

**Cestodes of the order
Onchoproteocephalidea II infecting
cartilaginous fishes (Elasmobranchii) in
South Africa**

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PREFACE AND ACKNOWLEDGEMENTS

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This MSc is disclaimed for purposes of Zoological Nomenclature in accordance with the International Code of Zoological Nomenclature, Fourth Edition Articles 8.2 and 8.3 (ICZN 1999). No new names or nomenclature changes are available from statements in this MSc thesis.

With this study I propose to lay the foundation for future research, especially proposing the integration of taxonomic parasitology along with ecology and conservation science, in hope to make a difference for our world's marine ecosystems. "The greatest danger to our planet is the belief that someone else will save it" – Robert Swan.

ABSTRACT

South Africa is globally classified as the third most biodiverse country on Earth. However, the immense deficiency of taxonomic marine based initiatives, especially regarding parasitological research, within this region leaves the majority of this hidden biodiversity unrecognised and unexplored. What's more, the current anthropogenic pressures placed on our marine ecosystems not only increase the threats posed on elasmobranch host survival, but increases the risk of the co-extinction of both host and parasites before their unique host-parasite system is discovered, and the benefits of their evolutionary relationship can be appreciated. Recognising the absence of taxonomic biodiversity research for cestode-elasmobranch systems in the ocean basins surrounding southern Africa, the present study was conducted to establish a foundation for future research within this field. By investigating the biodiversity of onchoproteocephalidean cestodes infecting elasmobranchs off the coast of South Africa, eight new onchoproteocephalidean species were discovered and described. These species are *Acanthobothrium microhabentes* Van Der Spuy, Smit et Schaeffner, 2020, *A. microtenuis* Van Der Spuy, Smit et Schaeffner, 2020, *A. crassus* Van Der Spuy, Smit et Schaeffner, 2020, *A. dolichocollum* Van Der Spuy, Smit et Schaeffner, 2020, *A. umbungus* Van Der Spuy, Smit et Schaeffner, 2022, *A. usengozinius* Van Der Spuy, Smit et Schaeffner, 2022, *A. ulondolozus* Van Der Spuy, Smit et Schaeffner, 2022 and *Phoreiobothrium* sp. A, seven of which have already been registered and published. The first four species of *Acanthobothrium* van Beneden, 1849, were reported to parasitise subsets of eight spotted skates, *Raja straeleni* Poll, whilst the following three species were reported to parasitise an endangered host species namely the white skate, *Rostroraja alba* (Lacépède). The last species, *Phoreiobothrium* sp. A, was reported from the spinner shark, *Carcharhinus brevipinna* (Valenciennes). This study was conducted by use of morphological methods (i.e. light microscopy and scanning electron microscopy). Furthermore, each species described was consequently distinguished from all other valid species worldwide, therefore ensuring the legitimacy of the new species. Although differing quite extensively from most congeners, five of the seven species of *Acanthobothrium* (*A. microhabentes*, *A. microtenuis*, *A. crassus*, *A. dolichocollum* and *A. ulondolozus*) seem to share a unique feature with merely 12 other species from the Indo-Pacific Ocean, suggesting that these might be each other's closest relatives. *Phoreiobothrium* sp. A could possibly be the same species found by Jensen and Bullard (2010) off the coast of Mississippi, U.S.A. However, without a molecular phylogenetic analysis at this point these assumptions could not be proven. Moreover, since these eight species were recovered from a previously unexplored locality, and in two cases unexplored hosts, these results expand the geographical distribution, biodiversity and host associations of the order. Given the fact that three species were recovered from an endangered host, it is apparent to not only address the biodiversity of onchoproteocephalidean cestodes, but also to determine the specific, unique and evolutionary

host-parasite interrelationships. By recognising these cestodes as affiliate species, researchers could gain more knowledge of the ecological importance these species hold, which might give threatened elasmobranch species a better chance of survival. Hence, this study additionally highlights the necessity for the incorporation of novel and multisource approaches of interdisciplinary fields such as ecology, conservation science and ecotoxicology for future research.

Keywords

Marine fish parasites, elasmobranchs, taxonomy, tapeworms, affiliate species, new species, biodiversity, threatened fauna, co-evolution, co-extinctions, conservation biology.

References

Jensen, K. & Bullard, A. 2010. Characterization of a diversity of tetraphyllidean and rhinebothriidean cestode larval types, with comments on host associations and life-cycles. *International Journal for Parasitology*, 40: 889-910.

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CHAPTER 1: GENERAL INTRODUCTION



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Species and populations from terrestrial, marine and other aquatic ecosystems all form part of our biological diversity on earth, sustaining all forms of life through various ecosystem services (Diaz *et al.*, 2006). Amongst these, aquatic ecosystems, including freshwater, estuarine and marine habitats, form the basis of all life on Earth (Selbach *et al.*, 2022).

The world's oceans represent the longest evolutionary history, constituting some of Earth's greatest habitats (Jackson, 2010). Marine organisms fundamentally determine the nature of planet Earth (Cavicchioli *et al.*, 2019). Most of the marine life, however, is confined to continental shelf waters (Spalding *et al.*, 2007). As such, oceans are divided in a nested system of 12 marine realms, covering coastal waters across the world (Spalding *et al.*, 2007). By combining coastal benthic and pelagic biotas, these waters become biogeographically distinct from one another along with adjacent deep benthic water environments (Ekman, 1953; Hedgepeth 1957; Briggs, 1974; Spalding *et al.*, 2007). The 12 realms are known as the Arctic, Temperate Northern Atlantic, Tropical Atlantic, Western Indo-Pacific, Central Indo-Pacific, Eastern Indo-Pacific, Tropical Eastern Pacific, Temperate South America, Temperate Southern Africa, Temperate Australasia and the Southern Ocean, which in total include 62 provinces along with 232 ecoregions worldwide (Spalding *et al.*, 2007).

The biogeographical realm of Temperate Southern Africa is divided into two diverse provinces known as the Agulhas and Benguela provinces (*sensu* Spalding *et al.*, 2007). The Agulhas Current dominates the Agulhas province as a western boundary current, flowing south along Africa's eastern coastline towards Cape Agulhas (Briggs, 1995; Van As *et al.*, 2012). The Benguela province, on the other hand, is dominated by the Benguela Current, flowing from Cape Agulhas northwards along the western coastline of South Africa up to Angola (Briggs, 1995; Van As *et al.*, 2012). This creates a unique and extremely biodiverse marine ecosystem (Branch *et al.*, 2016), since the Agulhas province is subject to warm, fast-flowing water, whilst the Benguela province is subject to cold slow-flowing water (Briggs, 1995). Consequently, the ocean basins surrounding southern Africa are considered a biodiversity hotspot for a large variety of marine organisms, including cartilaginous fishes of the class Chondrichthyes (Weigmann, 2016). The majority of chondrichthyans are defined to either biogeographical province, with 175 species inhabiting waters of the Agulhas province and 96 species inhabiting waters of the Benguela province (Ebert & van Hees, 2015). Additionally, the minority of the Southern African chondrichthyan diversity (i.e. 62 species) share an expanded distribution over both provinces (Ebert & van Hees, 2015).

Throughout the world, chondrichthyans are considered the most successful fishes in terms of historical endurance (Grogan & Lund, 2004). These fishes arose approximately 455 million years ago, classifying them amongst some of the oldest and most ecologically, biodiverse vertebrate lineages (Kriwet *et al.*, 2008; Boisvert *et al.*, 2019). The class Chondrichthyes is described as a monophyletic group, composed of the collection of two sister taxa. These taxa are comprised of the two subclasses Elasmobranchii and Holocephali, including sharks, rays and skates, and chimaeras, respectively (Grogan & Lund, 2004). According to Ebert and van Hees (2015), elasmobranchs inhabiting the ocean basins of southern Africa are well documented accounting for 18 % (204 of 1,154 species) of the world's known elasmobranch fauna.

Elasmobranchs and other fishes offer a wide array of habitats within and on their bodies, which are often occupied by other organisms such as parasites. Parasites, although often remaining unnoticed, are crucial in fulfilling key roles in their marine ecosystems, modulating host communities (Selbach *et al.*, 2022). Parasites are able to alter the activity and behaviour of grazers within the marine environment, consequently influencing the complexity of marine food webs, and ultimately the community structure (i.e., diversity and population density) of free-living invertebrates (Mouritsen & Poulin, 2005; Dunne *et al.*, 2013; Poulin *et al.*, 2016).

This is in consequence of either trait mediated effects or direct impacts on host survival during natural and anthropogenic pressures. Furthermore, parasites offer fundamental contributions to applied marine ecology acting either as indicator organisms for marine pollution and ecotoxicology or as biological tags of commercial fish stocks (Jankovská *et al.*, 2011; Nhi *et al.*, 2013; Poulin & Kamiya, 2014; Sures *et al.*, 2017). Therefore, parasites represent beneficial contributors that regulate the overall health of this entire interconnected system (Nhi *et al.*, 2013; Selbach *et al.*, 2022). Complex natural ecosystems result from multiple species interactions, including the co-evolution between host and parasites, ultimately providing researchers with vital ecological information regarding relevant host biology, and environmental requirements (Nhi *et al.*, 2013; Selbach *et al.*, 2022).

Several groups of protozoan and metazoan parasites infect chondrichthyan fishes, living either temporarily or permanently in close association with their hosts (Caira & Healy, 2004; Schaeffner & Smit, 2019). These parasites do not typically occupy one specific organ system, and not all organ sites are equally parasitised (Caira & Healy, 2004). There are typically six phyla of parasitic invertebrates infecting elasmobranchs, namely: Mollusca; Acanthocephala; Annelida; Nematoda; Arthropoda; and Platyhelminthes (Caira & Healy, 2004). It is, however, generally accepted that these parasites are characterised as either ectoparasites or endoparasites. Ectoparasites, inhabiting exterior body parts of their host, most commonly include copepods, monogeneans, leeches, arthropods and molluscs (Novelo *et al.*, 2018), whereas endoparasites, inhabiting interior

cavities, organs, ducts, and musculature of their hosts, commonly include acanthocephalans (with exceptions), nematodes, and mainly platyhelminths (Novelo *et al.*, 2018; Caira & Healy, 2004). Platyhelminthes, however, overshadows all other parasitic phyla known to parasitise elasmobranchs. The class Cestoda, for instance, offers an extremely rich diversity found within the spiral intestines of their hosts, vastly exceeding that of all other metazoan parasite groups (Caira & Healy, 2004).

Many elasmobranch species have been examined for intestinal parasites, yielding a diverse and rich cestode fauna (Reyda & Caira, 2006). Over the past two decades researchers have gained a remarkable new perspective on our understanding of elasmobranch-hosted cestodes (Caira *et al.*, 2017; Caira *et al.*, 2018). Cestodes of elasmobranchs are now known to constitute nine of the nineteen cestode orders (Caira & Jensen, 2014). After recent studies by Caira *et al.* (2018), Coleman *et al.* (2019), Palm *et al.* (2020) and Caira *et al.* (2021), a total of 203 genera are now recognised across these nine cestode orders. However, according to Schaeffner and Smit (2019), very little information of these parasites has been documented in South Africa, and reports of the parasitic species reported are solely based on opportunistic findings.

Almost all cestode orders are known to reside in the spiral intestines of the host species and are known to exhibit a spectacular variety of shapes and forms (Caira & Jensen, 2014). The disparate morphological forms exhibited by these cestodes has historically been used to group cestodes in higher-level classifications, resulting in major revisions, specifically concerning the order Onchoproteocephalidea (Caira *et al.*, 2014). Interestingly, prior to 2008, due to their hook-bearing nature, members of this order were historically believed to belong to the order Tetraphyllidea, in the family Onchobothriidae, which consisted of 17 valid genera (Caira *et al.*, 2014). General morphological characteristics such as pronounced segmentation, along with an armed scolex, consisting of four bothridia were used by Braun (1900) to assign members to this family (in Caira *et al.*, 2017). Since then, the characterisation of members within this family conflicted with Braun's descriptions, fieldwork expanded, leading to substantial revisions, consequently resulting in the erection of two new tetraphyllidean orders namely, Onchoproteocephalidea and Phyllobothriidea (Caira *et al.*, 2014). Additionally, members of the order Proteocephalidea were also designated to the new order Onchoproteocephalidea along with the members historically classified in the family Onchobothriidae, therefore recognising their hook bearing nature (Caira *et al.*, 2014).

The order Onchoproteocephalidea is the only order known to parasitise elasmobranchs along with various other vertebrate groups, including freshwater fishes, mammals, amphibians, lizards and snakes (Caira & Jensen, 2014). In light of this, the order Onchoproteocephalidea is further divided into two separate groups namely Onchoproteocephalidea I (Caira *et al.*, 2014) and Onchoproteocephalidea II (Caira *et al.*, 2017), facilitating the analysis between parasite biology,

distribution, host associations and diversity. Onchoproteocephalidea I comprise all genera infecting freshwater vertebrate groups, whereas Onchoproteocephalidea II comprise all genera infecting elasmobranchs (de Chambrier *et al.*, 2017; Caira *et al.*, 2017).

To date, the order Onchoproteocephalidea II consists of 12 valid genera parasitising elasmobranchs, with a global fauna estimated to be greater than 1,150 species (Caira *et al.*, 2017). Regarding the basic morphology, members of this order can generally be characterised by a scolex, consisting of four bothridia. Each bothridium has either one, two or three facial loculi (Caira *et al.*, 2017). Additionally, an anterior modification (i.e. a muscular pad) can be seen on each bothridium, with either a presence or absence of an accessory sucker (Caira *et al.*, 2017). Each bothridium contains a single pair of hooks (with exception to *Prosobothrium* Cohn, 1902) constituent to a specific genus, and are either bi- or tri-pronged (Caira *et al.*, 2017). The cephalic peduncle is covered with gladiate spinitriches, extending from the scolex throughout the length of the strobila (Caira *et al.*, 2017). Furthermore, members of this group are known to exhibit oioxenous host specificity, meaning that these parasites infect merely one exclusive elasmobranch host species (Euzet, 1954; Williams, 1964; Caira *et al.*, 2018). Members of this group are, however, hosted by a wide range of elasmobranch groups. They have been reported in four of the seven orders of sharks, being particularly dominant in carcharhiniform, heterodontiform and squaliform sharks. In addition, they are known from all but four of the 25 batoid families (Caira *et al.*, 2017). Furthermore, onchoproteocephalidean cestodes essentially have a cosmopolitan distribution, however records suggest that the majority of species predominantly infect elasmobranchs from tropical and subtropical waters (Caira & Jensen, 2014). According to studies by Caira *et al.* (2017), Baer and Euzet (1962), and Manger (1972), these cestodes are known from all twelve of Spalding *et al.*'s (2007) global marine realms.

According to Poulin and Morand (2000), parasite biodiversity constitutes 50 % of all species on Earth. Moreover, cestodes consist of more than half of the global diversity of all elasmobranch parasites (Randhawa & Poulin, 2020). Unfortunately, however, adequate research on cestode taxa along with their elasmobranch hosts is lacking, leaving multiple gaps in biological and taxonomic research, altering our comprehension on their unique and integral host-parasite system (Poulin *et al.*, 2016; Zaragoza-Tapia *et al.*, 2020). Consequently, parasitological observations have in many parts of the world gone greatly unexplored. This is grounded upon the fact that only 40 % of the global elasmobranch populations have been inspected for cestode infections (Caira *et al.*, 2017), leaving the majority of these cestodes and their host-associations unknown to science.

One region that potentially still hosts many undescribed onchoproteocephalidean species is the extremely biodiverse biogeographical realm of Temperate Southern Africa. Many hosts and

localities haven't been properly explored, for instance in waters off the Western Cape and KwaZulu-Natal Provinces of South Africa. Prior to this study there have been no species of onchoproteocephalidean cestodes reported from South Africa (Caira *et al.*, 2020). The diversity of cestodes in this region is hypothetically either equal to, or larger than the diversity of elasmobranch hosts (Schaeffner & Smit 2019). In South Africa, more than 200 species of elasmobranchs were recorded; however, only 18 of these species have been examined for cestode infections and only 31 cestode species have been discovered and described (Schaeffner & Smit, 2019; Oosthuizen *et al.*, 2021; Van Der Spuy *et al.*, 2020; 2022). This is particularly alarming, given the fact that the current and growing anthropogenic pressures placed on the world's marine ecosystems could result in an extensive loss of biodiversity, which limits the time to obtain specimens to be identified and classified. When accounting all other remaining cestode species parasitising elasmobranchs, the loss of parasite diversity might be even higher than that of their hosts (Dobson *et al.*, 2008). The potential loss of these cestodes is particularly daunting, since it has recently become obvious that host and parasite interactions play an imperative role in ecological function and structure, with significant impacts for the biodiversity of a specific region (Gomez & Nichols, 2013). The presence of parasites may even contribute to ecosystem conservation efforts, in the sense that they can be used as indicator organisms (Sasal *et al.*, 2007).

Unfortunately, the ongoing, extensive anthropogenic pressures on our ecosystems are leading to a massive decline in host species. Accordingly, researchers will never understand the natural history, phylogeny, host and parasite ecology, or essential dynamics contributing to multi-host life cycles and host specificity if these parasites become extinct along with their hosts (Gomez *et al.*, 2012). The lack in knowledge of parasites infecting elasmobranchs, as well as the specific services they provide, is startling and needs to be considered if conservation of these organisms is anticipated.

With this in mind, the present study was initiated in order to gain more knowledge on the biodiversity of onchoproteocephalidean cestodes infecting elasmobranchs off the coast of South Africa. This study was conducted implementing morphological methods and proposes the incorporation of novel and multisource approaches of interdisciplinary fields such as ecology, conservation science and ecotoxicology for future research.

1.1. Hypothesis

The general hypothesis of this study is that focussed research on the cestodes of selected elasmobranch species should uncover new and previously undetected species of onchoproteocephalidean cestodes infecting elasmobranchs in South African marine waters.

1.2. Aims of the study

The aim of this study was to determine the species diversity of onchoproteocephalidean cestodes infecting elasmobranchs from both ocean basins surrounding South Africa.

1.3. Objectives of the study

In order to achieve the above-mentioned research aim, the objectives of this study were to:

1. investigate the onchoproteocephalidean cestode diversity using morphological methods;
2. investigate host-parasite interrelationships, between elasmobranchs and onchoproteocephalidean, in the Western Cape and KwaZulu Natal provinces;
3. and to compare the onchoproteocephalidean cestode fauna to other zoogeographical regions.

1.4. Dissertation outline

The current chapter (Chapter 1) is a general introduction to the present study with the aim to provide the reader with a brief overview and background of the research study, as well as the problem statement, a research hypothesis, specific aim and objectives of this study. All the protocols followed for this research were adapted from that set forth by Ghoshroy and Caira (2001) and, in order to reduce repetition of the protocols used, are not discussed in a separate materials and methods chapter but rather in each of the three different results chapters. Chapters 2, 3 and 4 are constituent result chapters. Each of these three chapters provide the reader with a summary of the work done in that chapter (abstract), introduction, materials and methods, morphological descriptions of new onchoproteocephalidean taxa, and a discussion. Chapter 5 forms the concluding chapter of the thesis and provides the reader with recommendations for future studies. Both Chapters 2 and 3 have already been published in international peer reviewed journals and have been inserted in the thesis in their respective published formats, including specific journal editing requirements (see Appendix 1 and 2 for instructions to authors for the respective journals). The unpublished chapters 1, 4 and 5 follow prescribed NWU Harvard style of referencing. A reference list is provided for each chapter.

References

- Baer, J.G. & Euzet, L. 1962. Revision critique des cestodes tétraphyllides décrits par T. Southwell (1re partie). *Bulletin de la Société Neuchâteloise des Sciences Naturelles*, 85: 143–172.
- Boisvert, C., Johnston, P., Trinajstić, K. & Johanson, Z. 2019. Chondrichthyan evolution, diversity, and senses: Anatomical, functional, and developmental diversity in chordate evolution. *Heads, Jaws, and Muscles*, pp. 65–91.
- Branch, G., Griffiths C.L., Branch M.L. & Beckley L.E. 2016. *Two Oceans: A Guide to the Marine Life of Southern Africa*, Vol 4. Struik Nature, Cape Town, pp. 464.
- Briggs, J.C. 1974. *Marine Zoogeography*. New York: McGraw-Hill. 1995. *Global Biogeography*. Amsterdam: Elsevier.
- Briggs, J.C. 1995. *Global biogeography*, Vol 14. *Developments in Palaeontology and Stratigraphy*. Elsevier, Amsterdam, pp. 451.
- Caira, J.N. & Healy, C.J. 2004. In: Carrier, J.C., Musick, J.A. & Heithaus, M.R. (Eds.), *Biology of sharks and their relatives*. CRC press: Boca Raton, London, New York.
- Caira, J.N. & Jensen, K. 2014. A digest of elasmobranch tapeworms. *Journal of Parasitology*, 100: 373–391.
- Caira, J.N., Jensen, K. & Barbeau, E. (Eds.). 2020. *Global Cestode Database*. World Wide Web electronic publication. Accessed at: www.tapewormdb.uconn.edu. Date accessed: 13 Nov. 2020.
- Caira, J.N., Jensen, K. & Fyler, C.A. 2018. A new genus of tapeworm (Cestoda: Onchoproteocephalidea) from sawfish (Elasmobranchii: Pristidae). *Journal of Parasitology*, 104: 133–144.
- Caira, J.N., Jensen, K. & Ivanov, V.A. 2017. Onchoproteocephalidea II. In: J.N. Caira and K. Jensen (Eds.), *Planetary Biodiversity Inventory (2008–2017): Tapeworms from Vertebrate Bowels of the Earth*. The University of Kansas Natural History Museum. Special Publication no. 25, pp. 279–304.

Caira, J.N., Jensen, K., Waeschenbach, A., Olson, P.D. & Littlewood, D.T.J. 2014. Orders out of chaos – molecular phylogenetics reveals the complexity of shark and stingray tapeworm relationships. *International Journal for Parasitology*, 44: 55–73.

Carrier, J.C., Musick, J.A. & Heithaus, M.R. 2004. Biology of sharks and their relatives. CRC press: Boca Raton, London, New York.

Cavicchioli, R., Ripple, W.J., Timmis, K.N., Azam, F., Bakken, L.R., Baylis, M., Behrenfeld, M.J., Boetius, A., Boyd, P.W., Classen, A.T., Crowther, T.W., Danovaro, R., Foreman, C.M., Huisman, J., Hutchins, D.H., Jansson, J.K., Karl, D.M., Koskella, B., Welch, D.B.M., Martiny, D.B.H., Moran, M.A., Orphan, V.J., Reay, D.S., Remais, J.V., Rich, V.I., Singh, B.K., Stein, L.Y., Stewart, F.J., Sullivan, M.B., van Oppen, M.J.H., Weaver, S.C., Webb, E.A. & Webster, N.S. 2019. Scientists' warning to humanity: microorganisms and climate change. *Nature Reviews Microbiology*, 17: 569–586.

Coleman, G.M., Beveridge, I. & Campbell, R.A. New genera, species and records of rhinebothriidean cestodes (Platyhelminthes) parasitic in Australian stingrays (Elasmobranchii: Batoidea). *Systematic Parasitology*, 96: 347–368.

De Chambrier A., Scholz, T., Mariaux, J. & Kuchta, R. 2017: Onchoproteocephalidea I Caira, Jensen, Waeschenbach, Olson & Littlewood, 2014. In: J.N. Caira and K. Jensen (Eds.), Planetary Biodiversity Inventory (2008–2017): Tapeworms from Vertebrate Bowels of the Earth. University of Kansas, Natural History Museum, Special Publication No. 25, Lawrence, KS, USA, pp. 251–277.

Diaz, S., Fargione, J., Chapin, F.S. & Tilman, D. 2006. Biodiversity loss threatens human well-being. *PLOS Biology*, 4: 1300–1305.

Dobson, A., Lafferty, K.D., Kuris, A.M., Hechinger, R.F. & Jet, W. 2008. Homage to Linnaeus: How many parasites? How many hosts? *Proceedings of the National Academy of Sciences of the United States of America*, 105: 11482–11489.

Dunne, J.A., Lafferty, K.D., Dobson, A.P., Hechinger, R.F., Kuris, A.M., Martinez, N.D., McLaughlin, J.P., Mouritsen, K.N., Poulin, R., Reise, K., Stouffer, D.B., Thieltges, D.W., Ebert, D. & van Hees, K.E. 2015. Beyond jaws: rediscovering the 'lost sharks' of southern Africa. *African Journal of Marine Science*, 37: 141–156.

Ekman, S. 1953. Zoogeography of the Sea. London: Sidgwick and Jackson. *Annals and Magazine of Natural History*, 6: 63, 240.

Euzet, L. 1954. Parasites de poissons de mer ouest africains récoltés par J. Cadenat. I.—Cestodes tetraphyllides de sélaciens. (Note préliminaire). *Bulletin de l'Institut Français d'Afrique Noire*, A16: 126–138.

Ghoshroy, S. & Caira, J.N. 2001. Four new species of *Acanthobothrium* (Cestoda: Tetraphyllidea) from the whiptail stingray *Dasyatis brevis* in the Gulf of California. *Journal of Parasitology*, 87: 354–372.

Gomez, A. & Nichols, E. 2013. Neglected wild life: parasitic biodiversity as a conservation target. *International Journal for Parasitology*, 2: 222–227.

Gomez, A., Nichols, E.S. & Perkins, S. 2012. Parasite conservation, conservation medicine, and ecosystem health. Conservation influences in coral-reef lagoons: a case study of Apogonidae parasites in New-Caledonia. *Marine Pollution Bulletin*, 54: 1697–1706.

Grogan, E.D. & Lund, R. 2004. In: Carrier, J.C., Musick, J.A. & Heithaus, M.R. (Eds.), *Biology of sharks and their relatives*. CRC press: Boca Raton, London, New York.

Hedgepeth, J.W. 1957. Marine biogeography. *Memoir of the Geological Society of America*, 67: 359–382.

Jackson, J.B.C. 2010. The future of the ocean's past. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 365: 3765–3778. Medicine: Applied Cases of Ecological Health. Oxford University Press; New York: 67–81.

Jankovská, I., Miholová, D., Petrtyl, I.M., Romocuský, S., Kalous, L., Vadlejch, J., Cadková, Z. & Langrová, I. 2011. The intestinal parasite *Acanthocephalus lucii* (Acanthocephala) from European perch (*Perca fluviatilis*) as a bioindicator for lead pollution in the stream “Jevanský potok” near Prague, Czech Republic. *Bulletin of Environmental Contamination and Toxicology*, 86: 342–346.

Kriwet, J., Witzmann, F., Klug, S. & Heidtke, U.H.J. 2008. First direct evidence of a vertebrate three-level trophic chain in the fossil record. *Proceedings of the Royal Society B-Biological Sciences*, 275: 181–186.

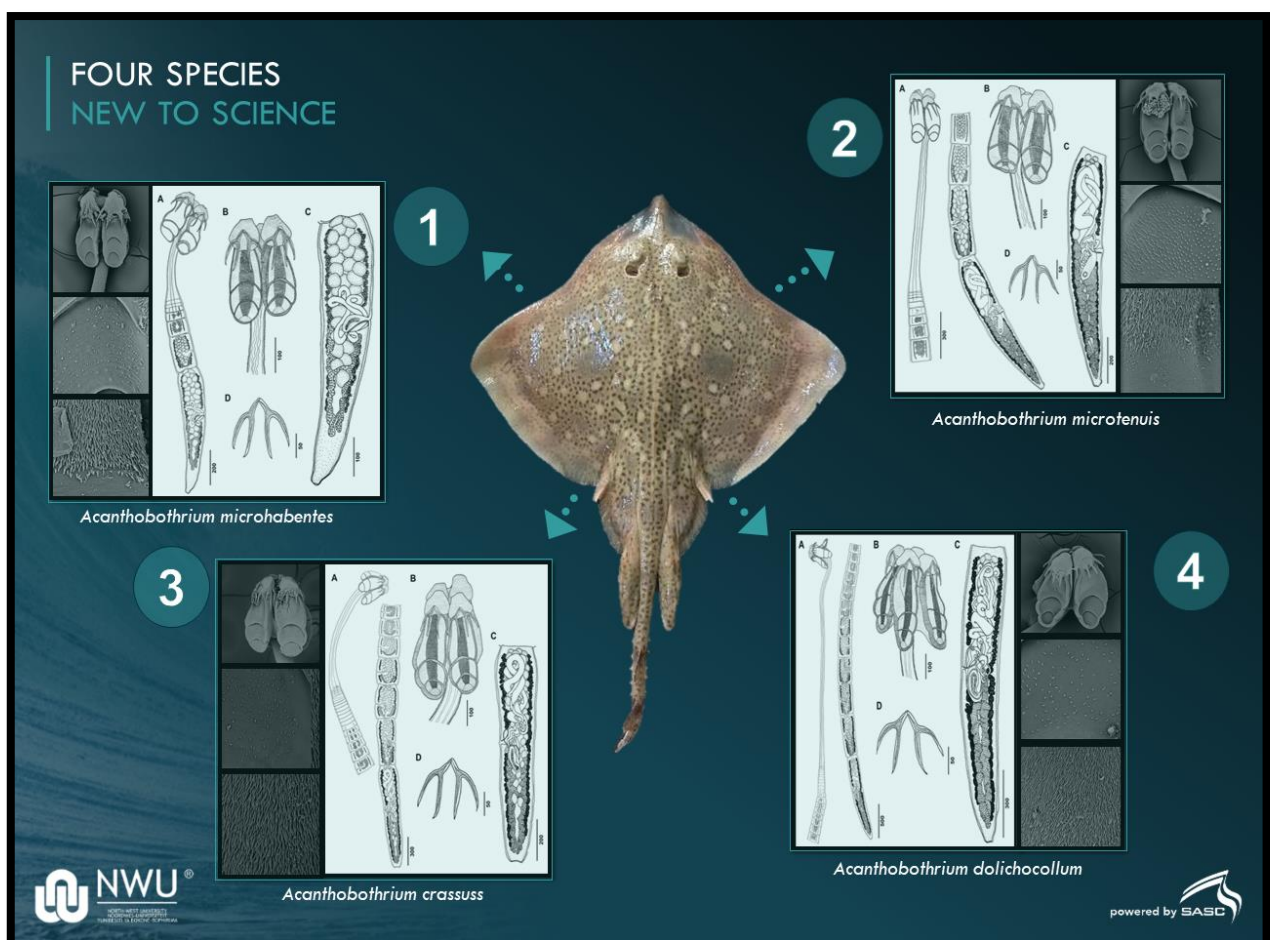
- Manger, B.R. 1972. Some cestode parasites of the elasmobranchs *Raja batis* and *Squalus acanthias* from Iceland. *Bulletin of the British Museum of Natural History (Zoology)*, 24: 161–181.
- Mouritsen, K.N. & Poulin, R. 2005. Parasites boost biodiversity and change animal community structure by trait-mediated indirect effects. *Oikos*, 108: 344–350.
- Nhi, T.T.Y., Shazili, N.A.M. & Harrison, F.S. 2013. Use of cestodes as indicator of heavy-metal pollution. *Experimental Parasitology*, 133: 75–79.
- Novelo, J.F.E., Escribano, R. & Oliva, M.E. 2018. Metazoan parasite communities of two deep-sea elasmobranchs: the southern lanternshark, *Etmopterus granulosus*, and the largenose catshark, *Aspristurus nasutus*, in the Southeastern Pacific Ocean. *Parasite*, 25: 53.
- Oosthuizen, G., Acosta, A.A., Smit, N.J. & Schaeffner, B.C. 2021. A new species of *Grillotia* Guiart, 1927 (Cestoda: Trypanorhyncha) from the spotted skate, *Raja straeleni* Poll, in South Africa. *Parasitology International*, 82: 102307.
- Poulin, R. & Kamiya, T. 2014. Parasites as biological tags of fish stocks: a meta-analysis of their discriminatory power. *Parasitology*, 145: 145–155.
- Poulin, R. & Morand, S. 2000. The diversity of parasites. *The Quarterly Review of Biology*, 75: 277–293.
- Poulin, R., Blasco-Costa, I. & Randhawa, H.S. 2016. Integrating parasitology and marine ecology: Seven challenges towards greater synergy. *Journal of Sea Research*, 133: 3–10.
- Randhawa, H.S. & Poulin, R. 2020. Tapeworm discovery in elasmobranch fishes: quantifying patterns and identifying their correlates. *Marine Freshwater Research*, 71: 78–88.
- Reyda, F.B. & Caira, J.N. 2006. Five new species of *Acanthobothrium* (Cestoda: Tetraphyllidea) from *Himantura uarnacoides* (Myliobatiformes: Dasyatidae) in Malaysian Borneo. *Comparative Parasitology*, 73: 49–71.
- Sasal, P., Mouillot, D., Fichez, R., Chifflet, S. & Kulbicki, M. 2007. The use of fish parasites as biological indicators of anthropogenic influences in coral-reef lagoons: A case study of Apogonidae parasites in New-Caledonia. *Marine Pollution Bulletin*, 54: 1697–1706.

- Schaeffner, B.C. & Smit, N.J. 2019. Parasites of cartilaginous fishes (Chondrichthyes) in South Africa - a neglected field of marine science. *Folia Parasitologica*, 66: 002.
- Selbach, C., Mouritsen, K.N., Poulin, R., Sures, B. & Smit, N.J. 2022. Bridging the gap: aquatic parasites in the One Health concept. *Trends in Parasitology*, 38: 109–111.
- Spalding, M.D., Fox, H.E., Allen, G.R., Davidson, N., Ferdaña, Z.A., Finlayson, M., Halpern, B.S., Jorge, M.A., Lombana, A., Lourie, S.A., Martin, K.D., McManus, E., Molnar, J., Recchia, C.A. & Robertson, J. 2007. Marine ecoregions of the world: a bioregionalization of coastal and shelf areas. *Bioscience*, 57: 573–583.
- Sures, B., Nachev, M., Selbach, C. & Marcogliese, D.J. 2017. Parasite responses to pollution: what we know and where we go in 'Environmental Parasitology. *Parasites and Vectors*, 10: 65.
- Van As, J., Du Preez, J., Brown, L. & Smit, N. 2012. The Story of Life and the Environment, an African Perspective. Struik Nature, Cape Town, pp. 456.
- Van Der Spuy, L., Smit, N.J. & Schaeffner, B.C. 2020. Four new species of *Acanthobothrium* van Beneden, 1849 (Cestoda: Onchoproteocephalidea) from the spotted skate, *Raja straeleni* Poll, off the Western Cape, South Africa. *Folia Parasitologica*, 67: 036.
- Van Der Spuy, L., Smit, N.J. & Schaeffner, B.C. 2022. Threatened, host-specific affiliates of a red-listed host: Three new species of *Acanthobothrium* van Beneden, 1849 (Cestoda: Onchoproteocephalidea) from the endangered white skate, *Rostroraja alba* (Lacépède). *International Journal for Parasitology: Parasites and Wildlife*, 17: 114–126.
- Weigmann, S. 2016. Annotated checklist of the living sharks, batoids and chimaeras (Chondrichthyes) of the world, with a focus on biogeographical diversity. *Journal of Fish Biology*, 88: 837–1037.
- Williams, H.H. 1964. Some new and little known cestodes from Australian elasmobranchs with a brief discussion on their possible use in problems of host taxonomy. *Parasitology*, 54: 737–748.
- Williams, R.J. & Zander, C.D. 2013. Parasites affect food web structure primarily through increased diversity and complexity. *PLOS Biology*, 11: e1001579.

Zaragoza-Tapia, F., Pulido-Flores, G., Gardner, S.L. & Monks, S. 2020. Host relationships and geographic distribution of species of *Acanthobothrium* Blanchard, 1848 (Onchoproteocephalidea, Onchobothriidae) in elasmobranchs: a metadata analysis. *ZooKeys*, 940: 1–49.

CHAPTER 2:

Four new species of *Acanthobothrium* van Beneden, 1849 (Cestoda: Onchoproteocephalidea) from the spotted skate, *Raja straeleni* Poll, off the Western Cape, South Africa



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Four new species of *Acanthobothrium* van Beneden, 1848 (Cestoda: Onchoproteocephalidea) from the spotted skate, *Raja straeleni* Poll, off the Western Cape, South Africa

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2.1. Abstract

The examination of eight spotted skates, *Raja straeleni* Poll, resulted in the discovery of four new species of *Acanthobothrium* van Beneden, 1848, namely *A. microhabentes* Van Der Spuy, Smit et Schaeffner, 2020, *A. microtenuis* Van Der Spuy, Smit et Schaeffner, 2020, *A. crassus* Van Der Spuy, Smit et Schaeffner, 2020, and *A. dolichocollum* Van Der Spuy, Smit et Schaeffner, 2020, located off the Western Cape of South Africa. With a total of over 200 valid species of *Acanthobothrium* recognised worldwide, the use of an integrative approach becomes imperative in the interest of simplifying interspecific comparisons between congeners. In accordance with this, the four new species were incorporated into the category classification system established by Ghoshroy and Caira in 2001, where they were identified as category 2 species, which, at present, includes 47 recognised species of *Acanthobothrium*. Nevertheless, each of the four new species exhibits postovarian testes, a most intriguing and highly unusual feature among *Acanthobothrium*, instantly differentiating them from most congeners. This feature has been reported in 12 congeners, which have previously been considered to be restricted to waters of the Indo-Pacific Ocean. Not only do the four new congeners represent the first species of *Acanthobothrium* reported from southern Africa, but they also represent the first reported species with postovarian testes from the southern Atlantic Ocean. Regarding the legitimacy of the four new species, only two other category 2 species are reported to exhibit this feature, namely *A. popi* Fyler, Caira et Jensen, 2009, and *A. bobconniorum* Fyler et Caira, 2010, to which the four congeners were compared. *Acanthobothrium microhabentes* is the smallest of the congeners and differs from *A. popi* and *A. bobconniorum* by having fewer testes and postovarian testes, a shorter body, fewer proglottids, a shorter scolex, and longer cephalic peduncle. *Acanthobothrium microtenuis* differs from *A. popi* and *A. bobconniorum* by having fewer testes and postovarian

testes, a shorter scolex, longer cephalic peduncle, and the possession of columnar spinitriches on the anterior region of the terminal proglottid. *Acanthobothrium crassus* differs from *A. popi* and *A. bobconniorum* by having fewer postovarian testes, a narrower cirrus-sac, larger vitelline follicles, and a longer cephalic peduncle. *Acanthobothrium dolichocollum* is the longest of the four new species and differs from *A. popi* and *A. bobconniorum* by having fewer postovarian testes, more postporal testes, a larger body, more proglottids, larger testes and vitelline follicles, and an exceptionally long cephalic peduncle. Apart from differences in overall size, the four new species differ in a combination of measurements for the scolex, vitelline follicles, muscular pad and cephalic peduncle, and the number of proglottids and testes. The four species were recovered from a previously unexplored host and locality, expanding the host associations and geographical distribution of the genus.

Keywords: Marine fish parasites, elasmobranchs, tapeworms, taxonomy, biodiversity, new species

2.2. Introduction

Over the past few decades, researchers have gained remarkable new perspectives on our understanding of the vast number of cestodes infecting elasmobranchs across the world's oceans (Caira and Jensen 2014, 2017). Of the nine cestode orders known to parasitise elasmobranchs, members of the Onchoproteocephalidea Caira, Jensen, Waeschenback, Olsen et Littlewood, 2014 are the only tapeworms known to parasitise other vertebrate groups such as bony fishes, reptiles and amphibians (Onchoproteocephalidea I) (de Chambrier et al. 2017) as well as elasmobranchs (Onchoproteocephalidea II) (Caira and Jensen 2014, 2017).

To date, the Onchoproteocephalidea II consists of 12 valid genera, with an estimated diversity of more than 1,154 species (Caira and Jensen 2017, Caira et al. 2018). Members of this order are known to exhibit an oioxenous host specificity, with each species infecting a single definitive host species (Euzet 1954, Williams 1964, Caira et al. 2018). They are, however, hosted by a wide range of elasmobranch groups, with the majority of species described to parasitise stingrays (Campbell and Beveridge 2002, Ivanov 2005, Fyler 2011, Maleki et al. 2015). Members of the Onchoproteocephalidea II are known from all but four of the 25 batoid families (Caira et al. 2017), and have been reported in four of the seven orders of sharks. Species parasitising sharks typically occur in carcharhiniform, heterodontiform and squaliform sharks (Caira et al. 2017).

Although having a cosmopolitan distribution, the majority of records of onchoproteocephalideans from elasmobranchs were derived from species of *Acanthobothrium* van Beneden, 1848 in

tropical and subtropical waters (Caira and Jensen 2014). *Acanthobothrium* is the species-richest genus of all elasmobranch-infecting cestode genera (Randhawa and Poulin 2010, Caira and Jensen 2017). The genus is characterised by a scolex with four bothridia, each containing one pair of bi-pronged hooks.

Despite the large number of valid species of the genus, it is estimated that an additional 800, if not more, species of *Acanthobothrium* are yet to be discovered worldwide (Caira et al. 2017). One region that potentially hosts many undescribed species of *Acanthobothrium* is the extremely biodiverse, biogeographical realm of Temperate southern Africa (*sensu* Spalding et al. 2007). The diversity, biogeography and biology of elasmobranchs off southern Africa have been well documented, with 204 elasmobranch species reported from this region (Ebert and van Hees 2015). However, only 18 of these species have been examined for cestode infections (Schaeffner and Smit 2019).

