A STUDY OF ICHTHYOPARASITES FROM IMPORTED ORNAMENTAL FISH FROM INDONESIA AND SRI LANKA AND POSSIBLE HOST-PARASITE INTERACTIONS

by

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DISSERTATION

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DECLARATION

I declare that the dissertation hereby submitted to the University of Limpopo, for the degree of MSc Zoology has not previously been submitted by me for a degree at this or any other university; that it is my work in design and in execution, and that all material contained herein has been duly acknowledged.

LP Lukhele	28/10/2023
Surname and initials	Date

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Abstract

The current study aimed to investigate the prevalence, mean intensity and parasite composition from fishes imported from Sri Lanka to determine the effectiveness of Sri Lanka's treatment strategy against parasite infestations, and to determine the host-parasite interactions between *Glossolepis incisus* from Indonesia and its associated parasites. The keeping of ornamental fish has become a popular hobby among millions of enthusiasts worldwide, including in South Africa. Most ornamental fish sold on the South African market are alien and imported from countries such as Sri Lanka and Indonesia. Ornamental fish along with their parasites can be introduced into local aquatic systems. The repercussions of introducing alien ichthyoparasites to native species have been documented. However, currently, in South Africa, the legislation does not require that imported fish destined for the pet industry be quarantined or treated against parasites. Sri Lanka has gained a reputation for effectively treating ornamental fish before exporting them to other countries. In this study, 11 ornamental fish species from nine families were imported via WCB Import from Sri Lanka (n = 10) and Indonesia (n = 1) and examined for parasites within 24 hours of their arrival in South Africa. In the laboratory, fish were sacrificed by percussive stunning and cervical transection, dissected, and the organs were placed in Petri dishes and scrutinised for parasites through a compound microscope, whilst skin smears were scrutinised under a stereo microscope. Observed parasites were collected and preserved using standard methods and their prevalence and mean intensity were determined.

Of the 10 species received from Sri Lanka and examined, five were found to be infected by parasites. Two groups of parasites were collected from the fishes, these being Branchiura and Monogenea, with monogeneans being the most dominant. Branchiura was represented by a single species, whilst Monogenea was represented by five genera and eight species. A single specimen of branchiuran was collected from *C. auratus* and identified as *Argulus japonicus*. Monogeneans collected from *C. auratas* were *Dactylogyrus baueri, Dactylogyrus intermedius, Gyrodactylus gurleyi* and *Gyrodactylus kobayashii.* In turn, *Hypostomus plecostomus* was infected by the monogenean *Heteropriapulus heterotylus* with *Metynnis hypsauchen, Pangasianodon hypophthalmus* and *Barbonymus schwanenfeldii* each infected by *Urocleidoides sinus*, *Thaparocleidus caecus* and *Dactylogyrus lampam* respectively. Branchiura recorded the lowest prevalence and

intensity at 6.7% and 1% respectively. The prevalence for monogenean parasites varied between 60% for *H. heterotylus* from *H. plecostomus* and 100% for *T. caecus* from *P. hypophthalmus*. The mean intensity for monogenean parasites varied between 5.1 for *H. heterotylus* from *H. plecostomus* and 224.2 for *T. caecus* from *P. hypophthalmus*.

In a separate trial, host-parasite interactions using *Glossolepis incisus* were investigated over nine weeks. Upon arrival at the University of Limpopo, Parasitology Laboratory, 15 specimens were sacrificed and examined for parasites. The next week another 15 specimens were examined. Thereafter, on a biweekly basis, 15 specimens that were housed in aquaria were randomly selected and examined for parasites. The nematode *Camallanus cotti* and a new monogenean species (Ancryocephalidae n. gen.) were collected from the fish examined. Throughout the trial, no trend in parasite numbers was observed for monogeneans. However, a high ammonia concentration and eutrophication were associated with an increase in monogenean numbers. A decrease in nematode numbers was observed due to these parasites having a lower survival rate under aquarium conditions thought to be due to the absence of the intermediate host. Water changes and reduced fish congestion were also linked to a decrease in both monogenean and nematode numbers. Monogeneans generally share very similar morphological features, and thus their identifications have to be genetically confirmed. Amongst the collected monogenean species in the current study, the 18S, ITS1 and 28S rDNA segments were successfully sequenced for only *T. caecus* and Ancyrocephalidae n. gen. Two haplotypes of *T. caecus* were revealed. Therefore, the current study recognises the need to re-evaluate the morphometry of the type-material of *T. caecus,* to establish which genotype represents *T. caecus* and which would be a new species of high morphological similarity to *T. caecus*. Ancyrocephalidae n. gen. was genetically confirmed to be a new monogenean species. The current study therefore presents the first DNA sequencing of 18S, ITS1 and 28S rDNA segments of monogenean parasites collected from the host species *G. incisus.*

Since some of the fishes examined in this study were observed to be infected with parasites, the risk of their introduction and possibly invasion into local systems still exists. The current study therefore recommends that the South African

authorities at customs put in place quarantine and treatment protocols for live fish imported into the country.

Ethical clearance

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ANIMAL RESEARCH ETHICS COMMITTEE CLEARANCE CERTIFICATE

MEETING: **30 March 2022**

PROJECT NUMBER: **AREC/05/2022: PG**

PROJECT:

Title:	A Study of Ichthyoparasites from Imported Ornamental Fish from Indonesia and Sri Lanka and Possible Host-Parasite Interactions.
Researcher:	LP Lukhele
Supervisor:	Prof JR Sara
Co-Supervisor/s:	N/A
School:	Molecular and Life Sciences
Degree:	Master of Science in Zoology

PROF JW NGAMBI
CHAIRPERSON: ANIMAL RESEARCH ETHICS COMMITTEE

The Animal Research Ethics Committee (AREC) is registered with the National Health Research Ethics Council, Registration Number: **AREC-290914-017**

Note:
i) i) **Should any departure be contemplated from the research procedure as approved, the researcher(s) must re-submit the protocol to the committee.**
ii) ii) **The budget for the research will be considered separately from the protocol.**
iii) **PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES.**

Study outputs

Published articles

1. Molokomme PS, Benovics M, Luus-Powell WJ, Lukhele LP, Přikrylová I. 2023. *Dactylogyrus* spp. (Dactylogyridae: Monogenea) from Tinfoil Barb, *Barbonymus schwanenfeldii* imported into South Africa: Morphometric and Molecular Characterisation. *Parasite* 30: 29.

Conference contributions (Published abstracts)

1. Lukhlele LP, Přikrylovál, Tavakol S, Luus-Powell WJ. 2021. A Study of Parasites of Three Imported Ornamental Fishes. 1st Electronic Conference on Biological Diversity, Ecology and Evolution. 15–31 March. (Oral Presentation).

2. Lukhlele LP,Přikrylová I, Tavakol S, Luus-Powell WJ. 2021. A Study of Parasites of Three Imported Ornamental Fishes. 49th Annual Parasitological Society of Southern Africa Conference, 14–16 September. Online. (Oral Presentation).

3. Lukhlele LP, Přikrylová I, Tavakol S, Luus-Powell WJ. 2021. Parasites of Imported Ornamental Fishes: Case of Concern or Potential for New Discoveries? FSA UL Postgraduate Research Dates, 6–8 October, Bolivia Lodge, Polokwane (Oral Presentation).

4. Přikrylová I, Lukhele LP, Molokomme PS, Sara JR, Smit WJ, Luus-Powell WJ. 2022. Transcontinental Travelers: Monogeneans from Ornamental Fish Imported into South Africa. XIV. Czech and Slovak Parasitological Day, May 9–13, 2022, Hotel Medlov, Czech Republic. Book of Abstract pp 38 (Oral Presentation).

5. Lukhele LP, Sara JR, Luus-Powell WJ, Smit WJ, Přikrylová I. 2022. Diversity of Parasites on Ornamental Fish Imported from Sri Lanka. 15th International Congress of Parasitology. 21–26 August 2022, Copenhagen, Denmark. (Oral Presentation).

6. Lukhele LP, Sara JR, Luus-Powell WJ, Přikrylová I. 2022. A Study of Ichthyoparasites from Imported Ornamental Fish from Sri Lanka. 4th

International Congress on Parasites of Wildlife and the 50th Annual Conference of the Parasitological Society of Southern Africa (PARSA). 11–15 September 2022, Skukuza, South Africa. (Oral Presentation).

7. Přikrylová I, Lukhele LP, Molokomme PS, Sara JR, Luus-Powell WJ. 2022. Monogenean Parasites from Ornamental Fish Imported into South Africa. 12th Postgraduate Research Day, Faculty of Science and Agriculture, University of Limpopo. 21–23 September, Bolivia Lodge, Polokwane, South Africa. (Oral Presentation).

8. Přikrylová I, Lukhele LP, Molokomme PS, Sara JR, Luus-Powell WJ. 2022. Parasites from Ornamental Fishes Imported into South Africa: So, Little is Known. World Aquatic Health Conference 2022. 4–7 December 2022, Future Africa Campus, University of Pretoria, Pretoria, South Africa. (Oral Presentation).

9. Přikrylová I, Molokomme PS, Lukhele LP, Sara JR, Luus-Powell WJ. (2023): Hidden Voyagers: Parasites of ornamental fish imported into South Africa. Southern African Society for Aquatic Scientists Annual Congress 2023. 25–29 June, Lord Charles Hotel, Somerset West, South Africa. (Oral Presentation).

10. Přikrylová I, Molokomme PS, Lukhele LP, Sara JR, Luus-Powell WJ. 2023. Parasites of Ornamental Fish imported into South Africa: Stowaways that we should be concerned about? 17th European Congress of Ichthyology. 4–8 September 2023, Prague, Czech Republic. (Oral Presentation).

11. Lukhele LP, Sara JR, Luus-Powell WJ, Přikrylová I. 2023. The Parasite diversity and Host-parasite Interactions of Ornamental Fishes from Sri Lanka and Indonesia. 13th Postgraduate Research Day, Faculty of Science and Agriculture, University of Limpopo. 20–22 September, Bolivia Lodge, Polokwane, South Africa. (Oral Presentation).

12. Lukhele LP, Sara JR, Luus-Powell WJ, Přikrylová I. 2023. Metazoan Parasites of Ornamental Fishes Imported from Sri Lanka and Indonesia. 40th Zoological Society of Southern Africa Conference. 25–29 September 2023, Champagne Sport Resort, South Africa. (Oral Presentation).

13. Molokommne PS, Benovics M, Luus-Powell WJ, Lukhele LP, Přikrylová I. 2023. *Dactylogyrus* spp. (Dactylogyridae: Monogenea) from Tinfoil Barb, *Barbonymus schwanenfeldii* imported into South Africa: Morphometric and Molecular characterisation. 9TH International Symposium on Monogenea, 8–11 August, Lucknow, India. Book of abstract p. 84. (Poster).

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Chapter 1: Introduction

1.1 Background

1.1.1 The history and origin of ornamental fishes

The hobby of keeping ornamental fish as pets has become popular among millions of enthusiasts worldwide, including in South Africa (Mouton et al. 2001; Teletchea 2016; Allen et al. 2017). The literature alludes to ancient China as being the country that pioneered the practice of keeping wild-caught fishes as pets (Novák et al. 2020). One of the most popular ornamental fish traded worldwide is the goldfish, *Carassius auratus* (Linnaeus 1758), which was selectively bred from the crucian carp *Carassius carassius* (Linnaeus, 1758) and has undergone the process of domestication for thousands of years (Komiyama et al. 2009; Chen et al. 2020). Moreover, the goldfish is thought to be the first ornamental species to be exported as early as the 16th century, circa 1506 and 1521 AD, from China to Japan (Jassim et al. 2012).

1.1.2 Rising popularity of ornamental fish keeping

Over the years the popularity of keeping ornamental fish has gradually increased (Dey 2016). In 1970, 1973, and 1980 approximately 8, 13 and 120 million freshwater fish specimens were imported into Brazil, Peru, and the United States of America respectively (Hemley 1984). By the early 1980s, the keeping of ornamental fish in home aquariums was popular in South Africa (Andrews 1990) and other countries such as Italy and Belgium, with about 5% of homes in the latter housing fish as pets (Raja et al. 2019). By the late 1980s the industry, as a whole, was making over 7 billion United States Dollars (USD) in profits (Andrews 1990) with the industry's net worth increasing yearly (Bartley 2000; Padilla and Williams 2004) with global profits increasing from 21.5 million USD in 1976 to 347 million USD in 2014 (Monticini 2010; Dey 2016).

1.1.3 Current state of ornamental fish keeping

Currently, there continues to be a high global demand for ornamentals (Alam et al. 2016) with over 125 countries trading in ornamental fish (Monticini 2010; Raja et al. 2019), a number that increased from 28 in 1976 to 104 in 2004 (Raja et al. 2019). Although there is no data on the exact number of ornamental fish being exported

annually, in recent years, an estimate exceeding 4000 freshwater species is thought to be traded annually (Whittington and Chong 2007), giving rise to a multibillion-dollar industry worldwide (Padilla and Williams 2004; Alam et al. 2016; Dey 2016) with most fish exported derived from Asian countries such as Singapore, Sri Lanka, and Indonesia (Kottelet and Whitten 1996). The harvesting and trade of ornamental fish provides a reliable source of income for rural communities in these countries (Wei et al. 2021) with most species bought and sold being freshwater species (Livengood and Chapman 2007).

1.2 Problem statement

1.2.1 The potential threat to freshwater ecosystems by the introduction of alien ichthyoparasites

The ornamental fish trade is mostly unregulated and has led to the invasion of more than 150 fish species into new ecosystems globally (Padilla and Williams 2004), making the goldfish, for example, have a nearly worldwide distribution in the wild by the 1980s (Andrews 1990). The detrimental effects caused by the introduction of alien fish on native fish are well documented (Kitchell et al. 1997; Attayde et al. 2007; Okun et al. 2008; Vitule et al. 2009; Sandilyan et al. 2018). However, the impact of parasites introduced along with alien fish has received less attention (Smit et al. 2017). The trade of fish via the ornamental trade provides a route for the introduction and translocation of foreign pathogens (Whittington and Chong 2007; Chang et al. 2009) and parasites (Gozlan 2008). Parasites introduced along with their fish hosts can become invasive (Lymbery et al. 2014). For a non-native species to be classified as being an “invasive species” the species must be a detriment to the health and survival of native species (Simberloff 2006) that ultimately can lead to the eventual extinction of the native species (Padilla and Williams 2004). According to Wilcove et al. (1998), invasive species are the second leading cause of extinction of native species worldwide. Specific to ichthyoparasites, an alien parasite species is considered to be invasive when it infects a native fish host (Lymbery et al. 2014).

Imported exotic ornamental fish can find their way, along with their parasites, into local aquatic systems due to hobbyists discarding the fish into the wild after the novelty of keeping ornamentals as pets has worn off (Freyhof and Korte 2005; Gertzen et al. 2008; Patoka et al. 2017) or be introduced accidentally from holding facilities owned

by those in the pet industry (Casimiro et al. 2018). This could have deleterious effects on native fish populations that have not co-evolved to develop an immune response against infection and infestation by alien parasites (Gozlan 2008; Ellender and Weyl 2014).

1.2.2 The repercussions of introducing alien ichthyoparasites to native species

The swim bladder nematode, *Anguillicoloides crassus* (Kuwahara, Niimi and Hagaki, 1974), which is reported to have a low infection intensity and causes negligible harm to the Japanese eel *Anguilla japonica* Temmick and Schlegel, 1846, was reported to greatly infect and cause disease in the European eel, *Anguilla anguilla* (Linnaeus, 1758), after *A. japonica* was imported into Europe for the consumption market (Lymbery et al. 2014). Similarly, the parasite, *Gyrodactylus salaris* Malmberg, 1957 first described from Atlantic salmon in Sweden and considered to be benign, caused severe disease in Atlantic salmon when introduced in Norway (Peeler et al. 2011). Although both the Sweden and Norway Atlantic salmon meet the criteria to be classified as a single species, they have been geographically isolated for thousands of years and consequently have some genetic differences between them (Peeler et al. 2011).

In South Africa, the introduction of the ciliated protozoan, *Chilodonella hexasticha* (Kiernik, 1909) has been the cause of mass mortalities of *Oreochromis mossambicus* (Peters, 1852) housed in aquaculture facilities (Du Plessis 1952; Paperna and Van As 1983), a fish species native to the northern and eastern regions of the country (Russell et al. 2012). Further mortalities caused by the infestation of *C. hexasticha* in captive-bred fish and those in the wild have not been reported (Smit et al. 2017). However, with the presence of the non-native Nile tilapia, *Oreochromis niloticus* (Linnaeus, 1758), which is host to *C. hexasticha* (Padua et al. 2013; Bastos Gomes et al. 2017) reported to be in some rivers in Limpopo and KwaZulu-Natal provinces (Van Rensburg et al. 2011), the likelihood that this parasite occurring in these rivers may be inferred. Furthermore, the ornamental fish *Poecilia reticulata* Peters, 1859, which is native to South America and has established populations within some local systems, is also a host to *C. hexasticha* (Koyuncu and Engin 2022). Currently, there are no studies documenting the extent *C. hexasticha* interacts with native fish species in local waters (Smit et al. 2017). Moreover, in South Africa, ornamental fish species that have

established feral populations in local systems have introduced alien parasites that are potential threats to the health and survival of other local species (Tavakol et al. 2017; Greeff-Laubscher et al. 2019).

1.2.3 Records of parasites and pathogens introduced into South Africa through the introduction of ornamental fish

In a study by Tavakol et al. (2017), *P. reticulata* sourced from various pet shops, a fishpond in Polokwane, and the Crocodile River at Komatipoort in Mpumalanga, were found to be infected by the nematode, *Camallanus cotti* Fujita, 1927. This was the first report of the parasite in Africa. The first report of the bacterium *Mycobacterium fortuitum* Da Costa Cruz, 1938 in South Africa was also discovered when Bragg et al. (1990) examined the guppy, and two other non-native ornamental fish; the oscar *Astronotus ocellatus* (Agassiz, 1831), and the blue/brown discus *Symphysodon aequifasciatus* Pellegrin, 1904 for pathogens. With *P. reticulata* having established feral populations in local freshwater systems after having been released from fish ponds and aquaria (Blackburn et al. 2011), future studies ought to investigate the interaction between guppy parasites and native fish species. In a separate study at an ornamental fish farm, in the Western Cape, South Africa, the Malawi endemic orange blotched peacock cichlid *Aulonacara* sp. was reported to be infected by *Achlya bisexualis* Coker and Couch, 1927 by Greeff-Laubscher et al. (2019) and was the first report of this parasite in South Africa.

Another example is the goldfish *C. auratus*, which has been reported by Trujillo-González et al. (2018a) to be infected worldwide by over 100 different ichthyoparasite species. In South Africa, Kruger et al. (1983) inferred *C. auratus* to be responsible for the introduction of the alien parasite, *Argulus japonicus* Thiele, 1900, a parasite later found to be infecting all native fish species in the Vaal River (Kruger et al. 1983; Avenant-Oldewage 1994). Similarly, indigenous species *Clarias gariepinus* Burchell, 1822 and *Labeo rosae* Steindachner, 1894 from Loskop Dam, which is situated on the mainstem of the Olifants River, were reported by Avenant-Oldewage (1994) to be infected by the same parasite. Goldfish are thought to play a role in the distribution of the Asian endemic parasite, *Ichthyophthirius multifiliis* Fouquet, 1876 in South Africa and in the rest of the world (Trujillo-González et al. 2018a). *Carassius auratus* has established feral populations in South African waters (McDowall 2004), but there is

little knowledge with regard to the possibility of parasites associated with this fish species spilling-over to indigenous species. However, before a parasite can invade a new habitat and host, it must overcome physical and biological barriers (Blackburn et al. 2011; Lymbery et al. 2014).

1.2.4 Barriers against ichthyoparasite invasion in the wild

According to Blackburn et al. (2011), to be classified as invasive, alien species generally have to overcome three physical and biological barriers, these being a geographic barrier, a barrier hindering survival and reproduction, and ultimately a barrier impeding the alien species dispersal in the new habitat. Blackburn et al. (2011) term an alien species "introduced" if it overcomes a geographical barrier, "established" if it is able to survive and reproduce and "invasive" if the species can overcome a barrier that hinders its dispersal into the new habitat. Lymbery et al. (2014) applied the framework from Blackburn et al. (2011) to explain the barriers foreign ichthyoparasites have to overcome to be invasive, as illustrated in Figure 1.1.

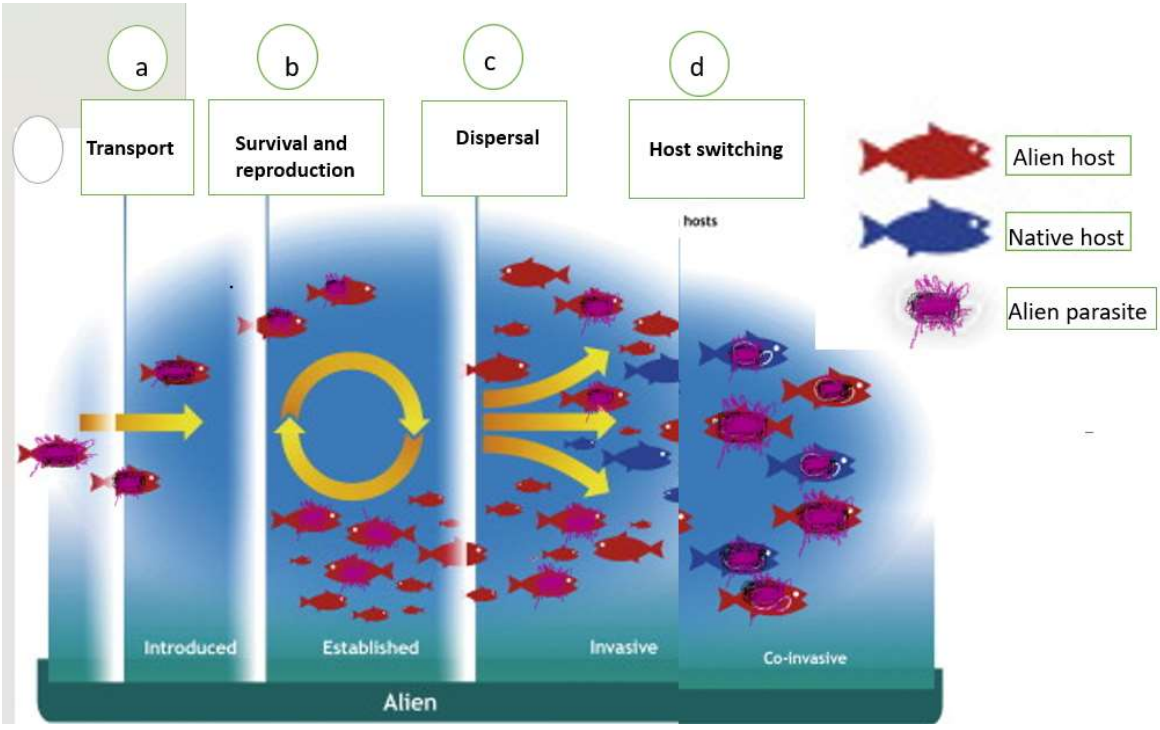

Figure 1.1: An illustration displaying the barriers an alien parasite has to overcome to be classified as invasive (sourced and modified from Lymbery et al. 2014).

As shown in Figure 1.1, a parasite along with its host must be introduced into a new habitat whereby the host must then survive conditions in the new environment to reproduce and grow to form a viable population. The alien parasite, in turn, needs to thrive along with its fish host to survive and reproduce. However, the survival of alien endoparasites is dependent on its host's immune system and environmental conditions in the case of ectoparasites (Buchmann and Bresciani 2006; Koskivaara et al.1992; Gilbert and Avenant-Oldewage 2021). The reproduction success of the alien parasite is dependent on the life cycle of the parasite (Yanong 2002). A parasite can either undergo a direct or an indirect life cycle (see Figure 1.2).

