Metazoan parasites of anurans from the Vhembe area, Limpopo, South Africa

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THIS DISSERTATION IS DEDICATED TO
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DON T BE A FISH; BE A FROG. SWIM IN THE WATER AND JUMP WHEN YOU HIT THE GROUND."
- KIM YOUNG-HA.

ABSTRACT

Amphibians were the first vertebrate group to appear on land about 350 m.y.a and have diversified and colonized all suitable continents and islands. Amphibians are hosts to vast numbers and an astonishing diversity of parasites, representing all parasitic groups. These include protozoans, nematodes, acanthocephalans, monogeneans, digeneans, cestodes, leeches and mites. This impressive parasite diversity can be explained by the fact that amphibians are closely associated with water, which facilitates parasite transmission and they play the middle role in food chains - being both predators and prey. Amphibian parasitic fauna is generally poorly understood and understudied; this is especially true in southern Africa, where over 170 amphibian species have been identified. This study will show that we discovered a vast diversity of parasites in 22 different anuran species, from the previously unstudied area.

A large number of parasites were found using morphological, molecular and statistical approaches and several of them appear to be distinct from all known species and are, most likely, new to science. During this project 269 specimens of frogs representing 22 species were collected, dissected and studied for parasites. This research revealed 35 species of metazoan parasites, including 24 nematode species, two cestode species, three trematodes, three monogeneans, one acanthocephalan, one mite and one annelid species. The most infected amphibian species were the common river frog, *Amietia delalandii* and the African clawed frog, *Xenopus laevis* which harboured six and five helminth species, respectively. Nematodes were the most common parasites, accounting for 69% of the faunal community (24 of 35 species). We discovered numerous species that are morphologically and genetically different in a rather small research region and within only 22 species of anurans. Understanding and researching host-parasite interactions in nature will help to close the knowledge gaps in disease and community ecology. Parasites are everywhere, diverse, and inventive and we believe that incorporating them into basic biological research will be a significant stride forward for decades to come.

Keywords: Africa, Amphibia, Anura, Metazoan parasites, Community analysis

OPSOMMING

Amfibieë was die eerste gewerwelde diere wat ongeveer 350 m.j. op land verskyn het, en het sedertdien gedeversifiseer en alle bewoonbare kontinente en eilande gekoloniseer. Amfibieë tree op as gashere vir groot getalle en 'n verstommende diversiteit van parasiete wat alle parasitiese groepe verteenwoordig. Dit sluit in protosoë, nematode, acanthocephale, monogeniër botte, digeniër botte, lintwurms bloedsuiers en myte. Hierdie indrukwekkende parasietdiversiteit kan verklaar word deur die feit dat amfibieë met water, geassosieer word wat die oordrag van parasiete vergemaklik. Verder beset hulle 'n middle trofiese vlak in voedselkettings, en tree op beide as roofdiere en prooi. Die parasietfauna van Amfibieë regoor die wêreld word relatief min bestudeer. Dit is dan ook die geval vir Suider-Afrika waar meer as 170 amfibieërs tans bekend is (Channing and Rodel, 2019), maar slegs enkele parasietopnames op paddas is al in Afrika Suid van die Sahara gedoen.

In hierdie studie poog ons om te wys dat binne 'n beperkte geografiese gebied ons in 22 paddaspesies 'n groot verskeidenheid parasiete gevind is, Van hulle is morphologies en geneties uniek en verskil van alle voorheen bekende spesies. Deur gebruik te maak van morfologiese, molekulêre en statistiese benaderings is 'n groot aantal parasiete geïdentifiseer en verskeie van hulle blyk nuut te wees tot die wetenskap.

Tydens hierdie projek is 269 ampibieë, wat 22 paddaspesies verteenwoordig, versamel, gedissekteer en vir parasiete bestudeer. In totaal het ons 35 spesies metazoïese parasiete tydens hierdie opname gevind, insluitend 24 spesies rondewurms, twee lintwrums, drie trematode, drie monogeniërs, een haakwurm, een myt en een annelied spesie. Die mees besmette padda spesies was die gewone rivierpadda, *Amietia delalandii* en die gewone Platanna, *Xenopus leavis* wat onderskeidelik ses en vyf parasiet spesies gehuisves het. Rondewurms was die mees volopste parasiete met 'n prevalensie van 69% (24 van 35 spesies). In 'n relatiewe klein studiegebied, en in slegs 22 spesies paddas, het ons talle parasietspesies gevind wat morfologies en geneties verskil van alle voorheen bekende spesies. Ons resultate toon dat parasiete, verskillende taksonomiese groepe verteenwoordig. Om die interaksiepatrone en determinante van die gasheer-parasiet-assosiasie te verstaan, is dit nodig om die kennisgapings in beide gemeenskap- en siekte-ekologie te vul. Parasiete is oral, divers en kreatief en ons glo dat hul integrasie in fundamentele biologiese studies, 'n groot stap sal wees vir dekades wat kom.

Sleutelwoorde: Afrika, Amfibieë, Anura, Metazoïese parasiete, Gemeenskapsanalise

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CHAPTER 1:

AMPHIBIAN INTRODUCTION AND PARASITES

1.1 STUDY RELATED BACKGROUND

Of the three extant Orders of amphibians only anurans are found in South Africa. Caecilians are found in central Africa; while salamanders do not occur in the Ethiopian Realm. Despite the fact that frogs are the only order in southern Africa, the region is blessed with an impressive diversity. They represent a wide range of shapes and sizes, as well as life histories, calls, habitats, colours and phylogenetic diversity. Anurans are very much a part of South Africa's natural heritage, representing a large portion of the vertebrate biodiversity (Measey, 2011). In terms of amphibian species richness, South Africa is the 27th most diverse country in the world, and it's the fifth most diverse country in the Afrotropical region (Blasco-Costa *et al.,* 2017; Stuart *et al*., 2005). The parasite fauna of Amphibians across the globe is poorly understood and understudied and this is especially true for southern Africa where more than 177 amphibian species are currently known (Du Preez & Carruthers, 2017). Prior to the 2010 evaluation, major threats to amphibians at the global and national levels revealed that invasive and other problematic species and genes account for 15.7 % of all global species and an incredible 37.1 % of all South African species (Measey, 2011).

The present study was not only aimed at identifying anurans and their harboured parasites, belonging to different taxonomic groups, it also assessed the parasite community from the Vhembe area, Limpopo Province, South Africa. Understanding the connections between environmental changes and developing diseases in wildlife populations is one of the most difficult tasks facing ecologists and parasitologists today (Koprivnikar *et al*., 2012). Nearly a third of all amphibian species are in decline (Koprivnikar *et al*., 2012), making them the most endangered vertebrate group on the planet. With this hazard exposed, parasitologists, have discovered that metazoan parasite infections also influence amphibian hosts (Koprivnikar *et al*., 2012). Parasites, for example, affect amphibian development and reproduction rates, which has a significant impact on frog population fluctuations (Holmstad *et al.,* 2005). Among the metazoan parasites that are widespread among amphibians are monogeneans, trematodes, cestodes (tapeworms), nematodes (roundworms) and acanthocephalans (spiny-headed worms). Investigating the diversity of helminths in amphibians, is a cheap and dependable way of learning about host distribution, activity and sensitivity to environmental changes (Byers *et al*., 2011). In both aquatic and terrestrial food webs, amphibians serve either as intermediate, paratenic or definitive hosts to a variety of parasites (Koprivnikar *et al*., 2012).

As a representative group, free-swimming trematode larvae (cercaria) emerge from snails (first intermediate hosts), enter a variety of tadpoles and grow into an encysted stage or another

mobile larva stage (Koprivnikar *et al*., 2012). Amphibians are frequently used as intermediate or paratenic hosts by these parasites. The most common modes of infection include direct penetration and/or ingestion of juveniles or eggs. Although endoparasites predominate in amphibians' parasite ecology, ectoparasites are also encountered. These include leeches, mites and monogeneans which can be typically found on the gills of tadpoles but later as adults in the bladder of amphibian hosts(Koprivnikar *et al*., 2012). Monogeneans have a direct life cycle that does not require the presence of an intermediate host. Arthropods, however, are known as the ectoparasites of amphibians and a variety of them such as mites, have been reported on adult frogs. The host's exposure to infections, transmission pathways, and parasite development can all be influenced by a range of environmental conditions (Koprivnikar *et al*., 2012). Because hosts and parasite diversity are closely related, parasites have a lot of potential to be used as markers of environmental stress, food web structure and biodiversity (Thieltges *et al*., 2011). Amphibians have long been regarded as sentinel species for environmental distresses because they live in a variety of habitats (both aquatic and terrestrial), have a biphasic life cycle and occupy a variety of trophic niches (Kerby *et al*., 2010). As a result, parasites were proposed to have even more potential as bio-indicators. The study of the overall richness and composition of amphibian metazoan parasite communities can reveal important details regarding food web structures and the connections between terrestrial and aquatic environments (Koprivnikar *et al*., 2012). Furthermore, Koprivnikar *et al*. (2012) added that research efforts on amphibian' parasites mainly focus on larval trematodes and nematodes, which provides a great opportunity for future research on other groups and species (Koprivnikar *et al*., 2012). For the terms nematodes, trematodes, cestodes, acanthocephalans and monogeneans, a search in the Web of Science database from August 1899 to 2011, yielded the following numbers of results: nematodes (1975), trematodes (1004), cestodes (293), acanthocephalans (211) and monogeneans (244).

1.2 AIMS AND OBJECTIVES OF THE STUDY

1.2.1 AIM

Within the Vhembe ecosystem in the Limpopo Province, South Africa, the study aims to provide insight into the diversity of metazoan parasites from anuran species and provide a detailed checklist of hosts and their metazoan parasites. According to the aims the following **objectives** arose:

1.2.2 OBJECTIVES

- collect helminthological material from representative samples of as many different frog species in Vhembe area, Limpopo, South Africa, as possible;

- identify collected parasites based on morphological features;
- obtain molecular data of nuclear and mitochondrial gene markers for selected parasites where taxonomic uncertainties exist;
- compile a detailed check list of hosts and their metazoan parasites;
- investigate and establish the geographical distribution of anuran species in the study area and correlate their metazoan parasite diversity by applying GIS tools;
- compile a report on the parasite communities in the Vhembe area.

1.3 CLASS AMPHIBIA

As far back as human history can be traced, amphibians have been part of human society. Ancient people discovered these four-legged creatures hiding beneath massive logs and trees (Wake and Koo, 2018). Amphibians are regarded as beneficent and harmless creatures that consume hazardous insects and provide an alternative food supply for some societies around the world. Following that, in the 1980's, amphibians were in a general decline all over the world, and as a taxon, they were in more danger of extinction than any other vertebrate group, which surprised most biologists. Amphibians are represented by three Orders: Anura, which includes frogs and toads; Caudata, which includes salamanders and newts and Gymnophiona, which includes limbless caecilians (Arnold and Ovenden, 2002).

As the oldest terrestrial vertebrates amphibians include forms dating back to the early Mesozoic. According to Wake and Koo (2018), amphibians appear to have survived the mass extinction which occurred at the end of the Cretaceous period, relatively unscathed. Amphibians are ectothermic, tetrapod animals that live in a wide range of habitats, with the majority of species inhabiting terrestrial or freshwater aquatic systems. Based on the palaeontological data, first amphibians originated from sarcopterygian fish with lungs and bony-limbed fins, which helped them adapt to dry land during the Devonian period, some 370 million years ago (Blackburn and Wake, 2011). Amphibians have evolved characteristics that allow them to survive in terrestrial environments for longer periods of time. The bones evolved to become stronger, allowing them to support the weight of their body on land.

According to Frost (2021), there are currently 8393 amphibian species known, with Anurans accounting for nearly 88% of them. The three Orders that make up the Class Amphibia will never be confused since their appearances are so different. However, they all have one thing in common: a moist skin region that serves as their major respiratory organ. Amphibians require a unique type of habitat in a moist environment to keep their skin protected. In the event of changes in environmental

conditions, amphibians are the first to perish especially when habitats are disrupted or contaminated with chemicals.

1.4 ORDER ANURA

The Order Anura meaning “without tail”, comprises mostly the frogs and toads. Anurans have extended hind limbs, shorter fore-limbs, webbed toes, large eyes and granular moist skin. Species representative of this order consisting of smooth skin are classified as frogs, while species with warty skins are known as toads. The order Anura is home to 88 percent of the world’s amphibian biodiversity (Frost, 2021). *Craugastoridae*, *Hylidae*, *Microhylidae* and *Bufonidae* are just a few of the 55 families represented. The *Bufonidae*, or true toads, have the greatest diversity of species of all Anura species (Frost, 2021).

Frog fossils have been discovered on every continent on the planet (Evans *et al.,* 2008). The bones of a 40-million-year-old helmeted frog were discovered by a group of vertebrate palaeontologists investigating Seymour Island on the Antarctic Peninsula in 2020. This discovery suggested that frogs, similar to those found in the South American Nothofagus woods, once lived on the Seymour Islands (Mörs, Reguero and Vasilyan, 2020; Joel, 2020; Darlington, 1948).

Although most species are connected with water and moist environments, some have evolved to live in trees or even deserts. Tropical rainforests are known for having a high concentration of anuran species. Frogs are found all across the world, from the tropics to the subarctic, although tropical rainforests have the largest diversity of anuran species (Evans *et al.,* 2008). Some of these species have become increasingly isolated as a result of climate change or an unwelcoming habitat, such as stretches of sea, mountain ridges, deserts, or other man-made barriers (Evans *et al.,* 2008). Because the vast majority of species have limited ranges and live in unique settings, they are disproportionately affected by shifting land use and habitat degradation (Sintayehu, 2018). Anurans rely on specific adaptations to survive in harsh conditions especially for breeding seasons.

Anurans serve as prey for a variety of predators in various food webs, making them an important component of most ecosystems (Wake and Koo, 2018). Frogs emit a vast range of vocalizations, especially during breeding seasons and engage in a wide range of complicated behaviours to attract mates, ward off predators and survive in general. Frogs are highly regarded as a food source for humans and they also frequently play a variety of cultural functions in literature, symbolism and religion. They're also environmental indicators, so when frog populations drop in a given location, it is generally taken as a sign of impending environmental damage or disruption (Aho, 1990). Adult frogs eat invertebrates such as arthropods, worms, snails and slugs, making them genuine carnivores. Other smaller frog species, small animals and fish are prey for larger frogs. Many predators attack fully-grown frogs, including herons, hawks, fish, giant salamanders, snakes, raccoons, skunks, mink, bullfrogs and other creatures. (Fig. 1)

Figure 1: Huntsman spider capturing tree frog after luring it into a leaf trap and then feeding on the frog. Adapted from https://www.livescience.com/madagascar-spiders-catch-frogs.html

1.5 SOUTH AFRICAN ANURANS

South Africa, which is part of the Afrotropical region, is rated ninth in the IUCN Red List of Threatened Species in terms of total native species. There are currently 118 anuran species on the Red List, 51 of which are endemic or limited to a specific geographic location. Eighteen frogs (35%) are endemic to South African regions and are listed as endangered by the IUCN.

Thirteen of the world's 33 anuran families can be found in southern Africa. These 13 families are grouped into 34 genera, each of which has a DNA link to a common ancestor. According to Du

Preez and Carruthers (2017) there are currently 170 species of frogs described in southern Africa, with new ones being discovered on a regular basis as new habitats are explored and technology improves. Southern African frogs can be classified into three primary geographical categories based on their distribution (Carruthers, 2016). The northern and north-western parts of South Africa are inhabited by tropical species, which account for 33% of all species. These species' range extends to Africa's tropical regions, with only a few species found south of the Orange River and Tugela River (Carruthers, 2016). Transition species which make up a total of 47% of all species, subsists in the eastern regions of South Africa, exposed to high rainfall, however, most of the Cape species are endemic to the southern parts of South Africa (Carruthers, 2016).

South Africa is a biologically rich country with diverse landscapes including nine major terrestrial biomes ranging from wet, tropical regions to deserts (Mucina and Rutherford, 2006). However, South Africa is still regarded as a semi-arid country with a low number of permanent wetlands consisting of localized stagnant water (Minter, 2011). Drinkrow and Cherry (1995) reported high anuran species richness around the KwaZulu-Natal coast, the northern portions of Durban, the arid savanna of northern and eastern Gauteng, the grassland biome near Pietermaritzburg and the Afromontane forest of the Natal Drakensberg (Drinkrow and Cherry, 1995).

Wetlands are the third most important life support system on Earth (Schrader, 1991). According to research conducted by Taylor *et al* (1995), South Africa has a small number of wetlands and more than a third of these habitats have been degraded or lost. They also discovered that in locations where vulnerable frogs congregate, nearly all wetlands disappeared (Taylor *et al.,* 1995). Natural events can cause some wetlands to become seasonal and biologically active at different periods, in addition to rainfall and seasonal change. Wetland habitats produce a vast range of biotic diversity, giving this ecosystem a high priority for species conservation and protection. Frogs have soft, permeable skin and while they have several adaptations to help them conserve water, they are generally restricted to moist areas when they are active. Except for those residing in permanent wetlands, most frog species spend a considerable portion of the year dormant due to the risk of desiccation and/or a lack of prey.

Some species in wetlands may burrow into the mud or damp subsoil of sites where water gathers during the wet season. Frogs also use reeds, grass tufts, rocks, rock crevices and the burrows of other animals such as mice, as shelter (Channing and Van Dijk, 1995). Small wetlands are particularly important for frogs and they have a larger impact in the metapopulation dynamics of some taxa than their small size would suggest (Gibbs, 1993). Despite the fact that many anurans are habitat selective and dependent, their sensitivity allows them to be utilized as markers of environmental stress and the consequences of changing land use (Davis *et al.,* 2019). Many anuran species in South Africa live in habitats other than big permanent wetlands (Russell and Downs, 2012). Some amphibians, such as the primarily aquatic clawed frog, *Xenopus laevis* (Daudin, 1802), require permanent water supplies, while others, such as the common river frog, *Amietia delalandii*

(Dumeril et Birbon, 1841) prefers shallow streams or rivers (Measey, 2011). Others like the African bullfrog, *Pixychepahlus adsp.esus* (Tschundi, 1838) exclusively inhabit wetlands or protected areas (Du Preez and Cook, 2004). Species, such as the Mozambique rain frog, *Breviceps* spp. do not need open water to survive and breed; instead, they live and breed in leaf litter, under fallen trees and in burrows (Maesey, 2011).

1.6 ANURAN THREATS

Amphibians tend to be vulnerable to environmental changes and the factors that contribute to amphibian extinction are varied and complicated. This includes causes such as habitat degradation, felling trees in indigenous forests for timber and replacement of these land uses with agricultural operations, as well as urbanization and wetland drainage. Other factors include the extensive use of pesticides and fertilizers, as well as the growing effects of climate change. According to Wake and Koo (2018), amphibians are key components of ecosystems throughout the world, playing the role of both predator and prey in food chains. According to a report published by Stuart *et al.* in 2004, 43 percent of amphibian species are in decline. Amphibian populations and species are endangered or declining in many places of the world (Scoccianti, 2004). Frog population declines have been documented throughout studies, with Stuart *et al*. (2004) and Netherlands *et al*. (2015) reporting that amphibians are still the most endangered vertebrate group, with considerable diversity declines since the 1970s. More research on the topic of frog population losses has revealed that various components and factors, as previously noted (Beebee and Griffiths, 2005), may contribute to the declines.

Amphibian diseases have an impact on anuran populations and variety and may be the cause of mass fatalities. However, while much research has focused on the fungal disease, chytridiomycosis (amphibian chytrid), anurans can also be hosts to a wide range of ecto- and endoparasites (Du Preez and Carruthers, 2009). Despite the fact that haemoparasites receive a lot of attention, there has not been much parasite research, or many frog surveys, carried out in southern Africa and some information concerning the host-parasite relationship is still unclear (Readel and Goldberg, 2010). More research on metazoan parasites is needed to fully comprehend the consequences parasites may have on their natural hosts. Only then can the impact that parasites may have on anurans' conservation, be determined (Netherlands *et al*., 2015). Another factor contributing to the decline of wetlands is human interaction with natural river ecosystems, which is gradually altering. As a result of the events described above, the natural dynamics of these water bodies have changed considerably. Introduction of new farming techniques, which result in the transformation or even elimination of substantial parts of structural elements such as hedges, woodlands, lakes and scrublands, is one of the most common dangers and issues for amphibian species. All of these structural features serve as primary habitats or microhabitats for most amphibians, allowing them to breed or feed (Scoccianti, 2004). As we know, for anurans to survive and complete a life cycle, they mainly depend on both aquatic and terrestrial habitats (Scoccianti,

2004). Thus, the deterioration and progressive disappearance of structural elements may have a dramatic negative effect on population conservation in many regions. Nowadays, agriculture constitutes one of the most serious and widespread sources of dispersion of synthetic toxic compounds into the environment. Because amphibians have a complicated life cycle, they might come into contact with these substances, in both the environments that they inhabit (Scoccianti, 2004). The taxonomic and geographical database for all South African vertebrates, excluding fish, is inadequately maintained and studied, according to Shackleton, (2000) and more than half of protected areas lack correct, or completed, species lists.

1.7 VHEMBE BIOSPHERE RESERVE

This 'one-of-a-kind' reserve is found in the north-eastern sections of South Africa's Limpopo Province, near the borders with Botswana, Mozambique and Zimbabwe (Linden *et al.,* 2013). The reserve includes the Kruger National Park, Mapungubwe National Park and World Heritage Site, as well as a variety of Provincial Nature Reserves, two acknowledged centres of biodiversity and endemism, such as the Soutpansberg and Blouberg Reserves and finally, there's the Makgabeng Plateau, which has tens of thousands of rock art locations (Linden *et al.,* 2013). However, the area has a relatively large and rapidly growing human population along with high unemployment rates. These pose a severe threat and negative impact on this reserve's natural resources, which are being harvested in an unsustainable manner by the locals (Evans, 2017).

The Vhembe Biosphere Reserve (Fig. 2) consists of three biomes, savannah, grassland and forest biomes; with four bio-regions home to 23 different vegetation types or biotopes (Linden *et al.,* 2013). Eight bio-regions are endemic to South Africa, out of the total of 23. There are 250 species of butterflies, 44 species of anurans, 140 species of reptiles, 542 species of birds and 152 species of mammals that live in the area. The Vhembe Biosphere Reserve is the largest biosphere reserve in South Africa, with a surface area of 30 701 km (Linden *et al.,* 2013).

According to Evans (2017) there are three key functions of the Vhembe Biosphere. First, the conservation which aims to identify areas that are important to "contribute to the conservation of the hierarchy of bio-diversity, including landscapes, eco-systems, species and genes". Second, the goal is to "promote socio-culturally and environmentally sustainable economic development." As a result, all Biospheres allow and encourage development within their boundaries. Finally, their research which aims to promote local, national, international conservation and development concerns through research, plant and animal species monitoring, education and information exchange.

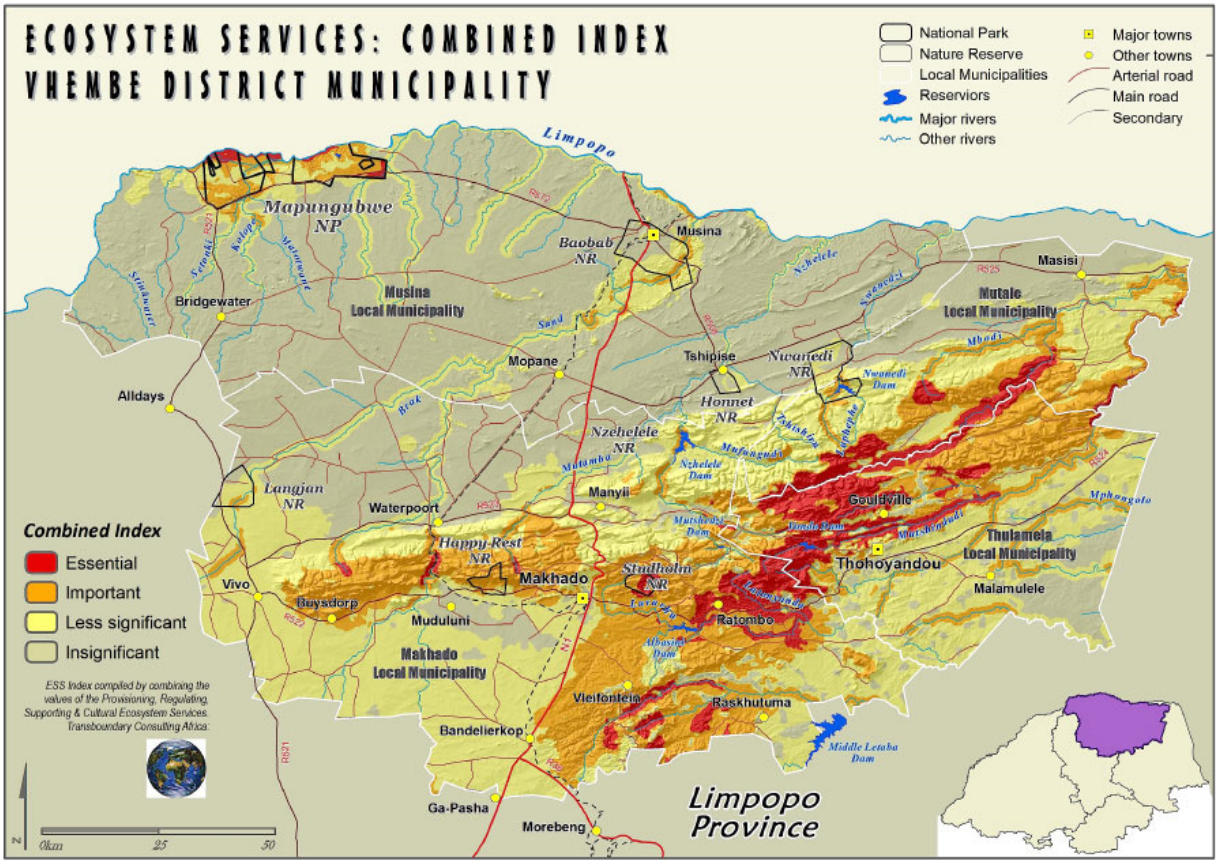


Figure 2: An assessment of land cover change as a source of information for conservation planning in the Vhembe Biosphere Reserve. Adapted from http://www.profwillemvanriet.com/blog/maps

1.8 ANURANS STUDIED

Of about 40 species that might be found in the study area, 22 were collected in representative (more than 10 specimens) samples and studied for parasites. Below is some basic information on each of the species researched.

Amietia delalandii (Duméril and Bibron, 1841) (common river frog) is a species of the southern African river frog. It can differ in colour from green to brownish and has dark stains along its back. The common river frogs have a definite pointed snout and a white smooth stomach; however, dark spots may appear. These frogs have large hind legs that help them avoid predators and catch prey. *Amietia delalandii* is found in the highlands of southern and eastern Lesotho and in the permanent mountain streams originating in the Drakensberg escarpment, with a wide distribution throughout South Africa.

Breviceps adsp.ersus Peters 1882, (bushveld rain frog) has a body similar to a toad and tends to bloat when threatened. These frogs are dark brown in colour with orange-brown stains. The stomach is smooth and white in colour; however, the males tend to have a dark throat. Most burrowing frogs and toads, such as the one mentioned above, have short stout limbs for mainly for digging in the subsoil. *Breviceps adsp.ersus* lays eggs underground (Carruthers, 2016). The Bushveld rain frog can be frequently found in suitable climate forests and in the grasslands of South

Africa. They are a terrestrial species that only reproduce during the wet season. The common rain frog, or bushveld rain frog, inhabits the bushveld northeast of the well-known Vaal river, in South Africa (Carruthers, 2016).

Breviceps sylvestris taeniatus (FitzSimons, 1930) (forest rain frog) is a robust frog, with females larger than the males. They have large heads, compared to the small bodies and a short-rounded snout. Colours of these frogs include orange, red, yellow, green, and even purple. *Breviceps s. taeniatus* is found in the Limpopo Province of South Africa, in the north-eastern part of the country (Gouws, 2019). These species have a terrestrial way of life in forests and grasslands and are often found in gardens or along roadsides (Nielsen *et al.*, 2018). Males call at dusk on prominent platforms during wet seasons and nocturnally from the cover of leaf litter. These species face threats such as deforestation, fire and agricultural practices. Despite this, this species also occurs in several protected areas, including Blouberg Nature Reserve, Thabina Nature Reserve and the Wolkberg Wilderness Area (Gouws, 2019).

Chiromantis xerampelina Peters, 1854 (southern foam-nest tree frog) colours comprise of a grey-yellow, brownish body colour with varying numbers of dark spots (Carruthers, 2016). These frogs tend to alter and become white during the daytime. However, the ventral side of these anurans is white and smooth with dark spots and stains underneath throat area. *Chiromantis xerampelina* does not have heavily build hind legs and their toes and fingers have unique suction pads (Carruthers, 2016). Frogs of this species lay eggs in foam nests on branches overhanging rivers and dams (Carruthers, 2016). *Chiromantis xerampelina* can be found in the bushveld of eastern subtropical parts of the lowveld and the Limpopo-valley northwards.

Cacosternum boettgeri (Boulenger, 1882) (common/ Boettger's caco) is known for consuming termites (Branch, 2010), however, Jurgens, (1979) stated that these frogs are predators of a variety of mosquito species in South Africa. *Cacosternum boettgeri* can be found in a variety of habitats. The Nama Karoo, Succulent Karoo, Savanna, Grassland, Fynbos, and Thicket biomes are all home to the Boettger's caco. Breeding can take place in any small temporary water body in grasslands, culverts and other rain filled objects. The following is a part of the original description from Boulenger (1882): "Skin perfectly smooth: a curved fold from the eye to the shoulder. Olive above: a light line from below the eye to the shoulder and belly generally white with round black spots."

Hyperolius marmoratus Rapp, 1842 (painted reed frog) inhabits great ranges of natural habitats such as forests, savannas, shrublands, grasslands, rivers and swamps. These frogs have yellow, black and orange stripes, dull brown or speckled with changing colour patterns. The toes of *H. marmoratus* are dark pink and the stomach is white.

Kassina senegalensis (Dumeril and Bibron, 1854) (bubbling Kassina) has a smooth skin, a rounded snout area and a white smooth stomach. These frogs are also known as the Senegal

running frog and classified in the family *Hyperoliidae*. They have distinguishing dark brown stripes against a greenish background (Carruthers, 2016). Male species have enlarged throat. Western and southern Africa are home to these species.

Phrynomantis bifasciatus (Smith, 1847) (banded rubber frog) possess a smooth skin and a rounded snout. These frogs have a bright orange and red colour stripe with a contrasting black body. They have mostly grey stomachs along with some white spots (Carruthers, 2016). Males tend to call on the banks of rivers, pools and pans. *Phrynomantis bifasciatus* can be found in the bushveld of South Africa and the subtropical lowveld. These frogs release a milky, toxic substance through their skin. This substance is toxic both to other frog species and humans.

Phrynobatrachus mababiensis FitzSimons, 1932 (Mababe puddle frog) ranges very widely in eastern and southern Africa from northern Tanzania south to eastern South Africa (IUCN SSC Amphibian Specialist Group, 2014). The Mababe puddle frog is a widespread and very common species that is found in huge numbers at breeding aggregations (IUCN SSC Amphibian Specialist Group, 2014). These frogs live in open and wooded savannah, less frequently grassland. *Phrynobatrachus mababiensis* breeds in marshes, vleis and ponds, slow-flowing streams, and other permanent, semi-permanent, and temporary bodies of water, along the edges of small pans and shallow stagnant water amongst emergent vegetation (Carruthers, 2016).

Poyntonophrynus fenoulheti (Hewitt and Methuen, 1912) (Northern pygmy toad) occurs from Zeerust, situated in the North West Province of South Africa, eastwards through the Limpopo Province. *Poyntonophrynus fenoulheti* inhabits the Savanna Biome and is occasionally found in grassland. Its distribution lies within the summer-rainfall region. Although the frogs are sometimes found in sandy environments, they are capable of hiding between rocks or under subsoil. In these extreme situations the Northern pygmy toad, will occur in groups of 5-6 individuals (Minter, 2011).

Ptychadena anchietae (Bocage, 1867) (plain grass frog) is distributed in the north-eastern parts of South Africa and has a distinctive rust brown colour along with dark stains (Carruthers, 2016). *Ptychadena anchietae* morphology consists of a short acute snout, a smooth white stomach and big muscular hind legs. These frogs call can be identified calling from shelters on river banks, banks of wetlands and dams (Carruthers, 2016).

Ptychadena uzungwensis (Loveridge, 1932) (Udzungwa ridged frog) can be found in Tanzania, Burundi, Rwanda, Zambia, Zimbabwe and in central Mozambique. This frog species can also be found in the Soutpansberg, Limpopo Province, South Africa (Dehling and Sinsch, 2013). The Udzungwa ridged frog inhabits medium-to-high altitude grasslands near pools and streams. This species is inactive during the dry season, however, can be found around waterbodies during wet-and-breeding seasons. *Ptychadena uzungwensis* mainly prey on insects and spiders but can ingest snails and other frogs. This frog is only known from a few localities and has an extremely peripheral distribution.

Pyxicephalus adsp.ersus Tschudi, 1838 (giant bullfrog) has a bulky, supple body with bright olive-green skin (Carruthers, 2016). Both males and females have yellow stomachs (Carruthers, 2016). The African bullfrog is a carnivore that eats insects, small rodents, reptiles, tiny birds and other amphibians, among other things. Females are half the size of males, which is unusual since in most amphibians females are larger than males, to benefit amplexus.

Pyxicephalus edulis Peters, 1854 (African bullfrog) is a large-bodied frog. The females are much smaller than males and are half the weight of an adult male bullfrog. The skin of an adult frog is olive green and smooth textured, with the males being greener than the more olive brown females. During most of the year, these frogs, same as the giant bullfrog, are buried in the substrate, only emerging when it rains (Perret, 1966). Both young and adult frogs make cocoons out of discarded skin layers and soil particles to help them survive the dry season (Carruthers, 2016). These frogs breed in temporary pools in central highveld areas (Carruthers, 2016).

Schismaderma carens (Smith, 1848) (African red toad) has a leathery skin and is a fairly large species, with females being slightly larger than males. Their skin is reddish-brown in colour, with two dark brown patches on it. Their stomachs are spotty with grey stains (Carruthers, 2016). These toads are only found in Africa's southern half. They can frequently be found in habitat types such as wooded savannah, agricultural land and grassland and it breeds in deep lakes, ponds and pools (Chen, 2008). *Schismaderma carens* breeds in persistent deep waters mostly in grassland biomes (Carruthers, 2016).

Sclerophrys garmani, (Meek, 1897) (eastern olive toad) is a species of toad in the family *Bufonidae*, which is native to eastern and southern Africa (Tandy *et al*., 2004). These toads inhabit arid savannas, wooded savannas and agricultural areas, breeding in temporary water (vleis, dams or pans), and sometimes in artificial pools and rivers (Carruthers, 2016). *Schleroprys garmani* faces daily threats due to environmental degradation resulting from human expansion, settlement and agricultural encroachment, however, this is a resilient species that is not regarded as endangered (IUCN, 2016).

Sclerophrys gutturalis (Power, 1927) (African common toad or guttural toad) can be located from Angola all the way to the northern parts of South Africa. This species grows into fairly large toads, with the females being larger than the male specimens. Males call all year, but the breeding season is primarily in October and November. The guttural toad is a widespread species that is expanding across southern Africa as its population grows (IUCN, 2016). These toads tend to survive a wide range of environments and are adaptable to changing conditions. They are not affected by major predators and do not suffer from habitat loss in certain parts of Africa (IUCN, 2016).

Sclerophrys poweri (Hewitt, 1935) (Kimberley toad, Power s toad) is a common species, and often locally abundant. (IUCN SSC Amphibian Specialist Group, 2016). *Sclerophrys poweri* is a denizen of the open savanna biome, wooded savannas, bushveld, river valleys and agricultural

lands. This toad breeds in temporary pools such as vleis, marshes, dams or pans (Carruthers, 2016) and sometimes artificial pools (IUCN SSC Amphibian Specialist Group, 2016). *Sclerophrys poweri* is a widely distributed toad and is not a species of concern, due to his ability to survive a variety of environmental conditions (IUCN SSC Amphibian Specialist Group, 2016). This species ranges from extreme southern Angola, through the northern two-thirds of Namibia, to Botswana, southward to central South Africa (Carruthers, 2016).