As part of a larger study on the biodiversity of marine fish parasites of southern Africa, specimens of *Acanthobothrium* were collected from *Raja straeleni* Poll (Rajidae) off the Western Cape of South Africa. This study provides information on the taxonomic status of species belonging to the genus *Acanthobothrium*, based on light and scanning electron microscopy, leading to the description of four species new to science.

2.3. Materials and Methods

Eight specimens of *Raja straeleni*, seven males and one female, were collected by longline. One specimen was collected during October 2018 from Vermont (-34.4927, 19.4136), whereas the other seven specimens were collected in February 2019 from Walker Bay, Hermanus (-34.5283, 19.4102), Hawston (-34.4419, 19.3697), and De Kelders (-34.5925, 19.2925). Figure 1 indicates the sampling localities from which each species was obtained. Ethical approval for this project was received from the North-West University Animal Care, Health and Safety, Research Ethics Committee (NWU-AnimCareREC) with ethics number NWU-00065-19-A5. Permits for the collection and possession of batoid specimens for the purpose of research were issued by the South African Department of Agriculture, Forestry and Fisheries (permit nos. RES2019/45 and RES2019/105 issued to BCS; RES2019/58 and RES2019/61 issued to the South African Shark Conservancy).

Skates were sacrificed by means of an adjunctive procedure of trauma to the neurocranium, followed immediately by pithing of the brain. The body cavity was opened with a mid-ventral incision to remove the spiral intestine. Spiral intestines and their contents were fixed in hot, 4% formalin for morphological observations, and ultimately transferred to 70% ethanol after a period

of two weeks. Specimens of *Acanthobothrium* were hand-picked from the spiral intestines and contents. They were hydrated in a graded ethanol series and stained in Delafield's haematoxylin, dehydrated in a graded ethanol series, cleared in clove oil, and permanently mounted on microscope slides using Canada balsam. Images of various internal organs and characteristic body structures used for measurements were acquired using a Nikon Y-TV55 video camera mounted on a Nikon ECLIPSE Ni microscope (Nikon, Tokyo, Japan). Image analysis software Image Pro Express (Nikon, Japan) was used to obtain all necessary measurements for descriptive analyses.

All measurements were taken according to specifications given by Ghoshroy and Caira (2001). Measurements are given both in text descriptions as well as in tabular form. Text descriptions include the range of measurements taken, whereas Table 1 presents metrical data as the mean, followed by standard deviation and number of specimens examined. Measurements are all presented in micrometres, with the exception of the total length, which is presented in millimetres. Drawings were made by the use of a drawing attachment tube.

Two to three specimens of each species were used for scanning electron microscopy (SEM). By use of a liner brush, each specimen selected for SEM observation was cleaned by lightly brushing the surface of the scolex. Cleaned specimens were then dehydrated in a graded ethanol series and either placed in hexamethyldisilane (HMDS) overnight, or dried by critical point drying. Specimens were mounted onto carbon tape, placed on aluminium stubs and sputter-coated with either 20 to 30 nm of gold/palladium (SPI-module, SPI Supplies, Pennsylvania, USA), or first with carbon (Emscope TB500, Quorum Technologies, Puslinch, Ontario, USA), followed by 20 to 30 nm gold/palladium (Eiko IB2 ion coater, Eiko, Japan).

Specimens were examined with a Phenom Pro Desktop scanning electron microscope (ThermoScientific, Waltham, Massachusetts, USA) and a FEI Nova NanoSEM 450 scanning electron microscope (FEI, Hillsboro, Oregon, USA). Images were taken of similar regions of the strobila and scolex, aiding in microthrix comparisons among species. Microthrix morphologies were described according to Chervy (2009). Images of microtriches of immature proglottids were taken directly posterior to the cephalic peduncle. The presence of additional microthrix morphologies on the terminal proglottid was noted in which case images are also provided. The specific regions of micrographs taken are indicated on SEM plates for each of the respective species.

Species allocation followed the category classification system developed by Ghoshroy and Caira (2001) to facilitate the differentiation among congeners. Following this classification system, species were characterised and assessed based on four morphological features: total length (< or > 15 mm), number of proglottids (< or > 50 proglottids), number of testes (< or > 80), and

symmetry or asymmetry of aporal and poral ovarian lobes. Allocating each species to a specific category consequently simplifies differentiating between congeners. Accordingly, the four new species were incorporated into this system and compared particularly to the described species assigned to the same category as each of the four new species. Since members of different categories differ from one another in either one or more of the four morphological features constituent of this classification system (Fyler and Caira 2006), this subsequently warrants the elimination of congeners designated to different categories.

Specimens have been deposited in the helminthological collection of the National Museum, Bloemfontein, South Africa (NMB), the Institute of Parasitology, Biology Centre of the Czech Academy of Sciences, České Budějovice, Czech Republic (IPCAS), and the Natural History Museum, Geneva, Switzerland (MHNG-PLAT). Specimens used for SEM were retained in the parasite collection of the Water Research Group, North-West University, South Africa.

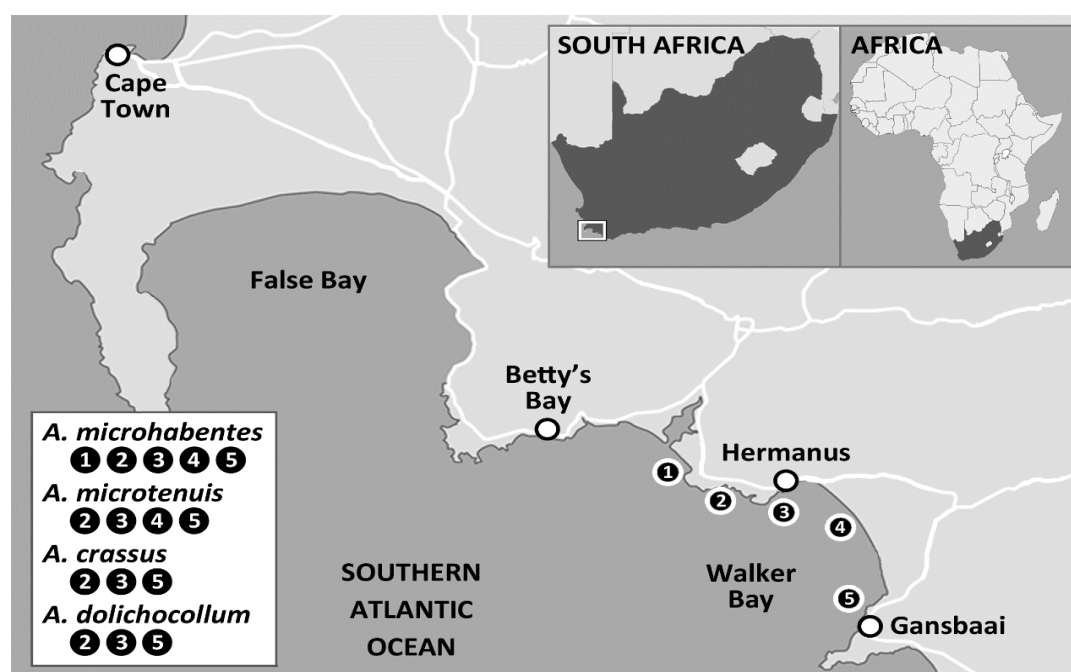


Fig. 1. Map indicating the sampling localities of species of *Acanthobothrium* van Beneden, 1849.

2.4. Results

Four morphologically distinct species of *Acanthobothrium* were collected from subsets of eight specimens of *Raja straeleni* measuring 64.9–74.2 cm in length, disc widths of 45.8–53.4 cm, and a weight of 1,820–6,630 g (Table 2). Of the eight skates, two were parasitised by all four morphotypes, two host specimens by three morphotypes, two by two morphotypes and two skates with a single morphotype. All four morphotypes were present in skates from Vermont, Walker Bay and De Kelders, but only a single morphotype was found in the skate collected at Hawston. The

only female caught during the present study was parasitised by only one of the four morphotypes (Table 2).

Table 1. Metrical information of the new species of *Acanthobothrium* van Beneden, 1849. Information is presented as the mean, followed by the standard deviation and the number of worms examined. All measurements are in micrometres, unless stated otherwise. *Abbreviations:* L – length; N– number; W – width.

Character	<i>Acanthobothrium microhabentes</i>	<i>Acanthobothrium microtenuis</i>	<i>Acanthobothrium crassus</i>	<i>Acanthobothrium dolichocollum</i>
Total length (mm)	2.13 ± 0.51; 25	3.22 ± 0.73; 14	6.62 ± 0.90; 5	9.15 ± 1.00; 6
Scolex L	303 ± 2; 25	314 ± 3; 14	446 ± 3; 5	413 ± 3; 6
Scolex W	232 ± 8; 22	261 ± 13; 14	324 ± 11; 4	353 ± 6; 6
Bothridium W	109 ± 3; 20	120 ± 6; 14	160 ± 14; 5	131 ± 3; 6
Anterior (A) loculus L	101 ± 3; 22	141 ± 8; 14	180 ± 4; 5	187 ± 10; 6
Middle (M) loculus L	47 ± 1; 22	52 ± 4; 14	82 ± 6; 5	80 ± 5; 6
Posterior (P) loculus L	51 ± 2; 22	63 ± 4; 14	81 ± 4; 5	67 ± 4; 6
Loculus L ratio (A: M: P)	1.0 : 2.2 ± 0.1 : 2.0 2.0 ± 0.1; 22	1.0 : 2.7 ± 0.3 : 2.2 2.0 ± 0.2; 14	1.0 : 2.2 ± 0.2 : 2.2 2.0 ± 0.2; 5	1.0 : 2.3 ± 0.1 : 2.80 2.0 ± 0.2; 6
Muscular pad L	70 ± 3; 23	70 ± 9; 13	95 ± 5; 5	80 ± 3; 6
Muscular pad W	104 ± 4; 21	110 ± 3; 13	105 ± 2; 5	123 ± 2; 6
Accessory sucker L	40 ± 2; 22	48 ± 2; 12	46 ± 2; 5	54 ± 2; 6
Accessory sucker W	36 ± 3; 22	41 ± 3; 12	43 ± 3; 5	49 ± 1; 6
Lateral hook A	42 ± 2; 21	46 ± 4; 14	44 ± 3; 5	41 ± 6; 4
Lateral hook B	81 ± 5; 21	85 ± 9; 14	77 ± 5; 5	72 ± 4; 4
Lateral hook C	72 ± 6; 21	77 ± 11; 14	68 ± 6; 5	61 ± 8; 4
Lateral hook D	117 ± 7; 21	122 ± 9; 14	114 ± 5; 5	105 ± 7; 4
Medial hook A'	41 ± 3; 25	47 ± 4; 14	43 ± 2; 5	45 ± 2; 4

Table 1. Continued

Medial hook B'	81 ± 5; 25	84 ± 6; 14	78 ± 7; 5	70 ± 6; 4
Medial hook C'	75 ± 6; 25	80 ± 6; 14	72 ± 5; 5	67 ± 6; 4
Medial hook D'	116 ± 6; 25	125 ± 6; 14	115 ± 8; 5	110 ± 8; 4
Cephalic peduncle L	521 ± 123; 22	1,044 ± 320; 14	1,685 ± 254; 5	2,998 ± 970; 6
Cephalic peduncle W	52 ± 3; 25	63 ± 3; 14	91 ± 2; 5	69 ± 2; 6
Proglottid N	8 ± 1; 20	13 ± 2; 9	29 ± 2; 5	37 ± 6; 6
Immature proglottid N	7 ± 1; 20	12 ± 2; 9	27 ± 2; 5	35 ± 6; 6
Mature proglottid N	1 ± 1; 20	1 ± 1; 9	2 ± 1; 5	2 ± 1; 6
Genital pore position (%)	69 ± 5; 22	61 ± 2; 11	59 ± 4; 5	60 ± 4; 6
Terminal proglottid L	770 ± 155; 22	886 ± 128; 12	1,107 ± 128; 5	1,227 ± 149; 6
Terminal proglottid W	156 ± 23; 22	163 ± 30; 12	222 ± 20; 5	234 ± 27; 6
Terminal proglottid ratio (L: W)	5 ± 1; 22	6 ± 1; 12	6 ± 2; 5	6 ± 3; 6
Cirrus-sac L	68 ± 6; 24	119 ± 5; 14	128 ± 5; 5	124 ± 2; 6
Cirrus-sac W	40 ± 4; 24	58 ± 6; 14	52 ± 3; 5	70 ± 1; 6
Testis N	27 ± 3; 23	29 ± 3; 12	43 ± 2; 5	36 ± 3; 6
Postporal testis N	5 ± 1; 20	8 ± 1; 12	9 ± 1; 5	11 ± 1; 6
Postovarian testis N	1 ± 1; 20	2 ± 1; 12	2 ± 1; 5	3 ± 1; 6
Testis L	42 ± 5; 23	54 ± 7; 14	49 ± 4; 5	57 ± 3; 6
Testis W	29 ± 4; 23	35 ± 4; 14	41 ± 3; 5	44 ± 3; 6
Poral ovarian arm L	354 ± 82; 19	413 ± 65; 11	542 ± 95; 5	589 ± 54; 6
Aporal ovarian arm L	386 ± 110; 19	477 ± 94; 11	585 ± 107; 5	600 ± 46; 6
Ovary W	68 ± 8; 20	106 ± 7; 12	109 ± 7; 5	116 ± 4; 6
Vitelline follicle L	9 ± 2; 24	16 ± 3; 14	17 ± 3; 5	20 ± 2; 6
Vitelline follicle W	16 ± 3; 24	27 ± 5; 14	35 ± 2; 5	38 ± 3; 6

Table 2. Information (sex, size, weight) on host specimens of *Raja straeleni* Poll (Rajiformes: Rajidae), including sampling sites and presence or absence of species of *Acanthobothrium* van Beneden, 1849.

Collection code	Sex	Length (cm)	Disc width (cm)	Weight (g)	Locality	<i>Acanthobothrium microhabentes</i>	<i>Acanthobothrium microtenuis</i>	<i>Acanthobothrium crassus</i>	<i>Acanthobothrium dolichocollum</i>
HE18-15	M	64.9	46.5	1,900	Vermont, SA	+	+	+	+
HE19-01	M	69.5	46.0	2,290	Hermanus, SA	+	+	-	+
HE19-02	M	68.0	48.0	2,550	Hermanus, SA	-	+	-	+
HE19-05	M	67.5	47.5	4,250	Hermanus, SA	+	+	+	-
HE19-09	F	73.1	53.4	6,630	Hermanus, SA	-	+	-	-
HE19-10	M	67.0	46.8	1,880	Hermanus, SA	+	+	-	-
HE19-11	M	74.2	48.6	2,250	Hawston, SA	+	-	-	-
HE19-12	M	68.2	45.8	1,820	De Kelders, SA	+	+	+	+

Abbreviations: + – present; - – absent.

2.4.1. *Acanthobothrium microhabentes* Van Der Spuy, Smit et Schaeffner, 2020 (Figs. 2, 3)

ZooBank number for species: urn: lsid: zoobank.org: act: 26E2521F-A19F-4DBB-B86D-7D65CED10E82

2.4.1.1. Description

Based on 25 whole mounts, 21 mature and four immature worms; three mature worms used for SEM observations: Worms 1.5–3.0 mm long; greatest width at level of scolex; 5–9 proglottids per worm; euapolytic. Scolex consisting of scolex proper and short cephalic peduncle. Scolex proper with four bothridia, 301–306 long by 215–248 wide. Bothridia free posteriorly, 105–115 wide; each with three loculi and specialised anterior region in form of muscular pad. Muscular pad 63–74 long by 97–113 wide, falciform in shape, with pronounced posterior margin, bearing accessory sucker and one pair of hooks at posterior margin; accessory sucker 32–41 long by 37–45 wide. Anterior loculus (A) 99–105 long; middle loculus (M) 42–48 long; posterior loculus (P) 48–56 long; loculus length ratio (A : M : P) 1 : 2.2 : 2; maximum width of scolex at level of middle loculus. Velum absent.

Hooks bi-pronged, hollow, with tubercle on proximal surface of axial prong; internal channels of axial and abaxial prongs continuous, smooth; axial prongs slightly longer than abaxial prongs; lateral and medial hooks approximately equal in size. Lateral hook measurements: A 38–46, B 69–90, C 61–82, D 102–128. Medial hook measurements: A' 34–45, B' 71–87, C' 62–84, D' 105–126. Bases of lateral and medial hooks approximately equal in length; base of lateral hooks slightly overlapping base of medial hooks along medial axis of bothridium (Fig. 2D); medial hook base slightly wider than lateral hook base. Thin layer of tissue covering essentially entire length of each prong of hooks. Long cephalic peduncle 352–695 long by 47–57 wide. Apical pad and distal bothridial surface covered with papilliform filitriches and sparsely interspersed gladiate spinitriches (Fig. 3C). Proximal bothridial surface and bothridial rims covered with gladiate spinitriches, interspersed with acicular filitriches (Fig. 3D). Cephalic peduncle densely covered with gladiate spinitriches (Fig. 3E). Strobila covered in papilliform filitriches (Fig. 3F).

Proglottids acraspedote. Immature proglottids 4–9 in number; one mature proglottid; gravid proglottids absent; terminal proglottid 484–1,093 long by 122–205 wide; terminal proglottid length to width ratio 3.5–7 : 1. Proglottids protandrous; genital pores marginal, irregularly alternating, 50–66% of proglottid length from posterior end.

Testes conspicuous in mature proglottids, irregularly oval in dorsoventral view, 32–55 long by 23–35 wide, arranged in two regular columns, one layer deep, 21–31 in total number, 4–7 in postporal field, 1–2 in postovarian field. Cirrus-sac ovoid, bent posteriorly, 57–80 long by 32–47 wide, containing armed cirrus; cirrus greatly expanded at base.

Vagina narrow, relatively thin-walled and straight proximally, extending from ootype along medial line of proglottid to posterior margin of cirrus-sac, then laterally to common genital atrium. Ovary takes up approximately half proglottid length, stopping short of posterior margin of proglottid, H-shaped in dorsoventral view, lobulated. Ovary asymmetrical, poral lobe 243–444 in length, aporal lobe 210–525 in length, both lobes stopping before cirrus-sac; width of ovary at level of ovarian isthmus 54–76; ovarian isthmus located slightly posterior to mid-level of poral lobe of ovary. Mehlis' gland posterior to ovarian isthmus.

Vitellarium follicular, in form of two lateral bands each consisting of two columns of relatively large follicles; bands extending from posterior margin of anterior-most testes to near posterior margin of ovary, interrupted by vagina and cirrus-sac; vitelline follicles 6–13 long by 10–24 wide. Vitelline follicle length relative to testis length 0.1–0.2 : 1. Uterus thin-walled, extending from ovarian isthmus to near anterior margin of proglottid. Eggs not observed.

2.4.1.2. Taxonomic summary

Type host: Spotted skate, *Raja straeleni* (Rajiformes: Rajidae).

Type locality: South-eastern Atlantic Ocean off De Kelders (-34.5925, 19.2925), South Africa.

Site of infection: Spiral intestine.

Prevalence of infection: 75% (six of eight skates examined).

Type material: Holotype deposited at NMB (P-604), paratypes in NMB (P-605–P-606), IPCAS (C-848) and MHNG (PLAT-137324, 137334).

Etymology: The species name “*microhabentes*” is derived from Latin (“micro”, small; “habentes”, stumpy) and relates to the small size of the specimens.

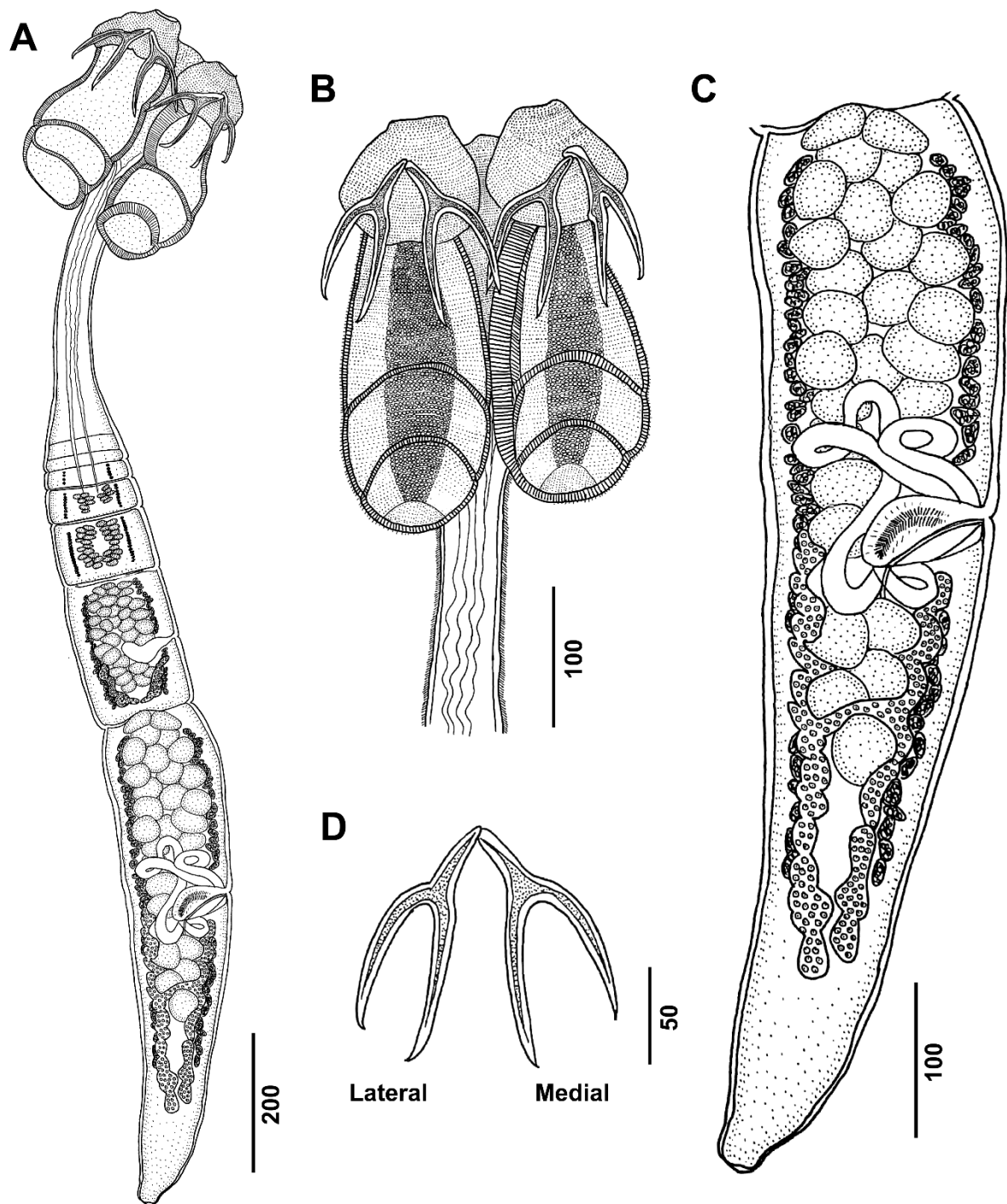


Fig. 2. Line drawing of *Acanthobothrium microhabentes* Van Der Spuy, Smit et Schaeffner, 2020. A – entire specimen; B – scolex; C – mature proglottid; D – hooks.

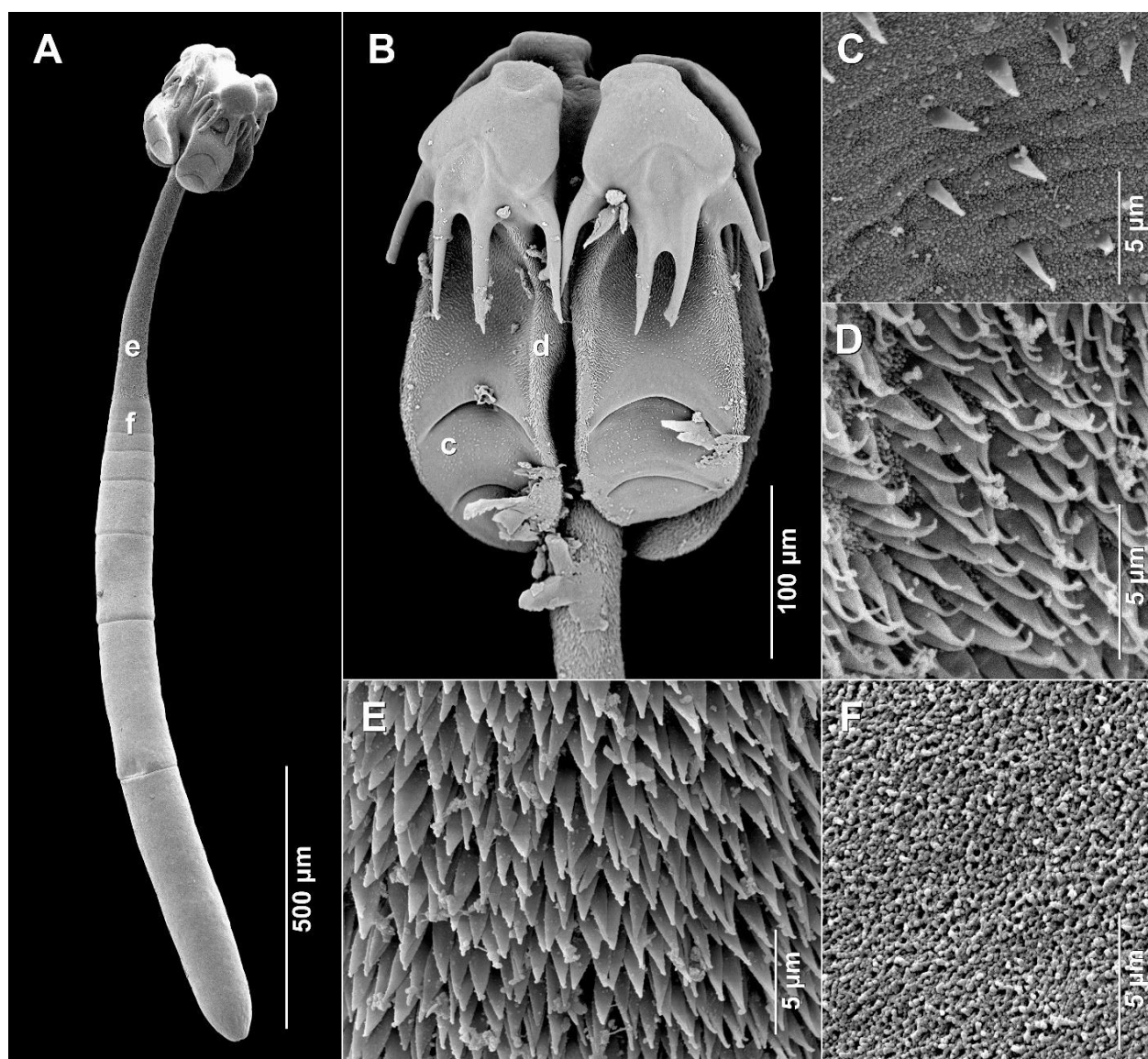


Fig. 3. Scanning electron micrographs of *Acanthobothrium microhabentes* Van Der Spuy, Smit et Schaeffner, 2020. A – entire specimen; letters indicate where micrographs of microtriches were taken; B – scolex, dorsoventral view; letters indicate where micrographs of microtriches were taken; C – distal bothridial surface; D – proximal bothridial surface near medial margin of bothridium; E – cephalic peduncle; F – first proglottid.

2.4.1.3. Remarks

Acanthobothrium microhabentes, is identified as a category 2 species according to specifications set by Ghoshroy and Caira (2001). At present, 47 species of *Acanthobothrium* recognised as category 2 species were reported worldwide (Maleki et al. 2013, Yang et al. 2016, Zaragoza-Tapia et al. 2019, 2020). Additionally, Franzese and Ivanov (2020) recognised two more species belonging to both category 1 and 2 as a result of intraspecific variability observed in ovarian symmetry. For the purpose of a more reliable comparison, the latter is considered as a category

2 species in this study. *Acanthobothrium microhabentes* features the presence of testes located posterior to the ovarian isthmus, therefore clearly distinguishing it from 45 of the recognised category 2 species, as well as the additional two species recognised by Franzese and Ivanov (2020).

Only 12 species of *Acanthobothrium* have been described to bear this unique characteristic feature, from which only two, *Acanthobothrium popi* Fyler, Caira et Jensen, 2009, and *Acanthobothrium bobconniorum* Fyler et Caira, 2010, fall into the category 2 (Fyler et al. 2009, Fyler and Caira 2010, Maleki et al. 2015). *Acanthobothrium microhabentes* is morphologically similar to *A. bobconniorum*. However, when comparing the unique trait responsible for associating these species, there are clear differences between *A. microhabentes*, *A. bobconniorum* and *A. popi* in the number of testes (less than 31 vs 36–49 and 37–50, respectively) and postovarian testes (1–2 vs 6–10 and 7–11, respectively). Furthermore, *A. microhabentes* differs from both *A. bobconniorum* and *A. popi* in the following characteristic features: a shorter body (less than 3 mm vs 3–5 mm and 3.9–7.1 mm, respectively), a shorter scolex (less than 306 μ m vs 387–490 μ m and 426–580 μ m, respectively), a shorter anterior loculus (less than 105 μ m vs 177–232 μ m and 193–282 μ m, respectively), a shorter middle loculus (less than 48 μ m vs 55–87 μ m and 53–93 μ m, respectively), a shorter posterior loculus (less than 56 μ m vs 62–110 μ m and 67–130 μ m, respectively), a narrower accessory sucker (less than 45 μ m vs 50–56 μ m and 50–88 μ m, respectively), fewer proglottids (less than 9 vs 10–14 and 14–20, respectively), a shorter cirrus-sac (less than 80 μ m vs 120–150 μ m and 108–152 μ m), and a narrower cirrus-sac (less than 47 μ m vs 60–117 μ m and 70–138 μ m).

2.4.2. *Acanthobothrium microtenuis* Van Der Spuy, Smit et Schaeffner, 2020 (Figs. 4, 5)

ZooBank number for species: urn: lsid: zoobank.org: act: B578A547-CB56-420E-9382-9B7F1422F9E9

2.4.2.1. Description

Based on 14 whole mounts, nine mature and five immature worms; three mature worms used for SEM observations: Worms 1.6–4.1 mm long; greatest width at level of scolex; 11–18 proglottids per worm; euapolytic. Scolex consisting of scolex proper and longer cephalic peduncle. Scolex proper with four bothridia, 311–318 long by 237–278 wide. Bothridia free posteriorly, 109–134 wide; each with three loculi and specialised anterior region in form of muscular pad. Muscular pad 64–76 long by 105–115 wide, falciform in shape, with pronounced posterior margin, bearing accessory sucker and one pair of hooks at posterior margin; accessory sucker 38–43 long by 44–

45 wide. Anterior loculus (A) 130–150 long; middle loculus (M) 47–56 long; posterior loculus (P) 59–68 long; loculus length ratio (A : M : P) 1 : 2.7 : 2.2; maximum width of scolex at level of middle loculus. Velum absent.

Hooks bi-pronged, hollow, with tubercle on proximal surface of axial prong; internal channels of axial and abaxial prongs continuous, smooth; axial prongs slightly longer than abaxial prongs; lateral and medial hooks approximately equal in size. Lateral hook measurements: A 40–52, B 69–97, C 54–94, D 105–135. Medial hook measurements: A' 41–54, B' 72–82, C' 69–89, D' 114–134. Bases of lateral and medial hooks approximately equal in length; base of lateral hooks slightly overlapping base of medial hooks along medial axis of bothridium (Fig. 4D); lateral hook base slightly wider than medial hook base. Thin layer of tissue covering essentially entire length of each prong of hooks. Long cephalic peduncle 695–1,858 long by 56–68 wide.

Apical pad and distal bothridial surface covered with papilliform filitriches and sparsely interspersed gladiate spinitriches (Fig. 5D). Proximal bothridial surface covered with gladiate spinitriches, sparsely interspersed with papilliform filitriches (Fig. 5E); papilliform filitriches pronounced along bothridial rims (Fig. 5E). Cephalic peduncle densely covered with gladiate spinitriches (Fig. 5F). Papilliform filitriches pronounced on strobila (Fig. 5G). Anterior part of terminal proglottid with prominent columnar spinitriches (Fig. 5H).

Proglottids acraspedote. Immature proglottids 8–18 in number; mature proglottids 1–2 in number; gravid proglottids absent; terminal proglottid 639–1,065 long by 134–211 wide; terminal proglottid length to width ratio 3.2–7.5 : 1. Proglottids protandrous; genital pores marginal, irregularly alternating, 58–64% of proglottid length from posterior end; testes conspicuous in mature proglottids, irregularly oval in dorsoventral view, 44–64 long by 25–41 wide, arranged in two regular columns, one layer deep, 24–33 in total number, 6–8 in postporal field, 1–2 in postovarian field. Cirrus-sac elongate, straight, 112–126 long by 48–72 wide, containing armed cirrus; cirrus greatly expanded at base.

Vagina narrow, relatively thin-walled and coiled proximally, greatly expanded distally, extending from ootype along medial line of proglottid to anterior margin of cirrus-sac, then laterally along anterior margin of cirrus-sac to common genital atrium. Ovary takes up approximately half of proglottid length, stopping short of posterior margin of proglottid, H-shaped in dorsoventral view, lobulated. Ovary asymmetrical, poral lobe 340–523 in length, aporal lobe 350–610 in length, both lobes extending past cirrus-sac; width of ovary at ovarian isthmus 95–116; ovarian isthmus located slightly posterior to mid-level of poral lobe of ovary. Mehlis' gland posterior to ovarian isthmus.

Vitellarium follicular, in form of two lateral bands, each consisting of two columns of relatively large follicles; bands extending from posterior margin of anterior-most testes to near posterior margin of ovary, interrupted by vagina and cirrus-sac; vitelline follicles 11–21 long by 20–34 wide. Vitelline follicle length relative to testes length 0.2–0.3 : 1. Uterus thin-walled, sacciform, extending from ovarian isthmus to near anterior margin of proglottid. Eggs not observed.

2.4.2.2. Taxonomic summary

Type host: Spotted skate, *Raja straeleni* (Rajiformes: Rajidae).

Type locality: South-eastern Atlantic Ocean off De Kelders (-34.5925, 19.2925), South Africa.

Site of infection: Spiral intestine.

Prevalence of infection: 88% (seven of eight skates observed).

Type material: Holotype deposited at NMB (P-607), paratypes in NMB (P-608–P-609), IPCAS (C-849) and MHNG (PLAT-137335, 137340).

Etymology: The species name “*microtenuis*” is derived from Latin (“micro”, small; “tenuis”, slender) and relates to the small and slender body shape of specimens.

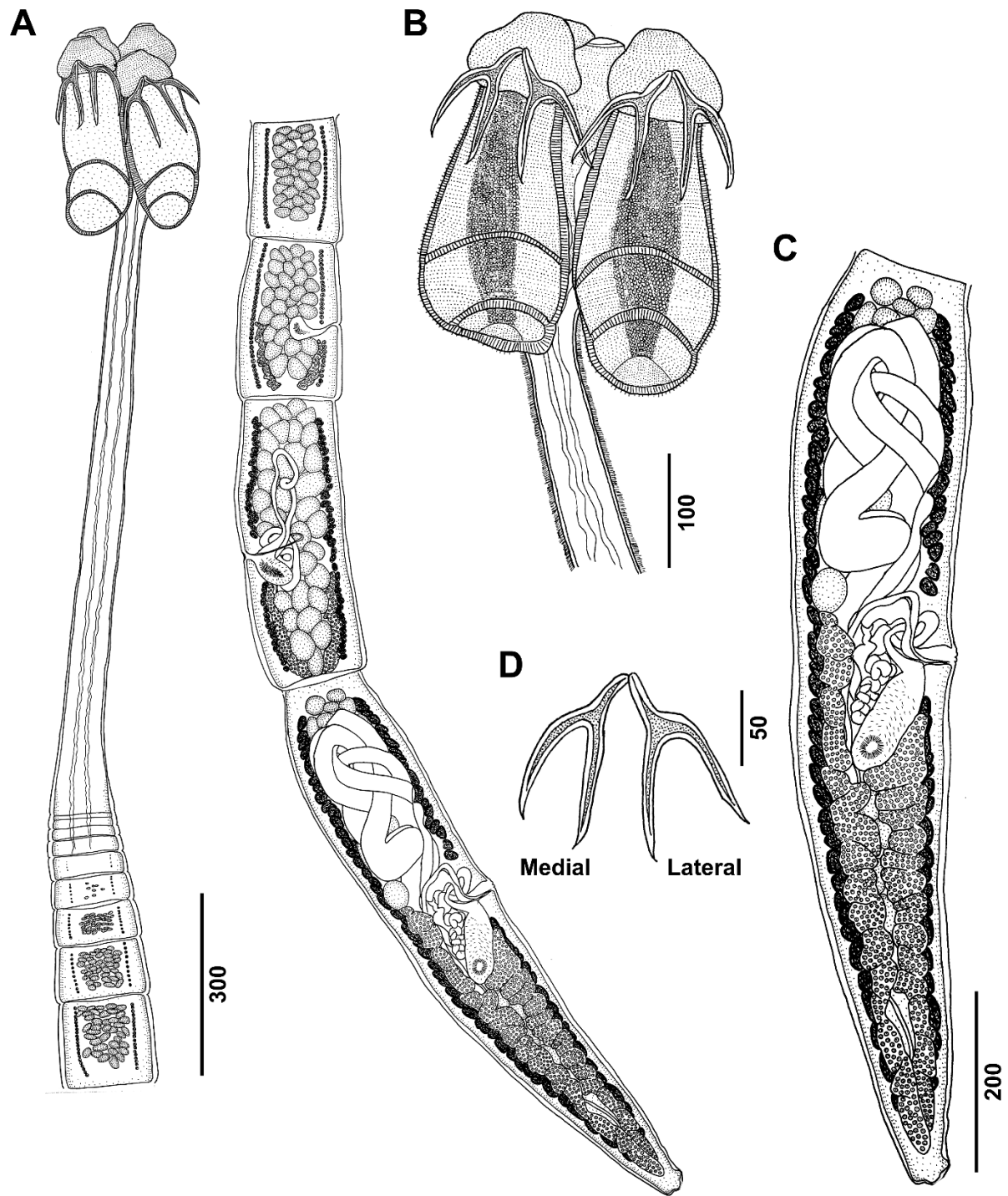


Fig. 4. Line drawing of *Acanthobothrium microtenuis* Van Der Spuy, Smit et Schaeffner, 2020. A – entire specimen; B – scolex; C – mature proglottid; D – hooks.

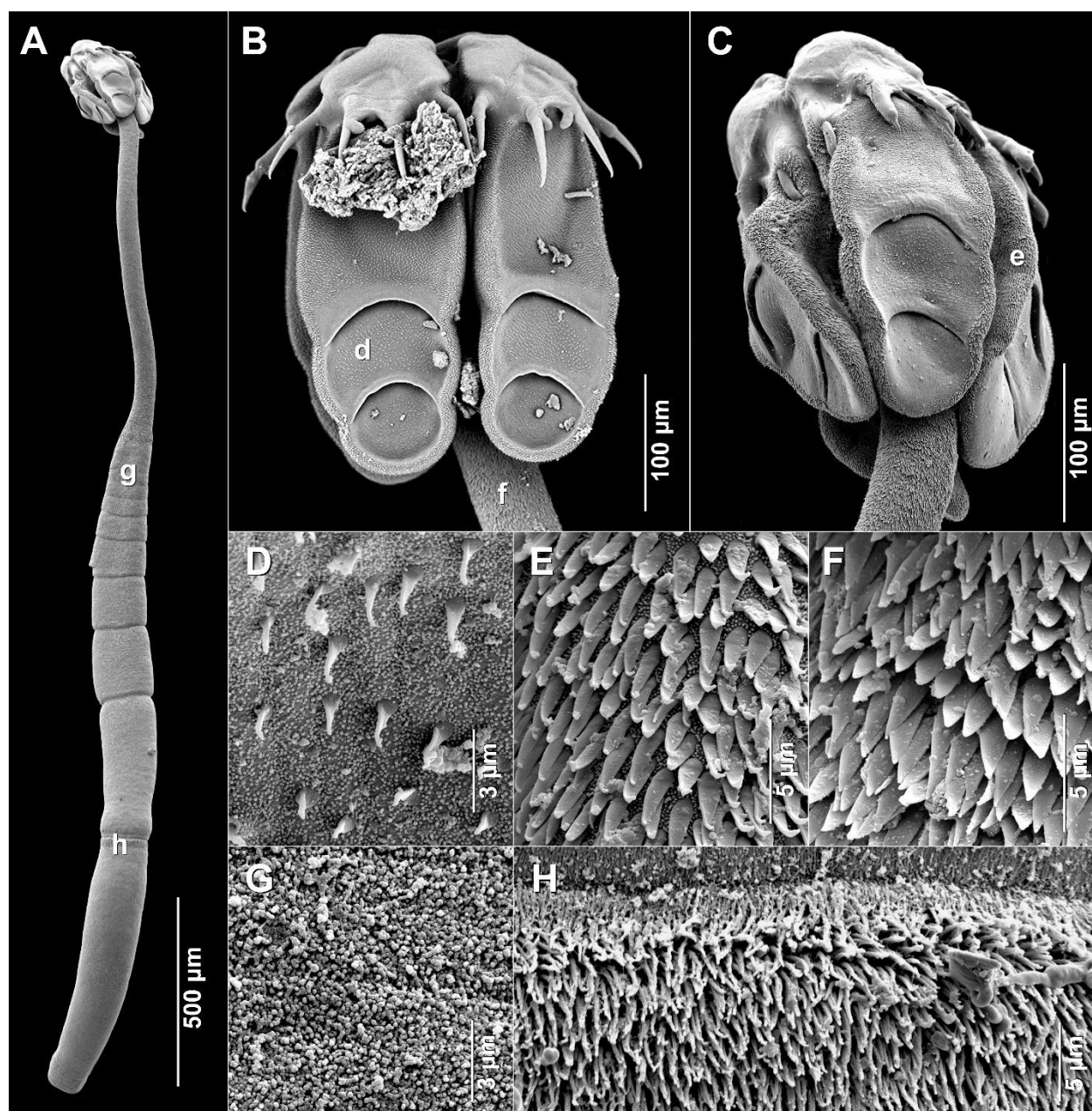


Fig. 5. Scanning electron micrographs of *Acanthobothrium microtenuis* Van Der Spuy, Smit et Schaeffner, 2020. A – entire specimen; letters indicate where micrographs of microtriches were taken; B – scolex, dorsoventral view; letters indicate where micrographs of microtriches were taken; C – scolex, lateral view; letter indicates where micrograph of microtriches was taken; D – distal bothridial surface; E – proximal bothridial surface near medial margin of bothridium; F – cephalic peduncle; G – first proglottid; H – anterior region of terminal proglottid.