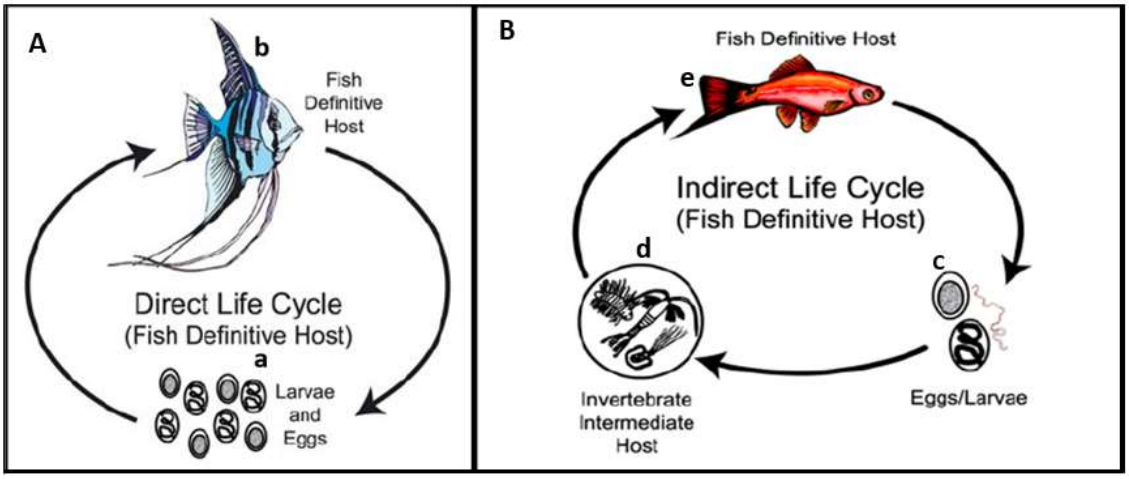

Figure 1.2: (A) A representation of a direct life cycle (a) = Larval stage of the parasite; (b) = definite host of the parasite. (B) A representation of an indirect life cycle. (c) = larval stage of the parasite; (d) = Intermediate host of the parasite; (e) = definite host of the parasite (Sourced and modified from Yanong 2002).

As shown in Figure 1.2 (A), some parasites undergo a direct life cycle whereby the larvae of the parasite emerge from the host. Depending on the species, the parasite will undergo different life stages before reinfecting the definitive host (Yanong 2002; Lymbery et al. 2014) becoming adults to reproduce and form larvae that will be released by the final host so that the cycle can be repeated (Yanong 2002; Lymbery et al. 2014). In the case whereby the parasite undergoes an indirect life cycle (see Figure 1.2 (B)), the larvae of the parasite emerge from the host. Depending on the parasite species, the parasite will then undergo different forms that will infect an intermediate host (Yanong 2002; Lymbery et al. 2014). In most cases, the intermediate host will then be ingested by the parasite's definitive host wherein the eggs or larvae

will mature, reproduce, and form larvae that will be released by the host to repeat the life cycle of the parasite (Yanong 2002; Lymbery et al. 2014).

If the alien fish along with the alien parasite become established, they have a final barrier to overcome to become invasive (Blackburn et al. 2011; Lymbery et al. 2014), that being a barrier that hinders the dispersal of the alien species within the new habitat according to Blackburn et al. (2011). However, being specific to alien ichthyoparasites, Lymbery et al. (2014) stated that for an alien fish parasite to be described as invasive, the parasite must be successful at changing hosts by infecting and thriving on or in native species. Host specificity of an alien fish parasite can reflect the probability that the alien ichthyoparasite infects native fish species. A parasite with a low host specificity has a higher probability of spilling-over to a new host as compared to a parasite with a high host specificity (Lymbery et al. 2014). The goldfish has been reported to be responsible for the invasion and distribution of parasites with both high and low host specificity.

The goldfish has been reported to distribute parasites with high host-specificity, such as *Dactylogyrus vastator* Nybelin, 1924, *Dactylogyrus formosus* Kulwiec, 1927, and *Dactylogyrus anchoratus* (Dujardin, 1845*)* in fish species within the same family, Cyprinidae (Trujillo-González et al. 2018a). South African cyprinids are therefore considered to be susceptible to infection from alien parasites with high host specificity should they make their way into local waters. A study by Bergmann et al. (2010) revealed goldfish to be a potential vector for koi herpesvirus (KHV), a pathogen especially fatal to the common carp *Cyprinus carpio* Linnaeus, 1758, but also harmful to other cyprinids. Moreover, *C. auratus* imported from Japan were reported to spread pathogenic bacteria with low host-specificity, such as *Aeromonas salmonicida* (Lehmann and Neumann, 1896) that caused a disease outbreak amongst several fish species housed in an Australian aquaculture facility (Whittington and Cullis 1988; Humphrey and Ashburner 1993).

1.2.5 Ornamental fish parasites and pathogens are a threat to the local aquaculture industry

Ornamental fish that are in high demand, such as the goldfish, guppy, and koi, often harbour parasites and are a nuisance and potential threat to aquaculture practices (Mouton et al. 2001; Florio et al. 2009; Rayamajhi and Kunwor 2017; Maftuch et al.

2018). In South Africa, for example, *Aeromonas* spp. which are common parasites of ornamental fish (Hossain and Heo 2021) are a stumbling block to the success of local aquaculture ventures, since they are displaying an increasing resistance to antimicrobial treatment (Jacobs and Chenia 2007; Gobi et al. 2018). *Aeromonas* spp. therefore limits the production of fish suitable for the local market, and thus negatively impacts the country's economy (Jacobs and Chenia 2007).

1.2.6 Policies established by different countries in relation to the importation of ornamental fish

Most ornamental fish in South Africa are imported from breeders and suppliers abroad (Mouton et al. 2001). These fish carry parasites that can be potentially introduced into local systems. To limit the introduction and invasion of non-native fish, South Africa has established an alien invasive species list under the National Environmental Management: Biodiversity Act 10 of 2004. According to their threat levels, listed species fall into four categories, namely, category 1a and 1b, category 2 and category 3. Only species assigned to category 2 may be imported into the country after receiving a permit from the relevant authorities. To date, there is no protocol that requires fish imported into South Africa be quarantined and treated for diseases and/or parasites. Hence, the discretion of treating and/or quarantining live imported fish against the introduction of pathogens and parasites rests with the local importer (Mouton et al. 2001). According to the information provided by the importing company, WCB Imports, located in Midrand, Gauteng, the following documents are required when importing fish from abroad.

These are the import, distribution, and sale permits, which are applied to and issued by the Department of Environmental Affairs to be submitted to the Department of Forestry, Fisheries and the Environment for authorization. Other countries employ similar yet different measures whereby they have established specific quarantine periods for certain ornamental fish prior to importation as seen in Table 1 below.

Table 1.1: Pre-importation requirements for ornamental fish in different countries. Table altered from Whittington and Chong (2007) where Y = Yes and N = No.

Country	Health certification required	Fish inspection required	Quarantine required	Quarantine duration
Australia	Y	Y	Y	Two to three weeks for freshwater species and one week for marine species
Mauritius	Y	Y	Y	One week
New Zealand	Y	Y	Y	Six weeks
Iceland	Y	Y	Y	Four weeks
South Africa	Y	Y	N	None

The implementation of strict protocols by the Australian authorities has shown to be effective at limiting the introduction of alien fish species and ichthyoparasites (Whittington and Chong 2007; Trujillo-González et al. 2019) into Australia. Therefore, the adoption of similar measures by South African policymakers could and should be considered whereby, similar to the Australian policies, South Africa could mandate the pre-import quarantining of fish and establish similar biosecurity protocols at places of entry (Whittington and Chong 2007; Trujillo-González et al. 2018b; Trujillo-González et al. 2019). Pre-import inspections of fish are usually visual, with inspectors only scanning for external parasites and for signs of disease and parasite infection (Thilakaratne et al. 2003; Trujillo-González et al. 2018b). However, even with stringent protocols in place, a high composition of parasites was reported by Trujillo-González et al. (2018b) from fish that were euthanized by Australian authorities responsible for the monitoring and control of live animals imported into the country. The study by Trujillo-González et al. (2018b) indicates that even with stringent protocols in place the possibility of parasites entering a country remains.

1.2.7 Ichthyoparasite identification and the role of genetics

Accurate identifications allow researchers to distinguish between parasite species linked to different fish diseases (Sterud et al. 2002; Besansky et al. 2003). The identification of parasites based on morphological features has been shown to be inefficient at distinguishing between species with similar phenotypes (Floyd et al. 2002; Ferri et al. 2009). Conversely, molecular identification has proven to be more accurate as this method does not require taxonomy-skilled individuals, is not subjective, and can be applied at any stage of a parasite's life cycle (Floyd et al. 2002; Ferri et al. 2009). The monogeneans *Gyrodactylus pakan* Razo-Mendivil, García-Vásquez, Rubio-Godoy, 2016 which is similar to *Gyrodactylus teken* Razo-Mendivil, García-Vásquez, Rubio-Godoy, 2016, and *Benedenia humboldti* Baeza, Sepúlveda, González, 2019 which is similar to *Benedenia seriolae* (Yamaguti, 1934), were revealed as being separate species after their DNAs were sequenced (Razo-Mendivil et al. 2016; Baeza et al. 2019). DNA sequencing also helps in establishing molecular markers that are unique to a specific species and can be utilized to identify the species and infer its evolutionary history (Wu et al. 2008; Šimková et al. 2013).

1.2.8 Studies on parasites from imported ornamental fish are lacking in South Africa

With knowledge of parasites associated with ornamental fish imported into South Africa being limited (Mouton et al. 2001; Tavakol et al. 2017), this study investigates the composition of ichthyoparasites from a selection of ornamental fishes imported from Sri Lanka, a country that is a prominent supplier of ornamental fishes to South Africa and globally (Evers et al. 2019) to determine the country's efficiency at treating fish against parasites before exportation. The current study also determines the host-parasite interactions between the ornamental fish species, *Glossolepis incisus* Weber, 1907 and its associated parasites.

1.3 Aim and objectives

1.3.1 Aim

The study aimed to identify and investigate the prevalence, mean intensity and composition of parasites collected from various ornamental fish species imported into South Africa from fish suppliers from Sri Lanka and to determine interactions between parasites and their host, *G. incisus* imported from Indonesia.

1.3.2 Objectives

The objectives of the study were to:

i. identify the parasites sampled from selected ornamental fishes imported from suppliers in Sri Lanka and Indonesia using a combination of morphometric and molecular methods.

ii. determine the effectiveness of the pre-export treatment of fishes imported from Sri Lanka by determining the prevalence, mean intensity and composition of parasites sampled from each fish species.

iii. investigate host-parasite interactions under aquarium conditions between *G. incisus* and its associated parasites in aquaria for nine weeks.

1.4 Research questions

i. Are morphometric methods sufficient to identify ichthyoparasites?

ii. To what effect do the pre-export treatments of ornamental fishes by fish suppliers from Sri Lanka eliminate ichthyoparasites?

iii. What are the host-parasite interactions between *G. incisus* and its associated parasites?

Chapter 2: Methodology and analytical procedures

2.1. Sampling of Fish

Ornamental fish were obtained from the company WCB Imports in Midrand, Gauteng, South Africa. WCB imports was responsible for all permits required to import fish into the country. Fishes were selected and imported according to availability from ornamental fish traders in Sri Lanka and Indonesia to conduct a quantitative study. Fish imported from Sri Lanka were selected to determine the prevalence, mean intensity and composition of parasites sampled from each host species. Host-parasite interactions were determined using *G. incisus* imported from Indonesia as a fish model. The following fish species (see Table 2.1) were selected:

Table 2.1: List of fish species selected and investigated for ichthyoparasites. IN – Indonesia, SL – Sri Lanka

Common name		Species name	No. of specimens	Country	Family
1	Wood catfish	*Trachelyopterus fisheri* (Eigenmann,1916)	26	SL	Auchenipteridae
2	Tetra silver dollar	*Metynnis hypsauchen* (Muller and Troschel, 1844)	15	SL	Characidae
3	Goldfish	*Carassius auratus* (Linnaeus, 1758)	15	SL	Cyprinidae
4	Tinfoil barb	*Barbonymus schwanenfeldii* (Boulenger, 1899)	24	SL	Cyprinidae
5	Suckermouth catfish	*Hypostomus plecostomus* (Linnaeus, 1758)	26	SL	Loricariidae
6	Rainbow kubutu	*Melanotaenia lacustris* Munro, 1964	15	SL	Melanotaeniidae
7	Red rainbowfish	*Glossolepis incisus* Weber, 1907	100	IN	Melanotaeniidae
8	Upside-down catfish	*Synodontis nigriventris* David, 1939	16	SL	Mochokidae
9	Giant gourami golden	*Osphronemus goramy* Lacepede, 1801	10	SL	Osphronemidae
10	Catfish pangasius	*Pangasianodon hypophthalmus* (Sauvage, 1878)	10	SL	Pangasiidae
11	Sailfin molly	*Poecilia latipinna* (Lesueur, 1821)	15	SL	Poeciliidae

2.1.1 A description of the host species

Trachelyopterus fisheri (Eigenmann,1916) (Figure 2.1), commonly known as the wood catfish belongs to the family Auchenipteridae. In the wild, *T. fisheri* is exclusively found in the Suico River in Colombia (Ferraris 2003). It is an omnivorous fish (Garcia et al. 2018) and can grow to a maximum length of about 28 cm (Ferraris 2003). This species

is listed as “least concern” under the IUCN Red List of Threatened Species (IUCN 2020). There are no published studies on parasites associated with this species.

Figure 2.1: *Trachelyopterus fisheri*

Metynnis hypsauchen (Muller and Troschel, 1844) (Figure 2.2), commonly known as the tetra silver dollar, belongs to the family Characidae. In the wild, it can be found in the Paraguay and Amazon Rivers (Zarske and Géry 1999). *Metynnis hypsauchen* is an omnivorous fish (Singh et al. 2007) and can grow to a maximum length of about 18 cm (Garcia-Ayala et al. 2014). This fish species is not listed in the IUCN Red List of Threatened Species (IUCN 2020). Parasites reported to infect this species are listed in Chapter 3, Table 3.1.

Figure 2.2: *Metynnis hypsauchen*

Carassius auratus (Figure 2.3) commonly known as the goldfish belongs to the family Cyprinidae. Initially limited to central Asia, China and Japan, this species has been introduced worldwide, inhabiting ditches, ponds, and lakes (Man and Hodgkiss 1981). It is an omnivorous fish (Brown et al. 2018) and can grow to a maximum length of about 12 cm (Allen 1991). The species is endangered in the wild (IUCN 2021) and has been recorded to be infected by over 100 parasite species (Trujillo-González et al. 2018a).

Figure 2.3: *Carassius auratus*

Barbonymus schwanenfeldii (Boulenger, 1899) (Figure 2.4), commonly known as the tinfoil barb belongs to the family Cyprinidae. In the wild, it is found in Asian rivers, streams, canals, and ditches (Rainboth1996) such as the Mekong and Chao Phraya rivers, and in the islands of Borneo and Sumatra (Kottelat et al. 1993). *Barbonymus schwanenfeldii* is predominantly herbivorous, occasionally feeding on insects and small fish (Rainboth 1996) and can grow to a maximum length of about 35 cm (Baird et al. 1999). The species is categorised under “Least concern” on the IUCN Red List of Threatened Species (IUCN 2021). Parasites recorded to infect this species are listed in Chapter 3, Table 3.1.

Figure 2.4: *Barbonymus schwanenfeldii*

Hypostomus plecostomus (Linnaeus, 1758) (Figure 2.5), commonly known as the suckermouth catfish belongs to the family Loricariidae. In the wild, it can be found in Guinan coastal drainages in South America and some Asian countries due to the ornamental trade (Baensch and Riehl 1985). The catfish's diet consists of algae and small crustaceans (Burgess 1989) and can grow to a maximum length of about 50 cm (Galvis et al. 1997). This species is not placed under any category on the IUCN Red List of Threatened Species (IUCN 2020). Parasites reported to infect this species are listed in Chapter 3 (see Table 3.1).

Figure 2.5: *Hypostomus plecostomus*

Melanotaenia lacustris Munro, 1964 (Figure 2.6), commonly known as the rainbow kubutu belongs to the family Melanotaeniidae. In the wild, it is found only in Lake Kubutu and the Soro River in Papua New Guinea (Allen 1991). *Melanotaenia lacustris*is is an omnivorous fish and can grow to a maximum length of about 10 cm (Allen 1991). The species is threatened in the wild (IUCN 2021). There are no published studies on parasites associated with this species.

Figure 2.6: *Melanotaenia lacustris*

Glossolepis incisus (Figure 2.7), commonly known as the red rainbowfish belongs to the family Melanotaeniidae. The species is found only in the Indonesian lake, Sentani in Western New Guinea (Siby et al. 2017). It is an omnivorous fish (Subamia et al. 2010) and can grow to a maximum length of about 12 cm (Allen 1991). The red rainbowfish is endangered in the wild (IUCN 2021). This species was recently reported to be infected by an undescribed monogenean species and the nematode *Camallanus cotti* Railliet and Henry, 1915 (see Lukhele 2021).

Figure 2.7: *Glossolepis incisus*

Synodontis nigriventris David, 1939 (Figure 2.8), commonly known as the upside-down catfish belongs to the family Mochokidae. In the wild, it can be found in the Congo River, including the Stanley Pool and the Ubangi and Kasai drainages (Seegers 2008). *Synodontis nigriventris* is an omnivorous fish (Mills and Vevers 1989) and can grow to a maximum length of about 9.6 cm (Gosse 1986). The species is placed under “least concern” on the IUCN Red List of Threatened Species (IUCN 2021). There are no published studies on parasites associated with this species.

Figure 2.8: *Synodontis nigriventris*

Osphronemus goramy Lacepede, 1801 (Figure 2.9), commonly known as the giant gourami golden and belongs to the family Osphronemidae. It is mostly found inhabiting rivers, lakes, and swamps (Frimodt 1995) in Indonesian islands and has been introduced to other countries through aquaculture practices (Roberts 1992). *Osphronemus goramy* is an omnivore (Ukkatawewat 1984) and can grow to a maximum length of about 70 cm (Rainboth 1996). This species is placed under the "least concern" category on the IUCN Red List of Threatened Species (IUCN 2020). This species has been reported to be infected by *Lernaea chackoensis* Gnanamuthu, 1951.

Figure 2.9: *Osphronemus goramy*

Pangasianodon hypophthalmus (Sauvage, 1878) (Figure 2.10), commonly known as the catfish pangasius belongs to the family Pangasiidae. In the wild, it is found in Asian large rivers (Rainboth 1996) such as the Mekong and Chao Phraya (Roberts and Vidthayanon 1991). It is omnivorous (Ukkatawewat 1984) and can grow to a maximum length of about 130 cm (Roberts and Vidthayanon 1991). This species is endangered in the wild (IUCN 2021). Parasites reported to infect *P. hypophthalmus* are listed in Chapter 3 (see Table 3.1).

Figure 2.10: *Pangasianodon hypophthalmus*

Poecilia latipinna (Lesueur, 1821) (Figure 2.11), commonly known as the molly sailfin belongs to the family Poecillidae. In the wild, it is naturally found in the Cape Fear River running through the USA and Mexico but has been introduced to different countries via the ornamental trade (Smith 1997). The species feeds mostly on algae (Robins and Ray 1986) and can grow to a maximum length of about 15 cm (Page and Burr 1991). The species is placed under the “least concern” category on the IUCN Red List of Threatened Species (IUCN 2020). Parasites reported to infect this species are listed in Chapter 3, Table 3.1.

Figure 2.11: *Poecilia latipinna.*

2.2 Fish handling

Prior to the arrival of the fishes, tanks were prepared, and the water was let to stand for 72 hours to let any aqueous chlorine evaporate and submersible heaters were used to heat the water at a constant temperature of 24˚C in each of the aquaria. To achieve the first and second objectives, between 10 – 25 specimens of each fish species (excluding the red rainbow fish which were used to investigate host-parasite interactions) were collected from the airport and upon arrival, transported to the University of Limpopo by vehicle. To reduce the amount of stress during travelling, each fish species was replaced in a relatively voluminous and oxygenated bag, with the package being sealed to ensure fish remained in the dark. On arrival at the University of Limpopo, Parasitology Laboratory, the fishes were acclimatised to the water in the 60 L glass tanks, following the protocol provided by the importing company, whereby the pH and temperature were measured from each of the water bags in which the fishes arrived, as well as for each tank prepared for the fishes. Each water bag was paired up with an aquarium that had similar pH and temperature readings. About 30% of the water was then removed from each water bag and replaced with water from the glass tank. The bags were then reoxygenated using air pumps, resealed, and then allowed to float in the aquaria selected to house a specific species for 20 minutes. This procedure was repeated twice. Thereafter the fishes were gently released into the glass tanks. Fish were kept in the tank for less than 24 hours before dissections commenced. Examination of fish was performed at the University of Limpopo, Parasitology Laboratory. A maximum of four species, at a time, were imported and examined.

Figure 2.12: Set up of aquaria for fish prior to their arrival.

2.3. Host-parasite examination

On removal from the aquarium, skin smears from the body surfaces and fins of each fish were taken using the edge of glass microscope slides and scrutinised for ectoparasites using a stereo microscope (Leica EZ4). Thereafter the total (cm), standard (cm) and fork length (cm), including body mass (g) of each fish specimen were recorded on a data sheet. Once measured and weighed, specimens were humanely killed by percussive stunning and cervical transection (Animal Ethics approval number AREC/05/2022: PG) and dissected whereby the internal organs (the digestive system and the brain) and gills were removed and placed into separate Petri dishes containing a small amount of distilled water. The presence of endoparasites in internal organs and monogeneans of the gills was examined using a compound microscope (Leica DM500).

2.4. Fixation and preservation of parasites

2.4.1 Branchiuran

Brachiuran parasites were removed using a dissection needle and blade and fixed in 70% ethanol for morphological identification.

2.4.2 Monogeneans

Monogeneans were detached from fins and gill filaments using dissection needles. The monogenean specimens were then placed on a microscope slide with a drop of water and were then covered with a cover slip. The corners of the cover slip were sealed using nail polish. A drop of glycerine ammonium picrate (GAP) (Malmberg 1957) was then added to the edges of the cover slip to ooze through the edges to the specimen for fixation for morphological identification. A few specimens were cut in half, with the posterior halves bearing the opisthaptor fixed on microscope slides as described above, and the anterior halves of the specimens were preserved in 96% alcohol for molecular identification.

2.4.3 Nematodes

Parasites were removed from the digestive tract of fish using needles and placed in a small amount of saline solution. Boiled water was poured over each specimen to ensure their body profiles were extended for ease of identification. Nematode specimens were then preserved in 70% ethanol. For the purpose of morphological identification, parasites were temporarily placed under a cover slip in a drop of lactophenol to clear the nematode and ease observation of internal organs.

2.5. Parasite identification

A compound microscope (Olympus BX50) was used to view and magnify parasites and the Stream Essentials 1.5.1 software was used to view the parasites on a computer screen. To identify parasite groups at species level, their morphological features were compared to those described in literature. For monogeneans, the shapes and measurement of the haptoral hard parts and the male copulatory organ (MCO) of each species were recorded and compared to the appropriate literature (Gussev 1985; Lim 1990; Mizelle and Kritsky 1969; Jogunoori et al. 2004; Omidzahir et al. 2012; Borisov 2013; Chaudhary et al. 2014; Tu et al. 2015; Zago et al. 2020). Haptoral sclerite measurements were taken using the Stream Essentials program. For the identification of nematodes, the overall appearance and components of the buccal

capsule and the shape of the posterior end were compared with those in the literature (Menezes et al. 2006). For branchiurans, the overall appearance of the parasite was compared to literature (Kruger et al. 1983).

In instances when parasites could not be identified using morphometric data, molecular identification was then used (Hansen et al. 2007). Molecular identification (see Chapter 5, Section 5.2) was undertaken in collaboration with the Water Research Group at North-West University.

2.6. Experimental trial to determine host-parasite interactions between the red rainbowfish and its parasites.

To investigate host-parasite interactions, *G. incisus* was identified as a suitable fish/parasite model based on the results previously obtained from an honours project undertaken in 2020 (Lukhele 2021). Lukhele (2021) imported *G. incisus* specimens from Indonesia and reported a 100% prevalence for monogeneans and nematodes infecting this fish species. The current study therefore inferred the chances that *G. incisus* imported from Indonesia harbour parasites to be high based on the results of the honours study (Lukhele 2021). A nine-week experimental trial was therefore run to determine the host-parasite interaction between *G. incisus* and its associated parasites, see Chapter 4, Section 4.2 for the experimental design. During the trial, aqueous levels of ammonia and nitrates were monitored regularly using NT Lab Aquarium-test kits. The concentrations of ammonia and nitrates were determined by comparing the colour of the aquarium water, which was collected using a 5 mL test tube wherein test kit reagents were added, with the colour chart provided in the test kit, see Figure 2.13.