Sclerophrys pusilla (Mertens, 1937) (flat-backed/ Striped-back Toad) is one of the "true toads", native to Africa (IUCN SSC Amphibian Specialist Group, 2016). This species can be found from the southern Democratic Republic of the Congo to southern Africa. It can be found in wet savannas, forest borders and degraded forest regions. It can also be found in agricultural areas (IUCN SSC Amphibian Specialist Group, 2016). *Sclerophrys pusilla* breeds in temporary rivers and commonly in streams (Carruthers, 2016). However, some populations face threats, such as environmental degradation in urban areas, because of expansions and development of infrastructures and agricultural development. It is widespread, adaptable and is not considered to be seriously at risk (Carruthers, 2016).

Tomopterna adiastola Channing and Du Preez, 2020 (southern sand frog) is a heavily build frog, reddish brown of colour without dark stains. These frogs have a blunt snout along with a smooth white stomach (Carruthers, 2016). Males of these species possess a dark brown, black throat which distinguishes them from the females. *Tomopterna adiastola* sings from river sandbanks in areas of South Africa's Kruger National Park and Zimbabwe.

Tomopterna tandyi Channing and Bogart, 1996 (Tandy s sand frog) belonging to the family *Pyxicephalidae*. The specific name "*tandyi*" honours Robert Mills Tandy, an American biologist, herpetologist, photographer and the collector of the type material (Beolens *et al.,* 2013). These frogs can be found in dry savanna regions, bushlands, grasslands and some survive in agricultural areas (IUCN, 2013). They are particularly associated with loose sandy soils associated with the forming of temporary pans. *Tomopterna tandyi* breeds in shallow water areas such as ditches, streams and dams. This species can be found from South Africa to Kenya in Africa.

Xenopus laevis (Daudin 1802) (common platanna, African clawed frog), has a viscous skin and a streamlined oval body with a greenish- grey stained colour. The stomach of these frogs is grey-white and sometimes freckled. The front legs are small in comparison to their big and powerful hind legs. The African clawed frog is known for having claws at the end of each toe. *Xenopus laevis* travels, when raining, to new breeding spots and stays permanently under water. They inhabit a wide range of habitats which include Sub-Saharan Africa, from Nigeria to South Africa and invaded Europe, North and South America (Weldon *et al.*, 2004).

1.9 PARASITES OF ANURANS

We need to understand the patterns of interaction and the factors of host-parasite relationship in order to bridge knowledge gaps in both community ecology and disease ecology (Campião *et al.*, 2015; Campião *et al.*, 2017). In addition to their diversity and life history strategies, amphibians harbour all known parasite taxa on both definitive and intermediate stages, making them an ideal model organism for studying parasite diversity. While amphibians are targeted for conservation, their parasites are poorly understood on a broad scale. Common metazoan parasites include various myxozoans, helminths (particularly monogeneans, trematodes, cestodes, acanthocephalans and nematodes) and arthropods (Herczeg *et al.*, 2021). Metazoan-induced diseases, like protozoan parasitic diseases, may be highly dependent on a host-related set of characteristics such as host age, condition and availability of compatible hosts (Densmore and Green, 2007; Koprivnikarr *et al.*, 2012). Many of the same metazoan parasites that infect fish also infect frogs in their aquatic phases.

It has been shown that many species of parasites have significant impact on the physiology, behaviour and even genetics of these animals (Bower *et al.,* 2017). For example, larvae of trematodes from the genus *Ribeiroia* Travassos 1939 are among the most widely known of the amphibian parasites (Densmore and Green, 2007). Rapid appearing of additional limbs in frogs makes them an easier prey for predators which are definitive hosts (Stopper *et al.*, 2002; Johnson and Sutherland, 2003). Parasitic trematodes can use amphibians as secondary, paratenic, intermediate, or definitive (final) hosts.

According to Campião *et al.* (2015) nematodes are the most common anuran parasites that infect frogs from egg to adult life stages and impact a variety of organs and tissues. Representatives of the genus *Rhabdias* Stiles and Hassall 1905, are lungworms, feeding on their hosts` blood. They are well-known problematic infective agents among captive anurans (Densmore and Green, 2007).

Cestodes (tapeworms) and acanthocephalans (spiny-headed worms) are two other helminths groups that have been documented as infecting amphibians and can cause diseases. They may cause significant gastrointestinal damage, gastrointestinal obstruction, and the transmission of overpowering diseases because of their various connecting organs (Wright, Wootton and Barber, 2006). Lastly, monogeneans commonly infect of the urinary bladders of adult amphibians and gills of tadpoles, where feeding on blood can have significant impact on their hosts' health (Densmore and Green, 2007).

Frogs are well-known species that are popular among the general public, are researched by scientists and have been the focus of medical and environmental studies. Conservation biologists are constantly working and researching to understand the causes of parasite and emerging fungal diseases among frogs. All wild reptiles and amphibians are thought to have parasites. In the wild, the delicate balance between parasite and host differs significantly from the connection between parasite and host in captivity. Creatures in the wild, such as frogs, are not trapped in a limited space

and parasite concentrations in the environment are often low. Parasites affect their hosts in numerous ways. Parasitized reptiles and amphibians have a shorter lifetime, are more susceptible to diseases and have an unattractive appearance in general. At the same time, parasites are an important component of each ecosystem and their absence may indicate decline of their host's populations or pollution of their environment.

Because frogs have permeable skin that allows toxins to easily enter their bodies, they are susceptible to contaminants released into freshwater habitats (Rohr *et al.,* 2011). The majority of chemical pollutants weaken the immune system, resulting in increased disease end-points (Martin *et al*., 2010).

1.9.1 MYXOSPOREAN PARASITES

According to Eiras (2005), myxosporeans are common parasites of various fish species with a sporadic presence among frogs, which are generally considered uncommon hosts. In 2005, nineteen species were described from amphibians belonging to the genera *Myxobolus* Theolan, 1892, *Myxidium* Buetschli, 1882, *Hoferellus* Berg, 1898, *Chloromyxum* Mingazzini, 1890, *Caudomyxum* Bauer 1954 and *Sp.haerosp.ora* Thélohan, 1892 (see Eiras, 2005). The biology of these parasites is nonetheless poorly understood and the details of the life cycles are completely unknown. Some infect the testes, oviducts and kidneys of anurans found in Australia. *Myxobolus bufonis* McAllister *et* Goldberg, 1992, infects the skin of anuran species, along with infecting the gall bladder. Other species include *Hoferellus anurae* Mutschmann, 2004 which infects the kidneys, intestine and urinary bladder of the anuran *Afrixalus dorsalis* (Peters, 1875) from Nigeria. It appears that amphibians and reptiles suffer from myxosporean infection, yet the pathophysiology of the infection remains unclear (Eiras, 2005). Eiras (2005) reported that myxosporeans infect 83 amphibian species, spanning numerous genera.

Eiras (2005) found that the geographical distribution of these parasites was extensive, ranging from Europe, North and South America, Africa, Asia and Australia with the majority of the hosts reportedly from Australia and U.S.A. Alternatively, this does not mean infection rates are more abundant in some places, but that research was more intense in some countries than in others (Eiras, 2005).

1.9.2 DIGENEANS

Adult trematodes are leaf-shaped flatworms with prominent oral and ventral suckers, which help feeding and maintain position in situ. These flatworms are classified within the phylum, Platyhelminthes. Trematodes are parasitic worms that live in molluscs and vertebrates (typically as their first intermediate hosts, second intermediate, parathenic, and definitive hosts) (Pace *et al.*, 2019). According to Pace *et al*. (2019) Digenea are metazoan parasites that affect a wide spectrum of vertebrate and invertebrate hosts

Hamann *et al.* (2013) wanted to see how different the trematodes were in amphibians with different lifestyles, such as terrestrial, semi-aquatic, fossorial and arboreal. Specimens were obtained in Argentina over a five-year span (Hamann *et al.,* 2013). A total of 19 species of amphibian trematodes were found in this area, with the majority of them being dominant in common species (Hamann *et al.,* 2013). They discovered that frogs with communal habitats, a generalist diet and an active foraging behaviour show patterns of similarity among species. These amphibians tend to have the highest infection rate. Lastly, their results suggested that amphibians in semi-aquatic environments, both extant in aquatic and terrestrial ranges, present a greater diversity of parasites as they tend to have a higher rate of exposure to a wider range of prey species and, thus, to diverse infective conditions (Hamann *et al.,* 2013).

Infections induced by trematodes seen in frogs, in particular, have recieived a lot of attention because they are linked to mass death (Rohr and Raffel, 2010), as well as hideous limb and body abnormalities (Johnson *et al*., 2012). From the clawed frogs, *X. laevis* in southern Africa, only cercaria of the genus *Tylodelphys* Diesing, 1850 from the pericardium and two adult species *Progonimodiscus doyeri* Ortlepp, 1926 and *Dollfuschella rodhaini* Vercammen-Grandjean, 1960 from the digestive system have been reported (Mbokane *et al.,* 2020).

1.9.3 MONOGENEANS

Fish and several other lower vertebrate groups serve as host for parasitic monogeneans. These flatworms are mostly ectoparasitic and live on fish skin, gills, and fins. They are hermaphrodites, measuring no more than 2 cm in length. Among parasitic platyhelminths, monogeneans have the simplest lifecycle. Though the terms, monogenetic trematodes and flukes, are often used to describe this group of parasites, both are incorrect because monogeneans are neither trematodes nor flukes (Cribb *et al.,* 2002). In fact, these organisms are distinct from other parasitic flatworms (Cribb *et al.,* 2002). Monogeneans attach to the host's external surfaces, although a few species can infect internal tissue including blood vessels or the digestive tract, as well as parasitize amphibians and freshwater turtles. The presence of a haptor, the posterior attachment organ, distinguishes Monogeneans from Trematoda and Cestoda. There are approximately 5000 species of monogeneans described worldwide, with over 4000 species consisting of parasitic

flatworms that belong to the Phylum Platyhelminthes. Located at the anterior part of the body, these hooks allow the organisms to attach themselves to the epithelial tissue so that they can start feeding. The world's fish fauna could potentially encompass 25000 monogenean species, of which only 3000–4000 have been described thus far (Whittington, 1998). According to Chaabane *et al.* (2019) polystomes (Monogenea, Polystomatidae) are found in semi-aquatic tetrapods like all three amphibian orders (anurans, salamanders, and caecilians), freshwater turtles and the hippopotamus. The African clawed frog, *X. laevis*, can be the definitive or intermediate host to 25 different parasite species, of which the class Monogenean is represented (Chaabane *et al.,* 2019). In the gut of the clawed frog, *X. laevis*, two species, *Protopolystoma xenopodis* Price, 1943 and *Gyrdicotylus gallieni* Vercammen-Grandjean, 1960, have been described. More than 10 species of polystomes can be found in their adult stages, in the urinary bladder of a wide variety of anuran hosts where they produce eggs (Theunissen *et al.,* 2014; Mbokane *et al.,* 2020).

1.9.4 CESTODES

Cestodes, known as tapeworms, are parasitic worms belonging to the phylum Platyhelminthes. Their morphology is characterized by a segmented body with proglottid segments. Proglottids are vital portions that contain eggs and part of male and female reproductive systems; with rare exceptions, adult cestodes dwell in the intestine of definitive hosts, while metacestodes may be found in various sites of intermediate hosts. According to Heyneman (1996) all Cestodes are parasitic and have complex life cycles. Cestodes are found comparatively rarely in amphibians, however, adult worms are found in the small intestines, skin, and can cause cysts in and on the liver, lungs and other organs, such as long bones and the nervous system (Heyneman, 1996). Adult worm pathology is caused by the physical presence and activity of big adult worm species. Cestodes that infect reptiles and amphibians, are hermaphroditic and often have no preference for a particular host.

Imasuen *et al*. (2012) investigated anurans as intermediate and paratenic hosts of helminth infections in different biotopes in southern Nigeria. According to the researchers, they detected a huge number of encysted helminth parasites that use anurans as intermediate or paratenic hosts (Imasuen *et al*., 2012). An encysted proteocephalid cestode was discovered on the walls of anurans gastrointestinal tracts from a typical savanna habitat (Imasuen *et al.,* 2012). The cyst contained cestode larvae with unsegmented bodies and four apparent suckers on the scolices (Imasuen *et al.,* 2012). This parasite was recorded in 15 anurans specimens from the rainforest, many of which were found in tree frogs (Imasuen *et al.,* 2012). *Cephalochlamys namaquensis* (Cohn, 1906) from the African clawed frog *X. laevis* and *Cylindrotaenia jaegerskioeldi* (Janicki, 1928) from various anurans are the only two species reported from frogs in southern Africa so far (Halajian *et al*., 2013; Schoeman *et al*., 2019).

1.9.5 NEMATODES

There are numerous species of parasitic nematodes and free-living species within the Nematode taxon (Martínez-Arce *et al.,* 2020). Nematodes are a taxon of worms that include parasitic and free-living species (Martínez-Arce *et al.,* 2020). Currently, roughly 27,000 nematode species have been formally classified based on morphological and ecological properties, but conservative estimates suggest that there are at least 100,000 species and other scientists predict that there will be more than one million (Hugot, 2002). The deceptively homogeneous structure of nematodes, on the other hand, makes fully appreciating true diversity and morphology-based taxonomy, a time-consuming and difficult effort, even for well-trained professionals (Martínez-Arce *et al.,* 2020). Vertebrate parasitic nematodes are an outstanding example of a category in which most species are recognised only by their morphology. They mostly infect their hosts intestines, although they can also infect the respiratory tract, body cavity and circulatory system (Halajian *et al*., 2013). As observed in the lungworm, *Rhabdias* spp. (Schrank, 1788) Stiles et Hassall, 1905, nematodes can cause a range of injury or modifications, which can lead to changes in host behaviour or metabolism (Finnerty *et al.,* 2018; Pizzatto and Shine, 2012; Goater *et al*., 1993).

Afrotropical nematodes have an unknown number of species, geographical distributions and host specializations (McAllister *et al.,* 2010; Aisien *et al.,* 2017;). Molecular techniques for species identification are currently unavailable in most genera with extensively widespread Afrotropical species, such as *Aplectana* (Stewart, 1914), *Falcaustra* Lane, 1915 and *Orneoascaris* Skrjabin, 1916 (Martínez-Arce *et al.,* 2020). The following is the currently known amphibian host species of the parasitic nematodes detected in studied frogs - *Orneoascaris chrysanthemoides* Skrjabin, 1916, commonly found in toads such as *Sclerophrys* spp. (Goldberg *et al*., 2021), *Gendria* (*Chabaudus*) *leberrei* (Bain and Philippon, 1969) found in *Sclerophrys* spp. (Sinsch *et al*., 2020), *Aplectana chamaeleonis* (Baylis, 1929) originally found in chameleons, but also reported from several amphibians (Aisien *et al.,* 2017) and lastly *Rhabdias collaris* Baker, 1987, detected in frog species (Kuzmin, 2013; Sinsch *et al*., 2020).

The lung-dwelling *Rhabdias* parasites are one of the few nematode genera having sufficient information based on morphology and genetic traits (Kuzmin *et al*., 2017). According to a study published in 2020, the *Rhabdias*idae Railliet, 1915 family contains more than 80 parasitic nematode species, 12 of which have been recorded from amphibians in the Afrotropical Realm and seven of them have been described in the last ten years (Junker *et al*., 2012; Svitin and Du Preez, 2018; Kuzmin *et al*., 2020). In total, 42 species of nematodes are known to parasitize frogs in southern Africa, of which 12 species and one genus were described within the last five years (Svitin *et al.,* 2018; Netherlands *et al*., 2020; Svitin *et al*., 2020; Kuzmin *et al*., 2021) and six were firstly reported from frogs in 2020 (Schoeman *et al*., 2020).

1.10 MODERN APPROACH TO PARASITOLOGIGAL STUDIES

A Geographical information system (GIS) is a useful and efficient tool for identifying and directing research to areas and species important in the conservation of biodiversity. It has the potential to assist in the selection of either a single, or a network, of suitable protected areas. Several centres of South African anuran species richness, endemism and RDB richness have been identified using the data currently available. In South Africa there are extensive areas where no distribution records exist and a concerted collecting effort is therefore required. GIS is a computer system that collects, saves, verifies and displays data on positions of the Earth's surface. GIS connects maps to databases, visualizes data, and enables an interaction between the map and the database data. GIS will be used in this study to identify anuran sampling locations along with their presented parasites.

Thereafter, parasite community analyses can be formulated using corresponding statistical analysis. The number of species in a community or region is an important statistic for analysing community and regional diversity (Gotelli and Colwell, 2001). It allows us to make quantitative comparisons between different ecosystem locations and it is on the basis of these comparisons that community and conservation ecology is built (Morris *et al.*, 2019). Species richness assessments can and have, benefited parasite diversity research because they provide information on the hidden biodiversity that organisms may be hiding (Dove and Cribb, 2006).

Huggins *et al.* (2017) established a non-invasive PCR-based technology aimed primarily at the detection and identification of parasitic nematodes, as parasite DNA is commonly found in the faeces of afflicted animals in the form of eggs or tissue pieces. There is a lack of molecular data for the majority of parasites from African frogs, so morphological studies as well as molecular analyses of adult stages are required before non-invasive treatments can be implemented (Huggins *et al.*, 2017).

1.11 REMARKS

Parasites have long been seen negatively, particularly when it comes to human and livestock health (Netherlands *et al.*, 2015). Parasites, on the other hand, may be viewed as an important component of a functioning and healthy ecosystem when they are found in their native habitat. Amphibians are prey for a range of predators, including birds, turtles, snakes and other reptiles and anurans (Imasuen *et al.,* 2012). Amphibians may be able to transfer parasitic illnesses to multiple trophic levels in the food web in this way. According to Sinsch *et al.* (2020) afrotropical nematode parasite diversity may be lower than previously presumed due to low host specificity development. On the other hand, latest discoveries of new species of parasitic nematodes is suggesting otherwise (Sinsch *et al.*, 2020). As a result, it is evident that parasite morphologists and molecular biologists should collaborate to build a long-term molecular library of DNA sequence data related to morphological identifications that may be used for both taxonomic and phylogenetic purposes in the future. In conclusion, southern Africa has escaped exotic amphibian invasions and South Africa is

employing national legislation to control domestic exotic invasions and reduce the possibility of new exotic amphibian invasions. This has the added benefit of reducing illness risk, which can only be effectively managed by lowering the risk of introduction.

CHAPTER 2:

MATERIALS AND METHODS

2.1 STUDY AREA AND SAMPLED ANURANS

Several field surveys conducted in the period 2019 – 2021, provided a great amount of material for screening of amphibians for metazoan parasites. Field work took place in five different transects along the northern and southern parts of the Soutpansberg within the Limpopo province, South Africa, located in the Vhembe Biosphere Reserve (Fig. 8). Frogs were collected by hand at night after obtaining the necessary collecting permission. Frogs were collected according to species and placed in clear plastic bags with damp vegetation to offer a hiding area, keep the frogs damp, and prevent the bag from inflating. Bags were clearly marked according to site and field numbers assigned. Bags containing frogs were then placed in a cool box and transported to the field laboratory. Specimens were then carefully sorted and evaluated to ascertain which needed to be dissected. In total 131 different sites were visited during several field trips in which 269 anuran specimens were collected (Fig. 8; Table 1).

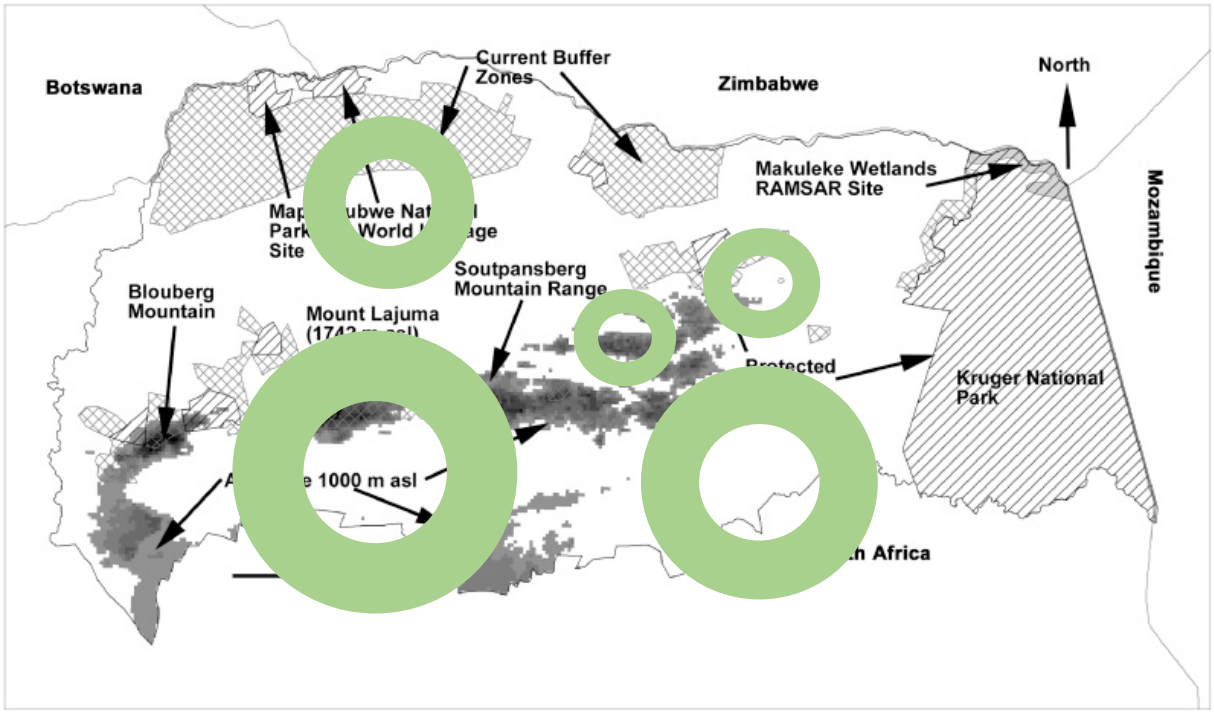


Figure 3: The study area along the Soutpansberg Mountain Range over five distinct transects in the Vhembe Biosphere Nature Reserve.

2.2 FULL PARASITE DISSECTION

All amphibian hosts were dissected after being humanely euthanized with tricaine ethyl-4-aminobenzoate (MS222) according to the approved standard operating procedure (NWU-00492-16-A5). All parasitic groups found within the hosts were removed from the external nares, eyes, buccal cavity, lungs, oesophagus, stomach, intestines, urinary bladder and cloacal opening (Fig. 4). The parasites were assigned to the parasitic species as follows: hot 70% Ethanol for all nematodes (Lutz *et al*., 2017) cold 70% Ethanol for acanthocephalans (Lutz *et al*., 2017) hot water (H_2O) for all trematodes and cestodes (Lutz *et al*., 2017), hot formalin for monogeneans, placed in 70% ethanol or 10 % NBF for further storage. Of each morphospecies, fixed in formalin, at least one specimen was fixed in ethanol for molecular studies. For a successful dissection the following equipment was used: dissecting microscope, dissecting tray to keep host in place, 0.06% amphibian saline solution, large and small petri dishes, 1ml insulin syringes with fixed needle, microscope slides, dissecting equipment containing at least scissors, forceps and needles, microscope slides, 2ml tubes 70% ethanol, 10% neutral buffered formalin (NBF), fixing tray and, lastly, a collection jar for all hosts dissected (Fig. 4).

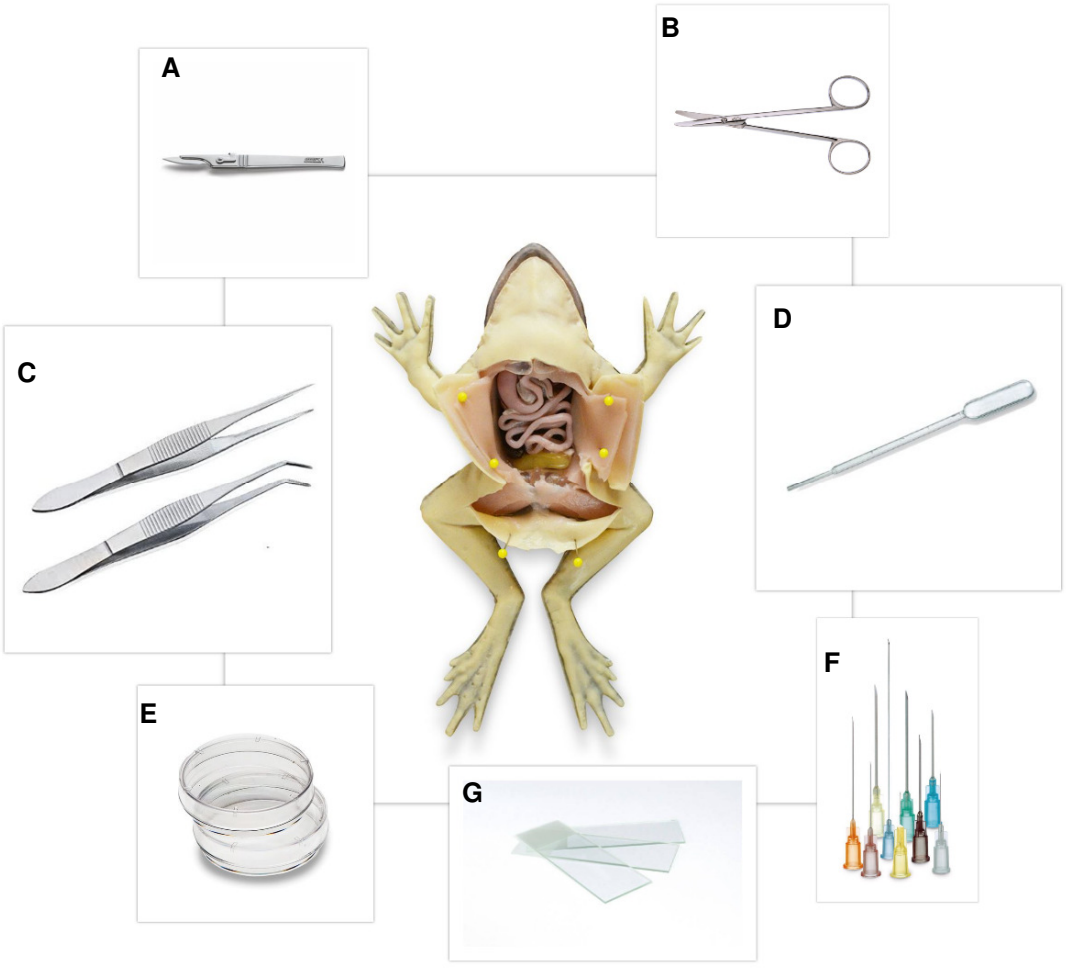


Figure 4: Diagram summarising most equipment needed for successful dissection. A, B, C -, forceps, scissors and needles respectively, D – glass or plastic pipets, E - large and small petri dishes, F - fixed needles, G - microscope slides.

2.3 OBTAINING A TISSUE SAMPLE

Each host and parasite were assigned a unique field number. A small piece of thigh muscle tissue and a small piece of liver were fixed in high quality 70% ethanol and each vial was labelled accordingly and stored at -80˚C. Following the dissection, a field tag, containing the assigned field number was attached to the thigh of the carcass and the carcass was then closed up and fixed in a natural position in 10% NBF, in a fixing tray for 24 hours and then transferred to a collecting jar containing 10% NBF. Fixed specimens were entered into the SAIAB collection (South African Institute for Aquatic Biodiversity). A collection number was assigned and all collecting data was entered on the SAIAB Specify Amphibian database.

2.4 MICROSCOPIC STUDIES

Prior to microscopic studies, depending on the parasitic group, the specimens were permanently mounted on slides or on temporary mounts. The fixed nematodes were rinsed in distilled water for about 20 minutes before being cleared in lactophenol (lactic acid, glycerine, phenol and distilled water in equal proportions). These specimens were investigated as lactophenol temporary mounts in lactophenol. All flatworms were studied on permanent mounts after staining according to following protocols.

To eliminate any leftover ethanol, the cestodes were rinsed in distilled water. The worms were then transferred to the Alum Carmine stain (Fig. 5) which had been diluted beforehand with distilled water. Most of the specimens were small and were stained for 5-7min. The specimens were then immersed in distilled water before being immersed in a 1% HCl solution. Specimens were examined and prepared for permanent slides using a light microscope. Specimens were conveyed through a series of ethanol with increasing concentrations of 70%, 80%, 90%, 95%, and 100%. After all the water has been removed from the specimens, they were transferred to a clearing agent, xylene or clove oil and subsequently mounted using Canada-balsam (Fig. 5) (Lutz *et al*., 2017).

As for digeneans, distilled water was added, keeping the worm at the bottom and repeated to ensure that all the ethanol was washed out. Thereafter, haematoxylin stain was added for 5-7 min. The stain was removed and replaced with HCl (1%) to ensure no stain was left. Ammonia (1%) was then added until the digenean stained blue/purple in colour. After five minutes, 70 percent ethanol was added, then removed and replaced with 80 percent ethanol twice. Thereafter, 100% ethanol was added and after five minutes removed and replaced with methyl-salicylate 100% ethanol (1:1) solution. Thereafter, 100% methyl-salicylate was added for two minutes. Before being examined under a microscope, the slides were carefully cleaned. Using ZEISS Axio ZII and Nikon AZ1000 compound microscopes, photomicrographs of all parasites were taken. All of those slides were permanent and could be used again in the future.

The *Ophionyssus* sp. Megnin, 1884 mite was placed on a lactophenol-coated microscope slide to allow for alterations while viewing under the Nikon AZ1000 compound microscope. The unidentified *Oligochaeta* Family gen. sp., annelid parasite species, was mounted on a microscope slide in distilled water to protect it from any damage caused by the cover slip.

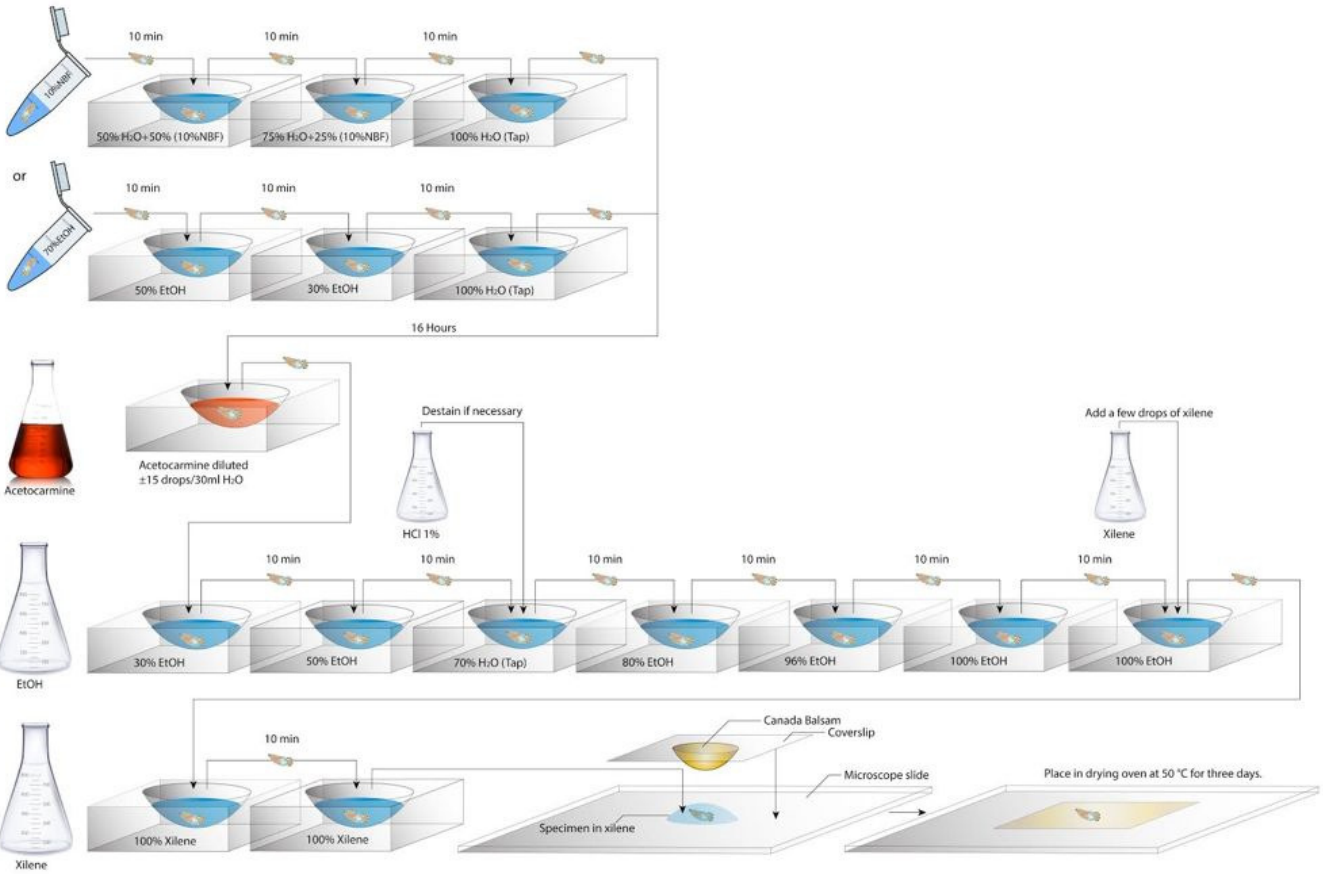


Figure 5: Alum Carmine stain following the correct protocol, designed by and adapted from Willie Landman (2022).

2.5 MOLECULAR STUDIES

3.5.1 NEMATODES

For molecular analysis, mid body sections as well as small anterior parts of nematodes were used. Following the manufacturer's instructions, DNA was extracted using the Zymo Research Quick DNA MiniPrep + Kit. The forward primer COIF (5′-TTTTTTGGTCATCCTGAGGTTTAT-3′) and the reverse primer COIR (5′-ACATAATGAAAATGACTA ACAAC-3′) were used to amplify cytochrome oxidase *c* subunit I (*cox1*) amplicons, and the thermocycling profile was as follows: three min at 94°C, 20 cycles at 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. COIR and COIF primer pair was used for *Aplectana*, *Cosmocerca*and some *Rhabdias* species. Another primer pair forward LCO1490 (5 -GGT CAA CAA ATC ATA AAG ATA TTG G-3) and reverse primer HCO2198 (5 -GGT CAA CAA ATC ATA AAG ATA TTG G-3) were used for *Rhabdias*, *Cosmocerca*, Neofoleyellides,

and *Contraceacum* species. The following conditions were used: three min denaturation at 94°C; 20 cycles of 94°C for 30 s, 45°C for 30 s, 72°C for 60 s, and 40 cycles of 94°C for 30 s, 51°C for 60 s, 72°C for 60 s for amplification; 72°C for 10 min for extension. The partial 28S rDNA gene and the internal transcribed spacer (ITS) region (ITS1+5.8S+ITS2) were amplified using the newly designed primer pair *Cosm*28Sf forward (5 – GCATGGCCGTTCTTAGTTGG – 3) and *Cosm*28Sr reverse (5 – TCGCCCCTATACCCAAGTCA – 3) and the thermocycling profile as follows: Denaturation for 2 minutes at 94°C, followed by 40 cycles of 30 seconds at 94°C, 30 seconds at 53°C, 2 minutes at 72°C and a final 7 minute extension at 72°C. The primers *Cosm*28S were designed using BLAST primer design tool with following manual editing for the amplification of *Aplectana* species. The primers ritf (5 - GCG GCT TAA TTT GAC TCA ACA CGG - 3) and 1500R (5 -GCT ATC CTG AGG GAA ACT TCG – 3) were used with the same PCR procedure for all *Rhabdias* species, as described by (Tkach *et al.,* 2014).

Forward 18S-F (5′-CGC GAATRGCTCATTACAACAGC-3′) and reverse primer 18S-R (5′-GGGCGGTATCTGATCGCC-3′) were used to amplify 18S rDNA gene segments. *Contracaecum*, *Aplectana*, and *Cosmocerca* were all amplified using the 18S-F and 18S-R primer combination.

