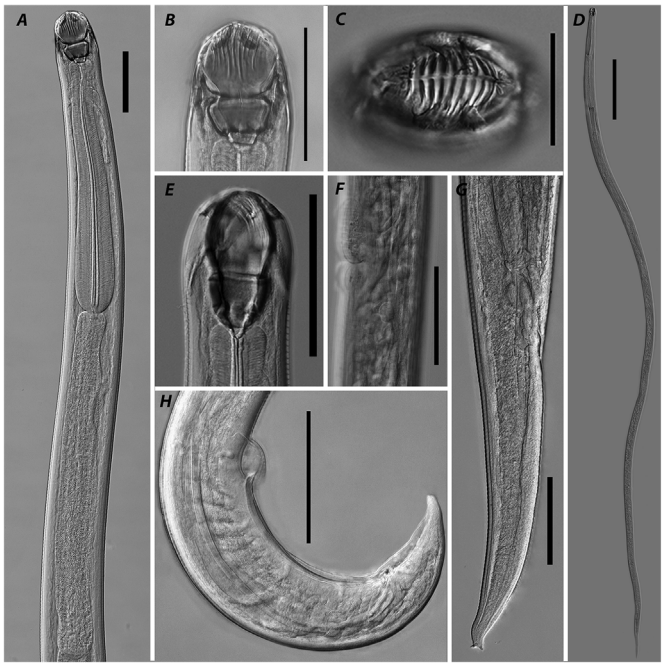

Fig. 3. *Paracamallanus cyathopgharynx*, photomicrographs. A – anterior part of body, male, lateral view; B – buccal capsule, male, lateral view; C – anterior part of body, male, apical view; D – female, general view; E - optical section at level of buccal capsule valves mid-length, male, dorsal view; F - part of body at vulva region, lateral view; G – posterior end of body, female, lateral view; H – posterior end of body, male, lateral view. Scale bars: A–C, E–H – 100; D – 1 mm.

Host: African sharptooth catfish *Clarias gariepinus* (Burchell, 1822).

Locality: Ndumo Game Reserve, KwaZulu-Natal Province, South Africa (32°30′69"E; 26°85′63"S).

Site of infection: Intestine.

Intensity: 1–8 (3.3).

Prevalence: 46% (seven of 15 infected).

Abundance: 1.5.

Representative DNA sequences: 18S [MN514775], COI [MN523683].

Description (Fig. 3).

General. Medium-sized nematode, body thin with maximum width at mid-length. Cuticle with conspicuous transverse striations along entire body. Apical: oral opening slit-like, surrounded by four cephalic plates, four conspicuous outer cephalic papillae, 4 min inner cephalic papillae and two amphids (Fig. 3C). Buccal capsule well sclerotised, divided in anterior and posterior parts. Anterior part consisting of two valves, each supported by nine longitudinal ridges and two tridents on dorsal and ventral sides (Fig. 3B,E). Each trident consisted of three posteriorly directed prongs of which central one somewhat longer than sublateral. Dorsal and ventral tridents equal in size and shape, beginning at level of buccal capsule anterior quarter and ending at level of oesophageal cup. Posterior part of buccal capsule shorter and narrower than anterior one with thick well-sclerotised walls. Oesophageal cup shorter than wide, poorly sclerotised. Muscular oesophagus evenly widened from anterior to posterior part. Glandular oesophagus almost cylindrical, slightly widened in middle third. Nerve ring encircling muscular oesophagus at level of its anterior third. Excretory pore opening somewhat posterior to level of nerve ring (Fig. 3A). Intestine and rectum straight, narrow. Tail tapering.

Males. Measurements based on nine specimens. Body 1.6–6.8 (5.4) mm long, 46–124 (102) wide. Anterior part of buccal capsule 51–61 (58) long, 53–58 (55) wide. Posterior part of buccal capsule 35–41 (38) long, 38–53 (46) wide. Oesophageal cup 4–8 (6) long, 8–18 (13) wide. Dorsal trident 65–76 (71) long, 9–12 (11) wide in lateral projection, ventral one 65–77 (71) long, 9–13 (11) wide. Muscular oesophagus 204–469 (384) long, 5.8–12.8 (7.5)% of body length; 31–47 (41), 30–62 (47) and 37–73 (57) wide at anterior, mid-length and posterior level, respectively. Glandular oesophagus 210–670 (531) long, 7.8–13.2 (10.2)% of body length; 33–63 (52), 44–72 (56) and 44–73 (54) wide at anterior, mid-length and posterior level, respectively. Nerve ring at 134–165 (147) from anterior end, 32.0–65.7 (40.0)% of muscular oesophagus. Excretory pore at 149–251 (196) from anterior end, 2.6–4.9 (3.4)% of body length.

Posterior end coiled ventrally. Caudal alae narrow, supported by papillae: five pairs of precloacal pedunculated papillae, two pairs of adcloacal papillae (anterior and posterior to cloaca), six pairs of postcloacal papillae (three pairs grouped posterior to cloaca, two pairs at mid-length of tail, one pair close to tail end). Spicules unequal. Right one longer, well-sclerotised, 177–271 (223) bearing short process on its tip, 27–69 (43) long. Left spicule shorter, less sclerotised, simple-shaped with sharpened tip, 37–70 (52) long. Tail tapering with rounded tip, 59–71 (66) long (Fig. 3H).

Females. Measurements based on seven gravid specimens. Body 9.5–15.8 (11.7) mm long, 130–204 (167) wide (Fig. 3D). Anterior part of buccal capsule 71–84 (76) long, 67–84 (75) wide. Posterior part of buccal capsule 48–56 (53) long, 63–70 (66) wide. Oesophageal cup 7–10 (9) long, 12–22 (17) wide. Dorsal trident 80–112 (93) long, 11–15 (13) wide in lateral projection, ventral one 78–112 (92) long, 11–16 (14) wide. Muscular oesophagus 535–681 (581) long, 3.5–5.8 (5.1)% of body length; 48–66 (58), 54–74 (68) and 70–110 (87) wide at anterior, mid-length and posterior level, respectively. Glandular oesophagus 680–955 (793) long, 6.0–7.4 (6.8)% of body length; 61–91 (71), 70–92 (80) and 76–119 (92) wide at anterior, mid-length and posterior level, respectively. Nerve ring at 173–213 (192) from anterior end, 28.8–38.4 (33.2)% of muscular oesophagus. Excretory pore at 227–420 (275) from anterior end, 1.6–4.2 (2.4)% of body length.

Vulva with slightly elevated lips at 3.7–8.5 (6.1) mm from anterior end, 37.1–56.3 (52.1)% of body length (Fig. 3F). Tail tapering, 291–507 (362) long, bearing three small mucrons on tip (Fig. 3G).

Remarks. The species has been found in many localities throughout Africa from clariid catfishes Mwita (2011); Madanire-Moyo and Barson (2010); Ajala and Fawole (2014); Moravec and Van As (2015c); Moravec and Jirků (2017) and was reported once from Israel (Paperna, 1964). Nevertheless, the morphology of the species was illuminated only in the latest redescription provided by Moravec and Van As (2015c). In the redescription the authors described an unusual shape of the right spicule consisting of two parts: thin elongated anterior and short well sclerotised posterior that has often been confused with the gubernaculum or the left spicule. At the same time, the left spicule was described as poorly sclerotised and needle-like. In present study, we also found a clearly visible right spicule consisting of two parts and poorly sclerotised (visible only on high magnification with DIC and when dissected) left one, both with slightly wider ranges of measurement values. Also, similar to that in the latest redescription, we found eight cephalic papillae on the anterior end of nematodes. Despite that papillae of inner circle are minute and often covered with host tissue, they were clearly observed under the light microscope using high magnification and DIC.

Rigby and Rigby (2014) proposed *Paracamallanus* as a junior synonym of the genus *Oncophora* based on the similarities in their buccal capsule morphology. These authors suggested that the only difference between genera is the greater width of the female posterior to the vulva in *Oncophora* and assumed it as not indicative of different genera. In our opinion, significance of the characters for generic differentiation should be confirmed with sufficient molecular analyses. Therefore, in the present study we prefer to assign found species to genus *Paracamallanus* following Moravec and Van As (2015c) and Moravec and Scholtz (2017).

Genus ***Procamallanus*** Baylis, 1923.

Procamallanus pseudolaeviconchus Moravec et Van As, 2015.

Host: African sharptooth catfish *Clarias gariepinus* (Burchell, 1822).

Locality: Ndumo Game Reserve, KwaZulu-Natal Province, South

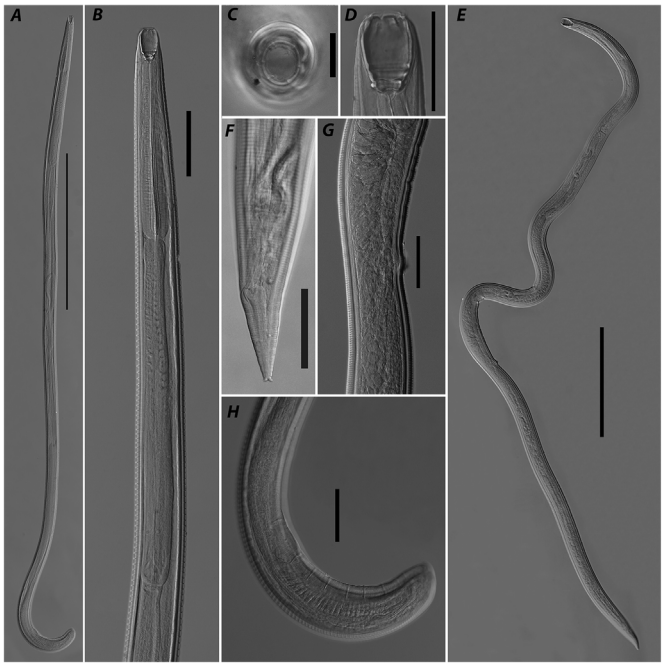

Fig. 4. *Procamallanus pseudolaeviconchus*, photomicrographs. A – male, general view; B – anterior part of body, male, lateral view; C – anterior part of body, male, apical view; D – buccal capsule, female, lateral view; E – female, general view; F – posterior part of body, female, lateral view; G – part of body at vulva region, lateral view; H – posterior end of body, male, lateral view. Scale bars: A, E – 1 mm, B, D, F–H – 100; C – 25.

Africa (32°30′69"E; 26°85′63"S).

Site of infection: Intestine.

Intensity: 1–2 (1.3).

Prevalence: 46% (seven of 15 infected).

Abundance: 0.6.

Representative DNA sequences: 18S [MN514770], 28S [MN525307], COI [MN523682].

Description (Fig. 4).

General. Body thin, elongated with maximum width at mid-body region. Cuticle with prominent transverse striations. Apical: oral opening rounded with unlobed peribuccal flange, surrounded by four inner submedian papillae, four outer submedian papillae and two amphids on lateral sides (Fig. 4C). Buccal capsule well sclerotised, longer than wide with two step-like folds and wide basal ring on its base (Fig. 4D). Oesophageal cup small, poorly sclerotised. Muscular oesophagus club-shaped with elongated posterior bulb. Glandular oesophagus almost two times longer than muscular one, almost cylindrical slightly widened posteriorly. Nerve ring encircling muscular oesophagus somewhat anterior to its mid-length. Excretory pore opening at level of muscular oesophagus posterior quarter (Fig. 4B). Minute deirids situated posterior to level of nerve ring. Intestine straight, narrow. Rectum straight, with thin walls. Tail tapering with rounded tip in both sexes.

Males. Measurements based on three specimens. Body 5.1–5.9 (5.5) mm long, 114–118 (116) wide (Fig. 4A). Buccal capsule 53–61 (56) long, 38–39 (39) wide. Basal ring 7–9 (8) long, 25–26 (26) wide. Oesophageal cup 7–7 (7) long, 10–12 (11) wide. Muscular oesophagus 365–388 (373) long, 6.5–7.2 (6.8)% of body length; 29–33 (31), 38–41 (40) and 48–58 (54) wide at anterior, mid-length and posterior level, respectively. Glandular oesophagus 667–824 (740) long, 13.1–13.9 (13.4)% of body length; 44–47 (45), 54–64 (58) and 51–62 (55) wide at anterior, mid-length and posterior level, respectively. Nerve ring at 173–189 (181) from anterior end of body, 47.4–49.2 (48.4)% of muscular oesophagus length. Excretory pore at 221–384 (326) from anterior end of body, 4.3–6.7 (5.8)% of body length.

Posterior end coiled ventrally with narrow caudal alae supported by papillae: nine pairs of precloacal pedunculated papillae, one pair of adcloacal papillae (anterior to cloaca) and four pairs of postcloacal papillae (Fig. 4H). Spicules unequal, simple-shaped with sharply pointed distal ends. Right spicule clearly visible, 112–126 (118) long; left one less sclerotised, 42–47 (45) long. Gubernaculum poorly sclerotised, 43 long (measured in one specimen). Tail tapering with rounded tip 46–54 (51) long.

Females. Measurements based on three gravid species. Body 4.9–9.1 (7.6) mm long, 110–186 (150) maximum width (Fig. 4E). Buccal capsule 58–67 (61) long, 54–56 (55) wide. Basal ring 9–10 (9) long, 26–35 (31) wide. Oesophageal cup 11–11 (11) long, 14–22 (18) wide. Muscular oesophagus 418–470 (448) long, 5.0–8.6 (6.3)% of body length; 27–37 (34), 32–56 (45) and 53–71 (62) wide at anterior, mid-length and posterior level, respectively. Glandular oesophagus 577–814 (711) long, 8.5–11.8 (9.8)% of body length; 37–58 (50), 53–62 (59) and 49–61 (57) wide at anterior, mid-length and posterior level, respectively. Nerve ring at 201–217 (209) from anterior end, 45.7–48.1 (46.7)% of muscular oesophagus. Excretory pore at 261–367 (315) from anterior end, 3.0–6.5 (4.5)% of body length. Vulva postequatorial, opening posterior to small projection of body wall at 3.0–6.5 (4.5) mm from anterior end, 58–63 (60)% of body length (Fig. 4G). Tail tapering with rounded tip, 89–114 (98) long, 1.0–1.8 (1.4)% of body length (Fig. 4F).

Remarks. The species was recently described by Moravec and Van As (2015a) based on material collected from the catfish *Cl. gariepinus* from Egypt and Botswana. The morphology and measurements of the specimens reported here from South Africa generally correspond with the original decription and the specimens represent a new geographical record.

Barson and Avenant-Oldewage (2006) reported *P. leaviconchus* from *Cl. gariepinus* in South Africa. Although, based on the provided SEM images it is clear that the peribuccal flange of the parasites is rounded, corresponding to that of *P. pseudolaeviconchus* (contrary to six-lobed in *P. leaviconchus*).

Genus ***Spirocamallanus*** Olsen, 1952.

Spirocamallanus daleneae (Boomker, 1993).

Host: Brown squeaker *Synodontis zambezensis* (Peters, 1852).

Locality: Ndumo Game Reserve, KwaZulu-Natal Province, South Africa (32°30′69"E; 26°85′63"S).

Site of infection: Intestine.

Intensity: 1–2 (1.6).

Prevalence: 32% (eight of 25 infected).

Abundance: 0.52.

Representative DNA sequences: 28S [MN525304], 18S [MN514771].

Description (Fig. 5).

General. Comparatively long nematodes, body thin with maximum width at mid-length. Cuticle with conspicuous transverse striations along entire body. Apical: oral opening rounded surrounded by 6 min papillae, four inner submedian papillae, four outer submedian papillae and two amphids (Fig. 5B). Buccal capsule sclerotised, longer than wide, with 9–14 (of which anterior and posterior ones usually incomplete) spiral ridges (Fig. 5E). Basal ring short and narrow, oesophageal cup poorly developed. Buccal capsule supported by six columns each consisting of four blocks (Fig. 5D,F). Muscular oesophgus club-shaped, almost cylindrical in anterior half with elongated posterior bulb. Glandular oesophagus somewhat shorter than muscular one, almost cylindrical along whole length, slightly widening posteriorly. Nerve ring encircling muscular oesophagus at level of its mid-length. Position of excretory pore varying within level of muscular oesophagus posterior quarter. Intestine and rectum strait, narrow. Tail tapering without mucrons.