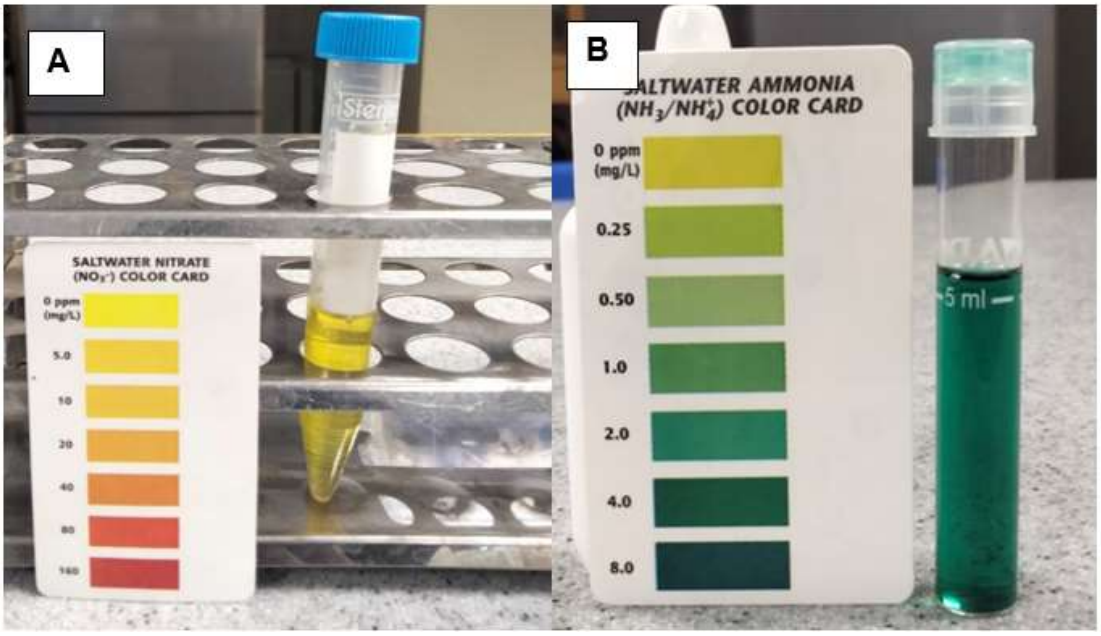

Figure 2.13: The NT Lab Aquarium-test kits for (A) ammonia and (B) nitrate concentrations.

2.7. Data analysis

The infestation indices prevalence (P%) and mean intensity (MI) were calculated according to Bush et al. (1997). Regarding the host-parasite interactions using *G. incisus*, the IBM SPSS Statistics, 29.0. program was used to test for the normality of data. The Kruskal-Wallis test was used to test if there was a significant change in parasite numbers throughout the nine-week trial.

Chapter 3: Parasite composition of ornamental fish imported from Sri Lanka

3.1 Introduction

3.1.1 A brief history of the Sri Lankan ornamental fish trade

Sri Lanka has a long history of breeding ornamental fish with Sri Lankan entrepreneurs reported to have bred and traded with ornamental fish from their homes since the early 1930s (Wijesekara and Yakupitiyage 2001). In 1952, the first public aquarium was constructed in Sri Lanka's capital, Colombo, to display ornamental fish (Jonklaas 1989). As the keeping of ornamental fish as pets became popular, fish bred in Sri Lanka were exported worldwide, providing more employment and business opportunities for the local populace (Wijesekara and Yakupitiyage 2001). By the late 1990s, Sri Lanka was exporting ornamental fish and aquatic plants to over 52 countries, generating an annual profit of about 8 million United States Dollars (USD) from the trade thereof (EDB 1999).

3.1.2 The composition of ornamental ichthyoparasites in Sri Lanka

Currently, Sri Lanka remains one of the prominent suppliers of ornamental fish worldwide, making over 12 million USD per year solely from the trade and export of ornamental fish (Raja et al. 2019). Sri Lanka imports non-native fish for the purpose of breeding exotic species for the export market (Wijesekara and Yakupitiyage 2001). Ornamental fish imported from various countries are often transported in densely stocked tanks (Trujillo-González et al. 2018b), thereby, creating conditions suitable for the rapid spread of ichthyoparasites between fish specimens.

Internationally Sri Lanka is known for trading ornamental fish of high quality (Wijesekara and Yakupitiyage 2001). To date, Thilakaratne et al. (2003) is the only study that has investigated the parasite composition of ornamental fish reared and housed in earthen ponds/aquaculture facilities in Sri Lanka. In the study, ornamental fish destined for the export market were collected from 26 Sri Lankan farms and examined for parasites. Out of the 26 farms examined, 23 harboured fish that comprised five groups of parasites i.e., Copepoda, Monogenea, Nematoda, Digenea, and Protozoa. Given that Sri Lanka has a tropical climate, the number of parasite species recorded by Thilakaratne et al. (2003) was lower than anticipated and was attributed to farm practices whereby the water supply to each pond was provided separately as a means to prevent cross-contamination between ponds and fish. Farm

ponds were also reported to be covered with wire nets so as to fend off the definitive hosts (i.e., birds) needed to complete the life cycle of some parasites. Moreover, fish were being chemically treated against parasite infections.

3.1.3 Selected ornamental fish and their parasite records

Of the ten ornamental fish species selected and imported from Sri Lanka, as listed in Chapter 2, Table 2.1, six have previously been reported to be infected by several of the nine parasite groups i.e. Acanthocephala, Branchiura, Copepoda, Cestoda, Digenea, Monogenea, Myxosporea, Nematoda, and Protozoa (see Table 3.1). Of the fish species studied, *C. auratus* was reported to have the highest parasite composition with over 150 parasite species collected. Moreover, among the selected species in the current study, *C. auratus* is the only species to have been reported to be infected by cestodes.

Table 3.1: Selected ornamental fish species along with the parasites recorded to infect them, where AC = Acanthocephala, BR = Branchiura, CO = Copepoda, DI = Digenea, MO = Monogenea, MY = Myxosporea, NE = Nematoda and PR = Protozoa.

Fish Species	Parasites recorded	References
Barbonymus schwanenfeldii	**AC**: *Acanthogyrus* (*Acanthosentis*) *kenyirensis* Mohd-Agos, Mohd-Husin, Zakariah, Yusoff, Wahab, Jones, Hassan, 2021, *Acanthogyrus (Acanthosentis) terengganuensis* Mohd-Agos, Mohd-Husin, Zakariah, Yusoff, Wahab, Jones, Hassan, 2021, *Acanthogyrus (Acanthosentis) tembatensis* Mohd-Agos, Mohd-Husin, Zakariah, Yusoff, Wahab, Jones, Hassan, 2021.	Chinabut and Lim (1993), Hafiza and Shaharom-Harrison (2020), Karim et al. (2021), Lim and Furtado (1986), Mohd-Agos et al. (2021)
	MO: *Dactylogyrus lampam* (Lim and Furtado, 1986), *Dactylogyrus tapienensis* Chinabut and Lim, 1993, *Dactylogyrus vitticulus* Chinabut and Lim, 1993	
	NE: *Cucullanus* sp.	
Carassius auratus	infected by over 150 parasite species, comprising the groups, AC, BR, Cestoda, CO, DI, MO, MY, NE, and PR.	Trujillo-González et al. (2018a)
Hypostomus plecostomus	**BR**: *Argulus multicolor* Stekhoven, 1937	Li and Huang (2012), Plaul et al. (2010), Neves and Tavares-Dias (2019), Ortega et al. (2009),
	CO: *Lernaea cyprinacea* Linnaeus, 1758	
	DI: *Centrocestus formosanus* Nishigori,1924.	
	MO: *Gyrodactylus* sp., *Heteropriapulus heterotylus* (Jogunoori, Kritsky and Venkatanarasaiah, 2004), *Unilatu sunilatus* Mizelle and Kritsky, 1967, *Gonocleithrum cursitans* Kritsky and Thatcher, 1983.	

	PR: *Trichodina* sp., *Trichophrya* sp., *Ichthyophthirius multifiliis* Fouquet, 1876.	Shoaibi-Omrani and Alinezhad (2019)
Metynnis hypsauchen	**AC**: Acanthocephala n. gen. sp.	Oliveira et al. (2015), de Oliveira et al. (2020), Figueredo et al. (2020), Lom et al. (1991)
	DI: *Dadayus pacuensis* Thatcher, Sey and Jégu, 1996.	
	MY: *Henneguya* sp., *Myxobolus* sp.	
	NE: *Procamallanus* (*Spirocamallanus*) *inopinatus* Travassos, Artigas and Pereira, 1928, *Spinoxyuris oxydoras* Petter, 1994, *Contracaecum* sp.	
	DI: *Haplorchis pumilio* (Looss, 1896), *Centrocestus formosanus* (Nishigori, 1924), *Stellantchasmus falcatus* Onji and Nishio, 1916.	
	MY: *Hennegoides longitudinalis* Lom, Tonguthai and Dykova, 1991.	
Osphronemus goramy	**CO**: *Lernaea chackoensis* Gnanamuthu, 1951.	Gnanamuthu (1951),
Pangasianodon hypophthalmus	**DI**: *Haplorchis pumilio* (Looss, 1896), *Haplorchis taichui* (Nishigori, 1924), *Centrocestus formosanus* (Nishigori, 1924), *Procerovum* sp., *Prosorhynchoides ozakii* (Nagaty, 1937).	Baska et al. (2009), Kumar et al. (2022), Mendoza-Franco et al. (2018), Thuy and Buchmann (2008),
	MO:*Thaparocleidus caecus* (Mizelle and Kritsky, 1969), *Thaparocleidus siamensis* (Lim 1990), *Dactylogyrus* sp.	

	MY: Myxosporean spp., including *Myxobolus miyairii* (Kudo 1920), *Ceratomyxa* sp., *Myxobolus hakyi* Landsberg and Lom, 1991.	Thuy et al. (2010)
	PR: *Ichthyophthirius multifiliis* Fouquet, 1876.	
Poecilia latipinna	**CO**: *Lernaea cyprinacea* Linnaeus, 1758.	Wold and Iversen (1978), McCallum (1986), Adel et al. (2015), Mirzaei (2015).
	MO: *Dactylogyrus* sp., *Gyrodactylus* sp.	
	MY: *Myxobolus latipinnacola* Word and Iversen, 1978.	
	PR: *Ichthyophtirius multifilus* Fouquet, 1876.	

3.2 Methods

In the current study, a total of 175 specimens from 10 fish species were imported and examined for the presence of parasites following the methods described in Chapter 2 (see Section 2.1 to Section 2.5).

3.3 Results

Of the 10 ornamental fish species examined, only five, namely, *C. auratus, B. schwanenfeldii, P. hypophthalmus, M. hypsauchen* and *H. plecostomus* were reported to be infected by parasites. The parasites retrieved from these fish species belonged to the groups Branchiura and Monogenea. Branchiurans were represented only by a single species, with monogeneans being represented by eight species. The monogenean *Dactylogyrus* spp. were found to be the most prevalent with the branchiuran, *Argulus* sp. and the monogenean *Gyrodactylus* sp. being the least (see Table 3.2). Of the infected fish species, *P. hypophthalmus* recorded the highest parasite load, followed by *C. auratus. Hypostomus plecostomus* was recorded to have the lowest intensity of monogeneans. A single specimen of *Argulus* sp. was recovered from *C. auratus.*

Table 3.2: Infestation indices for parasite groups collected from five of ten species of ornamental fish that were imported from Sri Lanka with MI indicating mean intensity of infection and P% indicating the percentage prevalence.

Fish species	Parasite	Intensity of infection		P%
Barbonymus schwanenfeldii	**Monogenea**	**MI**	**Min – max**	
	Dactylogyrus sp.	44.29	7 – 98	87
Carassius auratus	**Monogenea**			
	Dactylogyrus spp. (2 species)	77.42	23 – 222	87
	Gyrodactylus spp. (2 species)	6	6	6.7
	Branchiura			
	Argulus sp.	1	1	6.7
Hypostomus plecostomus	**Monogenea**			
	Heteropriapulus sp.	5.1	1 – 17	36
Metynnis hypsauchen	**Monogenea**			
	Urocleidoides sp.	15.63	1 – 40	95
Pangasianodon hypophthalmus	**Monogenea**			
	Thaparocleidus sp.	224.2	45 – 408	100

3.3.1 The composition of parasites collected from fishes imported from Sri Lanka

Of the five infected fishes, four were infected by a single genus of monogenean parasites, with *C. auratus,* infected by monogeneans of two genera and a branchiuran (see Table 3.2). Moreover, *C. auratus* was the host that recorded the highest number of parasite species (see Table 3.2). The current study recorded a lower number of parasite species as compared to previous studies on the same fish species (see Table 3.1 and Table 3.2).

The branchiuran species was identified based on its overall body morphology. In order to identify monogeneans to species level, the shapes and morphometric measurements of haptoral sclerites such as anchors, bars, and marginal hooks including the MCO were examined. From the fish infected, the following branchiuran and monogeneans were identified to species level, see Table 3.3.

Table 3.3: Parasite species identified from ornamental fishes imported from Sri Lanka.

Fish species	**Collected parasites species**
Barbonymus schwanenfeldii	**Monogenea***:* *Dactylogyrus lampam*
Carassius auratus	**Branchiura:** *Argulus japonicus* **Monogenea**: *Dactylogyrus intermedius* *Dactylogyrus baueri* *Gyrodactylus kobayashii* *Gyrodactylus gurleyi*
Hypostomus plecostomus	**Monogenea***:* *Heteropriapulus heterotylus*
Metynnis hypsauchen	**Monogenea:** *Urocleidoides sinus*
Pangasianodon hypophthalmus	**Monogenea:** *Thaparocleidus caecus*

3.3.2 Parasite body feature shapes and morphometric descriptions

Order Dactylogyridea Bychowsky, 1937

Family Dactylogyridae Bychowsky, 1933

Dactylogyrus baueri Gussev, 1985 (Figure 3.1)

Type host: *Carassius auratus* (Linnaeus, 1758)

Present host: *Carassius auratus* (Linnaeus, 1758)

Type locality: *D. baueri* was described in Italy from an ornamental goldfish imported from Sri Lanka

Present locality: Sri Lanka

Infection site: Gills

Description based on 15 specimens: Anchor (a) 41 – 54 µm long with miniscule outer root, anchor point 7 – 12 µm long. Anchor inner root, slender and well developed, 12 – 25 µm long (see Figure 3.1). Bar (b) 20 – 37 µm long, 1 – 3 µm wide, very gentle sloping V-shaped and widest at bar ends. Marginal hook (c) handle slightly demarcated mid-length leading towards marginal hook sickle. MCO (d, e) 29 – 37µm long and sickle-shaped. Measurements of haptoral sclerites are shown in Table 3.4.

The MCO was the most important feature for identification since it provided sufficient morphological differences to discriminate between similar *Dactylogyrus* spp. (Šimková and Morand 2008; Borisov 2013, Rahmouni et al. 2017b). The haptoral sclerites and the MCO for *D. baueri* collected in the current study (Figure 3.1) were morphologically identical to those referred to in the literature (Gussev 1985; Borisov 2013) used for identification. Most of the measurements from the current study of the haptoral sclerites and MCO of *D. baueri* fell within a range of the measurements done by Gussev (1985) and Borisov (2013), with the exception of anchor total length (ATL) and anchor inner root length (AIRL), see Table 3.4.

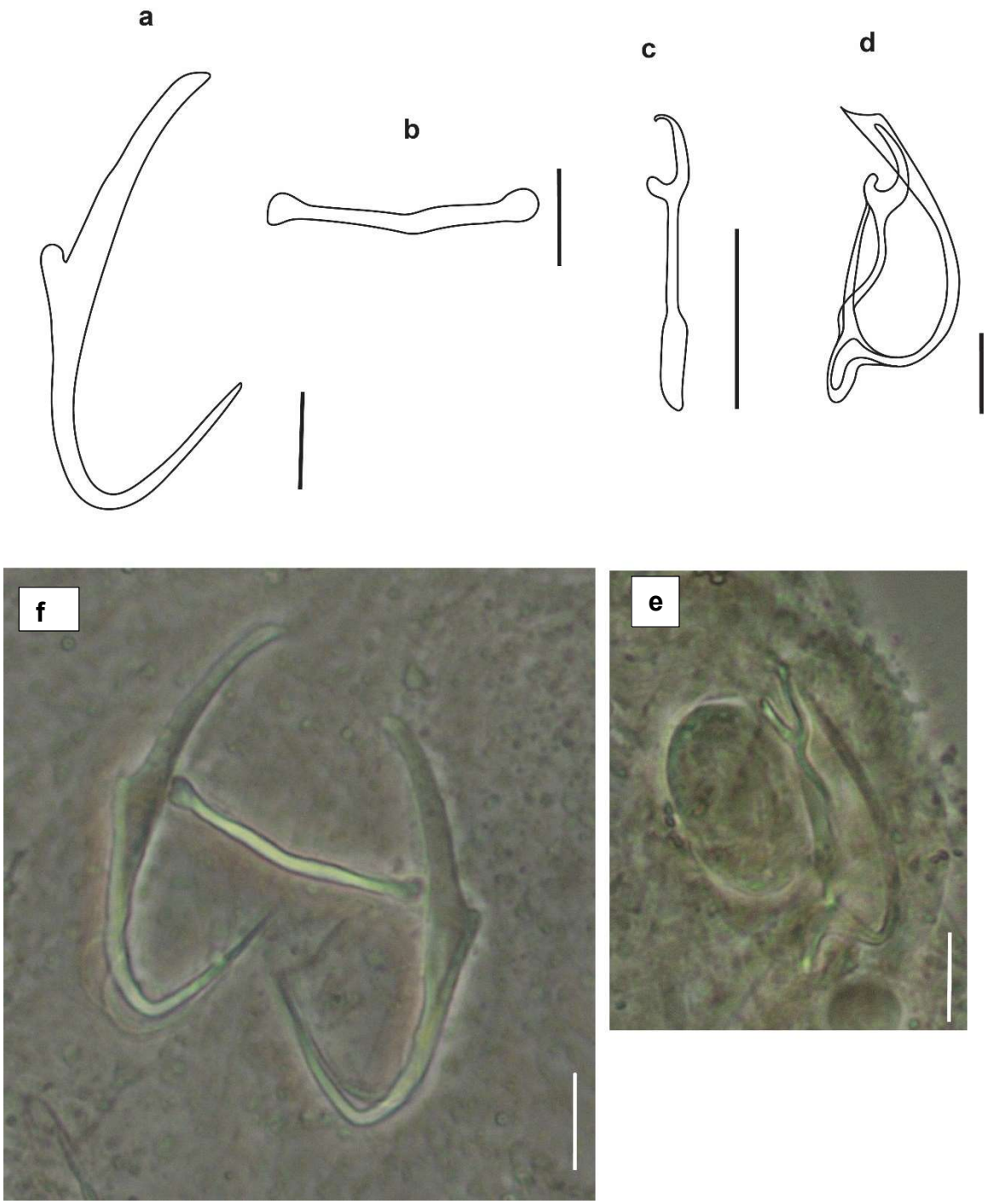

Figure 3.1: Line drawing and micrograph of haptoral hard parts of *Dactylogyrus baueri* ex *Carassius auratus*. (a) anchor, (b) bar, (c) marginal hook, (d, e) MCO, (f) overview of haptor. Scale bars = 10 µm.

Dactylogyrus intermedius Wegener, 1910 (Figure 3.2)

Type host: *Carassius auratus* (Linnaeus, 1758)

Present host: *Carassius auratus* (Linnaeus, 1758)

Type locality: Exact locality unknown, but thought to be in Singapore

Present study locality: Sri Lanka

Infection site: Gills

Description based on ten specimens: Anchor (a) 22 – 27 µm long, with two pronounced roots, unequal in length, anchor point 7 – 12 µm long. Inner root 10 – 15 µm long, outer root 3 – 7 µm long. Anchor with outward protrusion/ bulge mid-length, above the inner root (Figure 3.2). Bar (b) 17 – 23 µm long, 3 – 4 µm wide, linear at mid-length, widened and slightly arches at bar ends. Total length MCO (d, e) 33 – 43 µm with two tubules emerging from the entangled capsule. Measurements for haptoral sclerites are shown in Table 3.4.

The MCO provided enough morphological variability to discriminate *D. intermedius* from *Dactylogyrus* spp. with similar haptoral sclerites. Both the haptoral sclerites and MCO of specimens collected in the current study morphologically correspond to *D. intermedius* collected by Gussev (1985) and Borisov (2013). The measurements of haptoral sclerites and the MCO of *D. intermedius* collected in the current study fall within the size range indicated by Gussev (1985) and Borisov (2013).

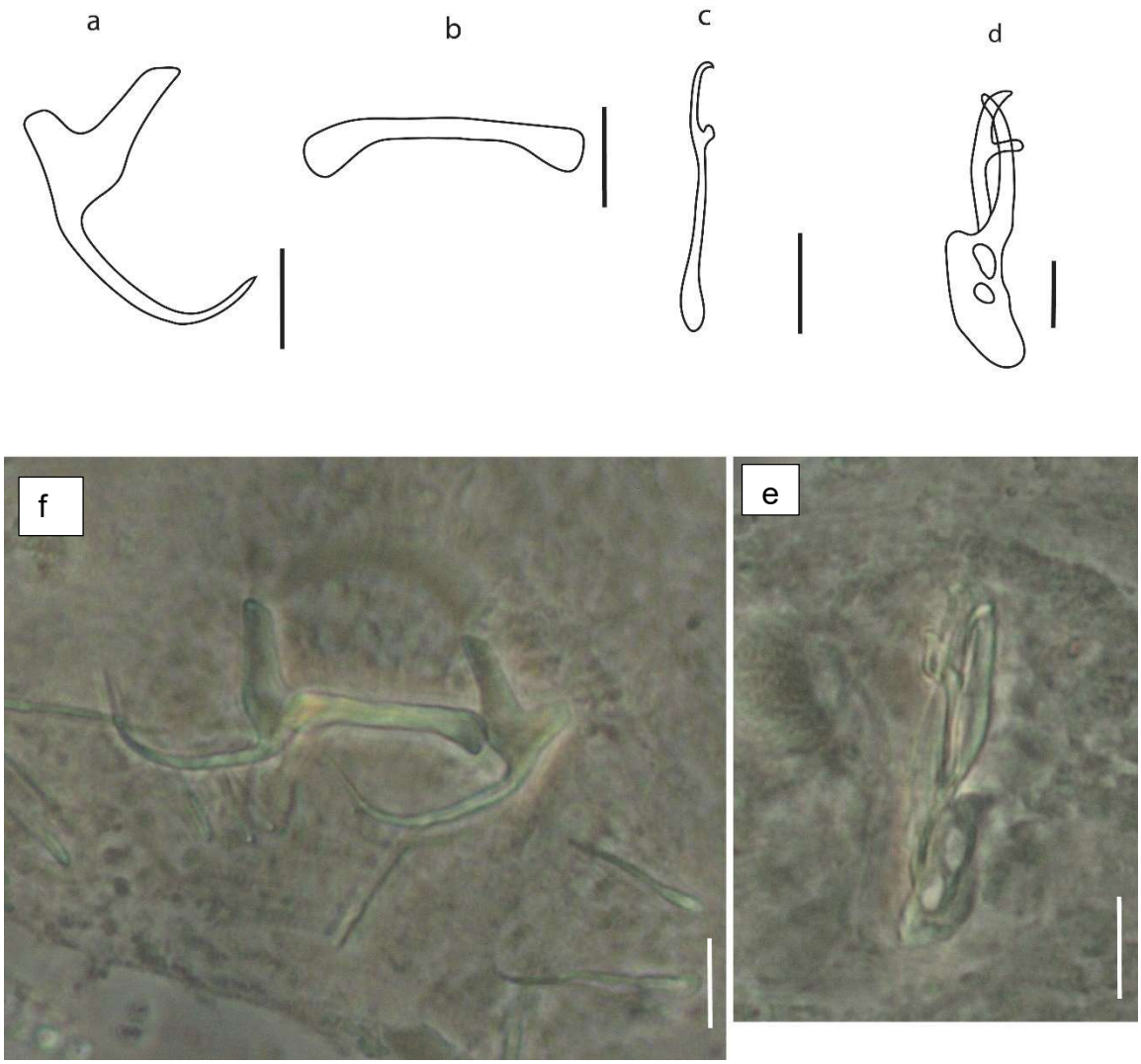

Figure 3.2: Line drawing and micrograph of haptoral hard parts of *Dactylogyrus intermedius* ex *Carassius auratus*. (a) anchor, (b) bar, (c) marginal hook, (d, e) MCO, (f) overview of haptor. Scale bars = 10 µm.