2.5.2 DIGENEANS

DNA was extracted from the trematode's posterior regions. Following that, DNA was extracted using the PCR Bio Rapid DNA Extraction Kit according to the manufacturer's instructions. The primer pairs 3S forward (5′-GGTACCGGTGGATCACGTGGCTAGTG-3′) and ITS2.2 reverse (5′-CCTGGTTAGTTTCTTTTCCTCCGC-3′) were used to amplify ITS2 using the PCR thermo-cycle profile: a single cycle of 95 °C denaturation for three minutes, 45 °C annealing for two minutes, 72 °C extension for 90 seconds, followed by four cycles of 95 °C denaturation for 45 seconds, 50 °C annealing for 45 seconds, 72 °C extension for 90 seconds, followed by 30 cycles of 95 °C denaturation for 20 seconds, 52 °C annealing for 20 seconds, 72 °C extension for 90 seconds, followed by a final 72 °C extension for five min. The Dig12 forward (5′-AAGCATATCACTAAGCGG-3′) and 1500 reverse (5′-GCTATCCTGAGGGAAACTTCG-3′) primer pairs were used to amplify the 28S genes, with the PCR thermo-cycle profile as follows: denaturation for five minutes at 95°C, followed by 40 cycles of 30 seconds at 95°C, 30 seconds at 55°C, two minutes at 72°C and a final seven minute extension at 72°C. Finally, the *cox1* (DICE) gene was amplified using the primer pairs Dice1F (5′-ATTAACCCTCACTAAATTWCNTTRGATCATAAG-3′) and Dice14R (5 - TAATACGACTCACTATACCHACMRTAAACATATGATG-3′) and the PCR thermo-cycle profile: denaturation at 94°C for four minutes; 40 cycles of 40 s at 94°C, 40 s at 51°C,

2.5.3 CESTODES

For cestodes, only a small piece of the worm was used for molecular studies and a piece after the scolex was obtained. DNA was extracted using the Zymo Research Quick DNA MiniPrep + Kit, just as it was for nematodes. The following genes were amplified *cox1*, 28S rDNA, 18S rRNA. The primer combination JB3 (5 – TAA AGA AAG AAC ATA ATG AAA ATG – 3) and JB4.5 (5 – TTT TTT GGG CAT CCT GAG GTT TAT = 3) were used to amplify cytochrome oxidase c subunit I (*cox1*) amplicons according to the Liu et al, 2011 methodology. The thermocycling profile was as follows: denaturation at 94°C for five minutes, followed by 36 cycles of 94°C for 30 s (denaturation), 55°C for 30 s (annealing), 72°C for 30 s for 36 cycles, followed by 72°C for five minutes (final extension) (Liu *et al*., 2011). Forward primer WormA5 – GCG AAT GGC TCA TTA AAT CAG – 3), reverse primer WormB (5 – CTT GTT ACG ACT TTT ACT TCC -3), forward primer 300F (5 – AGG GTT CGA TTC CGG AG – 3), and reverse primer 1270R (5 – CCG TCA ATT CCT TTA AGT – 3) were utilized for 18S. The following thermocycling process was devised: two minutes of denaturation at 94°C, followed by 40 cycles of 30s at 94°C, 30s at 54°C, two minutes at 72°C and seven minutes at 72°C (Timothy *et al*., 2008). The 28S rDNA gene was amplified using the forward primer ZX-1 (5 – ACC CGC TGA ATT TAA GCA TAT - 3), reverse primer 1500R (5 – GCT ATC CTG AGG GAA ACT TCG - 3), forward primer 300F (5 – CAA GTA CCG TGA GGG AAA GTT G - 3) and reverse primer ECD2 (5 – CTT GGT CCG TGT TTC AAG ACG GG - 3). The thermocycle consisted of five minutes of denaturation at 95°C, followed by 40 cycles of 30 seconds at 95°C, 30 seconds at 55°C, two minutes at 72°C and seven minutes of extension at 72°C (Littlewood *et al.,* 2008)

2.6 SEQUENCING

Sequences were obtained from impurified PCR products provided to a commercial sequencing company (Inqaba Biotechnical Industries (Pty) Ltd, Pretoria, South Africa) using BigDye® Terminator v3.1 Cycle Sequencing on an ABI3500XL sequencer. For sequencing of the nematodes, ITS-28S region, a pair of PCR primers were employed for each sample, as well as additional internal primers: ITS4 (5 -TCCTCCGCTTATTGA TATGC-3), ITS5 (5 -GAAAGTAAAAGTCGTA ACAAGG-3), ECD2 (5 -CTTGGTCCGTGTTTCAAGACGGG-3) and 300R (5 -CAACTTTCCCTCACGGTACTTG-3`). Geneious Prime software was used to put together contiguous sequences, which were then modified.

2.7 GEOGRAPHICAL INFORMATION SYSTEM

The geographical maps (Fig. 6) were configured with the help of an open-source QGIS that, the vector data was saved as point, line or polygon features. The longitude and latitude of each location were entered into the software program and plotted. Terrain features were especially essential for this study, thus showing the diversity of each site we visited from time periods 2019-2021. The maps presented in the results of this study are configured into annual periods when each site was visited and material collected during that year. Additionally, each site was given coordinates using a Garmin GPS and recorded in fieldnotes. Thereafter, coordinates were then transferred into an Excel spreadsheet and saved as a CSV UTF-8 (comma delimited) file.

2.7.1 MAP OF SAMPLING SITES

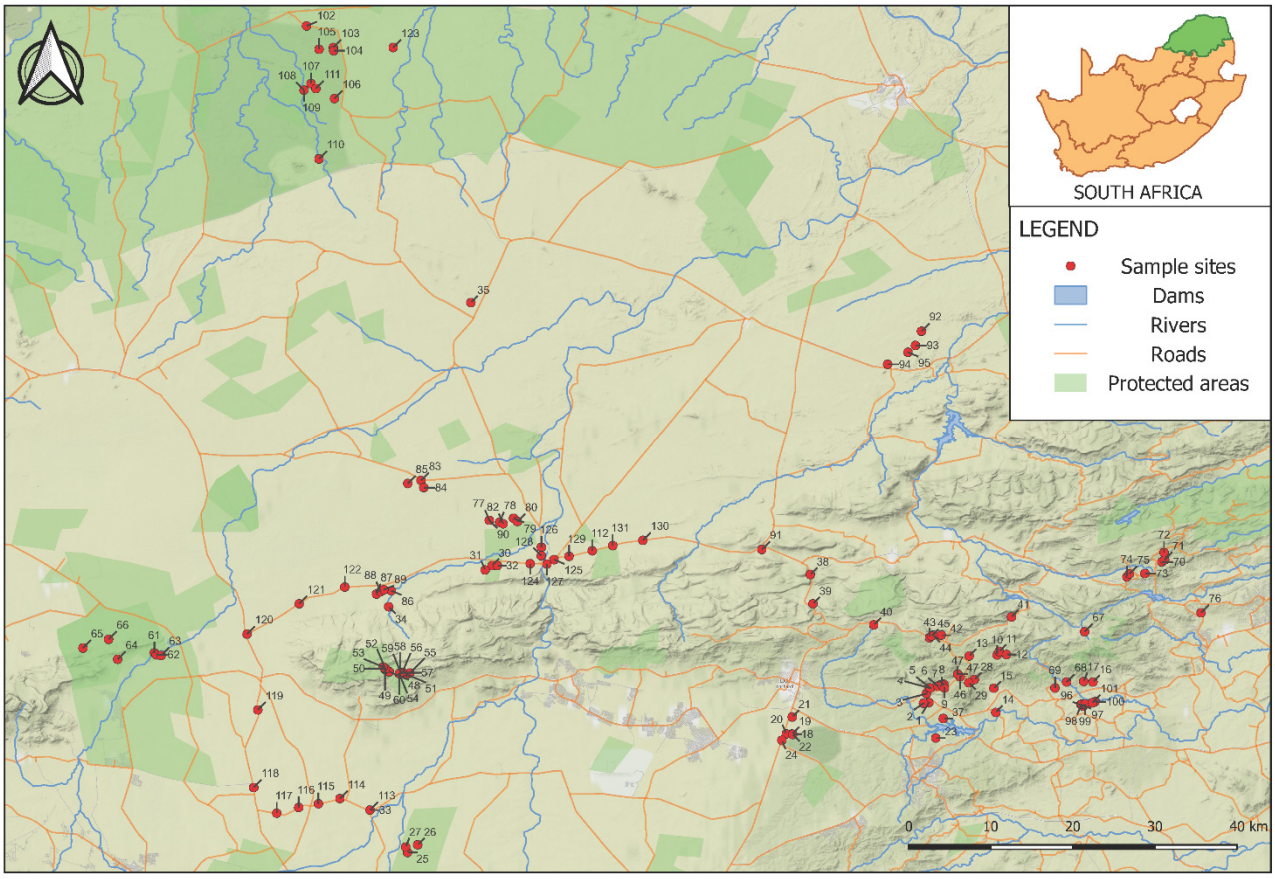


Figure 6: Five transects spanning the Vhembe Biosphere in Limpopo Province, South Africa, were used as sampling locations. Vivo, Waterpoort, Louis Trichardt, and the suburbs of Musina and Alldays are also close to sample locations. From February 2019 through February 2021, the reserve was sampled.

2.8 LOCALITIES OF SITES DURING 2019-2021

Table 1: Localities (Latitude and Longitude) and site numbers of areas.

2019	2020	2021
Longitude (E) /Latitude (S)	**Longitude (E) /Latitude (S)**	**Longitude (E) /Latitude (S)**
30.07083, -23.05502 (site4)	30.07268, -23.05526 (site 5)	29.54781, -2285586 (site 77)
30.07268, -23.05526 (site5)	29.90837, -23.11010 (site 18, 19)	29.56000, -22.85821 (site 78)
30.11901, -23.01718 (site 13)	29.45041, -23.25092 (site 25)	29.57663, -22.85349 (site 79)
30.25520, -23.04759 (site 17)	29.46246, -23.24177 (site 26)	29.58113, -22.85733 (site 80)
29.90886, -23.11048 (site 22)	29.44821, -23.24433 (site 27)	29.56423, -22.86007 (site 82)
30.06808, -23.06198 (site 3)	30.25520, -23.04759 (site 17)	29.46644, -22.80849 (site 83)
30.07930, -23.11484 (site 23)	30.12518, -23.04526 (site 28)	29.46972, -22.81697 (site 84)
29.90834, -23.08978 (site 21)	30.11842, -23.04906 (site 29)	29.45074, -22.81210 (site 85)
30.07105, -23.07283 (site 1)	29.55108, -22.90969 (site 30)	29.43107, -22.93965 (site 86)
30.06461, -23.07351 (site 2)	29.54277, -22.91499 (site 31)	29.41348, -22.94361 (site 87)
30.15228, -23.01608 (site 10)	29.55725, -22.90973 (site 32)	29.41830, -22.94076 (site 88)
29.90837, -23.11010 (site 18, 19)	29.40580, -23.20040 (site 33)	29.42223, -22.93853 (site 89)
30,08173, -23.05316 (site 6)	29.42800, -22.95870 (site34)	29.54771, -22.85585 (site 90)
30.08879, -23.05085 (site 7)	30.07930, -23.11484 (site 23)	29.87216, -22.89052 (site 91)
30.08643, -23.05157 (site 8)	29.88277, -22.99972 (site 35)	30.06220, -22.62986 (site 92)
30.08959, -23.05553 (site 9)	30.06888, -23.05944 (site 36)	30.05504, -22.64684 (site 93)
30.15477, -23.01183 (site 11)	29.89601, -23.11746 (site 24)	30.02192, -22.66917 (site 94)
30.16342, -23.01551 (site 12)	30.25542, -23.04736 (site 17)	30.04615, -22.65491 (site 95)
30.15063, -23.08441 (site 14)	30.25644, -22.98839 (site 67)	
30.14830, -23.05554 (site 15)	30.08808, -23.09164 (site 37)	
30.26634, -23.04831 (site 16)		
29.90127, -23.10990 (site 20)		

CHAPTER 3:

RESULTS

3.1 PREFACE

During the full parasitological survey, 35 species of metazoan parasites were recovered from the anurans collected. These included 24 nematodes, four cestodes, four trematodes, three monogeneans species, one acanthocephalan, one arthropod and one annelid (Table 2). Of recovered parasites we found seven species that are morphologically and genetically different from all previously known species and most likely belong to new species (Table 3).

Of 269 studied frogs, 209 (68%) were found infected with one or more metazoan parasite (Table 2). The parasites found in this study are described in the next chapter, which contains taxons, family names, and genus names, as well as illustrations.

3.1.1 PERCENTAGE INFECTION RATE OF PARASITES

Table 2: Percentage of the helminth community from the Vhembe Biosphere.

Nematoda	Cestoda	Digenea	Arthropoda	Monogenea	Annelida	Acanthocephala
68.57**%**	5.71%	8.57%	2.85%	8.57%	2.85%	2.85%

3.2 TAXONOMIC SEGMENT

3.2.1 NEMATODA

FAMILY: AMPHIBIOPHILIDAE
GENUS: ***Amphibiophilus* sp**. Skrjabin, 1916

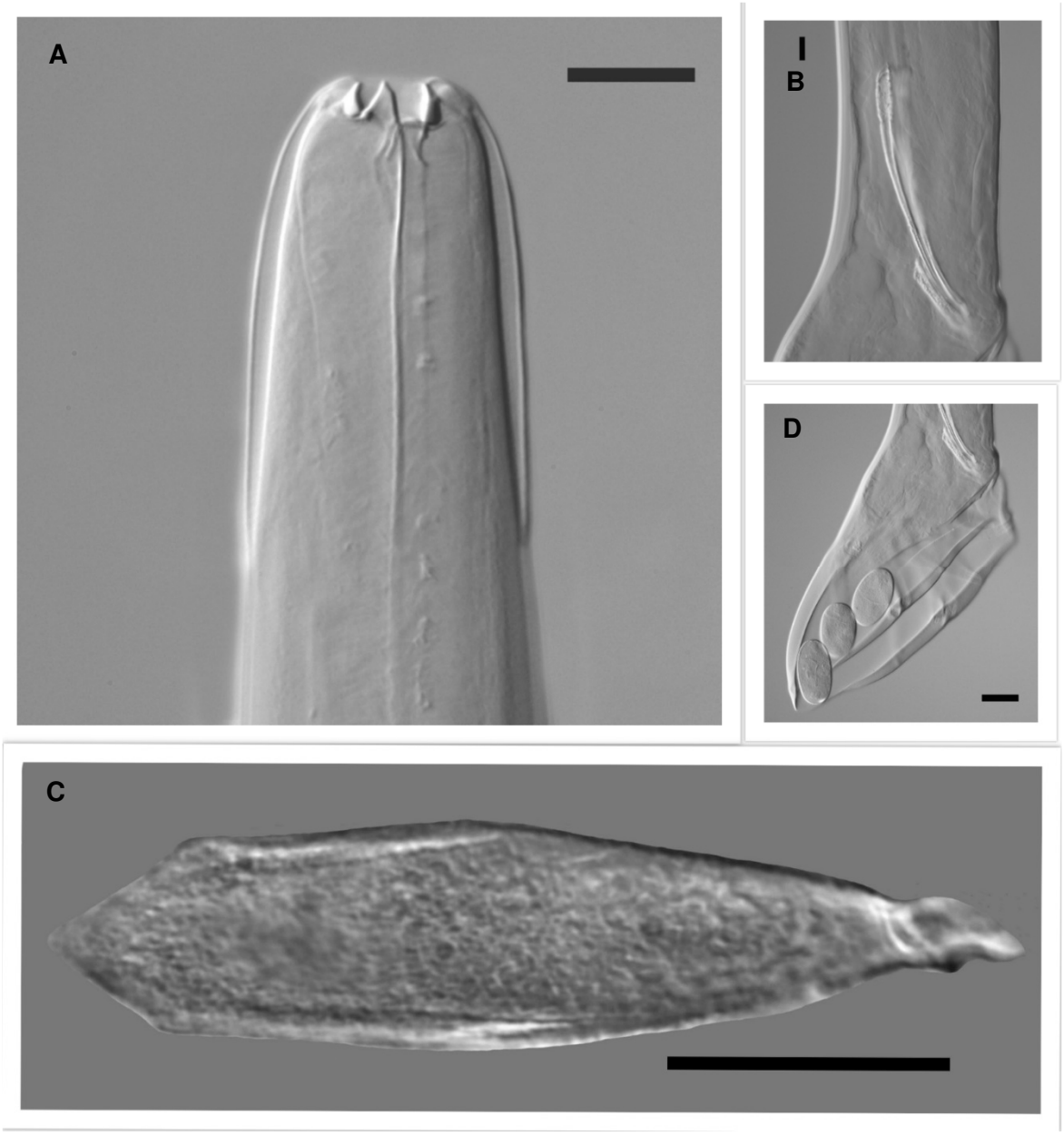


Figure 7: *Amphibiophilus* sp. parasitizing Common River frog, *Amietia delalandii* (Dumeril and Bibron), from South Africa. A - anterior end (showing dorsal oesophageal tooth), female, lateral view; B - posterior end of male (showing spicules and gubernaculum), lateral view; C – gubernaculum, ventral view; D - caudal bursa, lateral view. Scale bars: a, b, c, 20µm, d, 50µm.

Hosts - Common river frog, *Amietia delalandii* (Dumeril et Bibron, 1841) and the striped stream frog or striped grass frog, *Strongylopus fasciatus* (Smith, 1849).

Site in hosts - intestine.

Localities: *Amietia delalandii*: site 4, 5, 6, 13, 17, 22, 28, 34, 67, 90, 93.

Strongylopus fasciatus: site 32.

Infection parameters: *Amietia delalandii*: Intensity - 1 - 45 (12.82); Prevalence - 85%; Abundance 2. *Strongylopus fasciatus*: Intensity – 1-3 (1.6), Prevalence - 83%, Abundance – 0.025.

Remarks. Throughout most of the research region, several *Amphibiophilus* specimens were found in common river frogs (Fig. 7). The species was assigned to the genus *Amphibiophilus* because of the presence of two rings of labial papillae and a large cephalic vesicle, a buccal capsule with a well-developed oesophageal tooth (Fig. 7a), simple shaped spicules (Fig. 7b), a gubernaculum (Fig. 7c) and a well-developed caudal bursa with rays arrangements corresponding to the 2-3 type (Fig. 7d) (Skrjabin, 1916; Durette-desset *et al.*, 1994). *Amphibiophilus* spp. parasitise *A. delalandii* and *Strongylopus grayii* from South Africa, includes specimens from Louis Trichardt. In that study, the found specimens were morphologically very similar to other species, *Amietia natalensis* (Walton, 1935) (also described from *A. delalandii)* in the morphology of the anterior end of body, arrangements of rays of caudal bursa and female genital system (Baker, 1981), however, the form of the gubernaculum and the morphology of the distal end of females differed slightly. Because of morphological differences, the authors chose to classify these specimens as *Amphibiophilus* sp. instead of *Amphibiophilus natalensis*. Specimens from both *A. delalandii* and *S. fasciatus* from the present study appeared to be morphologically indistinct from that *Amphibiophilus* sp. 2, and moreover 100% corresponded in the 402 bp. long sequence of the *cox1* gene.

FAMILY : ONCHOCERCIDAE (Leiper 1911)

GENUS : ***Neofoleyellides*** Netherlands, Svitin, Smit et Du Preez, 2020

Neofoleyellides steyni Kuzmin, Netherlands, Du Preez, et Svitin, 2021

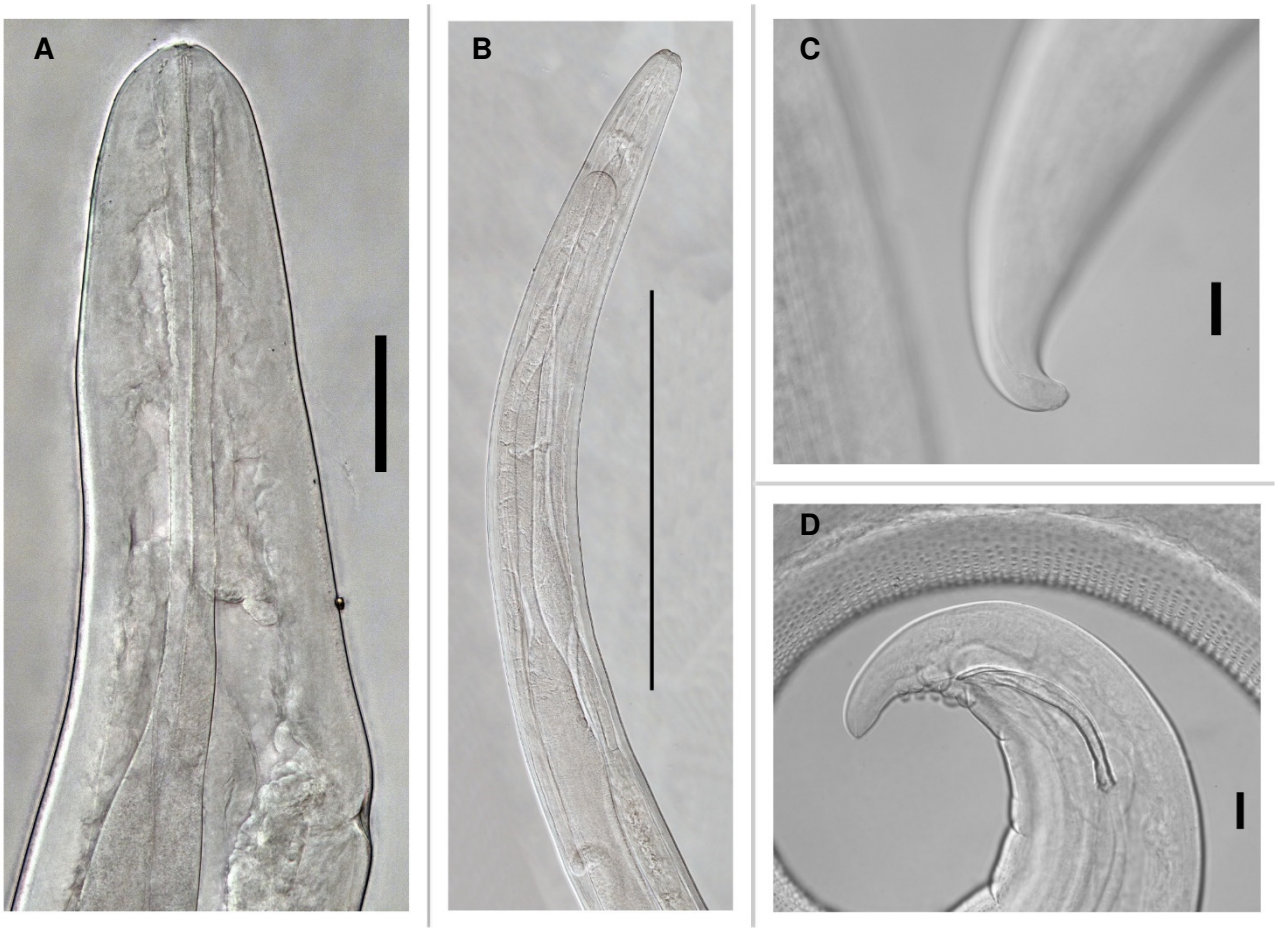


Figure 8: *Neofoleyellides steyni* parasitising *Amietia delalandii*, from Limpopo Province, South Africa. A - anterior end of body showing the shape of the oesophagus, male, lateral view; B –anterior end of body showing vulva, lateral view; C – posterior end of body, female, lateral view; D – posterior end of body showing spicules, caudal papillae and area rugosa, lateral view. Scale bars: A, 20µm, B, 1000µm, C, 50µm, D, 20µm.

Host: Common river frog, *Amietia delalandii* (Duméril and Bibron, 1841)

Site in host: body cavity, subcutaneous.

Localities: site 3, 5, 13.

Infection parameters: Intensity – 1-54 (10.38), Prevalence - 21% Abundance – 0.436

Remarks: The species assigned to the genus *Neofoleyellides* mostly based on the structure of the anterior end of body (Fig. 8D). Two lateral parastomal (Fig. 8A) structures, two small internal lateral papillae and two bigger external sublateral papillae surround the small oral orifice; based on male and female genital structures: in males - presence of caudal alae (Fig. 8D) wide plaque and unequal

spicules; in females - vulva situated at level of oesophagus (Fig. 8B,C) microfilariae with sheath (Netherlands *et al.*, 2020; Kuzmin *et al.*, 2021).

To date there are three species of *Neofoleyellides* described from four different frog species in South Africa (Aisien *et al.*, 2017, Helminthologia 54(2): 132-144, reported *Folleyellides* spp from some anurans). Because males had asymmetrical caudal alae and the number and position of genital papillae are asymmetrical, our specimens were classified to *N. steyni* because of the position of vulva within anterior part of oesophagus and the specific shape of the glandular portion of oesophagus (maximum width at the level of the first and second thirds border) (Kuzmin *et al.*, 2021). Moreover, our sequences of the *cox1* mitochondrial genetic markers are 100% corresponded to the sequences of the type material available in GenBank [MW598467].

FAMILY: COSMOCERCIDAE Travassos, 1925
GENUS: *Aplectana* Railliet and Henry, 1916

Several species were assigned to the genus *Aplectana* due to having oesophagus divided into three distinct parts (short pharynx, cylindrical corpus and wide posterior bulb with valve), lacking rosettes or plectanes on posterior end of males and the uteri and ovaries are anterior to the vulva in females (Baker, 1980).

Aplectana sp. 1

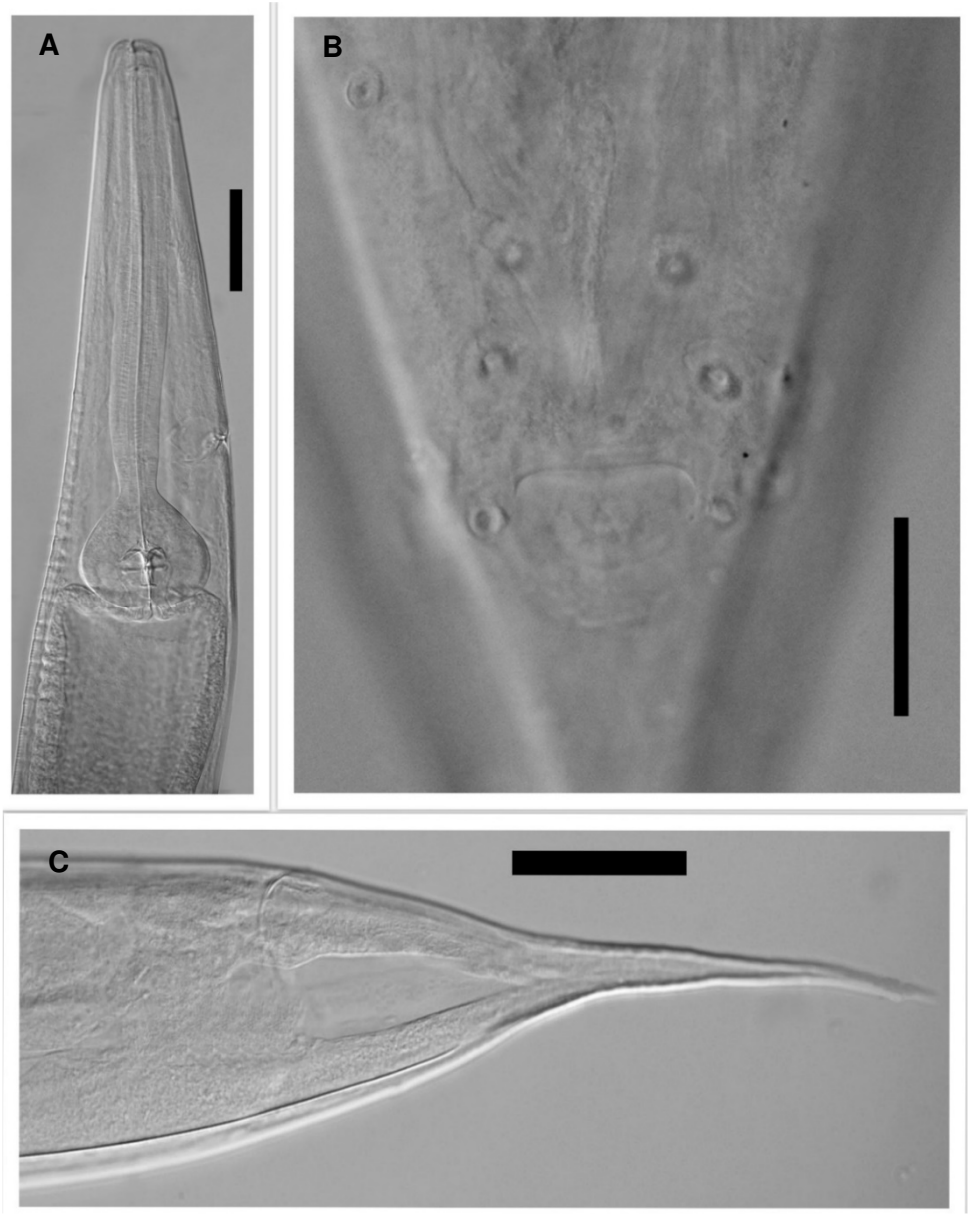


Figure 9: *Aplectana* sp. 1. parasitising Schismaderma carens, from Limpopo Province, South Africa. A – Anterior part of female showing the shape of the oesophagus; B – papillae of male posterior section, lateral view; C – Posterior part, tail of female, lateral view. Scale bars: A, 50µm, B, 20µm, C, 50µm.

Host: African red toad, *Schismaderma carens* (Smith, 1848).

Site in host: Intestine

Localities: site 1, 2, 21, 23, 26, 27, 29, 51.

Infection parameters: Intensity – 3-92 (50), Prevalence 83%, Abundance – 1.61

Remarks: Only one species – *Aplectana macintoshii* (Stewart, 1914) was previously discovered in African red toads. Despite the rather big sample of nematodes only several males were recovered. These males share some characters with *A. macintoshii* (Fig. 9) such as simple spicules (Fig. 9B) and numerous papillae especially in caudal region. However, such morphology is common across *Aplectana* spp. from different hosts but notable molecular differences are observed (see below). Therefore, we prefer not to rely solely on morphology and assign collected specimens to *A. macintoshii*, but rather identify them to *Aplectana* sp. 1 (Fig. 9).

Aplectana sp. 2

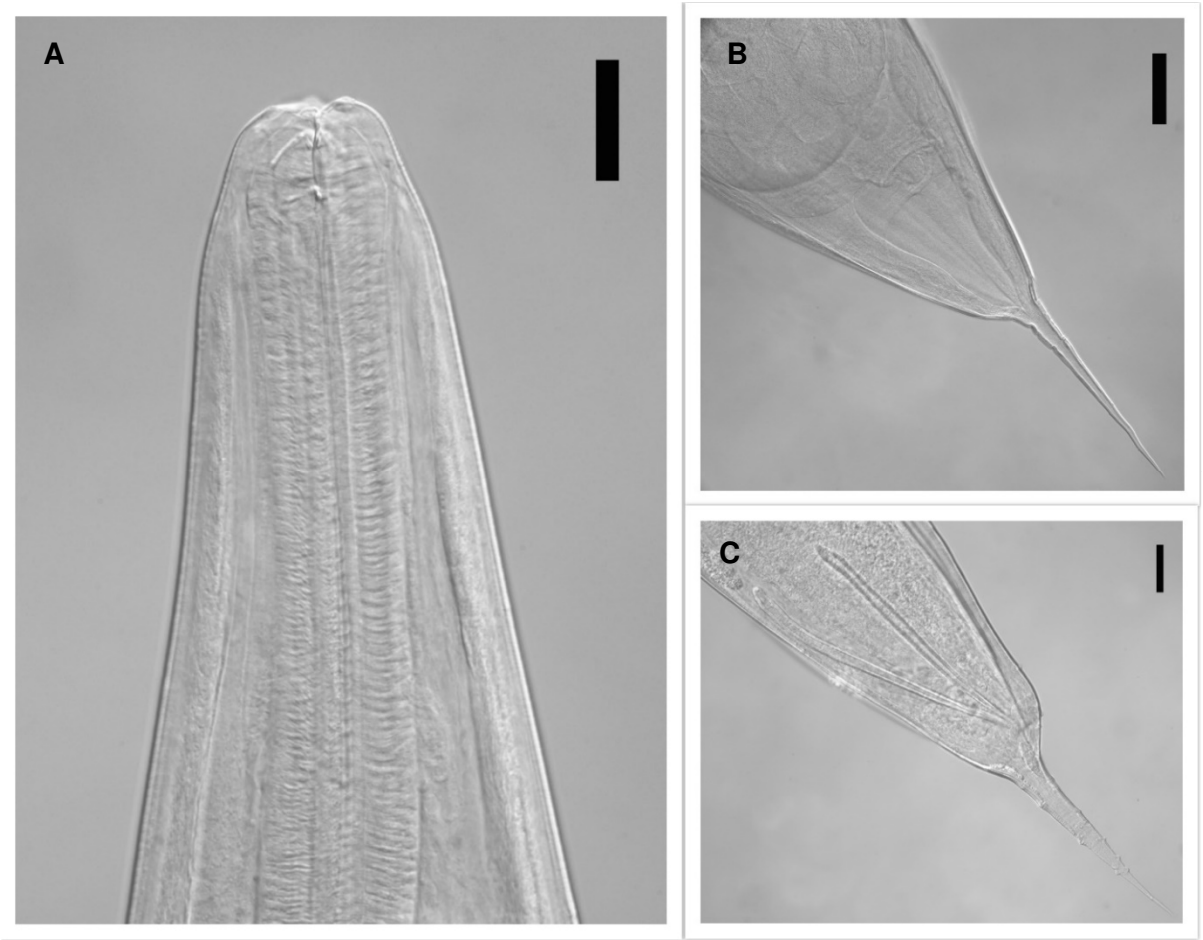


Figure 10: *Aplectana* sp. 2 parasitising the common river frog, *Amietia delalandii*, from South Africa. A – anterior end, male, lateral view; B –posterior end, female, lateral view; B – posterior end of male showing simple-shaped spicules, ventro-lateral view. Scale bars: A. 20µm, B, C, 50µm.

Host: Common river frog, *Amietia delalandii* (Duméril and Bibron, 1841)

Site in host: Intestine

Localities: site 1, 2, 4, 5, 17, 21, 22, 23, 26, 27, 28, 29, 51.

Infection parameters: Intensity – 1-29 (5.66), Prevalence – 24.5% Abundance – 0.275

Remarks: In most localities, specimens of *Aplectana* were collected from river frogs' intestines, often along with *Amphibiophilus* sp. These specimens also appeared to be morphologically close to *Aplectana macintoshii* in having simple shaped spicules (Fig. 10C) and numerous unpaired papillae at the pre- and post-cloacal regions. However, based on morphological similarity and genetic distinctness from *Aplectana* sp. 1 at the same time (3% in 1200 b.p. long ITS-28S alignment) we cannot assign found specimens to *A. macintoshii*. We hypothesize that found specimens might belong to a new (probably cryptic) species of *Aplectana*, although cannot confirm this fact without studying the type material of *A. macintoshii* and/or obtaining molecular data of *A. macintoshii* from its type host and type locality. Thus, in present study we prefer to assign specimens collected from common river frogs to *Aplectana* sp. 2 (Fig. 10).