2.4.2.3. Remarks

Acanthobothrium microtenuis, as a category 2 species, differs from all except three of the category 2 species, along with those described from Franzese and Ivanov (2020), in its possession of

postovarian testes. It resembles *A. bobconniorum*, *A. microhabentes* and *A. popi* morphologically but differs from these three species by its possession of an elongate cephalic peduncle (exceeding 700 μm vs 212–650 μm , 352–695 μm , and 328–450 μm , respectively). When comparing the length of the cephalic peduncle relative to the total body length for each species, the distinct individuality of *A. microtenuis* becomes even more prominent, as the cephalic peduncle equates 1/3 of the total length, whereas in *A. bobconniorum* and *A. popi*, the cephalic peduncle equates to 1/8 and 1/16 relative to the total body length, respectively. Apart from the cephalic peduncle, additional morphological differences between *A. microtenuis*, *A. bobconniorum* and *A. popi* can be observed in the following characteristic features: fewer testes (less than 33 vs 36–49 and 37–50, respectively), fewer postovarian testes (1–2 vs 6–10 and 7–11, respectively), a shorter scolex (less than 306 μm vs 387–490 μm and 426–580 μm , respectively), a shorter anterior loculus (less than 150 μm vs 177–232 μm and 193–282 μm , respectively), a shorter muscular pad (less than 76 μm vs 76–110 μm and 88–118 μm , respectively), and a narrower accessory sucker (less than 45 μm vs 50–65 μm and 50–88 μm , respectively).

Acanthobothrium microtenuis differs from *A. microhabentes* by a longer anterior loculus (exceeding 130 μm vs 99–105 μm), a longer posterior loculus (exceeding 60 μm vs 48–56 μm), greater number of proglottids (11–18 vs 5–9), a longer cirrus-sac (exceeding 112 μm vs 57–80 μm), and a wider ovary (exceeding 95 μm vs 54–76 μm). One additional morphological characteristic of *A. microtenuis*, contributing to the validity of this species, is the presence of columnar spinitriches observed on the anterior margin of the terminal proglottid (Fig. 5H). This feature has not been reported for any of the three congeners before.

2.4.3. *Acanthobothrium crassus* Van Der Spuy, Schaeffner et Smit, 2020 (Figs. 6, 7)

ZooBank number for species: urn: lsid: zoobank.org: act: D08145EC-E8B2-4FBD-9B40-3A66BEF3E45C

2.4.3.1. Description

Based on five whole mounts, five mature worms; two mature worms used for SEM observations: Worms 5.6–8.1 mm long; greatest width at level of scolex; 27–33 proglottids per worm; euapolytic. Scolex consisting of scolex proper and long cephalic peduncle. Scolex proper with four bothridia, 442–450 long by 318–337 wide. Bothridia free posteriorly, 138–173 wide; each with three loculi and specialised anterior region in form of muscular pad. Muscular pad 91–102 long by 102–108 wide, falciform in shape, with pronounced posterior margin, bearing accessory sucker and one

pair of hooks at posterior margin; accessory sucker 40–46 long by 44–49 wide. Anterior loculus (A) 175–185 long; middle loculus (M) 76–88 long; posterior loculus (P) 77–85 long; loculus length ratio (A : M : P) 1 : 2.2 : 2.2; maximum width of scolex at level of middle loculus. Velum absent.

Hooks bi-pronged, hollow, with tubercle on proximal surface of axial prong; internal channels of axial and abaxial prongs continuous, smooth; axial prongs slightly longer than abaxial prongs; lateral and medial hooks approximately equal in size. Lateral hook measurements: A 42–48, B 72–85, C 62–75, D 106–118. Medial hook measurements: A' 40–46, B' 71–89, C' 66–80, D' 107–126. Bases of lateral and medial hooks approximately equal in length; base of lateral hooks slightly overlapping base of medial hooks along medial axis of bothridium (Fig. 6D); lateral hook base slightly wider than medial hook base. Thin layer of tissue covering essentially entire length of each prong of hooks. Long cephalic peduncle 1,331–2,009 long by 88–94 wide.

Apical pad and distal bothridial surfaces covered with papilliform filitriches and sparsely interspersed gladiate spinitriches (Figs. 7C, D). Proximal bothridial surfaces covered with aristate gladiate spinitriches interspersed with acicular filitriches (Fig. 7E); capilliform filitriches pronounced along bothridial rims. Cephalic peduncle densely covered with gladiate spinitriches (Fig. 7F). Capilliform filitriches pronounced on strobila (Fig. 7G).

Proglottids craspedote. Immature proglottids 25–30 in number; mature proglottids 1–3 in number; gravid proglottids absent; terminal proglottid 980–1,285 long by 138–173 wide; terminal proglottid length to width ratio 4–7.5 : 1. Proglottids protandrous; genital pores marginal, irregularly alternating, 54–63% of proglottid length from posterior end. Testes conspicuous in mature proglottids, irregularly oval in dorsoventral view, 45–54 long by 36–43 wide, arranged in two regular columns, one layer deep, 40–46 in total number, 8–10 in postporal field, 1–3 in postovarian field. Cirrus-sac elongate, bent posteriorly, 123–133 long by 48–55 wide, containing armed cirrus; cirrus greatly expanded at base.

Vagina narrow, relatively thin-walled and coiled proximally, greatly expanded distally, extending from ootype along medial line of proglottid to anterior margin of cirrus-sac, then laterally to common genital atrium. Ovary takes up approximately half of proglottid, stopping short of posterior margin of the proglottid, H-shaped in dorsoventral view, lobulated. Ovary asymmetrical, poral lobe 440–652 in length, aporal lobe 464–711 in length; both lobes extending shortly past cirrus-sac; width of ovary at ovarian isthmus 98–115; ovarian isthmus located slightly posterior to midpoint of poral lobe of ovary. Mehlis' gland posterior to ovarian isthmus.

Vitellarium follicular, in form of two lateral bands, each consisting of two columns of relatively large follicles; bands extending from posterior margin of anterior-most testes to near posterior

margin of ovary, interrupted by vagina and cirrus-sac; vitelline follicles 12–19 long by 32–38 wide. Vitelline follicle length relative to testes length 0.2–0.3 : 1. Uterus thin-walled, sacciform, extending from ovarian isthmus to near anterior margin of proglottid. Eggs not observed.

2.4.3.2. Taxonomic summary

Type host: Spotted skate, *Raja straeleni* (Rajiformes: Rajidae).

Type locality: South-eastern Atlantic Ocean off De Kelders (-34.5925, 19.2925), South Africa.

Site of infection: Spiral intestine.

Prevalence of infection: 38% (three of eight skates examined).

Type material: Holotype deposited at NMB (P-610), paratypes in NMB (P-611), IPCAS (C-850) and MHNG (PLAT-137341).

Etymology: The species name “*crassus*” is derived from Latin (“*crassus*”, thick) and relates to the form of proglottids, which are considerably thicker in comparison to congeners from South Africa.

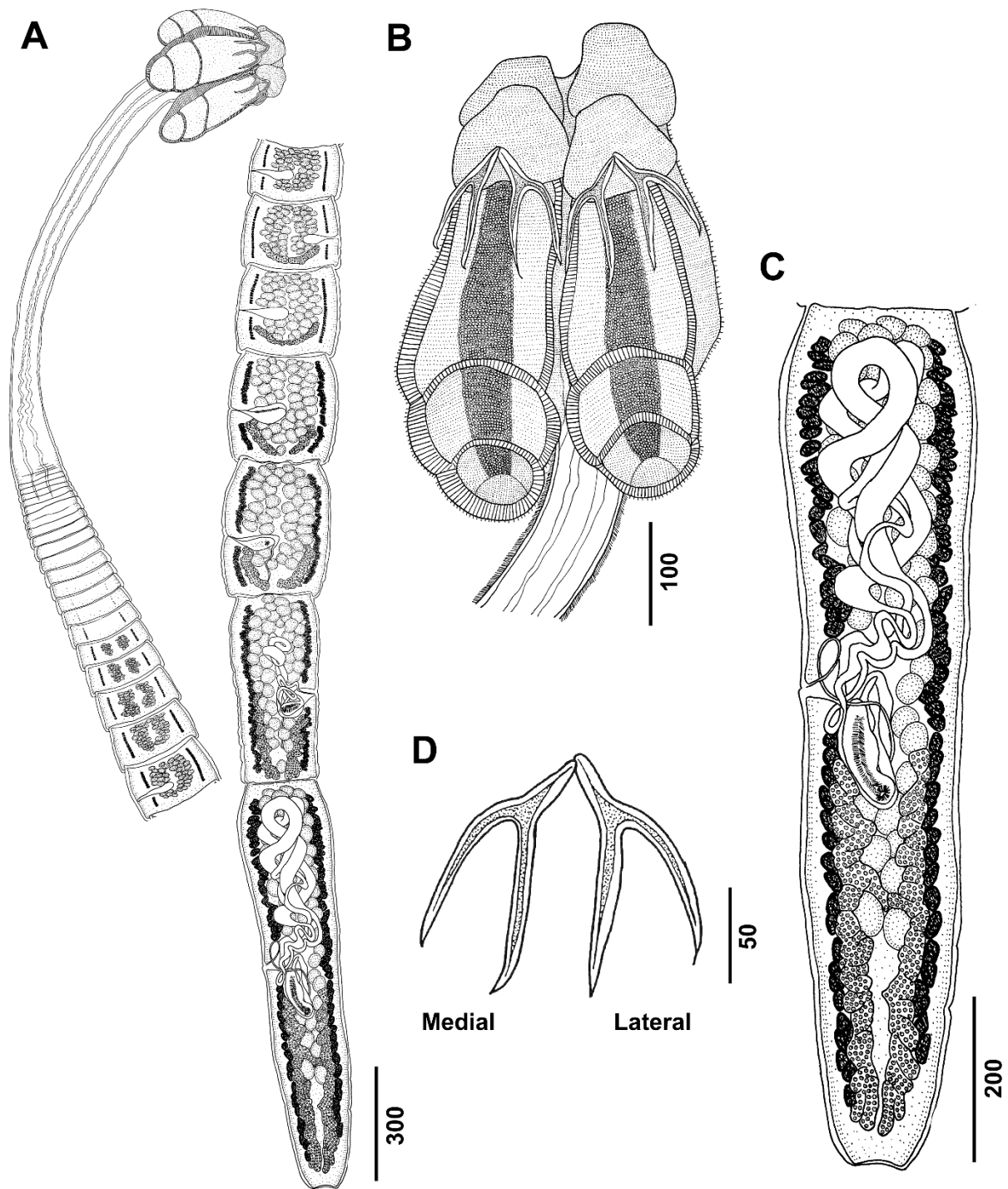


Fig. 6. Line drawing of *Acanthobothrium crassus* Van Der Spuy, Smit et Schaeffner, 2020. A – entire specimen; B – scolex; C – mature proglottid; D – hooks.

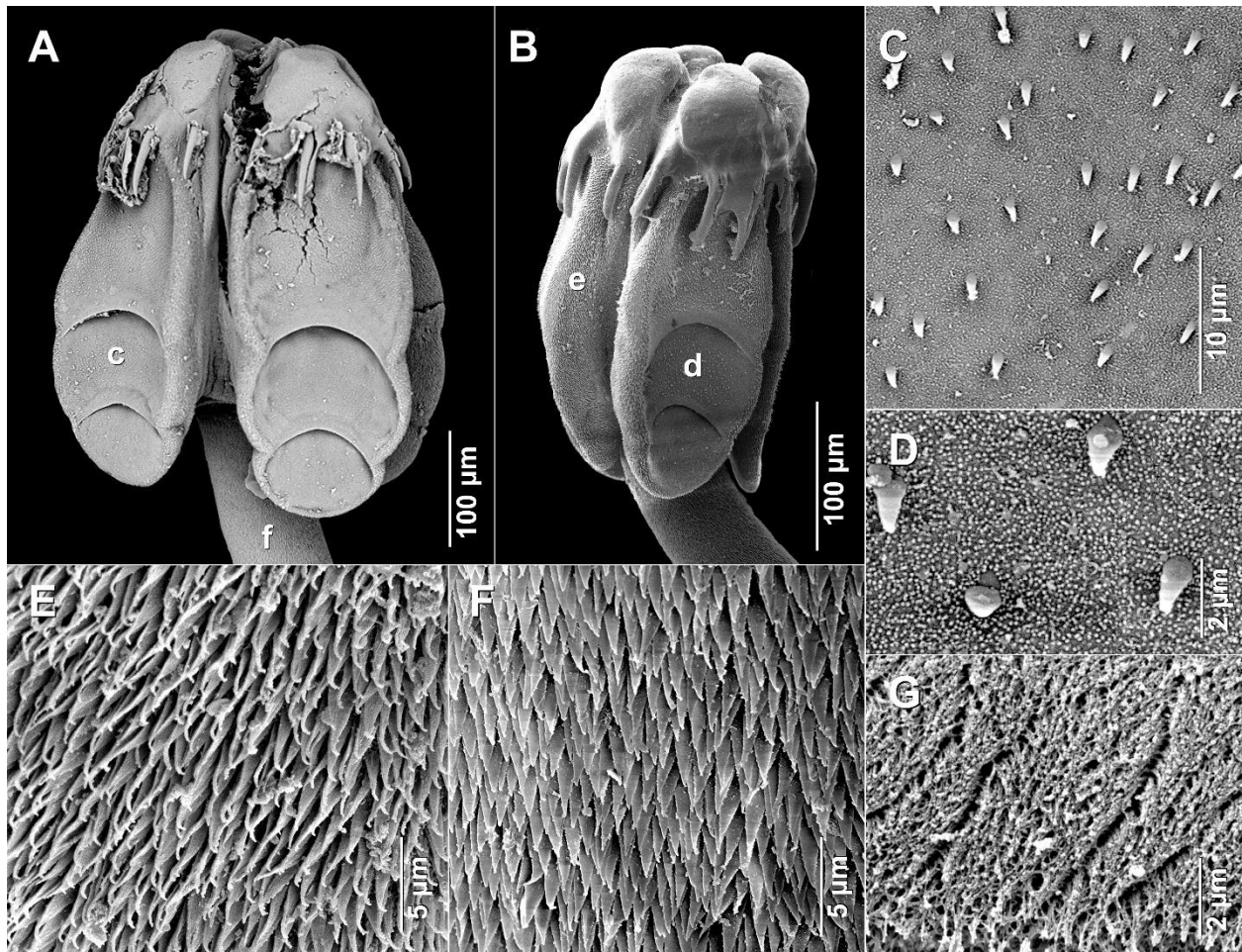


Fig. 7. Scanning electron micrographs of *Acanthobothrium crassus* Van Der Spuy, Smit et Schaeffner, 2020. A – scolex, dorsoventral view; letters indicate where micrographs of microtriches were taken; B – scolex, lateral view; letters indicate where micrographs of microtriches were taken; C – distal bothridial surface; D – detailed view of distal bothridial surface; E – proximal bothridial surface near medial margin of bothridium; F – cephalic peduncle; G – anterior strobila.

2.4.3.3. Remarks

Acanthobothrium crassus, following the classification criteria set by Ghoshroy and Caira (2001), is identified as a category 2 species. By including both *A. microhabentes*, and *A. microtenuis*, *A. crassus* is among the 50th category 2 species reported to date. *Acanthobothrium crassus* differs from all but four of its congeners in the possession of postovarian testes. *Acanthobothrium crassus* is distinguished from *A. bobconniorum*, *A. microhabentes*, *A. microtenuis*, and *A. popi* by a combination of morphological features. One such feature is the presence of craspedote proglottids in *A. crassus*, whereas its congeners possess acraspedote proglottids.

Acanthobothrium crassus has a larger body composed of more proglottids (i.e. 27–33), in comparison to congeners with a smaller body not exceeding 20 proglottids. In addition, the new species differs from *A. microhabentes* and *A. microtenuis* in the following characteristic features: a larger body (exceeding 6 mm vs 1.5–3 mm and 1.6–4.1 mm, respectively), a larger scolex (exceeding 442 $\mu\text{m} \times 320 \mu\text{m}$ vs 301–306 $\mu\text{m} \times 215$ –248 μm and 311–318 $\mu\text{m} \times 237$ –278 μm , respectively), a wider bothridium (exceeding 140 μm vs 105–115 μm and 109–134 μm , respectively), a longer muscular pad (exceeding 90 μm vs 63–74 μm and 64–76 μm , respectively), and a greater number of testes (exceeding 40 vs 21–31 and 24–33, respectively). The cephalic peduncle is considerably longer in *A. crassus* (exceeding 1,330 μm) than the one of *A. bobconniorum* (212–650 μm), *A. microhabentes* (352–695 μm) and *A. popi* (328–450 μm).

Additionally, *A. crassus* differs from *A. bobconniorum* by its body size (exceeding 6 mm vs 3–5 mm), a wider scolex (exceeding 320 μm vs 215–282 μm , respectively), a narrower cirrus-sac (less than 55 μm vs 60–117 μm , respectively), less postovarian testes (3 vs 6–10, respectively), longer testes (exceeding 45 μm vs 17–45 μm , respectively), and much wider vitelline follicles (exceeding 32 μm vs 12–25 μm , respectively).

Acanthobothrium crassus is morphologically similar to *A. popi*, but differs from the latter by having a narrower muscular pad (less than 108 μm vs 112–163 μm , respectively), a shorter lateral hook C (less than 75 μm vs 75–100 μm , respectively), a shorter medial hook C' (less than 80 μm vs 87–108 μm , respectively), a narrower cirrus-sac (less than 55 μm vs 70–138 μm , respectively), fewer postovarian testes (1–3 vs 7–11, respectively), and larger vitelline follicles (exceeding 32 μm vs 13–25 μm , respectively).

2.4.4. *Acanthobothrium dolichocollum* Van Der Spuy, Smit et Schaeffner, 2020 (Figs. 8, 9)

ZooBank number for species: urn: lsid: zoobank.org: act: 89F7BDDD-8036-4F56-B366-C96712CE1291

2.4.4.1. Description

Based on six whole mounts, six mature worms; two mature worms used for SEM observations: Worms 7.7–10.5 mm long; greatest width at level of scolex; 30–46 proglottids per worm; euapolytic. Scolex consisting of scolex proper and extremely long cephalic peduncle. Scolex proper with four bothridia, 410–416 long by 344–360 wide. Bothridia free posteriorly, 126–134 wide; each with three loculi and specialised anterior region in form of a muscular pad. Muscular

pad 77–84 long by 120–126 wide, falciform in shape, with pronounced posterior margin, bearing accessory sucker and one pair of hooks at posterior margin; accessory sucker 48–51 long by 51–56 wide. Anterior loculus (A) 175–202 long; middle loculus (M) 75–86 long; posterior loculus (P) 61–71 long; loculus length ratio (A: M: P) 1 : 2.3 : 2.8; maximum width of scolex at level of middle loculus. Velum absent.

Hooks bi-pronged, hollow, with tubercle on proximal surface of axial prong; internal channels of axial and abaxial prongs continuous, smooth; axial prongs slightly longer than abaxial prongs; lateral and medial hooks approximately equal in size. Lateral hook measurements: A 35–47, B 68–76, C 51–68, D 95–112. Medial hook measurements: A' 42–46, B' 61–74, C' 59–73, D' 98–116. Bases of lateral and medial hooks approximately equal in length; base of lateral hooks slightly overlapping base of medial hooks along medial axis of bothridium (Fig. 8D); lateral hook base slightly wider than medial hook base. Thin layer of tissue covering essentially entire length of each prong of hooks. Extremely long cephalic peduncle 2.07–4.78 long by 66–73 wide. Apical pad and distal bothridial surfaces covered with sparsely interspersed gladiate spinitriches (Fig. 9C). Proximal bothridial surfaces covered with gladiate spinitriches interspersed with capilliform filitriches (Fig. 9D); acicular filitriches prominent along bothridial rims. Cephalic peduncle densely covered with gladiate spinitriches (Fig. 9E). Strobila covered with papilliform filitriches (Fig. 9F). Anterior margin of terminal proglottid covered with acicular filitriches (Fig. 9G).

Proglottids acraspedote. Immature proglottids 28–44 in number; mature proglottids 2–4 in number; gravid proglottids absent; terminal proglottid 1,046–1,484 long by 182–260 wide; terminal proglottid length to width ratio 3.9–10.7 : 1. Proglottids protandrous; genital pores marginal, irregularly alternating, 56–66% of proglottid length from posterior end.

Testes conspicuous in mature proglottids, irregularly oval in dorsoventral view, 52–60 long by 41–49 wide, arranged in two regular columns, one layer deep, 33–39 in total number, 10–13 in postporal field, 2–4 in postovarian field. Cirrus-sac pyriform, bent anteriorly, 122–127 long by 68–72 wide, containing armed cirrus; cirrus greatly expanded at base.

Vagina narrow, relatively thin-walled and extensively coiled proximally, thick-walled and greatly expanded distally, extending from ootype along medial line of proglottid to anterior margin of cirrus-sac, then laterally along anterior margin of cirrus-sac to common genital atrium. Ovary takes up almost half of length of proglottid, stopping short of posterior margin of proglottid, H-shaped in dorsoventral view, lobulated. Ovary asymmetrical, poral lobe 538–692 in length, aporal lobe 565–689 in length, both lobes extending past cirrus-sac; width of ovary at ovarian isthmus 108–120; ovarian isthmus located slightly posterior to mid-level of poral lobe of ovary. Mehlis' gland posterior to ovarian isthmus.

Vitellarium follicular, in form of two lateral bands, each consisting of two columns of relatively large follicles; bands extending from posterior margin of anterior-most testis to near posterior margin of ovary, interrupted by vagina and cirrus-sac; vitelline follicles 17–23 long by 34–41 wide. Vitelline follicle length relative to testes length 0.3–0.4 : 1. Uterus thin-walled, sacciform, extending from ovarian isthmus to near anterior margin of proglottid. Eggs not observed.

2.4.4.2. Taxonomic summary

Type host: Spotted skate, *Raja straeleni* (Rajiformes: Rajidae).

Type locality: South-eastern Atlantic Ocean off Hermanus (-34.6355, 19.4933), South Africa.

Site of infection: Spiral intestine.

Prevalence of infection: 50% (four of eight skates observed).

Type material: Holotype deposited at NMB (P-612), paratypes in NMB (P-613), IPCAS (C-851) and MHNG (PLAT-137342, 137343).

Etymology: The species name “*dolichocollum*” is derived from Latin (“*dolicho*”, elongate; “*collum*”, neck) and relates to the unique morphological characteristic of the species i.e., its elongate cephalic peduncle.

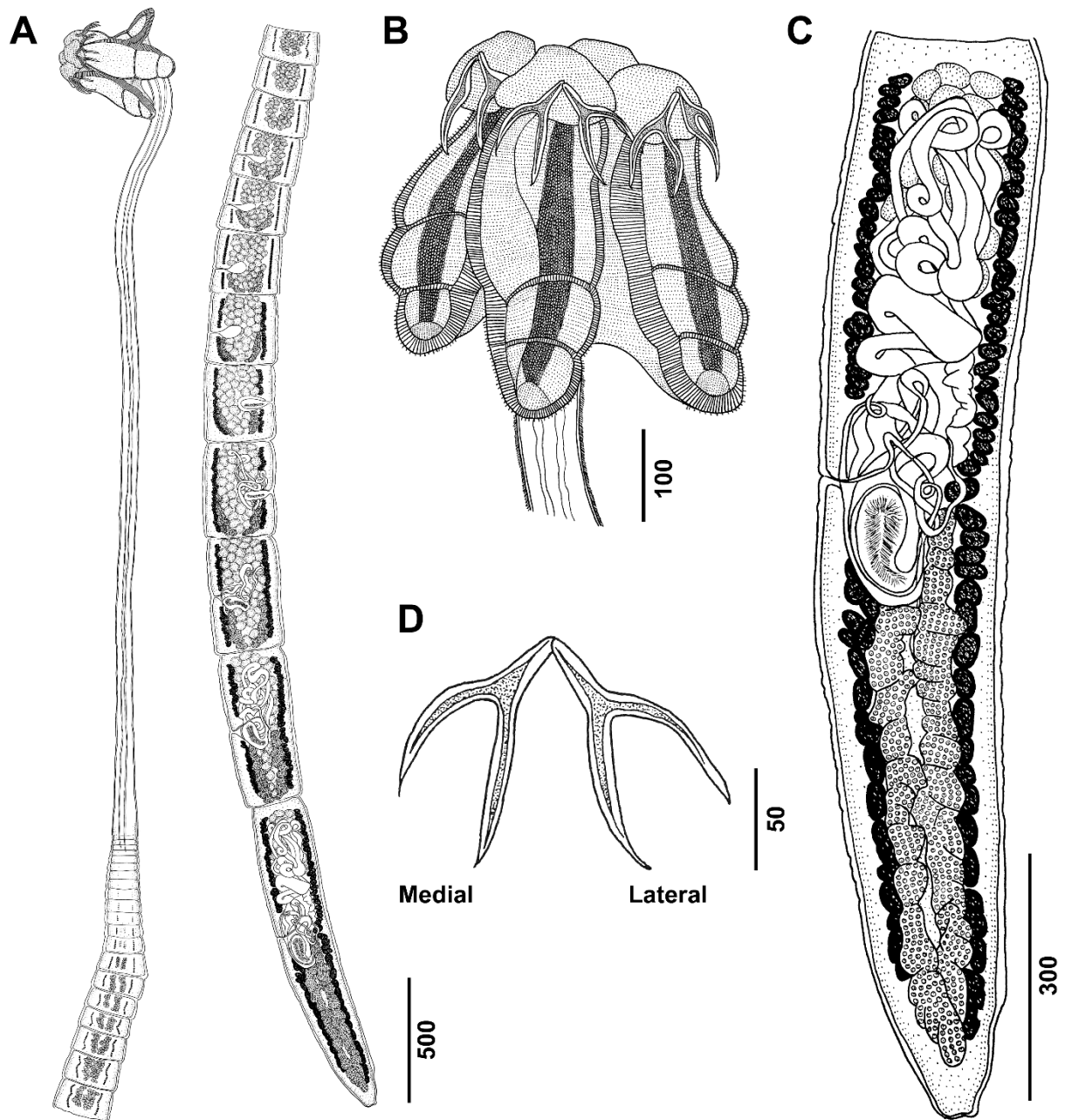


Fig. 8. Line drawing of *Acanthobothrium dolichocollum* Van Der Spuy, Smit et Schaeffner, 2020. A – entire specimen; B – scolex; C – mature proglottid; D – hooks.

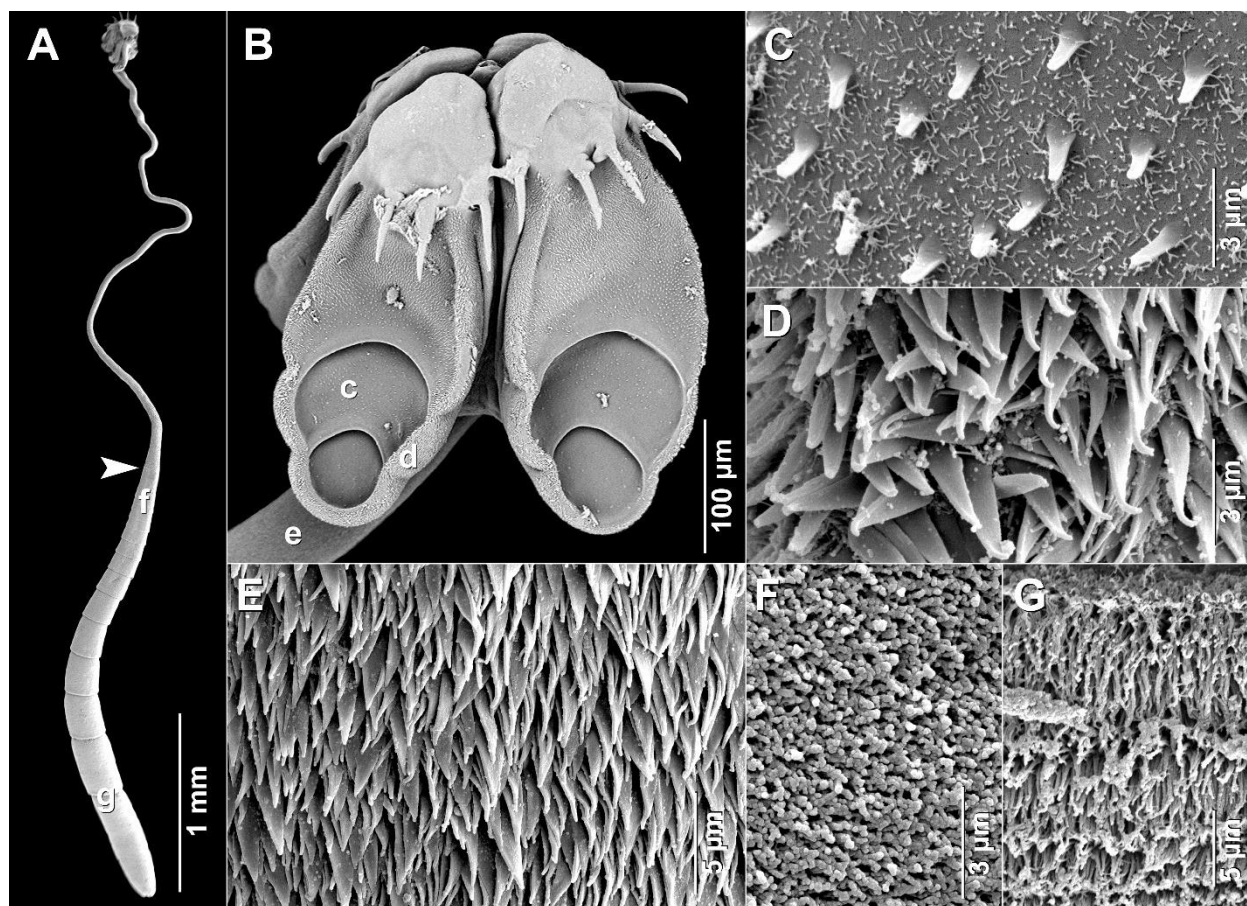


Fig. 9. Scanning electron micrographs of *Acanthobothrium dolichocollum* Van Der Spuy, Smit et Schaeffner, 2020. A – entire specimen; arrow indicates termination of cephalic peduncle, letters indicate where micrographs of microtriches were taken; B – scolex, dorsoventral view, letters indicate where micrographs of microtriches were taken; C – distal bothridial surface; D – proximal bothridial surface near medial margin of bothridium; E – cephalic peduncle; F – first proglottid; G – anterior region of terminal proglottid.

2.4.4.3. Remarks

Acanthobothrium dolichocollum, following the classification criteria set by Ghoshroy and Caira (2001), is identified as a category 2 species. With the description of *A. dolichocollum*, we now recognise 51 species of *Acanthobothrium* assigned to category 2. *Acanthobothrium dolichocollum* presents testes located posterior to the ovarian isthmus, differentiating it from 45 of the recognised category 2 species, as well as the two additional species described by Franzese and Ivanov (2020). *Acanthobothrium dolichocollum* differs from the remaining five category 2 species, *A. bobconniorum*, *A. popi*, *A. microhabentes*, *A. microtenuis* and *A. crassus*, by having more postporal testes (exceeding 10 vs 6–10, 5–9, 4–7, 6–8, and 8–10, respectively).

Another truly exceptional feature of *A. dolichocollum* is the possession of an extraordinarily long cephalic peduncle (exceeding 2 mm), equivalent to 1/3 of its total body length. The cephalic peduncle of *A. bobconniorum* and *A. popi* is much shorter (212–650 µm and 328–450 µm, respectively), equivalent to 1/8 and 1/17 of the body length, respectively. Furthermore, *A. dolichocollum* differs from *A. bobconniorum*, *A. popi*, *A. microhabentes* and *A. microtenuis* by having a larger body (exceeding 7.7 mm vs 3–5 mm, 3.9–7.1 mm, 1.5–3 mm and 1.6–4.1 mm, respectively), more proglottids (exceeding 30 vs 10–14, 14–20, 5–9 and 11–18, respectively), and wider vitelline follicles (exceeding 34 µm vs 12–25 µm, 13–25 µm, 10–24 µm and 20–34 µm, respectively).

Differences between *A. dolichocollum*, *A. microhabentes*, *A. microtenuis* and *A. crassus* can be seen in the scolex length (410–416 µm vs 301–306 µm, 311–318 µm and 442–450 µm, respectively), scolex width (344–360 µm vs 215–248 µm, 237–278 µm and 318–337 µm, respectively), muscular pad length (77–84 µm vs 63–74 µm, 64–76 µm and 91–102 µm, respectively), muscular pad width (120–126 µm vs 97–113 µm, 105–115 µm and 102–108 µm, respectively), number of testes (33–39 vs 21–31, 24–33 and 40–46, respectively), and testes width (41–49 µm vs 23–35 µm, 25–41 µm and 36–43 µm, respectively). *Acanthobothrium dolichocollum* differs from *A. bobconniorum* in a wider scolex (exceeding 344 µm vs 215–282 µm, respectively), smaller lateral hooks (A less than 47 µm vs 47–52 µm; B less than 76 µm vs 72–92 µm; C less than 68 µm vs 70–86 µm; D less than 112 µm vs 111–134 µm, respectively), less postovarian testes (4 vs 6–10, respectively), and longer testes (exceeding 52 µm vs 17–45 µm, respectively).

The new species also differs from *A. popi* in a shorter scolex (less than 416 µm vs 426–580 µm, respectively), a shorter muscular pad (less than 84 µm vs 88–118 µm, respectively), shorter lateral hooks (A less than 47 µm vs 47–65 µm; B less than 76 µm vs 71–120 µm; C less than 68 µm vs 75–100 µm; D less than 112 µm vs 111–173 µm, respectively), shorter medial hooks (A' less than 46 µm vs 48–60 µm; B' less than 74 µm vs 75–119 µm; C' less than 73 µm vs 87–108 µm; D' less than 116 µm vs 120–175 µm, respectively), less postovarian testes (4 vs 7–11, respectively), and larger testes (exceeding 52 µm vs 28–50 µm, respectively).

2.5. Discussion

Sampling efforts focussed on parasitological observations of elasmobranchs off South Africa have been extremely sparse (Smit and Hadfield 2015, Schaeffner and Smit 2019). This particularly applies to the spotted skate, *Raja straeleni*, a relatively common species along the western and southern coastlines of southern Africa that has only once been examined for cestode infections

(see Caira et al. 2014). Caira et al. (2014) discovered a new diphyllidean cestode, a species that has not been encountered in the present study. On a global scale, a total of 18 species of *Acanthobothrium* have thus far been reported from 12 of the 23 valid species of *Raja* Linnaeus (Global Cestode Database 2020), clearly expressing the fact that host species are not limited to the infection of merely a single species of *Acanthobothrium*. Not only has this been previously reported in multiple studies (Fyler et al. 2009, Fyler and Caira 2010, Maleki et al. 2015), but this observation was also reflected in the present study, where four new species have been described from a single host species.

The present study marks the first to report species of *Acanthobothrium* from South Africa. With the addition of these four species, a total of 207 valid species of *Acanthobothrium* are currently recognised worldwide (Caira et al. 2017, Franzese and Ivanov 2018, 2020, Maleki et al. 2018, 2019, Rodríguez-Ibarra et al. 2018, Zaragoza-Tapia et al. 2019, 2020), making the taxonomic classification of new species, and intrageneric comparisons between congeners within this genus taxing (Ghoshroy and Caira 2001). Hence, in the interest of simplifying this matter, Ghoshroy and Caira (2001) have developed a category classification system on the basis of four morphological characteristics. The South African species all measure less than 15 mm in total length, with fewer than 50 proglottids, fewer than 80 testes, and an asymmetric ovary, designating all of them as category 2 species.

Comparisons between the four species presented in the current study suggest the possibility that these congeners could be each other's closest relatives. This assumption, however, is not only grounded upon the fact that these species resembled each other in numerous morphological characteristics, but also due to the presence of four synhospitalic congeners parasitising the same host species (Caira and Tracy 2003, Caira et al. 2004, Fyler and Caira 2006). To ensure that the South African species are in fact new to science, interspecific comparisons were made between all category 2 species recognised worldwide.

With the addition of the South African species, the total number of species of *Acanthobothrium* allocated to category 2 increased to 51 species. With this still being quite a substantial number of species, the South African congeners are very easily distinguished from all except two of these congeners by the presence of postovarian testes, undoubtedly being a very interesting, yet highly unusual feature among species of *Acanthobothrium* (see Fyler et al. 2009, Fyler and Caira 2010, Maleki et al. 2015). In point of fact, this feature has been previously documented in merely 6% (12 out of 203) of all species of *Acanthobothrium* known to infect elasmobranchs (Subhapradha 1955, Robinson 1959, Campbell and Beveridge 2002, Fyler and Caira 2006, Reyda and Caira 2006, Twohig et al. 2008, Fyler et al. 2009, Fyler and Caira 2010, Maleki et al. 2015).

The descriptions of *Acanthobothrium microhabentes*, *A. microtenuis*, *A. crassus* and *A. dolichocollum* increases the total number of species of *Acanthobothrium* known to possess postovarian testes to 16 species. Interestingly, with respect to the 12 previously reported species of *Acanthobothrium* known to bear this feature, all of these species were reported from hosts in waters of the Indo-Pacific Ocean (Fyler et al. 2009, Fyler and Caira 2010, Maleki et al. 2015), consequently suggesting that this feature might be restricted to this particular geographical region (Fyler and Caira 2006, Reyda and Caira 2006, Twohig et al. 2008, Fyler et al. 2009, Maleki et al. 2015). However, this could be argued, because species with this same feature off the south-western coast of South Africa clearly contradict the earlier conception. To date, the majority of species reported to possess postovarian testes, however, seems to be identified as category 1 species, with seven species assigned to category 1, six to category 2, one to category 3, and two to category 4 (Twohig et al. 2008, Fyler et al. 2009, Fyler and Caira 2010, Maleki et al. 2015, present study). These congeners, including the South African species, all seem to parasitise host species from the families Dasyatidae and Rajidae. Seven species (*Acanthobothrium cannoni* Campbell et Beveridge, 2002, *A. foulki* Reyda et Caira, 2006, *A. larsoni* Reyda et Caira, 2006, *A. marymichaelorum* Twohig, Caira et Fyler, 2008, *A. saliki* Fyler et Caira, 2006, *Acanthobothrium popi* and *A. zimmeri* Fyler, Caira et Jensen, 2009) are reported from species of *Himantura* Müller et Henle (Campbell and Beveridge 2002, Fyler and Caira 2006, Reyda and Caira 2006, Twohig et al. 2008), six (*A. blairi* Campbell et Beveridge, 2002, *A. microhabentes*, *A. microtenuis*, *A. crassus*, *A. dolichocollum* and *A. wedli* Robinson, 1959) from species of *Raja* (Campbell and Beveridge 2002, Robinson 1959, present study), two (*Acanthobothrium bobconniorum* and *A. jamesi* Maleki, Malek et Palm, 2015) from species of *Rhynchobatus* Müller et Henle (Fyler and Caira 2010, Maleki et al. 2015), and one (*A. southweli* Subhapradha, 1955) from *Rhinobatos schlegelii* Müller et Henle (Subhapradha 1955).