Table 3.4: Measurements (µm) of the haptoral sclerites of *Dactylogyrus intermedius* and *Dactylogyrus baueri* collected from the gills of *Carassius auratus.*

Features	*D. intermedius*			*D. baueri*		
	Present study (*n* = 10)	**Gussev (1985)**	**Borisov (2013)**	**Present study** (*n* = 15)	**Gussev (1985)**	**Borisov (2013)**
ATL	22 – 27	23 – 31	16 – 25	38 – 51	41 – 54	39 – 49
APL	7 – 12	7–11	11 – 17	17 – 25	21 – 28	14 – 27
AIRL	10 – 15	9 –15	9 – 14	12 – 25.	20 – 30	14 – 25
AORL	3 – 7	3 – 5	2 – 6	–	–	–
BTL	17 – 23	23 – 30	14 – 24	20 – 37	26 – 36	17 – 38
BW	3 – 4	3 – 4	2 – 3	1 – 3	2 – 3	1 – 3
MCOTL	33 – 43	33 – 60	20 – 42	29 – 37	31 – 67	24 – 38

Note: ATL = anchor total length, APL = anchor point length, AIRL = anchor inner root length, AORL = anchor outer root length, BTL = bar total length, MCOTL = male copulatory organ length.

Dactylogyrus lampam (Lim and Furtado, 1986) (Figure 3.3).

Type host: *Barbonymus schwanenfeldii* (Bleeker, 1853)

Present host: *Barbonymus schwanenfeldii* (Bleeker, 1853)

Type locality: Bukit Merah Reservoir, Malaysia

Present locality: Sri Lanka

Infection site: Gills

Description based on 15 specimens: Anchor 9 (a) 22 – 26 µm long, with recurved point, and inner root 8 – 9 µm long and outer root 2 – 3 µm long. Dorsal bar 2 – 3 µm long, 15 – 16 µm wide and basin-shaped. Ventral bar (c) 16 – 18 µm long, 5 – 6 µm wide and V-shaped. The marginal hook (d) handle slightly demarcated leading to a marginal hook sickle. The MCO (e, g) consists of a single coiled tube. Haptoral sclerites measurements for *D. lampam* are shown in Table 3.5.

The shapes of both haptoral sclerites and the MCO of *D. lampam* from the current study were compared to and resembled those by Lim and Furtado (1986) and Mohanta and Chandra (2000). Haptoral sclerites measurements from the current study were more consistent with the original description by Lim and Furtado (1986).

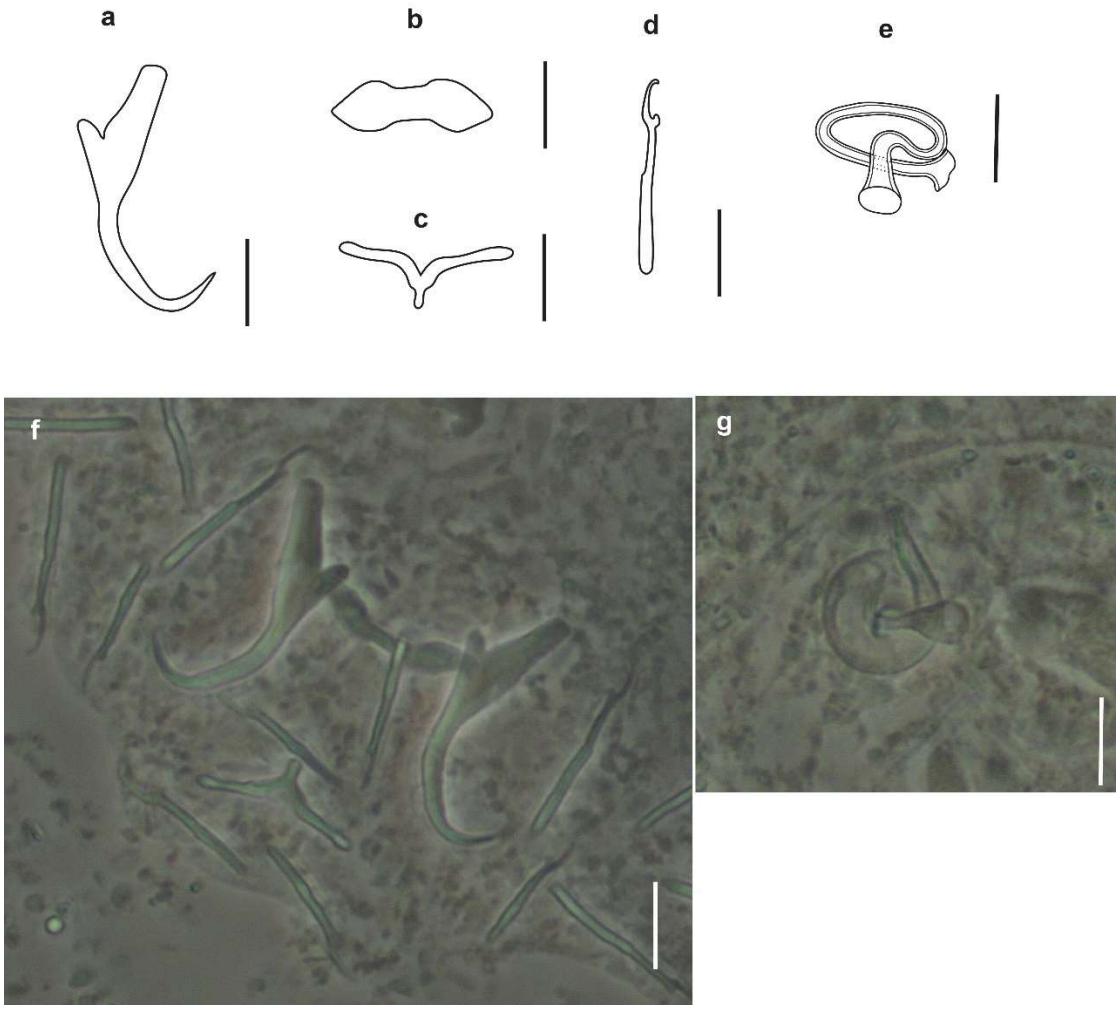

Figure 3.3: Line drawing and micrograph of haptoral hard parts of *Dactylogyrus lampam* ex *Barbonymus schwanenfeldii*. (a) anchor, (b) dorsal bar, (c) ventral bar, (d) marginal hook, (e) MCO, (f) overview of haptor, (g) MCO. Scale bars = 10 µm.

Table 3.5: Measurements (µm) of the haptoral sclerites of *Dactylogyrus lampam* collected from the gills of *Barbonymus schwanenfeldii*.

Features	Present study (*n* = 15)	Lim and Furtado (1986) (*n* = 3)	Mohanta and Chandra (2000)
ATL	22 – 26	30 – 34	36 – 40
APL	8 – 9	8 – 10	12 – 14
AIRL	8 – 9	8 – 10	12 – 16
AORL	2– 3	1 – 2	3 – 4
DBTL	2 – 3		3 – 4
DBW	15 – 16	24 – 27	22 – 25
VBTL	16 – 18	–	20 – 24
VBW	5 – 6	–	7 – 8

Note: ATL = anchor total length, APL = anchor point length, AIRL = anchor inner root length, AORL = anchor outer root length, DBTL = dorsal bar total length, DBW = dorsal bar width, VBTL = ventral bar total length, VBW = ventral bar width.

Thaparocleidus caecus (Mizelle and Kritsky, 1969) (Figure 3.4)

Type host: Unknown, but likely to be *Pangasius djambal* Bleeker, 1846

Present host: *Pangasianodon hypophthalmus* (Sauvage, 1878)

Type locality: unknown, as *T. caecus* was described in the United States of America from fish imported from Asia

Present study locality: Sri Lanka

Infection site: Gills

Description based on 15 specimens. Dorsal anchor (a) 40 – 48 µm long with recurved points, and stumpy outer root. Dorsal bar (b) 24 – 31 µm long, dome-shaped mid-length and linear at ends. Ventral anchor (c) 18 – 22 µm long, with recurved point, and underdeveloped roots. Ventral bars (d) 54 – 63 µm long and V-shaped (see Figure 3.4). Haptoral sclerite measurements are shown in Table 3.6.

The haptoral sclerites and the MCO for *T. caecus* specimens collected in the current study were morphologically similar to *T. caecus* specimens collected by Lim (1990), Mizelle and Kritsky (1969), and Chaudhary et al. (2014). The current study, however, recorded the largest sizes for dorsal anchor point length (DAPL) (25 µm), and VBTL (63 µm), but in general there was an overlap in the measurements (see Table 3.6) as compared to values from literature.

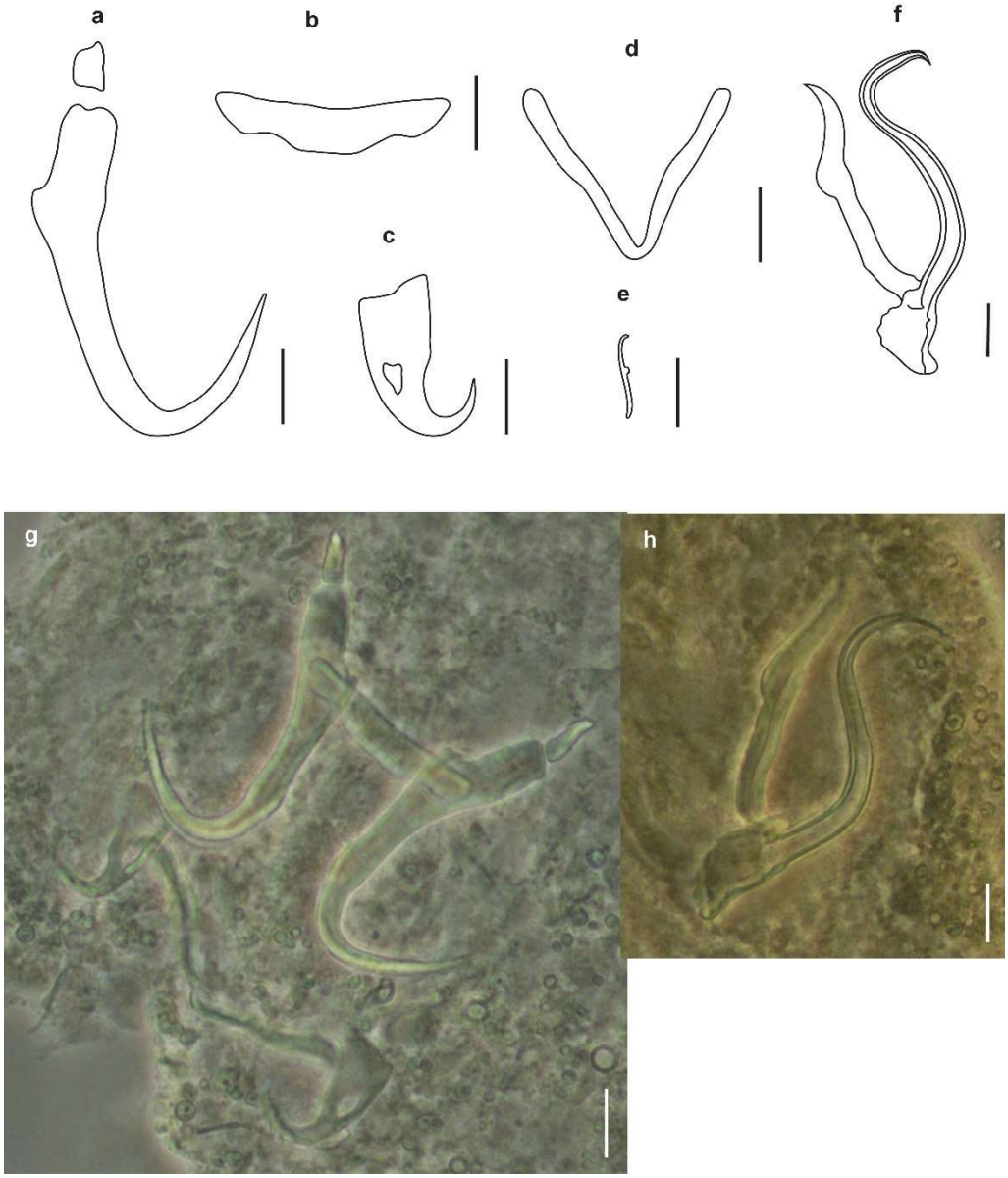

Figure 3.4: Line drawing and micrograph of haptoral hard parts of *Thaparocleidus caecus* ex *Pangasianodon hypophthalmus* (a) dorsal anchor, (b) dorsal bar, (c) ventral anchor, (d) ventral bar, (e) marginal hook, (f) MCO, (g) haptor overview, (h) MCO. Scale bars = 10 µm.

Table 3.6: Measurements (µm) of the haptoral sclerites of *Thaparocleidus caecus* collected from the gills of *Pangasianodon hypophthalmus*.

Features	**Lim (1990)** (*n* = 10)	**Mizelle and Kritsky (1969)** (*n* = 10)	**Chaudhary et al. (2014)** (*n* = 10)	**Present study** (*n* = 15)
DATL	–	45 – 48	50 – 54	40 – 48
DAPL	12 – 14	–	17 – 19	16 – 25
VATL	–	20 – 22	23 – 25	18 – 22
VAPL	8 – 10	–	8 – 10	8 – 12
DBTL	44 – 52	29 – 34	32 –38	24 – 31
VBTL	–	44 – 59	44 – 58	54 – 63

Note: DATL = dorsal anchor total length, DAPL = dorsal anchor point length, VATL = ventral anchor point length, VAPL = ventral anchor point length, DBTL = dorsal bar total length, VBTL = ventral bar total length.

Heteropriapulus heterotylus (Jogunoori, Kritsky and Venkatanarasaiah, 2004) (Figure 3.5).

Type host: *Hypostomus* sp.

Present host: *Hypostomus plecostomus* (Linnaeus, 1758)

Type locality: Hyderabad and Secunderabad twin cities, India

Present study locality: Sri Lanka

Infection site: Gills

Description based on ten specimens. Ventral anchor (a) 37 – 42 µm long with recurved point, ventral anchor base 10 – 12 µm wide. Ventral anchor shaft and point extend posteroventral from the whole haptor. Dorsal anchor (b) 31 – 36 µm long and have minuscule to non-existent outer roots. Dorsal anchor base 7 – 9 µm wide. Dorsal bar (c) 18 – 22 µm long and rod-shaped. Ventral bar (d) 38 – 47 µm long, bow-shaped with tapered ends (Figure 3.5). Measurements of haptoral sclerite features are shown in Table 3.7.

The shape of haptoral sclerites for *H. heterotylus* specimens collected in the current study were identical to those described by Jogunoori et al. (2004). The measurements of haptoral sclerite features for *H. heterotylus* from specimens sampled in the current

study were also consistent with those measurements of Jogunoori et al. (2004). The current study, however, recorded slightly higher measurements for the ventral anchor total length VATL (see Table 3.7).

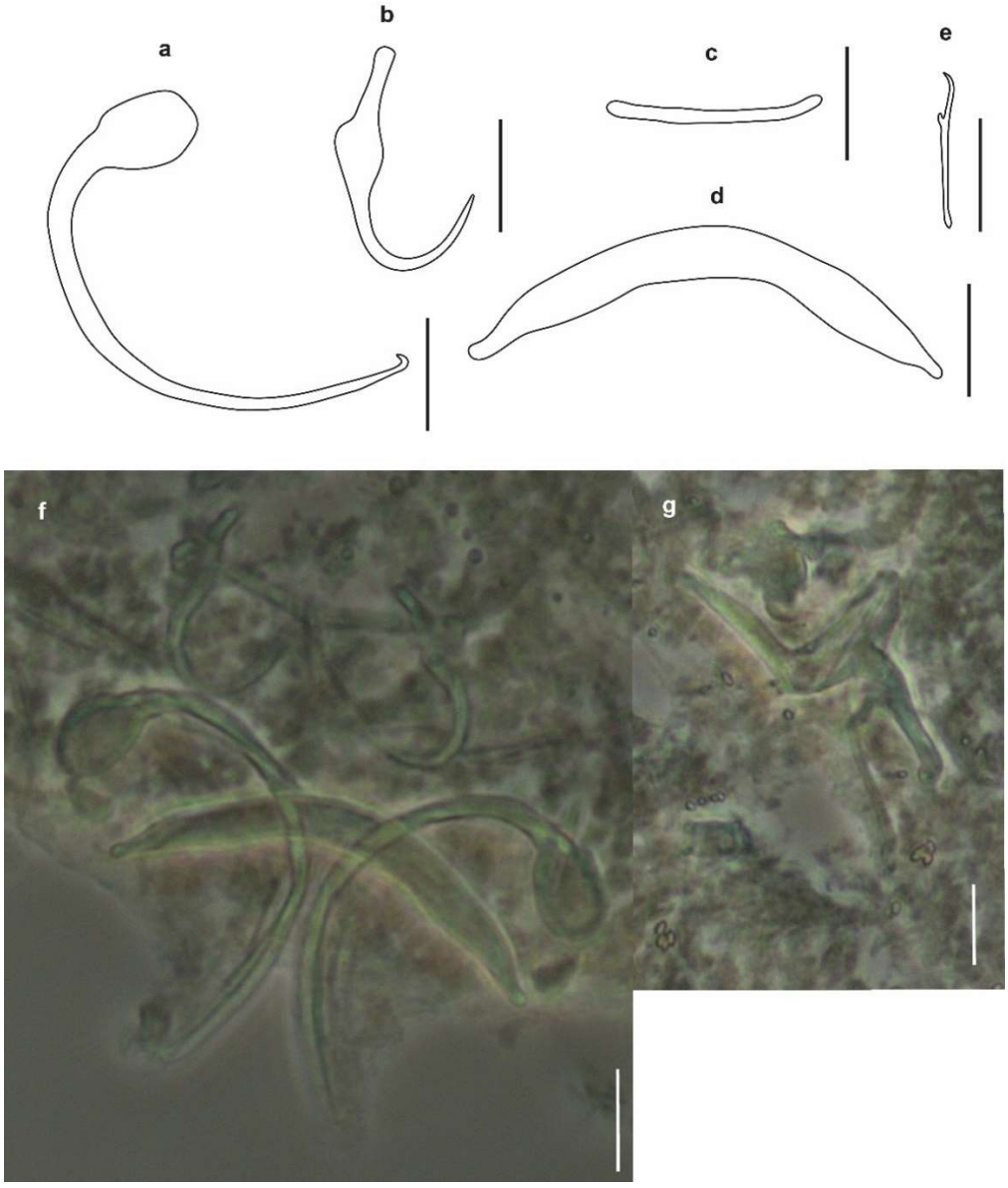

Figure 3.5: Line drawing and micrograph of haptoral hard parts of *Heteropriapulus heterotylus* ex *Hypostomus plecostomus.* (a) ventral anchor, (b) dorsal anchor, (c) dorsal bar, (d) ventral bar, (e) marginal hook, (f) haptor overview, (g) MCO. Scale bars = 10 µm.

Table 3.7: Measurements (µm) of the haptoral sclerites of *Heteropriapulus heterotylus* collected from the gills of *Hypostomus plecostomus.*

Features	Present study (*n* = 10)	Jogunoori et al. (2004)	Number examined
DATL	11 – 21	8 – 21	9
DABW	7 – 9	6 – 9	9
VATL	37 – 42	34 – 41	11
VABW	10 – 12	9 – 12	11
DBTL	18 – 22	18 – 23	9
VBTL	38 – 47	40 – 47	10

Note: DATL = dorsal anchor total length, DABW = dorsal anchor base width, VATL = ventral anchor total length, VABW = ventral anchor base width, length, DBTL = Dorsal bar total length, VBTL = ventral bar total length.

Urocleidoides sinus Zago, Yamada, de Oliveira Fadel Yamada, Franceschini, Bongiovani, da Silva, 2020 (Figure 3.6)

Type host: *Schizodon nasutus* Kner, 1858

Present host: *Metynnis hypsauchen* (Muller and Troschel, 1844)

Type locality: Sapucaí-Mirim River and Jurumirim Reservoir, Brazil

Present study locality: Sri Lanka

Infection site: Gills

Description based on ten specimens. Dorsal anchor (a) 31 – 36 µm long, 9 – 18 µm wide with asymmetrical, tapered roots. Ventral anchor (b) 25 – 29 µm long and 12 – 15 µm wide with asymmetrical, tapered roots. Ventral bar (c) 27 – 34 µm long, 5 – 10 µm wide, and open V-shaped. Dorsal bars 27 – 40 µm long, 8 – 16 µm wide and U-shaped. Marginal hook (see Figure 3.6 (e)) with recurved points. Haptoral sclerite measurements are shown in Table 3.8.

The shape of haptoral sclerite features was sufficient to distinguish *U. sinus* from other *Urocleidoides* spp. (Zago et al. 2020). The haptoral sclerites for *U. sinus* specimens

collected in the current study were morphologically identical to the *U. sinus* specimens described by Zago et al. (2020). The measurements of haptoral sclerite features of *U. sinus* collected in the current study were also consistent with the measurements of haptoral sclerite features of the specimens collected and described by Zago et al. (2020). *Urocleidoides sinus* from the current study recorded slightly larger measurements for dorsal anchor total length (DATL), dorsal anchor width (DAW), VATL, DBTL, DBW, VBTL, and VBW (see Table 3.8).

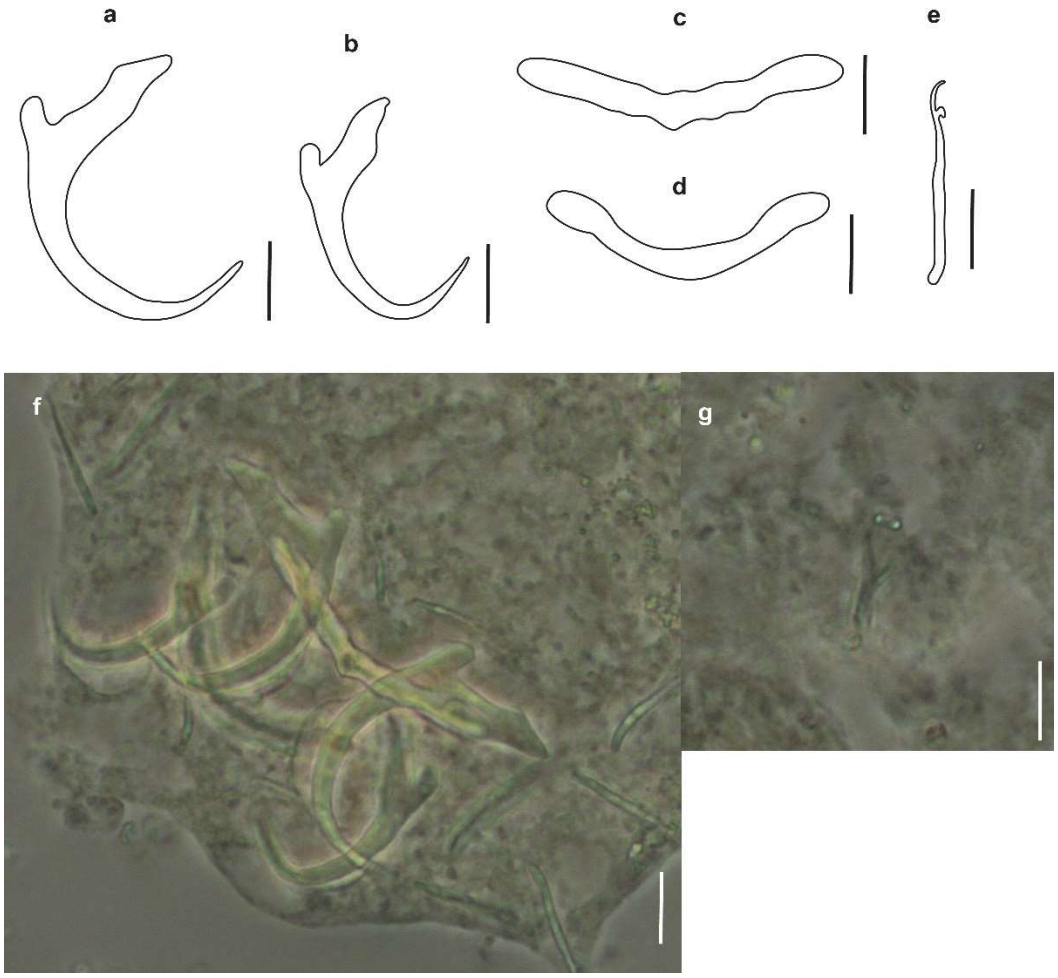

Figure 3.6: Line drawing and micrograph of haptoral hard parts of *Urocleidoides sinus* ex *Metynnis hypsauchen.* (a) dorsal anchor, (b) ventral anchor, (c) ventral bar, (d) dorsal bar, (e) marginal hook, (f) haptor overview, (g) MCO. Scale bars = 10 µm.