Aplectana sp. 3

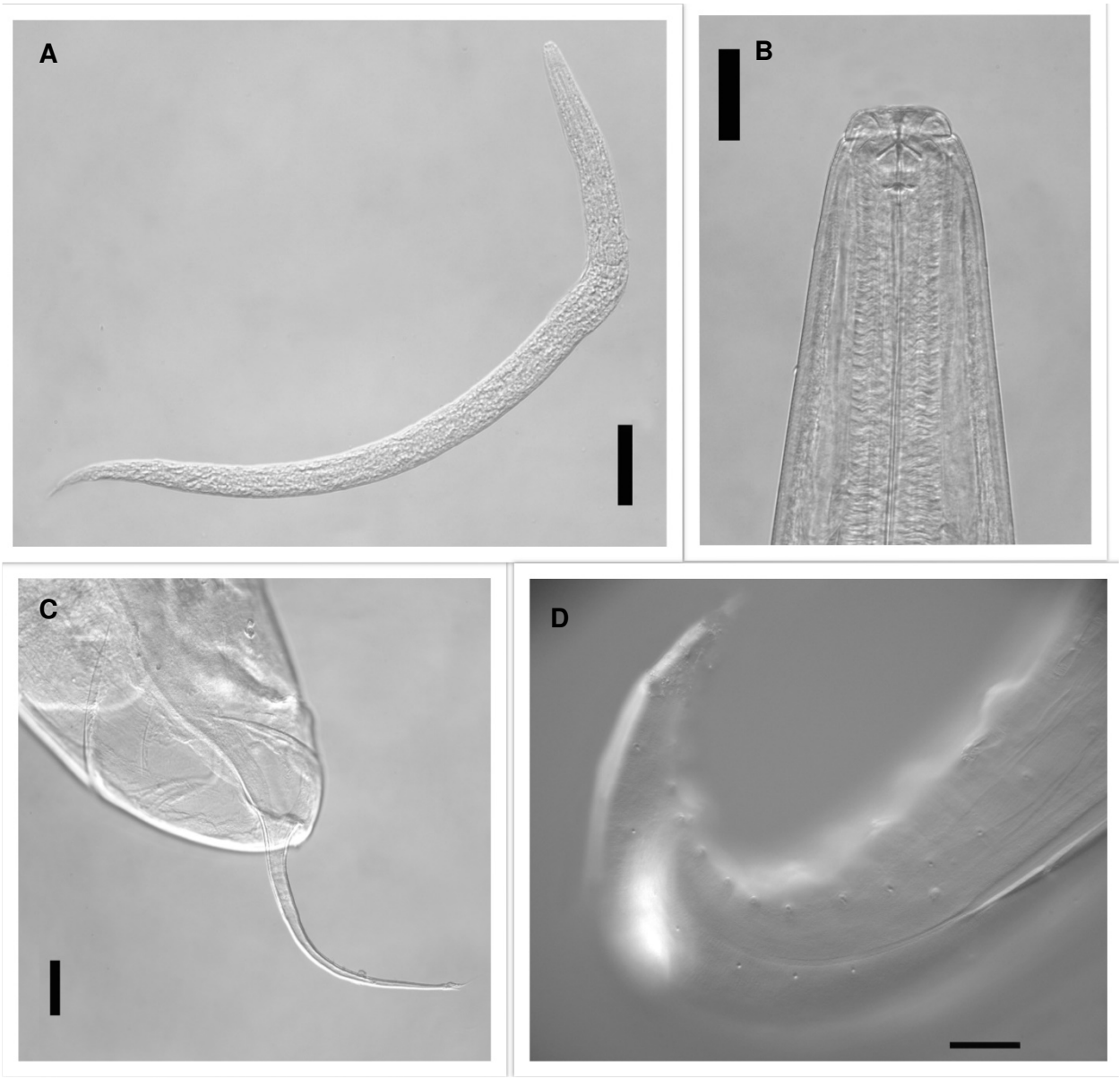


Figure 11: *Aplectana* sp. 3 parasitising *Sclerophrys pusilla* from Limpopo South Africa. A – whole body, female, lateral view; B – Anterior end, female, lateral view; C – posterior end, female tail, dorsal view; D - caudal end of male, lateral view. Scale bars: A, B, C, D, 50µm.

Hosts: Flat-backed/ striped-back toad, *Sclerophrys pusilla*, (Mertens, 1937), Eastern olive toad *Sclerophrys garmani*, (Meek, 1897) and African common toad or guttural toad, *Sclerophrys gutturalis* (Power, 1927).

Site in hosts: Intestine

Localities: *Sclerophrys pusilla:* site 13, 17, 34. *Sclerophrys garmani*: site 13, 21, 23. *Sclerophrys gutturalis:* site 22, 23.

Infection parameters: *S. pusilla*: Intensity – 3-12 (7), Prevalence - 40%, Abundance – 0.121

S. garmani: Intensity – 9-35 (25.3) Prevalence – 60%, Abundance – 0.245

S. gutturalis: Intensity – 1-79 (26.8), Prevalence – 45%, Abundance – 0.433

Remarks: Similar to the previous *Aplectana* sp. 1 and *Aplectana* sp. 2, specimens from all studied *Sclerophys* spp. morphologically resembled *A. macintoshii* (Fig. 11). Studied specimens from three toad species appeared to be 100% corresponded between each other in *cox1* gene sequences on one hand and on the other hand somewhat different from other *Aplectana* spp. recovered from *Amietia delalandii* and *S. carens* (3% and 4% in 1200 b.p. ITS-28S sequences, respectively). It is also different from congeners collected from *P. anchietae* and *Breviceps* spp. (see below). Thus, we assign these specimens to *Aplectana* sp. 3 (Fig. 11).

Aplectana sp. 4

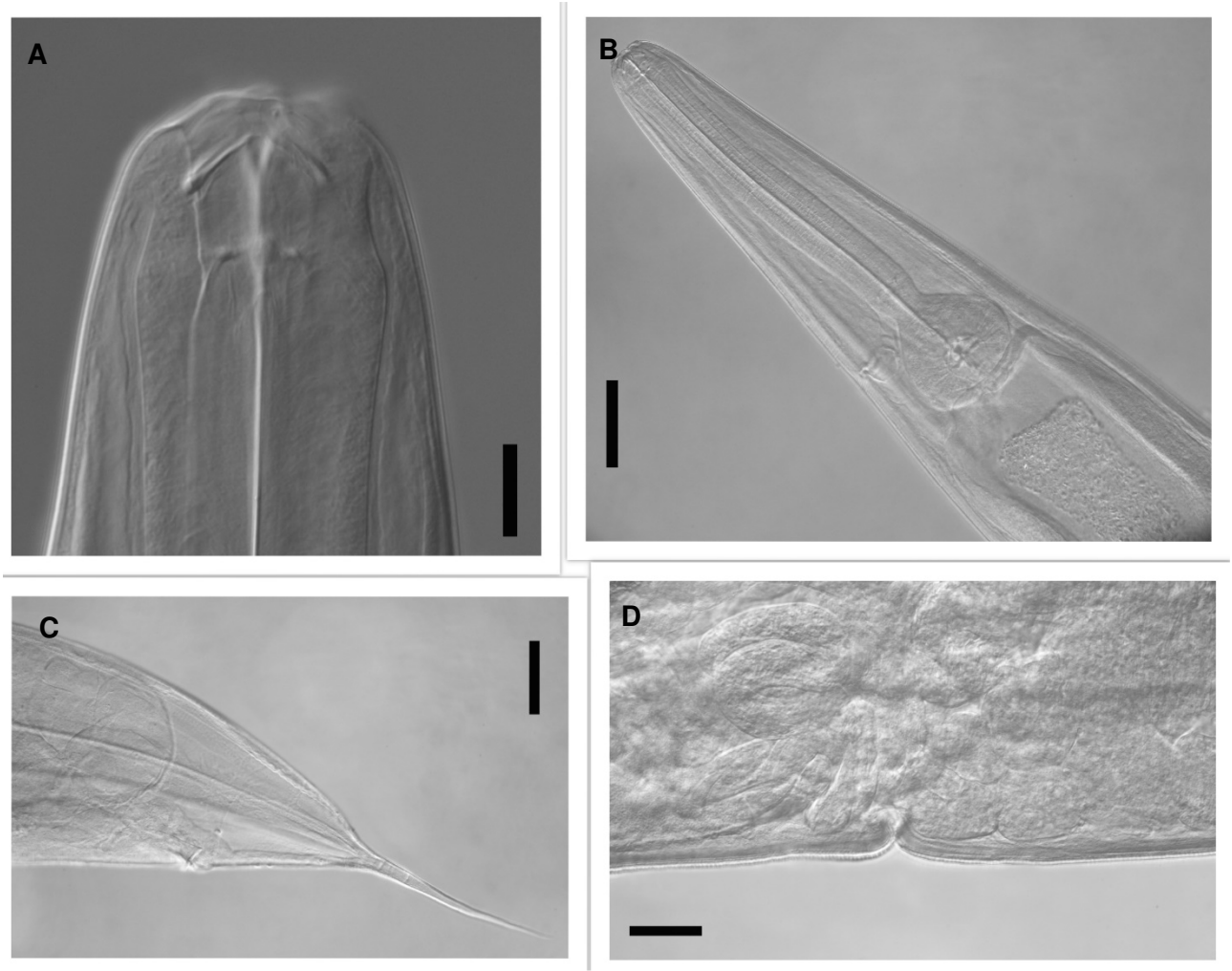


Figure 12: *Aplectana* sp. 4 parasitising *Ptychadena. anchietae*, from Limpopo Province, South Africa. A –fragment of body at anterior end, female, lateral view; B – anterior extremity, female, lateral view; C – posterior extremity, female tail; D – vulva mid-body length, female, lateral view. Scale bars: A, 20µm, B, C, 100µm, D, 50µm.

Host: Plain Grass Frog, *Ptychadena anchietae* (Bocage, 1867)
Site in host: Intestine
Localities: site 22, 78, 83.
Infection parameters: Intensity – 1-17 (4.22), Prevalence – 39%, Abundance – 0.122

Remarks: Several female specimens of cosmocercid nematodes were recovered from plain grass frogs in three distant localities (Fig. 12). Based on molecular analyses of partial 28S and *cox1* sequences, found specimens were assigned to the genus *Aplectana*. Unfortunately, due to lack of males and lack of molecular data, we could only identify these specimens only up to generic level. However, *cox1* sequences showed their distinctness (3-4 % in ITS-28S sequences) from other congeners collected in present study and led us to assign them to *Aplectana* sp. 4.

Aplectana sp. 5

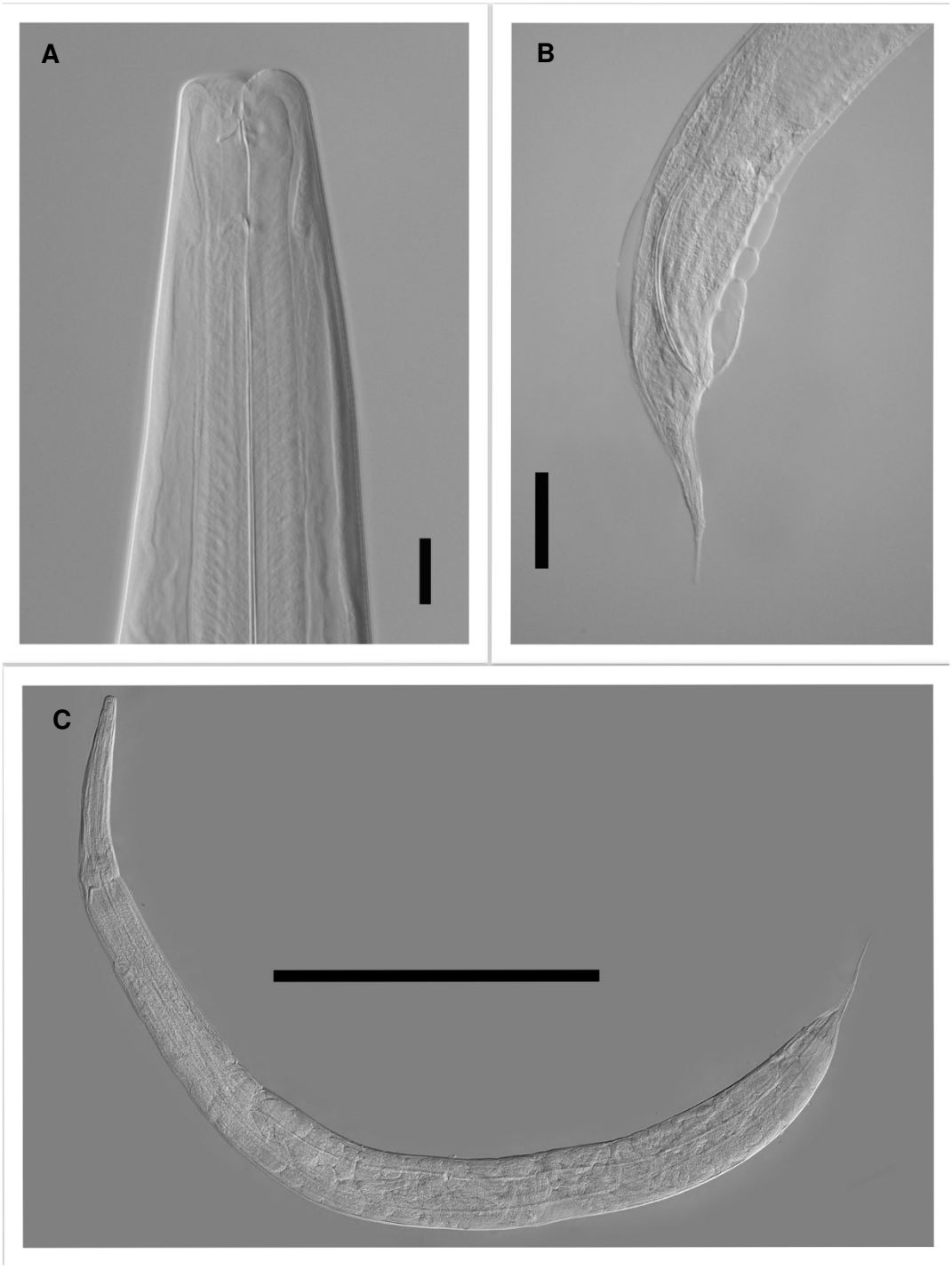


Figure 13: *Aplectana* sp. 5 parasitising *Breviceps adsp.ersus* from South Africa. A – Anterior end of body, male, lateral view; B – posterior end of male, lateral view; C – Whole nematode, lateral view, female. Scale bars: A, 20µm, B, 100µm, C, 1mm.

Host: Bushveld rain frog, *Breviceps adsp.ersus* Peters, 1882, and the forest rain frog, *Breviceps sylvestris* FitzSimons, 1930.

Site in host: intestine

Localities: *Breviceps adsp.ersus*: site 79. *B. sylvestris*: site 4.
Infection parameters: *Breviceps adsp.ersus*: Intensity – 1-17 (4.22), Prevalence – 39%, Abundance – 0.122. *Breviceps sylvestris*: Intensity – 21-40 (31.2), Prevalence – 1%, Abundance – 0.504

Remarks: Two species namely *Aplectana capensis* Baker, 1981 from *Breviceps rosei* Power, 1926 and *Breviceps montanus* Power, 1926 and *Aplectana degraaffi* Baker, 1981 from *B. sylvestris* have been previously described from *Breviceps* spp. from southern parts of South Africa (Baker, 1981). Specimens of *Aplectana* collected from two species *Breviceps* in present study differ from *A. capensis* in the morphology of spicules (Fig. 13b) (lacking cap on the proximal part of spicules) and from *A. degraaffi* caudal papillae arrangement (lacking large unpaired papillae on the dorsal lip of cloaca). These specimens also differ from other *Aplectana* spp. from present study in 4-6 % in ITS-28S. Differences in morphology and molecular data led us to assign them to *Aplectana* sp. 5 (Fig. 13).

GENUS: *COSMOCERCA Diesing*, 1861

Four different species were assigned to the genus *Cosmocerca* due to having an oesophagus divided into three distinct parts (short pharynx, cylindrical corpus and wide posterior bulb with valve), possession of plectanes, numerous papillae along the body and sexual dimorphism, since males are half the size of females (Baker, 1980).

Cosmocerca sp. 1 *(makhadoensis) Diesing*, 1861

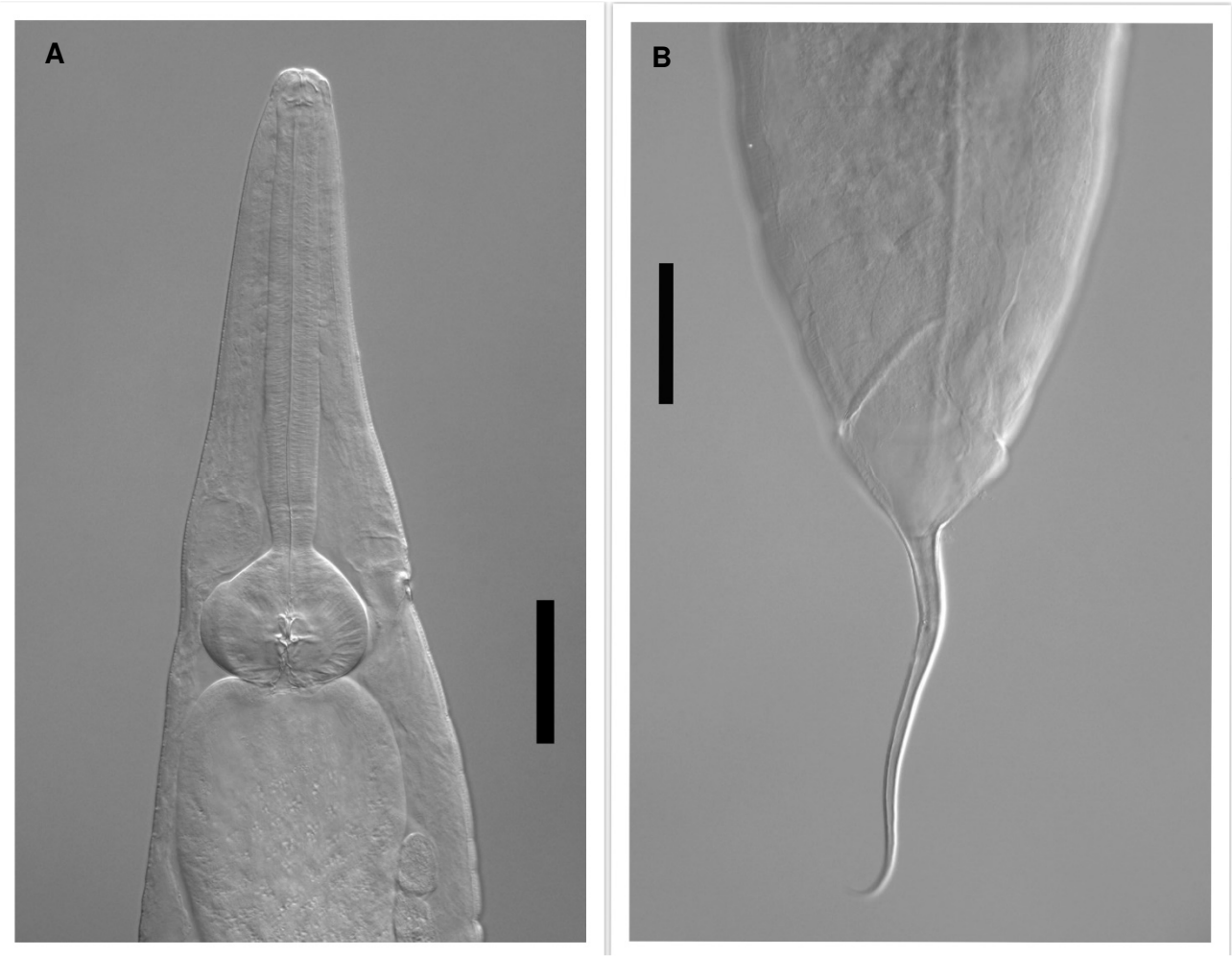


Figure 14: *Cosmocerca* sp. 1 (*machadoensis*) found in *Phrynomantis bifasciatus* from South Africa. A – fragments of anterior and mid-body sections of female, muscular oesophagus, lateral view; B –at posterior extremity of body. Scale bars: A, B, 100µm.

Host: Banded rubber frog, *Phrynomantis bifasciatus* (Smith, 1847)
Site in host: Intestine
Localities: site 22, 27, 80.
Infection parameters: Intensity – 4-136 (26.190), Prevalence – 84%, Abundance – 1.77

Remarks: Found species belongs to the genus *Cosmocerca* due to having an oesophagus divided into three distinct parts (Fig. 14A) (short pharynx, cylindrical corpus and wide posterior bulb with

valve), possession of plectanes, numerous papillae along the body and sexual dimorphism, with males half the size of females (Baker, 1980). *Cosmocerca makhadoensis* has been recently described based on the material from *P. bifasciatus* collected in the outskirts of Makhado town (Limpopo Province), close to the study area. Our specimens clearly corresponded to the original description in having the club-shaped gubernaculum, simple morphology of spicules, 10 pairs of plectanes and arrangement of the somatic papillae. Moreover, obtained sequences of *cox1* and partial 28S gene markers 100% corresponded to the sequences of type material (Fig. 14).

Cosmocerca sp. 2 *(daly n. sp.)*

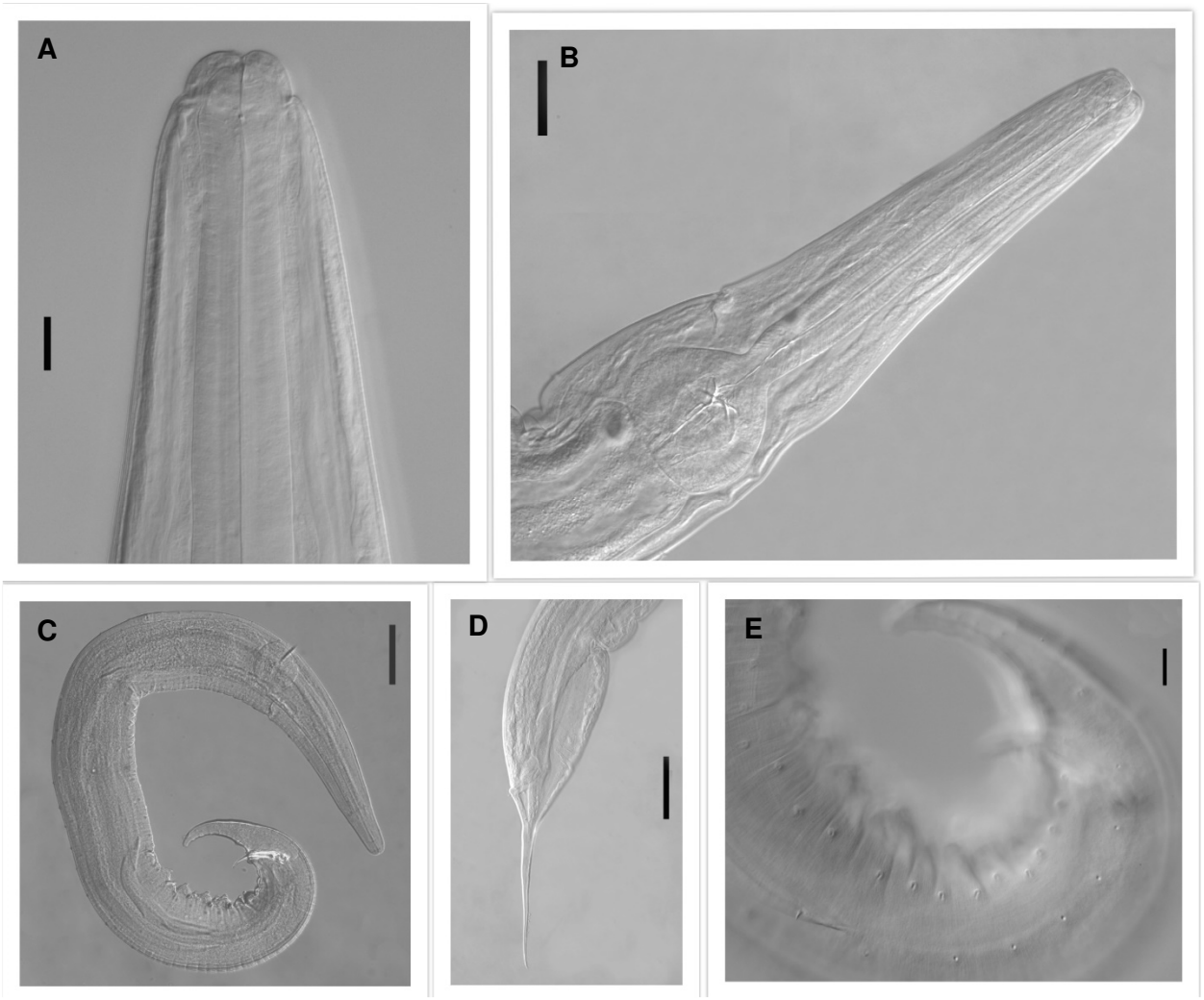


Figure 15: *Cosmocerca* sp (*daly n. sp)*, parasitising *Kassina senegalensis* from Limpopo Province, South Africa. A, B – anterior end of body, female, lateral view; C – whole body of male, lateral view, D – posterior end of body, female, lateral view, E – posterior end of body, male, lateral view. Scale bars: A, 20µm, B, 50µm, C, D, 100µm, E, 50µm.

Host: Bubbling Kassina, *Kassina senegalensis* (Dumeril and Bibron, 1854)
Site in host: Intestine
Localities: site 10, 22, 33.
Infection parameters: Intensity – 1-26 (7.58), Prevalence – 80%, Abundance – 0.294

Remarks: Our specimens match the original description of *Cosmocerca daly* n. sp. to a tee, in having a prominent structure – V-shaped gubernaculum (Fig. 15C) with hook-like structures on the margins, five pairs of plectanes and arrangements of somatic papillae (Fig. 15E). Additionally, obtained sequences of *cox1* and 28S gene markers 100% corresponded to the sequences of type material (Fig. 15)

Cosmocerca sp. 3

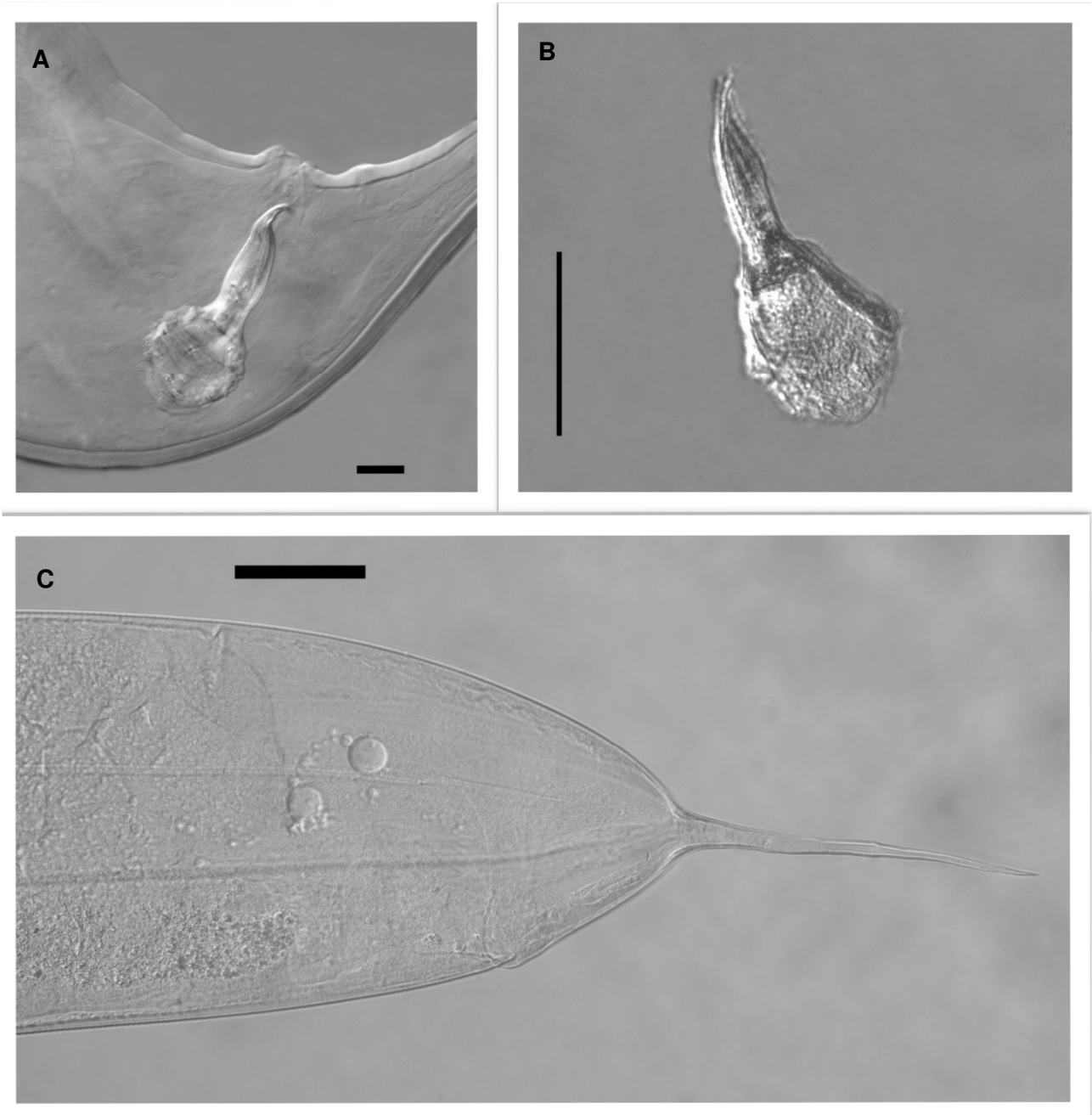


Figure 16: *Cosmocerca* sp3 parasitising *Poyntonophrynus fenoulheti*, from Limpopo, South Africa. A – male posterior end, gubernaculum, lateral view, posterior end; B – gubernaculum, at male tail, lateral view; C – fragments of posterior end of female tail. Scale bars: A, 20µm, B, 50µm, C, 100µm.

Host: Northern Pygmy toad, *Poyntonophrynus fenoulheti*, (Hewitt and Methuen, 1912)

Site in host: Intestine

Locality: site 34.

Infection parameters: Intensity – 1-33 (6.07), Prevalence – 68%, Abundance – 0.255

Remarks: The species was recovered only from *P. fenoulheti* in one locality. All found specimens appeared to be different from all *Cosmocerca* previously reported from Africa based on the shape of

the gubernaculum (wide Y-shaped with well sclerotised edges) (Fig. 16a, b). Also, our specimens differ by 3 – 5 % from other species reported from South Africa (*C. daily*, *C. makhadoensis* and *C. monicae*). In our opinion specimens from *P. fenoulheti* might belong to a new species which can be outlined in further studies and thus are assigned to *Cosmocerca* sp. 3 in present study (Fig. 16).

Cosmocercidae gen. sp.1

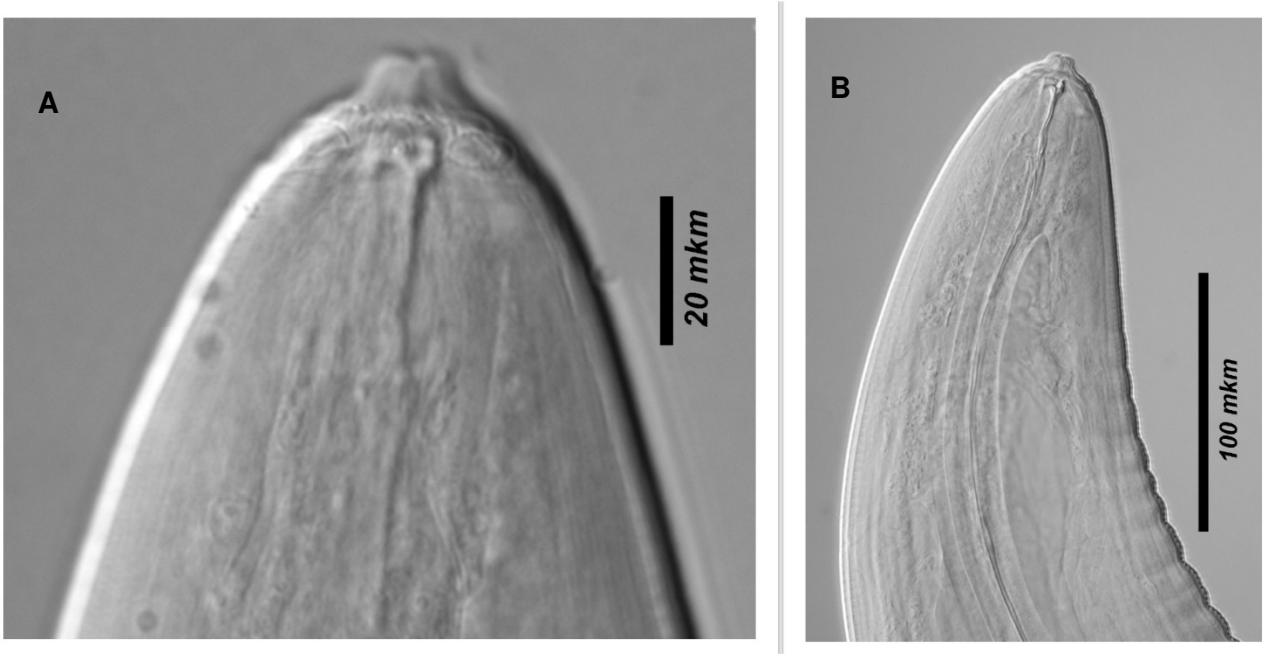


Figure 17: *Cosmocercidae* gen. sp.1 parasitising *Chiromantis xerampelina* from South Africa. A – anterior end of female, dorsal view; B – mid body with oesophagus visible of female anterior part. Scale bars: A, 20µm, b, 100µm.

Hosts: Southern foam-nest tree Frog, *Chiromantis xerampelina* Peters, 1854, and the African bullfrog, *Pyxicephalus edulis* Peters, 1854.
Site in hosts: Intestine
Localities: *Chiromantis xerampelina:* site 21. *Pyxicephalus edulis*: site 30, 81.
Infection parameters: *C. xerampelina*: Intensity – 1-5 (3), Prevalence – 95%, Abundance – 0.019
P. edulis: Intensity – 1-104 (35.33), Prevalence – 16%, Abundance – 0.343

Remarks: Several female cosmocercid nematode specimens were found in material taken from *C. xerampelina* and *P. edulis* in the indicated locations (Fig. 17). The morphological characteristics of available females were insufficient for genus assignment. Moreover, BLAST searching of the obtained sequences of ITS and partial 28S genetic markers placed found specimens in the family *Cosmocercidae*, however correspondence was not enough for assigning them into the genus *Cosmocerca* or *Aplectana*. Therefore, we can identify these specimens only to the family level until obtaining males in further studies.

Cosmocercidae gen. sp. 2

Host: Udzungwa ridged frog, *Ptychadena uzungwensi*s (Loveridge, 1932)
Site in host: Intestine
Locality: site 32.
Infection parameters: Intensity – 1, Prevalence – 12.5%, Abundance - 0.0032

Remarks: In all studied *P. uzungwensis* we have found a single non-gravid female of the cosmocercid nematode. Unfortunately, morphological characters of the immature female specimen were insufficient even for the genetic identification and the DNA extraction attempt failed. Therefore, we could only identify the found nematode to the family level.