The South African congeners not only parasitise the same host species, but also share the rare feature of postovarian testes with 12 other species of *Acanthobothrium*. This makes the possibility of their relatedness even more evident. Given the fact that two other species with postovarian testes have also been documented in two other species of *Raja* (i.e. *Zearaja nasuta* [Müller et Henle], and *Spiniraja whitleyi* [Iredale]), the four new congeners might not only be grouped together as each other's closest relatives, but also with the 12 species of *Acanthobothrium* exhibiting the same unique feature, endorsing the notion that these species might comprise their own monophyletic clade (Fyler and Caira 2010). Moreover, as this is the first time that species of *Acanthobothrium* have been reported in *R. straeleni*, we can assume that this is the result of a single host invasion. Conversely, molecular analyses conducted by Fyler et al. (2009), including numerous synhospitalic congeners, concluded that these species do not represent closely related lineages. Fyler and Caira (2010) conducted a similar study, subsequently reaching the same conclusion.

Phylogenetic trees do not only serve insights on host associations but also on the geographical distribution of species. According to earlier studies (Goldstein 1967, Williams 1969, Ghoshroy and Caira 2001, Fyler 2009), congeners displaying the same geographical affinities are more likely to group together. Surprisingly, species of *Acanthobothrium* parasitising the same host species of different geographical localities do not group in the same clade (Fyler 2009).

If a phylogenetic tree would display the four new species as distinct or independent lineages it would indicate that the infection of *R. straeleni* does not resemble a single host invasion as previously suggested, but rather be the result of multiple disparate host invasions (Fyler 2009). Regardless of the host's specific nature, extensive host substitutions may have occurred throughout a much longer evolutionary time, accordingly proceeding unnoticed during short-lived investigations (Fyler 2009). The combined effects of different geographical affinities as well as host substitution possibly resulted in an extraordinary host specificity, as they have been documented from eight of 11 elasmobranch orders (Caira et al. 2017).

This is truly intriguing, considering the fact that the South African species are the first species to possess postovarian testes outside of the Indo-Pacific realm. Furthermore, the host from which the species were obtained, *R. straeleni*, has only been documented to populate the Eastern Atlantic Ocean from the coastline of the western Sahara to southern Africa. Nevertheless, regardless of geographical affinities, the possibility of potential relatedness between species of *Acanthobothrium* possessing this rare feature still persists, supported by the fact that two of these species (i.e. *A. popi* and *A. zimмери*) have already grouped together, during a molecular study performed by Fyler et al. (2009). Unfortunately, as of yet, the degree of relatedness among these unique species is still unknown, recognising the need for further investigation and a collection of additional specimens of *R. straeleni* from the same localities. A more conclusive molecular analysis including both the South African and Indo-Pacific species is furthermore fundamental in defining their phylogenetic relationships, informing us on whether these species do, in fact, form a monophyletic clade.

In addition, a molecular phylogenetic analysis on newly collected specimens will reveal their phylogenetic position and will highlight new perspectives on the evolutionary relationships between these cestodes and their elasmobranch hosts. With merely 9% (18 of 204) of elasmobranchs (Ebert and van Hees 2015, Schaeffner and Smit 2019) off southern Africa examined for cestode infections, it is also expected that the species diversity of cestodes infecting elasmobranchs in South African waters is much higher than currently reported (Schaeffner and Smit 2019) and future large-scale surveys on elasmobranch parasites will, without any doubt, lead to numerous new species discoveries.

Caira and Jensen (2017) estimated that 5,126 species of cestodes infect approximately 1,269 elasmobranch species worldwide, representing an average of four cestode species per elasmobranch host. As a consequence, the diversity of cestodes in the waters of South Africa is predicted to be either equal to, or, more likely, greater than the diversity of the chondrichthyan hosts present in these waters (Schaeffner and Smit 2019). Nevertheless, the current extensive and rapid rate of loss in biodiversity, not just in this region, but globally, limits the time remaining for the discovery and description of the cestode diversity. However, since species of *Acanthobothrium* are known to parasitise multiple elasmobranch orders, along with the ability to switch hosts, parasites of this genus could possibly be able to avoid extinction. Nonetheless, when accounting other cestode orders and genera parasitising elasmobranchs (i.e. cestodes without the ability to switch hosts), the loss of parasite diversity would then likely be higher than that of their hosts (Dobson et al. 2008, Randhawa and Poulin 2010, Randhawa et al. 2015). This is alarming given this will likely result in the extinction of numerous cestode species before they have even been discovered. New, sustainable conservation schemes should be enforced, including a combination of multidisciplinary approaches. Hereby, information can be gained from parasites, providing us with a better understanding of the host, such as its evolutionary and demographic history and migratory patterns (Gomez and Nichols 2013). Collections of additional hosts and localities, such as the ocean basins surrounding southern Africa, which have previously not been properly explored, are required in this regard. New studies should also be concurrent with previously applied methods (i.e. taxonomy, molecular systematics) and the incorporation and implementation of novel, multisource approaches (i.e. biogeography, ecology, conservation science, ecotoxicology). When we consider to use these parasites as indicator organisms for pollution and global change, we may yield a much better understanding on the evolutionary interrelationships of host-parasite systems present within our world's remarkable ecosystems, contributing to various scientific fields, and ultimately contributing to the conservation of declining populations of endangered species and positive environmental change.

2.6. Declaration of funding

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References

- Caira J.N., Jensen K. 2014: A digest of elasmobranch tapeworms. *J. Parasitol.* 100: 373–391.
- Caira J.N., Jensen K. (Eds.) 2017: Planetary Biodiversity Inventory (2008–2017): Tapeworms from Vertebrate Bowels of the Earth. The University of Kansas Natural History Museum, Special Publication no. 25, Lawrence, pp. 463.
- Caira J.N., Jensen K., Fyler C.A. 2018: A new genus of tapeworm (Cestoda: Onchoproteocephalidea) from sawfish (Elasmobranchii: Pristidae). *J. Parasitol.* 104: 133–144.
- Caira J.N., Jensen K., Ivanov V.A. 2017. Onchoproteocephalidea II. In: J.N. Caira and K. Jensen (Eds.), Planetary Biodiversity Inventory (2008–2017): Tapeworms from Vertebrate Bowels of the Earth. The University of Kansas Natural History Museum. Special Publication no. 25, pp. 279–304.
- Caira J.N., Jensen K., Waeschenbach A., Olson P.D., Littlewood D.T.J. 2014: Orders out of chaos – molecular phylogenetics reveals the complexity of shark and stingray tapeworm relationships. *Int. J. Parasitol.* 44: 55–73.
- Caira J.N., Tracy R. 2003: Two new species of *Yorkeria* (Tetraphyllidea: Onchobothriidae) from *Chiloscyllium punctatum* (Elasmobranchii: Hemiscyllidae). *J. Parasitol.* 88: 1172–1180.
- Caira J.N., Tracy R., Euzet L. 2004: Five new species of *Pedibothrium* (Tetraphyllidea: Onchobothriidae) from the tawny nurse shark, *Nebrius ferrugineus*, in the Pacific Ocean. *J. Parasitol.* 90: 286–300.
- Campbell R.A., Beveridge I. 2002: The genus *Acanthobothrium* (Cestoda: Tetraphyllidea: Onchobothriidae) parasitic in Australian elasmobranch fishes. *Invertebr. Syst.* 16: 237–344.
- Chervy, L. Unified terminology for cestode microtriches: a proposal from the International Workshops on Cestode Systematics in 2002–2008. *Folia Parasitol.* 56: 199–230.
- De Chambrier A., Scholz T., Mariaux J., Kuchta R. 2017: Onchoproteocephalidea I Caira, Jensen, Waeschenbach, Olson & Littlewood, 2014. In: J.N. Caira and K. Jensen (Eds.), Planetary Biodiversity Inventory (2008–2017): Tapeworms from Vertebrate Bowels of the Earth.

University of Kansas, Natural History Museum, Special Publication No. 25, Lawrence, KS, USA, pp. 251–277.

Dobson A., Lafferty K.D., Kuris A.M., Hechinger R.F., Jet W. 2008: Homage to Linnaeus: How many parasites? How many hosts? *Proc. Natl. Acad. Sci.* 105: 11482–11489.

Ebert D., van Hees K.E. 2015: Beyond jaws: rediscovering the ‘lost sharks’ of southern Africa. *Afr. J. Mar. Sci.* 37: 141–156.

Euzet, L. 1954. Parasites de poissons de mer ouest africains récoltés par J. Cadenat. I.—Cestodes tetraphyllides de sélaciens. (Note préliminaire). *Bulletin de l'Institut Français d'Afrique Noire*, A16: 126–138.

Franzese S., Ivanov V.A. 2018: Hyperapolytic species of *Acanthobothrium* (Cestoda: Onchoproteocephalidea) from batoids off Argentina. *Parasitol. Int.* 67: 431–443.

Franzese S., Ivanov V.A. 2020: Two new species of *Acanthobothrium* Blanchard, 1848 (Cestoda: Onchoproteocephalidea) from rajiform batoids off Argentina. *Folia Parasitol.* 67: 016.

Fyler C.A. 2009: Systematics, Biogeography and Character Evolution in the Tapeworm Genus *Acanthobothrium* van Beneden, 1850. ProQuest Dissertations Publishing, University of Connecticut, pp. 200.

Fyler C.A. 2011: An extremely hyperapolytic *Acanthobothrium* species (Cestoda: Tetraphyllidea) from the Japanese wobbegong, *Orectolobus japonicus* (Elasmobranchii: Orectolobiformes) in Taiwan. *Comp. Parasitol.* 78: 4–14.

Fyler C.A., Caira J.N. 2006: Five new species of *Acanthobothrium* (Tetraphyllidea: Onchobothriidae) from the freshwater stingray *Himantura chaophraya* (Batoidea: Dasyatidae) in Malaysian Borneo. *J. Parasitol.* 92: 105–125.

Fyler C.A., Caira J.N. 2010: Phylogenetic status of four new species of *Acanthobothrium* (Cestoda: Tetraphyllidea) parasitic on the wedgefish *Rhynchobatus laevis* (Elasmobranchii: Rhynchobatidae): implications for interpreting host associations. *Invertebr. Syst.* 24: 419–433.

Fyler C.A., Caira J.N., Jensen K. 2009: Five new species of *Acanthobothrium* (Cestoda: Tetraphyllidea) from an unusual species of *Himantura* (Rajiformes: Dasyatidae) from northern Australia. *Folia Parasitol.* 56: 107–128.

Ghoshroy S., Caira J.N. 2001: Four new species of *Acanthobothrium* (Cestoda: Tetraphyllidea) from the whiptail stingray *Dasyatis brevis* in the Gulf of California. J. Parasitol. 87: 354–372.

Global Cestode Database 2020: World Wide Web electronic publication.
<http://tapewormdb.uconn.edu>. Accessed: 22 February 2020.

Goldstein R.J. 1967: The genus *Acanthobothrium* van Beneden, 1849 (Cestoda: Tetraphyllidea). J. Parasitol. 53: 455–483.

Gomez A., Nichols E. 2013: Neglected wild life: parasitic biodiversity as a conservation target. Int. J. Parasitol. 2: 222–227.

Ivanov V.A. 2005: A new species of *Acanthobothrium* (Cestoda: Tetraphyllidea: Onchobothriidae) from the ocellate river stingray, *Potamotrygon motoro* (Chondrichthyes: Potamotrygonidae), in Argentina. J. Parasitol. 91: 390–396.

Maleki L., Malek M., Palm H.W. 2013: Two new species of *Acanthobothrium* (Tetraphyllidea: Onchobothriidae) from *Pastinachus* cf. *sephen* (Myliobatiformes: Dasyatidae) from the Persian Gulf and Gulf of Oman. Folia Parasitol. 60: 448–456.

Maleki L., Malek M., Palm H.W. 2015: Four new species of *Acanthobothrium* van Beneden, 1850 (Cestoda: Onchoproteocephalidea) from the guitarfish, *Rhynchobatus* cf. *djiddensis* (Elasmobranchii: Rhynchobatidae), from the Persian Gulf and Gulf of Oman. Folia Parasitol. 62: 012.

Maleki L., Malek M., Palm H.W. 2019: Five new species of *Acanthobothrium* (Cestoda: Onchoproteocephalidea) from the longtailed butterfly ray, *Gymnura* cf. *poecilura* (Elasmobranchii: Gymnuridae), from the Persian Gulf and Gulf of Oman. Zootaxa 4609: 289–307.

Maleki L., Malek M., Rastgoo A. 2018: *Acanthobothrium chabahariense* n. sp. (Cestoda: Onchoproteocephalidea) in the cowtail stingray *Pastinachus* cf. *sephen* (Myliobatiformes: Dasyatidae) from the Gulf of Oman, Iran. J. Genet. Resour. 4: 114–121.

Randhawa, H.S., Poulin, R., Krkosek, M., 2015. Increasing rate of species discovery in sharks coincides with sharp population declines: implications for biodiversity. Ecography 38, 96–107.

Randhawa, H.S., Poulin, R., 2020. Tapeworm discovery in elasmobranch fishes: quantifying patterns and identifying their correlates. *Mar. Freshw. Res.* 71, 78–88.

Reyda F.B., Caira J.N. 2006: Five new species of *Acanthobothrium* (Cestoda: Tetraphyllidea) from *Himantura uarnacoides* (Myliobatiformes: Dasyatidae) in Malaysian Borneo. *Comp. Parasitol.* 73: 49–71.

Robinson E.S. 1959: Some new cestodes from New Zealand marine fishes. *Trans. R. Soc. N. Z.* 86: 381–392.

Rodríguez-Ibarra E., Pulido-Flores G., Violante-González J., Monks S. 2018: A new species of *Acanthobothrium* (Eucestoda: Onchobothriidae) in *Aetobatus* cf. *narinari* (Myliobatidae) from Campeche, México. *Braz. J. Vet. Parasitol.* 27: 67–74.

Schaeffner B.C., Smit N.J. 2019: Parasites of cartilaginous fishes (Chondrichthyes) in South Africa – a neglected field of marine science. *Folia Parasitol.* 66: 002.

Smit N.J., Hadfield K.A. 2015: Marine fish parasitology in South Africa: history of discovery and future direction. *Afr. Zool.* 50: 79–92.

Spalding M.D., Fox H.E., Allen G.R., Davidson N., Ferdaña Z.A., Finlayson M., Halpern B.S., Jorge M.A., Lombana A., Lourie S.A., Martin K.D., McManus E., Molnar J., Recchia C.A., Robertson J. 2007: Marine ecoregions of the world: a bioregionalization of coastal and shelf areas. *Bioscience* 57: 573–583.

Subhapradha C.K. 1955: Cestode parasites of fishes of Madras. *Ind. J. Helminthol.* 7: 41–132.

Tazerouti F., Kechemir-Issad N., Euzet L. 2009: *Acanthobothrium minus* n. sp. (Tetraphyllidea: Onchobothriidae) parasite de *Raja asterias* (Elasmobranchii: Rajidae) en Méditerranée. *Parasite* 16: 203–207.

Twohig M.E., Caira J.N., Fyler C.A. 2008: Two new cestode species from the dwarf whipray, *Himantura walga* (Batoidea: Dasyatidae), from Borneo with comments on site and mode of attachment. *J. Parasitol.* 94: 1118–1127.

Williams, H.H. 1964. Some new and little known cestodes from Australian elasmobranchs with a brief discussion on their possible use in problems of host taxonomy. *Parasitology*, 54: 737–748.

Williams H.H. 1969: The genus *Acanthobothrium* Beneden, 1849 (Cestoda: Tetraphyllidea). *Nytt Mag. Zool.* 17: 1–56.

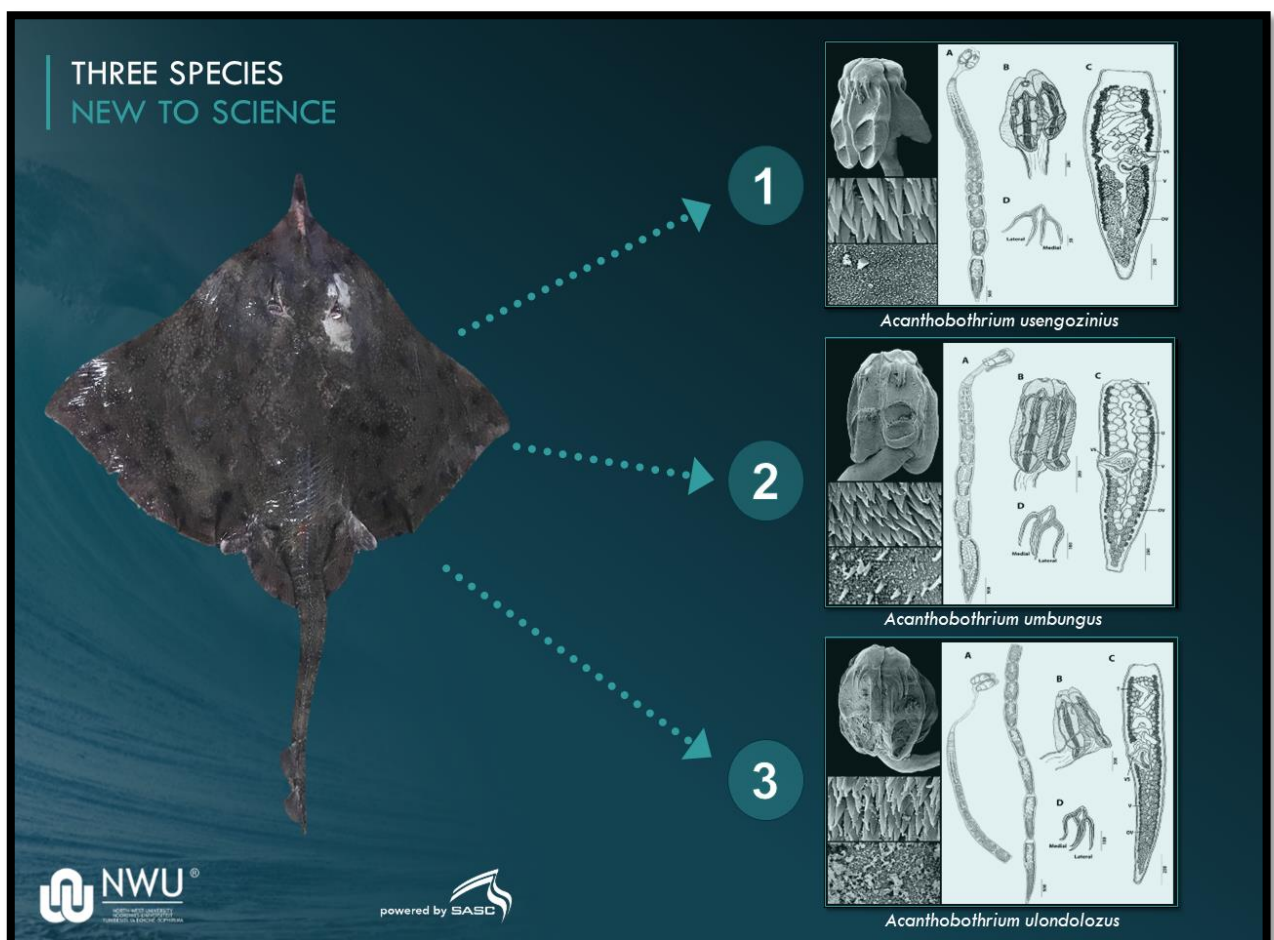
Yang C., Sun Y., Zhi T., Iwaki T., Reyda F.B., Yang T. 2016: Two new and one redescribed species of *Acanthobothrium* (Cestoda: Onchoproteocephalidea: Onchobothriidae) from *Dasyatis akajei* (Myliobatiformes: Dasyatidae) in the China Sea. *Zootaxa* 4169: 286–300.

Zaragoza-Tapia F., Pulido-Flores G., Monks S. 2020: Three new species of *Acanthobothrium* Blanchard, 1848 (Cestoda: Onchoproteocephalidea) in stingrays (Dasyatidae) from the Pacific coast in Mexico. *Zootaxa* 4766: 139–172.

Zaragoza-Tapia F., Pulido-Flores G., Violante-González J., Monks S. 2019: Two new species of *Acanthobothrium* Blanchard, 1848 (Onchobothriidae) in *Narcine entemedor* Jordan & Starks, 1895 (Narcinidae) from Acapulco, Guerrero, Mexico. *ZooKeys* 852: 1–21.

CHAPTER 3:

Threatened, host-specific affiliates of a red-listed host: Three new species of *Acanthobothrium* van Beneden, 1849 (Cestoda: Onchoproteocephalidea) from the endangered white skate, *Rostroraja alba* (Lacépède)



CHAPTER 3:

Threatened, host-specific affiliates of a red-listed host: Three new species of *Acanthobothrium* van Beneden, 1848 (Cestoda: Onchoproteocephalidea) from the endangered white skate, *Rostroraja alba* (Lacépède)

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3.1. Abstract

The white skate, *Rostroraja alba* (Lacépède), is listed as an endangered species, the second-highest category before being declared extinct in the wild, in the International Union for Conservation of Nature's (IUCN) Red List of Threatened Species. This species is heavily affected by anthropogenic impacts such as capture induced stress by overfishing and by-catch, habitat degradation and pollution that caused a drastic decline in populations in recent years. As part of a larger study on elasmobranch affiliates in southern Africa, two specimens of *R. alba* were screened. Three species of the tapeworm genus *Acanthobothrium* van Beneden, 1848 (Cestoda: Onchoproteocephalidea) were discovered. Application of Ghoshroy and Caira's classification system facilitated the differentiation of congeners through a combination of specific morphological characteristics. As a consequence, three species new to science are described herein, namely *Acanthobothrium umbungus* Van Der Spuy, Smit et Schaeffner, 2022, *Acanthobothrium usengozinius* Van Der Spuy, Smit et Schaeffner, 2022, and *Acanthobothrium ulondolozus* Van Der Spuy, Smit et Schaeffner, 2022. In light of these new discoveries from an endangered host, it is apparent to address the conservation status of its affiliate species, that co-evolved with their elasmobranch hosts for millions of years, thereby creating unique and intimate host-parasite interrelationships. Currently, altering environmental conditions caused by anthropogenic pressures have direct impacts on this host-parasite system with increasing risks of extinction. As merely 9% of elasmobranchs in South African waters have been examined for endohelminths and other affiliate taxa, extensive studies on these organisms and their hosts implementing

multisource approaches are needed. This will provide a better understanding on the intimate nature of host-parasite systems that may lead to new prospects in conservation science and the preservation of threatened host species, such as *R. alba*, together with their unique fauna of affiliate species.

Keywords: Elasmobranchs Affiliate species Taxonomy Threatened fauna Co-extinctions Conservation biology Marine parasites

3.2. Introduction

Elasmobranchs are currently facing alarming global population declines with 37% of species threatened with a higher risk of extinction, making them the most threatened group of vertebrates in the marine environment (IUCN, 2021). These apex marine predators are heavily affected by anthropogenic impacts, most notably through overfishing and by-catch, and habitat degradation (amongst others) that caused a drastic decline in populations in recent years (Siskey et al., 2019; Sousa et al., 2019). What makes it even more alarming is that elasmobranchs host a variety of affiliate species within and on their bodies, that make up a large proportion of the marine biodiversity (Caira and Healy, 2004; Randhawa and Poulin, 2010; Zaragoza-Tapia et al., 2020a).

Helminths such as cestodes have co-evolved with their elasmobranch hosts for millions of years (see for instance Dentzien-Dias et al., 2013), thereby creating unique and very intimate host-parasite interrelationships (Caira and Jensen, 2001, 2014). *Acanthobothrium* van Beneden, 1848 (Cestoda: Onchoproteocephalidea II, sensu Caira et al., 2017) is reported to be the most species-rich tapeworm genus known to infect elasmobranchs (Maleki et al., 2015; Caira et al., 2017), currently consisting of 207 valid species (Caira et al., 2017; Zaragoza-Tapia et al., 2019, 2020b; Van Der Spuy et al., 2020). Albeit already being an extremely diverse genus, Caira et al. (2017) state that an estimated 800 additional species of *Acanthobothrium* await future discovery. Species of *Acanthobothrium* are also known as synhospitalic taxa, exhibiting oioxenous host specificity (Fyler, 2009; Caira et al., 2017). This means that multiple species of *Acanthobothrium* infect a single definitive host species (Caira et al., 2017). This trait could represent a beneficial attribute in providing vital information on the host's biology, life conditions and environmental requirements (Nhi et al., 2013). In recent times, these organisms were also implemented as indicators for ecosystem health assessments (Jankovská et al., 2011; Nhi et al., 2013) and pollution studies (Sures et al., 2017).

With increasing threats to elasmobranch host populations and declines in biodiversity, these affiliate species might face even a greater risk of extinction, especially in the cases of highly host-

specific taxa such as species of *Acanthobothrium*. Therefore, declines in a single elasmobranch host species will, without a doubt, result in the co-extinction of several affiliate species with potential negative implications for marine ecosystems worldwide (Poulin and Presswell, 2016).

Unfortunately, concurrent with their elasmobranch hosts, research on cestodes and their unique interrelationships with their definitive hosts are ignored and not taken into consideration by conservation agencies. This leaves most of these taxa and their unique ecological services they may provide unknown to science, and render them even more vulnerable to extinction (Poulin and Presswell, 2016; Zaragoza-Tapia et al., 2020a). Acknowledging this research necessity, two specimens of the endangered white skate, *Rostroraja alba* (Lacépède), were screened for cestodes as part of a larger study on elasmobranch affiliates in the understudied ocean basins surrounding southern Africa. By use of light and scanning electron microscopy, we provide taxonomic information on species of *Acanthobothrium*, and describe three species new to science.

3.3. Materials and methods

Specimens for research purposes were obtained with the help of the South African Shark Conservancy (SASC). Ethical approval for this project was received from the North-West University Animal Care, Health and Safety, Research Ethics Committee (NWU-AnimCareREC) with ethics number NWU-00065-19-A5. Permits for the collection and possession of batoid specimens for the purpose of research were issued by the South African Department of Agriculture, Forestry and Fisheries (permit nos. RES2019/58 and RES2019/61 issued to the South African Shark Conservancy).

Two specimens of *Rostroraja alba* [specimen 1: female, mature, 1.85 m in length (tail missing), 1.70 m in disc width, approx. 70 kg in weight, sampling code HE-19-03, fin-clip NB714; specimen 2: female, mature, 2.11 m in length, 1.63 m in disc width, approx. 60 kg, sampling code HE- 19-04, fin-clip EC426] were collected by longline in February 2019 from Danger Point, Gansbaai, South Africa [34° 28' 50" S, 19° 19' 55" E]. Both skates were euthanised by use of an adjunctive procedure, inducing neurocranial trauma, pithing the brain immediately thereafter. The spiral intestine, and its contents, of each skate was removed by a mid-ventral incision, and fixed in hot, 4%, neutrally-buffered formalin for morphology and pure ethanol for molecular studies. No specimens were recovered in the ethanol-fixed samples. After a period of two weeks, the spiral intestines and contents were transferred from formalin to 70% ethanol and observed with a stereo microscope. Each individual specimen of *Acanthobothrium* was removed by use of picking tools from both the spiral intestine as well as its contents, and allocated to morphotypes. Specimens of

each morphotype were hydrated in a graded ethanol series and stained with Delafield's haematoxylin. Following staining, specimens were again dehydrated in a graded ethanol series to 70%. The overstain of each specimen was cleared in 1% hydrogen chloride. Specimens were then further dehydrated in a grade ethanol series to 100% ethanol, cleared in clove oil, and permanently mounted onto microscope slides in Canada balsam.

Morphological observations were conducted and images of each specimen's various characteristic body structures were acquired by use of a Nikon Y-TV55 video camera mounted on a Nikon ECLIPSE Ni light microscope (Nikon, Tokyo, Japan). These images were used to obtain measurements for descriptive analyses by use of image analyses software Image Pro Express (Nikon, Japan). Measurements of internal organs, body structures and hooks followed specifications given by Ghoshroy and Caira (2001); text descriptions provide the range of measurements only, whereas Table 1 provides additional metrical data including the mean, standard deviation and number of specimens examined. Besides the total length measured in millimetres, all other measurements are presented in micrometres. Line drawings of individual specimens were acquired by use of a drawing attachment tube.

Scanning electron microscopy (SEM) was performed on selected specimens in order to characterise microthrix patterns. Two specimens of each species were cleaned in 70% ethanol from host mucus, and dried by critical point drying. Specimens were then mounted onto carbon tape on aluminium stubs and sputter-coated with carbon (Emscope TB500, Quorum Technologies, Puslinch, Ontario, USA), followed by 20–30 nm gold/palladium (Eiko IB2 ion coater, Eiko, Japan). Each specimen was observed by use of a FEI Nova NanoSEM 450 scanning electron microscope (FEI, Hillsboro, Oregon, USA). Terminology on the microthrix morphology of different scolex regions and strobila follows Chervy (2009). Micrographs were also taken of both immature proglottids (directly posterior to the cephalic peduncle) and mature proglottids (the most anterior region of the terminal proglottid).

Following the most recent species descriptions of *Acanthobothrium*, species determination followed Ghoshroy and Caira's (2001) category classification system to facilitate species characterisations. Species were grouped and assessed based on the following four morphological features: total length of the cestode (<or >15 mm), number of proglottids (<or >50 proglottids), number of testes (<or >80) per proglottid, and the symmetry or asymmetry of aporal and poral ovarian lobes (see Ghoshroy and Caira, 2001). Congeners are distinguished only between members within the same category, as different categories already confirm their dissimilarity in various morphological features (Fyler and Caira, 2006).

All type material has been deposited in the following three helminthological collections: the National Museum, Bloemfontein, South Africa (NMB); the Institute of Parasitology, Biology Centre of the Czech Academy of Sciences, České Budějovice, Czech Republic (IPCAS); and the Natural History Museum, Geneva, Switzerland (MHNG-PLAT). Stubs containing specimens of each species used for SEM were retained in the parasite collection of the Water Research Group, North-West University, South Africa.

3.4. Results

Two, female specimens of the endangered white skate, *R. alba* have been examined for cestode infections. Three, morphologically-distinct species of *Acanthobothrium* were discovered; the first species was found to parasitise the first skate, while the other two species were found in the spiral intestines of the second skate. In addition, a few larval stages of a tentaculariid species belonging to the order Trypanorhyncha were obtained from the second *R. alba* (data not presented herein).

Table 1. Metrical information of the three new species of *Acanthobothrium* van Beneden, 1849. Information is presented as the mean, followed by the standard deviation and the number of worms examined. All measurements are in micrometres, unless stated otherwise. *Abbreviations:* L – length; N – number; W – width.

Character	<i>Acanthobothrium umbungus</i>	<i>Acanthobothrium usengozinius</i>	<i>Acanthobothrium ulondolozus</i>
Total length (mm)	4.23 ± 1.89; 17	7.80 ± 0.92; 7	11.25 ± 1.40; 7
Scolex L	465 ± 30; 17	569 ± 34; 7	561 ± 45; 7
Scolex W	288 ± 49; 17	448 ± 61; 7	409 ± 68; 6
Bothridium W	136 ± 13; 17	233 ± 12; 7	181 ± 10; 6
Anterior (A) loculus L	149 ± 15; 14	247 ± 8; 7	205 ± 6; 6
Middle (M) loculus L	84 ± 10; 14	134 ± 6; 7	109 ± 18; 6
Posterior (P) loculus L	78 ± 13; 14	102 ± 5; 7	107 ± 7; 5
Loculus L ratio (A: M: P)	1.0 : 1.7 ± 0.2 : 1.8 2.0 ± 0.3; 14	1.0 : 1.8 ± 0.1 : 2.4 2.0 ± 0.1; 7	1.0 : 1.8 ± 0.1 : 1.9 2.0 ± 0.1; 5
Muscular pad L	80 ± 9; 14	94 ± 14; 7	136 ± 10; 5
Muscular pad W	106 ± 9; 14	146 ± 10; 7	153 ± 8; 5
Accessory sucker L	24 ± 4; 12	25 ± 2; 7	32 ± 4; 5
Accessory sucker W	38 ± 7; 12	48 ± 5; 7	42 ± 5; 5
Lateral hook A	58 ± 6; 16	68 ± 4; 7	69 ± 6; 6
Lateral hook B	143 ± 9; 16	144 ± 7; 7	157 ± 3; 6
Lateral hook C	115 ± 6; 16	133 ± 5; 7	138 ± 5; 5
Lateral hook D	196 ± 7; 16	203 ± 10; 7	215 ± 3; 6
Medial hook A'	60 ± 5; 17	69 ± 3; 7	68 ± 5; 5
Medial hook B'	143 ± 10; 17	149 ± 10; 7	153 ± 10; 5
Medial hook C'	111 ± 6; 17	132 ± 7; 7	133 ± 9; 5
Medial hook D'	196 ± 11; 17	204 ± 15; 7	207 ± 4; 5

Table 1. Continued.

Cephalic peduncle L	457 ± 124; 15	749 ± 211; 7	1,244 ± 150; 7
Cephalic peduncle W	82 ± 10; 15	121 ± 2; 7	82 ± 8; 7
Proglottid N	21 ± 4; 17	38 ± 5; 7	40 ± 7; 7
Immature proglottid N	20 ± 4; 17	37 ± 5; 7	37 ± 7; 7
Mature proglottid N	1 ± 1; 17	1 ± 1; 7	3 ± 1; 7
Genital pore position (%)	46 ± 4; 11	49 ± 5; 7	46 ± 4; 7
Terminal proglottid L	907 ± 320; 11	1,147 ± 310; 7	1,565 ± 389; 7
Terminal proglottid W	273 ± 56; 12	333 ± 39; 7	320 ± 27; 7
Terminal proglottid ratio (L: W)	3 ± 1; 11	3 ± 1; 7	5 ± 1; 7
Cirrus-sac L	138 ± 19; 12	176 ± 33; 6	192 ± 20; 7
Cirrus-sac W	55 ± 12; 12	50 ± 5; 6	66 ± 6; 7
Testis N	33 ± 3; 17	52 ± 8; 6	54 ± 4; 7
Post-poral testis N	5 ± 1; 17	5 ± 1; 7	8 ± 1; 7
Postovarian testis N	0; 17	0; 7	1; 7
Testis L	43 ± 8; 12	54 ± 6; 6	55 ± 5; 7
Testis W	34 ± 3; 12	47 ± 4; 6	44 ± 5; 7
Poral ovarian arm L	386 ± 143; 12	624 ± 56; 6	683 ± 198; 7
Aporal ovarian arm L	434 ± 150; 12	685 ± 58; 6	773 ± 224; 7
Ovary W	95 ± 19; 12	157 ± 14; 6	126 ± 16; 6
Vitelline follicle L	11 ± 3; 17	30 ± 3; 7	17 ± 3; 7
Vitelline follicle W	22 ± 7; 17	19 ± 1; 7	34 ± 8; 7

3.4.1. *Acanthobothrium umbungus* Van Der Spuy, Smit et Schaeffner, 2022 (Figs. 1 and 2)

ZooBank number for species: urn: lsid: zoobank.org: act: CD024DAE-E2E6-4B6F-B6CF-CBE3A5FABB40.

3.4.1.1. Description

Based on whole mounts of 12 mature and five immature worms; two mature worms examined with SEM: Worms 2.4–8.9 mm long, greatest width at level of scolex, 15–32 proglottids per worm, euapolytic. Scolex consisting of scolex proper and cephalic peduncle. Scolex proper with four bothridia, 415–524 long by 225–385 wide. Bothridia free posteriorly, 118–154 wide; each bothridium with three loculi and specialised anterior region in form of muscular pad. Muscular pad 70–96 long by 93–116 wide, falciform in shape, with pronounced posterior margin, bearing accessory sucker and one pair of hooks at posterior margin; accessory sucker 20–30 long by 31–45 wide. Anterior loculus (A) 125–172 long; middle loculus (M) 65–110 long; posterior loculus (P) 60–105 long; loculus length ratio (A: M: P) 1.00 : 0.56: 0.52; maximum width of scolex at level of middle loculus. Velum absent.

Hooks bi-pronged, hollow, with tubercle on proximal surface of axial prongs; internal channels of axial and abaxial prongs continuous, smooth; axial prongs slightly longer than abaxial prongs; lateral and medial hooks approximately equal in size. Lateral hook measurements: *A* 49–69, *B* 127–158, *C* 102–125, *D* 183–208. Medial hook measurements: *A'* 48–71, *B'* 131–162, *C'* 101–121, *D'* 180–216. Bases of lateral and medial hooks approximately equal in length; base of lateral hook slightly overlapping base of medial hook along medial axis of bothridium (Fig. 1D); lateral hook base slightly wider than medial hook base. Tissue covering almost entire length of each prong of hooks. Short cephalic peduncle 291–834 long by 62–97 wide.

Cephalic peduncle densely covered with gladiate spinitriches, filitriches not observed (Fig. 2C). Apical pad and distal bothridial surface covered with acicular filitriches and sparsely interspersed gladiate spinitriches (Fig. 2D). Proximal bothridial surface and bothridial rims covered with gladiate spinitriches, interspersed with acicular filitriches (Fig. 2E). Entire strobila covered in acicular filitriches (Fig. 2F and G).

Proglottids acraspedote. Immature proglottids 16–30 in number; 1–2 mature proglottids; gravid proglottids absent; terminal proglottid 426–1460 long by 165–345 wide; terminal proglottid length to width ratio 2.3–5.4 : 1.0. Proglottids protandrous; genital pores marginal, irregularly alternating (Fig. 1A), 49–65% of proglottid length from posterior margin.

Testes conspicuous in mature proglottids, oval in dorsoventral view, 28–59 long by 27–40 wide, arranged in two to three irregular columns anterior to ovarian isthmus (Fig. 1C), one layer deep, 29–36 in total number, 5–6 in post-poral field. Cirrus-sac pyriform (Fig. 1C), 107–164 long by 35–71 wide, containing armed cirrus; cirrus greatly expanded at base.

Vagina narrow, relatively thin-walled and straight proximally, extending from ootype along medial line of proglottid to anterior margin of cirrus-sac, then laterally at anterior margin of cirrus-sac to common genital atrium. Vaginal sphincter prominent (Fig. 1C). Ovary occupying half of proglottid, almost reaching posterior margin of proglottid, H-shaped in dorsoventral view, lobulated (Fig. 1C), asymmetrical, 60–124 wide at level of ovarian isthmus; poral lobe 139–594 in length; aporal lobe 198–667 in length; ovarian lobes not reaching level of genital opening anteriorly; ovarian isthmus located posterior to mid-level of ovary. Mehlis' gland posterior to ovarian isthmus.

Vitellarium follicular; follicles in two lateral bands, 6–16 long by 10–32 wide, length relative to testis length 0.2–0.4 : 1.0; each band consisting of two columns, extending from posterior margin of anterior-most testes to near posterior margin of ovary (Fig. 1C). Uterus thin-walled, extending from ovarian isthmus to near anterior margin of proglottid (Fig. 1C). Eggs not observed.

3.4.1.2. Taxonomic summary

Type host: White skate, *Rostroraja alba* (Lacépède) (Rajiformes: Rajidae).

Type locality: Danger Point, Gansbaai, South Africa [34°28'50"S, 19°19'55"E].

Site of infection: Spiral intestine. Prevalence of infection: 50% (one of two skates examined).

Type material: Holotype deposited at NMB (Accession number: NMB P 833), paratypes in NMB (Accession numbers: NMB P 834 – NMB P 835), IPCAS (Accession numbers: IPCAS C-910) and MHNG (Accession numbers: MHNG-PLAT-013888).

Etymology: The species name "*umbungus*" is derived from "umbungu" [Xhosa; an indigenous language to the Eastern and Western Cape of South Africa] meaning "worm", referring to the species of tapeworm.

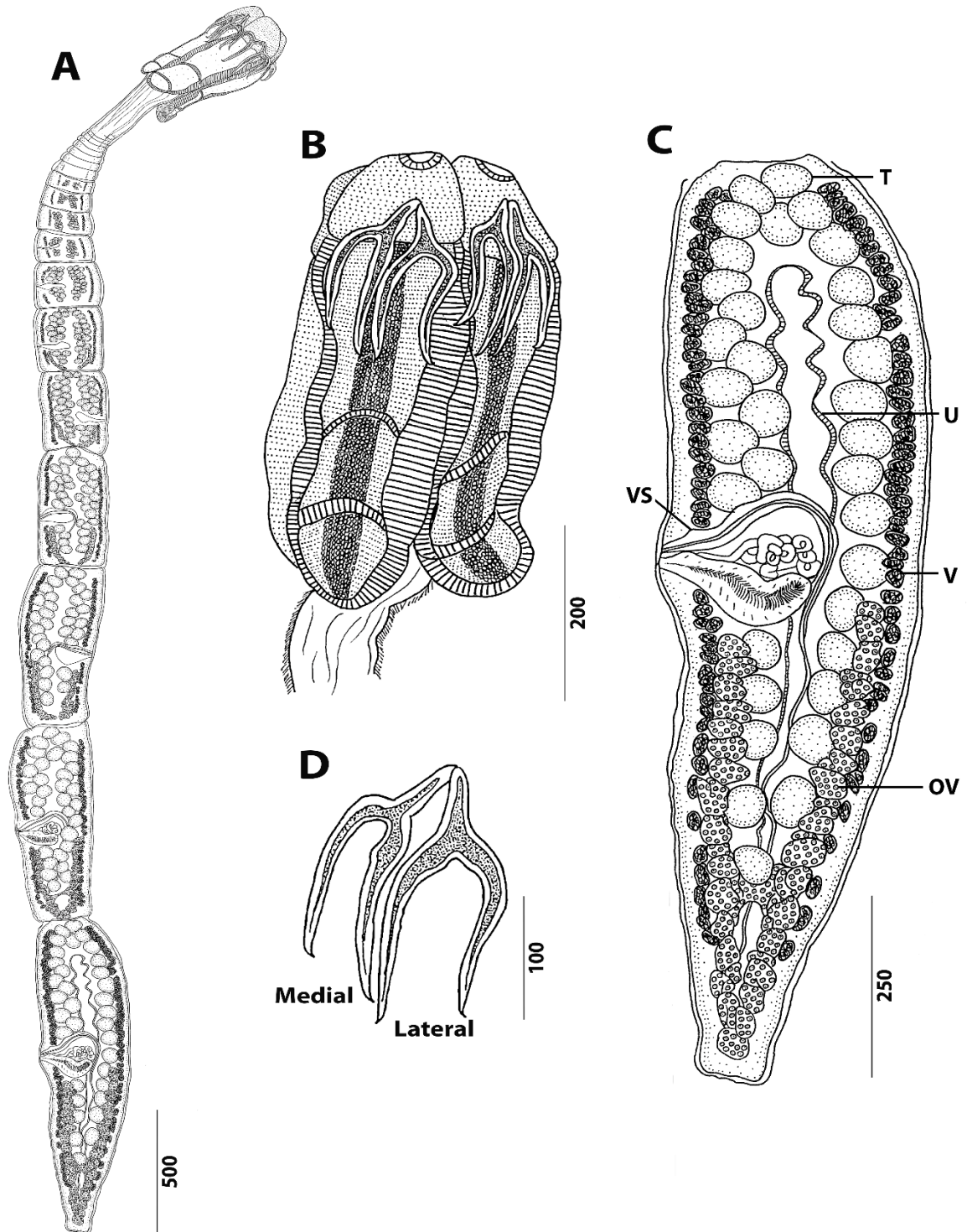


Fig 1. Line drawings of *Acanthobothrium umbungus* Van Der Spuy, Smit et Schaeffner, 2022. A – entire specimen; B – scolex; C – mature proglottid (VS, vaginal sphincter; T, testis; U, uterus; V, vitelline follicle; OV, ovary); D – hooks.