Males. Measurements based on six specimens. Body 1.6–2.0 (1.8) mm long, 246–354 (295) wide (Fig. 5A). Buccal capsule 83–107 (95) long, 77–95 (88) wide with 11–14 (13) ridges. Basal ring 9–14 (11) long, 43–55 (51) wide. Muscular oesophagus 704–737 (717) long,

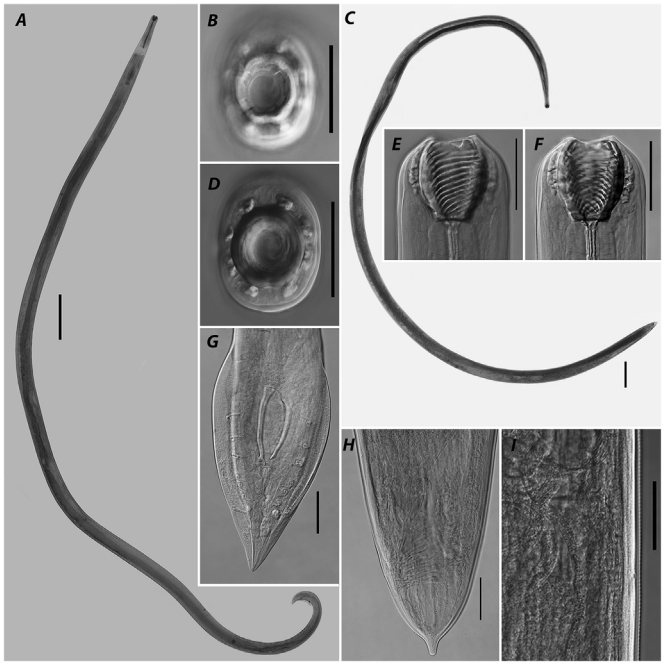

Fig. 5. *Spirocamallanus daleneae*, photomicrographs. A – male, general view; B – anterior part of body, male, apical view; C – female, genetal view; D – optical section at buccal capsule mid-length, male, apical view; E – optical sections at buccal capsule mid-width level, male, lateral view; F – optical section at buccal capsule 2/3 width level, male, lateral view; G – posterior end of body, male, ventral view; H – posterior end of body, female, lateral view; I – part of body at vulva region, lateral view. Scale bars: A, C – 1 mm; B, D–I – 100.

3.6–4.4 (4.1)% of body length; 54–69 (64), 51–72 (62) and 84–126 (104) wide at anterior, mid-length and posterior level, respectively. Glandular oesophagus 588–690 (628) long, 3.3–3.6 (3.5)% of body length; 65–91 (82), 91–129 (107) and 77–108 (96) wide at anterior, mid-length and posterior level, respectively. Nerve ring at 348–427 (373) from anterior end, 48.6–57.9 (52.1)% of muscular oesophagus length. Excretory pore opening at 473–652 (565) from anterior end, 2.4–3.7 (3.1)% of body length. Caudal end bended ventrally with narrow caudal alae supported by papillae: three pairs pre-anal pedunculated papillae, two pairs of sessile ad-anal papillae (one anterior and one posterior to cloaca), four pairs of post-cloacal papillae (of which posterior one situated close to alae margins) (Fig. 5G). Spicules unequal, poorly sclerotised. Right spicule larger, with bifurcated tip (with one branch somewhat longer), 163–231 (205) long; left one shorter, simple-shaped with sharpened tip, 132–199 (164) long. Tail 212–271 (235), 1.0–1.3 (1.1)% of body length.

Females. Measurements based on six gravid specimens. Body 1.2–3.0 (2.1) mm long, 227–589 (383) wide (Fig. 5C). Buccal capsule 59–123 (97) long, 63–118 (93) wide, supported by 9–13 (11) ridges. Basal ring 8–14 (10) long, 40–68 (55) wide. Muscular oesophagus 605–950 (798) long, 3.2–5.4 (4.2)% of body length; 49–78 (63), 52–77 (66) and 71–142 (107) wide at anterior, mid-length and posterior level, respectively. Glandular oesophagus 427–927 (670) long, 2.8–4.0 (3.4)% of body length; 68–101 (84), 72–136 (105) and 80–113 (97) wide at anterior, mid-length and posterior level, respectively. Nerve ring at 271–479 (348) from anterior end, 37.6–50.4 (43.8)% of muscular oesophagus length. Excretory pore at 392–766 (324) from anterior end, 2.3–4.6 (3.1)% of body length. Vulva small, often poorly visible, opening around mid-body level at 5.5–17.4 (10.4) mm from anterior end, 46.9–64.3 (53.7)% of body length (Fig. 5I). Tail conical, bearing short process with rounded tip (Fig. 5H).

Remarks. The morphology of the specimens collected from the Ndumo Game Reserve corresponds to the original description of *S. daleneae* from the Brown squeaker *Sy. zambezensis* collected in South Africa's Kruger National Park (Boomker, 1993). The only difference found is that our specimens have eight columns around the buccal capsule which are not reported (probably overlooked) in the original description. Nevertheless, all other morphological (number of buccal capsule ridges, shape of tail in females, number and arrangement of papillae on male caudal region) and morphometric data, as well as host species and geographical origin, led us to assign found specimens to *S. daleneae*. Outside South Africa, *S. daleneae* has been recorded from *Sy. acanthomias* Boulenger, 1899 in the Central African Republic (Moravec and Jirků, 2015b) and from *Sy. vanderwaali* Skelton et White, 1990 in Botswana (Moravec and Van As, 2015b). The authors assigned the studied specimens to *S. daleneae*, but mentioned that in their material all specimens possessed a nerve ring more anterior than that in the original description (Boomker, 1993). Moreover, Moravec and Jirků (2015) described five pairs of postcloacal papillae in males contrary to the four pairs presented in the original description. These authors assumed that Boomker (1993) overlooked one pair of caudal papillae in male and the nerve ring position. Nevertheless, all specimens in our material from the type host of *S. daleneae Sy. Zambezensis*, from South Africa possessed a nerve encircling muscular oesophagus posterior to its mid-length and all males possessed four pairs of postcloacal papillae. In our opinion, the specimens studoed by Moravec and Van As (2015b) and Moravec and Jirků (2015) might belong to a new species while *S. daleneae* might be a specific parasite of *Sy. zambezensis*.

Moravec and Jirků (2015) and Moravec and Van As (2015b) assigned the species to the genus *Procamallanus* and subgenus *Spirocamallanus*. In the present study, we prefer to assign the species to *Spirocamallanus* as a separate genus due to distant phylogenetic relationships between *S. daleneae* and *P. pseudolaeviconchus* (24% (189 nt) in the 28S rDNA gene) (see Table 1).

Batrachocamallanus xenopodis (Baylis, 1929).

Host: Muller's platanna *Xenopus muelleri* (Peters, 1844).

Locality: Ndumo Game Reserve, KwaZulu-Natal Province, South Africa (32°32′34"E; 26°93′11"S).

Site of infection: Stomach.

Intensity: 1–4 (2.7).

Prevalence: 100% (three of three infected).

Abundance: 2.7.

Representative DNA sequences: 28S [MN525305], 18S [MN514768], COI [MN523681].

Table 1
Genetic divergences between different species of Camallanidae based on 28S rDNA gene alignments. Presented as percent (number of nucleotides).

Name of species	1	2	3	4	5	6	7
1. *Cosmocercoides pulcher*							
2. *Spirocamallanus daleneae*	32 (253)						
3. *Procamallanus psedolaeviconchus*	32 (254)	24 (189)					
4. *Batrachocamallanus slomei*	32 (249)	23 (184)	14 (108)				
5. *Batrachocamallanus xenopodis*	32 (252)	23 (183)	14 (110)	3 (23)			
6. *Camallanus xenopodis*	33 (260)	26 (201)	20 (159)	19 (149)	19 (146)		
7. *Camallanus kaapstaadi*	34 (263)	25 (198)	21 (162)	19 (147)	19 (146)	4 (30)	
8. *Camallanus sodwanaensis* n. sp.	32 (248)	25 (197)	20 (157)	20 (157)	20 (156)	12 (92)	12 (96)

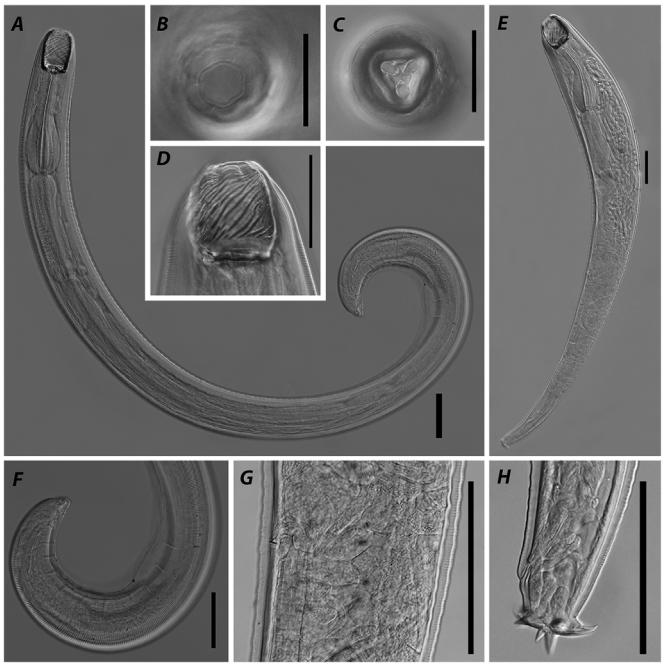

Fig. 6. *Batrachocamallanus xenopodis*, photomicrographs. A – male, general view; B – anterior part of body, male, apical view; C – optical section at base of buccal capsule level, male, apical view; D – buccal capsule, female, lateral view; E – female, general view; F – posterior part of body, male, lateral view; G – part of body at vulva region, lateral view; H – posterior part of body, female, lateral view. Scale bars: A, E, F–H – 100, B–D – 50.

Description (Fig. 6).

General. Small nematodes, body comparatively thick with maximum width at anterior quarter. Cuticle with conspicuous transverse striations along entire body. Apical: oral opening rounded surrounded by 6 min papillae, four inner submedian papillae, four outer submedian papillae and two amphids (Fig. 6B). Buccal capsule sclerotised, longer than wide, with 12–16 (most of which incomplete) spiral ridges (Fig. 6D). Three tooth-like projections situated at base of buccal capsule (Fig. 6C). Basal ring short and narrow, oesophageal cup poorly developed. Muscular oesophgus club-shaped, almost cylindrical in anterior half with elongated posterior bulb. Glandular oesophagus as long as muscular one, almost cylindrical along whole length, slightly widening posteriorly. Nerve ring encircling muscular oesophagus somewhat anterior to its mid-length. Position of excretory pore varying within level of muscular oesophagus posterior quarter. Intestine and rectum strait, narrow. Tail tapering in males and narrowing with six mucrons in females.

Males (Fig. 6A). Posterior end coiled ventrally, caudal alae relatively long, supported by papillae: 11 precloacal pedunculated papillae, two pair of adcloacal papillae (anterior and posterior to cloaca) and four pairs of postcloacal papillae (Fig. 6F).

Females (Fig. 6E). Vulva with poorly sclerotised walls, situated at mid-body level (Fig. 6G). Tail relatively short, narrowing, bearing six mucrons (Fig. 6H).

Remarks. Due to the lack of gravid specimens in our material we could not provide measurements for this species, although all morphological characters correspond to the redescription provided by Jackson and Tinsley (1995). Moravec et al. (2006) considered the genus *Batrachocamallanus* as junior synonym of *Procamallanus*. Contrary to that opinion we prefer to assign found species to the genus *Batrachocamallanus* due to the distant phylogenetic relationships between *B. xenopodis* and *P. pseudolaeviconchus* (14% (110 nt) in the 28S rDNA gene) and close relationships between *B. xenopodis* and the type species of the genus – *B. slomei* (4% (30 nt) in the 28S rDNA gene) (see Table 1).

3.2. Molecular analyses

During the present study, sequences for the partial 18S and 28S rRNA genes and the mitochondrial COI gene were generated for *B. xenopodis*, *S. daleneae* and *P. pseudolaeviconchus*, whereas only 18S rDNA and 28S rDNA were obtained for *C. sodwanaensis* and only 18S rDNA and COI sequences were obtained for *P. cyathopharynx*. Phylogenetic analyses were performed using separate datasets according to the gene fragment amplified.

Alignment 1 of the 28S rDNA dataset comprised four newly obtained sequences, as well as two sequences for *Camallanus* spp. and one sequence of *B. slomei* retrieved from GenBank. The outgroup selected for the analyses was *Cosmocercoides pulcher* Wilkie, 1930 (LC018444). Bayesian inference and maximum likelihood analyses yielded similar

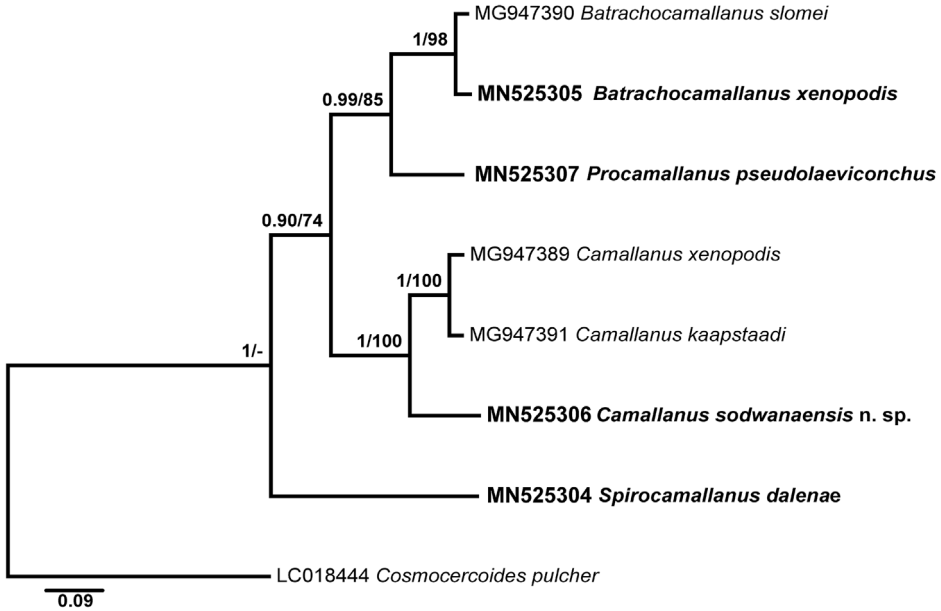

Fig. 7. Phylogenetic tree of Camallanidae nematodes based on 867 nucleotides long alignments of 28 rDNA gene. Nodal support presented for Bayesian Inference and Maximum Likelihood analyses (BI/ML).