FAMILY: ANISAKIDAE
GENUS: *Contraceacum* Raillet and Henry, 1912

Contracaecum sp. 1

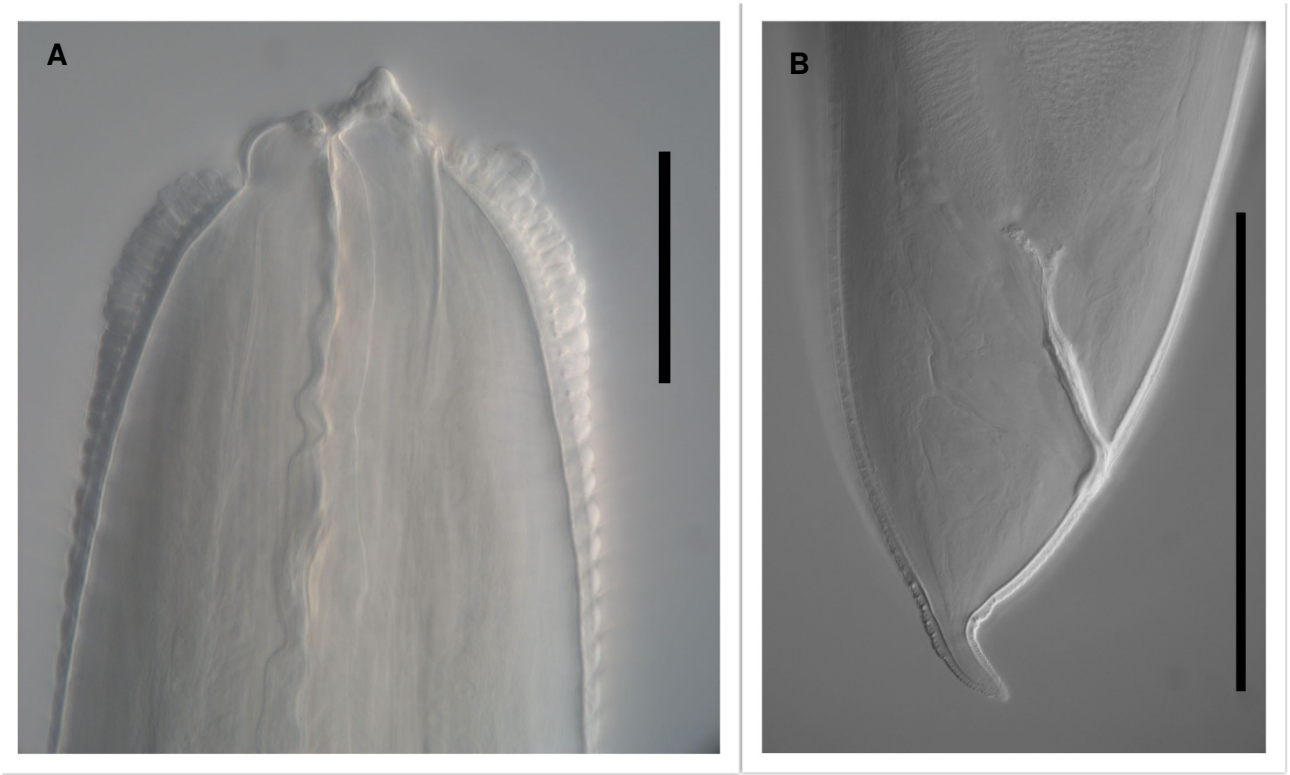


Figure 18: *Contraceacum* sp from *Amietia delalandii*, common river frog, from South Africa. A – Anterior end of female, dorsal view, with fragments of body. B – female posterior extremity, lateral view. Scale bars: A, 50 µm, B, 20 µm.

Host: Common river frog, *Amietia delalandii* (Duméril and Bibron, 1841)

Site in host: Body cavity, subcutaneous.
Localities: site 13, 34.
Infection parameters: Intensity – 0-5 (5), Prevalence – 16%, Abundance – 0.016

Remarks: Nematodes of the genus *Contracaecum* are common parasites of birds and mammals that often use fish or other aquatic vertebrates as obligate intermediate hosts (Moravec *et al.,* 2016). It is nearly impossible to identify the *Contracaecum* species collected in the intermediate host due to the indistinctness of the third stage larva between different *Contracaecum* spp. (Moravec *et al*., 2016) and a lack of molecular data (Fig. 18). Detailed descriptions and molecular data of *Contracaecum* sp. has been recently published for specimens parasitising African clawed frogs *Xenopus laevis* throughout South Africa (Schoeman *et al*., 2020).
Contracaecum third-stage larvae have been discovered encapsulated in the body cavities of a variety of common river frogs, *Amietia delalandii*, in the Limpopo Province of South Africa, including the towns of Louis Trichardt and Waterpoort. Although we cannot identify our specimens to the species level, the molecular data of the *cox1* gene showed clear distinctness of our specimens from previously recorded *Contracaecum* from *X. laevis* and therefore assigned to *Contracaecum* sp. 1

FAMILY: GNATHOSTOMATIDAE Railliet, 1895
GENUS: *Tangua* (Lowry and Fenwick, 1983)

Tangua sp.

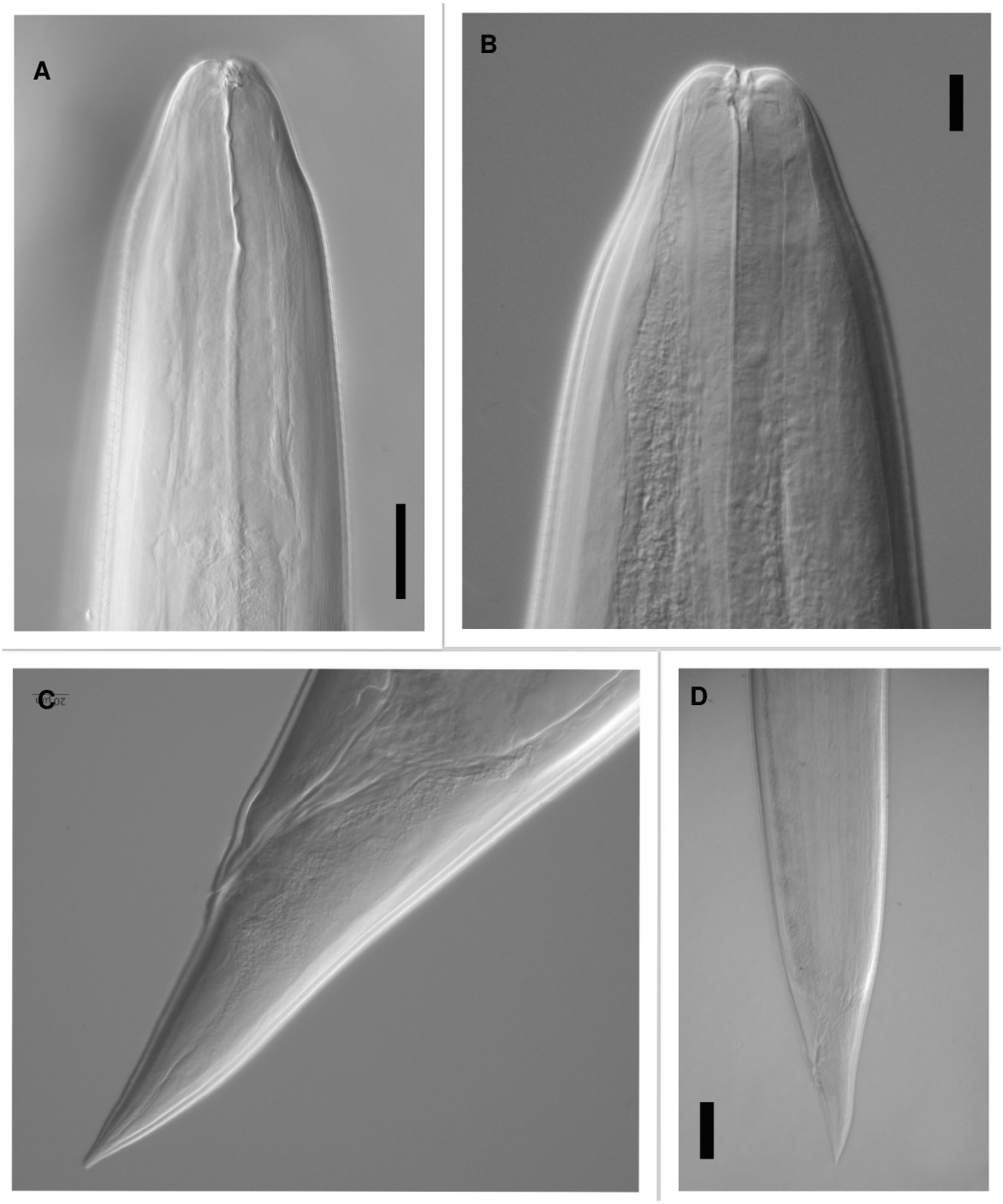


Figure 19: *Tangua* sp. larva from *Hyperoluis marmoratus*, found in South Africa. A – anterior end with tooth-like structures, dorsal view; B – anterior end with tooth-like structures, lateral view; C, D –posterior of body, lateral view. Scale bars: A, 50µm, B, C, 20µm, D, 100µm.

Host: Painted reed frog, *Hyperolius marmoratus* Rapp, 1842.

Site in host: Body cavity

Locality: site 34.

Infection parameters: Intensity -1 - 2 (1.5), Prevalence - 100% Abundance – 100.

Remarks: In one specimen of *H. marmoratus* two nematodes were found encapsulated in body cavity. Initially, these specimens were confused with *Contracaecum*, however, due to the presence of tooth-like features (Fig. 19a, b) on the front end, collected specimens were classified as larval stage of the genus *Tangua* under high magnification. Species of *Tangua* are common parasites of monitor lizards, *Varanus* Merrem, 1820, that has been recorded in clawed frogs recently (Schoeman *et al.,* 2020). We believe that the present finding is an occasional infection as the specimens of *Varanus niloticus L*. were commonly spotted in sampling sites (Fig. 19).

FAMILY: *RHABDIAS*IDAE Railliet, 1915
GENUS: *Rhabdias* Stiles et Hassal, 1905

In lungs of several frog species specimens of dark brownish nematodes were recovered. The presence of an inflated body cuticle, a small buccal capsule with thick walls, particular anatomy of the reproductive system and parasitism in the lungs of an amphibian host led to their classification as *Rhabdias*. In the Afrotropical realm, there are currently 23 species of the genus, 10 of which have been recovered from amphibian hosts.

Rhabdias engelbrechti Kuzmin, Halajian, Tavakol, Luus-Powell et Tkach, 2017

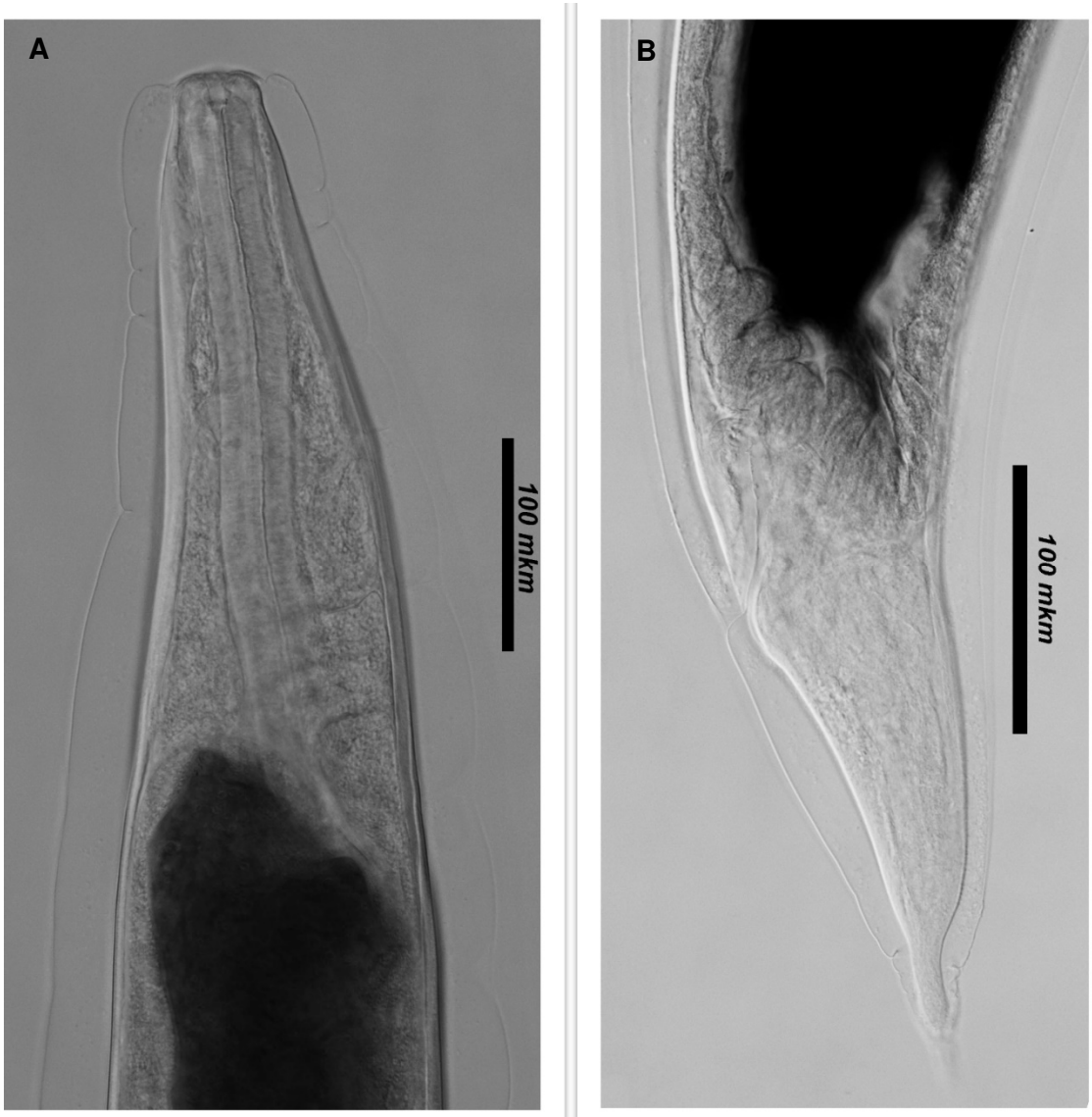


Figure 20: *Rhabdias engelberechti* parasitising *Phrynomantis bifasciatus*, from the Vhembe area, South Africa. A - anterior end, female lateral view; B - Posterior end of female, dorsal view. Scale bars: a, b, 100µm.

Host: Banded rubber frog, *Phrynomantis bifasciatus* (Smith, 1847)

Site in host: lungs.
Locality: site 27.
Infection parameters: Intensity -1 - 9 (5), Prevalence - 16% Abundance - 0.064

Remarks. *R. engelberechti* was recently described as a parasite of banded rubber frogs from the outskirts of Pietersburg (Limpopo Province, South Africa). Our specimens collected from the same host (even though from a rather distant locality) clearly corresponded to the original description in having small buccal capsule (Fig. 20) cuticular inflation and round on the anterior end of body, however, posterior end narrowing, body prominently inflated at anterior and posterior thirds and lacking of lateral pseudolabia.
Moreover, our sequences of the ITS-28S nuclear and *cox1* mitochondrial genetic markers are 100% corresponded to the sequences of type material available in GenBank (MG428406 for ITS-28S and MG428410 for *cox1*). Therefore, we assign all *Rhabdias* collected from *Phrynomantis bifasciatus* to the species *R. engelbrechti.*

Rhabdias africanus (Kuzmin, 2001)

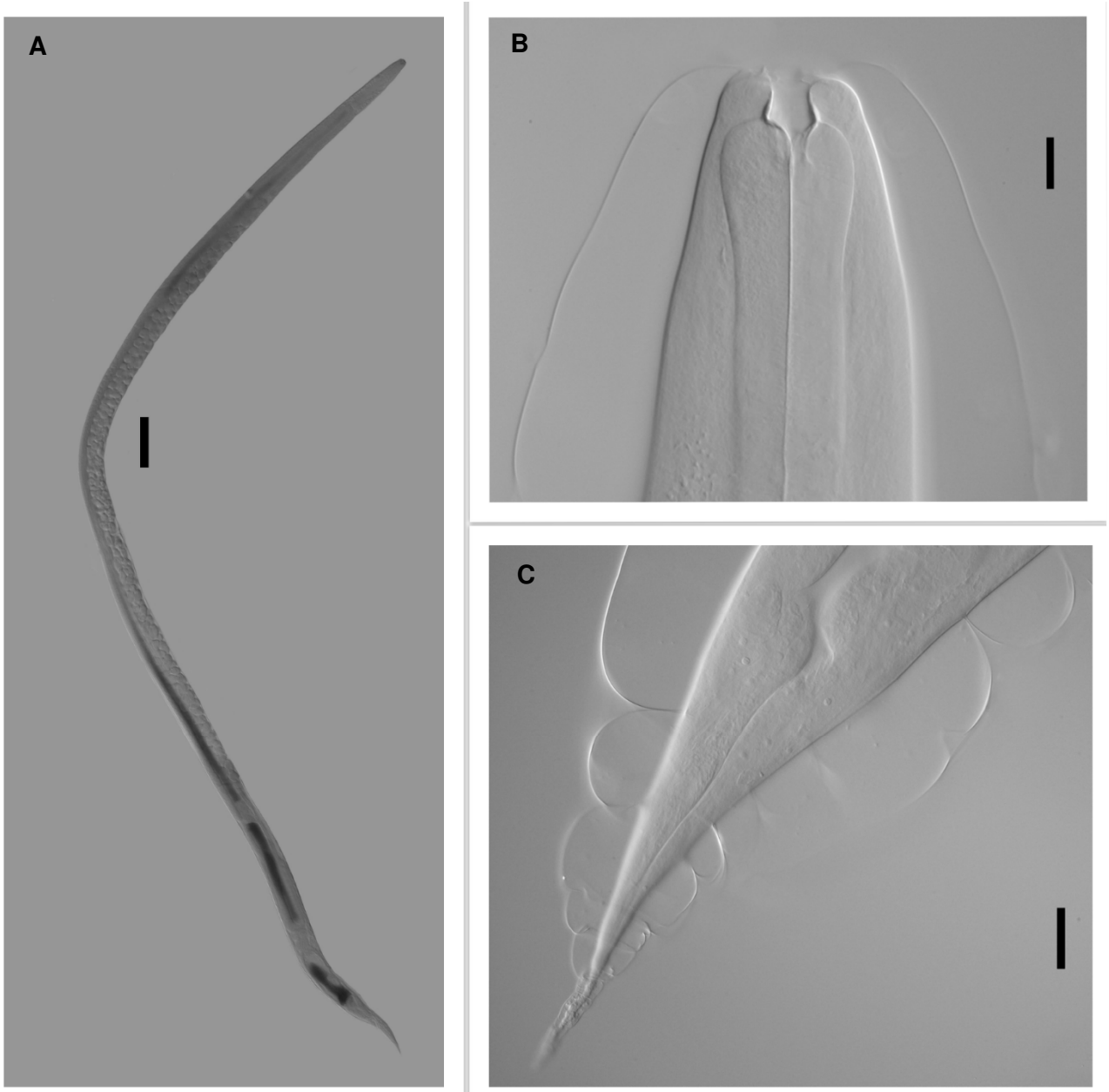


Figure 21: *Rhabdias africanus* parasitising *Sclerophrys pusilla* from South Africa. A – Whole body, anterior to posterior end, female, lateral view; B – anterior end, female lateral view; C – female tail, lateral view. Scale bars: A, 1mm, B, 20µm, C, 50µm.

Hosts: Eastern olive toad, *Sclerophrys garmani* (Meek, 1897) and the flat-backed/ striped-back, *Sclerophrys pusilla* (Mertens, 1937)

Site in hosts: Lungs

Localities: *Sclerophrys garmani*: site 21. *Sclerophrys pusilla*: site 34.

Infection parameters: *S. garmani*: Intensity – 1-2 (1), Prevalence – 20%, Abundance – 0.0064

S. pusilla: Intensity – 1-2 (1), Prevalence – 67%, Abundance – 0.0064

Remarks: The species was assigned to the genus *Rhabdias* also due to parasitism in the lungs of an amphibian host, the presence of inflated body cuticle (Fig. 22a) the reproductive system's small buccal capsule with robust walls and distinct shape. Collected specimens have body with irregular folds of swollen cuticle (Fig. 22b, c) anterior end rounded, two lateral pseudolabia clearly visible in lateral view, buccal capsule barrel shaped in lateral view, conical tail with phasmids located at its mid-length. The complex of such characters corresponds to the description of *R. africanus*. This species was described from *Sc. pusilla* (reported as *Bufo maculatus*) and *Sc. garmani* (reported as *Bufo garmani*) from the Kruger National Park (South Africa) and was only the third species of the genus described from African anurans. Subsequently, *R. africanus* was identified from its type hosts and other hosts such as guttural toad *Sclerophrys gutturalis* and the clicking stream frog *Strongylopus grayii* (Halajian *et al*., 2013; Kuzmin *et al*., 2017). The sequences of *cox1* and ITS-28S genetic markers in *Rhabdias* recovered from *Sclerophrys garmani* and *Sclerophys pusilla* in this study are 100 percent identical to each other and to those derived from GenBank [MG428411 for *cox1* and MG428407 for ITS-28S] of the species *R. africanus.* We consequently classify these specimens as *Rhabdias africanus* based on both morphological and genetic evidence (Fig. 22)

Rhabdias cf. *sylvestris* (Baker 1982)

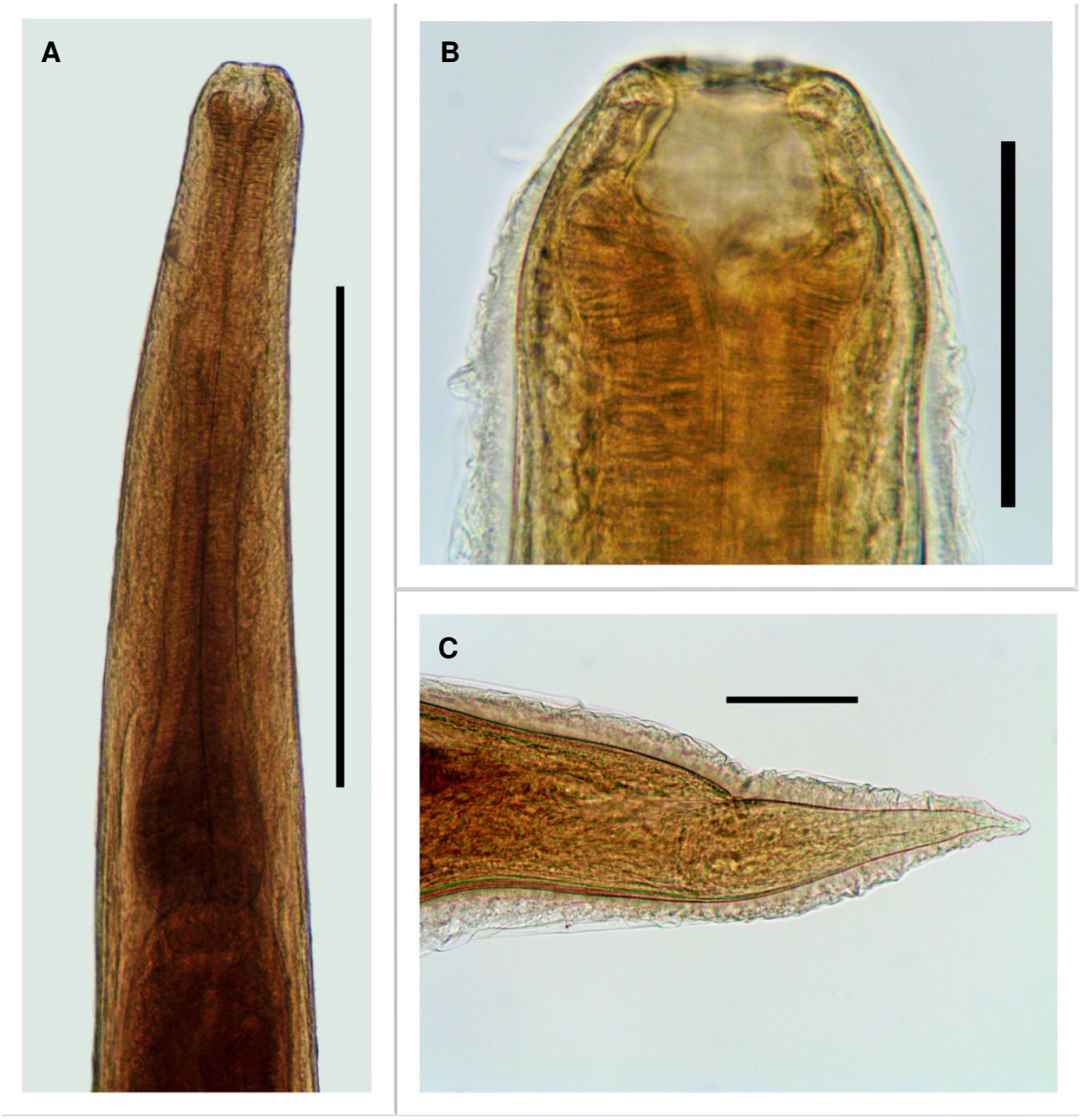


Figure 22: *Rhabdias* cf. *sylvestris* parasitising *Breviceps sylvestris* from South Africa. A – anterior part of female, with muscular oesophagus, lateral view; B – head part of female, dorsal view; C – Tail end of female, lateral view. Scale bars: A, 500µm, B, 20µm, C, 50µm.

Host: Forest Rain frog, *Breviceps sylvestris* FitzSimons, 1930.

Site in host: Lungs

Locality: site 4.

Infection parameters: Intensity – 1-4 (2), Prevalence – 6%, Abundance – 0.019

Remarks: Several specimens of large nematodes were collected from lungs of the forest rain frog from the forest on the top of the mountain. Morphologically, found specimens corresponded to the description of *Rhabdias sylvestris*, originally described from the frog *Breviceps sylvestris* in outskirts

of Polokwane (Limpopo Province, South Africa) by (Baker, 1982). The species was originally described as a member of the genus *Entomelas* Travassos, 1930 due to having a large buccal capsule with conspicuous teeth. However, with ongoing research and molecular analysis, this species clustered together with the members of *Rhabdias* from amphibian hosts and was thus reassigned to that genus (Tkach *et al.*, 2014). The same as *R. sylvestris*, our specimens have a large slender body (Fig. 22a), large buccal capsule subspherical in shape (Fig. 22b), with thick walls at anterior part, thin in posterior part. Although no morphological variations were found between our material and descriptions of *R. sylvestris*, there were some differences in *cox1* (5%) and ITS-28S (0.4%) sequences. Since we have data of this species from only two rather distant localities, we cannot evaluate the significance of the molecular diversity within the species and prefer to identify our material as *Rhabdias* cf. *sylvestris* until further studies (Fig. 22).

Rhabdias sp. 1

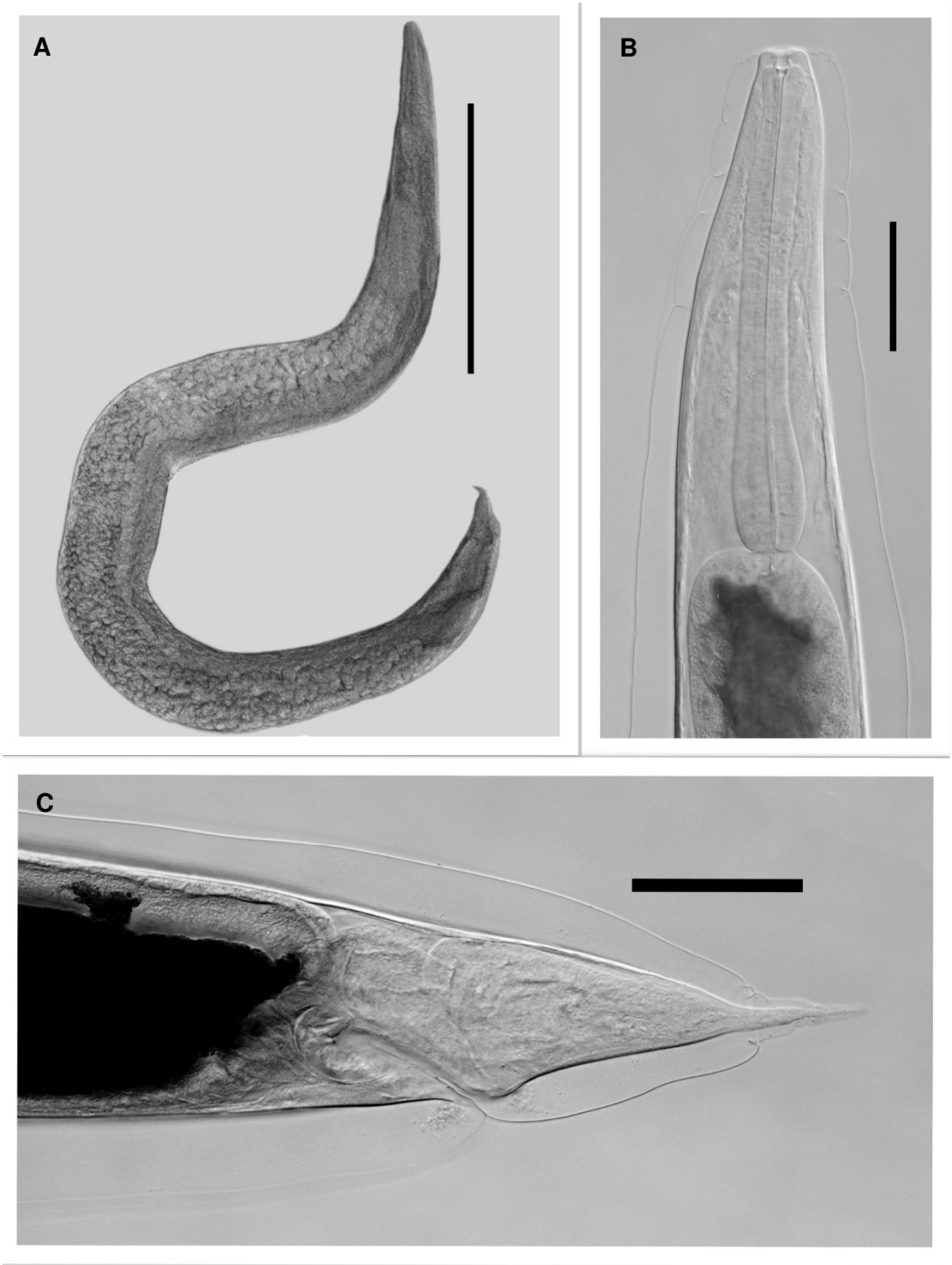


Figure 23: *Rhabdias* sp. 1 parasitising the Bubbling Kassina, *Kassina senegalensis* from the Limpopo province, South Africa. A – Whole body of female, lateral view; B – Anterior end of female, dorsal view; C – tail end of female worm, with dark spot, lateral view. Scale bars: 1000µm, B, C, 20µm.

Hosts: Bubbling Kassina, *Kassina senegalensis* (Dumeril and Bibron, 1854) and the African red toad, *Schismaderma carens* (Smith, 1848).

Site in hosts: Lungs

Localities: *Kassina senegalensis*: site 22, 95. *Schismaderma carens*: site 33.

Infection parameters: *K. senegalensis*: Intensity – 1-14 (5.77), Prevalence – 60%, Abundance – 0.168

S. carens: Intensity – 1-10 (10), Prevalence – 83%, Abundance – 0.032

Remarks: The specimens were collected from several specimens of *K. senegalensis* and from one *S. carens.* Morphologically, all of these specimens are very similar to *R. engelbrechti*, with a short buccal capsule (Fig. 23a) cuticular inflation and roundness on the anterior end of the body (Fig. 23b) but posterior end narrowing and lack of lateral pseudolabia (Fig. 23c). However, on the molecular level these specimens appeared somewhat different in *cox1* (2%) and ITS-28S (0.3%) alignments from sequences of *R. engelbrechti* (both, obtained in present study and retrieved from GenBank). Moreover, in one locality (Louis Trichardt) we found 3 of 8 infected *K. senegalensis* at exactly the same spot where nine *P. bifasciatus* (type host of *R. engelbrechti*) were found not infected. At the second locality (Waterpoort), we found specimens of *K. senegalensis* and *S. carens* infected with *Rhabdias* sp.1 and specimens of *P. bifasciatus* infected with *R. engelbrechti* also at the same spot. Therefore, based on molecular differences and host specificity, we prefer not to assign specimens from *K. senegalensis* and *S. carens* to R. *engelbrechti* but rather keep it as *Rhabdias* sp.1 until more data from different localities are obtained.

Rhabdias sp. 2

Figure 24: *Rhabdias* sp. 2 parasitising the Udzungwa ridged frog, *Ptychadena uzungwensis*. A – Anterior extremities of female, dorsal view; B – tail end of female, lateral view. Scale bars: A, 1000µm, B, 50µm.

Host: Udzungwa ridged frog, *Ptychadena uzungwensi*s (Loveridge, 1932)

Site in host: Lungs

Locality: site 32.

Infection parameters: Intensity – 1-11 (4), Prevalence – 100%, Abundance – 0.103

Remarks: Several specimens of *Rhabdias* were collected from *P. uzungwensis* in one locality. Collected specimens appeared to be significantly different from other congeners described in Africa by having a small body size with relatively bigger buccal capsule, well-developed excretory glands (Fig. 24a) and elongated conical tail (Fig. 24b). Based on the available molecular data, these

specimens also different in the *cox1* and ITS-28S genetic markers from other *Rhabdias* spp. recorded in southern Africa. Based on the clear molecular and morphological differences, collected specimens most likely belong to a new species and for the present study we keep them as *Rhabdias* sp. 2 (Fig. 24).

Rhabdias sp. 3

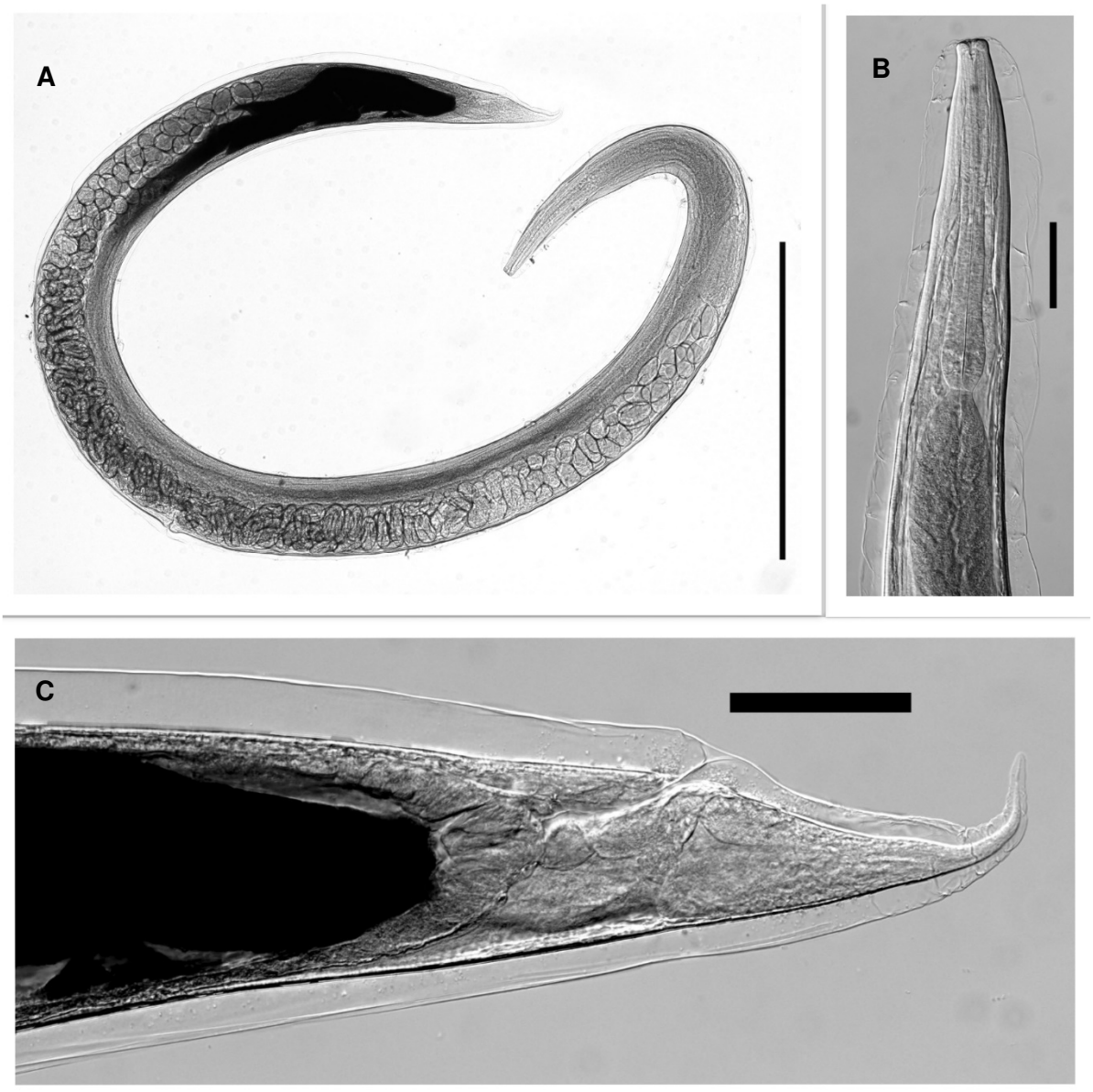


Figure 25: *Rhabdias* sp. 3 parasitising *Ptychadena anchietae* from the Limpopo province from South Africa. A – whole body of female, with dark spot at posterior extremities and eggs, lateral view; B – anterior end of female, dorsal view; C – tail, posterior part of female, with dark spot, lateral view. Scale bars: A, 200µm, B, 20µm, C, 50µm.