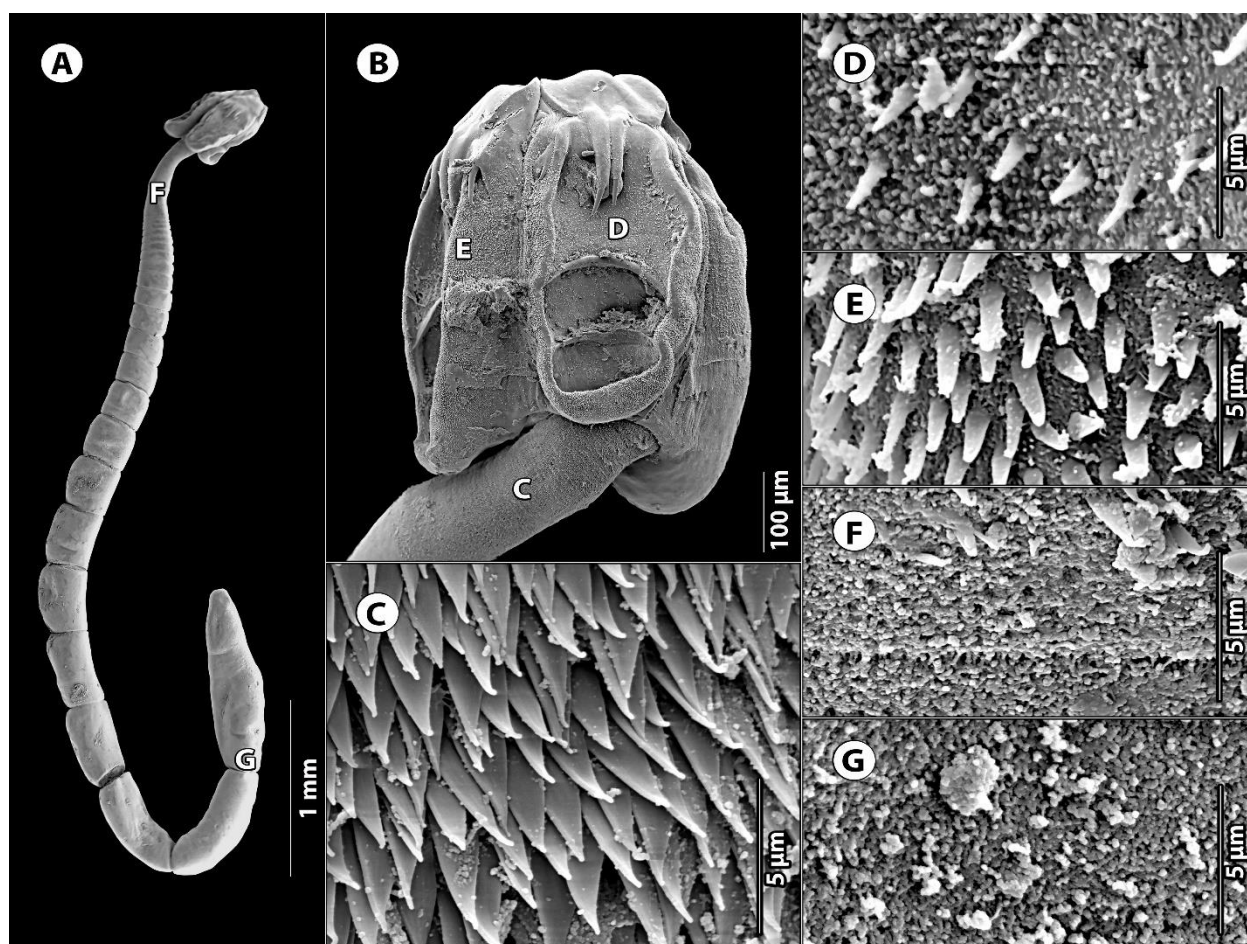


Fig. 2. Scanning electron micrographs of *Acanthobothrium umbungus* Van Der Spuy, Smit et Schaeffner, 2022. A – entire specimen, letters indicate where micrographs of microtriches were taken; B – scolex, letters indicate where micrographs of microtriches were taken; C – cephalic peduncle; D – distal bothridial surface; E – proximal bothridial surface, near medial margin of bothridium; F – first proglottid; G – anterior region of terminal proglottid.

3.4.1.3. Remarks

Following the description of four new species of *Acanthobothrium* by Van Der Spuy et al. (2020), a total of 207 valid species of *Acanthobothrium* are currently recognised worldwide. Ghoshroy and Caira (2001) developed a category classification system that facilitates the differentiation between congeners. *Acanthobothrium umbungus*, is a category 2 species (sensu Ghoshroy and Caira, 2001), with a relatively small body (<15 mm), few segments (<50 in number), few testes (<80 in number) and asymmetrical ovarian lobes. It was therefore compared to 51 congeners with the same category assignation. The most distinguishable feature of *A. umbungus* is its hooks, as the hooks of most congeners within this category are much smaller in size (lateral hooks: $B < 117 \mu\text{m}$ vs $127\text{--}158 \mu\text{m}$, $C < 102 \mu\text{m}$ vs $102\text{--}125 \mu\text{m}$, $D < 170 \mu\text{m}$ vs $183\text{--}208 \mu\text{m}$; medial hooks: $B' < 125 \mu\text{m}$ vs $131\text{--}162 \mu\text{m}$, $C' < 101 \mu\text{m}$ vs $101\text{--}121 \mu\text{m}$, $D' < 159 \mu\text{m}$ vs $180\text{--}216 \mu\text{m}$, respectively).

Based on the hook measurements, *A. umbungus* already differs from all but nine species, namely *A. annapienkensis* Carvajal et Goldstein (1971), *A. brayi* Campbell et Beveridge (2002), *A. domingae* Franzese et Ivanov, 2020, *A. gloveri* Campbell et Beveridge (2002), *A. guanghaiense* Yang, Sun, Zhi, Iwaki, Reyda et Yang (2016), *A. popi* Fyler, Caira et Jensen (2009), *A. ppdeleoni* Zaragoza-Tapia, Pulido-Flores et Monks, 2020, *A. tasajerasi* Brooks (1977), and *A. thomasaе* Campbell et Beveridge, 2002. The hooks of both *A. annapienkensis* and *A. domingae* exceed that of *A. umbungus* (lateral hooks: $B > 180\ \mu\text{m}$ vs $127\text{--}158\ \mu\text{m}$, $C > 125\ \mu\text{m}$ vs $102\text{--}125\ \mu\text{m}$, $D > 240\ \mu\text{m}$ vs $183\text{--}208\ \mu\text{m}$, respectively). Besides the differences in hook measurements, *A. brevissime* Linton (1908), *A. campbelli* Marques, Brooks et Monks, 1995, *A. edwardsi* Williams (1969), *A. lasti* Campbell et Beveridge, 2002, *A. minus* Tazerouti et al., 2009, *A. mooreae* Campbell et Beveridge, 2002, *A. quadripartitum* Williams (1968), *A. sphaera* Maleki, Malek et Palm, 2013, *A. stevensi* Campbell et Beveridge, 2002, *A. thomasaе*, *A. tripartitum* Williams (1968), and *A. zapteryicum* Ostrowski de Nunez, 1971, can also be differentiated from *A. umbungus* in the following features: a shorter body ($<2.4\ \text{mm}$ vs $2.4\text{--}8.9\ \text{mm}$, respectively), fewer proglottids (<13 vs $15\text{--}32$, respectively), and fewer testes (<25 vs $29\text{--}59$, respectively). By comparing the scolex length, *A. brevissime*, *A. campbelli*, *A. chisholmae* Campbell et Beveridge, 2002, *A. dujardini* van Beneden (1850), *A. edwardsi*, *A. hypanus* Zaragoza-Tapia, Pulido-Flores et Monks, 2020, *A. lasti*, *A. liliun* Baer et Euzet, 1962, *A. mashnihae* Fyler et Caira, 2006, *A. microhabentes* Van Der Spuy, Smit et Schaeffner, 2020, *A. microtenuis* Van Der Spuy, Smit et Schaeffner, 2020, *A. minus*, *A. mooreae*, *A. ppdeleoni*, *A. puntarenasense* Marques, Brooks et Monks, 1995, *A. sinaloensis* Zaragoza-Tapia, Pulido-Flores et Monks, 2020, *A. sphaera*, *A. tasajerasi*, *A. thomasaе*, *A. urotrygoni* Brooks et Mayes, 1980, *A. vargasi* Marques, Brooks et Monks, 1995, *A. walkeri* Campbell et Beveridge, 2002, and *A. zapteryicum*, all have a shorter scolex compared to *A. umbungus* ($<400\ \mu\text{m}$ vs $415\text{--}542\ \mu\text{m}$, respectively). Furthermore, *A. lasti*, *A. microhabentes*, *A. mooreae*, *A. puntarenasense*, *A. rajivi* Ghoshroy et Caira, 2001, *A. sinaloensis*, *A. sphaera*, and *A. urotrygoni* also differ from *A. umbungus* in the following features: bothridium width ($<112\ \mu\text{m}$ *A. rajivi*, respectively) and posterior loculus length ($<56\ \mu\text{m}$ vs $60\text{--}105\ \mu\text{m}$, respectively). *Acanthobothrium umbungus* can further be distinguished from *A. minus*, *A. mooreae*, *A. sphaera*, *A. tasajerasi*, and *A. thomasaе* as they all have a shorter cephalic peduncle ($<274\ \mu\text{m}$ vs $291\text{--}834\ \mu\text{m}$, respectively), whereas *A. chisholmae*, *A. cimari* Marques, Brooks et Monks, 1995, *A. crassus* Van Der Spuy, Smit et Schaeffner, 2020, *A. dolichocollum* Van Der Spuy, Smit et Schaeffner, 2020, and *A. dujardini* all have a longer cephalic peduncle ($>1000\ \mu\text{m}$ vs $291\text{--}834\ \mu\text{m}$, respectively). Furthermore, *A. carolinae* Franzese et Ivanov, 2020, *A. costarricense* Marques, Brooks et Monks, 1995, *A. guanghaiense* and *A. puntarenasense* all have a wider cephalic peduncle than that of *A. umbungus* ($>103\ \mu\text{m}$ vs $62\text{--}97\ \mu\text{m}$, respectively). Regarding the cirrus-sac and ovary, the length of the cirrus-sac of *A. campbelli*, *A. chisholmae*, *A. mashnihae*, *A. microhabentes*, *A. minus*, *A. soniae* Zaragoza-Tapia, Pulido-Flores, Violante-Gonzalez et Monks, 2019, *A. tetabuanense* Reyda et Caira, 2006, and *A. tripartitum* is $< 100\ \mu\text{m}$ while that of *A.*

umbungus measures 107–164 µm; the width of the cirrus-sac of *A. brachyacanthum* Riser (1955), *A. costarricense* and *A. olsenii* Dailey et Mudry, 1968 is > 84 µm versus that of *A. umbungus* with 35–71 µm; and the ovary width of *A. campbelli*, *A. costarricense*, *A. gloveri*, *A. semnovesiculum* Verma (1928), and *A. thomasa* are all <53 µm while that of *A. umbungus* ranges between 60 and 124 µm. *Acanthobothrium umbungus* can also be distinguished from *A. bobconniorum* Fyler et Caira, 2010, *A. crassus*, *A. dolichocollum*, *A. microhabentes*, *A. microtenuis*, and *A. popi* by its lack in testes posterior to the ovarian isthmus. Only *A. urotrygoni* and *A. woodsholei* Baer (1948) have a larger body size than *A. umbungus* with >12 mm versus 2.4–8.9 mm (respectively). Additionally, *A. annapienkensis*, *A. brayi*, *A. bullardi* Ghoshroy et Caira, 2001, *A. domingae* and *A. woodsholei* differ from *A. umbungus* in the following features: scolex length (>560 µm vs 415–542 µm, respectively), bothridium width (>176 µm vs 118–154 µm, respectively) and cirrus-sac width (>85 µm vs 35–71 µm, respectively). More species containing a wider bothridium than *A. umbungus* are *A. carolinae*, *A. chisholmae*, and *A. costarricense* (>161 µm vs 118–154 µm, respectively). *Acanthobothrium bobconniorum*, *A. cimari*, *A. costarricense*, *A. crassus*, *A. dujardini*, *A. hypanus*, *A. popi*, *A. puntarenenses* and *A. semnovesiculum* all have more testes than *A. umbungus* (>36 vs 29–36, respectively).

Acanthobothrium umbungus represents the third species of *Acanthobothrium* and the seventh cestode record from this host. In southern Africa, only four species of *Acanthobothrium* are currently known (Van Der Spuy et al., 2020). Including *A. umbungus*, it not only increases the number of species of this genus in the Eastern South Atlantic Ocean but also marks southern Africa as an understudied biogeographical region with the potential of an immense hidden parasite diversity.

3.4.2. *Acanthobothrium usengozinius* Van Der Spuy, Smit et Schaeffner, 2022 (Figs. 3 and 4)

ZooBank number for species: urn: lsid: zoobank.org: act: 4FD538E4-5659-4097-8164-A5B7E0E4E758.

3.4.2.1. Description

Based on whole mounts of five mature and two immature worms; two mature worms examined with SEM: Worms 6.3–8.8 mm long, greatest width at level of scolex, 32–45 proglottids per worm, euapolytic. Scolex consisting of scolex proper and cephalic peduncle. Scolex proper with four bothridia, 540–631 long by 372–508 wide. Bothridia free posteriorly, 215–245 wide; each bothridium with three loculi and specialised anterior region in form of muscular pad. Muscular pad 78–109 long by 139–163 wide, falciform in shape, with pronounced posterior margin, bearing

accessory sucker and one pair of hooks at posterior margin; accessory sucker 23–26 long by 43–54 wide. Anterior loculus (A) 235–257 long; middle loculus (M) 126–143 long; posterior loculus (P) 94–106 long; loculus length ratio (A: M: P) 1.00 : 0.54: 0.41; maximum width of scolex at level of middle loculus. Velum absent.

Hooks bi-pronged, hollow, with tubercle on proximal surface of axial prongs; internal channels of axial and abaxial prongs continuous, smooth; axial prongs slightly longer than abaxial prongs; lateral and medial hooks approximately equal in size. Lateral hook measurements: *A* 62–73, *B* 135–151, *C* 129–141, *D* 190–216. Medial hook measurements: *A'* 64–74, *B'* 139–162, *C'* 125–142, *D'* 193–228. Bases of lateral and medial hooks approximately equal in length; base of lateral hook slightly overlapping base of medial hook along medial axis of bothridium (Fig. 3D); medial hook base slightly wider than lateral hook base. Tissue covering almost entire length of each prong of hooks. Short cephalic peduncle 572–1080 long by 119–124 wide.

Cephalic peduncle densely covered with gladiate spinitriches, filitriches not observed (Fig. 4D). Apical pad and distal bothridial surface covered with acicular filitriches and very sparsely interspersed gladiate spinitriches (Fig. 4E). Proximal bothridial surface and bothridial rims covered with gladiate spinitriches, interspersed with acicular filitriches (Fig. 4F). Anterior region of strobila covered in acicular filitriches (Fig. 4G). Anterior region of terminal proglottid covered in capilliform filitriches (Fig. 4H).

Proglottids acraspedote. Immature proglottids 29–44 in number; 1–3 mature proglottids; gravid proglottids absent; terminal proglottid 795–1538 long by 275–394 wide; terminal proglottid length to width ratio 2.5–4.6 : 1.0. Proglottids protandrous; genital pores marginal, irregularly alternating (Fig. 3A), 42–58% of proglottid length from posterior margin.

Testes conspicuous in mature proglottids, oval in dorsoventral view, 42–63 long by 42–51 wide, arranged in one to two layers in intervacular field (Fig. 3C), 42–61 in total number, 0 in post-poral field of mature proglottids, 4–5 in post-poral field of immature proglottids. Cirrus-sac obpyriform (Fig. 3C), 151–234 long by 43–57 wide, containing armed cirrus; cirrus greatly expanded at base.

Vagina narrow, relatively thin-walled and straight proximally, extending from ootype along medial line of proglottid to anterior margin of cirrus-sac, then laterally to common genital atrium. Vaginal sphincter prominent (Fig. 3C). Ovary occupying about half of proglottid length, almost reaching posterior margin of proglottid, H-shaped in dorsoventral view, lobulated (Fig. 3C), asymmetrical, 143–180 wide at level of ovarian isthmus; poral lobe 548–679 in length; aporal lobe 616–757 in

length; ovarian lobes not reaching cirrus-sac anteriorly; ovarian isthmus located posterior to mid-level of ovary. Mehlis' gland posterior to ovarian isthmus.

Vitellarium follicular to lobulated; follicles in two lateral bands, 27–35 long by 17–20 wide, length relative to testis length 0.4–0.6 : 1.0; each band consisting of two columns, extending from posterior margin of anterior-most testes to near posterior margin of ovary (Fig. 3C). Uterus thin-walled, extending from ovarian isthmus to near anterior margin of proglottid. Eggs not observed.

3.4.2.2. Taxonomic summary

Type host: White skate, *Rostroraja alba* (Lacépède) (Rajiformes: Rajidae).

Type locality: Danger Point, Gansbaai, South Africa [34°28'50"S, 19°19'55"E].

Site of infection: Spiral intestine. Prevalence of infection: 50% (one of two skates examined).

Type material: Holotype deposited at NMB (Accession number: NMB P 836), paratypes in NMB (Accession numbers: NMB P 837 – NMB P 838), IPCAS (Accession numbers: IPCAS C-911) and MHNG (Accession numbers: MHNG-PLAT-013889).

Etymology: The species name "*usengozinius*" is derived from "usengozini" [Xhosa; an indigenous language to the Eastern and Western Cape of South Africa] meaning "endangered", referring to the threatened status of both the definitive host *Rostroraja alba*, as well as its host-specific parasite.

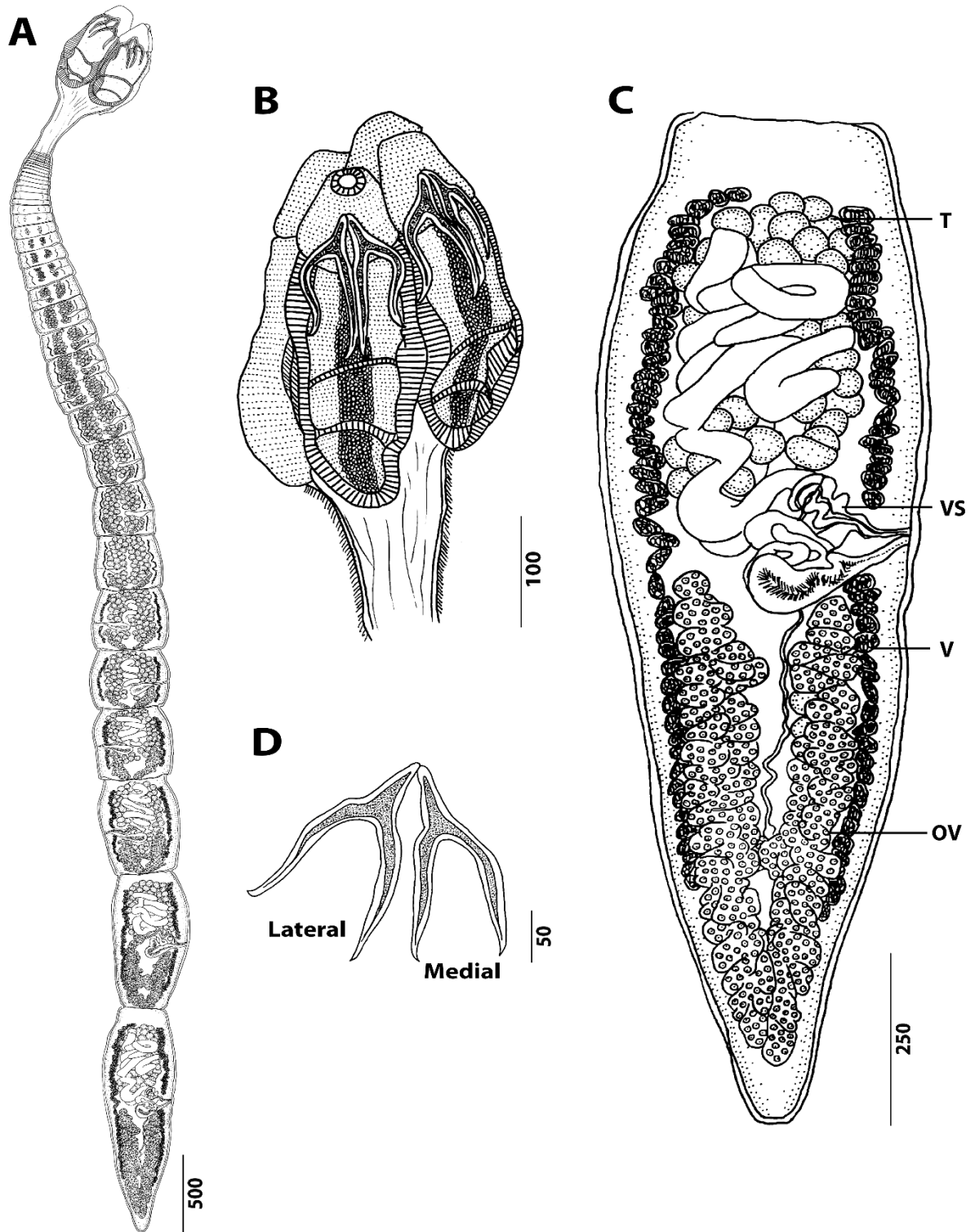


Fig. 3. Line drawings of *Acanthobothrium usengozius* Van Der Spuy, Smit et Schaeffner, 2022. A – entire specimen; B – scolex; C – mature proglottid (VS, vaginal sphincter; T, testis; V, vitelline follicle; OV, ovary); D – hooks.

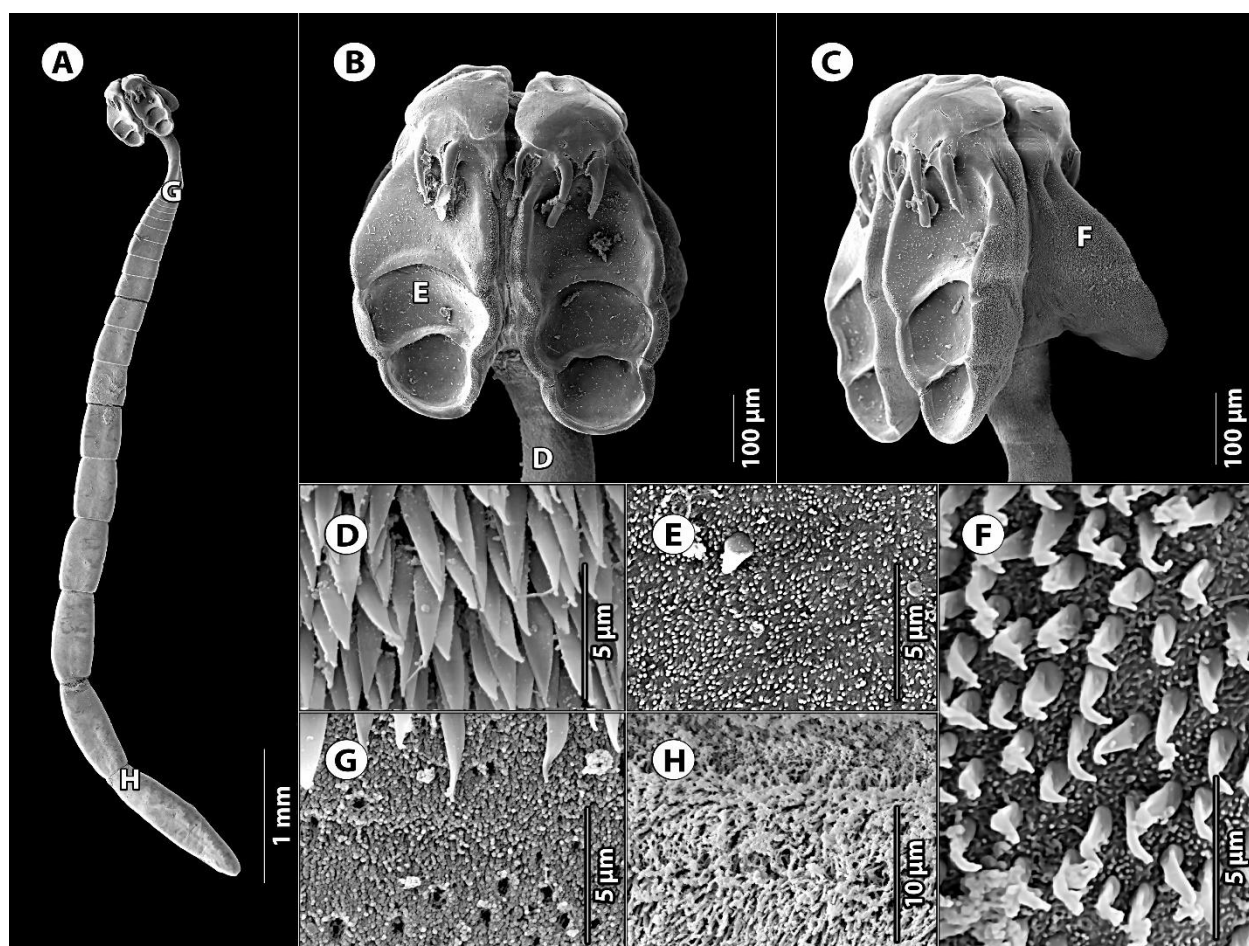


Fig. 4. Scanning electron micrographs of *Acanthobothrium usengozinius* Van Der Spuy, Smit et Schaeffner, 2022. A – entire specimen, letters indicate where micrographs of microtriches were taken; B – scolex, dorsoventral view, letter indicates where micrographs of microtriches were taken; C – scolex, lateral view, letters indicate where micrographs of microtriches were taken; D – cephalic peduncle; E – distal bothridial surface; F – proximal bothridial surface, near medial margin of bothridium; G – first proglottid; H – anterior region of terminal proglottid.

3.4.2.3. Remarks

Acanthobothrium usengozinius, is a category 2 species (sensu Ghoshroy and Caira, 2001). Similar to the description of *A. umbungus*, hooks of *A. usengozinius* are the most prominent feature, instantly distinguishing it from all but five (i.e. *A. annapienkensis*, *A. brayi*, *A. domingae*, *A. guanghaiense* and *A. umbungus*) of the 52 representatives within category 2. The remaining category 2 species present much smaller hook measurements than those of the new species, as follows: lateral hooks: A < 62 µm vs 62–73 µm, B < 130 µm vs 135–151 µm, C < 124 µm vs 129–141 µm, D < 178 µm vs 190–216 µm, respectively; medial hooks: A' < 64 µm vs 64–74 µm, B' < 136 µm vs 139–162 µm, C' < 122 µm vs 125–142 µm, D' < 193 µm vs 193–228 µm, respectively. The only species with larger hooks than *A. usengozinius* is *A. annapienkensis* (lateral hooks A

>73 µm vs 62–73 µm, *B* > 180 µm vs 139–162 µm, *C* > 160 µm vs 125–142 µm, *D* > 240 µm vs 193–228 µm, respectively). Apart from hook measurements, *A. benedenii* Lönnberg, 1889, , *A. bobconniorum*, *A. brachyacanthum*, *A. brayi*, *A. brevissime*, *A. campbelli*, *A. carolinae*, *A. chisholmae*, *A. dasi* Ghoshroy et Caira, 2001, *A. domingae*, *A. dujardini*, *A. edwardsi*, *A. gloveri*, *A. lasti*, *A. lilium*, *A. mashnihae*, *A. microhabentes*, *A. microtenuis*, *A. minus*, *A. mooreae*, *A. ocallaghani* Campbell et Beveridge, 2002, *A. olsenii*, *A. ppdeleoni*, *A. rajivi*, *A. sinaloansis*, *A. sphaera*, *A. stevensi*, *A. tasajerasi*, *A. tetabuanense*, *A. thomasaе*, *A. tripartitum*, *A. quadripartitum*, *A. vargasi*, *A. walkeri* and *A. zapterycum* can all be distinguished from *A. usengozinius* by a much smaller body size (<6 mm vs 6.3–8.8 mm, respectively), whereas *A. urotrygoni* and *A. woodsholei* are significantly larger (>12 mm vs 6.3–8.8 mm, respectively). Additionally, *A. benedenii*, *A. bobconniorum*, *A. brachyacanthum*, *A. brevissime*, *A. campbelli*, *A. cimari*, *A. costaricense*, *A. crassus*, *A. dolichocollum*, *A. dujardini*, *A. edwardsi*, *A. hypanus*, *A. lasti*, *A. lilium*, *A. mashnihae*, *A. microhabentes*, *A. microtenuis*, *A. minus*, *A. mooreae*, *A. ocallaghani*, *A. olsenii*, *A. ppdeleoni*, *A. quadripartitum*, *A. semnovesiculum*, *A. sinaloansis*, *A. soniae*, *A. sphaera*, *A. stevensi*, *A. tasajerasi*, *A. tetabuanense*, *A. thomasaе*, *A. umbungus*, *A. urotrygoni*, *A. vargasi*, *A. walkeri*, and *A. zapterycum* show differences in the following features: scolex length (<540 µm vs 540–631 µm, respectively), bothridium width (<214 µm vs 215–245 µm, respectively), cephalic peduncle width (<115 µm vs 119–124 µm, respectively), and ovary width (<116 µm vs 143–180 µm, respectively). In comparison *A. annapienkensis*, *A. bullardi* and *A. woodsholei* all have a longer scolex (>633 µm vs 540–631 µm, respectively). The poral ovarian lobe of *A. bobconniorum*, *A. brachyacanthum*, *A. brayi*, *A. campbelli*, *A. carolinae*, *A. chisholmae*, *A. dasi*, *A. domingae*, *A. gloveri*, *A. guanghaiense*, *A. hypanus*, *A. lasti*, *A. mashnihae*, *A. microhabentes*, *A. microtenuis*, *A. mooreae*, *A. ocallaghani*, *A. ppdeleoni*, *A. rajivi*, *A. sinaloansis*, *A. sphaera*, *A. stevensi*, *A. tasajerasi*, *A. tetabuanense*, *A. thomasaе*, *A. urotrygoni*, *A. vargasi*, and *A. walkeri* are shorter than that of *A. usengozinius* (<525 µm vs 548–679 µm, respectively). The same applies for the aporal lobe (<565 µm vs 616–757 µm, respectively). The following species all have a wider cirrus-sac than that of *A. usengozinius* (>60 µm vs 43–57 µm): *A. annapienkensis*, *A. bobconniorum*, *A. brachyacanthum*, *A. brayi*, *A. bullardi*, *A. costaricense*, *A. dolichocollum*, *A. domingae*, *A. mooreae*, *A. olsenii*, *A. popi*, *A. ppdeleoni*, *A. rajivi*, *A. semnovesiculum*, *A. soniae*, *A. thomasaе* and *A. urotrygoni*. *Acanthobothrium cimari*, *A. costaricense*, *A. crassus*, *A. dolichocollum*, *A. guanghaiense*, *A. hypanus*, *A. puntarenasense*, *A. semnovesiculum* and *A. soniae* also differ from *A. usengozinius* in a number of features such as: a narrower bothridium (<214 µm vs 215–245 µm, respectively), a shorter anterior loculus (<205 µm vs 235–257 µm, respectively), a shorter middle loculus (<90 µm vs 126–143 µm, respectively), a shorter posterior loculus (<91 µm vs 94–106 µm, respectively), and a narrower ovary (<120 µm vs 143–180 µm, respectively).

Acanthobothrium usengozinius marks the fourth species described from the endangered host, *R. alba*, expanding the remarkable host specificity within the genus. This discovery therefore subsequently marks the importance of dedicating appropriate research to endangered elasmobranch species, as macrohabitats of numerous parasitic organisms new to science that are threatened by co-extinction. *Acanthobothrium usengozinius* also marks the sixth species of this genus from the Eastern South Atlantic Ocean.

3.4.3. *Acanthobothrium ulondolozus* Van Der Spuy, Smit et Schaeffner, 2022 (Figs. 5 and 6)

ZooBank number for species: urn: lsid: zoobank.org: act: 94EA6A1C-A24B-4B9B-ADF3-13A6BFCE6D36.

3.4.3.1. Description

Based on whole mounts of seven mature worms; two mature worms examined with SEM: Worms 9.3–13.5 mm long, greatest width at level of scolex, 32–50 proglottids per worm, euapolytic. Scolex consisting of scolex proper and cephalic peduncle. Scolex proper with four bothridia, 486–599 long by 324–520 wide. Bothridia free posteriorly, 168–190 wide; each bothridium with three loculi and specialised anterior region in form of muscular pad. Muscular pad 128–150 long by 146–162 wide, falciform in shape, with pronounced posterior margin, bearing accessory sucker and one pair of hooks at posterior margin; accessory sucker 27–37 long by 35–48 wide. Anterior loculus (A) 201–212 long; middle loculus (M) 72–120 long; posterior loculus (P) 97–113 long; loculus length ratio (A: M: P) 1.00 : 0.53: 0.52; maximum width of scolex at level of middle loculus. Velum absent.

Hooks bi-pronged, hollow, with tubercle on proximal surface of axial prongs; internal channels of axial and abaxial prongs continuous, smooth; axial prongs slightly longer than abaxial prongs; lateral and medial hooks approximately equal in size. Lateral hook measurements: *A* 60–75, *B* 154–160, *C* 131–144, *D* 212–220. Medial hook measurements: *A'* 62–73, *B'* 144–165, *C'* 121–144, *D'* 203–211. Bases of lateral and medial hooks approximately equal in length; base of medial hook slightly overlapping base of lateral hook along medial axis of bothridium (Fig. 5D); medial hook base slightly wider than lateral hook base. Tissue covering almost entire length of each prong of hooks. Short cephalic peduncle 1044–1430 long by 76–98 wide.

Cephalic peduncle densely covered with gladiate spinitriches, filitriches not observed (Fig. 6C). Apical pad and distal bothridial surface covered with papilliform to acicular filitriches and extremely sparsely interspersed gladiate spinitriches (Fig. 6D). Proximal bothridial surface and bothridial

rims covered with gladiate spinitriches, interspersed with acicular filitriches (Fig. 6E). Anterior region of strobila covered in acicular filitriches (Fig. 6F). Anterior region of terminal proglottid covered in capilliform filitriches (Fig. 6G).

Proglottids acraspedote. Immature proglottids 29–48 in number; 2–3 mature proglottids; gravid proglottids absent; terminal proglottid 1063–2134 long by 284–356 wide; terminal proglottid length to width ratio 3.2–6.3 : 1.0. Proglottids protandrous; genital pores marginal, irregularly alternating (Fig. 5A), 48–59% of proglottid length from posterior margin.

Testes conspicuous in mature proglottids, oval in dorsoventral view, 50–61 long by 40–52 wide, arranged in one to two layers in intervascular field (Fig. 5C), 50–62 in total number, 8–9 in post-poral field; some segments with single testis posterior to ovarian isthmus. Cirrus-sac J-shaped, tilted posteriorly (Fig. 5C), 171–232 long by 60–73 wide, containing armed cirrus; cirrus greatly expanded at base.

Vagina narrow, relatively thin-walled and straight proximally, extending from ootype along medial line of proglottid to anterior margin of cirrus-sac, then laterally to common genital atrium. Vaginal sphincter prominent (Fig. 5C). Ovary occupying about half of proglottid length, almost reaching posterior margin of proglottid, H-shaped in dorsoventral view, lobulated (Fig. 5C), asymmetrical, 107–149 wide at level of ovarian isthmus; poral lobe 431–981 in length; aporal lobe 503–1133 in length; ovarian lobes not reaching cirrus-sac anteriorly; ovarian isthmus located posterior to mid-level of ovary. Mehlis' gland posterior to ovarian isthmus.

Vitellarium follicular to lobulated; follicles in two lateral bands, 13–21 long by 23–43 wide, length relative to testis length 0.2–0.4 : 1.0; each band consisting of two columns, extending from posterior margin of anterior-most testes to near posterior margin of ovary (Fig. 5C). Vitelline follicle length relative to testis length 0.2–0.4 : 1.0. Uterus thin-walled, extending from ovarian isthmus to near anterior margin of proglottid. Eggs not observed.

3.4.3.2. Taxonomic summary

Type host: White skate, *Rostroraja alba* (Lacépède) (Rajiformes: Rajidae).

Type locality: Danger Point, Gansbaai, South Africa [34°28'50"S, 19°19'55"E].

Site of infection: Spiral intestine. Prevalence of infection: 50% (one of two skates examined).

Type material: Holotype deposited at NMB (Accession number: NMB P 839), paratypes in NMB (Accession numbers: NMB P 840 – NMB P 841), IPCAS (Accession numbers: IPCAS C-912) and MHNG (Accession numbers: MHNG-PLAT -013889).

Etymology: The species name “*ulondolozus*” is derived from “ulondolozo” [Xhosa; an indigenous language to the Eastern and Western Cape of South Africa] meaning “conservation”, referring to the need for having better conservation plans for threatened elasmobranch species, which would also protect a wide variety of host-specific affiliate species facing an increased risk of co-extinction.

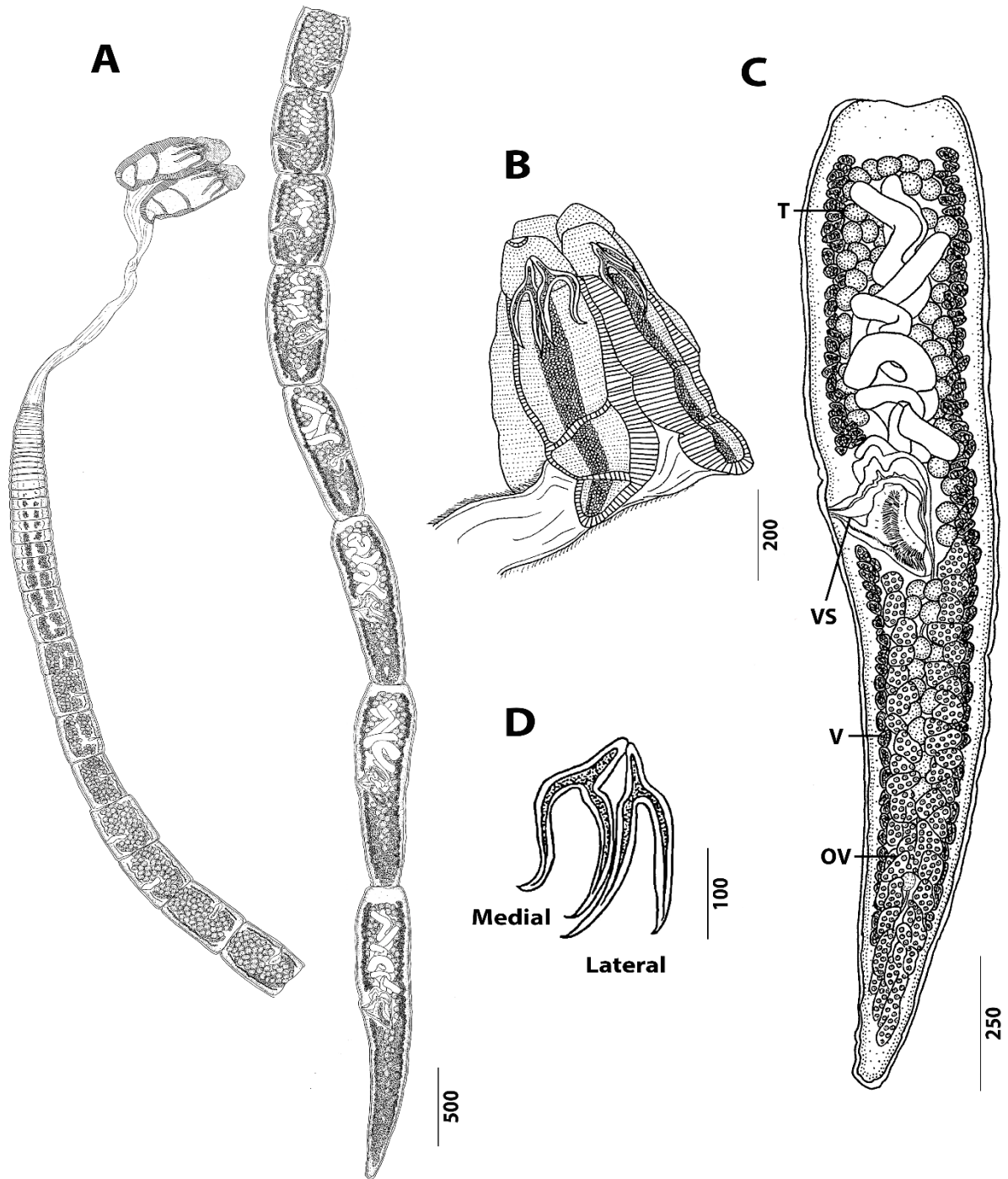


Fig. 5. Line drawings of *Acanthobothrium ulondolozus* Van Der Spuy, Smit et Schaeffner, 2022. A – entire specimen; B – scolex; C – mature proglottid (VS, vaginal sphincter; T, testis; V, vitelline follicle; OV, ovary); D – hooks.