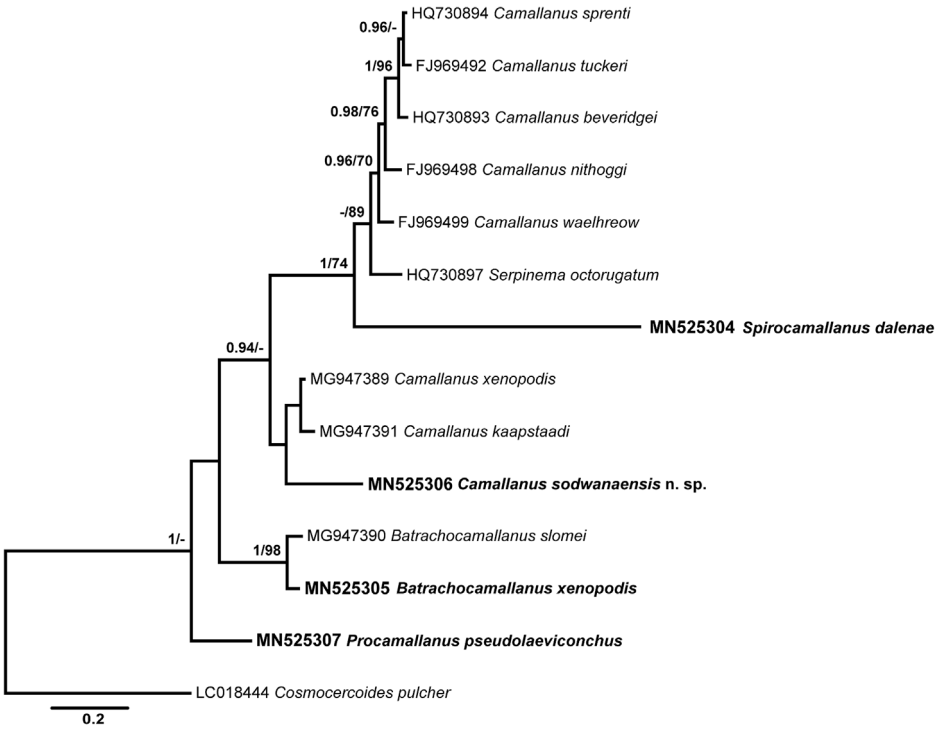

Fig. 8. Phylogenetic tree of Camallanidae nematodes based on 491 nucleotides long alignments of 28 rDNA gene. Nodal support presented for Bayesian Inference and Maximum Likelihood analyses (BI/ML).

phylogenetic topologies (Fig. 7). The novel sequence for *B. xenopodis* clustered with *B. slomei* in a highly supported clade with *P. pseudolaeviconchus*. The interspecific divergence between *Batrachocamallanus* spp. was 2.9% (23 nt) and between *Batrachocamallanus* spp. and *P. pseudolaeviconchus* it ranged from 13.8 to 14% (108–110 nt) (Table 1). The sequence of *C. sodwanaensis* n. sp. formed a basal branch to the clade formed by *C. xenopodis* and *C. kaapstaadi*. The genetic divergence between the new species and *Camallanus* spp. was 11.7–12.2% (92–96 nt) whereas differences between *C. kaapstaadi* and *C. xenopodis* was 3.8% (30 nt). The sequence for *S. daleneae* appeared at a basal position to the rest of the ingroup taxa.

Alignment 2 of the 28S rDNA dataset comprised four newly obtained sequences and seven sequences of *Camallanus* spp. plus the sequences for *B. slomei* and *Se. octorugatum* retrieved from GenBank. The outgroup used in the analyses was also *Co. pulcher* (LC018444). Bayesian inference and maximum likelihood analyses yielded similar phylogenetic topologies (Fig. 8). The sequence for *S. daleneae* appeared at the basal position within the subclade consisting of *Camallanus* spp. and *Se. octorugatum* from freshwater turtles. The positions of *C. sodwanaensis* n. sp. and *B. xenopodis* were identical to the positions on the tree based on Alignment 1. The new species formed a branch close to *Camallanus* spp. from African frogs. The sequences for *B. xenopodis* and *B. slomei* clustered together in a strongly supported clade. *Procamallanus pseudolaeviconchus*, in contrast to the results obtained in the analyses based on Alignment 1, appeared at the basal position to the ingroup taxa.

The tree based on shorter alignments (Alignment 2) also included two well supported clades. The first clade is comprised of two larger subclades: one formed by turtle-parasitising species and another formed by *C. sodwanaensis* n. sp. and two species from frogs similar to the tree based on Alignment 1. The second clade includes two species of *Batrachocamallanus* and *P. pseudolaeviconchus*. The position of *S. daleneae* remains unresolved as it appears without nodal support separate to all species in tree based on the BI analysis and is included in the clade of turtle-parasiting species in the tree based on the ML analysis.

The phylogenetic tree based on the 18S rRNA gene obtained in the present study consists of low supported genera-level clades (Supplementary file 1). Comparative sequence analysis based on the partial 18S rRNA gene revealed the identicalness of the isolates of *P. cyatopharynx* collected in our study from *Cl. gariepinus* is South Africa and an isolate of *P. cyatopharynx* (DQ813445) from Werner's catfish *Cl. werneri* Boulenger, 1906 collected in Tanzania and confirmed the differences between *P. laevionchus* (JF803934) and *P. pseudolaevionchus* comprising 1% (7 nt), and between *C. sodwanaensis* n. sp. and *C. carangis* (DQ442664) comprising 0.5% (3 nt).

The phylogenetic tree based on the COI gene consists of low supported clades with *Camallanus* and *Procamallanus* in one subclade, thus cannot be considered as adequate for rigorous analysis (Supplementary file 2).

4. Discussion

Despite the ample morphological characters in camallanid nematodes, their application for the delineation between species and genera is still complicated. The main character used to distinguish between the genera of the Procamallaninae is the presence or absence of additional structures supporting the buccal capsule, such as spiral ridges (*Spirocamallanus*), small spikes (*Punctocamallanus*), teeth (*Denticamallanus*), etc. (Rigby and Rigby, 2014). However, use of these characters is complicated due to the presence of sexual dimorphism, e. g. described in *P. iberingi* Travassos, Artigas et Pereira, 1928, *P. siluri* Osmanov, 1964, *P. pexatus* Pinto, Fabio, Noronha et Rolas, 1976 (females with spiral ridges and males with smooth buccal capsule) and *P. dentatus* Moravec et Thatcher (1997) (females with spiral ridges and males with conical teeth) (see Moravec and Thatcher, 1997). Moravec and Scholz (1991), Moravec and Thatcher (1997) suggested that taxonomy based solely on the structure of the buccal capsule is more or less artificial, does not reflect phylogeny of this group, and thus needs to be revised. Therefore, these authors considered all members of the Procamallaninae as subgenera of *Procamallanus* “for practical reasons” (Moravec and Thatcher, 1997). At the same time, Rigby and Rigby (2014) recognized the genera that Moravec and colleagues (1991,

1997) consider to be subgenera "for the sake of tradition and simplicity". Nevertheless, all authors agreed that only sufficient molecular studies can provide an answer to the question about the true taxonomic status and the value of the morphology of the buccal capsule for systematics.

Due to the small number of species, the present study does not support conclusions regarding the true taxonomic status of the different genera within the Camallanidae and to estimate the value of buccal capsule charachters. However, in the Procamallaninae clade, species with spiral ridges in the buccal capsule (*B. xenopodis* and *S. daleneae*) and without (*B. slomei* and *P. pseudolaevionchus*) clustered in the same clade. Absence of supported clades for *Procamallanus* and *Spirocamallanus* might be considered as evidence for the low value of buccal capsule ridges for generic differentiation. Therefore, in our opinion, division of the Procamallaninae into different genera or subgenera based on buccal capsule morphology might be equally inappropriate. However, in the present study, we prefer to follow the classification proposed by Anderson et al. (2009) recognizing most of the species in separate genera as they have been initially described. This was done due to the small number of species included in our analyses and also in order to avoid confusion in the species identification for future studies.

Other reliable characters concerned mostly the male caudal region. Several species of camallanid nematodes were described possessing only a right spicule. Although, using advanced microscopy, the inconspicuous left spicule was found in species initially described with only a right one (Moravec et al., 2006, 2016; Svitin et al., 2018). Moravec et al. (2006) also showed that the number of mucrons on the female tail can be different in larval, subgravid and gravid specimens and thus considered that this character can be used only for gravid females. In our material subgravid and even smaller larvigerous females of *C. sodwanaensis* n. sp. possessed two small mucrons while the largest females had rounded tail tips. Due to the high variability of some characters and inaccuracy in species descriptions, Moravec et al. (2006) stated that the most reliable character for species differentiation is the number and arrangement of caudal papillae, as none of the valid species was described with significantly varying number of caudal papillae. Despite the fact that in many descriptions phasmids were included in the number of postcloacal papillae (Moravec et al., 2016; Kuzmin et al., 2009), whereas in others the number of papillae were provided not including phasmids (Rigby et al., 1998), they can be easily found on the illustrations and text of the descriptions, thus can be compared between species. In case of *C. sodwanaensis* n. sp., the morphological difference between this species and *C. carangis* is only one pair of postcloacal papillae. Nevertheless, while most *Camallanus* spp. possess three anterior pairs of postcloacal papillae grouped together, *C. sodwanaensis* n. sp. bears only two pairs grouped, whereas the other papillae and phasmids situated similar to those of *C. carangis*. We agree with the opinion of Moravec et al. (2006) that the presence of one or two spicules cannot be considered as a significant character. The left spicule is often less sclerotised and poorly visible, thus might be overlooked. The left spicule of *C. sodwanaensis* n. sp. is almost indistinct and can be easily missed without DIC and high magnification, although it is clearly visible when dissected.

In the present study, all known species were found in the same hosts as previously reported: *Pa. cyathopharynx* and *P. pseudolaeviconchus* from *Cl. gariepinus*; *S. daleneae* from *Sy. zambezensis* and *B. xenopodis* from *X. muelleri*. Nonetheless, all species represent new geographical records and *C. sodwanaensis* n. sp. is the first *Camallanus* species described from marine fish in southern Africa. Unfortunately, studying the geographical distribution and host specificity of camallanid nematodes is highly complicated due to a number of species misidentifications. Therefore, in our opinion, all species records (even of well-known species) should be supported by short descriptions, illustrations and/or molecular data.

Informative phylogenetic trees were obtained only based on the partial 28S rDNA datasets. Use of partial 18S and COI sequences for analyses appeared not to be informative, probably due to the high level of conservatism of the studied fragment of 18S (630 out of 717 nt (88%) appeared to be identical for 26 species) and the variability of COI fragments (244 of 428 nt (57%) identical for nine species), respectively. In our opinion, using a combination of different nuclear (conservative) and mitochondrial (variable) genes of numerous Camallanidae species (including type species from each genus) is the only way to illuminate the real phylogenetic relationships between members of this group. Unfortunately, to date, 28S, 18S and COI sequences have been generated only for four species. Therefore, our knowledge of the phylogenetic relationships of camallanid nematodes is still at the stage of data accumulation and requires an in depth study of more species from all around the globe.

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Declaration of competing interest

No conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijppaw.2019.09.007.

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Chapter

Conserving Freshwater Biodiversity in an African Subtropical Wetland: South Africa's Lower Phongolo River and Floodplain

Aline Angelina Acosta, Edward C. Netherlands, Francois Retief, Lizaan de Necker, Louis du Preez, Marliese Truter, Reece Alberts, Ruan Gerber, Victor Wepener, Wynand Malherbe and Nico J. Smit

Abstract

Freshwater biodiversity is under constant threat from a range of anthropogenic stressors. Using South Africa's Phongolo River and floodplain (PRF) as a study case, the aim of this chapter is to provide an overview of the conservation and management of freshwater biodiversity in a highly diverse subtropical ecosystem. The PRF is the largest floodplain system in South Africa which is severely threatened by irregularly controlled flood releases from a large upstream dam, prolonged drought, deteriorating water quality, organic pollutants and the increasing dependence of the local communities. Based on a decade of survey of the PRF conducted from 2010 to 2020, this chapter highlights the current diversity of aquatic organisms (invertebrates, fishes, frogs and their parasitic fauna), followed by an overview of their biological and physical stressors. The current challenges in the management of the aquatic biodiversity of this region and a way forward to conservation strategies are also addressed in this chapter.

Keywords: conservation, aquatic organisms, biological stressors, physical stressors, management

1. Introduction

The Phongolo River (PR) originates in the South African Mpumalanga Province from where it flows first eastwards, before turning north through the Ubombo mountain ranges in South Africa's northern KwaZulu-Natal (KZN) [1]. The lower Phongolo River and associated floodplain (PRF) starts from where the river exits a gorge in the Ubombo mountains, which is known as the Pongolapoort, for approximately 80 km downstream to the confluence of the PR and Usuthu River (UR) at the South Africa/Mozambique border [2] (**Figure 1**). The PRF is about 10,000 increasing to 13,000 ha in full inundation and is characterised by its permanent and

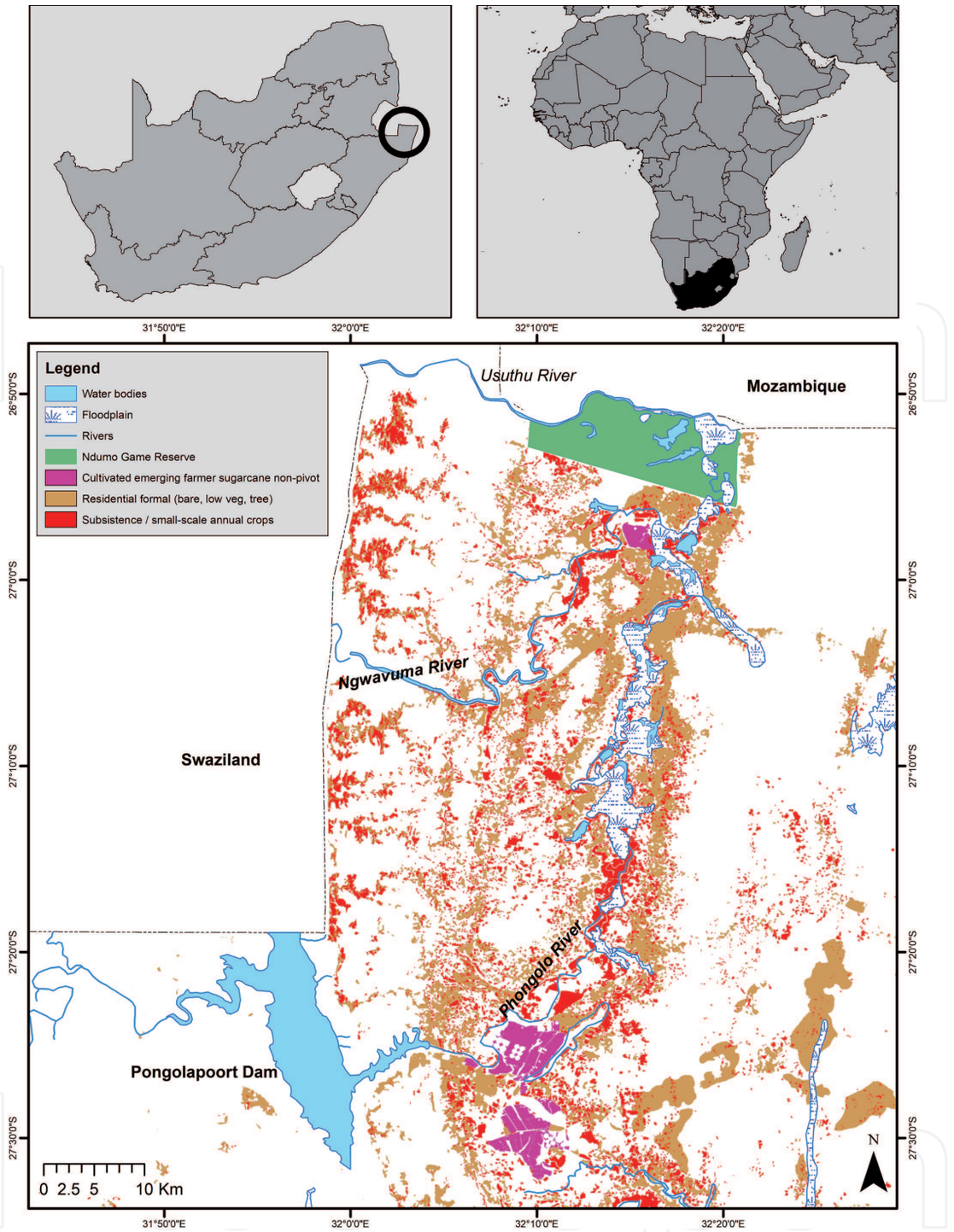

Figure 1.
Map of the Pongolapoort dam, the lower Phongolo River and its associated floodplain in North-Eastern South Africa.

temporary floodplain depressions (from here on further referred to as pans) that have either permanent, intermitted or no connectivity with the river. Natural flow regime determines the heterogeneity of the floodplain habitats. Pans that are river-fed function as spawning and breeding sites and as foraging areas for migratory species. The temporary pans are also known to harbour endemic species, therefore presenting a unique diversity. The connection between the river and floodplain also enables the exchange of organic matter, nutrients and aquatic vegetation. Thus, the flood pulse plays a key role in the health of the river ecosystem and productivity of the wetlands. The PRF constitutes one of the largest and the most biodiverse floodplain systems in South Africa.