Host: Plain Grass Frog, *Ptychadena anchietae* (Bocage, 1867)

Site in host: Lungs

Locality: site 83.
Infection parameters: Intensity –1-2 (1), Prevalence – 4%, Abundance – 0,08

Remarks: Similar to *Rhabdias* sp. 1 and *Rhabdias* sp. 2, our *Rhabdias* sp. 3 differs in the *cox1* and ITS-28S genetic markers from other *Rhabdias* spp. recorded in southern Africa. It also has a different oesophagus shape from two other species (Fig. 25b) (constricted just posterior to level of esophageal apex, comparatively narrower oesophageal bulb), the truncate shape of the anterior end of body (Fig. 25a). Based on the clear molecular and morphological differences, collected specimens most likely belong to a new species and for the present study are identified as *Rhabdias* sp. 3 (Fig. 25).

Rhabdias sp. *4*

Host: Mababe puddle frog, *Phrynobatrachus mababiensis* FitzSimons, 1932.
Site in host: Lungs
Locality: site 94.
Infection parameters: Intensity – 1, Prevalence – 16.6%, Abundance – 0.0032

Remarks: A single immature specimen was recovered from the lung of the puddle frog and as a whole used for the molecular analysis. The specimen most closely resembles *Rhabdias* sp. 1 and *R. engelbrechti* based on molecular data of *cox1* and ITS-28S sequences and differs from them by roughly 2% in *cox1* and 0.2 and 0.3 percent in ITS-28S alignments. Based on the molecular data we cannot identify this specimen to a species level and thus assign it to *Rhabdias* sp. 4

FAMILY: STRONGYLOIDIDAE Chitwood & McIntosh 1934.
GENUS: *Strongyloides* Speare, 1989

Strongyloides sp.

Host: Bubbling Kassina, *Kassina senegalensis* (Dumeril and Bibron, 1854)
Site in host: Intestine
Locality: site 33.
Infection parameters: Intensity 1-3 (2) – Prevalence – 66%, Abundance – 0.0097

Remarks: *Strongyloides* nematodes have been identified as a danger to amphibian conservation in both captive and free-ranging amphibians (Imai *et al*., 2009). *Strongyloides* spp parasites are very

small, about a few millimetres in length.Under the dissecting microscope, three members of the genus were identified and carefully fixed. Unfortunately, all specimens were accidentally suck to the label paper and got lost preventing the future identification.

FAMILY: CAMALLANIDAE

Batrachocamallanus slomei (Southwell et Kirschner, 1937).

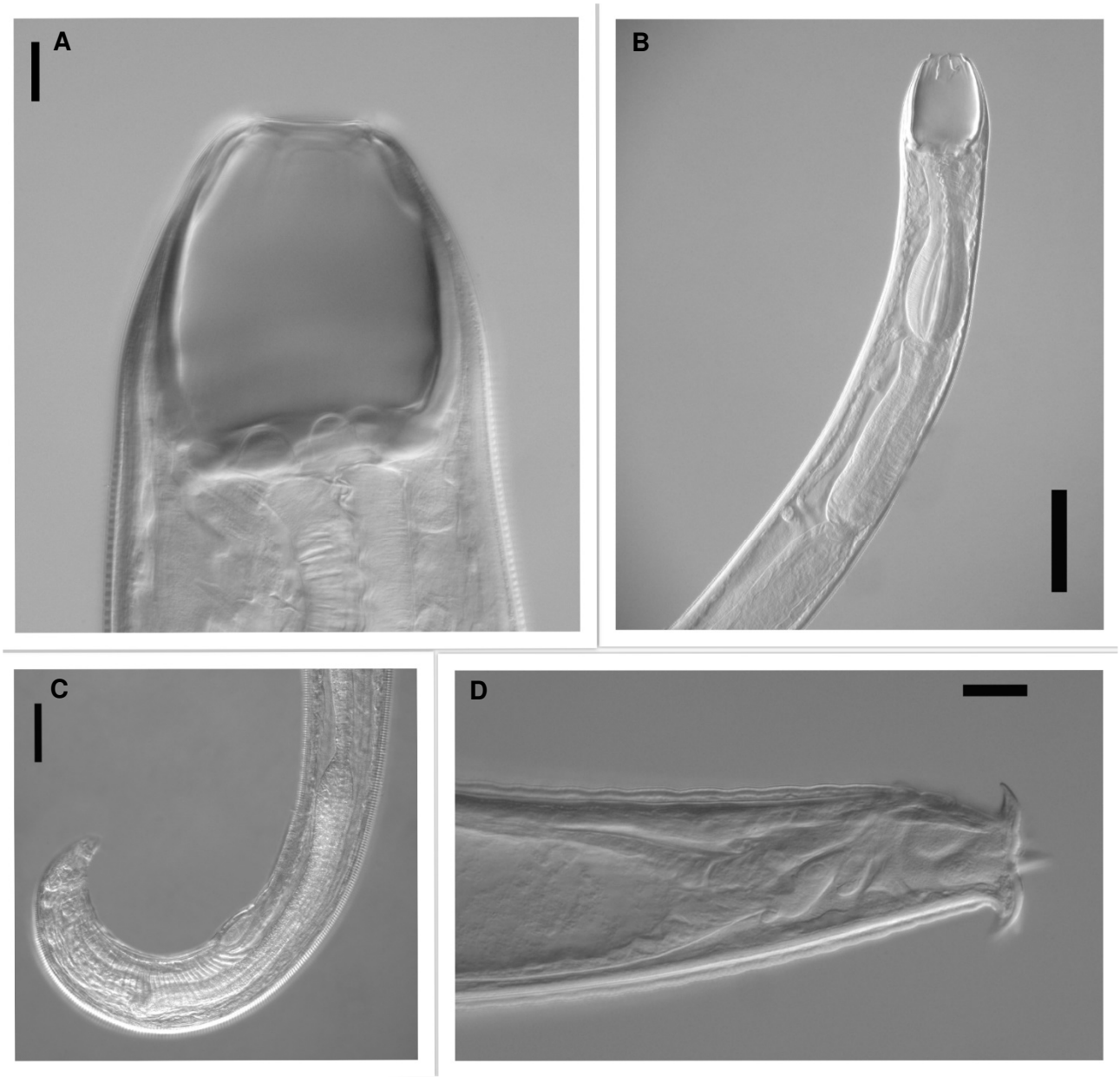


Figure 26: *Batrachocamallanus slomei*, parasitising *Xenopus laevis*, from South Africa. A - buccal capsule, female, lateral view; B – anterior end, with muscular oesophagus, female, lateral view; C – fragments of posterior end of male body; D – posterior end, tail of female, dorsal view. Scale bars: A, 20µm, B, 100µm, C, 50µm D, 20µm.

Host: Common platanna or African clawed frog, *Xenopus laevis* (Daudin 1802).

Site in host: Stomach

Localities: site 21, 25.

Infection parameters: Intensity – 9-11 (10), Prevalence – 41.7%, Abundance – 0.113

Remarks: The presence of a large number of mucrons (usually more than five) on the female tail end (Fig. 26D) the small body size of these species (Fig. 26B,C) the specificity to amphibian hosts, almost identical cephalic morphology, male caudal structures and the female reproductive system led us to assign collected specimens to the genus *Batrachocamallanus* (Jackson et Tinsley, 1995) Specifically, three species of camallanids are associated with *X. laevis* in South Africa: *Camallanus kaapstaadi* Southwell et Kirshner, 1937, *C. xenopodis* Jackson *et* Tinsley, 1995 and *B. slomei*. According to Svitin *et al*. (2019), *B. slomei* is primarily found in the stomach of amphibian hosts, alternatively also recovered from the oesophagus. Our specimen recovered from *X. laevis* clearly corresponds to *B. slomei* based on their morphology and sequences of 28S gene and *cox1* gene fragments. Thus, 100% corresponded to ones published recently (Svitin et al., 2019) (Fig. 26).

Camallanus kaapstaadi Southwell and Kirshner, 1937.

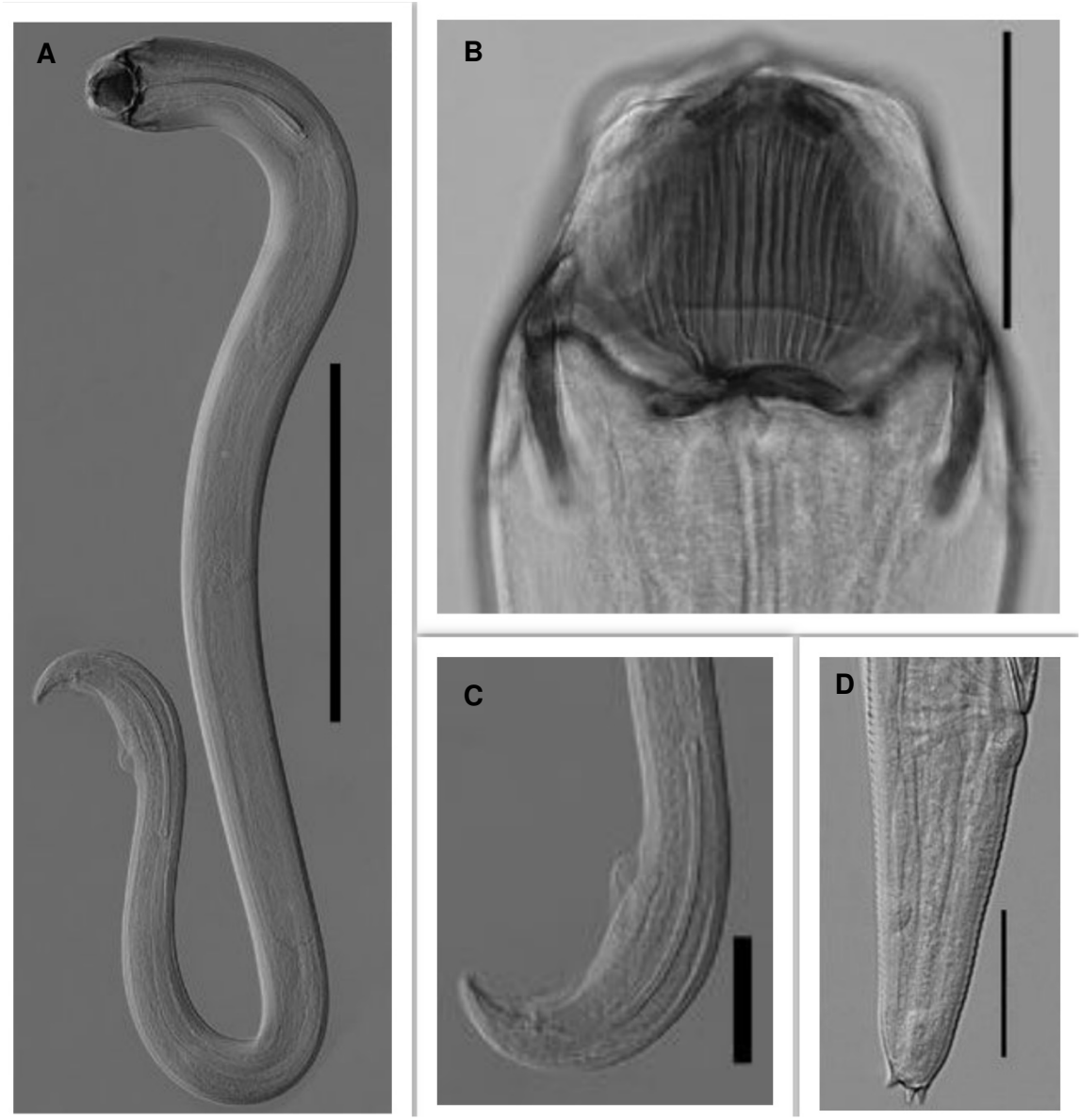


Figure 27: *Camallanus kaapstaadi* parasitising *Xenopus laevis* from South Africa. A – whole body, male, lateral view; B – anterior end, buccal capsule, club-shaped, female, lateral view; C - striations along whole body, posterior part, male, lateral view; D – posterior part ventral trident, female, lateral view. Scale bars: A, 500µm, B, 50µm, C, 20µm, D, 50µm.

Host: Common platanna or the African clawed frog, *Xenopus laevis* (Daudin 1802)

Site in host: Oesophagus

Locality: site 19.

Infection parameters: Intensity – 1, Prevalence – 8%, Abundance – 0.00236

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.

Found worms are medium-sized with a thick body looped dorsally and a maximum width at the anterior third level (Fig. 27A). The buccal capsule is made up of two valves, each with mostly finished ridges. At the base of the buccal capsule there is a thick, sclerotized basal ring (Fig. 27B). Muscular oesophagus (Fig. 27A) elongated posterior bulb and club-shaped. The granular section of the oesophagus is nearly as long as the muscular oesophagus. Three mucrons located on the tail tip of females (Fig. 27D). The data collected during the study, reports that our specimen collected from *Xenopus laevis*, from Vivo, corresponds to this of *C. kaapstaadi* (see Svitin, *et al.*, 2018; Mbokane *et al.*, 2020). The identification was also confirmed based on comparison of 28S gene fragments.

3.2.2 CESTODA

FAMILY: DIPHYLLOBOTHTRIIDAE

GENUS: *Cephaloclamys* (Cobbold, 1858)

Cephaloclamys namaquensis (Cohn 1906)

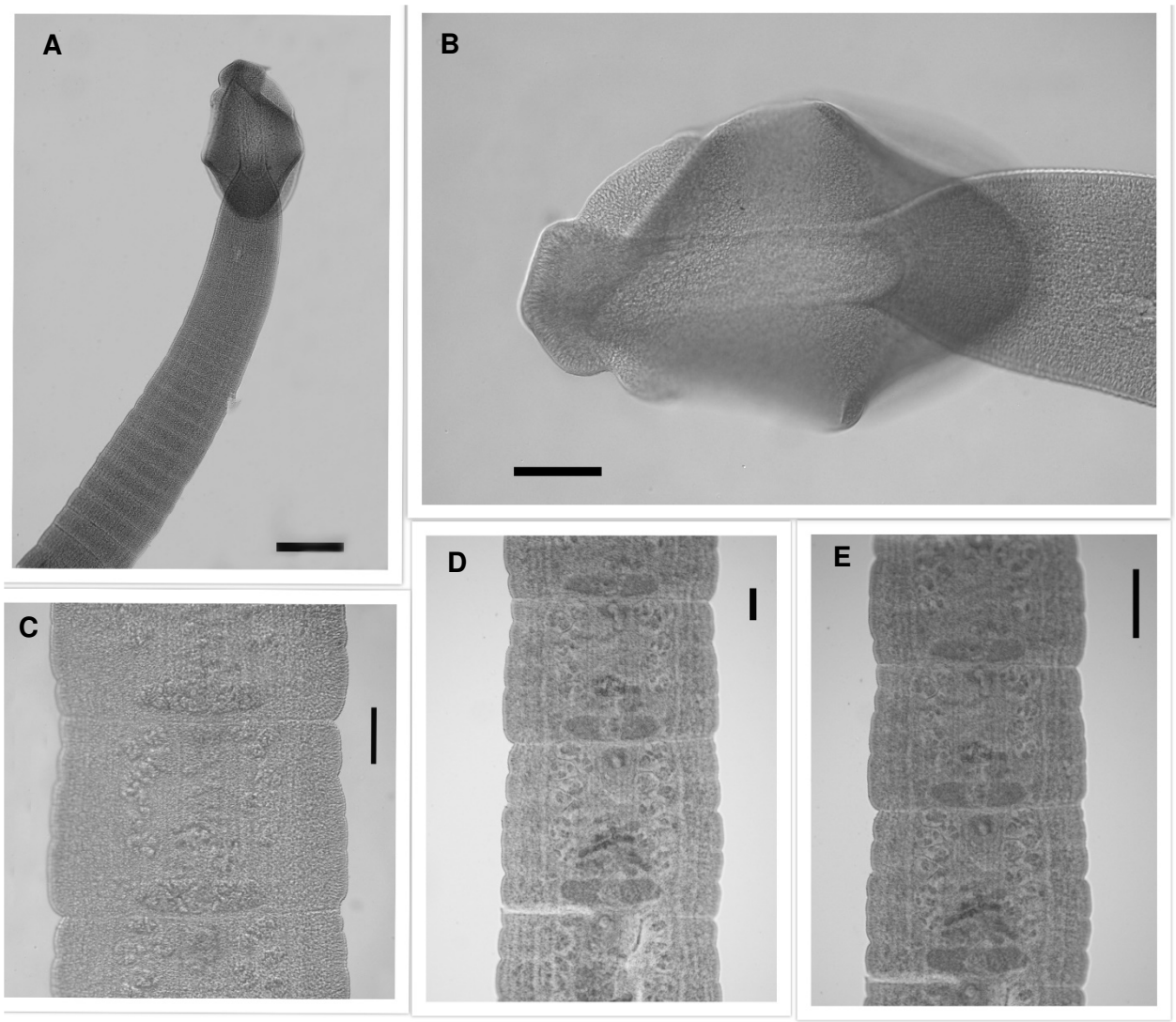


Figure 28: *Cephaloclamys namaquensis* parasitising the common platanna or the African clawed frog, from South Africa. A – anterior part with long scolex, with visible proglottids, dorsal view; B – anterior part with scolex, lateral view; C, D, E – mid-body with proglottids, dorsal view. Scale bars: a, b, c, 1000µm, d, e, 500µm.

Host: Common platanna or the African clawed frog, *Xenopus laevis* (Daudin 1802)

Site in host: Intestine

Locality: site 19.

Infection parameters: Intensity – 4-5 (4.5), Prevalence – 16.7%, Abundance – 0.029

Remarks: *Cephalochlamys namaquensis* is distributed throughout Africa and has been recorded several times from the African clawed frog, *Xenopus laevis*, in southern Africa (Ferguson and Appleton, 1988). Large living cestode, capable of extending, moderately relaxed. Large proglottids

at posterior part of strobila (Fig. 28A). Sucking plates created by constricted dorsal and ventral bothridia hold the duodenum to the wall. Bothridia with wide surface of thin tissue. Scolex with osmoregulatory canals. Dorsal osmoregulatory canal unbranched in scolex and strobila. Genitalia develop in segmented region. Proglottids do not subdivide once formed (Fig. 28C, D, E). Vagina opens internally, large, close to ovary. Egg walls are thin, non-operculate (Thurston, 1967). Based on the clear morphological correspondence and the molecular data of 18S rDNA gene marker, we assign our specimen recovered from several African clawed frogs, *Xenopus laevis*, to *C. namaquensis*.

FAMILY: CYCLOPHYLLIDEA

Nematoteniidae gen. sp. Liihe, 1910

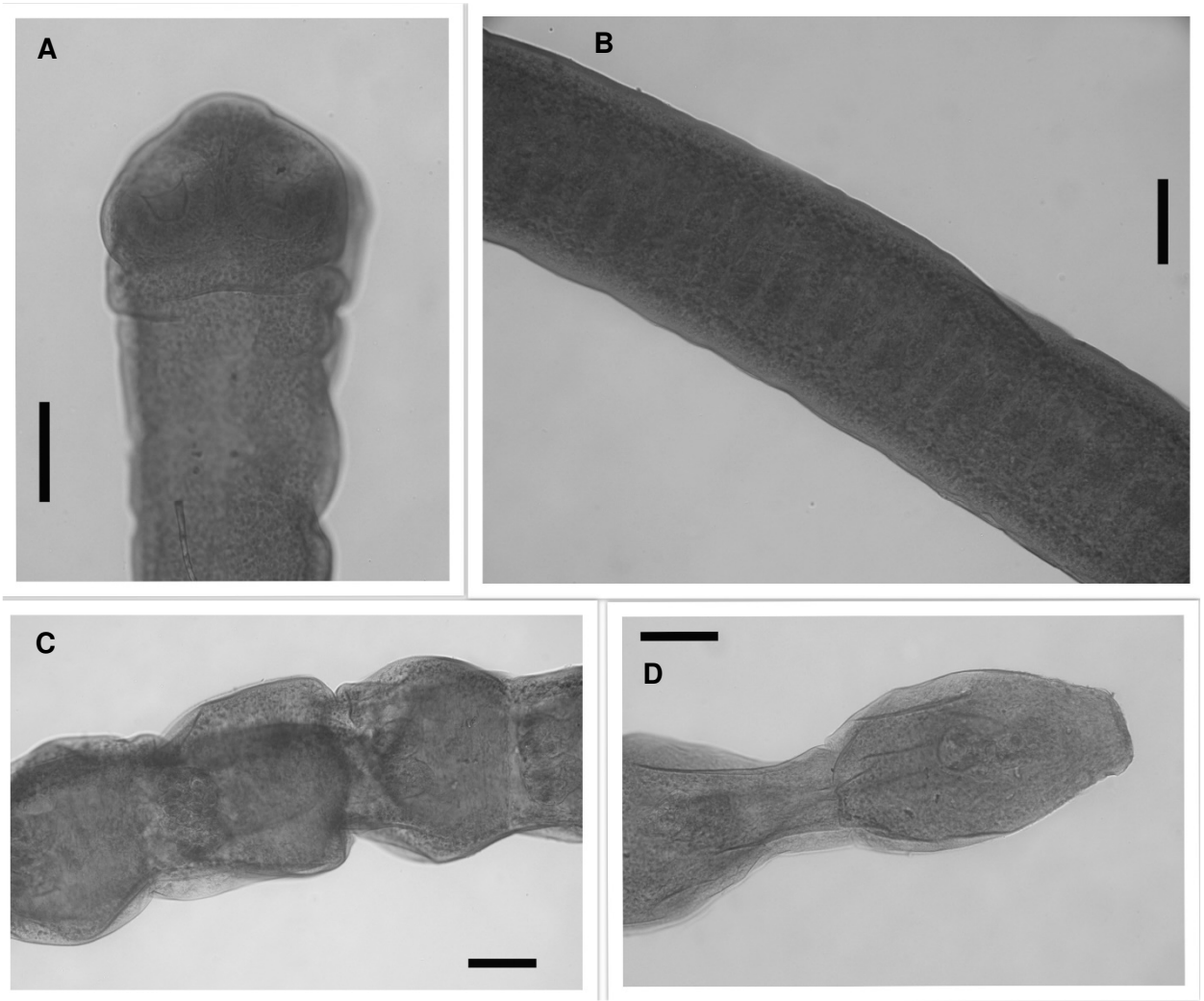


Figure 29: *Nematoteniidae* gen. sp. parasitising *Sclerophys pusilla*, from South Africa. A - simple scolex, lacks rostellum, lateral view; B – segmented mid-body, lateral view; C, D– posterior end of body, lateral view. Scale bars: a - d 1000µm.

Hosts: Common river frog, *Amietia delalandii* (Duméril and Bibron, 1841), Kimberley toad, Power s toad, *Sclerophrys poweri* (Hewitt, 1935), flat-backed/ striped-back toad, *Sclerophrys pusilla* (Mertens, 1937), African bullfrog, *Pyxicephalus edulis* Peters, 1854, the giant bullfrog, *Pyxicephalus adsp.ersus* Tschudi, 1838, Plain grass frog, *Ptychadena anchietae* (Bocage, 1867), the banded rubber frog, *Phrynomantis bifasciatus* (Smith, 1847) and the Southern sand frog, *Tomopterna adiastola* Channing and Du Preez, 2020.

Site in hosts: Intestine, stomach

Localities: site 5, 28, 33, 34, 78, 81, 83, 84, 94, 95.

Infection parameters: *Amietia delalandii*: Intensity – 1-15 (6.8), Prevalence – 8%, Abundance – 0.110. *Sclerophrys poweri*: Intensity – 1, Prevalence – 20%, Abundance –0.0023

Sclerophrys pusilla: Intensity – 1-6 (2.66), Prevalence – 0.049%, Abundance – 0.025
Pyxicephalus edulis: Intensity – 1, Prevalence – 0.055%, Abundance – 0.0032
Pyxicephalus adsp.ersus: Intensity – 2, Prevalence – 0.016%, Abundance – 0.0064
Ptychadena anchietae: Intensity – 4-15 (9.5), Prevalence – 0.086%, Abundance – 0.0614
Phrynomantis bifasciatus: Intensity – 1-4 (4), Prevalence – 0.080%, Abundance – 0.025
Tomopterna adiastola: Intensity – 1, Prevalence – 0.067%, Abundance – 0.00323

Remarks: *Nematoteniidae* is a tiny *cyclophyllidean* cestode family that has been found in reptiles and amphibians. According to Jones (1987), about 34 years ago, a total of 29 species of *Nematoteniids* were described. On the other hand, the taxonomic relationships between each species are poorly understood (Jones, 1987). Collected worms have simple scolex (Fig. 29a) lacking rostellum. Neck merges with the scolex and long in length (Fig. 29b). Scolex varies in size from species to species and it has only limited taxonomic relevance. The length-to-width ratio of segments varies at different stages of development (Fig. 29b) and is utilized to distinguish some species. In most cases, mature segments are wider than they are long. Elongated segments as it grows (Fig. 29c, d). The morphological characters and molecular data clearly place our specimens to the family *Nematotaeniidae*. However, none of the nematotaeniid representatives have been reported from African amphibians so far. Thus, we prefer not to assign our material to any of existing genera and identify them as *Nematotaeniidae* gen. sp. in present study.

3.2.3 TREMATODA

FAMILY: DIPLOSTOMIDAE

GENUS: *Tylodelphys* Diesing, 1850

Tylodelphys xenopi (Nigrelli and Maraventano, 1944)

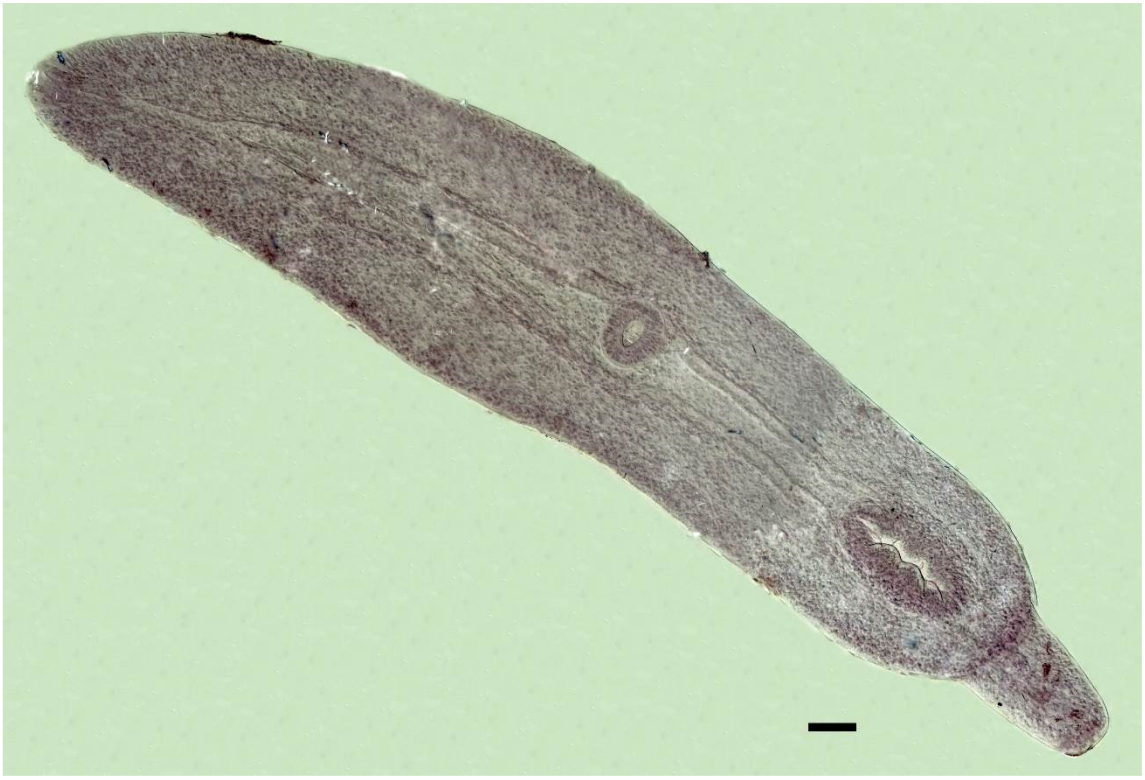


Figure 30: *Tylodelphys xenopi* parasitising *Xenopus laevis*, from the Limpopo province, South Africa. A – whole body, oval shaped, with oral sucker at anterior part and ventral sucker at posterior extremities. Short oesophagus, lateral view. Scale bar: 1mm.

Host: Common platanna or the African clawed frog, *Xenopus laevis* (Daudin 1802)

Site in host: lungs

Locality: Site 2.

Infection parameters: Intensity – 30, Prevalence – 0.083%, Abundance – 0.097

Remarks: Species of *Tylodelphys* Diesing, 1850, are digeneans parasitizing fish and sometimes amphibians. These parasites can infect the brain, or cranial cavity, pericardial sac and the body cavity of their second intermediate hosts which can be amphibians. *Tylodelphys* spp. consist of a complex three-host life cycle where fish and amphibian-eating birds serves as definitive hosts (Blasco-Costa *et al.,* 2017). Body short and oval-shaped (Fig. 30a). Lack spines on body surface. Poorly developed surface structures, uneven. The front section of the ventral surface has scale-like spines (Fig 30a). Body indistinctly divided into fore- and hind body. Oral sucker at anterior extremities. Pseudosucker on sides of oral sucker. Pseudosucker, edges flat or concave. Pharynx

elongated. Short oesophagus with a large intestinal cecum near the back of the neck. Ventral sucker, small, in middle of forebody, posteriorly. Testes large, posterior half of body. Ovary round and oval shaped, on posterior border (King and Van As, 1997). Specimens collected in previous study clearly fall under the morphological description of genus *Tylodelphys*. Since *T. xenopi* is the only species that have ever been recovered from *X. laevis* we feel confident to assign our material to that species.

FAMILY: MESOCOELIIDAE Faust, 1924

Mesocoelium sp. Odhner, 1910

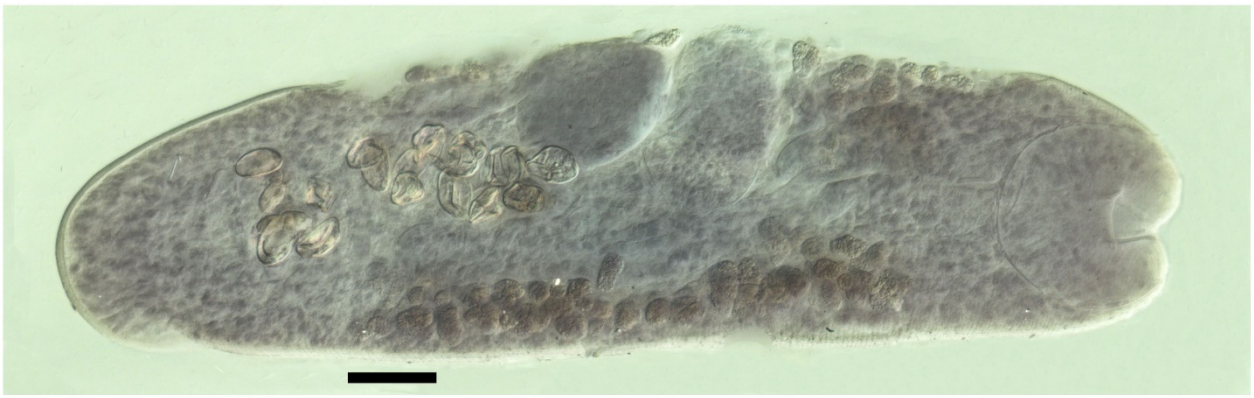


Figure 31: *Mesocoelium* sp parasitising *Tomopterna adiastola* from South Africa. A – whole body, with oral sucker at anterior extremities, ventral sucker visible at mid-body length. Scale bar: 1mm.

Host: Eastern olive Toad, *Sclerophrys garmani* (Meek, 1897), African common toad or guttural toad, *S. gutturalis* (Power, 1927), flat-backed/ striped-back Toad, *Sclerophrys pusilla* (Mertens, 1937), Tandy s sand frog, *Tompoterna tandyi* Channing and Bogart, 1996 and the Southern sand frog, *T. adiastola* Channing and Du Preez, 2020 and Udzungwa ridged frog, *Ptychadena uzungwensi*s (Loveridge, 1932)

Site in host: Intestine

Locality: site 21, 22, 32, 34, 95.

Infection parameters: *Sclerophrys garmani*: Intensity – 2, Prevalence – 20%, Abundance – 0.0064

Sclerophrys gutturalis: Intensity – 2, Prevalence – 9%, Abundance – 0.0064, *Tomopterna tandyi*: Intensity – 1-5 (3), Prevalence – 13%, Abundance – 0.019, *Tomopterna adiastola:* Intensity – 1, Prevalence – 3%, Abundance – 0.14, *Ptychadena uzungwensi*s: Intensity – 21-85 (53), Prevalence – 25%, Abundance – 0.343.

Remarks: These digeneans can be found in both marine and freshwater settings, as well as on land (Gomes *et al.*, 2013). The family Mesocoeliidae Faust, 1924, digeneans consists of a range of parasites that may be collected from the digestive tract and organs of amphibians and reptiles. One of two genera in the family is *Mesocoelium* Odhner, 1910. The taxonomy of the genus *Mesocoelium* is difficult, according to Gomes *et al*. (2013) and has been a source of debate since the 1960s.
The genus, *Mesocoelium* Odhner, 1910, have characteristics of the family but some differ from original description (Dronen *et al.,* 2012). Collected specimens have body elongated to oval in shape, rounded at both ends, transparent posteriorly. From the ventral sucker to the midline of the body, it is wide. With the mouth ventrally open, the preoral lobe is visible, as is the subterminal oral sucker. Pre-pharynx is short, muscular pharynx is smaller than oral sucker and the pharynx is larger (wider) than long. Oesophagus is present. A ventral sucker is located above the midline of the body at the anterior half of the body. Smaller than oral sucker. Testes rounded, laterally elongated and smooth. Side by side at level of ventral sucker. At the midpoint of the ventral sucker to the oral sucker, there is a genital pore. Ovary, oval to elongated in shape, smooth and creating a triangle with testes. The uterus is folded with ascending and descending limbs that fills the postovarian space. The vitelline follicles may reach the midpoint of the oral sucker anteriorly. Excretory vesicle Y-shaped, terminal to subterminal. *Mesocoelium* digeneans are known to infect terrestrial snails which serve as intermediate hosts, while arthropods may serve as second intermediate hosts. Morphological data along with the data from sequences based on *cox1* gene along with morphological data allowed us to only identify the digenean to genus level, thus we assign our specimen to *Mesocoelium* sp.