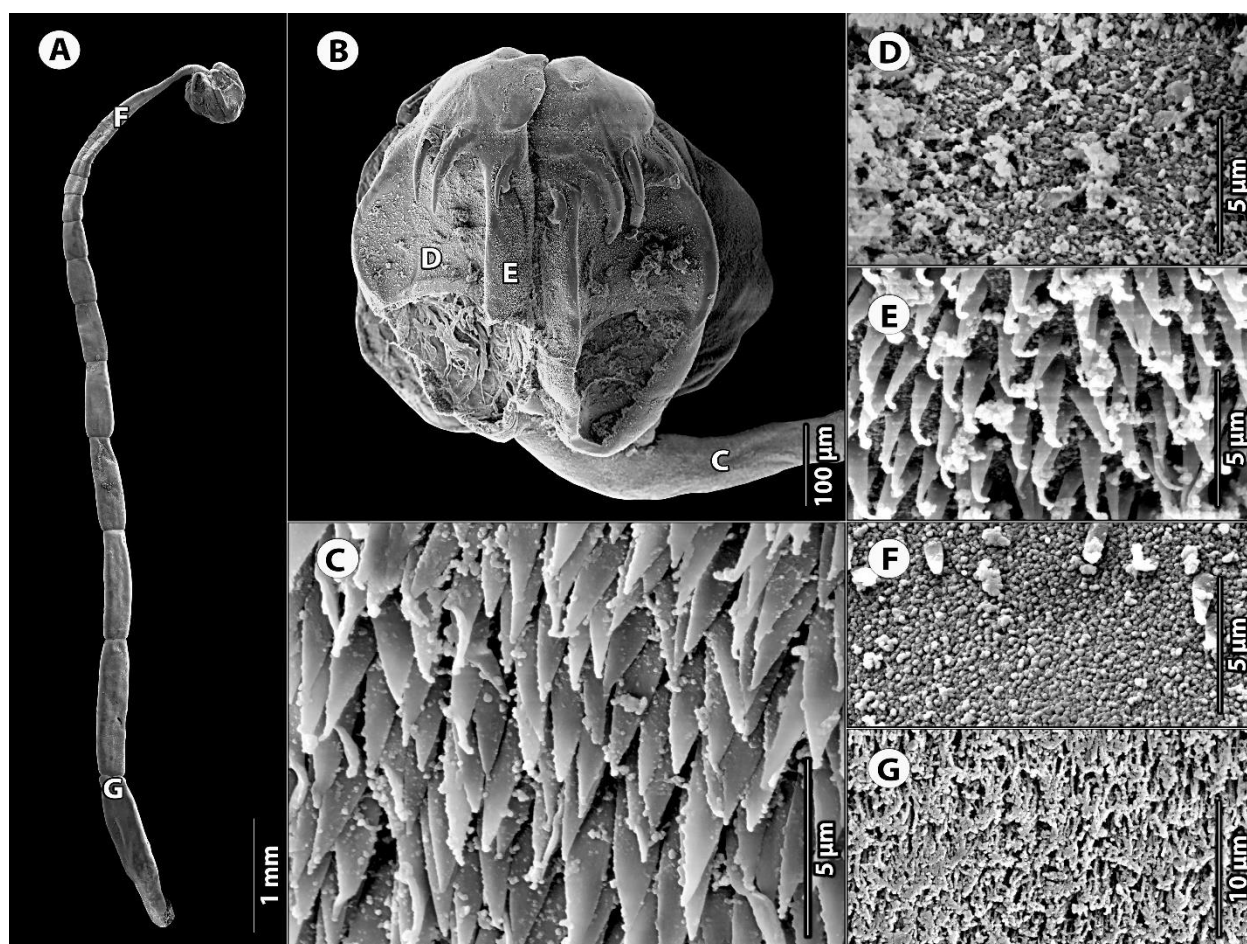


Fig. 6. Scanning electron micrographs of *Acanthobothrium ulondolozus* Van Der Spuy, Smit et Schaeffner, 2022. A – entire specimen, letters indicate where micrographs of microtriches were taken; B – scolex, letters indicate where micrographs of microtriches were taken; C – cephalic peduncle; D – distal bothridial surface; E – proximal bothridial surface, near medial margin of bothridium; F – first proglottid; G – anterior region of terminal proglottid.

3.4.3.3. Remarks

All of the new species described in the present study seem most distinguishable by their hooks. Just as *Acanthobothrium umbungus* and *A. usengozinius*, *A. ulondolozus*, is identified as a category 2 species. However, unlike the two new congeners, *A. ulondolozus* is the only species in the present study with occasional testes posterior to the ovarian isthmus. This is still regarded as an exceptional feature among species of *Acanthobothrium*, being present in less than 10% of all species recognised within this genus worldwide. Hence, *A. ulondolozus* can easily be distinguished from all but 16 congeners (across all categories). From the remaining 16 species of *Acanthobothrium* known to bear this feature, only six are identified as category 2 species, namely *A. bobconniorum*, *A. crassus*, *A. dolichocollum*, *A. microhabentes*, *A. microtenuis*, and *A. popi*. *Acanthobothrium microhabentes* and *A. microtenuis* were only recently described from the same

biogeographical region by Van Der Spuy et al. (2020). However, both species differ from *A. ulondolozus* in smaller metrical features (i.e. total length, scolex length, scolex width, bothridium width, lateral hooks, medial hooks, cephalic peduncle width, cirrus-sac length, poral and aporal ovarian length, ovarian width; see Van Der Spuy et al., 2020), while *A. bobconniorum*, *A. crassus*, *A. dolichocollum* and *A. popi* differ from *A. ulondolozus* in the following features: smaller lateral hooks ($A < 60 \mu\text{m}$ vs $60\text{--}75 \mu\text{m}$, $B < 120 \mu\text{m}$ vs $154\text{--}160 \mu\text{m}$, $C < 100 \mu\text{m}$ vs $131\text{--}144 \mu\text{m}$, $D < 175 \mu\text{m}$ vs $212\text{--}220 \mu\text{m}$, respectively), smaller medial hooks ($A' < 60 \mu\text{m}$ vs $62\text{--}73 \mu\text{m}$, $B' < 119 \mu\text{m}$ vs $144\text{--}165 \mu\text{m}$, $C' < 108 \mu\text{m}$ vs $121\text{--}144 \mu\text{m}$, $D' < 175 \mu\text{m}$ vs $203\text{--}211 \mu\text{m}$, respectively), a shorter terminal proglottid ($< 1320 \mu\text{m}$ vs $1663\text{--}2134 \mu\text{m}$, respectively), a narrower terminal proglottid ($< 260 \mu\text{m}$ vs $284\text{--}356 \mu\text{m}$, respectively), a shorter cirrus-sac ($< 152 \mu\text{m}$ vs $171\text{--}232 \mu\text{m}$, respectively) and smaller testes ($< 50 \mu\text{m}$ vs $50\text{--}61 \mu\text{m}$, respectively). Furthermore, *A. bobconniorum* and *A. popi* differ from *A. ulondolozus* in the following features: total length ($< 7.1 \text{ mm}$ vs $9.3\text{--}13.5 \text{ mm}$, respectively), cephalic peduncle length ($< 650 \mu\text{m}$ vs $1044\text{--}1430 \mu\text{m}$, respectively), and testes length ($< 50 \mu\text{m}$ vs $50\text{--}61 \mu\text{m}$, respectively). Both *A. crassus* and *A. dolichocollum* have a shorter scolex than *A. ulondolozus* ($< 450 \mu\text{m}$ vs $486\text{--}599 \mu\text{m}$, respectively). Additionally, the width of both the bothridium and cephalic peduncle can also be used to distinguish *A. ulondolozus* from *A. dolichocollum*, as the latter species has a narrower bothridium ($< 134 \mu\text{m}$ vs $168\text{--}190 \mu\text{m}$, respectively), and a narrower cephalic peduncle ($< 73 \mu\text{m}$ vs $76\text{--}98 \mu\text{m}$, respectively). Species of *Acanthobothrium* possessing testes posterior to the ovarian isthmus seem to be restricted to the families Dasyatidae (i.e. *Himantura* Müller et Henle), Rhinidae (i.e. *Rhynchobatus* Müller et Henle), Rhinobatidae (i.e. *Rhinobatos* Linck) and Rajidae (i.e. *Raja* Linnaeus), with the latter family including the host observed in the present study. The addition of yet another South African congener, *A. ulondolozus*, not only brings the total of species known to possess this remarkable feature to 17 species (across all categories), but also subsequently confirms Van Der Spuy et al.'s (2020) statement that this feature is not limited to the Indo-Pacific Ocean. Moreover, the plausibility regarding the relatedness of *A. ulondolozus* with other congeners only invites further research engagement. The fact that *A. ulondolozus* is synhospitalic with two other congeners and sharing a rare morphological feature with only 16 other species of *Acanthobothrium* worldwide (i.e. four from South Africa), further supports the need for future research into a more comprehensive phylogenetic analysis of these cestodes. Gathering information on the molecular phylogeny could prove if species presenting testes located in this region might in fact comprise their own monophyletic clade (Fyler and Caira, 2010), along with possible effects caused by regional geographical influences (i.e. the Eastern South Atlantic Ocean vs. the Indo-Pacific Ocean), given that adult species of *Acanthobothrium* are restricted to the same geographical limits portrayed by their hosts (Zaragoza-Tapia et al., 2020a).

Acanthobothrium ulondolozus is the fifth cestode species known to parasitise the endangered white skate, *R. alba*, and the first cestode species infecting this host bearing testes posterior to

the ovarian isthmus. This adds *R. alba* to the list of elasmobranch species known to host species of *Acanthobothrium* with this particularly rare morphological feature. Furthermore, *A. ulondolozus* represents the seventh species known from the south-eastern Atlantic Ocean off the coast of southern Africa, and the fifth species from this region with testes posterior to the ovarian isthmus, a feature previously thought to be restricted to the Indo-Pacific Ocean (Fyler et al., 2009; Fyler and Caira, 2010; Maleki et al., 2015).

3.5. Discussion

Cestodes constitute the most biodiverse metazoan parasite group infecting elasmobranchs worldwide (Caira and Healy, 2004). They fulfil vital roles in trophic systems and the health of a marine ecosystem, subsequently rendering them indispensable in ensuring healthy and more resilient marine ecosystems (Beer et al., 2019). However, researchers only dedicate limited attention to this group, leaving large gaps in taxonomic, biological, and ecological research as well as species conservation efforts (Caira and Healy, 2004; Poulin and Presswell, 2016; Randhawa and Poulin, 2020). With merely 40% of all known elasmobranch species examined for cestode infections (Caira and Jensen, 2017), parasitological research efforts have been extremely sparse in many hosts and regions of the world. This is grounded upon the fact that many research initiatives are greatly biased towards individual, more acknowledged elasmobranch host groups (Poulin and Presswell, 2016), and exacerbated by limited financial means and research expertise in specific regions, which are occupied by many endemic organisms with narrow geographical ranges (Randhawa et al., 2015; Randhawa and Poulin, 2020). This is especially true for biogeographical regions surrounding the South-eastern Atlantic Ocean.

The ocean basins surrounding Southern Africa present a high diversity of elasmobranch host species (Ebert and van Hees, 2015). However, only 19 out of 204 elasmobranch species (i.e. 9 % of the species diversity) reported from Southern Africa have been observed for parasites (Schaeffner and Smit, 2019; Van Der Spuy et al., 2020). Given that each elasmobranch species hosts several unique cestode species (Caira and Healy, 2004; Schaeffner and Smit, 2019), there is little doubt that future parasitological studies will reveal an immense hidden diversity of parasitic organisms, particularly cestodes, in this host group. This, in turn, might strengthen the conservation of large, apex-predator species and their numerous affiliated species, and ultimately the preservation of threatened host-parasite systems.

The present study describes three, host-specific species of onchoproteocephalidean cestodes of the genus *Acanthobothrium*. Merely 7% (16 of 207) of all species of this genus have been reported

from this region (see Zaragoza-Tapia et al., 2020a). The new species from South Africa further increase the diversity of this already very diverse genus, with a new total of 210 valid species worldwide (Caira et al., 2017; Franzese and Ivanov, 2018, 2020; Maleki et al., 2018, 2019; Rodríguez-Ibarra et al., 2018; Zaragoza-Tapia et al., 2019, 2020b; Van Der Spuy et al., 2020). Unfortunately, as a result of observing a limited number of specimens from an endangered host species, no specimens were recovered from ethanol-fixed material in order to conduct a proper molecular analysis. For this reason, the phylogenetic relationships of these congeners are presently unknown. Globally, there is still a great shortfall in molecular data regarding species belonging to this genus, supported by the fact that the GenBank database contain molecular sequences and records of merely 7.6% (16 of 210) of species of *Acanthobothrium* (Zaragoza-Tapia et al., 2020a). The information gained through phylogenetic analyses will not only address phylogenetic placement of parasite lineages, but further advance our knowledge on evolutionary host-parasite interrelationships, along with host-parasite co-speciation (Beer et al., 2019), and relevant niche expertise (Zaragoza-Tapia et al., 2020a).

The species described herein were recovered from an endangered host species, the white skate, *R. alba*. Apart from the alarming fact that this species is listed in the second-highest category in the International Union for Conservation of Nature's (IUCN) Red List of Threatened Species, with major implications on the conservation status and future conservation efforts of its affiliated (and host-specific parasite) species, it is a prime example that demands further research, contributing essential, valuable insights regarding deficient ecological data (Sousa et al., 2019). This study marks the first parasitological observation of this species in a different biogeographic region along its distributional range and the first from Southern Africa. Prior to this study, this endangered species has been sparsely screened for cestode parasites, exclusively under its former name *Raja marginata* Lacépède, 1803 and material collected in the Mediterranean Sea (see Baer, 1948; Euzet, 1959; Goldstein, 1967; Williams, 1969; Zaragoza-Tapia et al., 2020a). At present, only six, valid species of cestodes were reported from this host along its entire distributional range, including one diphyllidean species, *Echinobothrium affine* Diesing, 1863 (see Tyler, 2006), and three rhinebothriideans, namely *Echeneibothrium demeusiae* Euzet, 1959, *E. dubium* van Beneden, 1850, and *E. variabile* van Beneden (1850) (see Euzet, 1959). Only two additional species of *Acanthobothrium* were recorded from *R. alba*, *Acanthobothrium filicolle* (Zschokke, 1888) Yamaguti, 1959 and *A. rajaebatis* (Rudolphi, 1810) Euzet et al., 1959, both from *R. alba* (as *R. marginata*) from the Mediterranean Sea off France (Baer, 1948; Euzet, 1959). Although these species have been recorded from the same host species as *A. umbungus*, they originate from a different biogeographical region than the present specimens and furthermore fall into different categories of Ghoshroy and Caira (2001) classification system. Following Ghoshroy and Caira (2001) system, *A. filicolle* represents a category 1 species, with a body size <15 mm (i.e. 6–8 mm in Baer, 1948), <50 segments (i.e. 17 to 30 in Baer, 1948), <80 testes (i.e. 30 to 40 in

Baer, 1948; 24 to 56 in Euzet, 1959) and a symmetrical ovary (see illustrations in Baer, 1948 and Euzet, 1959). *Acanthobothrium rajaebatis* most likely represents a category 5 species, with a total length of 50–60 mm (see Euzet, 1959), >80 segments (i.e. 80 to 120 in Goldstein, 1967; more than 100 in Williams, 1969), between 58 and 85 testes (i.e. with a mean of 72 in Euzet, 1959), and a symmetrical ovary (see Goldstein, 1967 and illustration in Euzet, 1959). Yet, values for the number of testes superimpose the boundary of 80 testes of Ghoshroy and Caira (2001) system, which could also place this species into category 4. The placement of both congeners in different categories automatically excluded them from the species differentiations (above).

Species of *Acanthobothrium* are synhospitalic, possibly as a result of host-substitution events due to geographical and external environmental conditions (Fyler, 2009), causing lineage sorting, providing evidence of co-speciation (Beer et al., 2019), and therefore exhibiting an extraordinary specificity towards both their elasmobranch hosts as well as their environmental requirements (Nhi et al., 2013). By understanding the ecological importance of co-speciation events along with ecological factors, such as the hosts' specific diet, size, geographical location and depth, which ultimately shapes host-parasite systems, researchers might have a better chance of mitigating co-extinction events in the future (Beer et al., 2019). However, given the decline in host populations and steady increase in the number of threatened species, co-extinction events grow more and more likely, although unnoticed, leading to the loss of many parasites, or better affiliate species, including a vast number of yet undescribed species (Davidson and Dulvy, 2017). These co-extinctions might trigger a series of long-term and indirect negative effects, leaving detrimental repercussions, which are currently not fully understood.

The discovery of additional species of *Acanthobothrium* will, without a doubt, be beneficial in this regard, as genera with a higher parasite species diversity can be more sustainably used as biological and ecological indicators of their elasmobranch hosts' life conditions, aiding the conservation of specific host populations (Marcogliese, 2005; Nhi et al., 2013). These cestodes could play a vital role in ecosystem health assessments, when considered suitable indicators of pollution or other environmental changes on host species. Certain cestode species are able to accumulate pollutants such as heavy metals from their specific host tissues at high concentrations, possibly lowering the effects of these pollutants for vulnerable host species (Jankovská et al., 2011; Nhi et al., 2013). These parasites will therefore provide valuable insights to the vulnerability of specific host species, whereas understanding the host-parasite system may be just as vital providing insights to the vulnerability of the parasites to co-extinction with their host species (Beer et al., 2019).

Extensive studies on elasmobranch parasites and their hosts implementing integrative approaches (e.g., taxonomy, molecular systematics, biogeography, ecology, ecotoxicology) are

needed, in order to provide a better understanding on the intimate nature of this particular and ancient host-parasite system. This may ultimately lead to new prospects in conservation science and the preservation of threatened host species, such as *R. alba*, together with their unique parasite fauna. Furthermore, the conservation of endangered species, such as *R. alba*, harbouring cestode parasites is crucial to mitigate host-parasite co-extinction events, whereby conservation of the parasite species along with its host, contributes to the conservation of marine ecosystem, rather than merely a single species. Since these affiliate species provide numerous positive attributes to hosts and environment, which rely on their elasmobranch hosts as macroenvironments, they deserve consideration in modern conservation schemes. The incorporation of such affiliates in future conservation agendas is crucial and greatly encouraged, as the absence of these species in the “ecological theatre” (sensu Marcogliese, 2004) as well as on-going evolutionary processes, will be profoundly altered once extinction and co-extinction events occur.

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References

- Baer, J.G., 1948. Contributions a l'étude des cestodes de sélaciens I-IV. Bull. Soc. Neuchl. Sci. Nat. 71, 63–122.
- Baer, J.G., Euzet, L., 1962. Revision critique des Cestodes Tétraphyllides décrits par T. Southwell. Bull. Soc. Neuchl. Sci. Nat. 85, 143–172.
- Beer, A., Ingram, T., Randhawa, H.S., 2019. Role of ecology and phylogeny in determining tapeworm assemblages in skates (Rajiformes). J. Helminthol. 93, 738–751.
- Brooks, D.R., 1977. Six new species of tetraphyllidean cestodes, including a new genus, from a marine stingray *Himantura schmardae* (Werner, 1904) from Colombia. Proc. Helminthol. Soc. Wash. 44, 51–59.
- Brooks, D.R., Mayes, M.A., 1980. Cestodes in four species of euryhaline stingrays from Colombia. Proc. Helminthol. Soc. Wash. 47, 22–29.
- Caira, J.N., Healy, C.J., 2004. Elasmobranchs as hosts of metazoan parasites. In: Carrier, J.C., Musick, J.A., Heithaus, M.R. (Eds.), Biology of Sharks and Their Relatives. CRC Press, Boca Raton, FL, USA, pp. 523–551.
- Caira, J.N., Jensen, K., 2001. An investigation of the co-evolutionary relationships between onchobothriid tapeworms and their elasmobranch hosts. Int. J. Parasitol. 31, 960–975.
- Caira, J.N., Jensen, K., 2014. A digest of elasmobranch tapeworms. J. Parasitol. 100, 373–391.
- Caira J.N., Jensen K., Ivanov V.A. 2017. Onchoproteocephalidea II. In: J.N. Caira and K. Jensen (Eds.), Planetary Biodiversity Inventory (2008–2017): Tapeworms from Vertebrate Bowels of the Earth. The University of Kansas Natural History Museum. Special Publication no. 25, pp. 279–304.
- Campbell, R.A., Beveridge, I., 2002. The genus *Acanthobothrium* (Cestoda: Tetraphyllidea: Onchobothriidae) parasitic in Australian elasmobranch fishes. Invertebr. Systemat. 16, 273–344.

- Carvajal, G.J., Goldstein, R.J., 1971. *Acanthobothrium annapienkensis* n. sp. (Cestoda: Tetraphyllidae: Onchobothriidae) from the skate, *Raja chilensis* (Chondrichthyes: Rajiidae) from Chile. Zool. Anz. 186, 158–162.
- Chervy, L. Unified terminology for cestode microtriches: a proposal from the International Workshops on Cestode Systematics in 2002-2008. Folia Parasitol. 56: 199–230.
- Dailey, M.D., Mudry, D.R., 1968. Two new species of cestodes from California rays. J. Parasitol. 54, 1141–1143.
- Davidson, L.N.K., Dulvy, N.K., 2017. Global marine protected areas to prevent extinctions. Nat. Ecol. Evol. 1, 0040.
- Dentzien-Dias, P.C., Poinar Jr., G., de Figueiredo, A.E.Q., Pacheco, A.C.L., Horn, B.L.D., Schultz, C.L., 2013. Tapeworm Eggs in a 270-Million-Year-Old Shark Coprolite. PLoS One 8, e55007.
- Ebert, D., van Hees, K.E., 2015. Beyond jaws: rediscovering the 'lost sharks' of southern Africa. Afr. J. Mar. Sci. 37, 141–156. Euzet, L., 1959. Recherches sur les cestodes tétraphyllides des sélaciens des côtes de France. Doctoral Dissertation, Université de Montpellier, p. 263. Montpellier.
- Franzese, S., Ivanov, V.A., 2018. Hyperapolytic species of *Acanthobothrium* (Cestoda: Onchoproteocephalidae) from batoids off Argentina. Parasitol. Int. 67, 431–443.
- Franzese, S., Ivanov, V.A., 2020. Two new species of *Acanthobothrium* Blanchard, 1848 (Cestoda: Onchoproteocephalidae) from rajiform batoids off Argentina. Folia Parasitol. 67, 016.
- Fyler, C.A., 2009. Systematics, Biogeography and Character Evolution in the Tapeworm Genus *Acanthobothrium* Van Beneden, 1850. ProQuest Dissertations Publishing, University of Connecticut, p. 200.
- Fyler, C.A., Caira, J.N., 2006. Five new species of *Acanthobothrium* (Tetraphyllidae: Onchobothriidae) from the freshwater stingray *Himantura chaophraya* (Batoidea: Dasyatidae) in Malaysian Borneo. J. Parasitol. 92, 105–125.
- Fyler, C.A., Caira, J.N., 2010. Phylogenetic status of four new species of *Acanthobothrium* (Cestoda: Tetraphyllidae) parasitic on the wedge fish *Rhynchobatus laevis* (Elasmobranchii:

Rhynchobatidae): implications for interpreting host associations. *Invertebr. Systemat.* 24, 419–433.

Fyler, C.A., Caira, J.N., Jensen, K., 2009. Five new species of *Acanthobothrium* (Cestoda: Tetraphyllidea) from an unusual species of *Himantura* (Rajiformes: Dasyatidae) from northern Australia. *Folia Parasitol.* 56, 107–128.

Ghoshroy, S., Caira, J.N., 2001. Four new species of *Acanthobothrium* (Cestoda: Tetraphyllidea) from the whiptail stingray *Dasyatis brevis* in the Gulf of California, Mexico. *J. Parasitol.* 87, 354–372.

Goldstein, R.J., 1967. The genus *Acanthobothrium* van Beneden, 1849 (Cestoda: Tetraphyllidea). *J. Parasitol.* 53, 455–483. IUCN, 2021. The IUCN Red List of Threatened Species. <http://www.iucnredlist.org>. (Accessed 23 June 2021).

Jankovská, I., Míhlová, D., Petrýl, M., Romocuský, S., Kalous, L., Vadlejch, J., Cadková, Z., Langrová, I., 2011. The intestinal parasite *Acanthocephalus lucii* (Acanthocephala) from European perch (*Perca fluviatilis*) as a bioindicator for lead pollution in the stream “Jevansky’ potok” near Prague, Czech Republic. *Bull. Environ. Contam. Toxicol.* 86, 342–346.

Linton, E., 1908. Helminth fauna of the dry Tortugas. I. Cestodes. In: *Papers from the Tortugas Laboratory*. Carnegie Institution of Washington, Washington, D.C., pp. 157–190

Lönnberg, E., 1889. Bidrag till Kanendomen om i Sverige förs Kommande cestoder. Bihang till Kongl. K. Sven. vetensk. akad. handl. 14, 1–69.

Maleki, L., Malek, M., Palm, H.W., 2013. Two new species of *Acanthobothrium* (Tetraphyllidea: Onchobothriidae) from *Pastinachus cf. sephen* (Myliobatiformes: Dasyatidae) from the Persian Gulf and Gulf of Oman. *Folia Parasitol.* 60, 448–456.

Maleki, L., Malek, M., Palm, H.W., 2015. Four new species of *Acanthobothrium* van Beneden, 1850 (Cestoda: Onchoproteocephalidea) from the guitarfish, *Rhynchobatus cf. djiddensis* (Elasmobranchii: Rhynchobatidae), from the Persian Gulf and Gulf of Oman. *Folia Parasitol.* 62, 012.

Maleki, L., Malek, M., Palm, H.W., 2019. Five new species of *Acanthobothrium* (Cestoda: Onchoproteocephalidea) from the long-tailed butterfly ray, *Gymnura cf. poecilura*

(Elasmobranchii: Gymnuridae), from the Persian Gulf and Gulf of Oman. *Zootaxa* 4609, 289–307.

Maleki, L., Malek, M., Rastgoo, A., 2018. *Acanthobothrium chabahariensis* n. sp. (Cestoda: Onchoproteocephalidea) in the cowtail stingray *Pastinachus* cf. *sephen* (Myliobatiformes: Dasyatidae) from the Gulf of Oman, Iran. *J. Genet. Res.* 4, 114–121.

Marcogliese, D.J., 2004. Parasites: small players with crucial roles in the ecological theatre. *EcoHealth* 1, 151–164.

Marcogliese, D.J., 2005. Parasites of the superorganism: are they indicators of ecosystem health? *Int. J. Parasitol.* 35, 705–716.

Marques, F., Brooks, D.R., Monks, S., 1995. Five new species of *Acanthobothrium* van Beneden, 1849 (Eucestoda: Tetraphyllidea: Onchobothriidae) in stingrays from the Gulf of Nicoya, Costa Rica. *J. Parasitol.* 81, 942–951.

Nhi, T.T.Y., Shazili, N.A.M., Harrison, F.S., 2013. Use of cestodes as indicator of heavy-metal pollution. *Exp. Parasitol.* 133, 75–79.

Ostrowski de Núñez, M., 1971. Estudios preliminares sobre la fauna parasitaria de algunos elasmobranquios del litoral bonaerense, mar del plata, Argentina I. Cestodes y trematodes de *Psammobatis microps* (Gunther) y *Zapteryx brevirostris* (Muller Y Henle). *Physics* 30, 425–446.

Poulin, R., Presswell, B., 2016. Taxonomic quality of species descriptions varies over time and with the number of authors, but unevenly among parasite taxa. *Syst. Biol.* 65, 1107–1116.

Randhawa, H.S., Poulin, R., Krkosek, M., 2015. Increasing rate of species discovery in sharks coincides with sharp population declines: implications for biodiversity. *Ecography* 38, 96–107.

Randhawa, H.S., Poulin, R., 2020. Tapeworm discovery in elasmobranch fishes: quantifying patterns and identifying their correlates. *Mar. Freshw. Res.* 71, 78–88.

Reyda, F.B., Caira, J.N., 2006. Five new species of *Acanthobothrium* (Cestoda: Tetraphyllidea) from *Himantura uarnacoides* (Myliobatiformes: Dasyatidae) in Malaysian Borneo. *Comp. Parasitol.* 73, 49–71.

Riser, N.W., 1955. Studies on cestode parasites of sharks and skates. J. Tenn. Acad. Sci. 30, 265–311.

Rodríguez-Ibarra, E., Pulido-Flores, G., Violante González, J., Monks, S., 2018. A new species of *Acanthobothrium* (Eucestoda: Onchobothriidae) in *Aetobatus* cf. *narinari* (Myliobatidae) from Campeche, México. Rev. Bras. Parasitol. Vet. 27, 66–73.

Schaeffner, B.C., Smit, N.J., 2019. Parasites of cartilaginous fishes (Chondrichthyes) in South Africa - a neglected field of marine science. Folia Parasitol. 66, 002.

Siskey, M.R., Shipley, O.N., Frisk, M.G., 2019. Skating on thin ice: identifying the need for species-specific data and defined migration ecology of Rajidae spp. Fish Fresh. 20, 286–302.

Sousa, I., Baeyaert, J., Gonçalves, J.M.S., Erzini, K., 2019. Preliminary insights into the spatial ecology and movement patterns of a regionally critically endangered skate (*Rostroraja alba*) associated with a marine protected area. Mar. Freshw. Behav. Physiol. 52, 283–299.

Sures, B., Nachev, M., Selbach, C., Marcogliese, D.J., 2017. Parasite responses to pollution: what we know and where we go in 'Environmental Parasitology. Parasites Vectors 10, 65.

Tazerouti, F., Kechemir-Issad, N., Euzet, L., 2009. *Acanthobothrium minus* n. sp. (Tetraphyllidea: Onchobothriidae) parasite de *Raja asterias* (Elasmobranchii: Rajidae) en Méditerranée. Parasite 16, 203–207.

Tyler, G.A., 2006. Tapeworms of elasmobranchs (Part III). A monograph on the Diphyllidea (Platyhelminthes, Cestoda). Bull. Univ. Nebr. State Mus. 20, 1–142.

van Beneden, P.J., 1850. Recherches sur la faune littorale de Belgique. Les vers cestoïdes, considérés sous le rapport physiologique, embryogénique et zooclassique. Mem. Acad. r. Sci. Lett. Belg. 25, 1–204.

Van Der Spuy, L., Smit, N.J., Schaeffner, B.C., 2020. Four new species of *Acanthobothrium* van Beneden, 1849 (Cestoda: Onchoproteocephalidea) from the spotted skate, *Raja straeleni* Poll, off the Western Cape, South Africa. Folia Parasitol. 67, 036.

Verma, S.C., 1928. Some cestodes from Indian fishes including four new species of Tetraphyllidea and revised keys to the genera *Acanthobothrium* and *Gangesia*. Univ. Allahabad Stud. 4, 119–176.

Williams, H.H., 1968. *Acanthobothrium quadripartitum* n. sp. (Cestoda: Tetraphyllidea) from *Raja naevus* in the North Sea and English Channel. *Parasitology* 58, 105–110.

Williams, H.H., 1969. The genus *Acanthobothrium* van Beneden 1849 (Cestoda: Tetraphyllidea). *Nytt Magasin Zool.* 17, 1–56.

Yang, C., Sun, Y., Zhi, T., Iwaki, T., Reyda, F.B., Yang, T., 2016. Two new and one redescribed species of *Acanthobothrium* (Cestoda: Onchoproteocephalidea: Onchobothriidae) from *Dasyatis akajei* (Myliobatiformes: Dasyatidae) in the China Sea. *Zootaxa* 4169, 286–300.

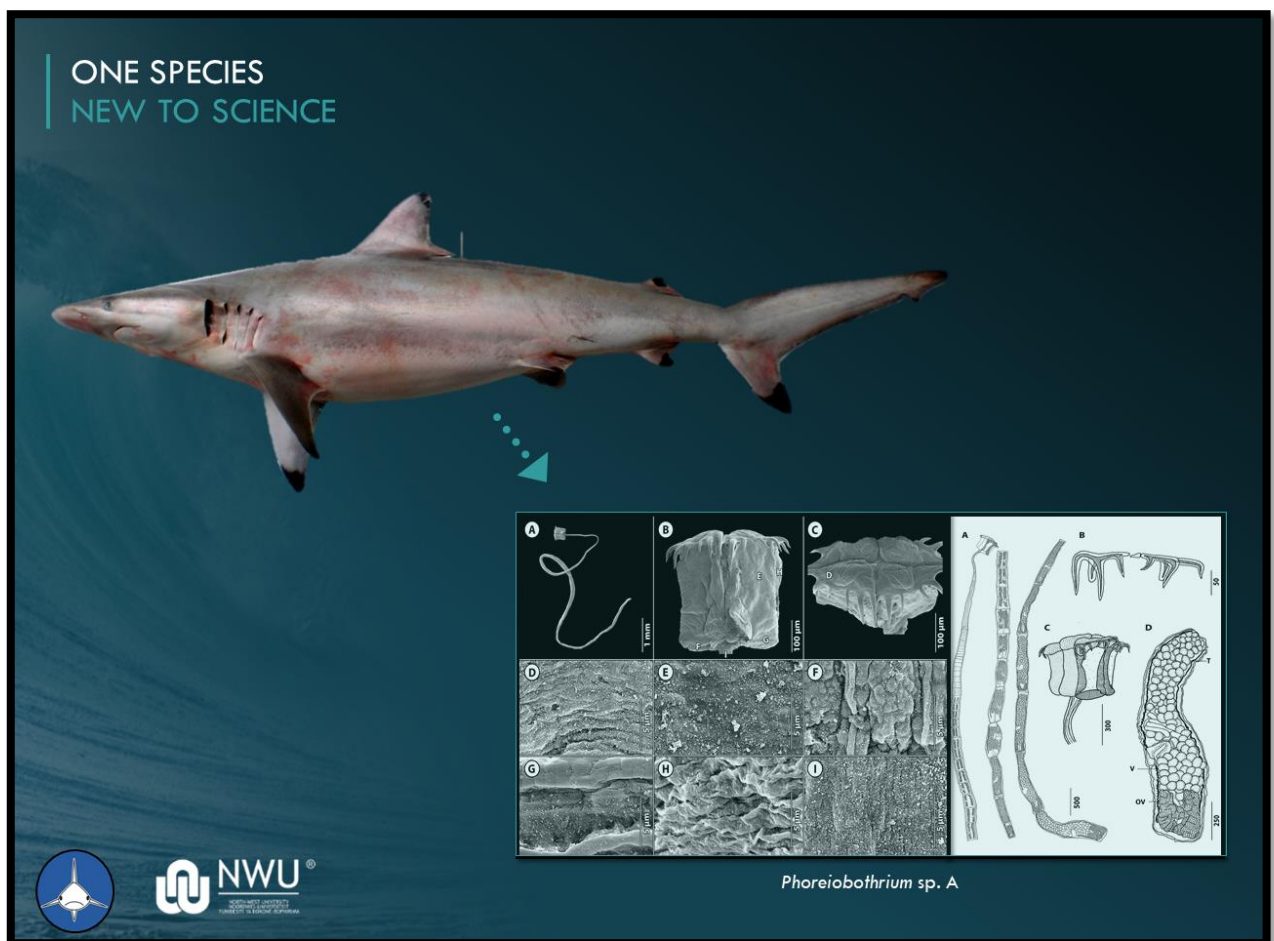
Zaragoza-Tapia, F., Pulido-Flores, G., Violante-González, J., Monks, S., 2019. Two new species of *Acanthobothrium* Blanchard, 1848 (Onchobothriidae) in *Narcine entemedor* Jordan & Starks, 1895 (Narcinidae) from Acapulco, Guerrero, Mexico. *ZooKeys* 852, 1–21.

Zaragoza-Tapia, F., Pulido-Flores, G., Gardner, S.L., Monks, S., 2020a. Host relationships and geographic distribution of species of *Acanthobothrium* Blanchard, 1848 (Onchoproteocephalidea, Onchobothriidae) in elasmobranchs: a metadata analysis. *ZooKeys* 940, 1–49.

Zaragoza-Tapia, F., Pulido-Flores, G., Monks, S., 2020b. Three new species of *Acanthobothrium* Blanchard, 1848 (Cestoda: Onchoproteocephalidea) in stingrays (Dasyatidae) from the Pacific coast in Mexico. *Zootaxa* 4766, 139–172.

CHAPTER 4:

A new species of *Phoreiobothrium* Linton, 1889 (Cestoda: Onchoproteocephalidea) from *Carcharhinus brevipinna* (Valenciennes) off the coast of KwaZulu-Natal, South Africa



CHAPTER 4:

A new species of *Phoreiobothrium* Linton, 1889 (Cestoda: Onchoproteocephalidea) from *Carcharhinus brevipinna* (Valenciennes) off the coast of KwaZulu-Natal, South Africa

Zoobank number for article: to be added.

4.1. Abstract

The extreme sparsity of collection efforts in many parts of the world, including southern Africa, leaves a vast hidden diversity of marine cestode species, such as species of *Phoreiobothrium*. The investigation of a common South African coastal-pelagic species, *C. brevipinna* (Valenciennes) resulted in the discovery of a new species of *Phoreiobothrium* Linton, 1889. *Phoreiobothrium* sp. A is undoubtedly different from all other congeners in several morphological characteristics, however, it is most easily distinguished from other species by the size of its hooks. Furthermore, this species is peculiar in the sense of its microthrix pattern. It only possesses filitriches on its proximal bothridial surface. Additionally, *Phoreiobothrium* sp. A also displays a scarcity of gladiate spinitriches on its cephalic peduncle. This has not been recorded in any other congener within this genus and could resemble a new, generic characteristic. The addition of *Phoreiobothrium* sp. A increases the total number of valid species of *Phoreiobothrium* to 18 worldwide. Furthermore, *Phoreiobothrium* sp. A marks the second onchoproteocephalidean genus to be described from southern Africa, therefore expanding the generic biogeographical representation and introducing new host associations. Apart from the description of this new species of cestode, the discovery of *Phoreiobothrium* sp. A as well as additional species of *Phoreiobothrium* is beneficial regarding future ecological investigations. With the high degree of host-specificity found amongst species of *Phoreiobothrium*, species such as *Phoreiobothrium* sp. A could be used as sentinel species for the accurate identification, separation and diagnosis of misidentified shark species, such as *C. brevipinna* and *C. limbatus* (Valenciennes).

Keywords: taxonomy, marine fish parasites, biodiversity, elasmobranchs.

4.2. Introduction

Research on elasmobranch cestodes has rapidly increased over the past two decades, specifically on taxonomic and phylogenetic advances of novel taxa (Caira & Jensen, 2014). The

discovery and description of new taxa, along with the analyses of novel molecular data helped resolve the inaccurate phylogenetic placement represented by taxa previously grouped in the order Tetraphyllidea (sensu Caira *et al.*, 2014). From this, two new orders, Onchoproteocephalidea Caira, Jensen, Waeschenback, Olsen et Littlewood, 2014 and Phyllobothriidea Caira, Jensen, Waeschenback, Olsen et Littlewood, 2014 emerged (Caira *et al.*, 2014). With these advancements, the unique host-parasite relationships between cestodes and elasmobranchs are currently well documented in most parts of the world (Caira & Jensen, 2014).