The previously mentioned gorge (Pongolapoort) in the Ubombo mountains provided the perfect structural position for the building of a dam and therefore it was no surprise that the building of a dam in the PR (**Figure 2A, B**), with the main aim to supply irrigation water for sugarcane and cotton crops, was commissioned in the 1960s. The Pongolapoort Dam (also referred to as the Jozini Dam) was completed in 1973, and although research on its possible effect on the PRF started before the completion of the dam, there have been continuous concerns over the past almost 50 years about the real impact thereof on the PRF. In order to mitigate the dam's impacts on the ecological integrity of and ecosystem services provided by the floodplain, a controlled flood release regime was developed to simulate natural flooding [3]; however, this was never fully implemented [1, 4]. The fragile ecosystem of the PRF has also, over the past 40 years, been further impacted by extreme climatic events such as a cyclone (1984) and severe droughts (1981–1983 and 2016–2020) [1].

The only officially protected section of the PRF lays within the 102 km^2 Ndumo Game Reserve (NGR) that was proclaimed in 1924 (**Figure 1**). In 1997, the game reserve was also declared a Ramsar site under the Wetlands of International Importance convention [2]. In addition to the PR and UR sections that fall inside the reserve, the NGR also provides protection to five distinct wetland types ranging between fresh, brackish and saline and permanent and intermittent rivers, lakes, pools and riparian/gallery forests. One of the largest water bodies and also the only naturally saline lake (approximately 5000 $\mu S \cdot cm^{-1}$) within NGR is Lake Nyamithi (183.4 ha) (**Figure 2C**). Nyamithi receives water largely from the PR through the annually controlled flood release from the Pongolapoort Dam, through rainfall during the wet season (summer) from its own small catchment and natural floods from the UR. Although the reserve is almost 100 years old, very little has been accomplished in terms of research since the reserve's establishment, and it is unknown whether the water that NGR receives is of a quality and quantity to support and protect this biodiversity hotspot.

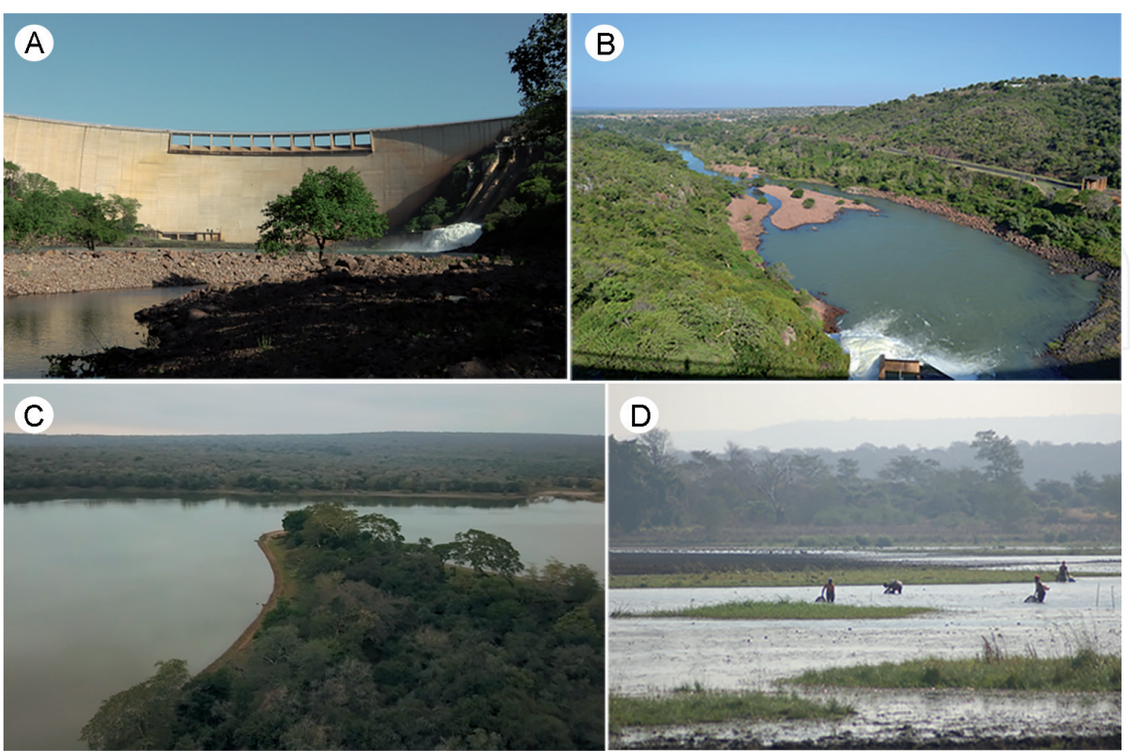

Figure 2.
(A) View of the dam wall from the Phongolo River; (B) downstream view from the dam wall; (C) Lake Nyamithi and (D) example of activity of people directly dependent on the floodplains for cultural and provisioning ecosystem services.

Figure 3.
View of the Phongolo River flowing North with Ndumo Game Reserve (NGR): protected site on the left (western) side, and invaded areas used for agriculture on the right (eastern) side.

The proximity to water has always been an important factor that governs the distribution of rural communities. The human population that is directly or indirectly dependent on the PRF has grown over the past 40 years from 30,000 to a projected 400,000 in 2020 [5, 6]. The majority of the people dwelling the floodplain live in very poor conditions, being directly dependent on the floodplain. The water is used for both humans and livestock existence as well as for other goods from the floodplain such as fish (**Figure 2D**), fruits, reeds, thatch grass and firewood. The indirect dependence on the PRF is shown by the increase of agricultural lands that were once covered by natural vegetation (**Figure 3**). Practices of subsistence agriculture in the PRF introduced fertilisers and chemicals into the area, decreasing water quality and aquatic biodiversity. This together with alterations in the natural flow regime that was brought about by the construction of the dam, including irregular flood releases, prolonged droughts, deteriorating water quality, and organic pollutants, all severely threaten the functioning and biodiversity of the PRF.

Therefore, the aim of this chapter is to provide information on the current status of the freshwater diversity of the PRF comprising invertebrates, fishes, frogs and their parasitic fauna as well as their biological and physical stressors. Furthermore, the challenges and a way forward in managing the aquatic biodiversity of the PRF and proposed research to inform management and conservation strategies will be addressed herein. This chapter is based on the results of a decade (2010–2020) of research on the PRF led by the North-West University's Water Research Group (WRG).

2. Biodiversity of aquatic organisms from the lower Phongolo River and associated floodplains

2.1 Diversity of aquatic invertebrates

Aquatic invertebrates are invertebrates that require aquatic habitats to complete either one, several or all of their life stages in the aquatic environment [7]. Many terrestrial insects have a larval aquatic life stage including dragonflies and damselflies (Odonata), mosquitoes and flies (Diptera) and mayflies (Ephemeroptera) [8–10] and are therefore considered aquatic invertebrates in their larval phase. Truly aquatic or 'permanent' aquatic invertebrates complete all of their life stages in the aquatic environment and include zooplankton such as water fleas (Cladocera), micro-crustaceans (Copepoda) and fairy shrimp (Anostraca) as well as larger macroinvertebrates such as freshwater snails (Mollusca) and crabs (Potamonautidae)

[10–12]. Aquatic invertebrates are present in most freshwater ecosystems and respond rapidly to a broad range of physical and chemical environmental conditions such as changes in habitat condition or water chemistry [7, 13]. They are also quite immobile, particularly those that complete all their life cycles in water, and are constantly in contact with both bottom sediments and the water column [14]. Aquatic invertebrates are also in the unique position to act as the transitional link between lower level producers (including diatoms and algae) that they feed on and higher level consumers (including larger invertebrates, fish and birds) that feed on them [15–17]. For these reasons, aquatic invertebrates are ideal indicators of change in the aquatic environment and ecosystem health, particularly in lotic habitats, and have been used as such in various ecological assessments (e.g. [13, 18, 19]).

Aquatic biodiversity research in the PRF has been undertaken since the late 1960s, but it was not until 2012 that aquatic invertebrates were also included in ecological assessments of the region (see [2, 20, 21]). As part of these assessments, aquatic invertebrates were collected from the PR as well as many floodplain and temporary pans, both within and outside NGR. Approximately 131 taxa of aquatic invertebrates from 70 families have been identified from the river, while 117 taxa from 49 families and 109 taxa from 54 families have been collected from the floodplain and temporary pans, respectively (e.g. **Figure 4**). Many of the aquatic invertebrates collected from the river and floodplain pans both within and outside NGR include sensitive biota [7] such as riffle beetles (Elmidae), brush-legged mayflies (Oligoneuriidae), caseless caddisflies (Philopotamidae) and spiny crawlers (Teloganodidae).

Lake Nyamithi forms an important part of the assessment of the aquatic invertebrates of the PRF. This lake is quite unique as it is a large naturally saline lake and the only permanent wetland-type ecosystem located within the NGR [3, 22]. Due to its permanence, this habitat acts as a refuge to many of the aquatic and (semi-)

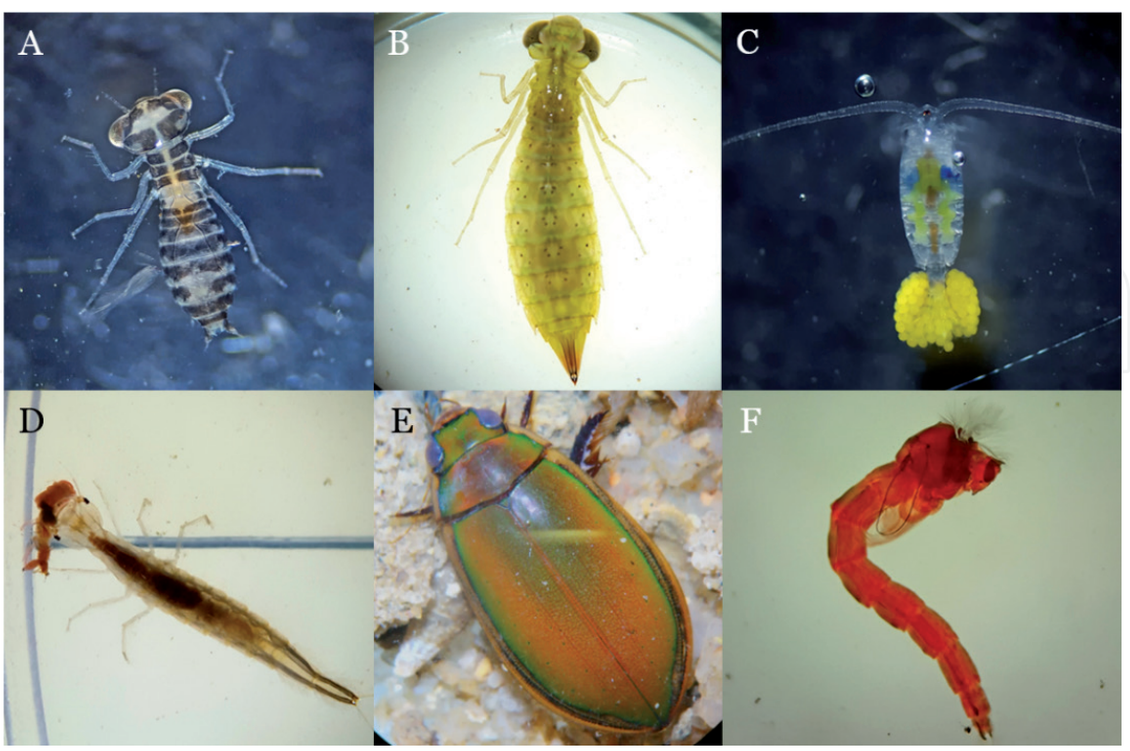

Figure 4.
Examples of aquatic invertebrates found in the lower Phongolo ecosystem. (A) Young dragonfly nymph (Aeshnidae); (B) mature dragonfly nymph (Aeshnidae); (C) female micro-crustacean with eggs (Cyclopoida); (D) larval predaceous diving beetle (Dytiscidae) feeding on a bloodworm (Chironomidae); (E) adult Dytiscidae beetle and (F) pupal stage of Chironomidae.

terrestrial biota of the region during the dry season when many of the pans dry out [2]. Even though it is naturally saline, the diversity and abundance of aquatic invertebrates found in this ecosystem (108 taxa from 47 families) is comparable to that found in the river and floodplain and temporary pans. Overall, the diversity of aquatic invertebrates in the PRF is greater than that of comparable lowland river (see [23, 24]), pan (see [25]) and saline lake (see [26]) ecosystems. These findings further demonstrate that the PRF is a biodiversity hotspot for aquatic invertebrates and of great ecological importance.

Worryingly, aquatic invertebrate assessments of this ecosystem have also indicated the possibility that the anthropogenic disturbances taking place outside NGR has a negative impact on the ecological integrity of the aquatic habitats of the PRF. Although biodiversity has generally been found to be greater in the river and pans located within NGR compared to outside the reserve, the river section that flows through the reserve is clearly not well protected given the types and abundances of certain aquatic invertebrates found. Several pollutant-tolerant taxa [7] such as the sharp-spired bladder snail, *Physa acuta*, and aquatic earthworms, Lumbriculidae, were found to be some of the most abundant taxa in the river inside NGR. Research found these types of taxa, particularly snails of the genus *Physa*, to flourish in systems that are nutrient enriched [27] suggesting that this may be the case for the PR. Additional stressors also include the invasive quilted melania snail, *Tarebia granifera*, which is distributed throughout the PR and is discussed further in Section 3.1.

Many of the pans outside the reserve are also threatened by anthropogenic stressors since they are utilised by local communities as a source of domestic and agricultural water and for subsistence fishing [2, 28, 29] all of which affects the integrity of pans. Ndumo Game Reserve therefore acts as a refuge for aquatic invertebrates of the PRF. This can be observed by the much higher number of invertebrate families that are present exclusively within NGR compared to outside (19 inside vs. 4 outside). Additionally, those invertebrate families present in high abundance in pans outside the reserve, namely rat-tailed maggots (Syrphidae), biting midges (Ceratopogonidae) and crane flies (Tipulidae) are known to be more tolerant of eutrophication and other forms of pollution [7] further demonstrating how these wetlands are being negatively affected.

2.2 Diversity of freshwater fishes

Fish have been regarded as one of the world's most important natural resources as they provide animal protein to billions of people annually, especially in developing countries [30]. Furthermore, fish also play an important role in freshwater ecosystem ecology where they can control prey such as zooplankton or be prey themselves for other reptiles, fish, mammals or birds. Fish communities within the PR and its associated floodplain wetlands are one of the most diverse found in South Africa [31] (see **Figure 5**). There have been 46 species recorded in this region from 12 different families (**Table 1**). Of these 46 species, *Oreochromis mossambicus* (Mozambique tilapia) (**Figure 5D**) is listed on the IUCN Redlist as a vulnerable species [40]. The South African Threatened or Protected Species List (TOPS) from 2013 [41] also included the *O. mossambicus* as a protected species in South Africa placing restrictions on its utilisation. This listing of *O. mossambicus* is due to the threat of hybridisation with the alien cichlid *Oreochromis niloticus* (Nile tilapia) [42] that could lead to a loss in genetic integrity. To date *O. niloticus* has not been recorded from the PRF thus making the *O. mossambicus* population from this region potentially one of only a few remaining genetically pure *O. mossambicus* populations left, although this needs confirmation through genetic studies. One of the unique fish species found in the PRF

is the annual killifish, *Notobranchius orthonotus* (spotted killifish). This fish species is found in the temporary pans, specifically within NGR, as it is able to withstand drying through dormant eggs that are deposited in the sediment [43].