Table 3.8: Measurements (µm) of haptoral sclerites of *Urocleidoides sinus* collected from the gills of *Metynnis hypsauchen.*

Features	**Present study** (*n* = 10)	**Zago et al. (2020)**
DATL	31 – 36	31 – 35
DAW	9 – 18	9 – 15
VATL	25 – 29	25 – 28
VAW	12 – 15	12 – 16
DBTL	27 – 40	27 – 34
DBW	8 – 16	8 – 13
DBMW	3 – 6	3 – 6
VBTL	27 – 34	27 – 33
VBW	5 – 10	5 – 9
VBMW	2 – 5	2 – 5

Note: DATL = dorsal anchor total length, DAW = dorsal anchor width, VATL = ventral anchor total length, VAW = ventral anchor base width, DBTL = dorsal bar total length, DBW = dorsal bar width, DBMW = dorsal bar median width, VBTL = ventral bar total length, VBMW = ventral bar median width.

Order: Gyrodactylidea Bychowsky, 1937

Family: Gyrodactylidae Van Beneden and Hess, 1863

Gyrodactylus gurleyi Price, 1937 (Figure 3.7)

Type host: *Carassius auratus* (Linnaeus, 1758)

Present host: *Carassius auratus* (Linnaeus, 1758)

Type locality: Type locality unspecified, likely in Japan

Present study Locality: Sri Lanka

Infection site: Fins

Description based on two specimens: Anchors (a) 44 – 53 µm long, curve into a point. Dorsal bar (b) 18 – 21 µm long, and gentle bending towards lateral spurs on each end. Ventral bar (c) 18 – 20 µm long, with well-developed but short lateral processes, tongue-shaped membrane present. Marginal hook (d, e) 24 – 28 µm long, with rounded sickle heel oblique to the marginal hook handle. Measurements of the haptoral sclerites are shown in Table 3.9.

The marginal hooks were the most important haptoral sclerite features in identifying and discriminating *G. gurleyi* from similar *Gyrodactylus* spp. (Harris and Cable 2000; Cable et al. 2005; Paetow et al. 2009). The current study's *G. gurleyi* possessed haptoral sclerites that were similar to those collected by Omidzahir et al. (2012). The size of haptoral sclerites of *G. gurleyi* collected in the current study were consistent with *G. gurleyi* described and recorded by Omidzahir et al. (2012).

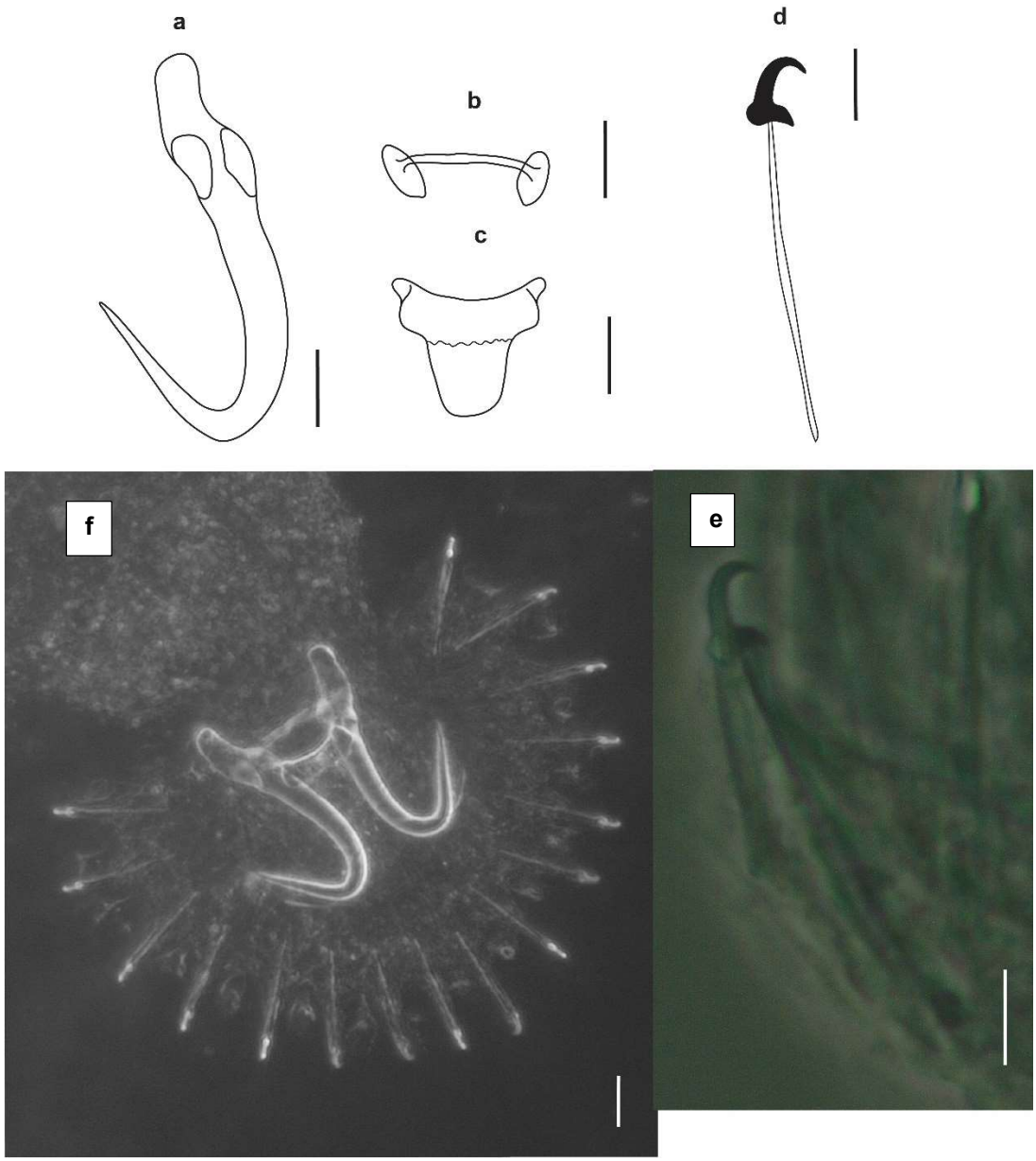

Figure 3.7: Line drawing and micrograph of haptoral hard parts of *Gyrodactylus gurleyi* ex *Carassius auratus*. (a) anchor, (b) dorsal bar, (c) ventral bar, (d, e) marginal hooks, (f) overview of haptor. Scale bars a, b, c, e = 10 µm with d and f = 5 µm.

Gyrodactylus kobayashii Hukuda, 1940 (Figure 3.8)

Type host: *Carassius auratus* (Linnaeus, 1758)

Present host*: Carassius auratus* (Linnaeus, 1758)

Type locality: Unspecified, likely in China

Present study locality*:* Sri Lanka

Infection site: Fins

Description based on four specimens: Anchors (a) 56 – 58 µm long, and curves into a tip. The dorsal bar (b) gently bending towards lateral spurs on each end. Ventral bar (c) 20 – 22 µm long, with pronounced membrane. Marginal hook (d, e) 24 – 26 µm long, with the sickle heel slightly laterally compressed and oblique to the marginal hook handle. The measurements of haptoral sclerites are shown in Table 3.9.

The marginal hooks of *G. kobayashii* provided enough variation to identify and distinguish *G. kobayashii* from similar *Gyrodactylus* spp. (Vanhove et al. 2011; García-Vásquez et al. 2011; Omidzahir et al. 2012; Tu et al. 2015).

Table 3.9: Measurements (µm) of the haptoral sclerites of *Gyrodactylus kobayashii* and *Gyrodactylus gurleyi* collected from the fins of *Carassius auratus.*

Features	*G. kobayashii*		*G. gurleyi*	
	Present study (*n* = 4)	**Tu et al. (2015)** (*n* = 4)	**Present study** (*n* = 2)	**Omidzahir et al. (2012)**
ATL	56 – 58	55 – 66	44 – 53	47– 53
APL	22 – 23	27 – 32	22 – 27	21– 26
ARL	21 – 23	19 – 25	11 – 12	10 – 17
ASL	43 – 45	37 – 45	34 – 42	35 – 41
ADSW	5 – 6	3 – 6	–	–
DBTL	–	–	18 – 21	19 – 23
VBTL	20 – 22	21 – 24	18 – 20	19 – 22
MHTL	24 – 26	25 – 30	24 – 27	24 – 28

Note: ATL = anchor total length, APL = anchor point length, ARL = anchor root length, ASL = anchor shaft length, ADSW = anchor distal shaft width, DBTL = dorsal bar total length, VBTL = ventral bar total length, MHTL = male hook total length.

The *G. kobayashii* specimens collected in the current study were morphologically similar to those collected by Tu et al. (2015). The measurements of haptoral sclerite features of *G. kobayashii* collected in the current study fell within the range of the *G. kobayashii* haptoral sclerite measurements by Tu et al. (2015).

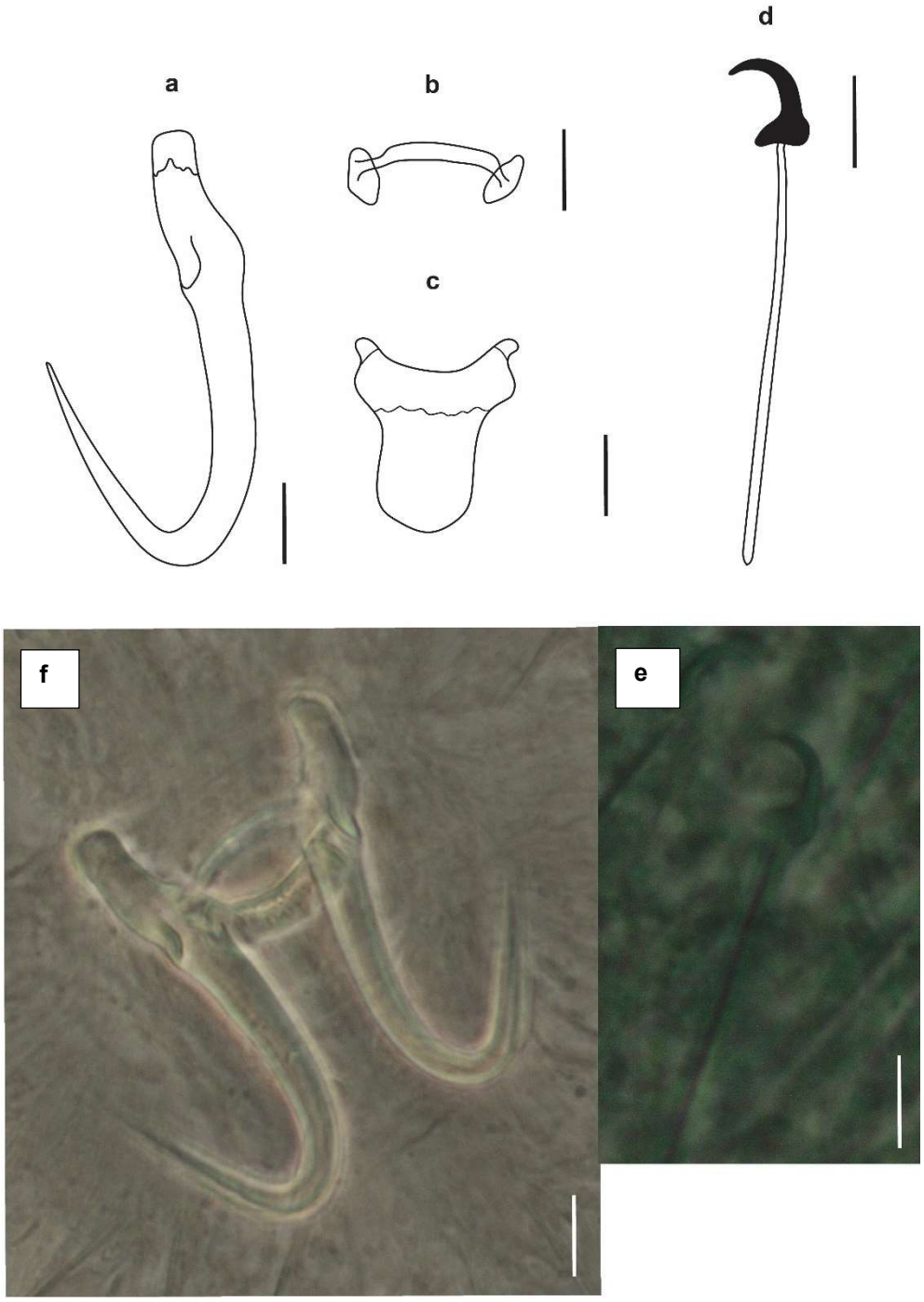

Figure 3.8: Line drawing and micrograph of haptoral hard parts of *Gyrodactylus kobayashii* ex *Carassius auratus* (a) anchor, (b) dorsal bar, (c) ventral bar, (d, e) marginal hooks, (f) overview of haptor. Scale bars a, b, c, e = 10 µm with d and f = 5 µm.

Order: ArguloidaYamaguti, 1963

Family: Argulidae Leach 1819

Argulus japonicus Thiele, 1900 (Figure 3.9)

Type host: *Carassius auratus* (Linnaeus, 1758)

Present host: *Carassius auratus* (Linnaeus, 1758)

Type locality: *Argulus japonicus'* exact type locality is unknown; however, it was first described in Japan

Present locality: Sri Lanka

Infection site: Fins

Description based on one specimen. Dorso-ventrally flattened segmented body, with anterior antennae, two anterior eyes with two circular suction disks below. Proboscis located below suck disks and four pairs of biramous legs following two testes and two abdominal lobes at the posterior end. Studying overall body features allowed for the identification and discrimination of *A. japonicus* from similar species (Pilgrim, 1967). The *A. japonicus* specimen from the current study resembles micrographs in literature used for identification (Kruger et al. 1983).

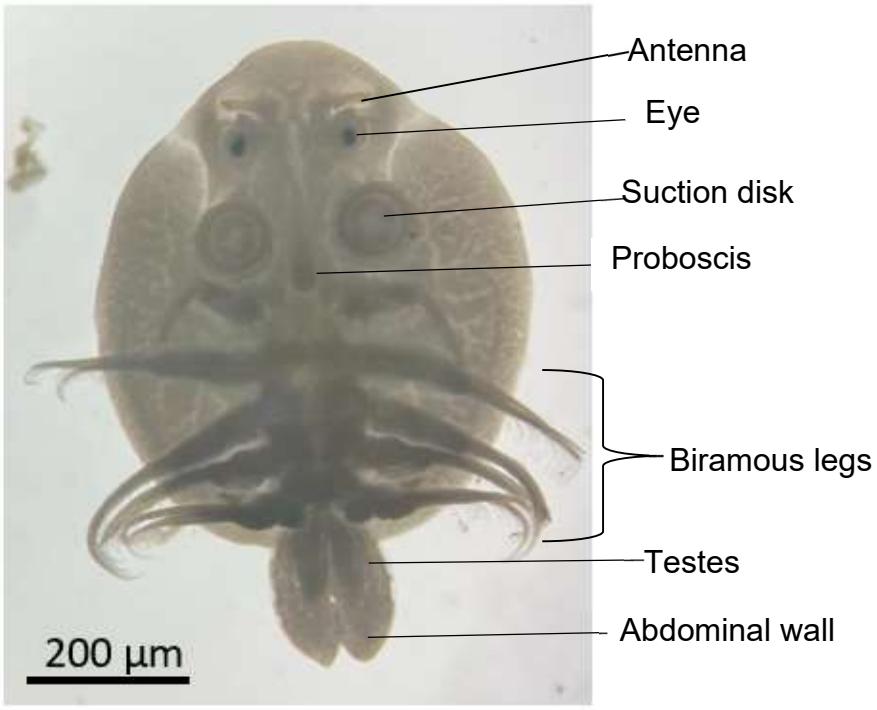

Figure 3.9: Image of *Argulus japonicus* collected from the caudal fin of *Carassius auratus.*

3.4 Discussion

Five of the 10 ornamental fish species examined in the current study were found to be infected by parasites, these being *B. schwanenfeldii*, *C. auratus*, *H. plecostomus*, *M. hypsauchen*, and *P. hypophthalmus*. Except for *C. auratus,* the infected fishes were solely infected by monogenean species. Conversely, *C. auratus* was infected by a branchiuran and monogeneans. Parasites were successfully identified at species level. Branchiuran species have a great resemblance to each other, however, studying their overall features is sufficient to identify and distinguish between species (Pilgrim, 1967). Alternatively, for monogeneans, a detailed examination of the haptoral sclerites and MCOs were required for their identifications. For the most part, the shape and size of haptoral sclerite features and the MCOs of identified monogeneans collected in the current study were in agreement with literature chosen for identification. Inconsistencies in haptoral sclerite size were recorded with slight inconsistencies observed from *Dactylogyrus* spp. in the current study, which could be attributed to the sizes of the haptoral sclerites and MCOs of *Dactylogyrus* spp. that have been shown to vary seasonally. This has, however, been proven with only two *Dactylogyrus* spp.,

Dactylogyrus crucifer Wagener, 1857 and *Dactylogyrus molnari* Ergens and Dulmaa, 1969 (Zolovs et al. 2012; Rastogi and Singh 2016). *Dactylogyrus* spp. have also been shown to vary in haptor and MCO sizes due to their geographic location and their host species (Chinabut and Lim 1993; Mohanta and Chandra 2000). Research is lacking on the factors that affect the size of haptor features and the MCOs for *Thaparocleidus* spp., *Urocleidoides* spp., and *Heteropriapulus* spp. The widths and lengths of haptoral sclerites of some *Gyrodactylus* spp. collected from the same fish host have been reported to change with a change in water temperature (Mo 1991; Dávidová et al. 2005). The *Gyrodactylus* spp. collected in the current study were, however, consistent with the literature utilised for identification.

Alternatively, no parasites were found from, *M. lacustris, O. goramy, P. latipinna, S. nigriventris,* and *T. fisheri* in the current study. Of these, only *P. latipinna* and *O. goramy* were previously recorded to be infected by parasites as shown in Table 3.1. Despite *M. lacustris, S. nigriventris,* and *T. fisheri* having not been previously recorded to be infected by parasites, it is likely that these fish species harbour parasites that are yet to be detected given that fish species from the same genera have been reported with parasites. For example, species of the genus *Melanotaenia* Gill, 1862 have been reported to be infected by nematodes, acanthocephalans, and protozoans (Gleeson et al. 2000; Smales 2009; Moravec and Adlard 2016). In turn species of the genus *Synodontis* Cuvier, 1816, have been reported to be infected by acanthocephalans, copepods, digeneans, nematodes, cestodes, monogeneans, and myxosporean parasites (Akinsanya et al. 2008; Basu et al. 2012; Eyo et al. 2013; Oldewage 2014; Mbondo et al. 2017) with species of the genus *Trachelyopterus* Valenciennes, 1840 reported to be infected by digeneans and monogeneans (Pantoja et al. 2016; de Oliveira Fadel Yamada et al. 2021).

The parasite composition recorded for infected fishes examined in this study was lower than previous studies' findings (see Table 3.1 and Table 3.2) for the same fish species. This could be due to Sri Lanka's reputed efficiency in treating ornamental fish before exportation to the international market (Wijesekara and Yakupitiyage 2001). As per information provided by Wijesekara and Yakupitiyage (2001), not only do Sri Lankan fish breeders treat against parasite infections but ornamental fish imported into Sri Lanka must be accompanied by a quarantine certificate from the country of origin. In instances when a quarantine certificate is not provided, imported fish are placed under

quarantine before being released from customs with the duration being at the discretion of the government officials responsible for the inspection and import of live fish (Wijesekara and Yakupitiyage 2001). Similarly, before fish can be exported from Sri Lanka to other countries, the fish have to be quarantined and inspected for signs of disease by local officials.

Ornamental fish imported into South Africa in this study were shown to have a lower parasite composition than those examined by Thilakaratne et al. (2003) from Sri Lankan farms. Thilakaratne et al. (2003) recorded three more parasite groups; Protozoa, Nematoda, and Digenea. Moreover, Thilakartane et al. (2003) recorded only a single fish specimen that was found not to be infected by parasites. This could be due to the following reasons. Firstly, the current study sampled different fish species with the goldfish being the only species common between the two studies. Secondly, Thilakaratne et al. (2003) had a much higher sample size of 1520 consisting of over 13 species. Lastly, Thilakaratne et al. (2003) sampled and examined fish species in Sri Lanka, and the lower parasite diversity recorded in the current study could be attributed to some parasites being lost during transit.

The goldfish was the only species common between the two studies. In the current study, *Dactylogyrus* spp. collected from goldfish were reported to have a higher prevalence of 87%, compared to Thilakaratne et al. (2003) with a prevalence of 30.8%. Thilakaratne et al. (2003) recorded a prevalence of 22.9% for *Gyrodactylus* spp., whereas a prevalence of only 6.7% was recorded here. The prevalence of *Argulus* sp. infecting goldfish was generally low in both studies, with a prevalence of 6.7% recorded in the present study and a prevalence of 2.6% recorded by Thilakaratne et al. (2003). The difference in prevalence can be attributed to differences in the sampling size used with Thilakaratne et al. (2003) examining a total of 153 goldfish as compared to the 15 in the current study. Moreover, Thilakaratne et al. (2003) most likely examined fish stock from different breeders. However, in both studies, *Dactylogyrus* spp. had the highest prevalence followed by *Gyrodactylus* and *Argulus* species. Since Thilakaratne et al. (2003) did not report on the intensity of infections for parasite groups, this parameter could not be compared. Besides the study by Thilakaratne et al. (2003), there is no other published research on the parasitic diversity of ornamental fish in Sri Lanka, prior to their exportation.

In a separate study, Trujillo-González et al. (2018b) examined eight freshwater ornamental fish species that were imported into Australia from Sri Lanka. Of the eight species examined, three were infected by parasites. Moreover, it is also important to add that Trujillo-González et al. (2018b) examined fish hosts for only monogeneans, and reported four species of monogeneans, whilst in the current study eight monogenean species were reported. Only monogeneans from the genera *Dactylogyrus* Diesing, 1850 and *Urocleidoides* Mizelle and Price, 1964 were common between the two studies. Trujillo-González et al. (2018b) reported a single *Dactylogyrus* species, while in the current study three were recorded. Both the current study and Trujillo-González et al. (2018b) recorded a single *Urocleidoides* species. Compared to the current study, Trujillo-González et al. (2018b) recorded a lower monogenean composition from ornamentals imported from Sri Lanka. This could be a reflection of Australia's mandatory quarantine protocols (Trujillo- González et al. 2018b). This could also, however, be due to the current study and Trujillo-González et al. (2018b) examining different ornamental fish species, without a single common species. To allow for more relatable results, future studies should examine the same fish species whereby the prevalence, mean intensity, and parasite diversity are being compared.

3.5 Conclusion

Out of the ten ornamental fish species selected and imported from Sri Lanka, only five species were reported to be infected by parasites. With the exclusion of *C. auratus* and *B. schwanenfeldii*, these fish species had monogenean parasite species that are new records in South Africa: these are *H. heterotylus*, *U. sinus* and *T. caecus*. The current study also presents the first report of *U. sinus* from *M. hypsauchen.* With monogeneans being morphologically similar, their identification had to be confirmed using genetic means (see Chapter 5). Ornamental fishes imported from Sri Lanka to South Africa in this study and those imported into Australia and examined by Trujillo-González et al. (2018b) recorded lower parasite compositions compared to most previous records on the same species (see Table 3.1 and Table 3.2; and Trujillo-González et al. 2018b). Based on these findings it can be deduced that the pre-export treatment provided by Sri Lankan traders is largely effective. However, since examined fishes were not completely free of parasites, the risk of their introduction and possible invasion into local systems still exists. However, with the majority of the recorded

parasites being the high-host specific gill monogeneans, the chances that these alien parasites infect local fish species are low. Moreover, with Trujillo-González et al. (2018b) recording a lower parasite composition as compared to the current study, the implementation of mandatory quarantine protocols, as conducted by the Australian authorities when importing live fish from abroad, is inferred to provide a means to limit and restrict the introduction of alien parasites.

Chapter 4: *Glossolepis incisus* and host-parasite interactions when housed in a closed system

4.1 Introduction

4.1.1 Host-parasite interactions

Host-parasite interactions refer to the multifactorial effects that the host and parasites have on each other, influencing the health of the host and the parasite numbers infecting the host (Buchmann and Lindenstrøm 2002; Khan 2012). Some of the factors that influence fish host-parasite interactions include the host's age, size, state of the host's immunity (Vincent and Font 2003; Wu et al. 2007; Khan 2012; Gilbert and Avenant-Oldewage 2021), the ichthyoparasite's nutritional requirements, rate of reproduction, and proficiency at evading the host's immune system (Sitjà-Bobadilla 2008; Rohlenová et al. 2011; Khan 2012). Environmental factors such as water temperature, water quality and the congestion of fish also affect host-parasite interactions (Khan 2012; Zargar et al. 2012; Florindo et al. 2017). Different parasites and fish species have distinctive responses to environmental changes (Khan 2012; Blanar et al. 2009). Therefore, the effects that environmental factors have on host-parasite interactions should not be generalised and must be linked to specific species of ichthyoparasites and their host(s). Studies have shown that when the conditions overly favour the parasite in a host-parasite interaction, it could lead to the demise of the host (Robertson 1979; Hansen et al. 2003; Barber and Scharsack 2010; Kim et al. 2002).