The order Onchoproteocephalidea II (sensu Caira *et al.*, 2017) currently includes 12 genera, all of which include one or two pairs of hooks on each of their four bothridia (with the exception of *Prosobothrium* Cohn, 1902) (Caira *et al.*, 2017). Amongst these is the genus *Phoreiobothrium* Linton, 1889 (Caira *et al.*, 2017). Although the World Register of Marine Species (WoRMS, 2022) lists 24 species of *Phoreiobothrium* worldwide, this genus only comprises 17 valid species (Caira *et al.*, 2021), since revisions by Caira *et al.* (2005) and Caira and Jensen (2015) concluded that a number of previously described species lack complete and vital descriptive data and are therefore considered *species inquirendae*. The majority of species belonging to this genus, with the exception of *P. exceptum* Linton, 1924 and *P. pectinatum* Linton, 1924 (Caira & Jensen, 2015) are extremely host-specific, exhibiting oioxenous specificity infecting merely one single host species (Caira *et al.*, 2017; Alijanpour Darvishi & Haseli, 2019). Species of *Phoreiobothrium* are known to explicitly parasitise sharks, with the majority of species (11/17) infecting sharks of the family Carcharhinidae, whilst the remainder (6/17) infect sharks from the family Sphyrnidae (Caira *et al.*, 2005; Caira *et al.*, 2021). Information regarding the life-cycles of cestodes belonging to this genus is however still insufficient (Caira & Reyda, 2005; Jensen, 2005). This is due to the fact that these cestodes often mature in their relevant elasmobranch hosts species (Jensen & Bullard, 2010). Larval cestodes of the genus *Phoreiobothrium* found to parasitise the intermediate hosts, such as teleost fishes, do not resemble the morphology of their adult stages (Caira & Reyda, 2005; Jensen & Bullard, 2010). Consequently, larval cestodes of this genus cannot accurately be assigned to species-level without the aid of molecular sequence data.

Given that the definitive hosts of the 17 currently accepted species of *Phoreiobothrium* only represent 21 % (11/52) of recognised carcharhinid and 66 % (6/9) of sphyrnid shark species (Froese & Pauly, 2021; Caira *et al.*, 2021), several species await scientific discovery. There are also sparse collection efforts of specific hosts for parasitic research in many parts of the world, including South Africa (Van Der Spuy *et al.*, 2020, 2022; Schaeffner & Smit, 2019). This situation is further impeded by the incorrect identification of elasmobranch hosts, which may cause complications regarding the accuracy of reported host-parasite interactions. Phylogenetic analyses from the NADH2 gene also revealed the existence of species-complexes within elasmobranchs (Naylor *et al.*, 2012). Species misidentifications are common, especially in the

carcharhinids, where, for instance, the spinner shark, *Carcharhinus brevipinna* (Valenciennes), is often misidentified as the blacktip shark, *C. limbatus* (Valenciennes) (Tillett *et al.*, 2012).

The present study introduces a new species of *Phoreiobothrium* from the spiral intestines of three spinner sharks, using morphological methods. Apart from the description of a new species of cestode, new host-parasite interrelationships are highlighted and a possible solution to the problems associated with either the identification or separation of shark species such as *C. brevipinna* and *C. limbatus* are presented, which could prove particularly valuable for future research in this field.

4.3. Materials and Methods

Three adult specimens of *C. brevipinna*, two males and one female ranging between 190 and 230 cm in total length, were collected by the KwaZulu-Natal Sharks Board in March 2020 at Umhlanga, KwaZulu-Natal, South Africa. All three specimens were recovered deceased in gill-nets set within the framework of the South African bather protection programme off the coast of Glenmore, Zinkwazi and Banana Beaches, and were subsequently frozen at -20°C in the laboratory at the Sharks Board Institute.

Prior to dissection, sharks were defrosted. Spiral intestines were removed by a mid-ventral incision and approximately two-thirds of each intestinal tract, along with the contents, was fixed in 4 % neutrally-buffered formalin. The intestines and contents were then transferred to 70 % ethanol after a period of two weeks. The remaining third of the intestinal tract was fixed in molecular grade ethanol. Preserved spiral intestines were scanned for cestodes using a stereo-microscope and specimens were manually removed. These specimens were hydrated in a graded ethanol series, stained with Delafield's haematoxylin, and dehydrated in a graded ethanol series again to 70 % ethanol. Following staining, specimens were placed in 1 % hydrogen chloride to clear the excess stain and then fully dehydrated in a graded ethanol series. The tissue of these specimens was cleared in clove oil, and each specimen was permanently mounted on microscope slides using Canada balsam.

Once the microscope slides were completely dry, morphological observations were conducted using a Nikon Y-TV55 video camera mounted on a Nikon ECLIPSE Ni light microscope (Nikon, Tokyo, Japan). Images of characteristic body structures were taken, from which measurements were obtained, using the image analysis software Image Pro Express (Nikon, Japan). The morphological characterisation of the specimens follows Van Der Spuy *et al.* (2020, 2022). Measurements of internal organs and body structures follows Caira *et al.* (2005). Measurements

are provided in the description as the range, followed by the mean and standard deviation, as well as the number of specimens examined. All measurements are presented in micrometres (μm), except for the total length presented in millimetres (mm). After acquiring all images and measurements, line drawings were made using a drawing attachment tube. All specimens studied resembled a single morphotype. Four specimens were subjected to scanning electron microscopy (SEM). Specimens were cleaned in 70 % ethanol from any remaining host tissue or mucus as thoroughly as possible by using a soft, liner brush. Specimens were dehydrated in a graded ethanol series to 100 % ethanol and placed in hexamethyldisilane (HMDS). Following the drying protocol, specimens were mounted on carbon tape on aluminium stubs and sputter-coated with carbon (Emscope TB500, Quorum Technologies, Puslinch, Ontario, USA), followed by 20 to 30 nm gold/palladium (Eiko IB2 ion coater, Eiko, Japan). Specimens were observed and micrographs of the various individual body regions were taken using a FEI Nova NanoSEM 450 scanning electron microscope (FEI, Hillsboro, Oregon, USA). Micrographs were used to conduct microthrix comparisons of scolex regions and the strobila following Chervy (2009).

Microscope slides of type specimens have been deposited in the following helminthological collections: the National Museum, Bloemfontein, South Africa (NMB); the Institute of Parasitology, Biology Centre of the Czech Academy of Sciences, České Budějovice, Czech Republic (IPCAS); and the Natural History Museum, Geneva, Switzerland (MHNG-PLAT). Stubs containing specimens used for SEM were retained in the parasite collection of the Water Research Group, North-West University, South Africa.

Ethical approval for this project was received from the Faculty of Natural and Agricultural Sciences' Ethics Committee (FNASREC) of the North-West University with ethics number NWU-01655-20-A9. Permits for the collection and possession of sharks for the purpose of research were issued by the South African Department of Agriculture, Forestry and Fisheries (permit no. RES2020/20 issued to the KwaZulu-Natal Sharks Board).

4.4. Results

4.4.1. *Phoreiobothrium* sp. A (Figs. 1–2)

ZooBank number for species: To be added

4.4.1.1. Description

Based on 11 whole mounts, six mature and five immature worms; four mature worms used for SEM observations: Worms 6.3–33.1 mm (16.85 ± 8.37 ; $n=11$) long; greatest width at level of scolex; 20–46 (33 ± 8 ; $n=7$) proglottids per worm; euapolytic. Scolex consisting of scolex proper and short cephalic peduncle. Scolex proper with four rectangular bothridia, 318–378 (349 ± 30 ; $n=11$) long by 262–299 (285 ± 13 ; $n=11$) wide. Bothridia free posteriorly, 126–148 (137 ± 7 ; $n=11$) wide; each bothridium with two loculi and specialised anterior region in form of muscular pad. Muscular pad 70–75 (73 ± 3 ; $n=2$) long by 122–125 (123 ± 2 ; $n=2$) wide, falciform in shape, with pronounced posterior margin. Anterior prehook loculus (A) 138–215 (191 ± 20 ; $n=7$) long; posterior posthook loculus (P) 38–63 (50 ± 7 ; $n=7$) long; posterior loculus subdivided into 20–22 (22 ± 1 ; $n=7$) subloculi; subloculi 7–10 (8 ± 2 ; $n=7$) wide.

Hooks tri-pronged, with blunt talon embedded in muscular pad of scolex; talon of medial hook longer than talon of lateral hook (Fig. 1B). Hook prongs and talon hollow. Internal channels of all hook prongs continuous, smooth (Fig. 1B). Axial prongs longest. Lateral hook lengths: A 65–84 (74 ± 9 ; $n=7$), B 80–94 (88 ± 6 ; $n=7$), C 82–94 (90 ± 7 ; $n=7$), D 117–119 (118 ± 1 ; $n=7$), E 53–61 (56 ± 3 ; $n=7$), F 45–57 (52 ± 5 ; $n=7$). Medial hook measurements: A' 69–79 (75 ± 4 ; $n=7$), B' 73–87 (79 ± 7 ; $n=7$), C' 79–90 (85 ± 4 ; $n=7$), D' 117–120 (118 ± 1 ; $n=7$), E' 49–56 (53 ± 3 ; $n=7$), F' 45–51 (48 ± 2 ; $n=7$). Bases of medial and lateral hooks approximately equal in length; in close proximity to each other. Thin layer of tissue covering essentially entire length of each prong of hooks. Cephalic peduncle 744–5,274 ($2,319 \pm 1,453$; $n=11$) long by 32–60 (45 ± 10 ; $n=11$) wide, with inconspicuous posterior margin.

Anterior muscular pad covered in papilliform filitriches (Fig. 2D); devoid of spinitriches. Distal surfaces of bothridia (Fig. 2E, F), apex of scolex proper, and prehook locular region covered with papilliform filitriches; devoid of spinitriches. Boundary between anterior and posterior loculi with muscular ledge and papilliform filitriches (Fig. 2G). Proximal surface of bothridia covered with papilliform filitriches (Fig. 2H), devoid of spinitriches. Cephalic peduncle covered in papilliform filitriches and sparsely interspersed with gladiate spinitriches (Fig. 2I).

Proglottids acraspedote. Immature proglottids 27–44 (32 ± 7 ; $n=11$) in number, initially wider than long, becoming longer with maturation; mature proglottids 0–4 (2 ± 2 ; $n=11$) in number, longer than wide; gravid proglottids absent; terminal proglottid 974–2,177 ($1,381 \pm 470$; $n=6$) long by 280–318 (294 ± 16 ; $n=6$) wide; terminal proglottid length to width ratio 1: 3.42–7.75 (1: 4.73 ± 1.74 ; $n=6$). Proglottids protandrous; genital pores marginal, irregularly alternating (Fig. 1A), 51–68 (59 ± 7 ; $n=11$) % of proglottid length from anterior margin.

Testes conspicuous in mature proglottids, oval in dorsoventral view, 34–56 (44 ± 7 ; $n=7$) long by 32–50 (40 ± 6 ; $n=6$) wide, arranged in four regular columns (Fig. 1D), two layers deep, 88–96 (92 ± 4 ; $n=6$) in total number, 26–31 (28 ± 2 ; $n=6$) in post-poral field. Cirrus-sac ovoid, bent posteriorly (Fig. 1D), 90–106 (106 ± 6 ; $n=6$) long by 30–50 (42 ± 8 ; $n=6$) wide, containing coiled cirrus; cirrus greatly expanded at base.

Vagina narrow, relatively thin-walled and straight proximally, extending from ootype along medial line of proglottid to anterior margin of cirrus-sac, then laterally to common genital atrium. Ovary takes up approximately one-quarter of proglottid length, stopping short of posterior margin of proglottid, H-shaped in dorsoventral view, lobulated (Fig. 1D). Ovary symmetrical, 228–314 (285 ± 39 ; $n=6$) in length; poral and aporal lobes not reaching cirrus-sac anteriorly; width of ovary at level of ovarian isthmus 134–224 (165 ± 51 ; $n=3$); ovarian isthmus located posterior to mid-level of poral lobe of ovary. Mehlis' gland posterior to ovarian isthmus.

Vitellarium follicular, in form of two lateral bands each consisting of two columns of follicles; bands extending from posterior margin of anterior-most testes not reaching posterior margin of ovary, interrupted by vagina and cirrus-sac (Fig. 1D); vitelline follicles 10–13 (11 ± 1 ; $n=6$) long by 6–9 (7 ± 1 ; $n=6$) wide. Vitelline follicle length relative to testis length 0.22–0.30 : 1.0. Uterus thin-walled, extending from ovarian isthmus to near anterior margin of proglottid (Fig. 1D). Eggs not observed.

4.4.1.2. Taxonomic summary

Type host: Spinner shark, *Carcharhinus brevipinna* (Valenciennes) (Carcharhiniformes: Carcharhinidae).

Type locality: Glenmore Beach, KwaZulu-Natal, South Africa (31°00'42"S; 30°15'54"E),

Additional localities: Zinkwazi Beach (29°17'21"S; 31°26'51"E) and Banana Beach (30°39'37"S; 30°31'16"E), KwaZulu-Natal, South Africa.

Site of infection: Spiral intestine.

Prevalence of infection: 100 % (of three sharks examined).

Type material: Holotype deposited at NMB (Accession number: to be added), paratypes in NMB (Accession numbers: to be added), IPCAS (Accession numbers: to be added) and MHNG (Accession numbers: to be added).

Etymology: This species epithet is in memory to the late Mr. Martin de Klerk Jnr, in life the partner of the first author, who provided his dedicated support for her research throughout her MSc studies.

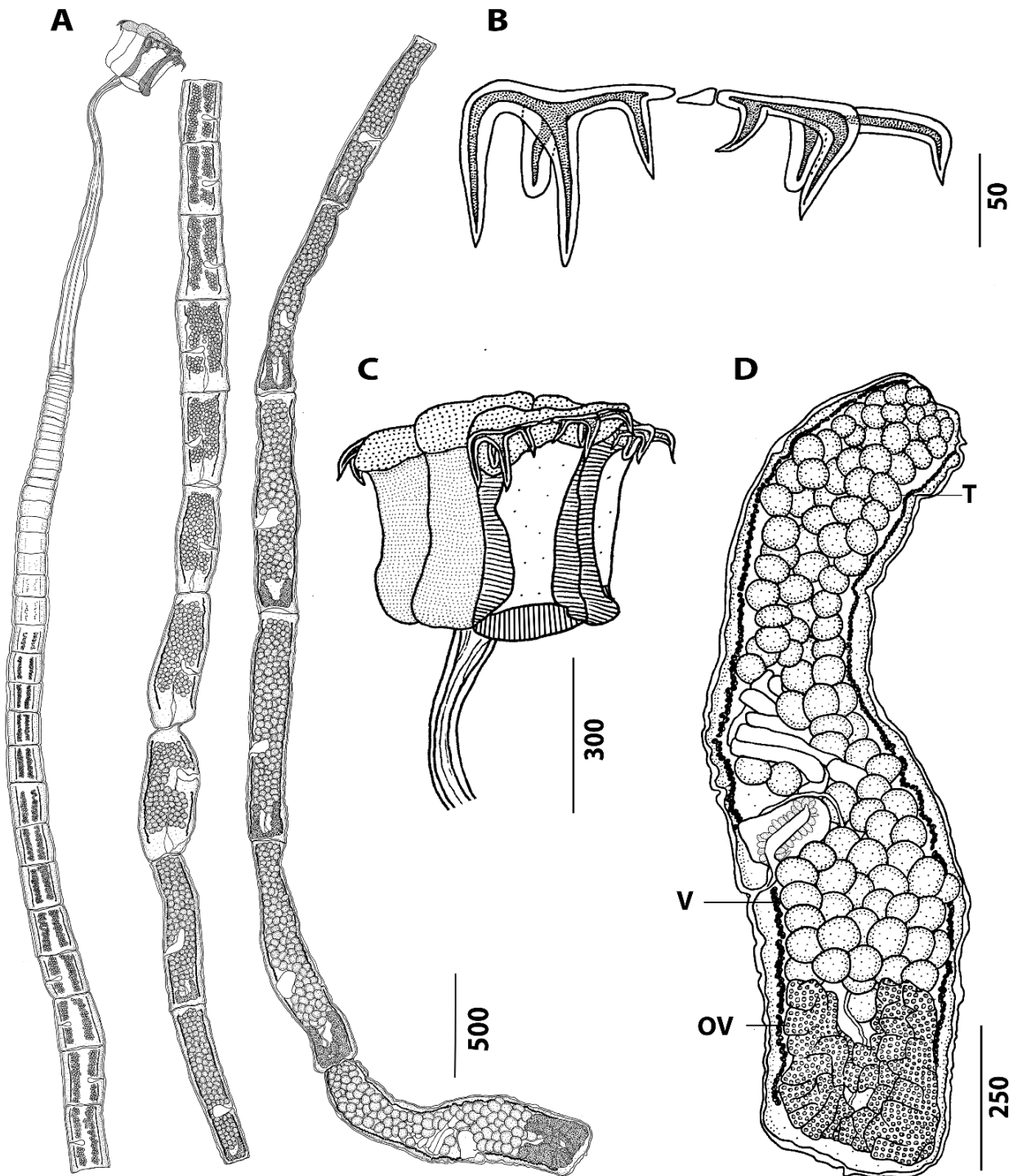


Fig. 1. Line drawing of *Phoreiobothrium* sp. A. A – entire specimen; B – hooks; C – scolex; D – mature proglottid (T, testis; V, vitelline follicle; OV, ovary).

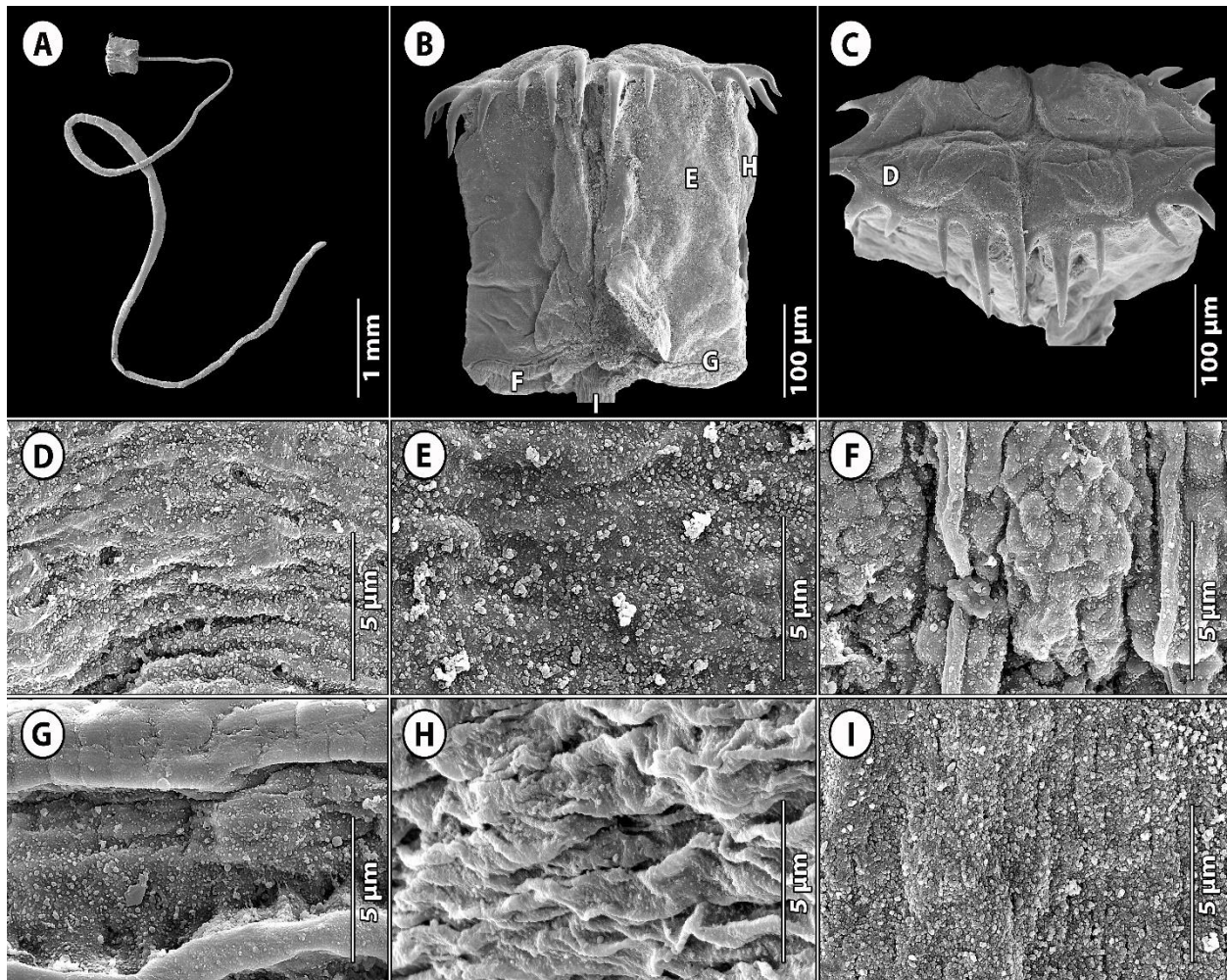


Fig. 2. Scanning electron micrographs of *Phoreiobothrium* sp. A. A – entire specimen; B – scolex, letters indicate where micrographs of microtriches were taken; C – anterior muscular pad and hooks, letters indicate where micrographs of microtriches were taken; D – papilliform filitriches on anterior muscular pad; E – distal bothridial surface: anterior locus; F – distal bothridial surface: posterior locus; G – distal bothridial surface: boundary between anterior and posterior locus; H – proximal bothridial surface; I – cephalic peduncle.

4.4.1.3. Remarks

The most distinguishing characteristic of *Phoreiobothrium* sp. A is its hooks, differing from 14 of the 17 other species worldwide. The hooks of *P. anticaporum* Caira, Richmond et Swanson, 2005, *P. golchini* Alijanpour, Darvishi et Haseli, 2019, *P. jahki* Caira et Jensen, 2015, *P. lewinense* Caira, Richmond et Swanson, 2005, *P. manirei* Caira, Healy et Swanson, 1996, *P. nadiae* Caira et Jensen, 1924, *P. pectinatum* Linton, 1924, *P. perilocrocodilus* Caira, Richmond et Swanson, 2005, *P. puriensis* Srivastav et Capoor, 1982, *P. robertsoni* Caira, Richmond et Swanson, 2005, *P. rozatii* Alijanpour Darvishi et Haseli, 2019, *P. sorrahcola* Masoumi Ganjgah et Haseli, 2020, *P. swaki* Caira et Jensen, 2015 and *P. tiburonis* Cheung Nigrelli et Ruggieri, 1982 are all smaller in

size from *Phoreiobothrium* sp. A (lateral hooks: *A* less than 53 μ m vs. 65–84 μ m, *B* less than 78 μ m vs. 80–94 μ m, *C* less than 62 μ m vs. 82–94 μ m, *D* less than 97 μ m vs. 117–119 μ m, *E* less than 38 μ m vs. 53–61 μ m, *F* less than 35 μ m vs. 45–57; medial hooks: *A'* less than 67 μ m vs. 69–79 μ m, *B'* less than 68 vs. 73–87, *C'* less than 68 μ m vs. 79–90 μ m, *D'* less than 102 μ m vs. 117–120 μ m, *E'* less than 40 μ m vs. 49–56 μ m, *F'* less than 41 μ m vs. 45–51 μ m, respectively). The hooks of *P. blissorum* Caira, Richmond et Swanson, 2005, and *P. exceptum* Linton, 1924, on the other hand are much larger than those of *Phoreiobothrium* sp. A (lateral hooks: *A* more than 107 μ m vs. 65–84 μ m, *B* more than 98 μ m vs. 80–94 μ m, *C* more than 102 μ m vs. 82–94 μ m; medial hooks: *A'* more than 78 μ m vs. 69–79 μ m, *B'* more than 93 vs. 73–87, *C'* more than 93 μ m vs. 79–90 μ m, *D'* more than 120 μ m vs. 117–120 μ m, respectively). Besides having larger hooks, *P. blissorum* and *P. exceptum* also differ from *Phoreiobothrium* sp. A in the following characteristics: number of proglottids (more than 55 vs. 20–46, respectively), scolex width (wider than 450 μ m vs. 262–299 μ m, respectively), scolex length (longer than 750 μ m vs. 318–378 μ m, respectively), bothridium length (longer than 425 μ m vs. 216–318 μ m, respectively), bothridium width (wider than 250 μ m vs. 126–148 μ m, respectively), anterior loculus length (longer than 340 μ m vs. 138–215 μ m, respectively), number of testes (more than 103 vs. 90–106, respectively), cirrus-sac length (longer than 138 μ m vs. 90–106 μ m, respectively), and cirrus-sac width (wider than 50 μ m vs. 30–50 μ m, respectively). Furthermore, *P. lewinense*, *P. puriensis*, *P. rozatii*, *P. swaki* and *P. tuberonis* all present a smaller body than that of *Phoreiobothrium* sp. A (less than 6 mm vs. 6.3–33.1 mm, respectively). Additionally, *P. lasium* Linton, 1889, *P. rutnagiriensis*, and *P. robertsoni* also differ from *Phoreiobothrium* sp. A in the following characteristics: scolex length (longer than 378 μ m vs. 318–378 μ m, respectively), scolex width (wider than 305 μ m vs. 262–299 μ m, respectively), bothridium length (longer than 341 μ m vs. 216–148 μ m, respectively), bothridium width (wider than 158 μ m vs. 126–148 μ m, respectively), number of testes (more than 96 vs. 88–96, respectively), cirrus-sac length (longer than 113 μ m vs. 90–106, respectively), and cirrus-sac width (wider than 50 μ m vs. 30–50 μ m, respectively). On the other hand, *P. golchini*, *P. jahki*, *P. pectinatum*, *P. puriensis*, *P. swaki* and *P. tiburonis* differ from *Phoreiobothrium* sp. A in the following features: scolex length (less than 205 μ m vs. 262–299, respectively), scolex width (less than 184 μ m vs. 318–378, respectively), bothridium length (less than 196 μ m vs. 216–318 μ m, respectively), bothridium width (less than 125 μ m vs. 126–148, respectively), posterior loculus length (less than 32 μ m vs. 38–63 μ m, respectively), number of subloculi (less than 15 vs. 20–22, respectively), and number of postovarian testes (less than 18 vs. 26–23, respectively). *Phoreiobothrium puriensis* also shows striking differences to *Phoreiobothrium* sp. A in the following morphological reproductive characteristics: number of testes (125–140 vs. 88–96, respectively), cirrus-sac length (192–240 μ m vs. 90–106 μ m, respectively), cirrus-sac width (120–176 μ m vs. 30–50 μ m, respectively), and ovary length (510–720 μ m vs. 228–314 μ m, respectively). Furthermore, *P. anticaporum*, and *P. perilocrocodilus* differ from *Phoreiobothrium* sp. A in the following characteristics: posterior loculus size (less than 38 μ m vs. 38–63 μ m,

respectively), number of subloculi (less than 19 vs. 20–22, respectively), number of testes (less than 75 vs. 88–96, respectively), number of postvaginal testes (less than 25 vs. 26–31, respectively), and ovary width (less than 123 μm vs. 134–224 μm , respectively). *Phoreiobothrium rozatii* and *P. sorrahcola* also differ from *Phoreiobothrium* sp. A in the number of subloculi (12–15 and 12–14 vs. 20–22, respectively) and the number of postvaginal testes (5–12 and 5–13 vs. 26–31, respectively). One particularly interesting difference became obvious in the scanning electron micrographs of the cephalic peduncle of *Phoreiobothrium* sp. A compared to all the other species of *Phoreiobothrium*. All congeners display densely packed gladiate spinitriches on their cephalic peduncle, while *Phoreiobothrium* sp. A mostly displays aciculate filitriches interspersed with few small gladiate spinitriches (Fig. 2).

Phoreiobothrium sp. A marks the first species of the genus to be described from South Africa, and increases the number of species of *Phoreiobothrium* to 18 worldwide (Caira *et al.*, 2021).

4.5. Discussion

In accordance with the revision of the genus *Phoreiobothrium* by Caira *et al.* (2005), there is a general microthrix pattern within this genus. The new South African species does however differ from most in a few aspects. The South African species most closely resembles the microthrix pattern of *P. lewinense*. These are the only two species described to possess only filitriches on their proximal bothridial surfaces, while a combination of filitriches and gladiate spinitriches are presented by all other congeners (Caira *et al.*, 2005). The most surprising and peculiar feature of *Phoreiobothrium* sp. A, however, is the scarcity of gladiate spinitriches found on the cephalic peduncle. This has not been recorded in any other congener within this genus and could resemble a new morphological characteristic for this genus. However, this feature might also be artificial that resulted from the freezing process of the host after it was obtained, or the effects on parasites through the disintegration of the host tissue prior to its collection. For this reason, this feature must be regarded with caution, and collection of new specimens from fresh hosts are required to confirm the microthrix patterns reported herein.

In a previous study on the diversity of rhinebothriidean cestode larval types by Jensen and Bullard (2010), an undescribed species of *Phoreiobothrium* (*Phoreiobothrium* sp. 1A) was detected in a spinner shark, *C. brevipinna*, collected off the coast of Mississippi in 2006, and used for molecular analyses (GenBank nos.: GQ470064–66, 82, 95). However, this species has never been officially described, and as such *Phoreiobothrium* sp. A is the first descriptive record of a species of *Phoreiobothrium* from *C. brevipinna* (Pollerspöck & Straube, 2022). Each of the valid species of *Phoreiobothrium*, excluding *P. exceptum* and *P. pectinatum* (Caira & Jensen, 2015), is extremely

host specific (oioxenous), known to parasitise a single host species. Given that known species of *Phoreiobothrium* exhibit such tight associations towards their definitive hosts (Alijanpour Darvishi & Haseli, 2019), the species found by Jensen and Bullard (2010) might prove to be the same species described herein. However, without a comprehensive molecular analysis, this assumption or the possibility that *Phoreiobothrium* sp. A might have undergone co-speciation within *C. brevipinna*, as reported for congeners, cannot be proven at this time and requires additional research. Furthermore, given the geographical distance between *Phoreiobothrium* sp. A and the species of Jensen and Bullard (2010), they might be congeners rather than conspecifics. The spinner shark, *C. brevipinna*, exhibits a wide distribution, inhabiting warm temperate and tropical waters of the Atlantic, Mediterranean Sea and Indo-West Pacific Ocean (Compagno, 1984; Froese & Pauly, 2021). This host has also been recorded from the southwestern Indian Ocean ranging between southern Mozambique and the western coast of Madagascar (Allan & Cliff, 2000). In South Africa, *C. brevipinna* is a common coastal-pelagic species found off the coast of KwaZulu-Natal, ranging as far south as Mossel Bay (Smith, 1951; Allan & Cliff, 2000). Although having a wide distribution, this shark species prefers shallower inshore waters over the continental shelf with seasonal migratory patterns moving into shallower bays (Froese & Pauly, 2021). Given the rather restricted movement pattern of these sharks, it is unlikely that the specimens observed by Jensen and Bullard (2010) off the coast of Mississippi represent the same species. This means that *Phoreiobothrium* sp. 1A and *Phoreiobothrium* sp. A might form part of the “exception category” including *P. exceptum* and *P. pectinatum* known to exhibit synhospitalic congeners. Once again, this only endorses the need for future comprehensive molecular analyses.

The biogeographical realm including the ocean basins of South Africa still holds the potential of an immense hidden cestode diversity (Van Der Spuy *et al.*, 2020; 2022), with a large proportion of the regional cestode fauna awaiting scientific discovery. This is not only supported by the present study but also by the fact that studies by Van Der Spuy *et al.* (2020; 2022) and Oosthuizen *et al.* (2021) have reported and described new species from this region within the last two years, adding to the diversity of cestodes from this region and further highlighting southern Africa as a neglected biogeographic area for marine parasites with the potential of a large hidden diversity. Albeit a limited sample size, the study found all three sharks sampled, infected with the new cestode species described herein, suggesting that *Phoreiobothrium* sp. A is very prevalent in *C. brevipinna* in South African waters. Apart from increasing the total number of species of *Phoreiobothrium* to 18 worldwide, the description of *Phoreiobothrium* sp. A marks only the second onchoproteocephalidean genus reported from southern Africa, therefore expanding the generic biogeographical representation and introducing new host associations, which might contribute to environmental and ecological research in future (Caira & Jensen, 2015; Alijanpour Darvishi & Haseli, 2019).

The present study provides insights to support the degree of high host-specificity exhibited by the genus. These results may prove useful as this species could be used as a potential biological tag for the accurate identification of *C. brevipinna*, since it has often been misidentified as *C. limbatus*, due to their overall body similarity. This is supported by recent studies, where highly host-specific onchoproteocephalideans, especially species of *Phoreiobothrium*, were used to aid in the identification of host species belonging to the *Rhizoprionodon acutus* Rüppel species complex (Alijanpour Darvishi & Haseli, 2019; Ganjgah & Haseli, 2020). The discovery of additional species of *Phoreiobothrium* is likely, with additional sampling efforts of host species that have been either misidentified or which have never been part of a parasitological investigation. This will also be beneficial as the assessment of the species diversity has the potential to provide the resources for future ecological investigations (Caira & Jensen, 2014, 2015; Alijanpour Darvishi & Haseli, 2019). With the high degree of host-specificity found amongst species of *Phoreiobothrium*, these species can be applied as sentinel species for an accurate identification and diagnosis of elasmobranch hosts (Alijanpour Darvishi & Haseli, 2019). The lack of molecular and phylogenetic analyses within this field, renders a great vacuity regarding both phylogenetic relationships amongst congeners, as well as any information on the specific life-cycles of these marine cestodes. Future large-scale surveys, incorporating interdisciplinary approaches in ecology, taxonomy and molecular biology will undoubtedly lead to numerous scientific advances in the field of marine parasitology in South Africa.

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References

- Alijanpour Darvishi, F. & Haseli, M., 2019. Two new species of *Phoreiobothrium* Linton, 1889 (Cestoda: Onchoproteocephalidea) off southern Iran, completing the puzzle of *Phoreiobothrium* faunas in *Rhizoprionodon acutus* species complex. *Parasitology Research*, 118: 2557–2566.
- Allan, B.R. & Cliff, G. 2000. Sharks caught in the protective gill nets off KwaZulu-Natal, South Africa. 9. The spinner shark *Carcharhinus brevipinna* (Müller and Henle). *African Journal of Marine Science*, 22: 199–215.
- Caira, J.N. & Jensen, K. 2014. A digest of elasmobranch tapeworms. *Journal of Parasitology*, 100: 373–391.
- Caira, J.N. & Jensen, K. 2015. Insights on the identities of sharks of the *Rhizoprionodon acutus* (Elasmobranchii: Carcharhiniformes) species complex based on three new species of *Phoreiobothrium* (Cestoda: Onchoproteocephalidea). *Zootaxa*, 4059: 335–350.
- Caira, J.N., Jensen, K. & Barbeau, E. Editors. 2021. Global Cestode Database. World Wide Web electronic publication. www.tapewormdb.uconn.edu. Date accessed: 10 Dec. 2021.
- Caira, J.N., Jensen, K. & Ivanov, V.A. 2017. Onchoproteocephalidea II Caira, Jensen, Waeschenbach, Olson & Littlewood, 2014. In: Caira JN, Jensen K (eds) Planetary biodiversity inventory (2008–2017): tapeworms from vertebrate bowels of the earth. University of Kansas, Natural History Museum, Special Publication No. 25, Lawrence, KS, USA, pp. 279–304.
- Caira, J.N., Jensen, K., Waeschenbach, A., Olson, P.D. & Littlewood, D.T.J. 2014. Orders out of chaos—molecular phylogenetics reveals the complexity of shark and stingray tapeworm relationships. *International Journal for Parasitology*, 44: 55–73.
- Caira, J.N. & Reyda, F.B. 2005. Eucestoda (true tapeworms). In: Rohde, K. (Ed.), *Marine Parasitology*. CSIRO Publishing, Collingwood, pp. 92–104.
- Caira, J.N., Richmond, C. & Swanson, J. 2005. A revision of *Phoreiobothrium* (Tetraphyllidea: Onchobothriidae) with descriptions of five new species. *Journal of Parasitology*, 91: 1153–1174.
- Chervy, L. Unified terminology for cestode microtriches: a proposal from the International Workshops on Cestode Systematics in 2002–2008. *Folia Parasitologica*, 56: 199–230.

Compagno, L.J.V. 1984. F.A.O. species catalogue. 4. Sharks of the world. An annotated and illustrated catalogue of shark species known to date. (2) Carcharhiniformes. F.A.O. *Fisheries Synopsis*, 125: 250–655.

Froese, R. & Pauly, D. Editors. 2022. FishBase. World Wide Web electronic publication. Accessed at: www.fishbase.org. Date accessed: 04 Feb. 2022.

Ganjgah, N.M. & Haseli, M. 2020. A new onchoproteocephalidean cestode species from the spottail shark, *Carcharhinus sorrah* (Müller et Henle), in the Persian Gulf. *Acta Parasitologica*, 65: 518–524.

Jensen, K. 2005. A monograph on the Lecanicephalidea (Platyhelminthes, Cestoda). *Bulletin of the University of Nebraska State Museum*, 18: 1–241.

Jensen, K. & Bullard, A. 2010. Characterization of a diversity of tetraphyllidean and rhinebothriidean cestode larval types, with comments on host associations and life-cycles. *International Journal for Parasitology*, 40: 889–910.

Naylor, G.J., Caira, J.N., Jensen, K., Rosana, K.A.M., White, W.T. & Last, P.R. 2012. A DNA sequence–based approach to the identification of shark and ray species and its implications for global elasmobranch diversity and parasitology. *Bulletin of the American Museum of Natural History*, 367: 1–262.

Oosthuizen, G., Acosta, A.A., Smit, N.J. & Schaeffner, B.C. 2021. A new species of *Grillotia* Guiart, 1927 (Cestoda: Trypanorhyncha) from the spotted skate, *Raja straeleni* Poll, in South Africa. *Parasitology International*, 82: 102307.

Pollerspöck, J. & Straube, N. 2022. Accessed at: www.shark-references.com. World Wide Web electronic publication, Version 2021. Date accessed: 01 Feb. 2022.

Schaeffner B.C. & Smit N.J. 2019. Parasites of cartilaginous fishes (Chondrichthyes) in South Africa - a neglected field of marine science. *Folia Parasitologica*, 66: 002.

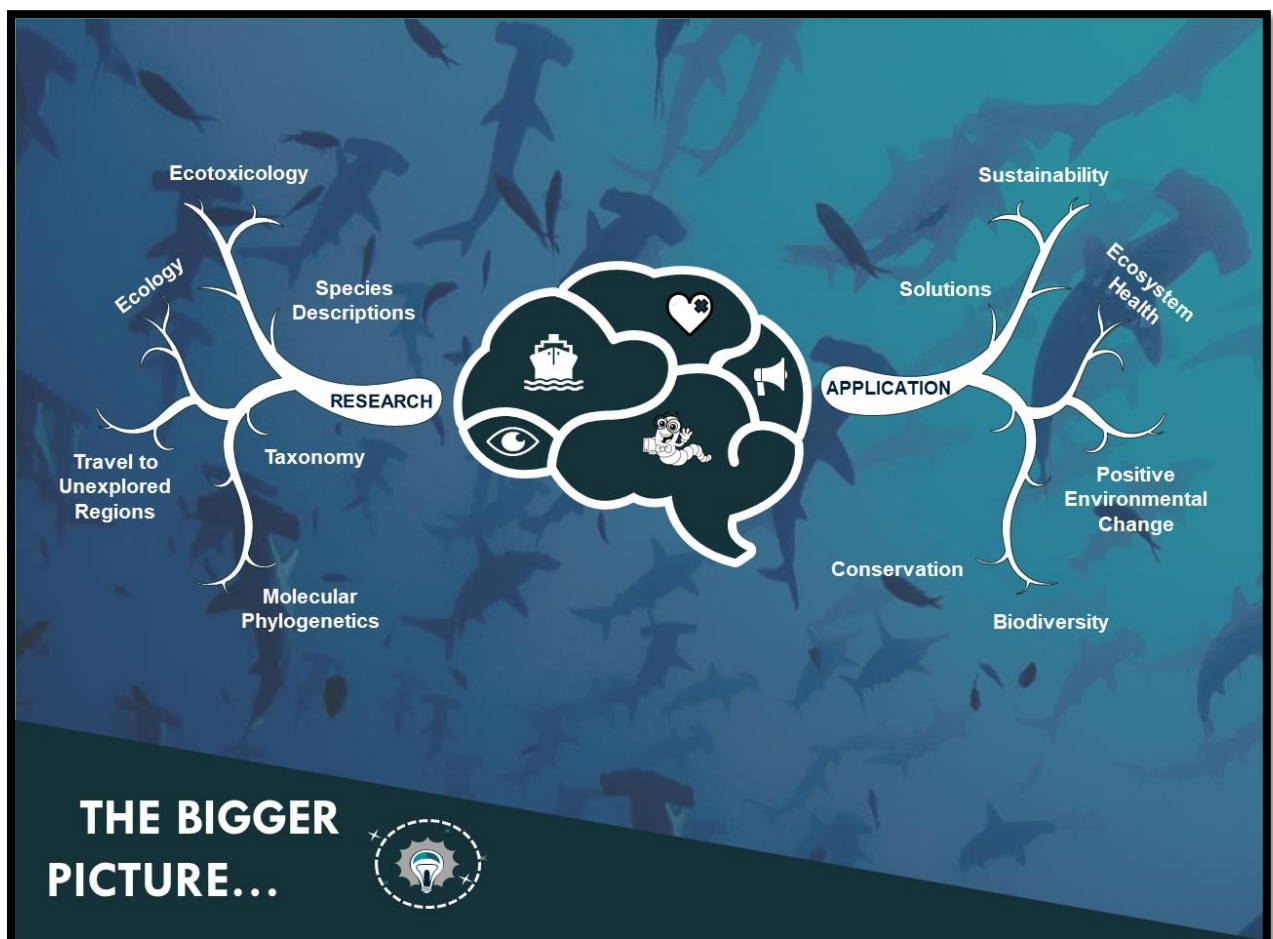
Tillett, B.J., Field, L.C., Bradshaw, C.J.A., Johnson, G., Buckworth, R.C., Meekan, M.G. & Ovenden, J.R. 2012. Accuracy of species identification by fisheries observers in a north Australian shark fishery. *Fisheries Research*, 127: 109–115.