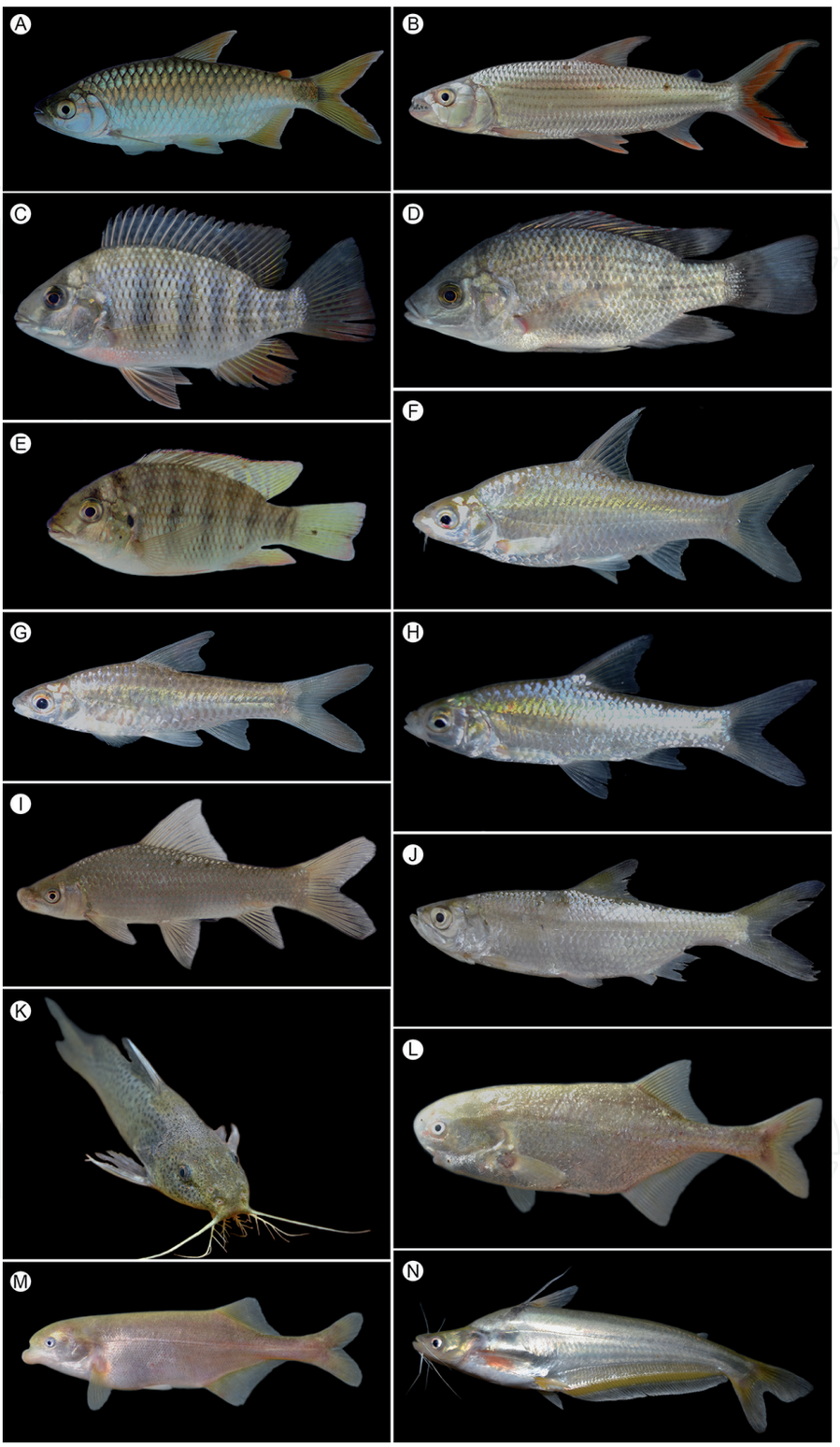

Figure 5.
Selection of fishes collected from the lower Phongolo River and floodplain: (A) Brycinus imberi *(max. length 19.8 cm); (B)* Hydrocynus vittatus *(max. Length 105 cm); (C)* Coptodon rendalli *(max. length 45 cm); (D)* Oreochromis mossambicus *(max. length 39 cm); (E)* Tilapia sparrmanii *(max. length 23.5 cm); (F)* Enteromius afrohamiltoni *(max. length 17.5 cm); (G)* Enteromius annectens *(max. length 7.5 cm); (H)* Enteromius paludinosus *(max. length 15 cm); (I)* Labeo rosae *(max. length 40 cm); (J)* Megalops cyprinoides *(common length 30–45 cm); (K)* Synodontis zambezensis *(max. length 43 cm); (L)* Petrocephalus wesselsi *(max. length 11.4 cm); (M)* Marcusenius macrolepidotus *(max. length 32 cm); (N)* Schilbe intermedius *(max. length 50 cm). Data on maximum length extracted from [32].*

Species	1974–1976	1983	1984	1993–1994	2012–2014	2016–2019
Alestidae						
Brycinus imberi (*B. lateralis*)	x	x	x	x	x	x
Hydrocynus vittatus	x	x	x	x	x	x
Micralestes acutidens	x	x	x	x	x	x
Anguillidae						
Anguilla bengalensis labiata (*A. nebulosa*)	x	x		x	x	x
Anguilla bicolor bicolor	x	x		x	x	
Anguilla marmorata	x	x		x	x	x
Anguilla mossambica	x		x	x	x	
Cichlidae						
Coptodon rendalli (*Tilapia rendalli*)	x	x	x	x	x	x
Oreochromis mossambicus	x	x	x	x	x	x
Oreochromis placidus					x	
Pseudocrenilabrus philander	x	x	x	x	x	x
Tilapia sparrmanii	x	x	x	x	x	x
Clariidae						
Clarias gariepinus	x	x	x	x	x	x
Clarias ngamensis	x	x	x	x		
Cyprinidae						
Enteromius afrohamiltoni	x	x	x	x	x	x
Enteromius annectens	x	x	x	x	x	x
Enteromius pallidus				x	x	
Enteromius paludinosus	x	x	x	x	x	x
Enteromius radiatus	x	x	x	x	x	x
Enteromius toppini	x	x	x	x	x	x
Enteromius trimaculatus	x	x	x	x	x	x
Enteromius unitaeniatus				x	x	
Enteromius viviparus	x	x	x	x		x
Cyprinus carpio[*]				x	x	x
Labeo congoro	x	x	x	x		x
Labeo cylindricus	x	x	x	x	x	x
Labeo molybdinus	x	x	x	x	x	
Labeo rosae	x	x	x	x	x	x
Labeobarbus marequensis	x	x				x
Engraulicypris brevianalis	x	x	x	x		x
Opsaridium peringueyi (*zambezense*)				x		
Cyprinodontidae						
Nothobranchius orthonotus	x			x	x	x

Species	1974–1976	1983	1984	1993–1994	2012–2014	2016–2019
Gobiidae						
Awaous aeneofuscus				x	x	x
Glossogobius callidus				x	x	x
Glossogobius giuris	x	x	x	x	x	x
Redigobius dewaali	x	x	x	x	x	
Megalopidae						
Megalops cyprinoides				x	x	
Mochokidae						
Chiloglanis paratus	x		x	x	x	x
Chiloglanis swierstrai	x	x	x	x	x	x
Synodontis zambezensis	x	x	x	x	x	x
Mormyridae						
Marcusenius macrolepidotus	x	x	x	x	x	x
Petrocephalus wesselsi (*P. catostoma*)	x	x	x	x	x	x
Schilbeidae						
Schilbe intermedius	x	x	x	x	x	x
Sparidae						
Acanthopagrus berda						x
Syngnathidae						
Microphis fluviatilis				x		
Total no. of species	35	32	30	42	37	35

[*] *Invasive species.*

Table 1.
Comparative species list of the fishes collected from the lower Phongolo River and floodplain (data from [2, 33–39], and the present study).

Research on the fish community have been extensive since the 1970s during the construction of the Pongolapoort Dam. Fish surveys were completed in various decades from the 1970s to the most recent surveys from 2016 to 2019 (**Table 1**). The species diversity found from the 1970s to the current surveys show variation, potentially due to fluctuating rainfall, the flooding regime and management of flood releases from Pongolapoort Dam. Of the 46 expected species, only the intensive surveys of [34–39] came close to sample all of the expected species (42 species collected), highlighting how the species diversity varies depending on many different physical and biological aspects. Interestingly, a total of 43 species (one more than the number recorded during the intensive surveys in the 1990s) were recorded by the WRG research teams between 2012 and 2019.

The occurrence and diversity of fish in the floodplains are driven by their dependence on receiving enough water during the summer rainfall season. Often the dominance of cichlids (*O. mossambicus* and *Coptodon rendalli* [redbreast tilapia]) occur during periods of lower flows while a more equal distribution of species and biomass are present during higher flow periods [3, 33]. The success of the cichlids during lower flow periods are attributed to its resilience to survive in systems with higher electrical conductivity. Furthermore, of the various important fish species in the system, these are the only species able to spawn without increased flow and flow

velocity triggering spawning events [3]. Smit et al. [2] indicated that two species [*Labeobarbus marequensis* (large-scale yellowfish) and *Engraulicypris brevianalis* (river sardine)] were potentially locally extinct, especially as the *Lb. marequensis* were not sampled during the 1990s and 2012–2014 surveys (**Table 1**). However, both these species were collected during the recent (2016–2019) surveys showing the resilience of the fishes and the system. There were eight species not collected during 2016–2019, which were collected during 2012–2014 (see **Table 1**), that might be the result of the extreme drought during the latter surveys. Furthermore, as no flood releases from the Pongolapoort Dam have been possible during this drought (since 2016), it is unlikely that these species are extinct from the PR, but should return from refugee areas in the Usuthu and Maputo Rivers once increased flows and flood releases occur.

The fish community in the PRF are important both ecological and economically. Firstly, many of the fish found in the PRF represent the southernmost distribution range of these species, making the system very important for biodiversity conservation. Secondly, the fish from the floodplain wetlands are extensively utilised (third most popular animal protein [44]) as a major source of protein by the local communities. Heeg and Breen [3] estimated that 400 tonnes of fish was harvested every year. The main fish species identified for consumption were *O. mossambicus* (**Figure 5D**), *C. rendalli* (**Figure 5C**), *Glossogobius giuris* (tank goby), and *Glossogobius callidus* (river goby). These species are also important fodder fish for *Hydrocynus vittatus* (tigerfish, **Figure 2B**), a popular recreational angling species in the PRF as well as in the Pongolapoort Dam [2, 45]. Thirdly, the fish communities are a crucial link in the food chain within the floodplain wetlands and especially in Lake Nyamithi where the abundances of fish serve as food sources to the unique and extremely diverse water bird community [2].

The 2012–2014 study [2] on the PRF investigated the Present Ecological State (PES), using the Fish Response Assessment Index. The results indicated that the fish community was in a seriously modified ecological state (Ecological Category of D/E); indicating an extensive or serious loss of natural habitat, biota and basic ecosystem functioning. This impacted state was especially evident downstream of the Pongolapoort Dam where high flow velocities are experienced during flood releases. The major threats to the fish communities were identified as physical impacts (unseasonal flood releases, poor water quality, water abstraction), over utilisation of fish from floodplains outside NGR, and biological threats (invasive species) [2].

2.3 Diversity of anurans

Amphibians are a diverse group that have adapted to a variety of habitats throughout the world. According to Frost [46], there are more than 8,203 known amphibian species globally, with more than 178 species in southern Africa. In recent decades, amphibians have suffered sudden, high mortality rates with many species becoming extinct [47, 48]. Although many factors contribute to this decline in amphibian populations, the most significant cause is habitat loss and fragmentation through anthropogenic disturbances. Paradoxically, the realisation that frog numbers are in decline sparked a renewed interest in amphibian biodiversity studies. The use of modern molecular and bioacoustic techniques and increased scientific surveys in remote areas, have resulted in new species being discovered and described frequently. Since 1985, the total number of globally recognised species has increased by over 60% [48–51].

In terms of amphibian diversity at a global scale, South Africa is currently ranked as the 27th country with the highest known species richness [52]. Within South Africa, the highest amphibian diversity can be found in the eastern part of the country and in particular, the province of KZN [53]. This province is an

essential refuge for several endangered and endemic species. However, environmental stress, due to anthropogenic activities, poses a severe threat to the survival of amphibians, with safe havens becoming more critical for the conservation of amphibian species richness in South Africa. Currently, there are 71 different anuran species (excluding subspecies) in KZN; these account for 40% of the total frog diversity that occur in southern Africa [50, 54]. Northern KZN or Zululand has two humid, subtropical regions, namely, the Maputaland and the KZN coastal area. These areas are transition zones between tropical and temperate climates, characterised by high anuran species richness (see **Figure 6**) [52, 53, 55, 56].

Figure 6.
Frogs of the lower Phongolo river and floodplain. (A) Arthroleptis stenodactylus*; (B)* Leptopelis mossambicus*; (C)* Breviceps adspersus*; (D)* B. mossambicus*; (E)* Poyntonophrynus fenoulheti*; (F)* Schismaderma carens*; (G)* Sclerophrys garmani*; (H)* Scl. gutturalis*; (I)* Scl. pusilla *(J)* Hemisus marmoratus*; (K)* Afrixalus aureus*; (L)* Afr. delicates*; (M)* Afr. fornasini*; (N)* Hyperolius argus*; (O)* Hyp. marmoratus*; (P)* Hyperolius poweri*; (Q)* Hyp. pussilus*; (R)* Hyp. tuberilinguis*; (S)* Kassina senegalensis*; (T)* Phlyctimantis maculatus*; (U)* Phrynomantis bifasciatus*; (V)* Phrynobatrachus acridoides*; (W)* Phry. mababiensis*; (X)* Phry. natalensis*; (Y)* Xenopus laevis*; (Z)* X. muelleri*; (AA)* Hildebrandtia ornata*; (BB)* Ptychadena anchietae*; (CC)* Pty. mossambica*; (DD)* Pty. nilotica*; (EE)* Pty. oxyrynchus*; (FF)* Pty. porosissima*; (GG)* Amietia delalandii*; (HH)* Cacosternum boetgeri*; (II)* Pyxicephalus edulis*; (JJ)* Strongylopus fasciatus*; (KK)* Tomopterna adiastola*; (LL)* T. krugerensis*; (MM)* T. natalensis *and (NN)* Chiromantis xerampelina*. Photos not to scale.*

According to Du Preez and Carruthers [50], frogs inhabit almost every environment and habitat on the subcontinent. However, even though frogs are found in a variety of habitats, most species are specific to the particular habitat in which they are able to survive and more importantly, reproduce. Because of this specialisation many frog species are vulnerable to changing habitats and are directly affected by environmental disturbances [57].

The PRF is an area recognised as a biodiversity hotspot for amphibians, offering a great diversity of habitats suitable for amphibians ranging from big rivers, streams, pans, pools, swamps, marshland, rain-filled depressions and terrestrial habitats [58]. Currently, this selection of habitats caters for the 41 species known from the area (**Figure 6**) [59]. Furthermore, as a result of the survey work conducted during the current project the previously unknown Ndumo rain frog (*Breviceps passmorei*) (**Figure 7**) was discovered and described, the common name referring to the type locality [60].

Amphibians are a sensitive group, especially to rapidly changing environments, with many species only adapted to survive in specific habitat types. To gain a better perspective of how additional stressors such as habitat loss and fragmentation affect amphibians within the PRF in comparison to historical data, the current study undertook an extensive survey using both active and passive sampling techniques. While most anuran species are nocturnal, some species have prolonged breeding seasons whereas others are explosive breeders and are only active following rainfall events. Due to the unpredictability of weather and breeding activity, certain species are often overlooked during traditional active biodiversity surveys. Furthermore, each frog species has unique vocalisation calls serving as a valuable identification aid. In the current project, passive acoustic monitoring (PAM) was utilised in the NGR, with automated recorders were set up at two selected wetlands and set to record from 18:00 till 5:00 the following morning for 13 months. Findings, in combination with active sampling, were used to monitor biodiversity and breeding activity of frog species associated with the selected endorheic habitats.

In the current study, 83% (34/41) of the expected frog species were recorded based on the 75 years (1929–2004) of historical data. In the NGR alone, a total of 32 frog species were recorded, stressing the importance and value of natural protected areas and how they support not only specific species but whole communities. These results indicate that even though there are significant global amphibian declines, areas such as the NGR still provide a haven and refugia for frog species to flourish in, and should remain protected at all costs.