4.1.2 The red rainbowfish and parasites associated with this species

Rainbowfish from the family Melanotaenidae are freshwater fish characterised by diverse body colours (Allen 1980). In 1907 the first rainbowfish was discovered in Australia (Ohee 2013) with a further 95 different species of rainbowfish discovered in subsequent years in other countries (Tappin 2010). Among these, the red rainbowfish *G. incisus*, which is native to Indonesia, is the most popular and one of the most valuable species within the ornamental fish trade (Kadarini et al. 2018). This species is named according to the bright red colour displayed by males, with females often displaying a chartreuse colour (Kadarini et al. 2018). Despite its popularity as an ornamental fish, the literature is porous about parasites associated with this species, with Lukhele (2021) being the only study available.

The study by Lukhele (2021) revealed that 15 red rainbowfish, imported from Indonesia, were found infected by a single nematode species and two monogenean species. The nematode identified was *Camallanus cotti* Fujita, 1927, a parasite native to Asia (Levsen and Berland 2002) and synonymous with ornamental fish (Evans and Lester 2001; Menezes et al. 2006). Due to the ornamental trade and the species popularity amongst hobbyists, *C. cotti* has nearly gained a worldwide distribution (Kim et al. 2002; Wu et al. 2007) with its presence first reported in South Africa from the guppy *Poecilia reticulata* Peters, 1859 by Tavakol et al. (2017). This parasite is reported to cause severe intestinal lesions by feeding on the blood and parts of the intestinal walls of its host (McMinn 1990). As a consequence, high infestation of this parasite has been associated with the death of its host (Kim et al. 2002).

In a study by Kim et al. (2002), the guppy, from a fish farm in Korea, reported a mortality of 30% that was attributed to a high infestation of *C. cotti,* whereby these parasites were observed protruding through the anuses of some dead individuals. The ciliate *Tetrahymena corlissi* (Thompson, 1955) was also stated to contribute to the fatalities to a degree. Kim et al. (2002) also pointed out that in combination with a high parasite load, poor water quality could have also contributed to the high mortalities.

Monogeneans recorded by Lukhele (2021) represented a new genus and species. To date, there are no studies as to how these monogeneans interact with their host. Gill monogeneans attach to gill filaments and feed on their host's mucus and blood resulting in severe anaemia (Yoshinaga et al. 2001; MacKay 2010). Depending on the parasite load, gill monogeneans can drastically decrease the efficiency of gas exchange between the environment and the host (Montero et al. 2004; MacKay 2010) and cause fatalities of the host (Kritsky and Heckmann 2002; Hansen et al. 2003; Rubio-Godoy et al. 2003). There are no studies on host-parasite interactions including the red rainbowfish *G. incisus* and its parasites. The current study, therefore investigated parasites associated with *G. incisus*, and if found, the interactions between *G. incisus* and its parasites.

4.2 Methods

To determine the host-parasite interactions between the red rainbowfish and its parasites, a total of 100 juveniles with a mean total length of 5.16 cm and body mass of 0.98 g were imported from Indonesia using the same import company. Using the

methods described in Chapter 2 (Section 2.1 to Section 2.5), upon arrival at the University of Limpopo, Parasitology Laboratory, 15 specimens were humanely killed and examined for parasites. The remainder was distributed into five aerated 60 L tanks with 15 specimens housed in each tank, equipped with rudimentary mechanical and chemical filters to maintain water quality. Tanks were kept at a constant temperature of 24˚C throughout the trial using submerged heaters. Fish were fed standard commercial pet flakes (Micro Pellets, Hikari) to satiation once a day. A week after their arrival, a further 15 individuals were randomly selected and examined for parasites. Thereafter on weeks three, five, seven, and nine, a further 15 fish were randomly sampled from each of the tanks, humanely killed, and examined for parasites. This was done to determine if there was a change in the number and composition of parasites throughout the nine-week trial. The parasite numbers (total counts), percentage prevalence (P%) and mean intensity (MI) of parasite groups collected for each time interval were recorded. Linear regression was utilised to see if fish length had any effect on parasite numbers. During the duration of the trial, aqueous levels of ammonia and nitrate were measured daily using a NT Lab Aquarium-test kit, and water replacements were also done once every three days for the first three weeks, and then daily for the remainder of the trial when fish mortalities were observed to increase by a small fraction.

4.3 Results

4.3.1 The trend of monogenean and nematode numbers throughout the trial

During the nine-week trial, a total of two parasite groups were collected and recorded from the red rainbowfish sampled. These were Monogenea and Nematoda. The Kruskal-Wallis test revealed monogenean and nematode numbers to be consistent from week zero to week seven ($p > 0.05$). The ninth week was not included in the statistical analysis due to fish mortalities, only seven (instead of 15) specimens were examined that week. Monogenean and nematode prevalence and mean intensity are shown in Table 4.1.

Table 4.1: Weekly infestation indices of parasites collected from *Glossolepis incisus*. MI = mean intensity and P% = percentage prevalence where n = number of fish specimens examined.

Week	Monogenea			Nematoda		
	P%	Intensity of infection		P%	Intensity of infection	
		MI	Min – max		MI	Min – max
0 (n = 15)	73.33	23.00	5 – 77	73.33	4.36	1 – 10
1 (n = 15)	86.67	27.85	1 – 102	73.33	3.83	1 – 12
3 (n = 15)	73.33	9.18	1 – 24	86.67	5.31	1 – 10
5 (n = 15)	80.00	11.42	2 – 49	86.67	4.77	1 – 12
7 (n = 15)	86.67	31.69	2 – 148	53.33	2.00	1 – 5
9 (n = 7)	0.00	0.00	-	42.86	1.33	1 – 2

The highest prevalence recorded was 86.67% for both monogeneans and nematodes. The lowest mean intensity was recorded at 9.18 for monogeneans and 1.33 for nematodes. The numbers of monogeneans and nematodes detected among red rainbowfish individuals examined varied widely. Monogeneans saw an irregular pattern of increase and decrease in infection indices throughout the trial, with nematodes displaying an overall downward pattern, see Figure 4.1.

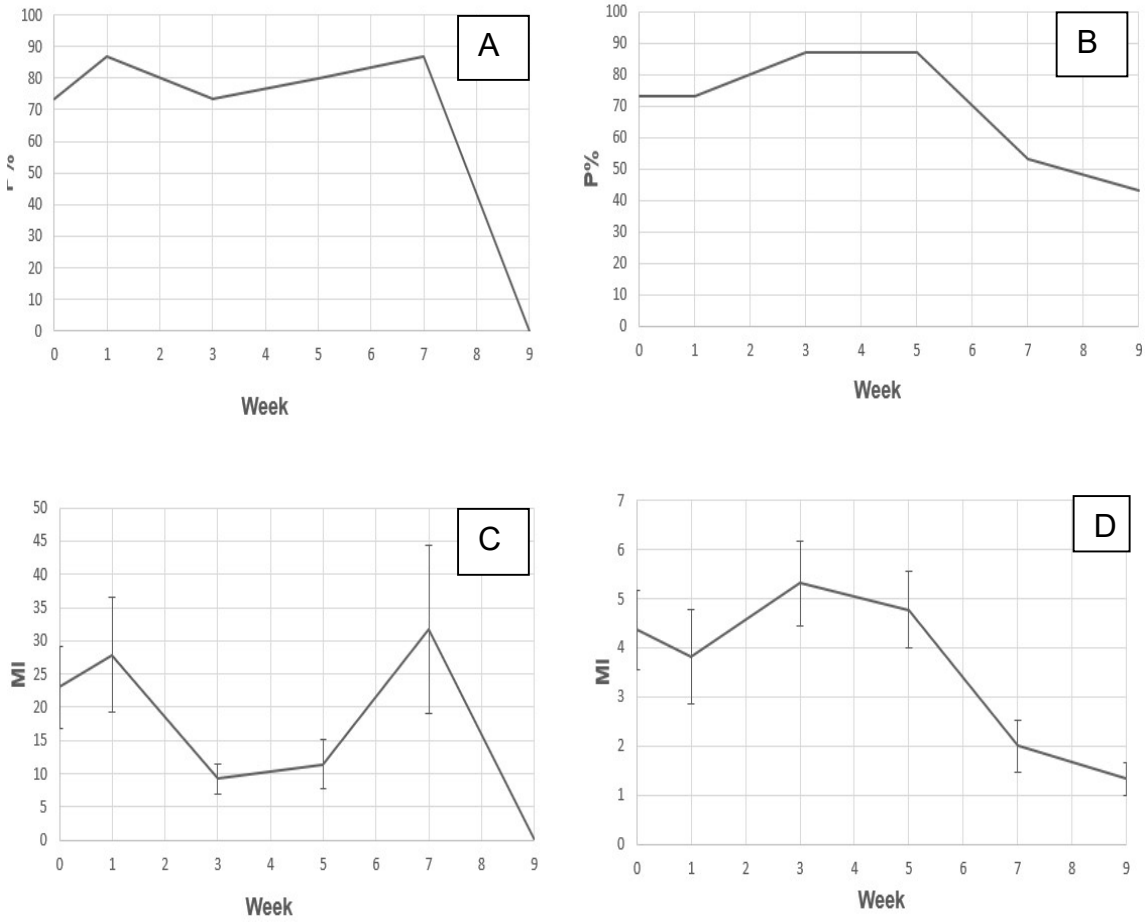

Figure 4.1: The pattern of infestation indices of parasites collected from *Glossolepis incisus*, with bars representing standard error. Percentage prevalence (P%) of (A) monogeneans and (B) nematodes. Mean intensity of (C) monogeneans and (D) nematodes.

Throughout the trial, monogenean prevalence (P%) remained relatively constant, alternating between 73.33% (11 out of 15 specimens infected) and 86.67% (13 out of 15) from week zero to week seven. However, in the nineth week, no parasites were recorded and P% went down to zero. Nematode P% also remained relatively constant, alternating between 73.33% and 86.67% from week zero to week five. However, from the seventh to the ninth week, prevalence decreased to 53.33% (8/15 specimens infected) and decreased further to 42.86% (3/7 specimens infected). Only seven fish specimens were alive on the ninth week and the aquarium was less congested, limiting the spreading of parasites, likely resulting in the decrease in P% for both monogeneans and nematodes. Based on linear regression, monogenean and nematode numbers revealed a very weak relationship between fish length and

parasite load with a R^2 of 0.017 reported for both nematode and monogeneans, see Figure 4.2.

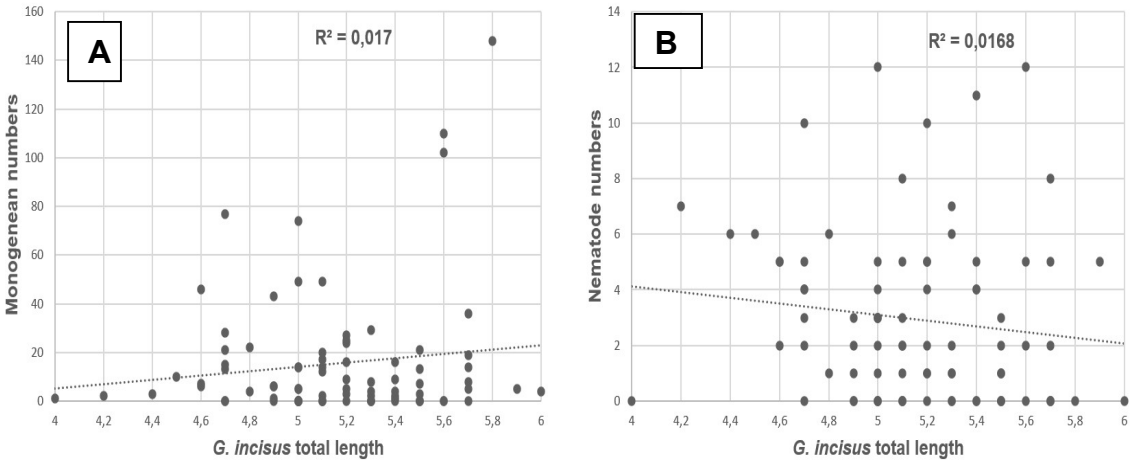

Figure 4.2: Regression analysis of the interrelation between the total length (cm) of the red rainbowfish and the numbers of (A) monogeneans and (B) nematodes reported.

Monogenean MI was shown to increase a week after initiating the experiment. However, by the third week, the lowest MI value of 9.18 was recorded. By week seven the MI peaked at 31.69. A drastic change was observed in the ninth week, whereby monogenean numbers dropped to zero. A week after the initiation of the trial, nematode MI went down from 4.36 to 3.82. The MI then peaked at 5.3 on week three steadily decreasing thereafter to 1.33 by the end of the trial. Moreover, some *G. incisus* specimens were observed with nematodes protruding through their anuses (see Figure 4.3), parasites that were often observed floating in the water after aquaria were subjected to water replacements.

Figure 4.3: Image of nematode protruding through the anus of a *Glossolepis incisus* specimen.

Moreover, some nematode specimens were seen to be carrying larvae when viewed under a microscope (see Figure 4.4). This confirmed this species to be viviparous. Nematode larvae were not included when parasites were counted as they were yet to be released by the adults.

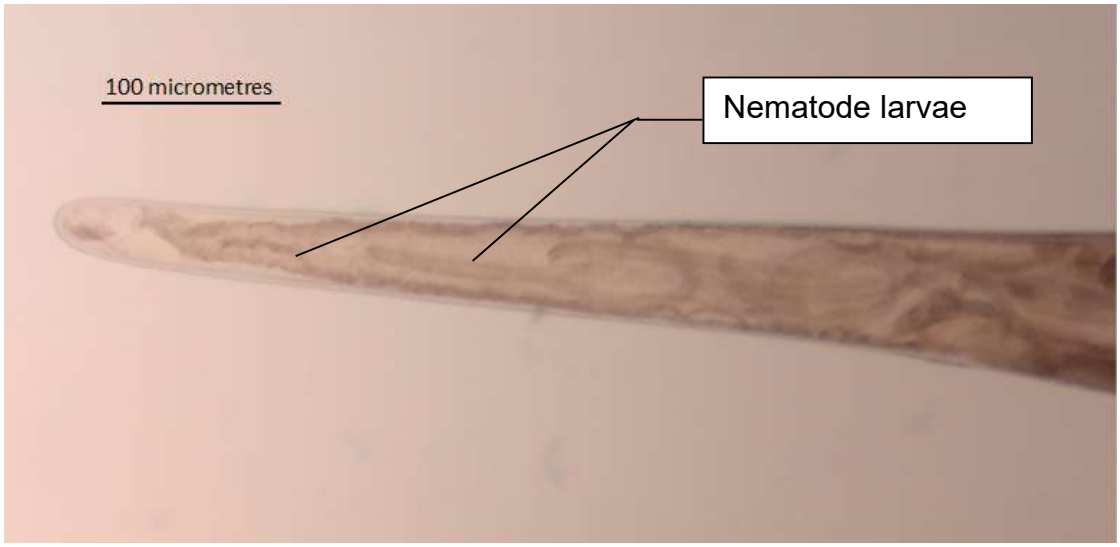

Figure 4.4: Image of a female nematode specimen carrying larvae.

4.3.2 Recorded fish mortality and water quality

Throughout the experiment, a total of 18 fish died before being humanely killed and examined. Of the 18, 12 were observed to be infected by nematodes (P% = 66.67%) with the remaining having no parasites. Nematodes collected from the dead

specimens had an MI of 9.30. No monogeneans were detected or collected from dead fish since monogeneans quickly dissociate after the death of a host (Reed et al. 2009). The highest fish mortalities were recorded from week three to week six whereby three specimens died every week. Daily water replacements were then done, which resulted in a decrease in fish fatalities.

With regard to water quality, throughout the trial, daily testing using the Aquarium-test kit revealed high (> 0.50 mg/L) ammonia concentrations and low (< 25 mg/L) nitrate concentrations (Medeiros et al. 2016; Maulini et al. 2022), even when water replacements were done daily. In fact, by comparing sampled aquarium water wherein the tests were administered, it could be observed that the concentration of ammonia reached the highest concentration that could be detected using the test kit, 8 mg/L, and that of nitrates was 0 mg/L. Ammonia concentrations remained consistently high throughout the trial.

Fish hosts and parasite groups have been reported to present similar host-parasite interactions (Skinner 1982; Thoney and Hargis 1991; Zargar et al. 2012; Florindo et al. 2017). However, some fish-parasite interactions can display trends unique to the host and parasite species involved (Khan 2012; Blanar et al. 2009). Therefore, the identification of both host and parasites to species level is crucial to link host-parasite interactions to the specific host and parasite species. The current study therefore identified the collected monogenean and nematode specimens to species level.

4.3.3 Parasite identifications based on the shapes and morphometrics of their body features

Order Dactylogyridea Bychowsky, 1937

Family Ancyrocephalidae Bychowsky and Nagibina, 1968

Ancyrocephalidae sp. 1 (Figure 4.5)

Host: *Glossolepis incisus* Weber, 1907

Locality: Indonesia

Infection site: Gills

Description based on 4 specimens. Dorsal bar (A) 26.79 – 29.84 µm long with auricles. Ventral bar (C) 21.14 – 24.71 µm long and concave. Dorsal anchor (E) 29.63 – 35.26 µm long, with 10.25 – 13.35 µm long inner root and 3.26 – 6.60 µm long outer root. Ventral anchor (G) 19.87 – 23.09 µm long, with 6.95 – 8.71 µm long inner root and 2.42 – 3.49 µm long outer root (Figure 4.5). Haptoral sclerite measurements are shown in Table 4.2.

Ancyrocephalidae sp. 2 (Figure 4.5)

Host: *Glossolepis incisus* Weber, 1907

Locality: Indonesia

Infection site: Gills

Description based on 4 specimens. Dorsal bar (B) 22.80 – 25.30 µm long with auricles. Ventral anchor (D) 19.68 – 21.20 µm long and concave. Dorsal anchor (F) 27.15 – 30.58 µm long. The dorsal anchor inner root is 9.17 – 10.76 µm and the outer root is 4.60 – 5.91 µm long. Ventral anchor (H) 18.56 – 20.61 µm long. Ventral anchor inner root 5.85 – 7.57 µm, outer root 1.74 –3.73 µm long. Measurements of haptoral sclerites are shown in Table 4.2.

The haptoral sclerites of the Ancyrocephalidae species from the current study (Ancyrocephalidae n. gen.) are most similar to those of some species from the genus

Cichlidogyrus Paperna, 1960. The similarities to *Cichlidogyrus* are stocky dorsal bars with upward extensions (auricles), concave ventral bars, anchors with arched blades and asymmetrical bases with the shaft (inner root) being shorter than the guard (outer root) at the anchors' bases (Ergens 1981; Douëllou 1993; Pariselle and Euzet 1994; Geraerts et al. 2020). The dorsal bar auricles of Ancyrocephalidae n. gen is significantly shorter (appearing as bumps) than those of *Cichlidogyrus,* and the region between the auricles (dorsal face) is gently sloped in the case Ancyroecephalidae n. gen., whilst the dorsal face is dome-shaped in *Cichlidogyrus* species (Ergens 1981; Douëllou 1993; Pariselle and Euzet 1994; Maneepitaksanti and Nagasawa 2012; Abdul-Ameer 2017; Rahmouni et al. 2017a, Geraerts et al. 2020).

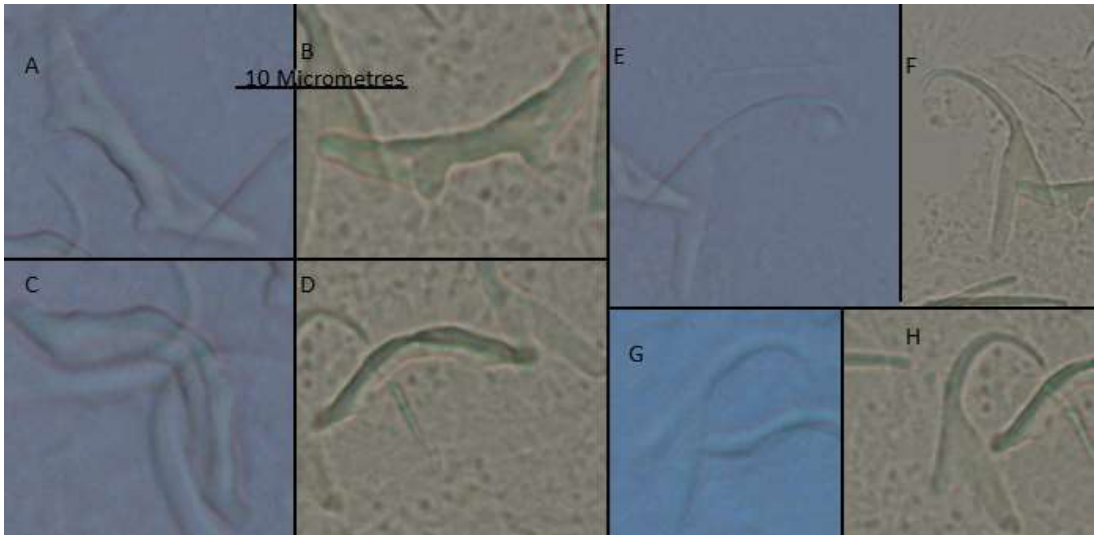

Figure 4.5: Micrograph of the haptoral sclerites of Ancyrocephalidae sp. 1 and sp. 2, collected from the gills of red rainbowfish. (A) Ancyrocephalidae sp.1, dorsal bar; (B) Ancyrocephalidae sp. 2, dorsal bar; (C) Ancyrocephalidae sp. 1, ventral bar; (D) Ancyrocephalidae sp. 2, ventral bar; (E) Ancyrocephalidae sp. 1, dorsal anchor; (F) Ancyrocephalidae sp. 2, dorsal anchor; (G) Ancyrocephalidae sp. 1, ventral anchor; (H) Ancyrocephalidae sp. 2, ventral anchor. The scale provided is relevant for all images.

The shapes and measurements of haptoral sclerites of Ancyrocephalidae n. gen. from the current study were consistent with those from the study by Lukhele (2021). Measurements from both studies revealed Ancyrocephalidae sp. 1 to possess longer haptoral sclerites as compared to Ancyrocephalidae sp. 2. Both studies revealed both of the monogenean species to possess dorsal anchors that are longer than their ventral anchors. These species were separated due to Ancyrocephalidae sp. 1's MCO

being found with an excess of spiralling tubules as compared to Ancyrocephalidae sp. 2's MCO (see Figure 4.6).

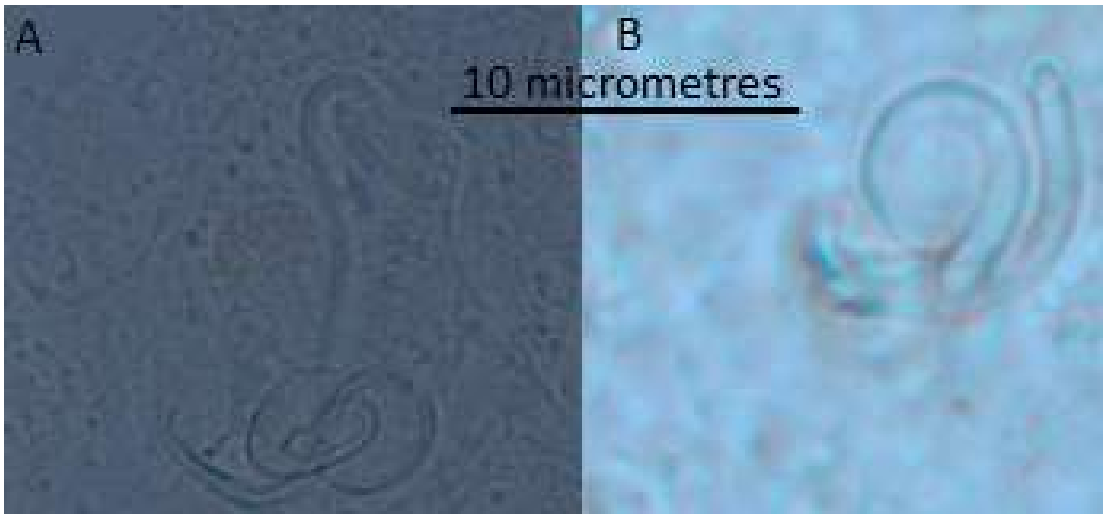

Figure 4.6: Micrograph of a male copulatory organ (MCO) for (A) Ancyrocephalidae sp. 1 and (B) Ancyrocephalidae sp. 2 collected from the gills of red rainbowfish. The scale provided is relevant for all images.