FAMILY: TELORCHIIDAE Looss 1899

Oligolecithus sp. Vercammen-Grandjean, 1960

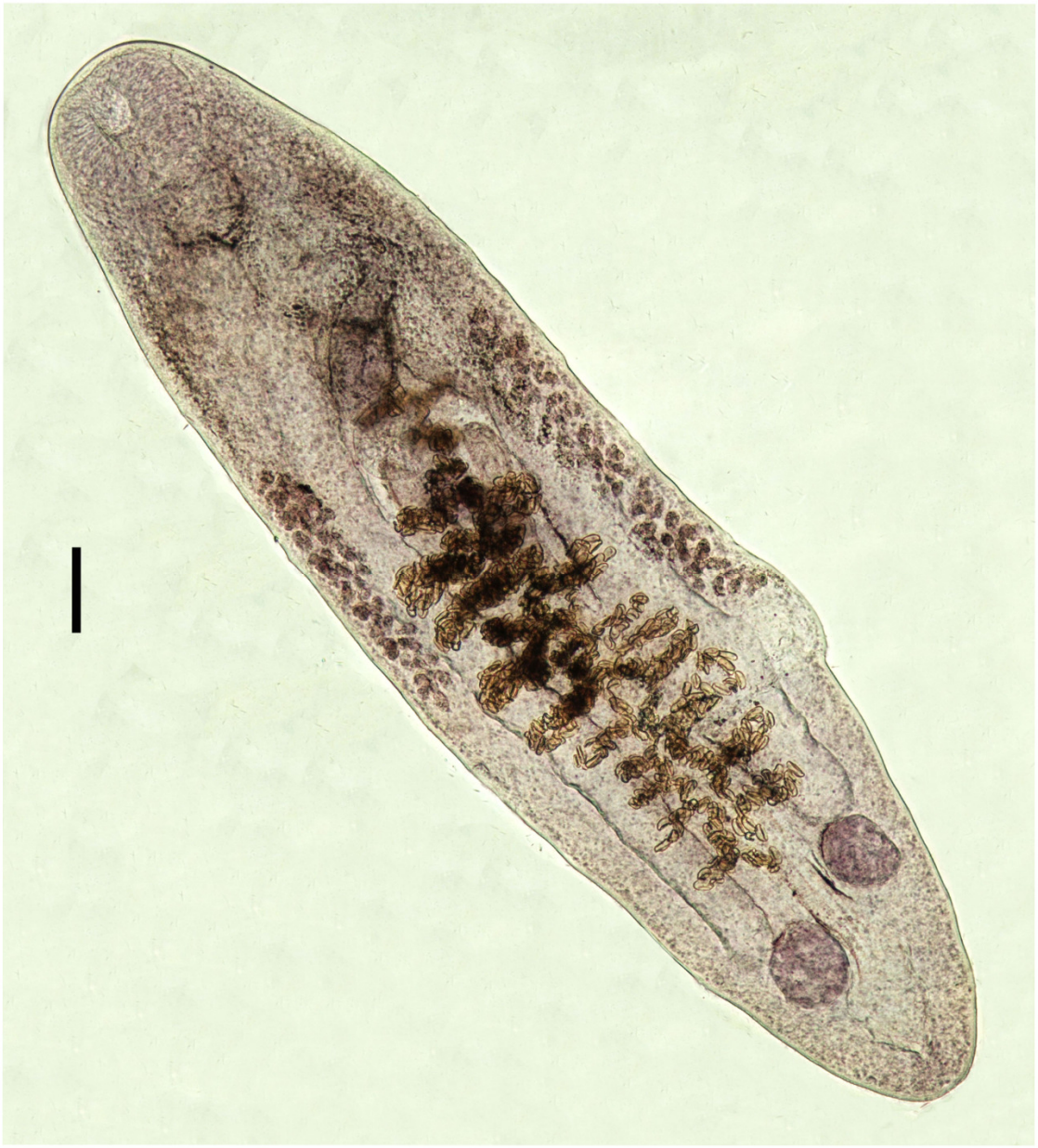


Figure 32: *Oligolecithus* sp. parasitising the African bullfrog, *Pixycephalus edulis*, from South Africa. A – whole body, oral sucker found at anterior end, ventral sucker at mid-body, definite testes at posterior end and Uterus form descending and ascending coils. Scale bar: 1mm.

Host: African bullfrog, *Pixycephalus edulis* Peters, 1854.

Site in host: Intestine

Locality: site 30.

Infection parameters: Intensity – 1, Prevalence – 50%, Abundance – 0.00232

Remarks: Species of *Oligolecithus* Vercammen-Grandjean, 1960, are commonly found in the intestine of African amphibians and parasitise a variety of Anurans from southern Africa. These species differ from other genera in the sub-family, by a long neck-shaped cirrus-sac that is situated in the forebody. The collected trematodes have tongue-shaped body (Fig. 32) with a larger ventral sucker than the oral sucker. Prepharynx present with short oesophagus (Fig. 32a). Round testes with irregular margins and diagonal. Cirrus-sac anterior to ventral sucker. Flask-shaped. Genital pore midway between caecum and lateral body. Round ovary, posteriolateral to ventral sucker. Seminal receptacle hidden by uterus. The uterus forms descending and ascending coils which do not create regular transverse folds because they overlap. Vitelline follicles are large, with lateral fields in the body's central third. Excretory vesicle Y-shaped. According to Keys to Trematodes (Vol. 3) these digeneans typically infect the intestines of African amphibians. Based on *cox1* sequences, morphological characteristics from Bray *et al.* (2008) we assign collected specimens to *Oligolecithus* sp. (Fig. 32a)

3.2.4 MONOGENEA

FAMILY: POLYSTOMATIDAE Gamble, 1896.
GENUS: *Protopolystoma* Bychowsky, 1957

Protopolystoma xenopodis (Price, 1943) Bychowsky, 1957

Figure 33: *Protopolystoma xenopodis* commonly found in the Common platanna or the African Clawed frog, *Xenopus laevis*, from South Africa. A – whole body, elongated, dorsal view. Scale bar: 1mm.

Host: Common platanna or the African Clawed frog, *Xenopus laevis* (Daudin 1802)
Site in host: Urinary bladder
Locality: site 21.
Infection parameters: Intensity - 1 Prevalence – 8%, Abundance – 0.00232

Remarks: *Protopolystoma xenopodis* is a parasite of *Xenopus laevis*, the African clawed frog (Fig. 33a). In the urinary bladder of hosts, these adult parasites can produce up to 15 eggs per 24 hours. Elongated body shape, cylindrical. Capable of considerable elongation and contraction. Posterior part oval, elongated longitudinally. The intestine is composed of two lateral caeca. Crystalline lenses can be found in two pairs of eyes in front of the pharynx. There are a lot of nerve ganglia and commissures. A large mass of nerve tissue spreads transversely dorsal to the pharynx between the anterior and posterior eye sites. Haptor present, cup-shaped, opening ventrally. 16 larval hooklets or marginals, lie in oval domus. Based on morphology, we assign our specimen recovered from the African clawed frog to *P. xenopodis* (Fig. 33a).

Eupolystoma sp. Kaw, 1950

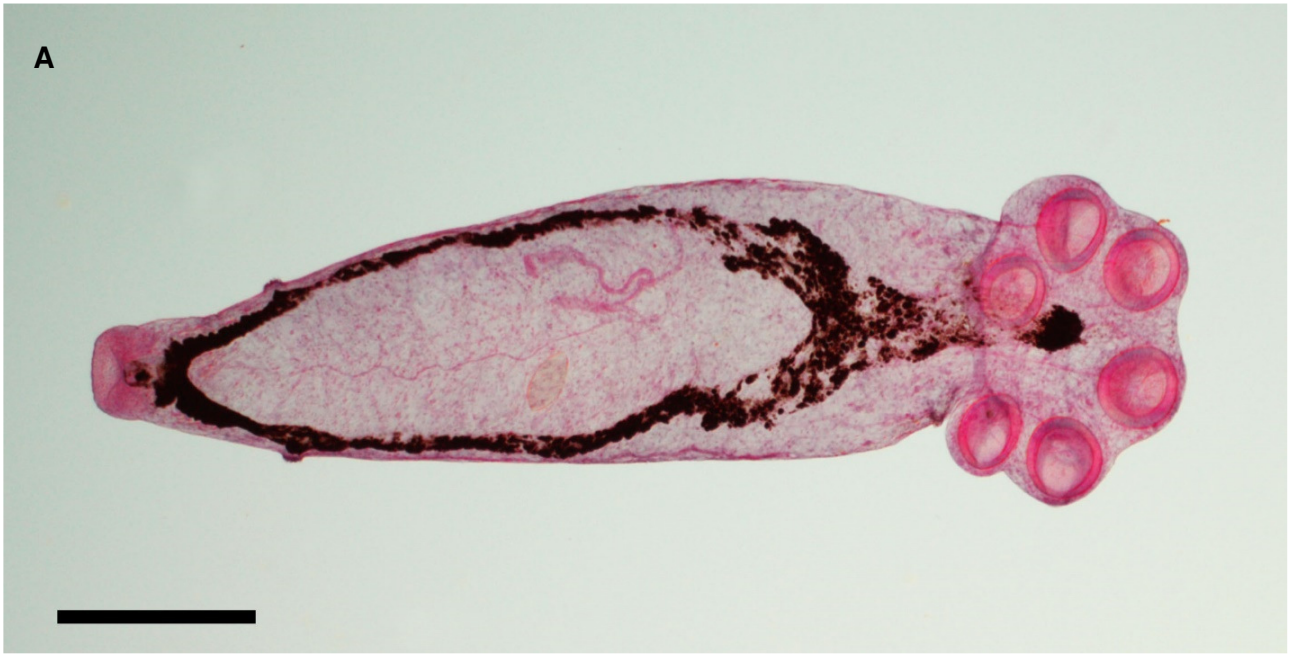


Figure 34: *Eupolystoma* sp. recovered from *Poyntonophrynus fenoulheti*, from the Limpopo province, South Africa. A – whole body, lacks hamuli, ovary and intestine visible, lateral view. Scale bar: 1mm.

Host: Northern pygmy toad, *Poyntonophrynus fenoulheti*, Hewitt and Methuen, 1913.
Site in host: Urinary bladder
Locality: site 34
Infection parameters: Intensity – 3-5 (4), Prevalence – 10.5%, Abundance – 0.025

Remarks: Polystomatids from anurans in Africa and South Africa, includes the genera *Polystoma* Zeder, 1800, *Metapolystoma* (Combes, 1976), *Protopolystoma* (Bychowsky, 1957.) and *Eupolystoma* (Bychowsky, 1957.) *Eupolystoma* is only known and described from Africa and India (Du Preez *et al.,* 2003) (Fig. 34a). *Eupolystoma* does not have hamuli, thus after close investigation we assigned our specimen to that of *Eupolystoma* sp. 1

Polystoma mashoni Beverley-Burton, 1962.

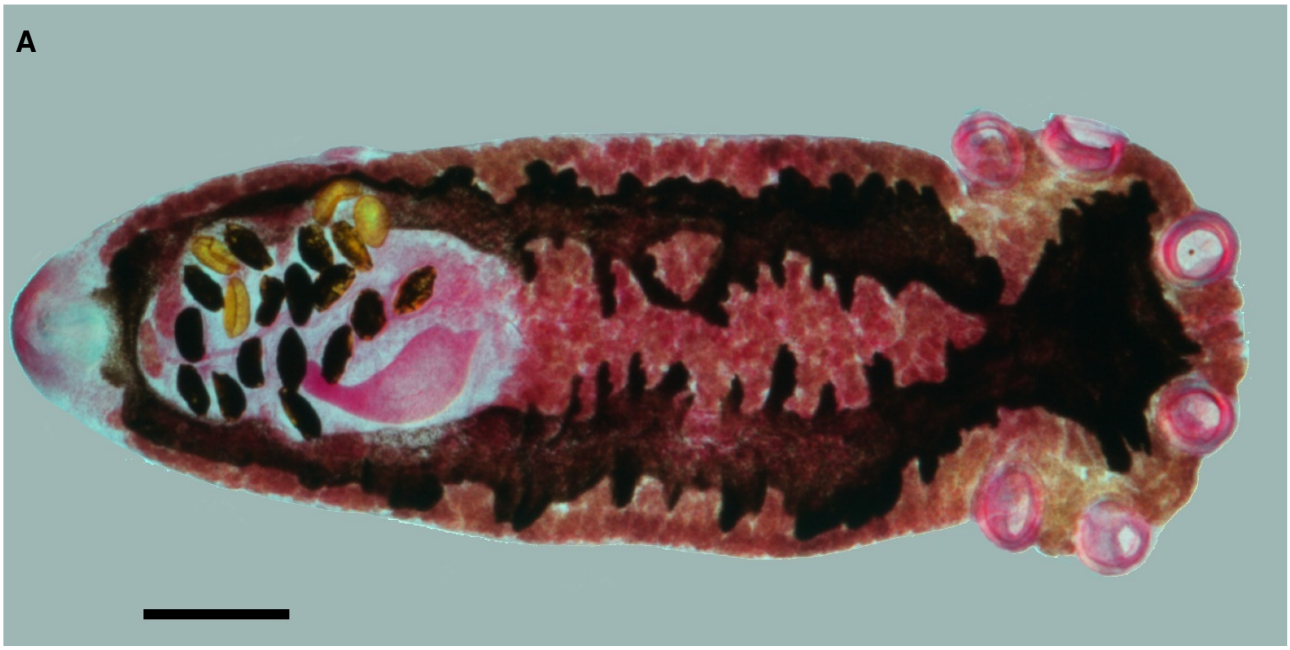


Figure 35: *Polystoma mashoni* parasitising the guttural toad, from South Africa. A – whole body, lateral view. Scale bar: 1mm.

Host: African common toad or guttural toad, *Sclerophrys gutturalis* (Power, 1927).
Site in host: Urinary bladder
Locality: site 32.
Infection parameters: Intensity – 10, Prevalence – 9%, Abundance – 0.032

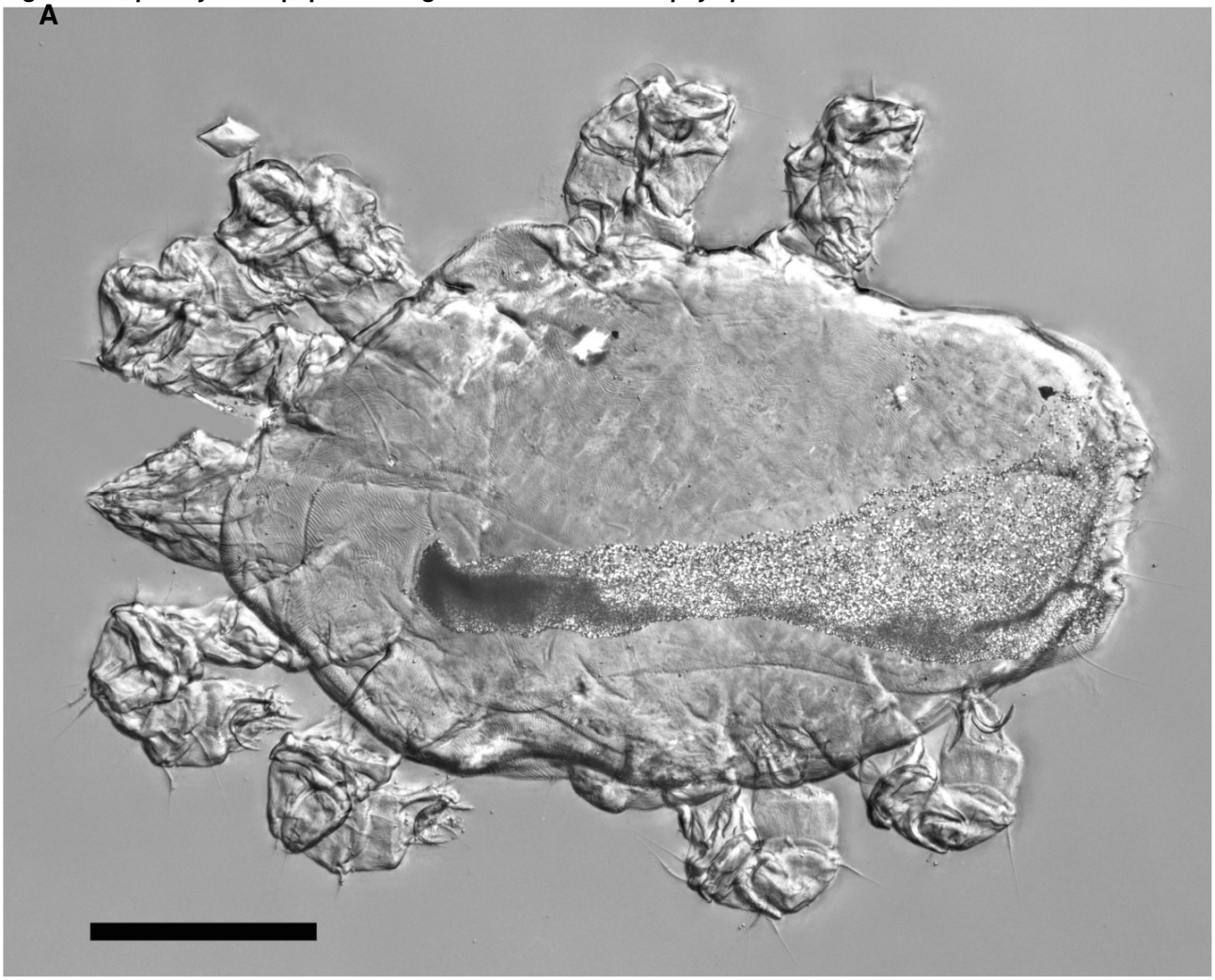
Remarks: *Polystomatidae* Gamble. 1896, a family of amphibious tetrapods that infects anurans, salamanders and, to a lesser extent, the African hippopotamus, has a variety of characteristics, including unusual host-parasite interactions. Polystomes, are found all over the world, with the exception of deserts and Antarctica (Mörs *et al.,* 2020). *Polystoma* is the most diverse genus among the 20 *Polystomatidae* genera currently recognized (Du Preez and Verneau, 2020). These species are categorized by strict host specificity. Polystomes have a simple life cycle that can be completed in either the gills of young tadpoles or the urinary bladder of adult frogs. Opisthaptor with six circular peripheral suckers. Haptoral hooks not observed (Fig. 35a). Wide intestine, posteriorly anastomose of the intestinal ceca and a ring-like gut with posterior expansion. Testis not observed. Genital pore observed directly behind intestinal bifurcation. Penial coronet of hooks presents in species. Ovary posterior. Vaginae present. Uterus mainly periovarian with embryos. Vitellaria has follicular, extracecal and transverse vitelline ducts (Beverley-Burton, 1962). Our specimens clearly match the species description and have been recognized as *P. mashoni* (Fig. 35).

3.2.5 ARTHROPODA

FAMILY: MACRONYSSIDAE Oudemans 1936

Ophionyssus sp. Megnin, 1884

Figure 36: *Ophionyssus* sp. parasitising the nostrils of *Sclerophys poweri* from South Africa. A – lateral view of whole body. Scale bar: 100µm.



Host: Power s toad, *Sclerophrys poweri* (Hewitt, 1935)
Site in host: Nostrils
Localities: site 78, 84.
Infection parameters: Intensity – 1-14 (7.5), Prevalence – 40%, Abundance – 0.0485

Remarks: *Ophionyssus* mites parasitize a variety of reptiles and are the most common and most pathogenic of all mites found within reptiles. *Ophionyssus* is a genus of bird mites of the *Macronyssidae*. Propodosomatal shield is generally lemon-shaped and about the same length as it is wide. The propodosomatal shield is rhomboidal in shape with a rectangular posterior projection

(Fig. 36a). The literature data on these parasites from African amphibians is unavailable and we prefer not to assign our specimens to any species found in lizards or snakes.

3.2.6 ANNELIDA

FAMILY: NAIDIDAE

unidentified Oligochaeta

Host: Banded rubber frog, *Phrynomantis bifasciatus* (Smith, 1847).
Site in host: Urinary bladder, eye
Localities: site 84, site 95
Infection parameters: Intensity – 1-4 (2.5), Prevalence – 4%, Abundance – 0.016

Remarks: *Oligochaete* worms are supposedly free-living worms from the *Naididae* Ehrenberg, 1828, family that live in frogs' cloaca, urinary bladder, Wolffian duct (ureter), and eye. The ubiquitous Oligochaetes are very rarely documented as internal parasites of vertebrates, according to (Sinsch *et al*., 2019). Internal parasites like cestodes and trematodes thrive in warm, humid environments, thus more oligochaetes should have been able to follow the same path to obligate parasitism. In previous studies, *Nais bauchensis,* was found parasitising an African frog, *Phrynomantis bifasciatus* in the anterior canthus of the eye, the lachrymal sac and lastly the uterus. Although, representatives from the family Naiidae are common throughout South Africa, we were not able to assign our specimens to this family. Thus, we prefer to keep them as unidentified Oligochaeta until more data is obtained.

3.2.7 ACANTHOCEPHALA

Corynosoma sp.

Figure 37: *Corynosoma* sp. parasitising the Striped stream frog, or striped grass frog, *Strongylopus fasciatus*, from the Limpopo Province, South Africa. A – anterior end of body with spike-like structures. Scale bars: 50µm.

Host: Striped stream frog, *Strongylopus fasciatus* (Smith, 1849)
Site in host: Body cavity
Locality: site 32
Infection parameters: Intensity – 5, Prevalence – 16.7%, Abundance – 0.016

Remarks: In the intestine of the one stripped stream frog we recovered five cysts containing acanthocephalan larvae (Fig. 37a). After attempts of excysting, larvae broke very easy which complicated any morphological identification. Thus, identification of the genus was mostly based on the *cox1* sequence which corresponded to the genus *Corynosoma* Lühe, 1904 (92% correspondence based on BLAST search). There were no surveys on acanthocepalans in or near the study area and thus we cannot hypothesise the origin of the infection of the dissected frog (Fig. 37).

3.3 CHECK LIST OF HOSTS AND METAZOAN PARASITES

Table 3: List of all anuran species collected and screened throughout the investigation, along with the names of representative metazoan parasite species.

Anuran species name	Metazoan parasite species name
Amietia delalandii	*Neofoleyellides steyni*
	Amphibiophilus sp.
	Aplectana sp. 2
	Contracaecum sp. 1
	Nematoteniidae gen. sp.
Breviceps adsp.ersus	*Aplectana* sp. 5
Breviceps sylvestris	*Aplectana* sp. 5
	Rhabdias cf. *sylvestris*
Chiromantis xerampelina	*Cosmocercidae* gen sp. 1
Cacosternum boettgeri	*Free of parasites*
Hyperoluis marmoratus	*Tangua* sp.
Kassina senegalensis	*Cosmocerca* sp. 2
	Rhabdias sp. 1
	Strongyloides sp.
Phrynomantis bifasciatus	*Cosmocerca* sp. 1
	Rhabdias engelbrechti
	Nematoteniidae gen. sp.
	unidentified oligochaeta
Phrynobatrachus mababiensis	*Rhabdias* sp. 4
Poyntonophrynus fenoulheti	*Cosmocerca* sp. 3
	Eupolystoma sp.

Ptychadena anchietae	*Aplectana* sp. 4
	Rhabdias sp. 3
	Nematoteniidae gen. sp.
Ptychadena uzungwensis	*Cosmocercidae* gen. sp. 2
	Rhabdias sp. 2
	Mesocoelium sp.
Pyxicephalus adsp.ersus	*Cosmocerca* sp. 1
	Nematoteniidae gen. sp.
Pyxicephalus edulis	*Cosmocercidae* gen sp. 1
	Nematoteniidae gen. sp.
	Oligolecithus sp.
Schismaderma carens	*Aplectana* sp. 1
	Rhabdias sp. 1
Schleroprys garmani	*Aplectana* sp. 3
	Rhabdias africanus
	Mesocoelium sp.
Schlerophrys gutturalis	*Aplectana* sp. 3
	Mesocoelium sp.
	Polystoma mashoni
Sclerophrys poweri	*Nematoteniidae* gen. sp.
	Ophionyssus sp.
Sclerophrys pusilla	*Aplectana* sp. 3
	Rhabdias africanus
	Nematoteniidae gen. sp
	Mesocoelium sp.
Strongylopus fasciatus	*Amphibiophilus* sp.
	Corynosoma sp.

Tomopterna adiastola	*Nematoteniidae* gen. sp
	Mesocoelium sp.
Tomopterna tandyi	*Mesocoelium* sp.
Xenopus laevis	*Batrachocamallanus slomei*
	Camallanus kaapstaadi
	Cephaloclamys namaquensis
	Tylodelphis xenopi
	Protopolystoma xenopodis

3.4 COMMUNITY ANALYSIS AND DISCUSSION

3.4.1 PREFACE

The focus of research on amphibian helminth communities has turned to the mechanisms that shape them today (Gustafson *et al.,* 2013). According to Aho (1990) amphibians are host generalists and many parasites can infect a wide range of amphibian species, nevertheless, sympatric amphibians do not share helminth species and do not transmit helminths evenly (Aho, 1990). Thus, studying the parameters that determine the prevalence and intensities of helminth species between and within hosts may aid in identifying the host responsible for helminth transmission within a population. Due to the habitat preferences, distinct host species will most likely alter helminth compound communities (Brooks *et al.*, 2006).

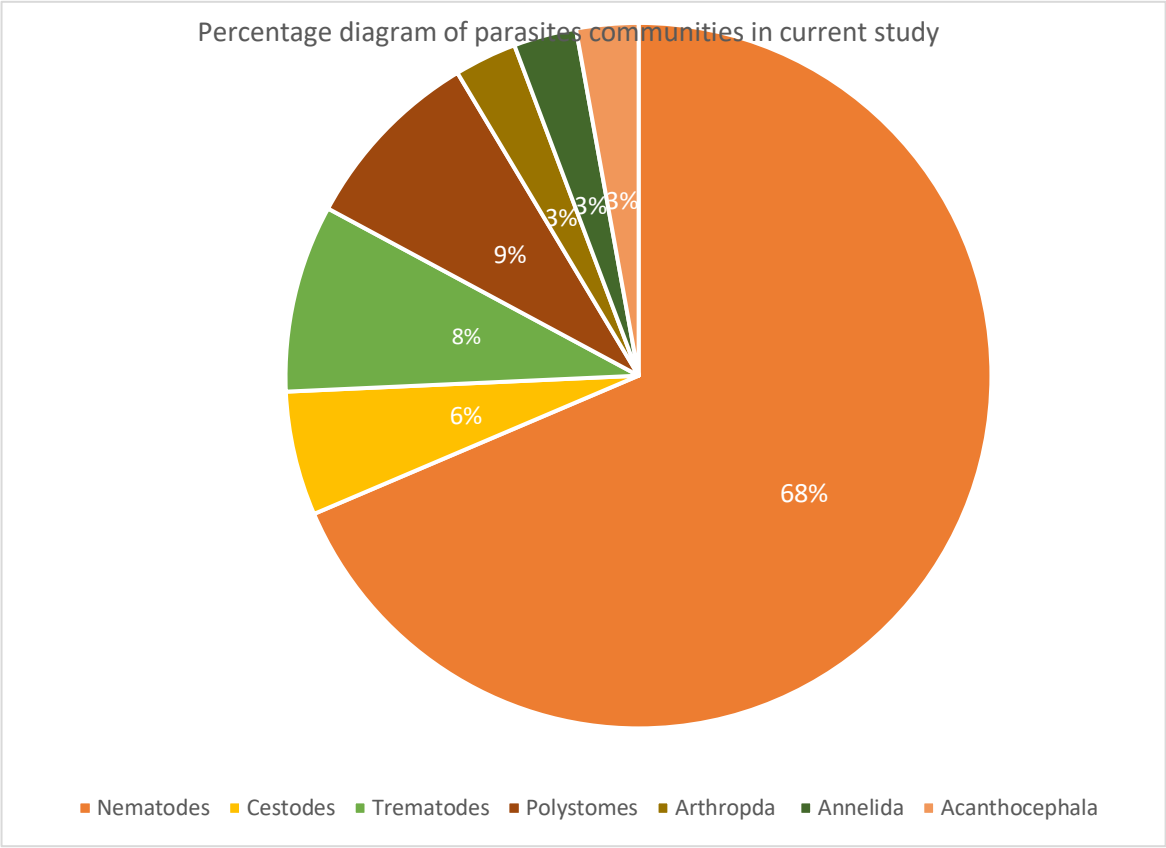


Figure 38: A percentage diagram of all parasite species found in the current study's frog community.

Nematodes have varied and complex lifecycles within different hosts (Fig. 38). As previously noted, anurans are the definitive host for various taxa of helminth parasites, including Nematoda species. Camallanid nematodes have been detected in every *Xenopus* species and they, too, utilise cyclopoids as intermediary hosts. A total of more than 50 species in the genus *Aplectana* is known,

thus expanding the diversity found within amphibians. In the Afrotropical Realm, 12 new *Rhabdias* species have been recently discovered in amphibians. Bringing the total number of nematodes that parasitise anurans only from southern Africa, to an astonishing 42 species.

Thus, the abundance of Nematodes in this study can be explained by their unique and biochemical adaptations to live in harsh environments, as well as their utilization of a variety of reproduction techniques. The number of nematode species and individuals varies depending on the host species; however, nematodes often dominate the helminth community. According to Sapir (2021) previous studies have shown that nematodes are the most abundant metazoan taxon in samples collected in extreme environments. Nematodes have been recovered from environments such as deep-subsurface habitats, hot or polar regions and parts of intestine, and anaerobic environments (Kitazume *et al.*, 2018; Sapir, 2021). Their abundance can be further explained by their cylindrical body with almost no appendages, which is advantageous when surviving in the intestine of anurans. Dietary requirements for nematodes are also minimal. Finally, they provide a variety of adaptations for surviving situations of adversity (Wright *et al.,* 2006). Thus, we believe that the wide variety of ecological niches that resulted in a high diversity of amphibians in South Africa is also reflected in the diversity of nematodes that adapted, not only to their hosts, but also to the environment their intermediate stages have to face.

Tapeworms are a diverse collection of ubiquitous endoparasites with a two-phase life cycle in which vertebrates serve as their final hosts (Fig. 38). According to Heyneman (1996) all cestodes are parasitic and have complicated life cycles. They are rarely found in amphibians; however, adults can be recovered from intestines of anuran hosts or encysted in the liver. Tapeworms, according to Wright (2006), can cause gastrointestinal ulcers, blockages, and mortality in severe cases. Ingestion of an intermediate host can result in transmission. *Cephaloclamys namaquensis* was only found in *X. laevis* in our study, however, it has been found in a variety of naturally occurring ranids (Fig. 28; Fig 38). Despite its ability to infect amphibians from far away, *C. namaquensis* has a very limited distribution among African pipids. This species has a geographical range in Sub-Saharan Africa that stretches over 40 degrees latitude and has a diverse spectrum of biotypes. Cestodes can be found in a variety of environments, from the temperate Mediterranean climate zone at South Africa's tip to highland savanna at lower latitudes. *Cephalochlamys* has a two-host transmission cycle, with intermediate hosts being cyclopoid copepods. While pipids usually feed underwater, most anurans are specialist terrestrial feeders, therefore this dependency on a copepod intermediate clearly limits the capacity to infect non-pipid anurans (Jackson and Tinsley, 2001).

According to Amin *et al.* (2012), *Nematotaeniidae* (Fig. 29) occur in almost every faunal region, but are least common in Palearctic areas. Species of this family infect anurans and are also found in varanid lizards, snakes and other reptiles (Amin *et al.,* 2012). Surprisingly, in the present study we found a vast number of different amphibians infected with cestodes previously unreported from southern Africa. It is clear that they belong to a new species and probably even to a new genus.

We believe that further studies may reveal that the true diversity of this parasitic group is more than it was supposed and molecular data will help to specify their taxonomic status.

Life cycles of trematodes are indirect and involves two or more hosts and are commonly found in African fish, reptiles. In our study *Oligolecithus sp.* (Fig. 33) was only observed in the African bullfrog with a prevalence of 50%. However, they are common parasites in the intestines of some southern African sand frogs and are common in *X. laevis*, found from southern and central Africa. Following that, studies revealed that *Oligolecithus* species have previously been distinguished from other subspecies of *X. laevis* at locations separated by over 4000 kilometers. *Tylodelphys* sp. parasites are more commonly found in the African clawed frog, and infect the body cavity, brain and intestine. *Xenopus laevis* was not heavily infected with numerous trematode species in the study, as expected from the literature data. However, *Tylodelphys* (Fig. 31) infects freshwater fish throughout Africa and share common habitats with the aquatic *X. laevis*. This infection can be explained by their closely related interaction in freshwater areas, thus making parasite transmission from fish such as the sharptooth catfish, *Clarias gariepinus* (Burchell 1822) to frogs (*X. laevis*), unchallenging.

Although monogeneans are primarily parasitic on fish, the *Polystomatidae* family can be commonly infects the urinary bladders of frogs. Monogeneans have a variety of adaptations to survive host environments, which include hamuli (anchors) that are used to attach to the host by penetration of the host skin or organ. They also have haptoral suckers providing the suction ability to attach to hosts. Because of their relationship with freshwater settings that favour parasite transmission, these host organisms are ecologically connected (Theunissen *et* al., 2014). Anuran polystomes in Africa are strictly host specific which may indicate the low infection rates in the present study. During the investigation, 12 *X. laevis*, 17 *Poyntonophrynus fenoulheti*, and 11 *Sclerophys gutturalis* frogs were dissected, revealing 24 polystomes (Fig. 36).

Approximately 15 families of mites are known (Mullen and O Connor, 2018). Some mites can be found in the respiratory canals, of the nasal passages, nasal sinuses and pulmonary tissue. The infection in the current study could be accidental because the *Bufonidae* frogs are terrestrial and occur along streams and in ploughed fields across wide areas, which can explain the interaction and infection of the *Ophionyssus* mite in the nostrils of the Power's toad (Fig. 38).

In the family *Naididae*, only species of the genus *Dero* Oken, 1815 uses vertebrates as hosts, which include toads and frogs for phoresis or parasitize the urinary bladder and eyes. *Dero bauchiensis* (Stephenson, 1930) has been established to be the only endoparasitic Dero known to infect African frogs, infecting their eyes and the Harderian glands (Sinsch *et al.*, 2019). They (Sinsch *et al.*, 2019) gave the first report of *Dero rwandae* Sinsch, Dehling, Scheid and Balczun, 2019, of an African *Dero* species to infecting the urinary bladder of anurans (Sinsch *et al.*, 2019). Our specimen had a total of five Oligochaete worms - one within the urinary bladder and four found in the eye. The relationship between the frog host and the worms has been classified as either parasitism or commensalism. It was later determined, that it is a case of parasitism because the worms injured

their host by rupturing the ureter, which can result in death in some circumstances (Andrews *et al.*, 2015). *Dero bauchiensis*, found in Nigeria and Mozambique, is the only Dero that infects *Phrynomantis bifasciatus* eyes and Harderian glands. There are currently nine free-living species recognized from Sub-Saharan Africa (Stephenson, 1930) (See Figure 36). Specimens found in present study need further investigation for the clear identification, especially on the molecular front.

No acanthocephalans, cystacanths, or adults have been documented from African frogs and toads, (Smales, 2005) (Fig. 37). Acanthocephalans are thorny-headed or spiny-headed worms which form part of a tiny group of obligate endoparasites found in vertebrates' alimentary tracts (Fig. 39). They have indirect life cycles and require an arthropod as intermediate host, these endoparasites inhabit the gastrointestinal tract of anurans, and may cause weight loss in some frogs. *Strongylopus fasciatus* had a total of five acanthocephalans encysted in its body cavity (Fig. 37) and their origin remains unknown.

3.5 DIFFERENT LIFE CYCLES OF PARASITES

3.5.1 PREFACE

All parasites have life cycles that include a length of time spent in a host organism, which is then separated into growth, reproduction and transmission phases. In this regard, parasite life cycles are split into two categories: direct (monoxenous) (Fig. 39) and indirect (heteroxenous) (Fig. 39). In the direct category, the life cycle is completed in a single host; they lack an intermediate host. Indirect have at least two host stages, one of which is a definitive host and the other an intermediate host. The definitive host is required for parasite replication and adult life. Parasite development takes place within the intermediate from which the parasite is passed to a definitive host.