Figure 7.
*The Ndumo rain frog (*Breviceps passmorei*).*

Results from PAM indicated that the peak breeding season for the majority of the species, with 79% (15/19) calling males recorded, was in the southern hemisphere summer between December 2013 and January 2014. Of the 19 species of frogs recorded the hourly calling activity and intensity differed among species, with only four species, namely, *Sclerophrys pusilla*, *Phlyctimantis maculatus*, *Phrynobatrachus mababiensis* and *Phrynomantis bifasciatus* were recorded reaching an average call intensity of 5/5 (**Figure 8**). These findings are indicative of explosive breeders, with a high calling intensity for only a few weeks a year in correlation with rainfall patterns. Six species were recorded calling in all 12-h slots, namely, *Chiromantis xerampelina*, *Hemisus marmoratus*, *Hyperolius marmoratus*, *Phly. maculatus*, *Kassina senegalensis* and *Phry. mababiensis*. Furthermore, four species (*Afrixalus aureus*, *Afr. delicatus*, *Hyp. marmoratus* and *K. senegalensis*) call intensity and thus breeding activity peaked between 18:00 and 00:00, and for six species, (*Scl. pusilla*, *Phry. mababiensis*, *Phry. natalensis*, *Phryn. bifasciatus*, *Ptychadena anchietae* and *Pty. mossambica*) call intensity peaked between 00:00 and 5:00 (**Figure 8**).

As mentioned previously, the PRF area experienced a massive influx of people over the past four decades that resulted in a transformation of the landscape and fragmentation of natural habitats. These alterations place enormous pressures on the environment. Furthermore, the introduction of the invasive redclaw crayfish (*Cherax quadricarinatus*) (see Section 3.1) poses a severe risk to tadpole population density and should be considered high priority threat to biodiversity. However, the

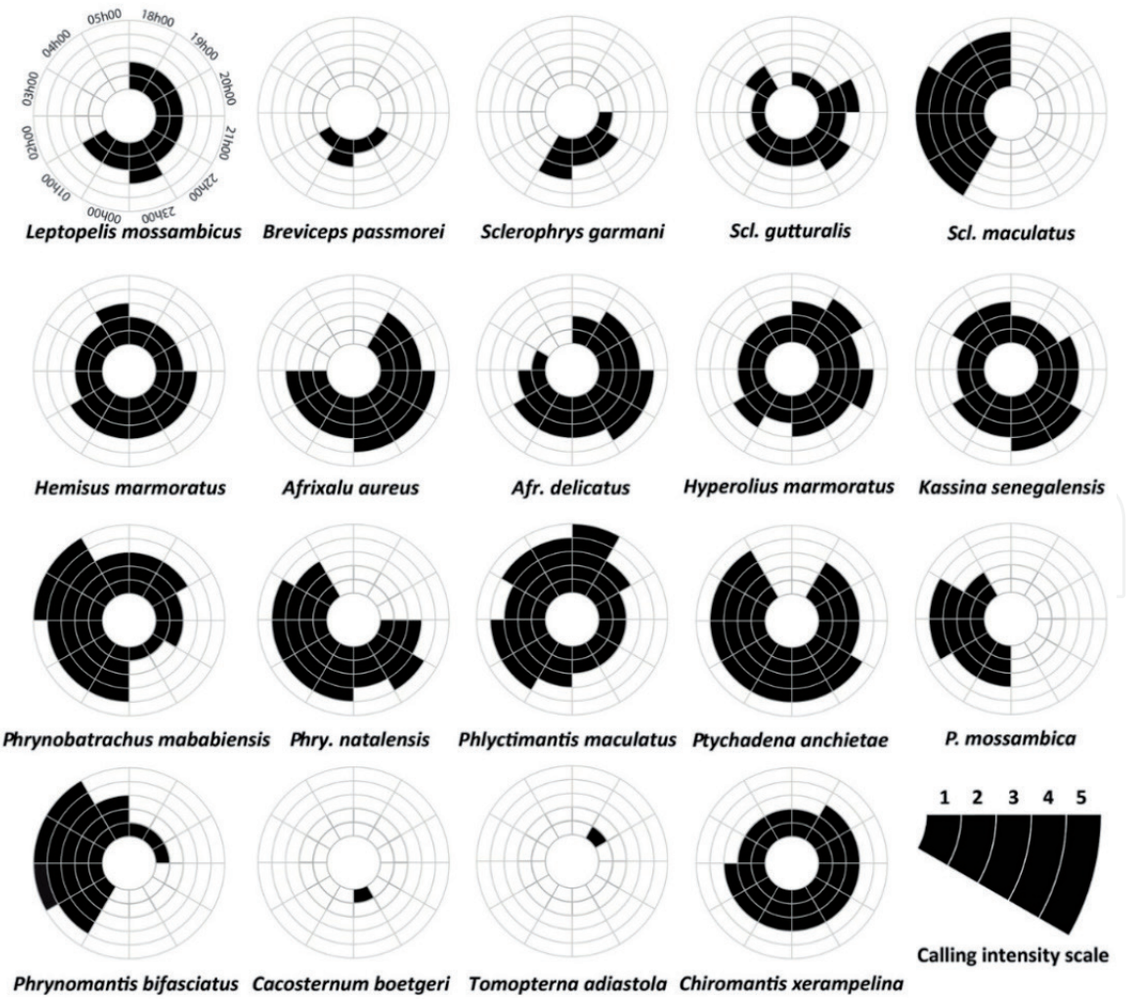

Figure 8.
The average hourly activity and intensity of male frog species. Displayed are the data on the average calling activity and intensity of each frog species recorded by the song metre for the first 10 min of each hour (18:00 to and including 05:00). The scale bar represents the hourly calling intensity.

use of educational posters and the field guide to the frogs of Zululand [59] helped inform and educate local communities on the importance of conserving the environment future generations.

2.4 Diversity of parasites of aquatic organisms

Parasites constitute a fundamental component of the global biodiversity, accounting for one third of the species on Earth, playing a key role in ecosystem functioning by regulating host population density and abundance, and being an integral part of food webs. However, parasites are still a neglected component in biodiversity surveys [61, 62]. Parasite inventories can provide knowledge to the comprehension of life cycles, pathological impacts, and evolution of host–parasite systems in aquatic environment [63].

Ectoparasites live on the surface of hosts, while endoparasites live internally [64]. The life cycle of parasites can be direct (monoxenous), requiring only one host to be completed, or indirect (heteroxenous), requiring one or more intermediate hosts to be completed [65]. A definitive (or final) host is the one in which the parasite is sexually mature; while the intermediate host is the one required for the development of parasitic stages without reaching sexual maturity. Paratenic or transport hosts are the ones in which parasites do not develop further but remains alive and infective [64]. Parasites exhibit variable degrees of host specificity, with some infecting only a single species or related species (specialists), and some infecting many unrelated species (generalists) [66].

A myriad of metazoan parasitic groups is found in aquatic organisms, including Protista, Myxozoa, Platyhelminthes [Monogenea, Trematoda (subclasses Aspidogastria and Digenea), Cestoda], Acanthocephala, Nematoda and Crustacea. Some species infecting aquatic organisms present zoonotic potential, such as certain digeneans, cestodes and nematodes. All parasitic groups have species that can harm aquatic hosts, especially when present at high intensity of infections that normally occurs in aquaculture scenarios or in the case of invasive parasites.

Parasites that naturally occur in wild fish normally do not cause negative impacts on host populations. The opposite is usually true as a high diversity of parasites in freshwater fishes in natural conditions are used as indicators of a healthy and functional ecosystem [67]. Moreover, parasites are very sensitive to environmental alterations such as pollutants, providing relevant information about the quality of a given system. Some parasites have been identified as sentinels for chemical pollution because these organisms can accumulate chemicals at a higher level compared to their hosts [68, 69]. Monogeneans ectoparasites are in direct contact with the environment, thus more sensitive to changes in water parameters. Studies on monogeneans of fishes as biomarkers have been conducted under different approaches, such as effects of high concentrations of effluents, hydrological cycle of floodplain areas, heavy metal concentrations, trophic concentrations of reservoirs, and prevalence and abundance in lotic and lentic environments of a river basin ([70] and references therein).

Research on freshwater fish parasites in the PRF is relatively new and still scant. Before the present study the only fish parasites recorded from the PR was two monogenean species by Price et al. [71] from the gills of *Tilapia sparrmanii* and *Enteromius trimaculatus*, respectively (see [72]). Svitin et al. [73] studied the diversity of camallanid nematodes from two catfishes, providing novel information on their morphology and genetic data; Hoogendoorn et al. [74] studied the diversity of digenean metacercariae (Diplostomidae) and also provided novel information on the morphology and genetic data of these parasites from freshwater fishes; Smit et al. [75] recorded for the first time trypanosomes in freshwater fish and in their leech vectors from this region, providing morphological and molecular characterisation

of the haemoparasites; and recently, Schaeffner et al. [76] described a new cestode species from *S. zambezensis*. Information on the abovementioned records are shown in **Table 2** and some of the fish parasites are shown in **Figure 9**.

Parasite species	Locality	Host species	Reference
Trypanosomatida			
Trypanosoma mukasai	PR (NGR)	*Clarias gariepinus*, *Coptodon rendalli*, *Oreochromis mossambicus*, *Synodontis zambezensis*	[75]
Monogenea			
Characidotrema auritum	PR	*Brycinus imberi*	[77]
Pseudodactylogyrus anguillae	PR (NGR)	*Anguilla marmorata*	[78]
Cichlidogyrus papernastrema	PR	*Tilapia sparrmanii*	[71]
Dactylogyrus myersi	PR,	*Enteromius trimaculatus*	[71]
Macrogyrodactylus clarii	KP (NGR),	*C. gariepinus*	[79]
Macrogyrodactylus congolensis	UR (NGR)	*C. gariepinus*	[79]
Macrogyrodactylus karibae	KP, UR (NGR)	*C. gariepinus*	[79]
Quadriacanthus sp. 1	LN, KP, PR (NGR)	*C. gariepinus*	Present study
Quadriacanthus sp. 2	LN, KP, PR (NGR)	*C. gariepinus*	Present study
Digenea			
Diplostomum sp.	PR (NGR)	*S. zambezensis*	[74]
Diplostomum sp. 14	PR (NGR)	*Anguilla bengalensis labiata*, *O. mossambicus*, *S. zambezensis*	[74]
Cestoda			
Barsonella lafoni	LN	*C. gariepinus*	Present study
Tetracampos ciliotheca	LN, PR	*C. gariepinus*	Present study
Wenyonia gracilis	PR (NGR)	*S. zambezensis*	[76]
Nematoda			
Paracamallanus cyathopharynx	NL	*C. gariepinus*	[73]
Procamallanus pseudolaeviconchus	NL	*C. gariepinus*	[73]
Spirocamallanus daleneae	PR	*S. zambezensis*	[73]
Hirudinea			
Batracobdelloides tricarinata	PR (NGR)	*C. gariepinus*, *S. zambezensis*	[75]

Abbreviations: KP—KuShokwe Pan; LN—Lake Nyamithi; NGR—Ndumo Game Reserve; PR—Phongolo River; UR—Usuthu River.

Table 2.
Diversity of freshwater fish parasites naturally occurring in the lower Phongolo River and floodplain.

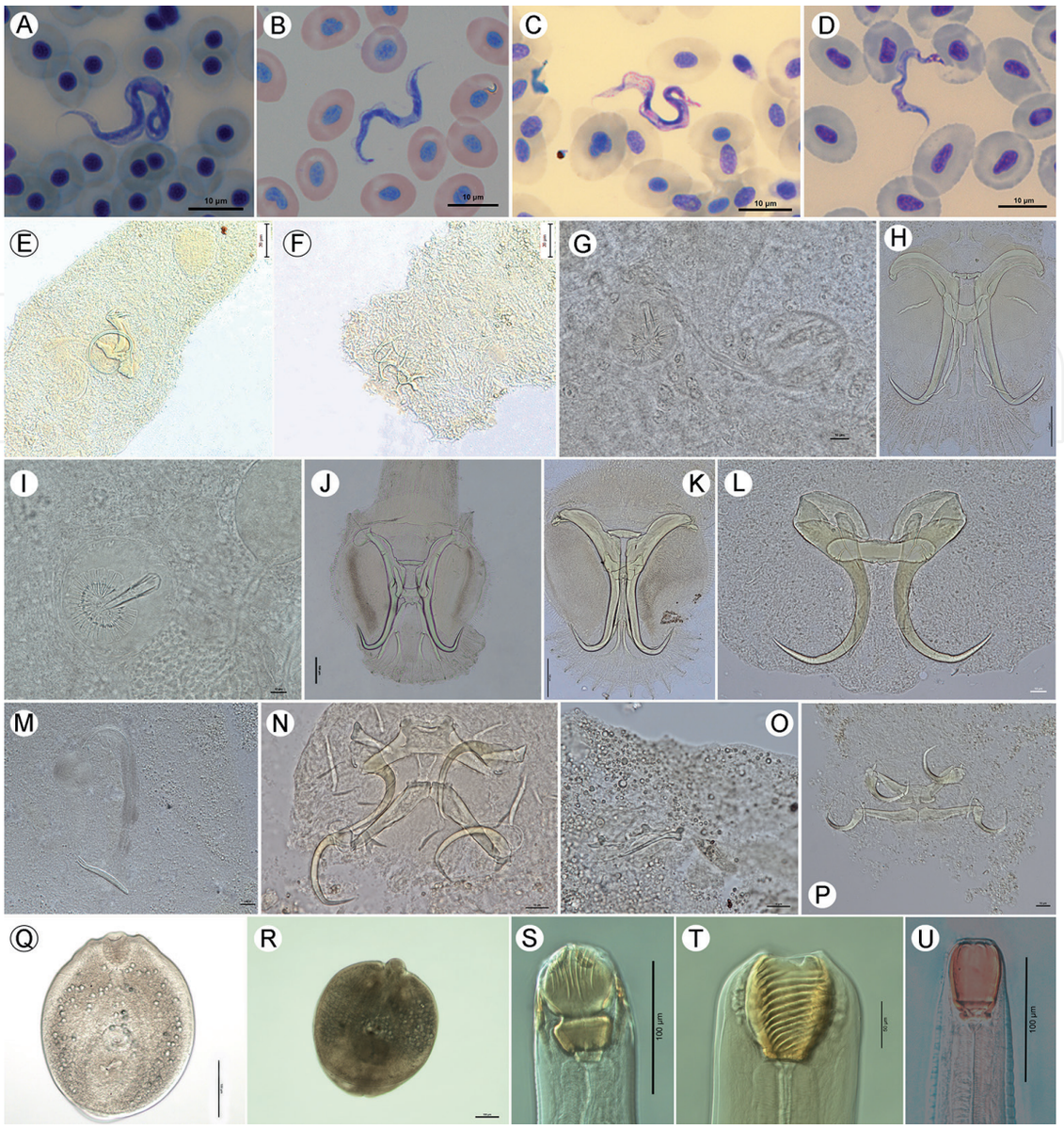

Figure 9.
Photomicrographs of some helminth parasites from freshwater fishes from the Phongolo River and floodplain. Trypanosoma mukasai *from: (A)* Clarias gariepinus*; (B)* Coptodon rendalli*; (C)* Oreochoromis mossambicus*; (D)* Synodontis zambezensis*.* Characidotrema auritum *(Monogenea: Dactylogyridae) from* Brycinus imberi*: (E) male copulatory complex (MCO); (F) haptor.* Macrogyrodactylus clarii *from* C. gariepinus*: (G) MCO; (H) haptor.* Macrogyrodactylus congolensis *from* C. gariepinus*: (I) MCO and (J) haptor.* Macrogyrodactylus karibae *from* C. gariepinus*: (K) haptor.* Pseudodactylogyrus anguillae *from* Anguilla marmorata*: (L) haptor; (M) MCO.* Quadriacanthus *sp. 1 from* C. gariepinus*: (N) haptor; (O) MCO.* Quadriacanthus *sp. 2 from* C. gariepinus*: (P) haptor.* Diplostomum *sp. 14 from* O. mossambicus*: (Q) total view.* Diplostomum *sp. from* S. zambezensis*: (R) total view.* Paracamallanus cyathopharynx *from* C. gariepinus*: (S) anterior end with detail of buccal capsule.* Spirocamallanus daleneae *from* S. zambezensis*: (T) anterior end with detail of buccal capsule.* Procamallanus pseudolaeviconchus *from* C. gariepinus*: (U) anterior end with detail of buccal capsule.*

Amphibians are well suited hosts for parasites, as most parasites rely on aquatic infective stages for transmission and reproduction, much in the same way that amphibians depend on extended exposure to aquatic systems for reproduction and their life history. This in turn increases the host–parasite contact rates. Frogs serve as host for most parasite groups including protozoans, monogeneans, digeneans, cestodes, acanthocephalans, nematodes and mites [50]. Furthermore, frogs are often infected with metacercaria where the frog serve as an intermediate host for a definitive reptile or bird host. However, parasites of frogs and other ectotherms have co-evolved over a long period of time and although these parasites are still true parasites per

definition, they seldom inflict adverse effects on their hosts, as compared to endotherm hosts [80, 81]. Parasites that have thus far been encountered in frogs from the PRF are presented in **Table 3**. Additionally, parasites reflect their host species' environmental interactions, revealing feeding behaviour, geographical ranges and social systems [87]. Blood parasites present good examples of these interaction based on their various transmission strategies, such as via consumption of an infected intermediate host or directly from a blood feeding vector. Frogs from the PRF were shown to harbour a number of protozoan blood parasites ranging from intracellular apicomplexan parasites to extracellular euglenozoan flagellates (see **Figure 10**) [85, 88].