Table 4.2: Measurements (µm) of haptoral sclerites of monogeneans collected from the gills of *Glossolepis incisus*. DA = dorsal anchor, VA = ventral anchor, DB = dorsal bar, VB = ventral bar, MH = marginal hook, TL = Total length, IRL = inner root length, ORL = outer root length, and PL = point length.

	Lukhele (2021) study		**Present study**	
	Ancyrocephalidae sp. 1 (n = 4)	**Ancyrocephalidae sp. 2** (n = 5)	**Ancyrocephalidae sp. 1** (n = 4)	**Ancyrocephalidae sp. 2** (n = 4)
DA				
TL	29.72 – 33.56	28.08 – 30.45	29.63 – 35.26	27.15 – 30.58
IRL	10.21 – 13.07	9.13 – 10.82	10.25 – 13.35	9.17 – 10.76
ORL	3.76 – 6.39	4.40 – 5.98	3.26 – 6.60	4.60 – 5.91
PL	4.31 – 5.91	4.70 – 5.90	4.27 – 5.78	4.50 – 5.81
VA				
TL	19.91 – 23.21	18.86 – 20.42	19.87 – 23.09	18.56 – 20.61
IRL	6.93 – 8.51	5.78 – 7.57	6.95 – 8.71	5.85 – 7.57
ORL	2.52 – 3.54	1.70 – 3.8	2.42 – 3.49	1.74 –3.73
PL	4.90 – 6.52	4.79 – 6.13	4.92 – 6.61	4.88 – 6.09
DB				
TL	26.89 – 29.88	22.71 – 25.00	26.79 – 29.84	22.80 – 25.30
VB				
TL	21.26 – 24.58	19.56 – 21.33	21.14 –24.71	19.68 – 21.20
MH				
I	13.48 – 15.07	10.63 – 13.49	13.32 – 14.93	10.43 – 13.29
II	23.70 – 25.39	20.95 – 23.65	22.92 – 25.79	21.15 – 23.70
III	31.26 – 35.22	29.01 – 31.21	31.30 – 35.12	28.93 – 31.29
IV	39.63 – 44.71	38.13 – 41.51	39.59 – 45.88	37.33 – 40.56
V	16.00 – 17.48	15.80 – 16.80	16.12 – 17.53	15.85 – 16.83
VI	23.34 – 25.25	20.29 – 21.63	23.54 – 25.20	20.13 – 21.83
VII	28.93 – 33.34	26.07 – 32.00	29.10 – 33.34	26.17 – 31.83

Order Camallanida Ralliet and Henry, 1915

Family Camallanidae Ralliet and Henry, 1915

Camallanus cotti Fujita, 1927 (Figure 4.7)

Type host: unspecified

Present host: *Glossolepis incisus* Weber, 1907

Type locality: Japan

Present locality: Indonesia

Infection site: intestine

Description based on two specimens. Reddish body with dorsoventrally flattened anterior buccal capsule (Figure 4.7A and C) consisting of longitudinal ridges. Female with blunt and long posterior ending (Figure 4.7B), and male with hooked, short posterior ending (Figure 4.7D). Identifications are according to Rigby et al. (1997), and specimens in the current study are similar to those reported by Lukhele (2021).

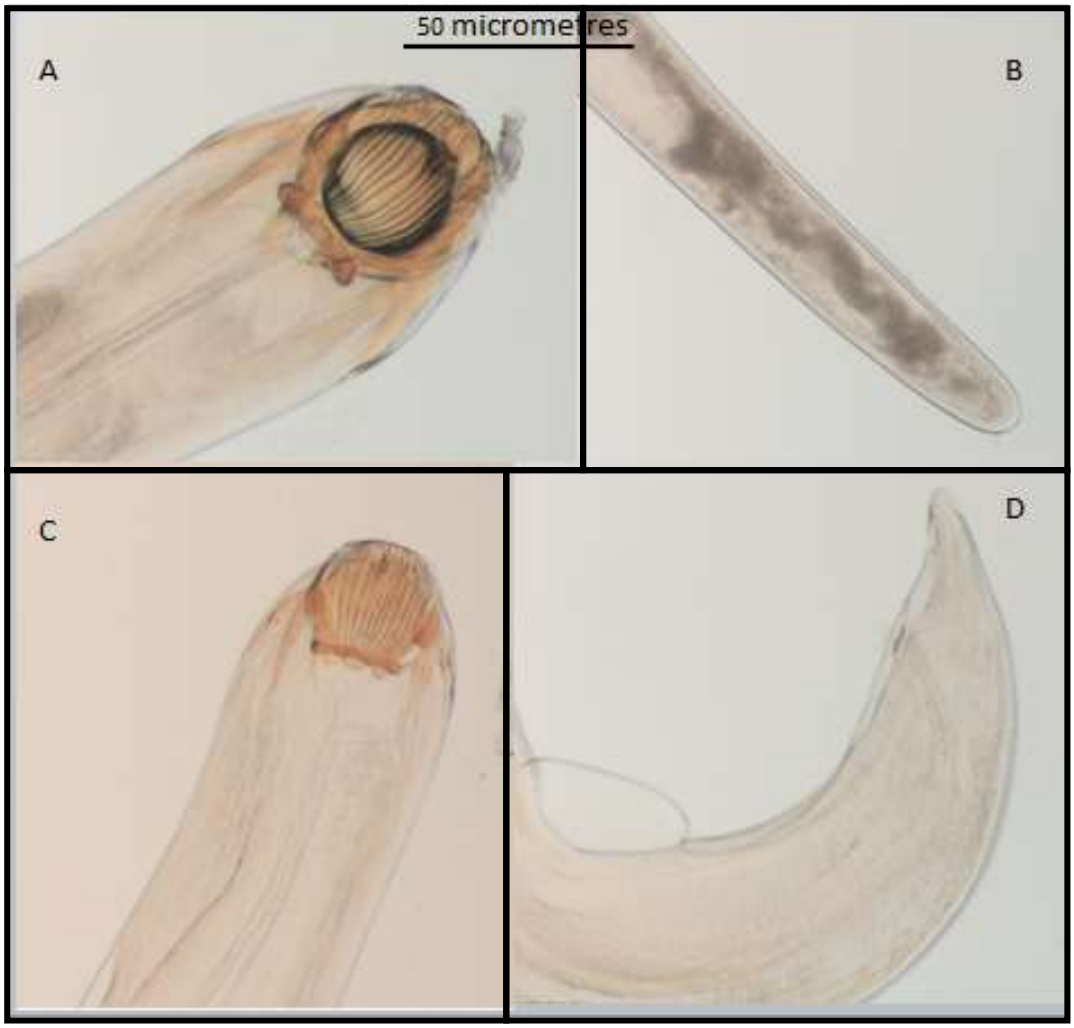

Figure 4.7: Images of identifying features of *Camallanus cotti* female, (A) anterior end and (B) posterior end. *Camallanus cotti* male, (C) anterior end and (D) posterior end collected from the intestine of the red rainbowfish. The scale provided is relevant for all images.

4.4 Discussion

Throughout the trial, an irregular pattern of increase and decrease in indices was observed for monogeneans. Monogenean numbers are controlled by chemical and physical changes in water (Buchmann and Bresciani 2006; Bayoumy et al. 2008). Studies on monogeneans have revealed a positive correlation between the majority of monogenean species and water parameters such as temperature, ammonia concentration, degree of eutrophication and turbidity (Skinner 1982; Thoney and Hargis 1991; Zargar et al. 2012; Florindo et al. 2017). In general, poor water quality is associated with a high monogenean infestation level (Skinner 1982; Khan and Kiceniuk 1988; Dzika et al. 2007). Water parameters such as salinity, turbidity, and

dissolved oxygen were, however, not measured throughout the current study. However, all fish tanks were well aerated and hence a shortage of dissolved oxygen was unlikely.

The temperature of water in fish tanks was also set and maintained at 24˚C throughout the trial using heaters. Thus, a temperature change is also an unlikely factor that could have influenced a change in monogenean numbers. The micro-pellet that the fish were fed contained a small amount of algae (as stated on the package) which can lead to a mild increase in eutrophic water conditions, so as to increase the stress response and disease resistance of fish (King 1970; Norambuena et al. 2015). Eutrophic water, however, also favours an increase in monogenean numbers (Zargar et al. 2012; Florindo et al. 2017). Therefore, similar to the majority of monogeneans, the monogenean species in the current study (Ancyrocephalidae sp.1 and sp. 2) most likely also grew in number under eutrophic conditions (Zargar et al. 2012; Florindo et al. 2017).

Nitrate concentration readings were constantly low throughout the experiment and ammonia concentration readings were constantly high. A high ammonia concentration has been reported to lead to an increase in monogenean numbers (Skinner 1982; Zargar et al. 2012), and thus the high ammonia concentration maintained throughout the study was likely in favour of an increase in monogenean numbers. However, the ammonia concentration test utilised could only detect concentrations up to a maximum of 8 mg/L, a value that was reached after each test.

Nematodes being endoparasites, respond differently to a change in water parameters as compared to ectoparasites. Ectoparasites, such as monogeneans, are directly affected by water parameter changes since they are in direct contact with their environment (Koskivaara et al.1992; Gilbert and Avenant-Oldewage 2021). However, endoparasites alone are indirectly affected when changes in water parameters impact the physiology and immunity of their fish host (Buchmann 2012; Gilbert and Avenant-Oldewage 2021). Free-living nematodes are directly affected by a change in water parameters (Khanna et al. 1997; Clavijo et al. 2016), however, parasitic nematodes are mostly affected by the altered physiological and physical factors of their hosts (Vincent and Font 2003; Wu et al. 2007; Buchmann 2012).

The endoparasite, *C. cotti*, has been shown to increase in numbers with an increase in temperature in other host-parasite interaction studies (Vincent and Font 2003; Wu et al. 2007). With the temperature kept constant throughout the trial in the current study, it is unlikely that this parameter affected *C. cotti* numbers. *Camallanus cotti* numbers have also been shown to increase with an increase in their host's length (Vincent and Font 2003; Wu et al. 2007). However, this trend was not observed in the current study, with host length revealed to not affect *C. cotti* numbers.

Some *C. cotti* specimens were seen carrying offspring when observed under a microscope. The temperature maintained throughout the trial provided the optimum environment for *C. cotti* offspring to develop, be released and infect new hosts (Levsen 2001). This could be an explanation for the initial increase in *C. cotti* numbers observed. In the wild, *C. cotti* uses copepods as intermediate hosts, however, in aquarium conditions this species is reported to directly infect its hosts but at lower intensities (Levsen and Jakobsen 2002). Moreover, *C. cotti* infecting aquarium fish have lower survival rates as compared to those in the wild (Levsen and Jakobsen 2022). Therefore, the subsequent decline in *C. cotti* numbers observed over time in the current study is attributed to this species' decrease in fitness under aquarium conditions as was reported in a study by Levsen and Jakobsen (2002) whereby *C. cotti* directly infected their hosts due to the absence of intermediate hosts.

From the third to sixth week, fish mortalities went up by a small fraction, and thus more frequent water changes were made. In some fish species, stress brought about by changes in water parameters is reported to make the fish hosts more susceptible to parasite infestations (Gómez-Laplaza and Morgan 2003) and suppress immune functions (Bly and Clem 1992; Hernández and Tort 2003; Bowden 2008). However, in the current study, frequent water changes were followed by a decrease in fish mortalities and parasite infestations. In fact, after water changes, *C. cotti* were often observed suspended in the water. Water changes could therefore also have played a role in decreasing monogenean and nematode numbers.

Regarding fish that had died without being humanely killed, both parasite infestations and high ammonia concentration could have contributed. Monogenean infestation levels could not be determined from fish that had died, since the parasites quickly dissipated. Monogenean infestations not being linked to the fish deaths is, however,

unlikely, since some killed fish were found infected by over 100 monogeneans. Fish that died without being killed recorded the highest MI for nematodes. It is therefore likely that nematodes may have also played a role in fish mortalities. The high MI could also be due to nematodes releasing offspring after the death of their host.

Ammonia levels remained high throughout the trial, even after water replacements. The toxicity of ammonia and nitrates differs among fish species, and it can be influenced by factors such as the length of exposure to the toxicant and the age of a fish species (Allan et al.1990; Tilak et al. 2007). No studies on the tolerance of *G. incisus* to ammonia and nitrates could be found, however, in general, to maintain good fish health, aquarium water is usually kept at a concentration that is below 0.5 mg/L for ammonia, and below 25 mg/L for nitrates (Medeiros et al. 2016; Maulini et al. 2022). Ammonia concentration reached 8 mg/L in the current study. The high ammonia concentration is likely to be due to the micro-pellets fed to the fish as the feed used had a high crude protein content. Feed with a high crude protein content has been associated with high aqueous ammonia levels due to increased ammonia excreted by fish (Florindo et al. 2017). Due to possible overfeeding, fish feed and faecal matter were also seen floating in the water.

Unconsumed fish feed and faecal matter break down into toxic ammonia instead of the relatively non-toxic ammonium (Putra et al. 2020). Bacteria then either convert the ammonia to toxic nitrites or relatively non-toxic nitrates (Kroupova et al. 2005). Ammonia converts into nitrites under poor oxygen levels (Azim and Little 2008) and nitrates under high or sufficient oxygen levels (Jiménez-Ojeda et al. 2018). Nitrite concentrations were not measured in this study. However, considering that all aquaria were well-aerated, nitrite concentrations were assumed to be low.

4.5 Conclusion

To investigate host-parasite interactions using *G. incisus* as a fish model, the current study first examined this species for parasites. Two parasite groups were found infecting the red rainbowfish, these being, monogeneans and nematodes. The length of the host was shown to not affect the host-parasite interactions between *G. incisus* and its associated monogeneans. An environment with a high ammonia concentration most likely favoured an increase of monogenean numbers on *G. incisus.* Similar to the majority of monogenean species, the number of monogeneans infecting the red

rainbowfish also most likely increases under eutrophic conditions. In the current study, monogenean numbers were observed to drastically decrease after water replacements were done more frequently. The current study therefore infers good water quality to limit parasite infestations. The infection of *Camallanus cotti* reported here under aquarium conditions was revealed to have a lower survival fitness as compared to what has been reported for *C. cotti* infecting fish species in the wild. The numbers of this nematode were also revealed to not be affected by the length of *G. incisus*, which is a trend that is not observed in other host fish species. Nematode numbers might also be affected by water and biological parameters not measured in the current study. Both water parameters and parasite infestations were inferred to be responsible for the deaths of red rainbowfish that were not humanely killed in the current study. The current study determined the host-parasite interactions between *G. incisus* and two of its associated parasite groups (Monogenea, Nematoda), but only under a few measured water parameters. The current study therefore realises the importance of measuring a wide range of water parameters to better explain trends observed when conducting experiments concerning fish host-parasite interactions.

Chapter 5: Molecular Characterisations of Monogeneans

5.1 Introduction

5.1.1 Phylogenetics background

One of the key topics in evolutionary biology has been the search for patterns and processes of evolution to better comprehend parasite speciation (Poulin 2002; Ŝimková et al. 2004). In phylogenetics, molecular and morphological data are used to infer evolutionary history in the form of a phylogenetic tree (Wu et al. 2008; Šimková et al. 2013; Chero et al. 2021). The use of ribosomal DNA molecular makers has been accepted as the best way of deducing phylogenies (Fox et al. 1977; Woese 1987; Patwardhan et al. 2014). Popular molecular markers used in constructing phylogenies of eukaryotes are the 18S, ITS1 and 28S ribosomal DNA regions (Patwardhan et al. 2014). The 28S region is mostly used in constructing phylogenetic trees due to this segment being relatively conserved with the 18S, ITS1 region mostly being used for identification (Chaudhary and Singh 2012).

5.1.2 Phylogeny of monogeneans

Monogenea is one of the most diverse parasitic groups in terms of species richness (Šimková et al. 2013). Monogeneans can speciate as a consequence of switching hosts (Ŝimková et al. 2013). The majority of monogeneans are host-specific (Ŝimková et al. 2013) and evolved within a host (duplication) or co-evolved with the host (Desdevises et al. 2002; Ŝimková et al. 2004). In the past, phylogenetic trees for monogenean species were determined solely using morphological characteristics (Lim et al. 2001; Pouyaud et al. 2006). Morphological separation of monogenean species and their inferred phylogeny were, however, greatly influenced by the subjective perceptions of researchers (Wu et al. 2008). Molecular characterisation and comparison of DNA sequences have allowed scientists to generate less biased phylogenies (Patwardhan et al. 2014) as some monogenean species can share highly similar morphological features (Razo-Mendivil et al. 2016; Baeza et al. 2019). The use of molecular phylogeny can therefore assist with the appropriate classification of morphologically similar or newly discovered monogenean species (Razo-Mendivil et al. 2016; Benovics et al. 2018; Chero et al. 2021). The sequence database GenBank (Benson et al. 2017) serves as a source of sequences for a range of organisms, which

can be utilised for comparative molecular identification and can be selected and used to infer phylogenies.

5.2 Methods

5.2.1 Monogenean collection and identification

Monogeneans were collected from the gills of *B. schwanenfeldii*, *C. auratus, G. incisus, H. plecostomus, M. hypsauchen* and *P. hypophthalmus*. Monogenean specimens were then cut in half, and their anterior portions consisting of the haptoral hard parts were fixed on a microscope slide for morphological identification, with the posterior portions being placed in microtubes with 96% ethanol for molecular characterisation. Morphological identifications of monogenean specimens were done as described in Chapter 3 (see Section 3.3).

5.2.2 DNA extraction, amplification and sequencing

Before DNA extractions the microtubules containing monogenean samples were placed in a vacuum centrifuge to evaporate the 96% ethanol rapidly and safely in the microtubes as per the methodology described by Přikrylová et al. (2013). Thereafter, DNA was extracted using DNeasy® Blood and Tissue kit - QIAGEN following the instruction protocol. For amplification of the 18S, ITS1 rDNA region the following primers were used, forward primer S1 (5′ - ATTCCGATAACGAACGAGACT - 3′) and reverse primer IR8 (5′ - GCTAGCTGCGTTCTTCATCGA-3′) (Šimková et al. 2013). For the 28S rDNA region, the forward primer C1 (5′ - ACCCGCTGAATTTAAGCA - 3′) and reverse primer D2 (5′ - TGGTCCGTGTTTCAAGAC - 3′) were used (Hassouna et al. 1984). A PCR mix of a total volume of 25 µl, was prepared by mixing 10.5 µl of the master mix with 7.5 µl of Milli-Q pure water, 2 µl of forward primer, 2 µl reverse primer and 3 µl of the DNA. Amplification of the partial 18S and entire ITS1 regions were done according to the cycling profile: denaturation at 95°C for 4 minutes, 35 cycles of amplification (95°C for 1 minute, 55°C for 1:30, and 72°C for 1:30), and 10 minutes extension hold at 72°C. For the 28S region, the cycling profile was: 94°C for 4 minutes, 40 cycles of amplification (94°C for 20 seconds, 56°C for 30 seconds, and 72°C for 1:30), and 10 minutes extension hold at 72°C following the protocol of Benovics et al. (2021) and Molokomme et al. (2023). The PCR products were visualized on a 1% agarose gel. Positive DNA samples were sent to Inqaba Biotechnical Industries (Pty)

Ltd for sequencing in both directions. The quality and chromatogram of the sequences were generated and analysed using Geneious v. 7.1.3 (Kearse et al. 2012).

5.2.3 Alignment of sequences and phylogeny estimation

Newly assembled sequences were submitted to a BLAST search in GenBank to find similar sequences. Sequences that were included in alignment for phylogenetic analysis were selected based on their similarities to the current study's sequences, i.e., their coverage and percentage identity as displayed on GenBank. Alignment and trimming of sequences were done using the MEGA11 software (Tamura et al. 2021). The MUSCLE tool from MEGA11 was used for aligning sequences. Before analysis the model test selection was performed in MEGA11. Phylogenies were erected through Bayesian inference using MrBayes 3.2 (Ronquist et al. 2012), Maximum likelihood using PhyML 3.0 (Guindon et al. 2010) and neighbour joining using MEGA 11 (Tamura et al. 2021). Genetic differences between sequences in phylogenies were calculated using uncorrected p-distances in MEGA 11 (Tamura et al. 2021).

5.3 Results

The DNA was successfully extracted and the 18S rDNA segments successfully amplified from a single specimen of *D. lampam* collected from *B. schwanenfeldii*, four specimens of *Dactylogyrus* spp. (*D. baueri* and *D. intermedius*) collected from *C. auratus*, six specimens of Ancryocephalidae n. gen., five specimens of *U. sinus* and four specimens of *T. caecus*. However, not all the extracted DNA from these monogenean specimens had positive PCR results (see Table 5.1). For the majority of samples analysed, the resultant PCR bands were of poor quality providing ambiguous results (see Figure 5.1).

Table 5.1: Number of specimens from which DNA was extracted with the PCR results for each monogenean species.

Monogenean species	**Number of specimens**	**PCR**
Dactylogyrus lampam	1	Negative
Dactylogyrus spp.	4	All positive
Ancyrocephalidae n. gen.	6	All positive
Urocleidoides sinus	5	Only 3 positive
Thaparocleidus caecus	4	All positive

All DNA extractions for *Dactylogyrus* spp. collected from *C. auratus,* Ancyrocephalidae n. gen. and *T. caecus* had a positive PCR. The PCR was unsuccessful for *D. lampam* collected from *B. schwanenfeldii.* Of the five DNA extractions for *U. sinus*, three had a positive PCR result, but the sequencing occurred only in a good quality sequence in the forward direction. The resulting DNA strands post-PCR are displayed in Figure 5.1.

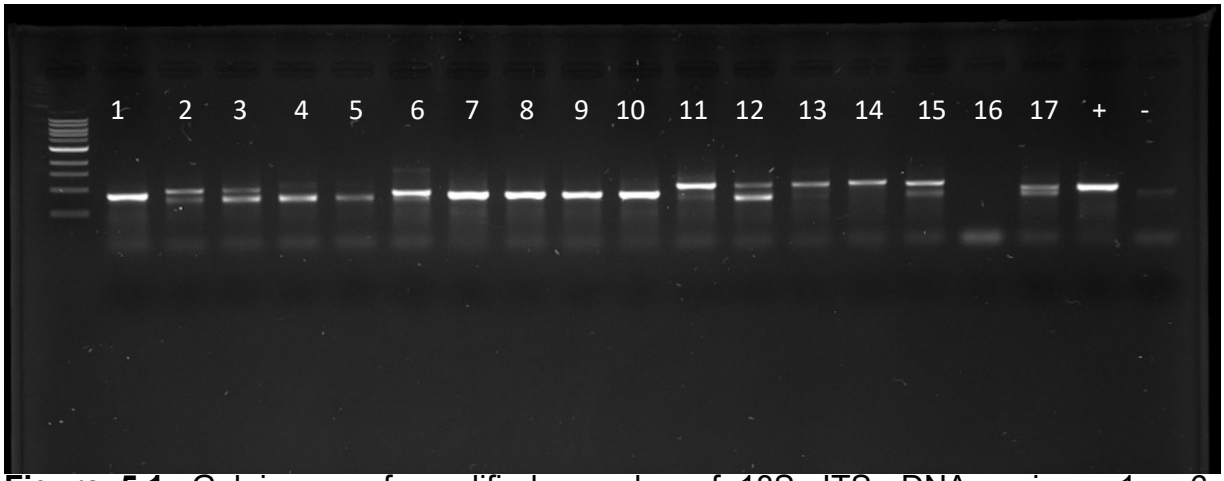

Figure 5.1: Gel image of amplified samples of 18S, ITS rDNA regions, 1 – 6 Ancyrocephalidae n. gen. ex *Glossolepis incisus*, 7 – 10 *Thaparocleidus caecus* ex *Pangasianodon hypophthalmus*, 11 – 14 *Dactylogyrus* spp. ex *Carassius auratus*, 15 – 17 *Urocleidoides sinus* ex *Metynnis hypsauchen*. Positive control = high quality previously sequenced Dactylogyridae DNA, negative control = distilled water.

Thaparocleidus caecus had the highest quality of distinct bands, with all extractions providing readable results that revealed base lengths to be similar. Ancyrocephalidae n. gen. had only two good bands that were similar in length. A single clear band was observed for *Dactylogyrus* spp. infecting *C. auratus*. The 28S rDNA segments were also amplified. The 18S, ITS1 and 28S rDNA segments were then sequenced and the summary of the success in sequencing of these regions is shown in Table 5.2.