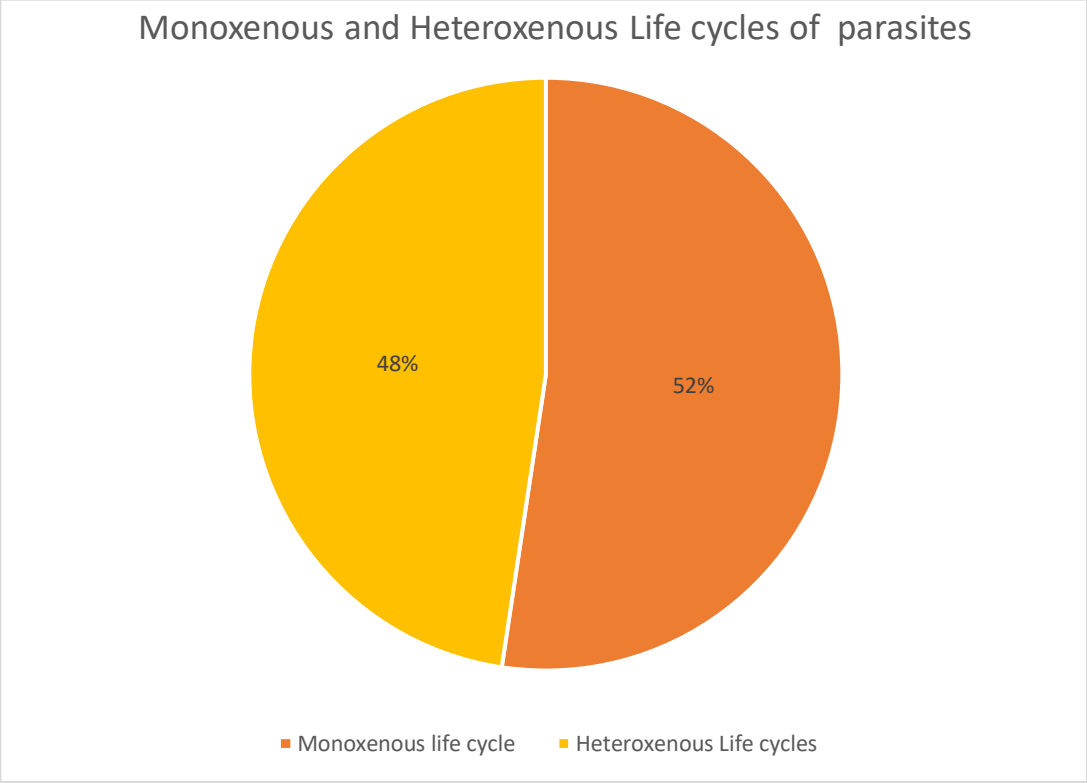


Figure 39: A pie chart depicting the different parasite life cycles of parasites recovered in the study.

During the current study, the following parasite species were classified not requiring intermediate hosts in their life cycles: *Amphibiophilus* sp*, Aplectana* sp*. 1, Aplectana* sp*. 2, Aplectana sp. 3, Aplectana* sp*. 4, Aplectana* sp.*5, Cosmocerca* sp.1*, Cosmocerca* sp. 2, *Cosmocercidae gen.* sp. 1, *Cosmocercidae* gen. sp. 2*, Rhabdias africanus, Rhabdias* cf. *sylvestris, Rhabdias engelbrechti, Rhabdias* sp.1*, Rhabdias* sp. *2, Rhabdias* sp. 3*, Rhabdias* sp. 4*. Contraceacum* sp. 1, *Strongyloides* sp*., Tangua* sp*., Eupolystoma* sp*., Polystoma mashoni, Ophionyssus* mites and lastly an unidentified *oligochaeta*. (Fig. 39) (Table 4).

The parasites observed in the study with more than one host in their life cycle observed in the study are represented as follows: *Cephaloclamys namaquensis, Nematoteniidae* gen. sp*., Mesocoelium* sp*., Oligolecithus* sp*., Tylodelphis* sp*., Protopolystoma xenopodis, Batracamallanus slomei, Camallanus kaapstaadii, Neofoleyellides steyni* (Fig. 39) (Table 4). Such prevalence of monoxenous of parasites in most of the frog's species shows their low involvement in the food chains in studied area (Fig. 39).

Table 4: Table demonstrating which types of helminths belong to which life cycles.

Monoxenous life cycle	**Heteroxenous Life cycles**
Aplectana	*Mesocoelium* sp.
Cosmocerca	*Nematoteniidae* gen. sp.
Rhabdias	*Cephaloclamys namaquensis*
Amphibiophilus sp.	*Tylodelphis* sp.
Contracaecum sp.1	*Acanthocephalan*
Strongyloides sp.	*Protopolystoma xenopodis*
Eupolystoma sp.	*Batracamallanus slomei*
Polystoma mashoni	*Camallanus kaapstaadii*
Ophionyssus sp.	*Neofoleyellides steyni*
Tangua sp.	*Oligolecithus* sp.
unidentified oligochaeta	

3.6 STATISTICAL ANAYLSIS

3.6.1 STATISTICAL REPRESENTATION OF ENTIRE SAMPLE

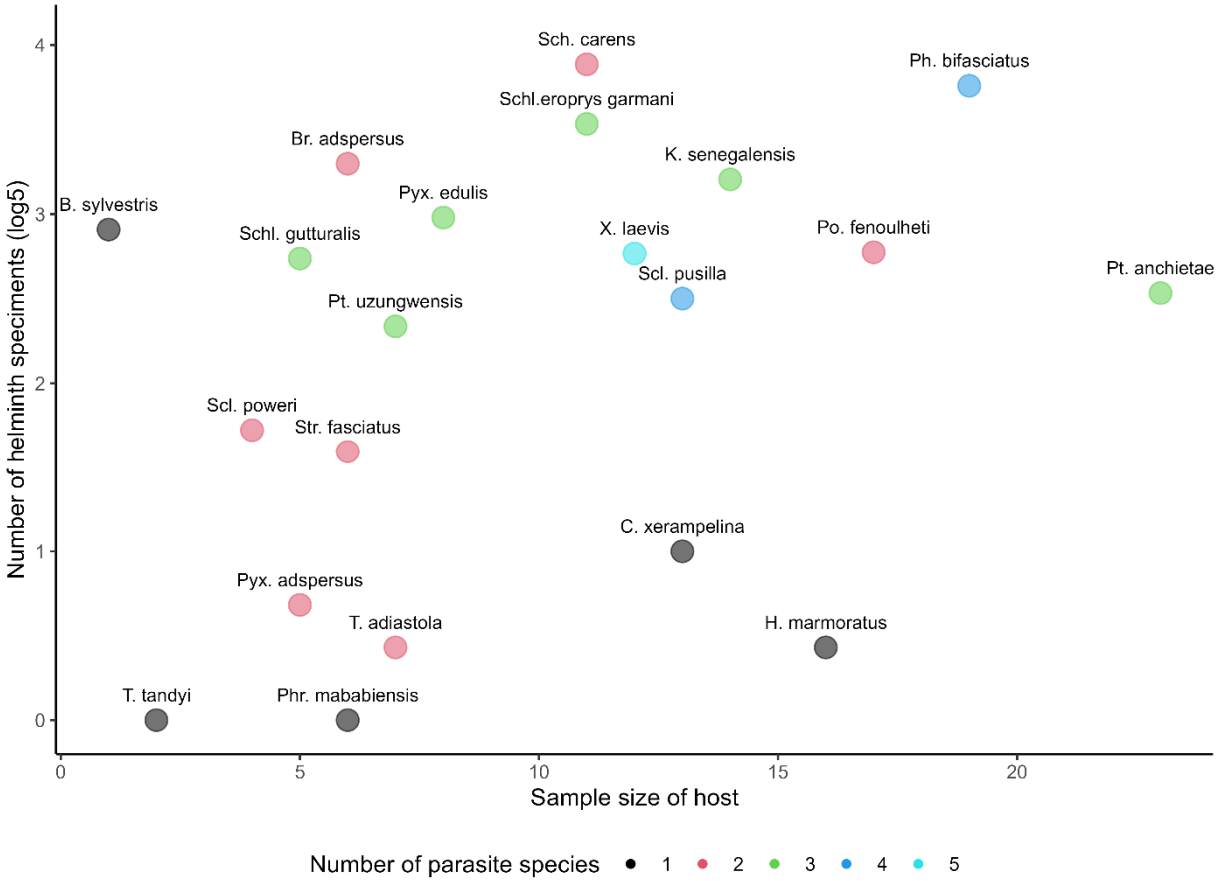


Figure 40: Statistical representation of all samples taken between 2019 and 2021. The various colours show the number of helminth species infections.

All amphibian species were included in the study and only *Amietia delalandii* was excluded from the visualization as this anuran species was the most prevalent in the sample collection. A total of 63 specimens were dissected harbouring a total of five different parasite species. Taking into account, all the sites visited during the period of 2019-2021, *Amietia delalandii* was captured at most sites, including all five trasect areas of research. Parasites with solely monoxenous life cycles were found in the common river frog. When there was a lot of competition within the host, site expansion (or asexual replication) was sometimes slowed down (for both simple life cycles parasites and complex life cycle parasites).

More than 40 species of parasites are known from *Xenopus laevis*, however, only five species were encountered in the 12 dissected frogs (Fig. 40). *Xenopus laevis* was only captured at four sites, including only two researched transects. The African clawed frog's widespread success as an invader can be attributed to a unique blend of characteristics. We hypothesise that if more clawed

frogs were dissected during this study, a greater parasite diversity would have been recorded in them (Fig. 40).

Most of the other host species were infected with only two helminths species, with a total of host dissections ranging between 2 and 17 (Fig. 40). Because *Poyntonophrynus fenoulheti* was screened 17 times, the chance that these different specimens would harbour a great number of helminths species, was favourable (Fig. 40). A total of 11 red toads were screened for parasites in this study and they harboured a great number of helminth species, with *Aplectana* sp. 1 (Fig. 10) being most dominant in this host species. Seven and five times, respectively, *Tomopterna adiastola* and *Pyxcephalus adspsersus* were examined. With only a few host samples, the volume and variety of helminth species could not be determined (Fig. 40).

Chiromantis xerampelina and *H. marmoratus* were only infected by one species despite the sample size being bigger than 10 frogs. *Chiromantis xerampelina* is a rhacophorid consisting of arboreal frogs. The foam nest tree frog, as their name implies, inhabits branches of trees and shrubs, grass tussocks and rocks, as well as artificial structures such as dam walls, bridges and buildings and the near-vertical rock walls of flooded quarries. Thus, it does not share common environments or habitats with *Phrynomantis bifasciatus* for example, that lives mainly underneath rocks and in shrubs on ground level and is heavily infected with a variety of helminth species. Focusing on *H. marmoratus*, these frogs tend to move around the canopy of surrounding trees or bask and sometimes also on trees, grasses, bushes and floating vegetation. Both *C. xerampelina* and *H. marmoratus* share no common parasite species, but the number of helminth species, is minimal (Fig. 40).

Dots on the far-right side of the graph (Fig. 40) display high sample sizes and a great number of helminth specimens. This shows that when several host species (anurans) are dissected and screened, the number of helminth specimens within the host species tend to be higher than when a single host species is analysed.

3.6.2 DISTRIBUTION OF SAMPLES

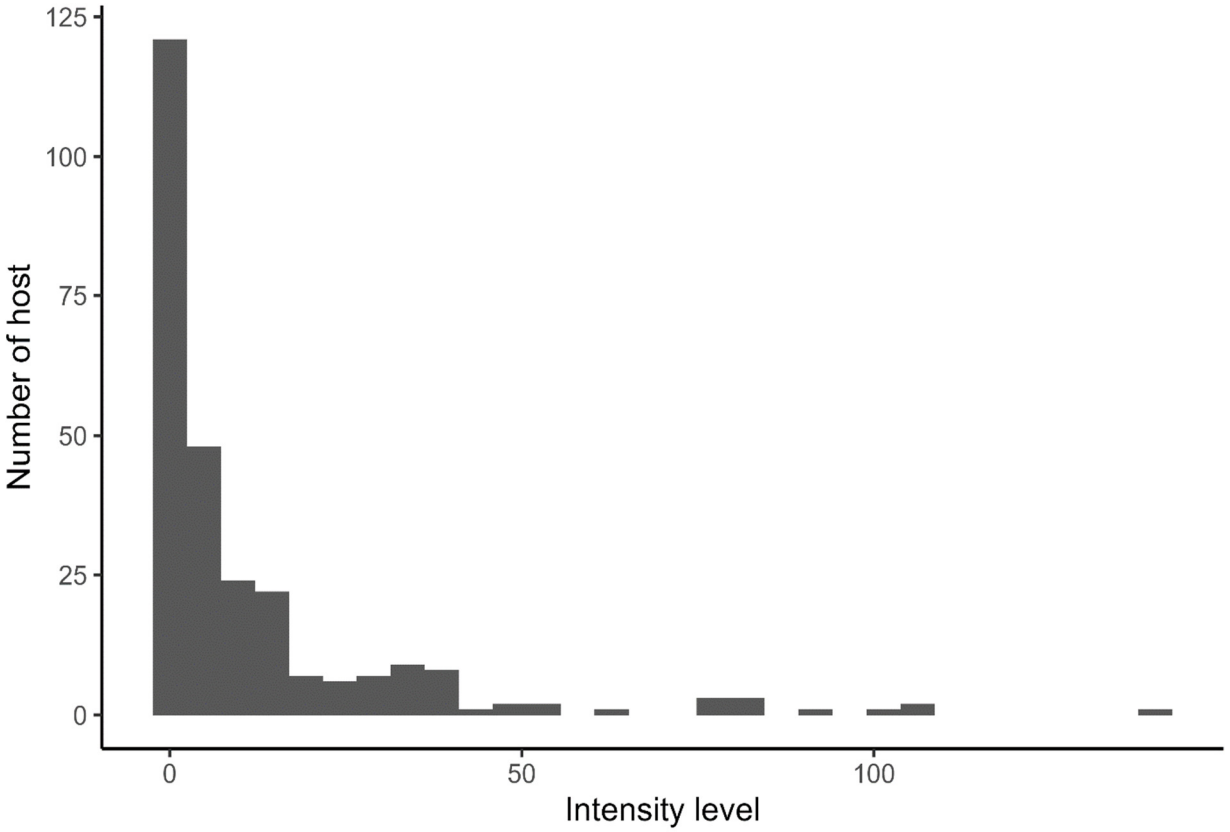


Figure 41: Showing the distribution of samples throughout the study an indicates of the helminth intensity level as a function of the number of hosts per sample.

Parasites are unevenly distributed among their hosts, with the majority of hosts having no parasites and only a few having a considerable number. The peak represents most individual hosts with no parasites, while many individual hosts have only one parasite (middle) and to the right, a few hosts harbour many parasites. The main reason for this is probably, either that some hosts are more exposed to parasites, or that they are more vulnerable to them. The observed pattern indicates that most individual hosts are free, or almost free, of parasites, while a minority carry a large number of parasites. Frogs scatter themselves across a wide range of habitats and environments, across a great area, at random, however, parasites may be concentrated in high densities in some areas and are scarces elsewhere (Fig. 41)

Because many parasites have complex life cycles, the different members of a parasite population dwell in completely distinct habitats. The abundance of a species is usually strongest in areas of its range where conditions are close to optimal and lowest elsewhere, resulting in an uneven distribution of abundance across its geographical range. When the parameter k of the negative binomial distribution is utilized, the same pattern emerges: with the exception of a few examples, the vast majority of populations have k values approaching zero, indicating the high aggregation (Fig. 41). The most likely distribution is negative binomial distribution which corresponds to general

tendency for such data. The higher the prevalence of infection, the greater the dispersion of parasites among available hosts and thus the lower the aggregation (Fig. 41).

3.6.3 THE SIMILARITY OF HELMINTH FAUNA COMPOSITION

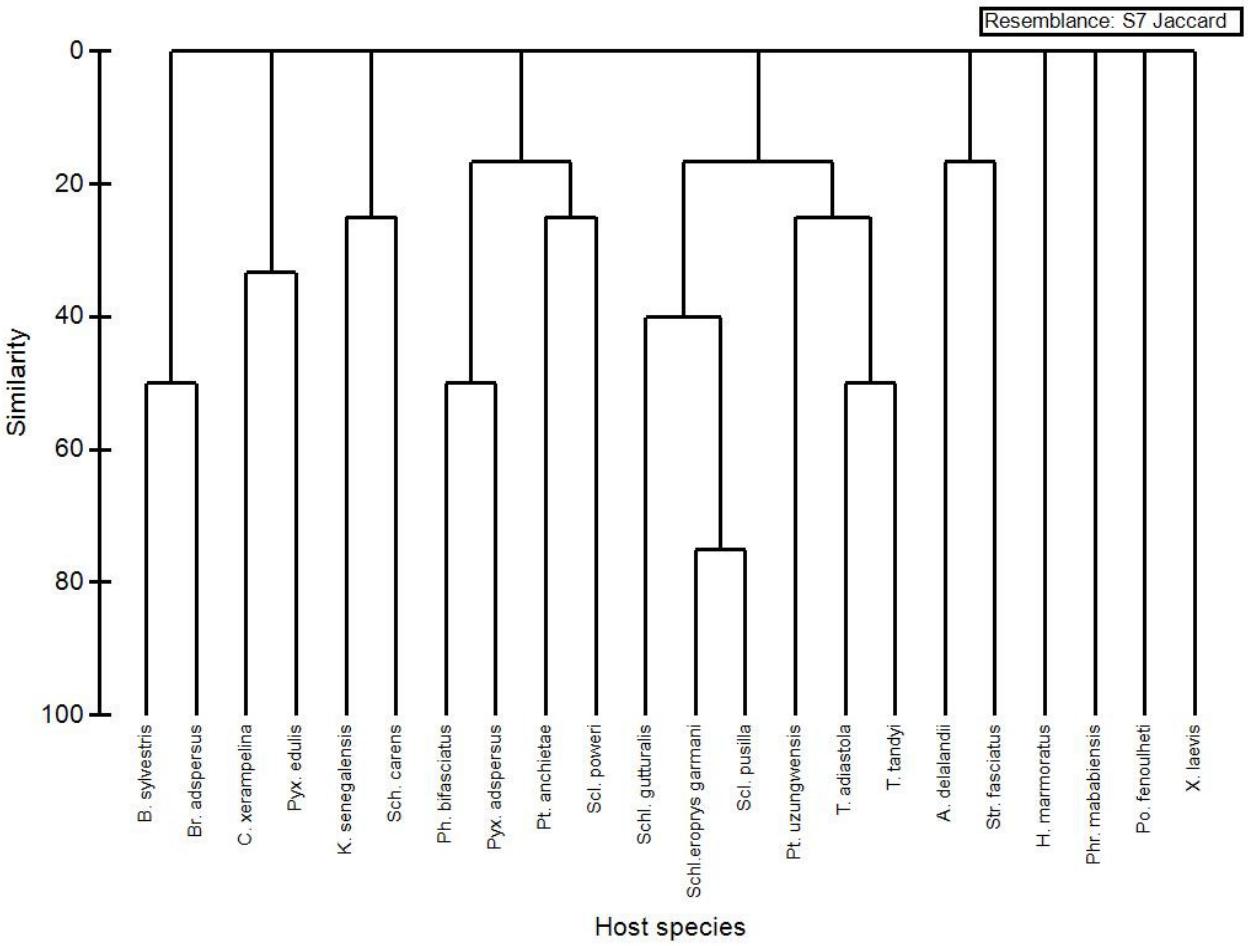


Figure 42: The similarity of helminths fauna composition indicating clusters in the parasite community.

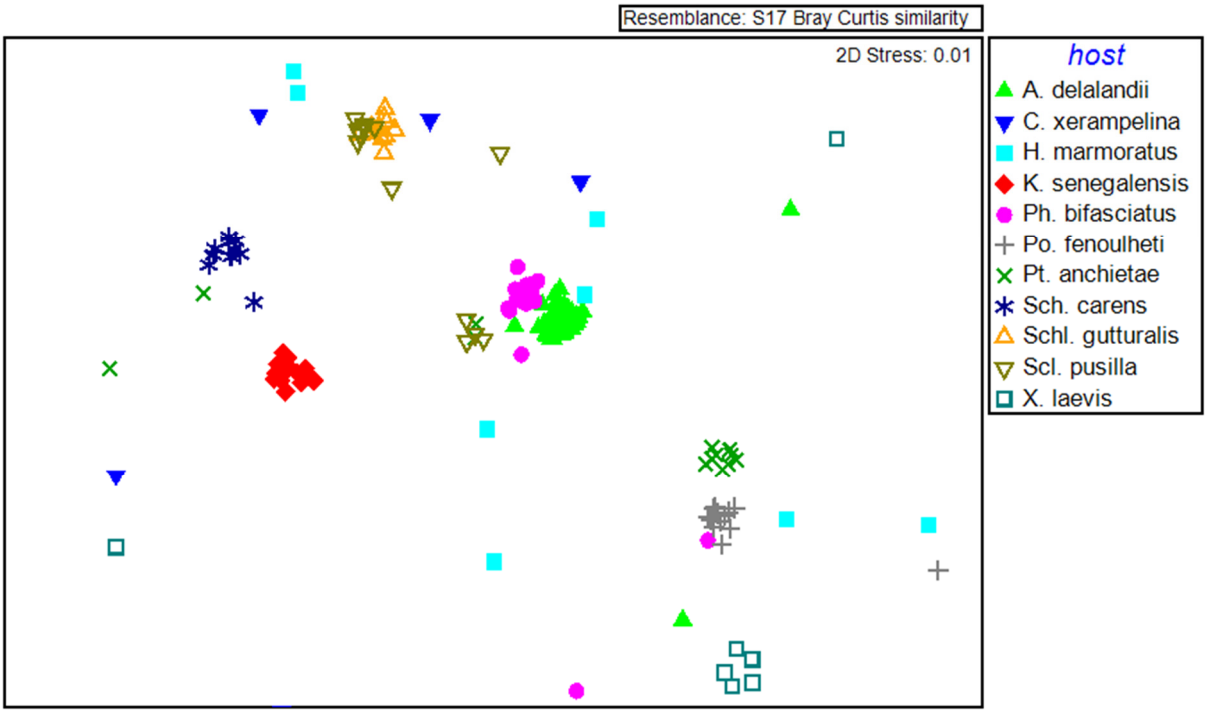
All frog species with fewer than ten individuals were removed from the study. The analysis found six groups (Fig. 42), none of which had a common parasite species. In this study, the helminth fauna makeup of most anuran hosts was comparable. Nevertheless, some frogs did not show any sign of clustering or similarity in the parasites found in them and these hosts included *Hyperolius marmoratus*, *Phrynobatrachus mababiensis*, *Poyntonophrynus fenoulheti* and lastly *Xenopus laevis*.

Parasites found within *Sclerophys garmani* and *Sclerophys pusilla* were most similar compared to other helminths parasites in that same cluster (Fig. 42). They share the following parasite species*, Aplectana sp..3, Rhabdias africanus* and *Mesocoelium sp..*, with a similarity of 75% (Fig. 42). *Ptychadena tandyi* and *Pthychadena anchietae*, also from the same cluster, share 50% similarity; the parasite species included, *Mesocoelium* sp. (Fig. 31). Because anuran hosts share

only one common parasite species within a cluster, they tended to have smaller similarity, compared to hosts sharing two or more parasite species.

Only one parasite species, *Rhabdias* sp. 1, was shared by *Kassina senegalensis* and *Schismaderma carens* (Fig. 23), thus indicating that the similarity is quite minimal. *Kassina senegalensis* did not share other parasite species with other anuran hosts, except *Schismaderma carens*. Cluster four, which included four anuran hosts, all share one common parasite, namely *Nematotaeniidae gen. sp.*., thereafter, the cluster indicates how similarity increases as those four anurans share even more common parasites with each other.

3.6.4 VISUALIZATION OF THE INFRACOMMUNITIES



Figures 43: Resemblance according to S17 Bray Curtis similarity, with clusters showing similarity in helminth composition.

Sclerophrys gutturalis and *Sclerophrys pusilla*, share common parasite species, thus representing one cluster (Fig. 43). These toads share habitat, food resources and ecological niches within the same area. Their helminth similarity can be explained by their close interaction with one another and dietary needs, making parasite transmission easier from one host to the other. As seen in graph (Fig. 42) they shared a 40% similarity in helminth community, nevertheless showing no parasite similarity with *Schismaderma carens* or with *Kassina senegalensis*. *Schismaderma carens*, had a unique parasite composition, as it shares only one common parasite with *K. senegalensis*.

Aplectana sp. 1 (Fig. 9) found in *S. carens*, occurred also in the red toad. However, *Rhabdias* sp. 1 (Fig. 24) occurred in both *S. carens* and in *K. senegalensis*. *Rhabdias* sp. was found in abundance in the bubbling Kassina, resulting in cluster, indicating similarity in parasite communities in the representative frogs. *Amietia delalandii, Phrynomantis bifasciatus*, and *Sclerophrys pusilla* formed another cluster, showing that these three frogs have the same parasite community. They shared a common parasite species, Nematotaeniidae gen. sp. (Fig. 29). As *Rhabdias* sp. 3 (Fig. 25) was only recovered from these frogs, *Ptychadena anchietae* formed a cluster on its own, demonstrating no similarity with other anuran groups and a unique parasite composition, Though, *P. anchietae* can be seen sharing the parasite *Nematotaeniidae* gen. sp, with *Amietia delalandii, Phrynomantis bifasciatus*, and *Sclerophrys pusilla*, *Poyntonophrynus fenoulheti* share no common parasite with other frogs, indicating no similarity within the parasite community. *Eupolystoma* sp. (Fig. 36), found in the bladder of *P. fenoulheti*, during this study, is host specific thus making the composition of parasites found within these frogs, extremely unique. The African clawed frog, *Xenopus laevis*, did not harbour a single parasite with a direct life cycle infecting it, but only ones with indirect life cycles. This frog had no parasite composition similarity comparable to other parasite compositios in the study (Fig. 43).

The toads, *Sclerophys gutturalis* and *Sclerophrys pusilla,* show parasite community similarity, with a high infection rate of nematodes. The prevalence of nematodes can be explained by their unique and biochemical adaptations to survive harsh environments, as well as their employment of a variety of reproductive techniques, as previously indicated. It is probable that toads share a parasite species that has adapted to exist in these harsh situations.

The key abiotic parameters that limit the distribution and abundance of parasitic nematode soil stages are soil temperature, moisture, and composition. Depending on the environment and site visited during a certain period, the intensity and parasite community of the African red toad may vary (Fig. 43).

3.7 ESTIMATING THE AMOUNT OF HELMINTH SPECIES IN THE AREA OF RESEARCH

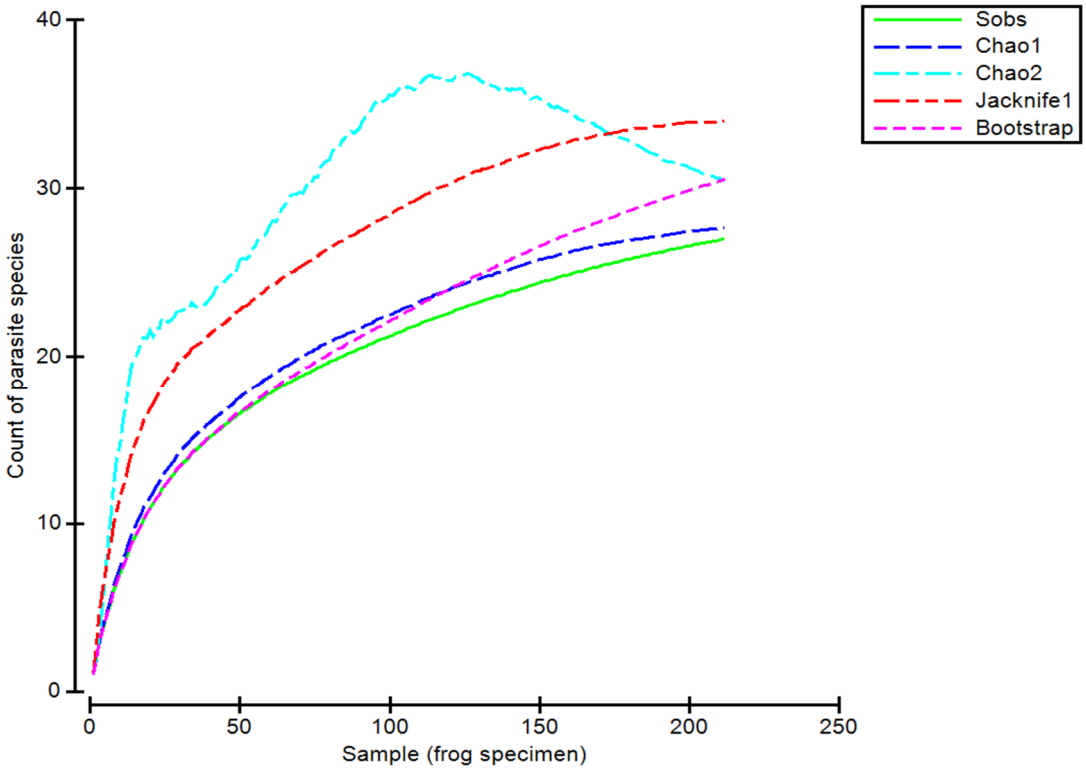


Figure 44: Sobs, Chao1, Chao2, Jacknife1, and Bootstrap curves used to estimate the number of helminth species in the research area.

All frog species with fewer than ten individuals were removed from the study. The actual species accumulation curve (S) is shown in green (Fig. 44) (SAC); the others are predicted SAC on the base of different estimators. Estimator Chao1 (Fig. 44) gives more weight to prevalent species; other estimators used here are focused on rare species rather than on prevalent ones. The green curve represents the actual approach when sampling and how we find new parasite species in real life. This curve shows that sampling is natural or random when visiting different sites. Chao1 is an estimator based on abundance. This means that the data it requires refer to the abundance of individuals belonging to a certain class in a sample. Since cure S (Sobs) and Chao1 almost overlap, we can suppose that all (or at least the great majority) of the prevalent parasite species are found within studied amphibians from the Vhembe Biosphere Reserve (Fig. 44).

CHAPTER 4:

DISCUSSION AND CONCLUSION

In this study, a total of 269 South African anurans were studied and a total of 169 hosts were infected. Of the 170 identified South African anurans, twenty-two screened anuran species (13 percent) have now been shown to host helminths (summary Table). From all the anurans screened in the timeframe of 2019-2021, a total of 35 species of metazoan parasites were encountered (Table 3). These parasites included, 24 Nematode species (*Amphibiophilus sp.., Neofoleyellides steyni, Aplectana* sp*. 1, Aplectana* sp*. 2, Aplectana* sp*..3, Aplectana* sp*. 4, Aplectana* sp*. 5, Cosmocerca sp. 1, Cosmocerca sp. 2, Cosmocercasp.. 3, Cosmocercidae* gen sp.1, *Cosmocercidae* gen. sp. 2, *Contracaecum* sp..1*, Tangua* sp., *Rhabdias engelbrechti, Rhabdias africanus, Rhabdias cf. sylvestris, Rhabdias* sp. 1, *Rhabdias* sp. 2, *Rhabdias* sp. 3, *Rhabdias* sp. 4*, Strongyloides* sp., *Batrachocamallanus slomei* and *Camallanus kaapstaadi*), three digenetic trematodes, (*Tylodelphys* sp., *Mesocoelium* sp. and *Oligolecithus* sp.), three species of Monogenea, (*Protopolystoma xenopodis, Eupolystoma* sp*.,* and *Polystoma mashoni*), two Cestodes species, (*Cephaloclamys namaquensis* and *Nematoteniidae* gen. sp.), one species of Acanthocephala (*Acanthocephalus criniae*), one species of Arthropoda (*Ophionyssus* sp.) and one species of Annelid, recovered from the frog`s eyes and urinary bladder (unidentified *Oligochaeta Family* gen. sp.) (Fig. 45).

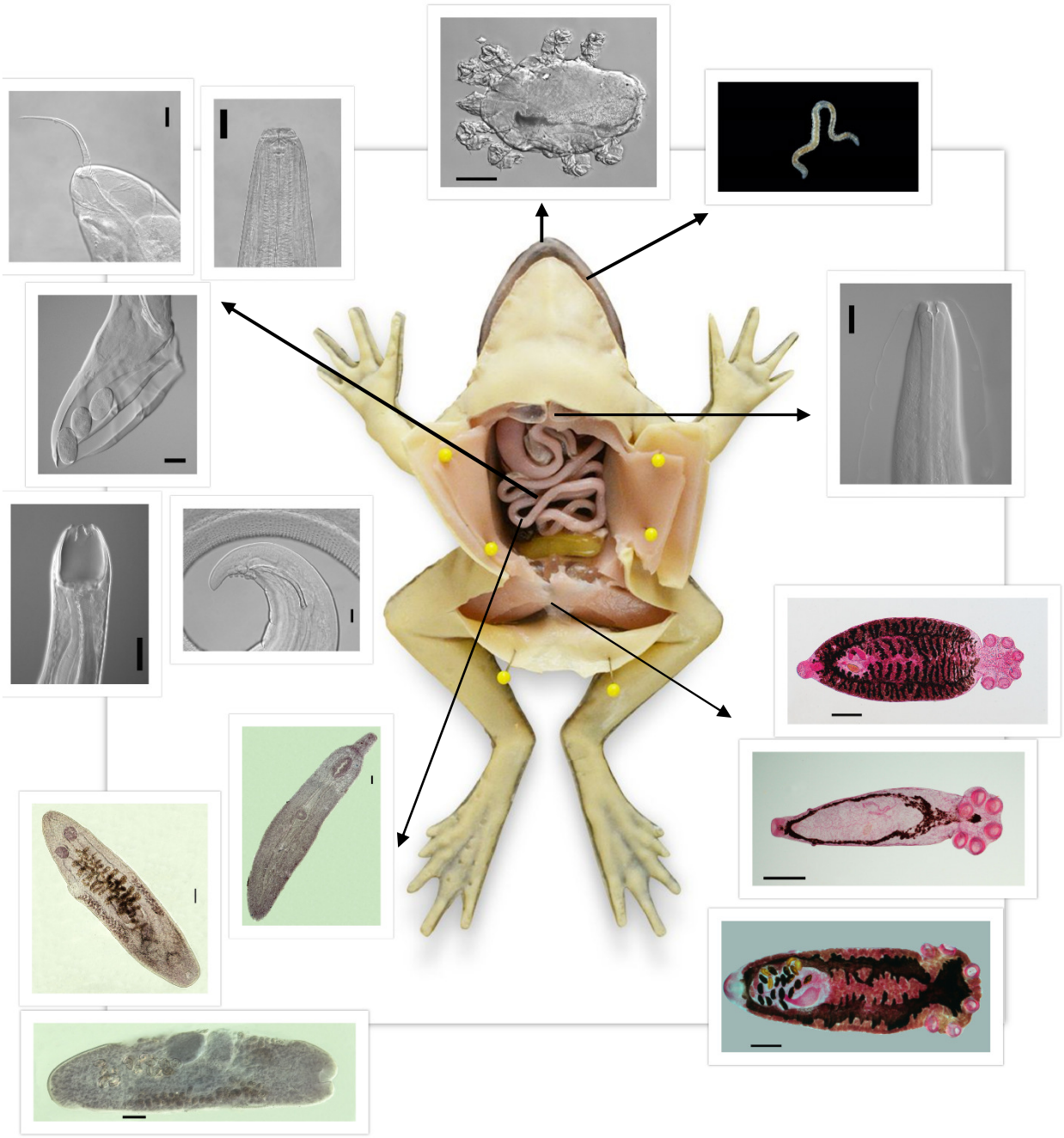


Figure 45: The helminth community discovered in anurans from the Vhembe Biosphere Reserve is depicted in this diagram.

The percentage infection rates of all the classes of parasites found in the study is as follow: Nematoda, 68.57%; Cestoda, 5.71%; Digenea, 8.57%; Monogenea, 8.57%; Arthropoda, 2.85% and Acanthocephala and Annelida, 2.85% (Table 2) (Fig. 45).

Cestodes, monogeneans and trematodes are among the most varied parasites found in aquatic organisms, with several species belonging to each taxon. The great parasite variety and richness currently observed in aquatic ecosystems reflects the long evolutionary history of both host and parasite life in freshwater and marine habitats (Rohde, 2005).

The results from this study show that frogs are low on the food chain but are, nevertheless, significantly involved because all the parasites recovered from this study indicate that frogs share parasites. Nonetheless, additional study needs to be done before the anuran helminth community in South Africa can be properly recognized and identified. This impressive parasite diversity can be explained by the fact that amphibians are closely associated with water, which facilitate parasite transmission.

In a relatively small study area and in only 22 species of anurans we found several species that are morphologically and genetically different from all previously known species. Our results demonstrate that anurans harbour parasites of different taxonomic groups. To solve knowledge gaps in community and disease ecology, researchers must first understand the patterns and factors of the host-parasite connection.

"I D KISS A FROG EVEN IF THERE WAS NO PROMISE OF A PRINCE CHARMING POPPING OUT OF IT. I LOVE FROGS.

- CAMERON DIAZ.

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