Considering the diversity of fishes and amphibians of the lower PRF, future studies on the diversity of their parasites can reveal unique ecological characteristics of the environment, more insights on the drivers of host–parasite relationships like

Parasite species	Locality	Host species	Reference
Protista			
Dactylosoma kermiti	PR (NGR)	*Ptychadena anchietae*	[82]
Hepatozoon involucrum	PR	*Hyperolius marmoratus*	[83]
Hepatozoon ixoxo	PR (NGR)	*Hemisus marmoratus*, *Sclerophrys garmani*, *Sclerophrys gutturalis*, *Sclerophrys pusilla*, *Ptychadena mossambica*, *Ptychadena nilotica*	[84, 85]
Hepatozoon tenuis	PR	*Afrixalus fornasini, Hyperolius argus, Hyp. marmoratus*	[83]
Hepatozoon thori	PR	*Hyp. argus, Hyp. marmoratus*	[83]
Hepatozoon sp.	PR (NGR)	*Ptychadena anchietae*,	[85]
Haemococcidia sp.	PR (NGR)	*Pty. anchietae*, *Phrynobatrachus mababiensis*	[86]
Trypanosoma spp.	PR (NGR)	*Afr. fornasini*, *Hem. marmoratus*, *Hyp. argus, Hyp. marmoratus*, *Hyperolius tuberilinguis*, *Pty. anchietae*, *Pty. mossambica*, *Scl. gutturalis*, *Scl. pusilla*	[85]
Monogenea			
Polystoma vernoni	NGR	*Ptychadena oxyrhynchus*	Present study
Protopolystoma orientalis	NGR	*Xenopus muelleri*	Present study
Cestoda			
Cephaloclamys sp	NGR	*Xenopus muelleri*	Present study
Nematoda			
Cosmocerca sp.	NGR	*Kassina senegalensis*	Present study
Cosmocerca sp.	NGR	*Ptychadena anchietae*	Present study
Cosmocerca sp.	NGR	*Tomopterna tandyi*	Present study
Aplectana sp.	NGR	*Breviceps passmorei*	Present study
Camallanus kaapstaadi	PR (NGR)	*Xenopus mulleri*	Present study
Batrachocamallanus xenopodis	PR (NGR)	*Xenopus mulleri*	[73]

Abbreviations: NGR—Ndumo Game Reserve; PR—Phongolo River.

Table 3.
Diversity of parasites occurring in frogs in the lower Phongolo River and floodplain.

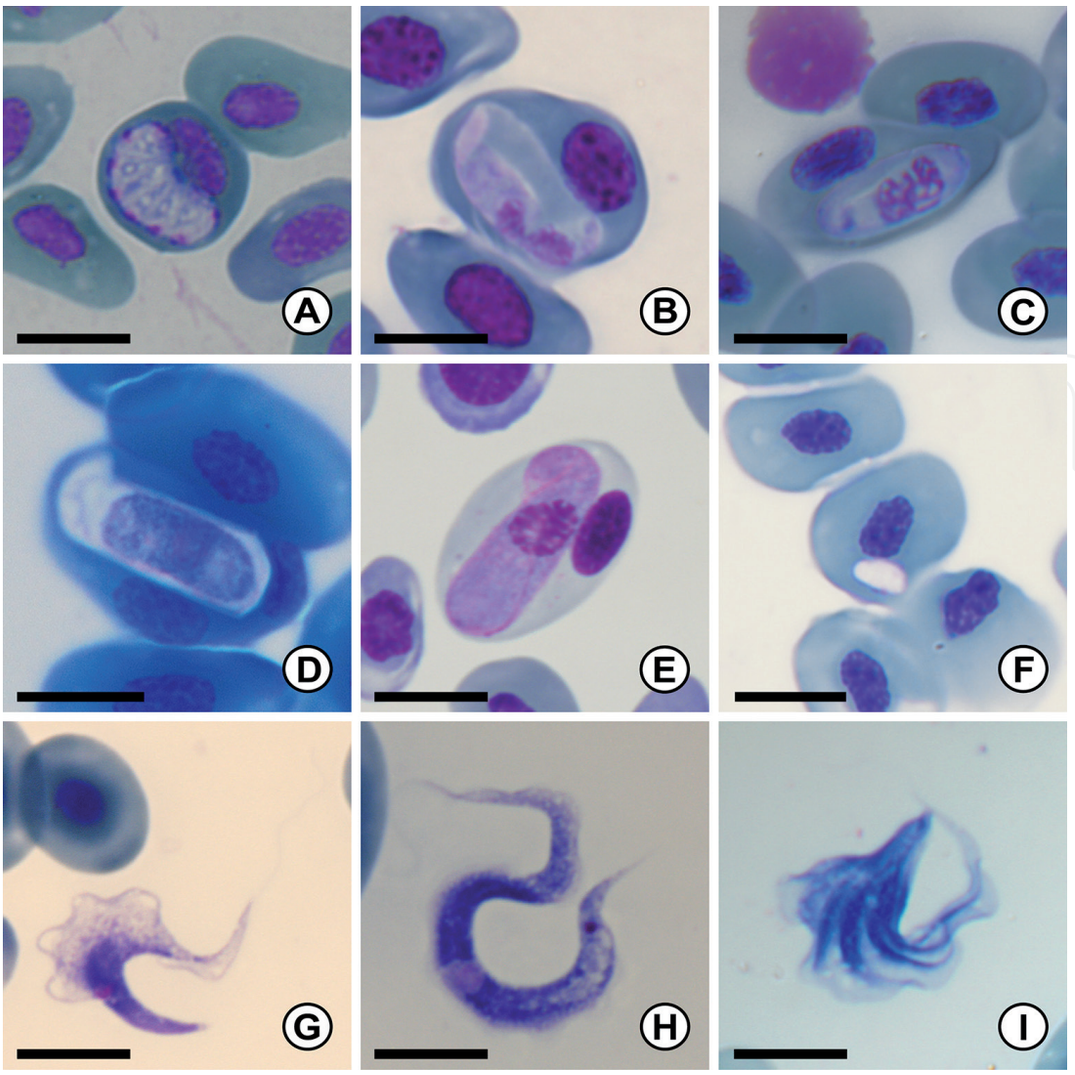

Figure 10.
Photomicrographs of various frog blood parasites encountered in the Phongolo River and floodplain. (A) Dactylosoma kermiti *infecting* Ptychadena anchietae*; (B)* Hepatozoon involucrum *found infecting* Hyperolius marmoratus*; (C)* Hepatozoon ixoxo *infecting* Sclerophrys gutturalis*; (D)* Hepatozoon thori *infecting* Hyperolius argus*; (E)* Hepatozoon *sp. infecting* Pty. anchietae*; (F)* Haemococcidia *sp. infecting* Ptychadena anchietae*; (G)* Trypanosoma *sp. infecting* Pty. anchietae*; (H)* Trypanosoma *sp. infecting* Scl. gutturalis *and (I)* Trypanosoma *sp. infecting* Sclerophrys pusilla*. Scale bar 10 μm.*

patterns of host-specificity specially for monogeneans, as well as unravel parasite species that are still new to science, thus increasing the knowledge about the aquatic biodiversity of South Africa.

3. Biological and physical stressors of aquatic organisms from the lower Phongolo River and associated floodplains

3.1 Invasive and alien aquatic species

Aquatic organisms are intentionally moved and introduced through several pathways and occasionally their introduction is accidental. Natural introductions of alien species occur when dispersed beyond its natural distribution range with connectivity of two geographic regions during floods (pans, rivers and streams) or migration of species that host parasitic organisms. Human mediated introductions are more common and deliberate when introduced for breeding, ornamental and recreational purposes (angling) or as a food source (aquaculture). Meanwhile, accidental

introductions arise with escapes from captivity (aquaculture), uncontrolled releases of angling and ornamental species, inter-basin water transfer schemes, underlying contaminants or as stowaways on vessels and aquaria or farmed species [89–91].

Contaminants, pathogens or parasites accompanying introduced host species such as aquatic plants, molluscs, crustaceans and fish species are only noticed with increased infection, disease or mortality of the associated hosts. Often when an alien host is introduced it loses some of its native parasites (enemy release), however alien species may acquire native parasites and amplify transmission of pathogens or parasites of the native hosts (spillback). Alien host species can also 'dilute' transmission and infection of native pathogens and parasites (see [92, 93]). In contrast to this, parasites and pathogens that are co-introduced only infect the alien host and is co-invasive once it spills over to native biota (see [94] and references therein, [95]). Once alien species are introduced and established in the novel environment, they can become pests or threaten native biota and are then invasive or co-invasive (for parasites).

Alien or invasive species can impact aquatic ecosystem services and biological diversity through predation, parasitism, competition, hybridisation, habitat use and food web alterations [96, 97]. Livelihoods dependent on freshwater ecosystem and its resources can also be affected if aquatic diseases arise because of alien or invasive species, influencing marketability and commercial value for species used as a staple by local communities.

To date, eight invasive species have been recorded from the PRF (see **Table 4**). The first invasive to be recorded in the lower Phongolo was the common carp *Cyprinus carpio* in 1993 [34]. Apart from competing for resources, impact on water quality and threat to larval populations of macro-invertebrates, *C. carpio* is a known host to a variety of co-invasive parasites. The widespread Asian tapeworm *Schyzocotyle acheilognathi* and the anchor worm *Lernaea cyprinacea* has been co-introduced into the PRF with *C. carpio* and has spilled over to two native small barb species *Enteromius annectens* (broad-stripped barb) and *Enteromius bifrenatus* (hyphen barb), and to the vulnerable native *O. mossambicus*, respectively (see **Table 4**). The presence of these two co-invaders is concerning as infections with *S. acheilognathi* is associated with high infection intensities and pathology of the gut lumen, while *L. cyprinacea* cause haemorrhagic ulcers on the body surface of fish, leading to increased susceptibility to secondary infections [100 and references therein]. A recent study in the lower Phongolo also confirmed that the overall health state of fishes can be compromised when heavy infestations with *L. cyprinacea* occur [102]. This is of concern since *O. mossambicus* is a vulnerable native species and plays important economic and ecological roles in the PRF (see Section 2.2).

Furthermore, three invasive freshwater snail species, a crayfish and its co-invasive parasite inhabits the waters of the lower Phongolo. The reticulate pond snail *Lymnaea columella*, *P. acuta* and *T. granifera* directly competes with and affect the distribution of three native freshwater snails species: the common pond snail *Lymnaea natalensis*, *Bulinus africanus* and the red-rimmed melania *Melanoides tuberculata* [2, 101, 103]. A single niche competitor, the invasive *C. quadricarinatus*, which escaped from aquaculture farms in Swaziland, was first recorded from the PRF in 2013 [99]. In addition to posing a threat through direct predation on tadpoles, habitat modification and the resident native Natal freshwater crab *Potamonautes sidneyi*, the *C. quadricarinatus* in the PRF also hosts a co-invasive temnocephalan *Diceratocephala boschmai* (see **Table 4**) [99]. To date, no spillover of this temnocephalan to native freshwater crab, shrimp and other branchiopods have been noted. However, it has been experimentally proven that one of three co-invasive temnocephalan species infecting invasive freshwater crayfish species in South Africa can utilise native freshwater crabs as a host [91]. Some of the invasive organisms mentioned are shown in **Figure 11**.

Species	Vector/pathway into RSA	Presence	Infection of native species	Reference
Diceratocephala boschmai (Platyhelminthes: Temnocephalida: Diceratocephalidae)	*Cherax quadriacanthus* (crayfish) escapes from aquaculture farm in Swaziland [98]	LN	No record of spread to native freshwater crabs, shrimps and other Branchiopoda.	[99]
Schyzocotyle acheilognathi (Cestoda: Bothriocephalidae)	*Cyprinus carpio* (common carp)	LN, PR, P	*Enteromius annectens*; *Enteromius bifrenatus*	[100]
Lymnaea columella (Gastropoda: Lymnaeidae)	Stowaway on aquarium plants		–	[2]
Physa acuta (Gastropoda: Physidae)	Stowaway on aquarium plants	LN, PR	–	[2, 101]
Tarebia granifera (Gastropoda: Thiarinae)	Stowaway on aquarium plants	LN, PR	–	[2, 101]
Lernaea cyprinacea (Arthropoda: Lernaeidae)	*Cyprinus carpio*	LN, PR	*Oreochromis mossambicus*; *Coptodon rendalli*	[2, 102]
Cherax quadricarinatus (Arthropoda: Parastacidae)	Escape from aquaculture farm in Swaziland [98]	LN, PR, UR	–	[99]
Cyprinus carpio (Cypriniformes: Cyprinidae)	Recreational angling	Various sites in lower PR	–	[34]

Abbreviations: LN—Lake Nyamithi; PR—Phongolo River; P—Pumphouse; UR—Usuthu River.

Table 4.
Introduced aquatic species present in the lower Phongolo system.

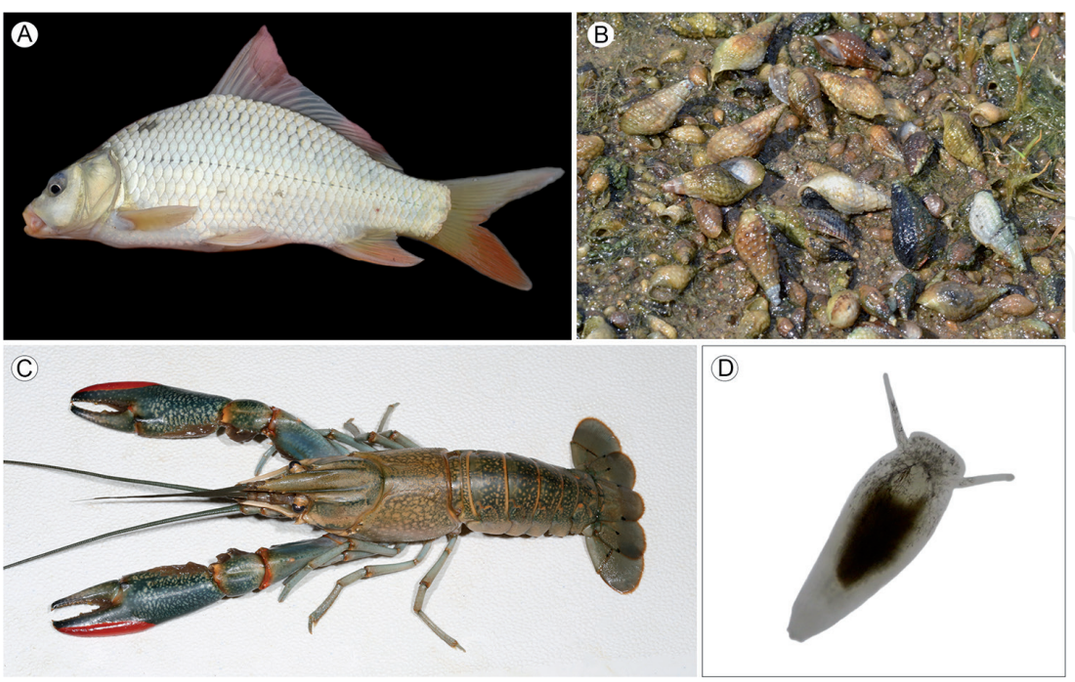

Figure 11.
Invasive species in the lower Phongolo River and floodplain: (A) Cyprinus carpio *(Cypriformes: Cyprinidae) (common length 31 cm); (B)* Tarebia granifera *(Gastropoda: Thiarinae); (C)* Cherax quadricarinatus *(Arthropoda: Parastacidae) (max. Length 35 cm) from Ndumo game reserve; (D)* Diceratocephala boschmai *(Platyhelminthes: Temnocephalida: Diceratocephalidae) (length 832–1500 μm) parasite from* C. quadricarinatus*. Information on length extracted from [32, 104, 105], respectively.*

3.2 Physical and chemical stressors

The construction of the Pongolapoort Dam (Jozini Dam) in the early 1970s is arguably the greatest agent of change in the PRF. Following the construction of the dam, the downstream terrestrial physical and chemical template of the system was irrecoverably altered with a complicated interaction between the benefits and threats derived from the damming of the PR. Prior to the construction of the dam, the subsistence fisheries and agriculture practices were synchronised with the natural floods and their flooding and alluvial sediment deposit regimes of the floodplain pans [3]. Since its construction, there have been various 'flood release' strategies to simulate the natural floods in the floodplain with varying degrees of success [4]. Recently, Brown et al. [106] developed a set of holistic environmental flow recommendations that would supposedly meet the ecosystem and agricultural water requirements. However, these have yet to be evaluated since the region has been in the grip of a long-term drought that has negated water releases, other than base flows of 8 $m^3 \cdot s^{-1}$, since 2015 to the present [1].