Van Der Spuy, L., Smit, N.J. & Schaeffner, B.C. 2020. Four new species of *Acanthobothrium* van Beneden, 1849 (Cestoda: Onchoproteocephalidea) from the spotted skate, *Raja straeleni* Poll, off the Western Cape, South Africa. *Folia Parasitologica*, 67: 036.

Van Der Spuy, L., Smit, N.J. & Schaeffner, B.C. 2022. Threatened, host-specific affiliates of a red-listed host: Three new species of *Acanthobothrium* van Beneden, 1849 (Cestoda: Onchoproteocephalidea) from the endangered white skate, *Rostroraja alba* (Lacépède). *International Journal for Parasitology: Parasites and Wildlife*, 17: 114–126.

WoRMS. 2022. *Phoreiobothrium* Linton, 1889. Accessed at: <https://www.marinespecies.org/aphia.php?p=taxdetails&id=415807>. Date accessed: 10 Feb. 2022.

CHAPTER 5: SUMMATIVE CONCLUSION



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5.1. Introduction

As part of a larger study on the biodiversity of marine fish parasites of southern Africa, the diversity of cestodes of the order Onchoproteocephalidea II Caira, Jensen, Waeschenback, Olsen et Littlewood, 2014 infecting cartilaginous fishes (Elasmobranchii) of South Africa was determined. This was achieved by use of morphological methods (i.e. light microscopy and scanning electron microscopy, SEM) from which the present study provides information on the taxonomic status of onchoproteocephalidean cestodes belonging to the genera *Acanthobothrium* van Beneden, 1848 and *Phoreiobothrium* Linton, 1889. Furthermore, the study provides the reader with a better understanding on the unique host-parasite interrelationship between cestodes and elasmobranchs in the Western Cape and KwaZulu-Natal provinces, as well as significant comparisons between cestodes described in the present study and cestode fauna from other zoogeographical regions. This study introduces and describes eight onchoproteocephalidean species new to science from the previously understudied ocean basins surrounding southern Africa.

In order to facilitate the reader, each specific objective is set out, discussed and concluded in separate subheadings. Furthermore, the reader will be provided with recommendations regarding future studies within this field. Thereafter a brief general conclusion will follow.

5.2. Research findings and recommendations

5.2.1. Onchoproteocephalidean cestode diversity

To date, the order Onchoproteocephalidea II consists of 12 valid genera (Caira *et al.*, 2017), constituting 275 (Caira *et al.*, 2022) species infecting elasmobranchs worldwide. These genera include *Megalonchos* Baer et Euzet, 1962, *Triloculatum* Caira et Jensen, 2009, *Platybothrium* Linton, 1890, *Prosobothrium* Cohn, 1902, *Uncibilocularis* Southwell, 1925, *Onchobothrium* de Blainville, 1828, *Pinguicollum* (Sleggs 1927) Riser, 1955, *Acanthobothroides* Brooks, 1977, *Potamotrygonocetus* Brooks et Thorson, 1976, *Maticestus* Caira, Jensen et Fyler, 2018, *Phoreiobothrium* Linton, 1889, and *Acanthobothrium* van Beneden, 1848. Of these, the latter genus is by far the most species-rich of all the onchoproteocephalidean cestode fauna, constituting 73 % (201 of 275) of species within this genus (Caira *et al.*, 2022). It is quite obvious that the diversity of species of *Acanthobothrium* greatly exceeds that of any other genus within this order since the second most species rich genus, *Phoreiobothrium*, only constitutes 8 % (17

of 201) of species (Caira *et al.*, 2022). Despite the immense diversity already present within this order, Caira *et al.* (2017) still estimate the global fauna of these cestodes to be greater than 1,150 species, since merely 40 % of all elasmobranchs have been examined for cestodes. Considering that onchoproteocephalidean cestodes predominantly infect batoids, this estimate only includes 269 species in Selachioidea, compared to 885 species in Batoidea (Caira *et al.*, 2017). These extreme estimates are not only due to a lack of host observations but also due to unexplored and financially challenged regions, where the establishment of many research initiatives become futile, along with the fact that particular endemic host species often go unnoticed (Poulin & Presswell, 2016; Randhawa & Poulin, 2020). Subsequently, the true diversity of these cestodes, coupled with their unique host-parasite interrelationships (see subheading 5.2.) remain largely unknown in many parts of the world, including the ocean basins surrounding South Africa. This is also grounded upon the fact that the majority of onchoproteocephalidean species have been reported from the north-west Atlantic Ocean (Caira *et al.*, 2022). Prior to this study, *A. edwardsi* Williams, 1969, was the only other *Acanthobothrium* species that has been described from the eastern Atlantic Ocean off the west coast of the U.K. (Williams, 1969). Moreover, there has previously not been a single report of onchoproteocephalidean cestodes present off the coast of South Africa. This is particularly peculiar, since South Africa is considered a biodiversity hotspot for chondrichthyan fishes, and the third most biodiverse country in the world (Weigmann, 2016; SANBI, 2022). Less than 10 % of all elasmobranchs within this region have been screened for parasites (Ebert & van Hees, 2015; Schaeffner & Smit, 2019; Van Der Spuy *et al.*, 2020), suggesting that South Africa holds an immense hidden diversity of onchoproteocephalidean cestodes (amongst numerous other parasites), based on the assumption that the parasite diversity should be at least equal to that of host species present within these waters (Caira & Healy, 2004; Schaeffner & Smit, 2019). Therefore, acknowledging this problem, the present study adds information on the cestode diversity of the extremely neglected region, and contributes to the biodiversity assessment.

The present study reports the first and only onchoproteocephalidean species found off the coast of southern Africa. Not only does this study report the first species from this region, but it also introduces eight new species within two separate genera, infecting three different host species. Seven new species of *Acanthobothrium* are described along with one new species of *Phoreiobothrium*, which endorses the fact that *Acanthobothrium* is the most specious genus within the order. The first four species of *Acanthobothrium* (i.e. *A. microhabentes*, *A. microtenuis*, *A. crassus* and *A. dolichocollum*) were described from a previously unexplored host, *Raja straeleni*, whilst the latter three species of *Acanthobothrium* (i.e. *A. umbungus*, *A. usengozinius* and *A. ulondolozus*) were described from an endangered host, *Rostroraja alba*, off the Western Cape province of South Africa. *Phoreiobothrium* sp. A. on the other hand, was described from *Carcharhinus brevipinna* of the coast of KwaZulu Natal, South Africa. With the new total of 208

valid species of *Acanthobothrium* and 18 valid species of *Phoreiobothrium*, the global fauna of onchoproteocephalidean cestodes has increased to 283 species. The addition of these species and newly discovered South African diversity could provide researchers with valuable insights regarding the intimate nature of their host-parasite interrelationships, leading to new prospects for the identification and conservation of host species affected by anthropogenic pressures.

5.2.2. Host-parasite interrelationships off the coast of South Africa

Since onchoproteocephalideans are found to parasitise both sharks and batoids, these cestodes display an extremely wide host range (Caira *et al.*, 2017). The majority of onchoproteocephalideans (i.e. *Acanthobothrium*) is particularly dominant in batoid rays, since 84 % (21 of 25) of all batoid families have been reported to harbour onchoproteocephalidean cestodes (Caira *et al.*, 2017). Although not as dominant as batoids, they have also been reported from 57 % (4 of 7) of shark orders, and are primarily found within carcharhiniform sharks (Caira *et al.*, 2017). This is also preliminarily supported by the present study, with the majority of species described from batoids, in comparison to the single species found in the order Carcharhiniformes.

Onchoproteocephalideans also exhibit oioxenous specificity towards their hosts, and it is not uncommon for individual host species to harbour synhospitalic onchoproteocephalidean congeners (Fyler, 2009; Caira *et al.*, 2017). The nature of host specificity amongst onchoproteocephalidean cestodes is often determined on genus level since the substantial advances made in generic monophyly (Caira & Jensen, 2014). As seen in the present study, the newly described species of *Acanthobothrium* all represent synhospitalic congeners, since three to four species of *Acanthobothrium* were found within a single host species. Species of *Phoreiobothrium* on the other hand are extremely host specific, parasitising a single definitive host species. With the exception of *P. exceptum* and *P. pectinatum*, a host species is not often occupied by congeners of *Phoreiobothrium* (Caira & Jensen, 2015).

In the present study, eight spotted skates, *Raja straeleni*, were examined for onchoproteocephalidean cestodes yielding four new species of *Acanthobothrium*. Additionally, two endangered white skates, *Rostroraja alba*, were examined, resulting in the discovery of three new species of *Acanthobothrium*. The discovery of *Phoreiobothrium* sp. A resulted from the examination of the spinner shark, *Carcharhinus brevipinna*. Since no valid onchoproteocephalidean species have been described from two of these hosts, the new species reported herein expand the host associations within the order. Albeit only on species level, one new host species, *R. straeleni*, is added to the family Rajidae, and one new host species, *C. brevipinna*, has been added to the family Carcharhinidae, respectively.

According to Caira and Jensen (2001; 2014), and Beer *et al.* (2019), cestodes and elasmobranchs have co-specified and co-evolved for eons. By understanding the diversity and specific host associations of a specific order or genus is extremely beneficial, not only to determine future estimates of undescribed biodiversity but also to determine the specific and intimate relationships these species share (Nhi *et al.*, 2013; Caira & Jensen, 2014). When researchers consider co-speciation events between known host-parasite associations, these host-parasite systems could provide valuable information regarding life-cycles and environmental requirements (Nhi *et al.*, 2013). Furthermore, studies by Jankovská *et al.* (2011) and Nhi, *et al.* (2013) concluded that cestodes are capable to accumulate high concentrations of heavy metal pollutants from their specific elasmobranch host tissues, conceivably lowering the repercussions of these pollutants for susceptible host species. As a result, these cestodes could play a pivotal role for the ecosystem health, providing that they could simultaneously be considered as biological tag species for the accurate identification of host species (Alijanpour Darvishi & Haseli, 2019), and as indicators of pollution or other environmental changes which impact host species (Marcogliese, 2005; Nhi *et al.*, 2013; Beer *et al.*, 2019).

5.2.3. Comparisons to the cestode fauna of other zoogeographical regions

Planet Earth consists of twelve marine realms (Spalding *et al.*, 2007), constituting comparative zoogeographical regions. Amongst these, the Indo-Pacific, Eastern Pacific, Western Atlantic and Eastern Atlantic Ocean regions were defined by Ekman (1953) and Briggs (1974; 1995) to be the most relevant marine biogeographical regions of the world.

In order to ensure the validity of the eight newly described species, all of them were compared to congeners from other marine zoogeographical regions across the globe, considering that species found within the same zoogeographical region are supposedly conspecific (Goldstein 1967, Williams 1969, Ghoshroy & Caira, 2001). However, since these reports represent the first species to be reported from this region and given the extremely high species-diversity of valid *Acanthobothrium* species, the category classification system developed by Ghoshroy and Caira (2001) was used to facilitate the differentiation between congeners. By use of this classification system it became clear that all species of *Acanthobothrium* found in the ocean basins of southern Africa were identified as category 2 species (Van Der Spuy *et al.*, 2020; 2022). Prior to this study, there were already 47 other *Acanthobothrium* species designated to this category (Maleki *et al.*, 2013; Yang *et al.*, 2016; Zaragoza-Tapia *et al.*, 2019; 2020), which hindered direct species comparisons.

The first four species of *Acanthobothrium* (*A. microhabentes*, *A. microtenuis*, *A. crassus* and *A. dolichocollum*) were only distinguished from 12 other species of *Acanthobothrium* worldwide, due

to the unique feature of postovarian testes; this feature was previously thought to be restricted to taxa from the Indo-Pacific Ocean (Fyler *et al.*, 2009; Fyler & Caira, 2010; Maleki *et al.*, 2015). Amongst these, only two congeners, namely *Acanthobothrium popi* Fyler, Caira and Jensen, 2009, and *Acanthobothrium bobconniorum* Fyler and Caira, 2010, were placed in the same category as the South African species (Fyler *et al.*, 2009; Fyler & Caira, 2010; Maleki *et al.*, 2015). Although differing in a combination of characteristic features, only the most pronounced differences are discussed. Both *A. microhabentes* and *A. microtenuis* differ from the above-mentioned congeners by having a longer cephalic peduncle, shorter scolex, and fewer postovarian testes. *Acanthobothrium microtenuis* is also quite unique in its possession of columnar spinitriches on its terminal proglottid. Additionally, *A. crassus* and *A. dolichocollum* also differ by having acraspedote proglottids and an extremely long cephalic peduncle, respectively. Since *A. umbungus* and *A. usengozinius* do not possess postovarian testes, their comparisons were aggravated, since they had to be distinguished from a total of 51 category 2 species within *Acanthobothrium*. *Acanthobothrium umbungus* and *A. usengozinius* were most easily distinguished by their hooks being of a much smaller size than the ones of a majority of congeners. Apart from hook measurements, *A. umbungus* and *A. usengozinius* are also easily distinguished from all congeners and each other by a combination of the body size, number of testes, scolex length, bothridium width, and the length of the cephalic peduncle. *Acanthobothrium ulondolozus* on the other hand was also noted to possess testes posterior to the ovarian isthmus. Accordingly, *A. usengozinius* differs from *A. bobconniorum*, *A. crassus*, *A. dolichocollum*, *A. microhabentes*, *A. microtenuis*, and *A. popi* in combination of the overall body size, scolex size, hook measurements, cephalic peduncle length, and ovary size.

By referring to each respective remarks section along with the discussions from both chapters two and three, it became obvious that the South African congeners from the south-eastern Atlantic most closely resemble species found in the Indo-Pacific off the coast of Australia (Caira *et al.*, 2022). Moreover, research on the possible relatedness of five of the South African species possessing postovarian testes with congeners from the Indo-Pacific could render intriguing results regarding the occurrence of monophyly between these two distinct regions (Van Der Spuy *et al.*, 2020).

Unlike the results generated for species of *Acanthobothrium*, chapter four suggests that *Phoreiobothrium* sp. A from the south-western Indian Ocean most closely resembles a congener from the north-western Atlantic off the coast of the United States of America. This species was not divided into a category classification system and consequently distinguished from 17 congeners worldwide. It seems that the South African species are all unique regarding hook measurements, as *Phoreiobothrium* sp. A can also be easily distinguished from other species by the size of its hooks. Furthermore, this is the first onchoproteocephalidean species to display a

scarcity of gladiate spinitriches on its cephalic peduncle, which may either represent a new generic characteristic or an artefact.

Currently, there is limited information available on the molecular phylogenetics of onchoproteocephalidean cestodes (Franzese & Ivanov 2018). The results of the present study therefore only emphasise the importance and the necessity of extensive future molecular analyses. Phylogenetic trees do not only provide insights on host associations but also reveal the biogeographic distribution of species (Fyler, 2009). Consequently, without a comprehensive molecular analysis, the phylogenetic status and evolutionary relatedness between the new South African species to each other and to congeners still remains a mystery.

5.2.4. Future recommendations

Throughout the present study, a number of complications occurred regarding host collections, fixation protocols and ultimately molecular analyses. The reason that only one host species was included in the study from the KwaZulu-Natal province, is based on the fact that sharks and batoids are collected after suffocation occurred in the shark nets. These species are collected by the KwaZulu-Natal Sharks Board and subsequently frozen, causing most parasites to die and disintegrate. For this reason, many other species collected from this region yielded either no parasites, or merely fragments of undistinguishable species. Future collections should focus on fresh host samples, followed by the immediate dissection and screening of organs for parasites. Molecular analyses were attempted during the study, however, due to complications that arose working on dead host specimens along with previously frozen, disintegrated parasite material, only DNA sequence fragments have been obtained, which rendered phylogenetic analyses unfeasible. Fixing parasitic specimens as soon as possible in the correct media for molecular and morphological studies is highly recommended. Specimens for morphology should be carefully handpicked and placed in 4 % neutrally buffered formalin from where they should be transferred after a period of two weeks to 70 % ethanol. Specimens for molecular analyses should be placed in molecular grade ethanol, avoiding all contact with formalin. Specimens collected for SEM should be cleaned by lightly brushing their surface before placing them in any media, after which it can be placed in 80 % ethanol. This research would have greatly benefited from a comprehensive molecular analysis, which should be anticipated in the future.

5.3. Conclusion

The biogeographical realm of Temperate Southern Africa conceals a vast, hidden cestode diversity. Collections of additional hosts and localities, which have previously not been properly

explored, such as the ocean basins surrounding southern Africa are required, which will undoubtedly lead to numerous new species discoveries, which will contribute to an already biodiverse region. Albeit the discovery of eight species new to science, the present study only covers the tip of an extraordinary iceberg, that awaits future research and discovery. The lack of molecular and phylogenetic analyses within this field, renders a great vacuity regarding both phylogenetic relationships amongst congeners, as well as any information on the specific life-cycles of these marine cestodes. Given the current anthropogenic threats posed on elasmobranch populations, this study highlights the need for the incorporation of novel and integrative approaches of interdisciplinary fields such as ecology, conservation science and ecotoxicology for future research. When we consider to not only explore the species diversity, but to additionally use these parasites as indicator organisms for pollution and global change, we may yield a much better understanding on the evolutionary host-parasite interrelationships present within our world's remarkable ecosystems, contributing to various scientific fields, and ultimately contributing to the conservation of declining populations of endangered species and positive environmental change.

References

- Alijanpour Darvishi, F. & Haseli, M. 2019. Two new species of *Phoreiobothrium* Linton, 1889 (Cestoda: Onchoproteocephalidea) off southern Iran, completing the puzzle of *Phoreiobothrium* faunas in *Rhizoprionodon acutus* species complex. *Parasitology Research*, 118: 2557–2566.
- Beer, A., Ingram, T. & Randhawa, H.S. 2019. Role of ecology and phylogeny in determining tapeworm assemblages in skates (Rajiformes). *Journal of Helminthology*, 93: 738–751.
- Briggs, J.C. 1974. Marine Zoogeography. New York: McGraw-Hill. 1995. Global Biogeography. Amsterdam: Elsevier.
- Briggs, J.C. 1995. Global biogeography, Vol 14. Developments in Palaeontology and Stratigraphy. Elsevier, Amsterdam, pp. 451.
- Caira, J.N. & Jensen, K. 2014. A digest of elasmobranch tapeworms. *Journal of Parasitology*, 100: 373–391.
- Caira, J.N. & Healy, C.J. 2004. Elasmobranchs as hosts of metazoan parasites. In 'Biology of Sharks and their Relatives'. (Eds. J. C. Carrier, J. A. Musick, and M. R. Heithaus.) CRC Press: Boca Raton, FL, U.S.A., pp. 523–551.
- Caira, J.N. & Jensen, K. 2001. An investigation of the co-evolutionary relationships between onchobothriid tapeworms and their elasmobranch hosts. *International Journal for Parasitology*, 31: 960–975.
- Caira, J.N. & Jensen, K. 2015. Insights on the identities of sharks of the *Rhizoprionodon acutus* (Elasmobranchii: Carcharhiniformes) species complex based on three new species of *Phoreiobothrium* (Cestoda: Onchoproteocephalidea). *Zootaxa*, 4059: 335–350.
- Caira, J.N., Jensen, K. & Barbeau, E. (Eds.) 2022. Global Cestode Database. World Wide Web electronic publication. Accessed at: www.tapewormdb.uconn.edu. Date accessed: 01 Feb. 2022.
- Caira, J.N., Jensen, K. & Ivanov, V.A. 2017. Onchoproteocephalidea II. In: J.N. Caira and K. Jensen (Eds.), Planetary Biodiversity Inventory (2008–2017): Tapeworms from Vertebrate

Bowels of the Earth. The University of Kansas Natural History Museum. Special Publication no. 25, pp. 279–304.

Ebert, D. & van Hees, K.E. 2015. Beyond jaws: rediscovering the ‘lost sharks’ of southern Africa. *African Journal of Marine Science*, 37: 141–156.

Ekman, S. 1953. Zoogeography of the Sea. London: Sidgwick and Jackson. *Annals and Magazine of Natural History*, 6: 63, 240.

Franzese S., Ivanov V.A. 2018. Hyperapolytic species of *Acanthobothrium* (Cestoda: Onchoproteocephalidea) from batoids off Argentina. *Parasitology International*, 67: 431–443.

Fyler C.A. 2009. Systematics, Biogeography and Character Evolution in the Tapeworm Genus *Acanthobothrium* van Beneden, 1850. ProQuest Dissertations Publishing, University of Connecticut, pp. 200.

Fyler C.A., Caira J.N. & Jensen K. 2009. Five new species of *Acanthobothrium* (Cestoda: Tetraphyllidea) from an unusual species of *Himantura* (Rajiformes: Dasyatidae) from northern Australia. *Folia Parasitologica*, 56: 107–128.

Fyler, C.A. & Caira, J.N. 2010. Phylogenetic status of four new species of *Acanthobothrium* (Cestoda: Tetraphyllidea) parasitic on the wedge fish *Rhynchobatus laevis* (Elasmobranchii: Rhynchobatidae): implications for interpreting host associations. *Invertebrate Systematics*, 24: 419–433.

Ghoshroy, S. & Caira, J.N. 2001. Four new species of *Acanthobothrium* (Cestoda: Tetraphyllidea) from the whiptail stingray *Dasyatis brevis* in the Gulf of California, Mexico. *Journal of Parasitology*, 87: 354–372.

Goldstein R.J. 1967. The genus *Acanthobothrium* van Beneden, 1849 (Cestoda: Tetraphyllidea). *Journal of Parasitology*, 53: 455–483.

Jankovská, I., Míhlová, D., Petrý, I.M., Romocuský, S., Kalous, L., Vadlejch, J., Cadková, Z. & Langrová, I. 2011. The intestinal parasite *Acanthocephalus lucii* (Acanthocephala) from European perch (*Perca fluviatilis*) as a bioindicator for lead pollution in the stream “Jevanský potok” near Prague, Czech Republic. *Bulletin for Environmental Contamination and Toxicology*, 86: 342–346.

Maleki, L., Malek, M. & Palm, H.W. 2013. Two new species of *Acanthobothrium* (Tetraphyllidea: Onchobothriidae) from *Pastinachus* cf. *sephen* (Myliobatiformes: Dasyatidae) from the Persian Gulf and Gulf of Oman. *Folia Parasitologica*, 60: 448–456.

Maleki, L., Malek, M. & Palm, H.W. 2015. Four new species of *Acanthobothrium* van Beneden, 1850 (Cestoda: Onchoproteocephalidea) from the guitarfish, *Rhynchobatus* cf. *djiddensis* (Elasmobranchii: Rhynchobatidae), from the Persian Gulf and Gulf of Oman. *Folia Parasitologica*, 62: 012.

Marcogliese, D.J. 2005. Parasites of the superorganism: are they indicators of ecosystem health? *International Journal for Parasitology*, 35: 705–716.

Nhi, T.T.Y., Shazili, N.A.M. & Harrison, F.S. 2013. Use of cestodes as indicator of heavy-metal pollution. *Experimental Parasitology*, 133: 75–79.

Poulin, R. & Presswell, B. 2016. Taxonomic quality of species descriptions varies over time and with the number of authors, but unevenly among parasite taxa. *Systematic Biology*, 65: 1107–1116.

Randhawa, H.S., Poulin, R., 2020. Tapeworm discovery in elasmobranch fishes: quantifying patterns and identifying their correlates. *Marine Freshwater Research*, 71, 78–88.

SANBI. 2022. Accessed at: <https://www.sanbi.org/biodiversity/>. Date accessed: 10 Feb. 2022.

Schaeffner, B.C. & Smit, N.J. 2019. Parasites of cartilaginous fishes (Chondrichthyes) in South Africa - a neglected field of marine science. *Folia Parasitologica*, 66: 002.

Van Der Spuy, L., Smit, N.J. & Schaeffner, B.C. 2020. Four new species of *Acanthobothrium* van Beneden, 1849 (Cestoda: Onchoproteocephalidea) from the spotted skate, *Raja straeleni* Poll, off the Western Cape, South Africa. *Folia Parasitologica*, 67: 036.

Van Der Spuy, L., Smit, N.J. & Schaeffner, B.C. 2022. Threatened, host-specific affiliates of a red-listed host: Three new species of *Acanthobothrium* van Beneden, 1849 (Cestoda: Onchoproteocephalidea) from the endangered white skate, *Rostroraja alba* (Lacépède). *International Journal for Parasitology: Parasites and Wildlife*, 17: 114–126.

Weigmann, S. 2016. Annotated checklist of the living sharks, batoids and chimaeras (Chondrichthyes) of the world, with a focus on biogeographical diversity. *Journal of Fish Biology*, 88: 837–1037.

Williams, H.H. 1969: The genus *Acanthobothrium* van Beneden 1849 (Cestoda: Tetraphyllidea). *Nytt Magasin for Zoologi*, 17, 1–56.

Yang, C., Sun, Y., Zhi, T., Iwaki, T., Reyda, F.B. & Yang, T. 2016. Two new and one redescribed species of *Acanthobothrium* (Cestoda: Onchoproteocephalidea: Onchobothriidae) from *Dasyatis akajei* (Myliobatiformes: Dasyatidae) in the China Sea. *Zootaxa*, 4169: 286–300.

Zaragoza-Tapia, F., Pulido-Flores, G., Gardner, S.L. & Monks, S. 2020. Host relationships and geographic distribution of species of *Acanthobothrium* Blanchard, 1848 (Onchoproteocephalidea, Onchobothriidae) in elasmobranchs: a metadata analysis. *ZooKeys*, 940: 1–49.

Zaragoza-Tapia, F., Pulido-Flores, G., Violante-González, J. & Monks, S. 2019. Two new species of *Acanthobothrium* Blanchard, 1848 (Onchobothriidae) in *Narcine entemedor* Jordan & Starks, 1895 (Narcinidae) from Acapulco, Guerrero, Mexico. *ZooKeys*, 852: 1–21.

APPENDICES

APPENDICES 1: FOLIA PARASITOLOGICA – GUIDE TO AUTHORS

1. PREPARATION OF MANUSCRIPTS – GENERAL INFORMATION

1.1. Language. Manuscripts should be written in simple and concise scientific English using British spelling. Authors whose mother tongue is not English are strongly urged to have their manuscripts checked and improved linguistically by a native speaker (preferably, a life scientist) before submission. In fact, editorial office does not take responsibility of the quality of the English. Professional provider of pre-submission editing services, such as Enago (www.enago.com) or Edanz (www.edanzediting.com) can be consulted. Submissions written in poor English will be returned immediately.

1.2. Names. Scientific names of taxa up to the generic level should be italicised throughout. Current, valid names of hosts should be used (with synonyms, such as used in cited papers, given in parentheses). Scientific names of parasites and hosts studied should be given with authorities (and, for parasites, year of description) in the Abstract and, again, when first mentioned in the main body of text, and also in tables and figures legends. Otherwise, citing these authorities is to be avoided or used sparingly. Authority and year of description should also be given for parasites mentioned in taxonomic comparison. References to authors of names should not be included in the References section. The following style is required: *Fasciola hepatica* Linnaeus, 1758, *Allocreadium patagonicum* Shimazu, Urawa et Coria, 2000. (Note that names of all authors are given; 'et' connects two last authors' names; and last author's name is separated from year by comma.).

1.3. Numerals. All numerals from one to ten are spelled out in the text except for morphological descriptions.

1.4. Taxonomic descriptions. Authors should follow all requirements of the current International Code of Zoological Nomenclature (1999). This journal requires that at least holotype be deposited in an institution that provides long-term care of collections and access for study of deposited materials. One of such institutions is the Institute of Parasitology, Biology Centre ASCR (publisher of this journal). Whenever possible, manuscripts containing description of new species of helminths or arthropods are expected to be accompanied with at least one paratype of each new taxon for scrutiny by reviewers and for subsequent deposition in the collection of the Institute (postal address: Tomáš Scholz, Curator, Institute of Parasitology, Biology Centre CAS, Branišovská 31, 370 05 České Budějovice, Czech Republic). Within taxonomic treatment the following order of data is preferred: species name, author with year of description (if not new species), reference to figures; synonymy; description in telegraphic style (i.e. omitting articles and as many verbs as can be elided without loss of meaning); after description: data for the material studied or taxonomic summary (type host, other hosts, type locality, other localities, site, prevalence, specimens deposited and their collection numbers); etymology; differential diagnosis (best headed as 'Remarks'); other comments if appropriate.

1.5. Citations. For papers by two authors, names should be connected with 'and', e.g. Koskivaara and Valtonen (1992). For papers by more than two authors, the first author should be cited with 'et al.', e.g. Iglesias et al. (1997). Names and years should not be separated by commas, e.g. (Koskivaara and Valtonen 1992); commas, not semicolons, should separate citations in parentheses, e.g. (Koskivaara and Valtonen 1992, Iglesias et al. 1997a,b). In the text, references should be listed in chronological order; papers published in the same year are ordered alphabetically.

1.6. References to illustrations and tables. Examples: Fig. 1. Figs. 1, 2. Figs. 2A,B. Figs. 1–3. Table 1. Use fig. 1, i.e. do not capitalise, for figures in other papers.

2. PREPARATION OF MANUSCRIPTS – ORGANISATION

The standard order of constituent parts for original full papers is: Title page (with Title, Author name[s], Running header, Abstract, Keywords, Address for correspondence); Introduction (which, however, does not bear this heading); Materials and Methods; Results; Discussion; Acknowledgements; References; Tables; Figure legends. The layout of research notes is similar to that of full papers but section headings (except for Abstract, Keywords and References) are omitted. For your convenience *Folia Parasitologica* has developed template word file to help you prepare your manuscript.

2.1. Title. Title should not mention authors of taxa and years of description unless in special cases (e.g. taxonomic revisions and redescriptions). Title is expected to contain names of higher taxa (such as order and family) accommodating the parasites and hosts under study; the names of higher taxa may not be appropriate for multiple parasites/hosts or widely used models in papers of experimental nature.

2.2. Authorship. Author names should be given in bold with full first names and with institutional addresses. Start each different address (not postal, i.e. without details such as street and ZIP code) on a separate line and separate them with semicolon.

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2.4. Abstract. It should be a factual summarisation of the main results and conclusions so that it can be published in abstracting journals without change. Abstracts, written without paragraphs, should not exceed 300 words (reviews and full papers) or 150 words (research notes). Abstracts of taxonomic papers should mention all nomenclatural acts and newly proposed nominal taxa with their brief differential diagnoses.

2.5. Keywords. Suggest a set of keywords (index terms). The set should be complete but keep it as short as possible (typically, under 10 words/phrases). Words from the title should not be repeated.

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2.10. Discussion. It should discuss new data in relation to existing information. It should be straightforward, not wordy, and perspectives of future research should be outlined.

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2.12. References. Entries should be listed alphabetically by names of all authors and, subordinately, chronologically. Author names should **not** be typed in capital letters. Title of journal article should be cited in full, followed by abbreviated name of journal as listed in *Serial Sources for the BIOSIS Data Base* (BioSciences Information Service, Philadelphia), volume and pagination (example 1). Issue number should be given (after

volume, in parentheses) only if volume is not continuously paginated. Title and chapter pagination are required for chapters in monographs (2). Publisher, place where published and number of pages are required for monographs (3). Titles in languages other than English, German, French, Spanish, Portuguese and Italian should be translated into English and their original language stated in parentheses. Titles whose English version is not that given in the original publication should be in square brackets (4). Electronic publication should be cited with the most recent version (5). Examples:

- (1) Moravec F., Bilal S.J., Abdullah S.M.A. 2012: Two species of *Rhabdochona* (Nematoda: Rhabdochonidae) from the cyprinid fish *Luciobarbus kersin* (Heckel) in northern Iraq, including *R. (Globochona) kurdistanensis* sp. n. *Folia Parasitol.* 59: 139–147. [Note that dash (–), not hyphen (-), should be used.]
- (2) Scholz T., Kuchta R., Williams C. 2012: *Bothriocephalus acheilognathi* Yamaguti, 1934. In: P.T.K. Woo and K. Buchmann (Eds.), *Fish Parasites: Pathobiology and Protection*. CABI, Wallingford, pp. 292–307.
- (3) Ružek D. (Ed.) 2011: *Flavivirus Encephalitis*. InTech, Rijeka, 478 pp.
- (4) Lykova K.A., Gulyaev V.D., Melnikova Yu.A., Karpenko S.V. 2006: [On the species independence of *Mathevolepis larbi* Karpenko, 1982 (Cyclophyllidae: Hymenolepididae: Ditestolepidini).] *Parazitologiya* 40: 299–305. (In Russian.)
- (5) Froese R., Pauly D. (Eds.) 2016: *FishBase*. World Wide Web electronic publication, www.fishbase.org, 1/2016.
- (6) Beveridge I. 2014: A review of the genus *Paramoniezia* Maplestone et Southwell, 1923 (Cestoda: Anoplocephalidae), with a new genus, *Phascolocestus*, from wombats (Marsupialia) and redescription of *Moniezia mettami* Baylis, 1934 and *Moniezia phacochoeri*. *Folia Parasitol.* 61: (in press).

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2.13. Legends. All abbreviations and symbols appearing in figures should be explained in legends or (if too numerous and frequently repeated) collected in a list (preferably in Materials and Methods). For composite plates, a summary statement, if possible, should precede specific explanations to separate figures. Parasite (with authority and year) and host names (with authorities but not year) should be spelled out completely in each legend.

2.14. Tables. Tables, typed on separate files and numbered consecutively with Arabic numerals, should be self-explanatory, i.e. their headings and explanations should make them fully understandable without reference to text.

2.15. Author Contributions statement. Authors should include a brief Author Contributions statement at the end of the paper in which they describe their specific contributions to the published work. Contributions could include, but are not limited to:

- designed research
- performed research
- contributed new reagents or analytical tools
- analysed data
- wrote the paper

3. PREPARATION OF MANUSCRIPTS – FIGURES

3.1. Illustrations. Illustrations should be numbered consecutively with Arabic numerals in left upper corner. Whenever possible, individual figures should be grouped together in a single block (composite figure) of rectangular shape, using space efficiently. Without change or after reduction, illustrations should neatly fit into one or two columns. The maximum printing area is 165 × 235 mm, but an appropriate space below figures should be left for legends. The elements of composite figure can be identified with consecutive Arabic numerals or with letters in Arial (in upper left corner). A scale bar is required for each figure (on right side), with units directly in figure.

3.2. Graphs. Two-dimensional black-and-white graphics are preferred. Lettering (in Arial) should be of such size that the height of characters, after possible reduction (often to one-column width, 80 mm), will be at least 2.5 and 1.25 mm (upper and lower case, respectively). The same font style and lettering size should be used for all graphs.

3.3. Photographs. It is optimal to submit photographs in the same size as they will appear in the journal. Space should be used efficiently; individual figures should be trimmed to contain only relevant information but areas of major interest (as well as labelling) should not be too close to the edges of figures. Individual figures should be composed in plates of rectangular shape. Magnification should be indicated by scale bars. All labels (i.e. figure identification, symbols, letters and scale bars) should be in Arial.

4. POLICY ON THE ACCESSIBILITY OF MOLECULAR DATA

Molecular data supporting publication results should be made available for scrutiny during the review process and for future use. Specifically, alignments of DNA sequences or RNA datasets are required to be made available either as Supporting information on the Folia Parasitologica web site or at publicly accessible repositories (e.g. <http://datadryad.org/>). Supporting information should be provided with sufficient details so that the data can be interpreted correctly by a third party. Supporting data will be made publicly available at the time of publication. Upon request, embargos may be exceptionally granted by the editorial team for data containing sensitive information (e.g. human medical data).

5. SPECIFIC REMARKS

Some specific remarks are listed below (in the case of any queries or doubts, please do not hesitate to request editorial assistant at folia@paru.cas.cz):

- Mean $\pm$ standard deviation (or standard error) should be provided only if $n > 30$; otherwise, mean with range should be presented.
- Symbols for protein-coding genes should be italicised (e.g. *cox1*), whereas symbols for proteins should not be italicised (e.g. *cox1*). The formatting of symbols for RNA-coding genes should not be italicised (e.g. 18S rDNA, 12S rRNA gene, ITS or SSU rDNA).
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- [sic!]

APPENDICS 2: INTERNATIONAL JOURNAL FOR PARASITOLOGY: PARASITES AND WILDLIFE – GUIDE TO AUTHORS

INTRODUCTION

The International Journal for Parasitology: Parasites and Wildlife (IJP:PAW) publishes the results of original research on parasites of all wildlife, invertebrate and vertebrate. This includes free-ranging, wild populations, as well as captive wildlife, semi-domesticated species (e.g. reindeer) and farmed populations of recently domesticated or wild-captured species (e.g. cultured fishes). Articles on all aspects of wildlife parasitology are welcomed including taxonomy, biodiversity and distribution, ecology and epidemiology, population biology and host-parasite relationships. The impact of parasites on the health and conservation of wildlife is seen as an important area covered by the Journal especially the potential role of environmental factors, for example climate. Also important to the journal is 'one health' and the nature of interactions between wildlife, people and domestic animals, including disease emergence and zoonoses.

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Study title: Morphological and molecular characterisation of Onchoproteocephalidean cestodes of South African elasmobranchs.
Study Leader/Supervisor: Prof NJ Smit
Student: L Van Der Spuy

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 - to ask further questions, seek additional information, require further modification or monitor the conduct of your research or the informed consent process;
 - withdraw or postpone approval if:
 - * any unethical principles or practices of the study are revealed or suspected;
 - * it becomes apparent that any relevant information was withheld from the FNASREC or that information has been false or misrepresented;
 - * submission of the annual (or otherwise stipulated) monitoring report, the required amendments, or reporting of adverse events or incidents was not done in a timely manner and accurately; and / or
 - * new institutional rules, national legislation or international conventions deem it necessary.
- FNASREC can be contacted for further information or any report templates via Roelof.Burger@nwu.ac.za 018 299 4269

The FNASREC would like to remain at your service as scientist and researcher, and wishes you well with your study. Please do not hesitate to contact the FNASREC or the NWU-SCRE for any further enquiries or requests for assistance.

Yours sincerely,

Prof Roelof Burger
Chairperson Faculty of Natural and Agricultural Sciences Ethics Committee (FNASREC)

APPENDICES 4: SIGNED SOLEMN DECLARATION



NWU Higher Degrees Administration

SOLEMN DECLARATION AND PERMISSION TO SUBMIT

1. Solemn declaration by student

I, Linda Van Der Spuydeclare herewith that the thesis/dissertation/mini-dissertation/article entitled (**exactly as registered/approved title**),

Cestodes of the order Onchoproteocephalidea II infecting cartilaginous fishes (Elasmobranchii) in South Africa

which I herewith submit to the North-West University is in compliance/partial compliance with the requirements set for the degree:

Master of Science in Environmental Sciences at the North-West University

is my own work, has been text-edited in accordance with the requirements and has not already been submitted to any other university.

LATE SUBMISSION: If a thesis/dissertation/mini-dissertation/article of a student is submitted after the deadline for submission, the period available for examination is limited. No guarantee can therefore be given that (should the examiner reports be positive) the degree will be conferred at the next applicable graduation ceremony. It may also imply that the student would have to re-register for the following academic year.

Ethics number:

NWU-01655-20-A9

ORCID:

0000 - 0003 - 4492 - 6588

Signature of Student

University Number

27014452Signed on this 21 day of March of 2022

2. Permission to submit and solemn declaration by supervisor/promoter

The undersigned declares that the thesis/dissertation/mini-dissertation/article:

- ☒ complies with the A-rules and the technical requirements provided for in the Manual for Master's and Doctoral studies and in faculty rules;
- ☒ has been checked by me for plagiarism (by making use of TurnItIn software for example) and a satisfactory report has been obtained;
- ☒ and that the work was language edited before submission for examination.

Faculty specific requirements as per A-rules: 1.3.2, 433, 4.2.4, 4.10.4, 5.3.2

- ☒ complies with regards to faculty rules on submission or acceptance by an accredited scientific journal;
- ☒ complies with regards to faculty rules on peer reviewed conference proceedings;
- ☒ the student is hereby granted permission to submit his/her article/mini-dissertation/ dissertation/thesis for examination.

Signatures of supervisor(s) and Promoter(s): (*only compulsory in cases where there are co- or assistant- supervisor(s/promoters)*)

Supervisor/Promoter

Nico J. Smit
Co-Supervisor/Co-Promoter

Assistant-Supervisor
Assistant-Promoter

Original details: Marietjie Ackermann/10512187/MSDN/HDA-HGA/HDA Toolbox/Forms/13.SOLEMN DECLARATION AND PERMISSION TO SUBMIT.docm 1 Oct 2019.

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