As mentioned previously, the assurance of water resources and potential for sustained larger-scale agriculture resulted in exponential growth in the human population that is directly or indirectly dependent on the PRF. These communities are considered to be among southern Africa's poorest and traditionally highly dependent on harvesting natural resources. For these impoverished communities, living on or near the PRF allows them to maintain subsistence agricultural activities, provide for livestock, water collection for household usage, religious activities, a source of protein and sustenance through fishing and lastly the harvesting of plants such as water lilies, reeds and thatching grass [2, 5].

The conversion of natural land cover to agricultural landscapes is one of the main causes of environmental degradation and is a known driver of pollution of surface waters and subsequent loss of habitats and biodiversity [107]. Between 1955 and 2003, 40% of the natural floodplain vegetation was transformed into agricultural land [5]. The inadequate accessibility to water leads to further exploitation of the limited water resources of the region. Recent years have seen a marked increase in informally installed pumps along the length of the river, mostly used to irrigate the fields of the subsistence farmers. The water systems of the floodplain are further contaminated by oil and fuel leaks from the aforementioned pumps and washing of clothes. Waste dumping is common practice in South Africa and is not solely limited to urban areas, as is evident throughout the rural communities surrounding the floodplain and this poses significant risks to human and environmental health. Such practices even created waste management issues within the NRG.

The excessive reliance on the floodplain and its resources could be considered unsustainable and thus leads to significant biodiversity losses. Less than 10% of the entire floodplain is formally conserved in the NGR. Outside of the reserve, the riparian forests and vegetation are removed, being the wood used as fuel and the cleared areas for subsistence agriculture (see **Figure 3**). In 2008, the communities along the eastern boundary took down the 11 km long fence to gain access to 1000 hectares of the reserve for resources and establishing subsistence agriculture. As such, the only intact riparian zone is a 3-km stretch along the western bank of the active channel of the PR within the reserve.

The altered water releases, drought and increased pesticide use in the floodplain has resulted in increased chemical stressors being released into surface and groundwater of the system. Historically, the floodplain has been subjected to fluctuations in drought periods and flood inundation. As a consequence, highly saline conditions (total dissolved solids in excess of 5000 $mg \cdot l^{-1}$) have been reported in the floodplain pans that are prone to seepage of salts from the underlying marine cretaceous

geological structure [3]. The salinities subsequently decrease when the pans are flooded during the next flood event. Due to the prolonged drought from 2014 and the absence of flooding since 2016, there has been a steady increase in electrical conductivity and nutrients. However, these levels still seem very similar to the historical conductivity and nutrient concentrations [1]. It would thus seem that the system is highly resilient to altered flow conditions with large fluctuations in water quality.

Large-scale organic pesticide application has been in place on the PRF for many decades. Dichlorodiphenyltrichloroethane (DDT) has been used for malaria vector control since the 1930s [108]. [109] studied the levels of pesticides in surface- and groundwater of water bodies of the PRF. They attributed the presence of pyrethroids (cypermethrin, λ-cyhalothrin and cyfluthrin), organophosphates (fenthion and fenitrothion) organochlorines (DDT and its metabolites—pp-DDD and pp-DDE) and carbamates (carbosulfan and carbofuran) to agricultural and malaria vector control applications. The first records of DDT in fish and other wildlife from the PRF were reported by [110]. [111] concluded that the high levels of DDT and its metabolites found in human serum and breast milk from the PRF could be attributed to a combination of consuming contaminated fish and direct exposure through spray drift.

Pesticide usage and subsequent spillage and run-off remain a major concern due to the numerous negative impacts on wildlife and ecosystems. Organochlorine pesticides (OCPs) are a group of pesticides which have been banned globally due to their persistence, attested by the quantifiable concentrations even though usage has been banned for over two decades, as well as consequent negative impacts on the environment [2, 112, 113]. [114] highlighted the renewed focus on in quantifying OCPs in aquatic ecosystems in and around the floodplain. These chemicals accumulate throughout the food web and represent a significant threat to the aquatic diversity of the floodplain. The floodplain and its surrounding area are still classified as a malaria endemic area and as such has consistently been sprayed with DDT for the purpose of malaria vector control. General pesticide use has also increased due to increasing agriculture activities. Recently, [114] recorded a range of pesticides, including discontinued pesticides such as lindane in five of the most economically and ecologically important fish species of the floodplain. Contamination levels in the floodplain were lower than other regions across South Africa. They are however a reason for concern due to the risks posed to both humans and animals reliant on the floodplain, including important protected and red data listed species such as the Nile crocodile (*Crocodylus niloticus*), pelicans (*Pelecanus onocrotalus*) and Saddle billed storks (*Ephippiorhynchus senegalensis*). As anticipated, DDT and its metabolites were the dominant OCPs and were shown to magnify through the food chain. DDTs are associated with several harmful effects on fish, crocodile and bird populations across the world, including but not limited to reproductive impacts, endocrine disruptions, eggshell thinning and ensuing population declines. Importantly, as OCPs do not adhere to any type of border, conservation efforts that are implemented within the region should strive towards better management and increased public education.

4. Conceptualising conservation approaches for the PRF

The previous sections explain the biodiversity and conservation importance of the PRF whilst also highlighting particular conservation challenges. In order to conserve this unique biodiversity, different conservation governance and/or management approaches exist. Therefore, this section briefly explains the different

approaches available in South Africa, for consideration in the Phongolo context. Although we explain the different approaches, we do not prescribe any specific approach. The entire suite of options needs to be considered to inform the design of a combined or hybridised tapestry of approaches, best suited to achieve the conservation objectives of the PRF. There is therefore no silver bullet or single solution when it comes to identifying conservation governance and management approaches. We frame our brief discussion around approaches and instruments already developed within the South African context more generally (for a more detailed discussion of these approaches and instruments see [115]). Three broad approaches are distinguished as illustrated by the three circles in **Figure 12**, namely: command- and control (CaC)-based, fiscal-/market-based and civil-based approaches. In relation to each approach, different so-called governance and management instruments are identified. Some of these instruments are considered hybridised instruments nesting between more than one approach, illustrated by the overlapping areas between the three circles numbered A, B, C and D.

The CaC approach includes those management instruments provided for by legal means. This is the most basic or classical approach centred on the understanding that the best way to control human behaviour is to enact laws and then enforce them—or the 'stick approach'. In terms of conservation, South Africa has a complex legal framework (for a detailed discussion of the legal framework see [116, 117]) and a myriad of instruments covering strategic and project level decision making. Strategic level instruments inform decision making at a policy and planning level such as the National Biodiversity Framework, Bioregional Plans, Biodiversity Management Plans and the proclamation of different protected areas (PAs). An example of these with in the PRF, is the formally proclaimed NGR. At project level CaC-based instruments include the issuing of permits and/or prohibition notices for legally defined 'restrictive activities' in relation to listed ecosystems and species (threatened or alien invasive). The protection of species through CaC has obvious relevance to the wealth of biodiversity in the PRF, highlighted in previous sections. While the CaC arrangements are considered critical for any conservation regime, failures of CaC instruments are well documented in the literature [118, 119]. The strengths of CaC are that it provides a high level of certainty by defining for example boundaries of PAs and prescribing behaviour in relation to listed species and ecosystems. The weaknesses relate to the resources required and time it takes

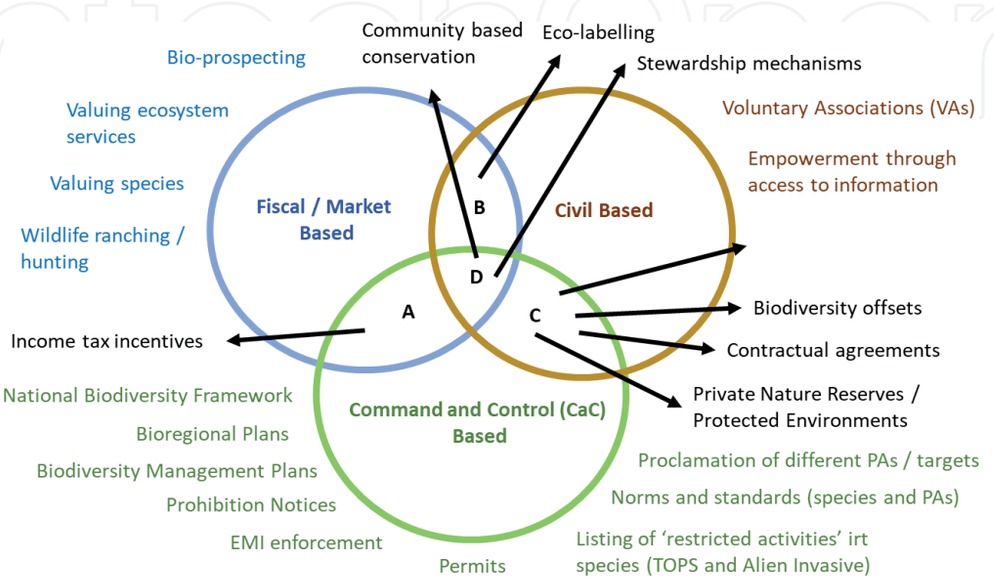

Figure 12.
Different conservation governance and management approaches within the south African context.

to ensure enforcement of these legal mechanisms. For this reason, additional approaches have emerged to supplement and support CaC arrangements such as fiscal- and civil-based approaches.

Fiscal-based approaches aim to use the market or price mechanism to change or incentivise certain behaviour, also sometimes referred to as the 'carrot approach'. In the case of conservation this refers to valuing ecosystem services and/or species within a market-based economy to incentivise their protection. The wildlife and bioprospecting industries are typically integral to this market-based system. The strengths of market-based instruments are the instant effect it seems to have on behaviour, unlike CaC-based instruments. However, pricing ecosystems and species have been controversial, especially in relation to the trade in endangered species – ivory and rhino horn a case in point. Market-based instruments are not well developed in the PRF and South African in general. This is mainly due to the significant inherent challenges in commodifying ecosystems and wildlife [120].

Civil-based instruments centre around empowerment of civil society in order to affect behaviour. The need for civil-based instruments derives from the acknowledgement that governments alone cannot deliver on conservation goals and objectives. Civil-based instruments include opportunities for civil society to organise themselves through for example voluntary associations (VAs) and empowerment of civil society through access to information on for example conservation-related matters. The PRF is an example of a highly complex civil society context with a range of civil society actors such as tribal authorities, farming unions, water user associations, etc. The advantage for the study area is that civil society seems well organised and represented. However, the challenge is to achieve a common understanding and general agreement on the future of conservation in the area amidst the range of actors.

In an ever-changing complex world, it is also evident that many of the most effective management instruments do not fit neatly into a specific approach but rather are designed as so-called hybrid instruments. For example, income tax incentives for landowners promoting conservation on their land is a clear hybrid between CaC and market-based approaches—see 'A' in **Figure 12**. This is because the tax incentive is incorporated into law, but the incentive is market based. We are not aware of any example within the PRF where this instrument has been used. Furthermore, instruments such as eco-labelling aims to make certain products more appealing to civil society or consumers based on their environmental performance—see 'B' in **Figure 12**. The hybrid approaches between civil- and CaC-based approaches shown as 'C' in **Figure 12** have a high level of potential relevance to the study area. These include biodiversity management agreements or contractual agreements between private or communal landowners and the state. The conservation status of private or communal land can also be formalised trough different kinds of protected areas such as Private Nature Reserves (PNRs) and Protected Environments [121]. An extension of the latter in recent years has been so-called community-based conservation (CBC) and conservation stewardship programmes that are considered the most integrated conservation instruments combining CaC, fiscal- and civil-based approaches—see 'D' in **Figure 12**. For example, the stewardship programme combines different levels of legal protection with money saving incentives and strong community involvement and ownership. Much research has been conducted on the success and failures of CBC generally, but also within the KZN Province, which could inform the future application within the study area (see [122–124]).

There are two guiding principles when considering these approaches, firstly the use of multiple approaches and instruments is preferred especially within

a complex and multi-faceted context such as the PRF. Multiple approaches also enhance the redundancy effect by ensuring multiple possible solutions for a range of conservation challenges. Secondly, hybrid approaches are preferred (as represented by A, B, C, and D in **Figure 12**) that aim to optimise and merge the strengths of different approaches into single instruments. Ultimately the design and selection of conservation approaches for the PRF will depend on the agreed context specific conservation goals and objectives.

5. Conclusions

It is clear from the information presented in the preceding sections that South Africa's PRF is, in terms of aquatic organisms, highly diverse and of national and international importance. However, the aquatic ecosystem of the PRF is also under extreme pressure from a vast array of human activities, ranging from broadscale influences such as climate change and associated extreme weather events to local-scale impacts such as pollutants, over utilisation and invasive species. Interestingly, the PRF ecosystem has also exhibited tremendous resilience in dealing with all these anthropogenic stressors. Despite more than 10-fold increase in the human population depending on the ecosystem services provided by the PRF, a 5-year ongoing below-rainfall period with no flood release from the Pongolapoort Dam and reduction in the size of the area under formal protection, there is currently no evidence that points towards a loss in species diversity. However, at least for fishes, Smit et al. [2] showed that although all species are still present, there has been a clear shift in the community structures and dominance. The main question for the PRF therefore still remains; how long, and especially in the light of the continued lack of flooding events and increasing human settlement and activities, can the socio-ecological system of the PRF stay resilient before complete collapse?

To answer this very important question, we propose that future research into this and similar systems in Africa and globally should follow the One Health approach. The One Health approach deals with a multidisciplinary and collaborative approach to ensure optimum health of humans, animals and the environment. The importance of, and need for, this approach has really come to the front during the Covid-19 pandemic of 2020 where the world was brought to a standstill due to a virus that originated from an animal (zoonotic disease). Therefore, studies on specifically environmental health are urgently required in order to gain a better understanding of the causes and consequences of anthropogenic activities, and how these in return have an impact on human health. Future research should thus aim at investigating the possible natural hazards associated with effects of climate change (i.e. droughts and floods), in combination with environmental pollutants, on the severely threatened aquatic ecosystem of the PRF. Specific research objectives should include:

- Determine the risk and possible impact of climate change on water quantity and quality, and food security.

- Identify different water quality governance approaches and instruments to ensure environmental conservation and human health protection amidst a changing climate.

- Identify dimension of vulnerability and characteristics of resilience that make local communities more, or less, susceptible to water-related disaster risks as a result of climate change.

- Monitoring the introduction and spread of invasive aquatic animals (both free-living and parasitic).

- Develop and validate holistic environmental flows to maintain and support ecosystem structure and functions.

- Develop and apply a risk assessment framework that integrates the ecological, human and wildlife factors to evaluate the socio-ecological consequences of climate change-induced changes in water quality and quantity.

Although the abovementioned recommendations are specifically proposed for the PRF, these research questions, aims and objectives are applicable to all threatened floodplain systems globally.

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Conflict of Interest

The authors declare no conflict of interest.

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