Table 5.2: Summative results for DNA sequencing for 18S ITS1 and 28S DNA regions.

Species	DNA Extraction	DNA Sequencing		Action Needed
		18S rDNA	28S rDNA	
Dactylogyrus lampam	1	Unsuccessful	Unsuccessful	Repeat PCR
Dactylogyrus spp.	4	One partially sequenced	Not sequenced	Concentrate DNA, repeat PCR
Ancyrocephalidae n. gen.	6	One complete, three partially sequenced	One complete	Repeat PCR for 3 samples with partial sequence
Urocleidoides sinus	5	One partially sequenced	One partially sequenced	Concentrate DNA, repeat PCR
Thaparocleidus caecus	4	Completed	Completed	No action needed

Of the monogenean specimens, only *T. caecus* and Ancyrocephalidae n. gen. DNA extractions and PCRs led to the generation of at least a single complete DNA sequence for both 18S, ITS1 and 28S molecular makers. *Urocleidoides sinus* underwent partial sequencing for both molecular markers, whilst *Dactylogyrus* spp. collected from *C. auratus* underwent partial sequencing for the 18S, ITS1 segment, and the 28S segment was not sequenced. The PCRs will be repeated for DNA extractions that either partially generated or failed to generate DNA sequences. However, for the time being, only phylogenies including *T. caecus* and Ancyrocephalidae n. gen. were erected using the 18S, ITS1 and 28S molecular markers. Species selected from GenBank to infer phylogenies are listed in Table 5.3 for *T. caecus* 18S, ITS1 segment (794 bp), Table 5.5 for *T. caecus* 28S segment (758 bp), Table 5.7 for Ancyrocephalidae n. gen. 18S, ITS1 segment (861 bp), and Table 5.9 for Ancyrocephalidae n. gen. 28S segment (748 bp).

Table 5.3: List of parasite sequences, along with their accession numbers and hosts, included in the phylogenetic comparison of *Thaparocleidus* spp. based on 18S, ITS1 rDNA regions. *Thaparocleidus caecus* sequence obtained in the current study is in bold.

Host Species	Parasite sequence and accession number	Country
Pangasianodon hypophthalmus (Sauvage, 1878)	***Thaparocleidus caecus***	Sri Lanka (commercially obtained)
	FJ493152.1 *T. caecus*	Borneo (Malaysia, Brunei and predominantly Indonesia)
	MK464252.1 *T. caecus*	Singapore (commercially obtained)
Pangasius microneme Bleeker, 1850	FJ493147.1 *T. sinespinae*	Indonesia, Musi River
	FJ493165.1 *T. lebrunae*	Indonesia, Batang Hari River
Pangasius nasutus (Bleeker, 1863)	FJ493153.1 *T. caecus*	Borneo
	FJ493145.1 *T. citreum*	Indonesia, Musi River
Pangasius polyuranodon Bleeker, 1852	FJ493159.1 *T. turbinatio*	Indonesia, Batang Hari River
	FJ493160.1 *T. legendrei*	
	FJ493158.1 *T. levangi*	
Pangasius djambal Bleeker, 1846	FJ493154.1 *T. komarudini*	
	FJ493155.1 *T. combesi*	
Siluru sasotus Linnaeus, 1758	OP394151.1 *T. magnicirrus*	
	MK271093.1 *Thaparocleidu*s sp.	China
-	MG601540.1 *T. asoti*	
Bagrus docmak (Fabricius, 1775)	KX713993.1 *Quadriacanthus bagrae*	Sudan

Note:(-) denotes an unknown host due to a parasite sequence from an unpublished source being directly submitted to GenBank

Fourteen *Thaparocleidus* spp. specimens from six known fish hosts were selected to be included in the phylogenetic analysis. *Thaparocleidus* spp. were sourced from over six countries, with the majority of the specimens being from Indonesia. As observed from Table 5.3 above, the current study's *T. caecus* sequence was collected from the same host as FJ493152.1. *T. caecus* and MK464252.1. *T. caecus*, that being, *P. hypophthalmus.* However, the sequence FJ493153.1. *T. caecus* was collected from a different host, that being*, P. nasutus.* The phylogeny construed using the selected *Thaparocleidus* spp. is shown in Figure 5.2.

The phylogram revealed two lineages, lineage A consisting of *Thaparocleidus* spp. from fish species from the family Siluridae and lineage B infecting fish from the family Pangasiidae. Within lineages B, are clusters b1 and b2. Cluster b1 consists of *Thaparocleidus* spp. that were retrieved from Indonesia only, whilst cluster b2 consists of *T. caecus* specimens*,* and *T. spinespinae,* with the former being collected from Sri Lanka, Borneo, and Singapore respectively, and the latter collected from Indonesia. *Thaparocleidus* spp. sister taxa included in the phylogram did not form sister groupings according to their country of origin, or host species. The sister grouping of *T. citreum* and *T. caecus* cl 2 is the only one whereby the taxa involved infect the same host species.

Thaparocleidus caecus specimens extracted from *P. nasutus* by Šimková et al. (2013) (FJ493153 *T. caecus*), *P. hypophthalmus* by Taner et al. (2022) (MK464252 *T. caecus*) and in the current study were not clustered with *T. caecus* extracted from *P. hypophthalmus* (FJ493152 *T. caecus* cl 2) by Šimková et al. (2013). The genetic distances between these *Thaparocleidus* spp. are shown in Table 5.4.

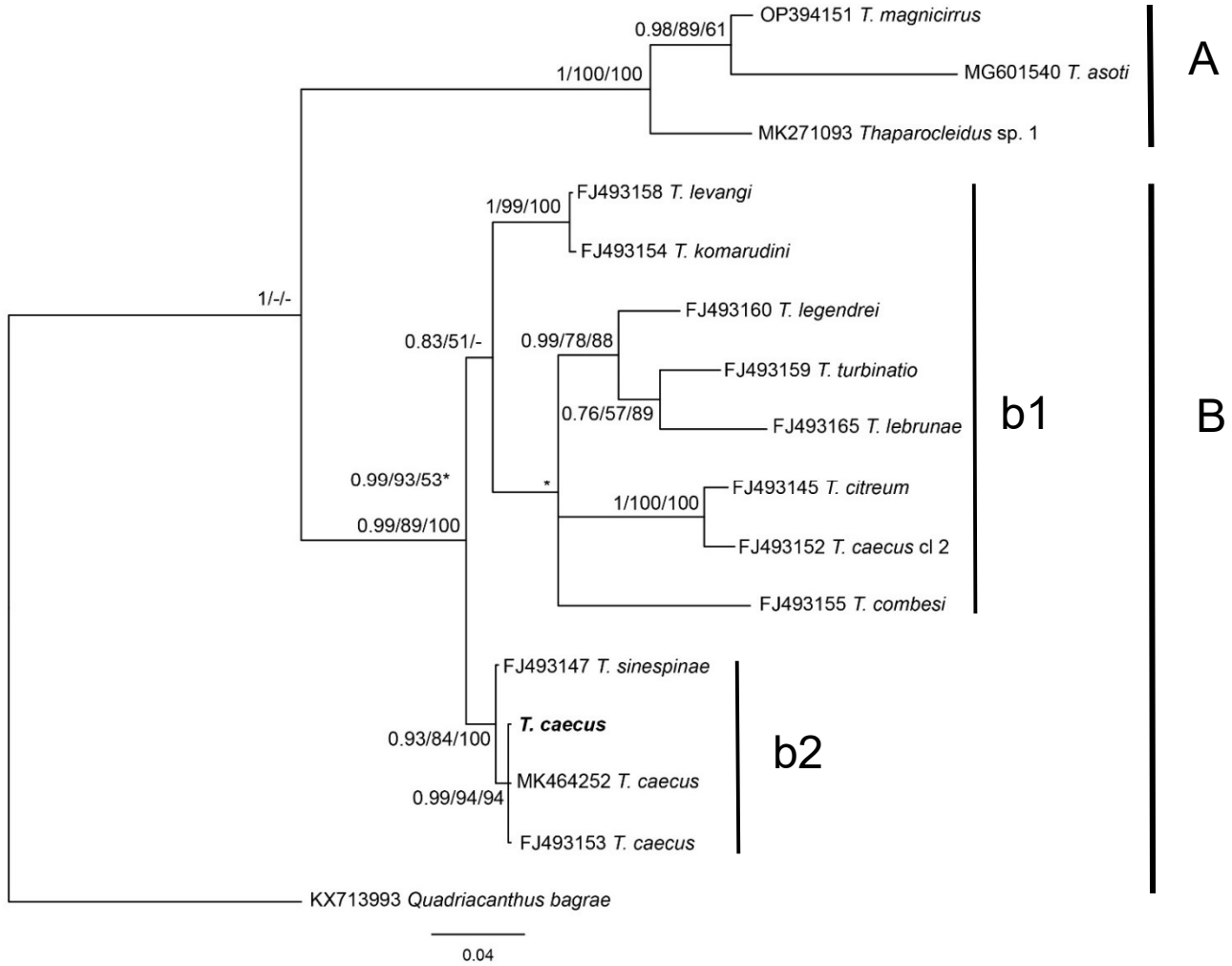

Figure 5.2: Bayesian inference consensus phylogram of *Thaparocleidus caecus* sequenced in the current study and *Thaparocleidus* spp. selected from GenBank. Consensus phylogram is generated based on 782bp long18S rDNA, ITS1 sequences alignment. Posterior probabilities for Bayesian inference, followed by bootstrap values for Maximum likelihood and Neighbour joining respectively are displayed behind branches. *Thaparocleidus caecus* sequenced in the current study is in bold. *Quadriacanthus bagrae* was selected as an outgroup.

Table 5.4: Uncorrected pairwise distances (p-distances) between *Thaparocleidus* spp. sequences included in the phylogeny inferred based on a 782 bp long18S rDNA, ITS sequences alignment. P-distances are in percentage. The current study's *Thaparocleidus caecus* is in bold.

Species	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**
1. T. caecus															
2. *T. caecus*	0.0														
3. *T. caecus*	0.0	0.0													
4. *T. sinespinae*	0.5	0.5	0.5												
5. *T. levangi*	5.3	5.3	5.3	4.8											
6. *T. komarudini*	5.4	5.4	5.5	5.5	0.2										
7. *T. legendrei*	6.7	6.7	6.5	6.2	8.1	8.3									
8. *T. citreum*	9.3	9.3	9.2	9.1	10.2	10.4	10.5								
9. *T. turbinatio*	9.5	9.5	9.3	9.3	9.7	9.8	5.4	9.9							
10. *T. caecus* cl 2	9.6	9.6	9.4	9.4	10.2	10.4	11.0	2.2	10.8						
11. *T. lebrunae*	10.2	10.2	10.1	9.8	10.1	10.3	7.3	10.2	6.0	10.6					
12. *T. magnicirrus*	16.9	17.2	17.0	16.7	16.7	16.9	18.2	18.6	18.8	18.0	18.3				
13. *Thaparocleidus* sp.	16.4	16.6	16.5	16.6	17.3	17.4	18.0	18.1	18.8	17.7	18.1	5.9			
14. *T. combesi*	10.7	10.7	10.6	10.2	10.0	10.1	10.1	11.1	10.9	11.4	10.7	19.8	19.2		
15. *T. asoti*	18.1	18.2	18.1	18.3	18.7	18.9	20.1	21.7	20.6	20.4	20.5	7.4	10.2	21.3	
16. *Q. bagrae*	18.2	18.4	18.4	17.9	18.5	18.8	19.8	21.3	20.1	21.1	21.7	20.2	20.4	20.7	22.2

Thaparocleidus caecus in the current study had a smallest -to- largest p-distance range of 16.4 – 18.1 % against species clustered in the lineage A, 5.3 – 10.7% against species in the cluster b1, and 0 – 0.5 % between species forming cluster b2 (see Figure 5.2). As observed in Table 5.4, *T. caecus* (ex *P. hypophthalmus*) from the current study, *T. caecus* (accession K464252 ex *P. hypophthalmus*) from Taner et al. (2022), and *T. caecus* (accession FJ493153 ex *P. nasutus*) from Šimková et al. (2013) were identical. *Thaparocleidus caecus* extracted from *P. hypothalamus* by Šimková et al. (2013) (Accession FJ493152) was, however, found to have a genetic distance of over 9% from the current study's *T. caecus*.

Table 5.5: List of parasite sequences, along with their accession numbers and hosts, included in the phylogenetic comparison of *Thaparocleidus* spp. based on 28S rDNA regions. The current study's *Thaparocleidus caecus* sequence is in bold.

Host species	Parasite sequence and accession number	Country
Pangasianodon hypophthalmus	***T. caecus***	Sri Lanka
	EF100546.1 *T. campylopterocirrus*	China
Pangasius sp.	KP056249.1 *Thaparocleidus* sp.	Czech Republic
Silurus asotus Linnaeus, 1758	OP453102.1 *T. magnicirrus*	China
	MK271090.1 *Thaparocleidus* sp.	
	EF100548.1 *T. infundibulovagina*	
	DQ157668.1 *T. varicus*	
	EF100547.1 *T. cochleavagina*	
Silurus glandis Linnaeus, 1758	AJ969940.1 *T. siluri*	Morava River, Central Europe
-	MG601547.1 *T. asoti*	China
Limnothris samiodon (Boulenger, 1906)	MH620705.1. *Kapentagyrus limnotrissae*	Zimbabwe

Note:(-) denotes an unknown host due to a parasite sequence from an unpublished source being directly submitted to GenBank.

For phylogenetic analysis using the 28S rDNA segment, 10 *Thaparocleidus* spp. collected from five known host species were selected. The majority of *Thaparocleidus* spp. were collected from the host species *Silurus asotus* Linnaeus, 1758. Selected *Thaparocleidus* spp. were sourced from over four countries, with the majority of monogenean specimens collected from China. The phylogeny inferred using the selected *Thaparocleidus* spp. is shown in Figure 5.3.

The phylogram revealed *Thaparocleidus* spp. to form two separate lineages. Lineage A, which consisted of taxa infecting fish species from the family Siluridae and lineage B consisting of taxa infecting fish species from the family Pangasiidae. Within lineage A are the clusters, a1 and a2. Cluster a1 is formed by *Thaparocleidus* spp. found to be infecting *S. asotus* only. Cluster a2 consisted of species infecting an unknown host, *S. asotus* and *Silurus glandis* Linnaeus, 1758. The country of origin of the monogeneans did not appear to affect the grouping. The majority of taxa forming sisters grouping were infecting *S. asotus*. The uncorrected p-distance between sequences included in the phylogram is shown in Table 5.6.

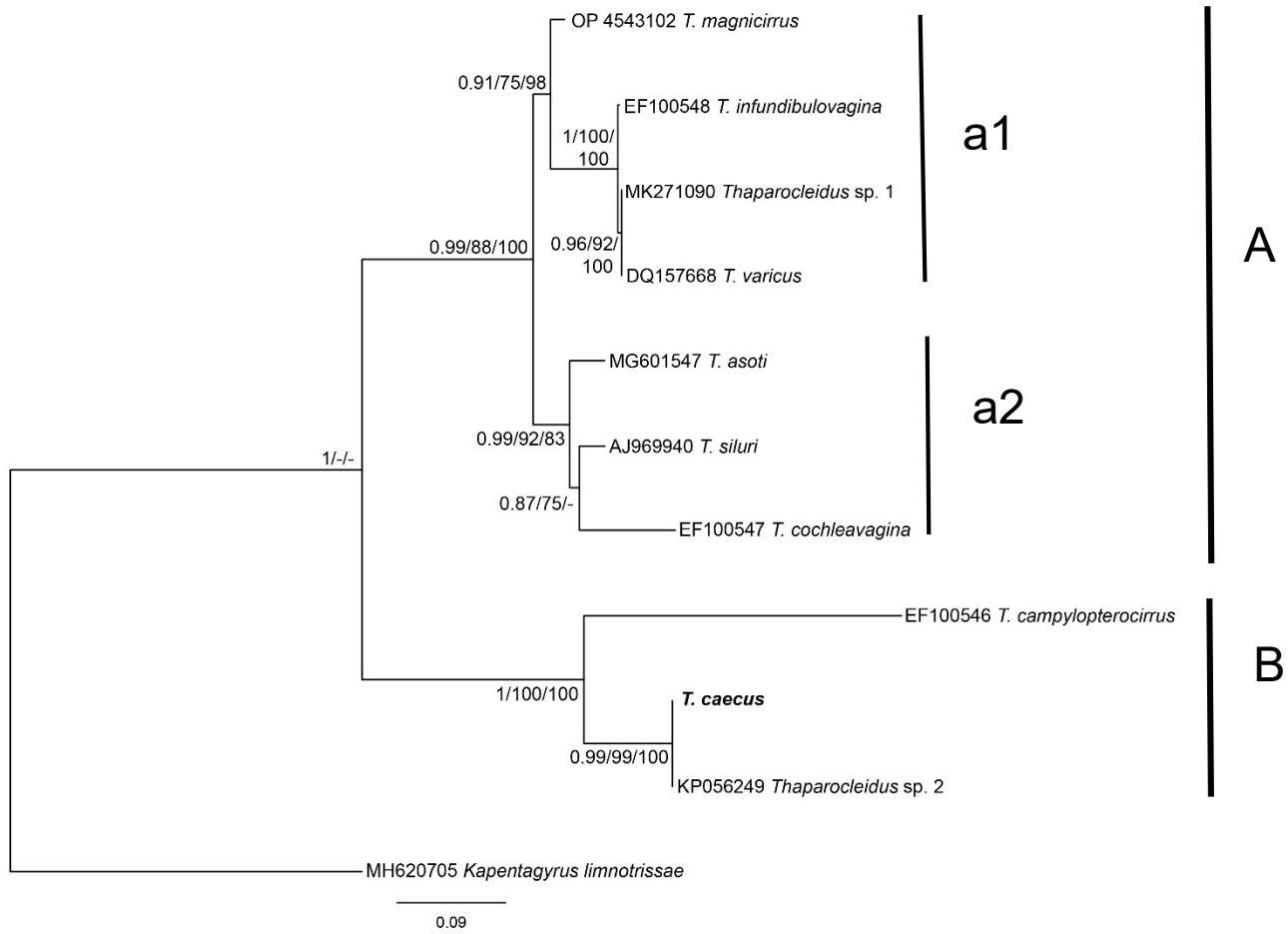

Figure 5.3: Bayesian inference consensus phylogram of *Thaparocleidus caecus* sequenced in the current study and *Thaparocleidus* spp. sequences selected from GenBank. Consensus phylogram is generated based on the 794bp-long alignment of 28S molecular sequences. Posterior probabilities for Bayesian inference, followed by bootstrap values for Maximum likelihood and Neighbour joining respectively are displayed behind branches. *Thaparocleidus caecus* sequenced in the current study is in bold. *Kapentagyrus limnotrissae* was selected as an outgroup.

Thaparocleidus caecus from the current study had a p-distance range of 23.4 – 23.7% against species from cluster a1, and a range of 24.2 – 26.8% against species from cluster a2 (see Figure 5.3). As observed in Table 5.6, the *T. caecus* sequence in the current study is identical to the unidentified *Thaparocleidus* sp. 2 (Accession KP056249.1). Moreover, the unidentified sequence of *Thaparocleidus* sp. (Accession MK271090) is also revealed to be identical to *Thaparocleidus varicus* Lim, 1996.

Table 5.6: Uncorrected pairwise distances (p-distances) between *Thaparocleidus* spp. sequences included in the phylogeny inferred based on a 794bp long 28S rDNA sequence alignment. P-distances are in percentage The current study's *T. caecus* is in bold.

Species	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
1. ***T. caecus***										
2. *Thaparocleidus* sp. 2	0.0									
3. *T. campylopterocirrus*	20.1	20.1								
4. *T. magnicirrus*	23.4	23.2	28.3							
5. *T. infundibulovagina*	23.4	23.3	28.5	6.3						
6. *Thaparocleidus* sp. 1	23.7	23.6	28.5	6.3	0.4					
7. *T. varicus*	23.7	23.6	28.5	6.3	0.4	0.0				
8. *T. siluri*	24.2	24.1	28.9	7.3	10.4	10.4	10.4			
9. *T. asoti*	24.8	24.7	28.3	7.4	10.9	10.6	10.6	5.3		
10. *T. cochleavagina*	26.8	26.7	30.0	11.2	13.5	13.3	13.3	8.6	9.5	
11. *K. limnotrissae*	30.9	31.0	33.6	29.1	31.0	31.3	31.3	29.9	30.1	30.3

Table 5.7: List of parasite sequences, along with their accession numbers and hosts, included in infering the phylogenetic relationship of Ancyrophelidae n. gen. to similar species based on 18S rDNA, ITS1 regions.

Host species	**Parasite sequence and accession number**	**Query cover (%)**	**Similarity (%)**	**Country**
Glossolepis incisus Weber, 1907	Ancyrophelidae n. gen.			Indonesia
Astronotus ocellatus (Agassiz, 1831)	ON368042.1 *Gussevia astronoti*	51	92.79	Peru
	ON368041.1 *Gussevia asota*	51	92.79	Peru
Carassius auratus (Linnaeus, 1758)	MZ358872.1 *Dactylogyrus*	51	92.52	India
Cichla monoculus Agassiz, 1831	ON368065.1 *Sciadicleithrum umbilicum*	50	92.45	Peru
Geophagus surinamensis (Bloch, 1791)	ON368064.1 *Sciadicleithrum* sp.	51	92.58	Colombia
Luciobarbus zayanensis Doadrio, Casal-Lopez and Perea, 2016	MW218671.1 *Dactylogyrus marocanus*	51	92.06	Morocco
Oreochromis mossambicus (Peters, 1852)	MN592985.1 *Enterogyrus mashegoi*	52	91.72	South Africa
	MN592986.1 *Enterogyrus multispiralis*	52	91.72	South Africa
Sarotherodon galilaeus (Linnaeus, 1758)	HE792790.1 *Cichlidogyrus halli*	51	91.80	Senegal
Tilapia guineensis (Bleeker, 1862)	HE792785.1 *Cichlidogyrus cubitus*	51	92.33	Senegal
Rhinogobius sp.	LC494518.1 *Gobioecetes biwaensis*			Japan

The species selected for phylogenetic analysis in Table 5.7 were those with the highest similarities to Ancyrocephalidae n. gen. identified on GenBank. However, all species found and selected were compared only to about half (query cover about 50%) of the 18S, ITS1 segment for Ancyrocephalidae n. gen. The eleven parasite sequences included in the phylogenetic analysis consist of *Gussevia* spp., *Dactylogyrus* spp., *Sciadicleithrum* spp*. Enterogyrus* spp*., Cichlidogyru*s spp., and *Gobiocetes biwaensi* and are based on a 748 bp long alignment. None of the selected parasite sequences were collected from the same host as Ancyrophelidae n. gen. MN592985.1. The phylogeny inferred using the selected sequences is shown in Figure 5.4.

The phylogenetic position of the newly found genus was not resolved. The only well-supported cluster found was that grouping *Gussevia* spp. and *Sciadicleithrium* spp., both parasites of South American cichlids. Ancyrocephalidae n. gen. was revealed to be a sister taxon to *Enterogyrus* spp. MN592985.1. *Enterogyrus mashegoi* Luus-Powell, Madanire-Moyo, Matla and Přikrylová, 2020, and MN592986.1. *Enterogyrus multispiralis* Luus-Powell, Madanire-Moyo, Matla and Přikrylová, 2020, were collected from the same host, *Oreochromis mossambicus* but with weak Bayesian inference support. The similarities in the shape of haptoral sclerites and the MCO also appeared not to mainly affect the grouping of the taxa. The specimens of the newly found Ancyrocephalidae genus have hooklets shaped most similar to *Cichlidogyrus* spp., but the position of the latter is not resolved. The p-distances between taxa included in the phylogram are shown in Table 5.8.

The monogenean species included in the phylogeny highly diverged from Ancyrocephalidae n. gen. with all species having a p-distance above 26% from the new monogenean genus. Ancyrocephalidae n. gen. was found to be most genetically similar to *Sciadicleithrum umbiliicum* Kritsky, Thatcher and Boeger, 1989 with a 26.9 % p-distance between the two monogenean species. Ancyrocephalidae n. gen. was found to be least similar to *Dactylogyrus marocanus* El Gharbi, Birgi and Lambert, 1994 with a p-distance of 30.9% between the two species. Selected taxa were also distant from each other, wherein, most selected taxa had a 20% plus p-distance between each other. Species that were closest to each other were *Gussevia astronoti* Kritsky, Thatcher and Boeger, 1989 and *Gussevia asota* Kritsky, Thatcher and Boeger, 1989 (2.5% p-distance), followed by *E. mashegoi* and *E. multispiralis* (9% p-distance).

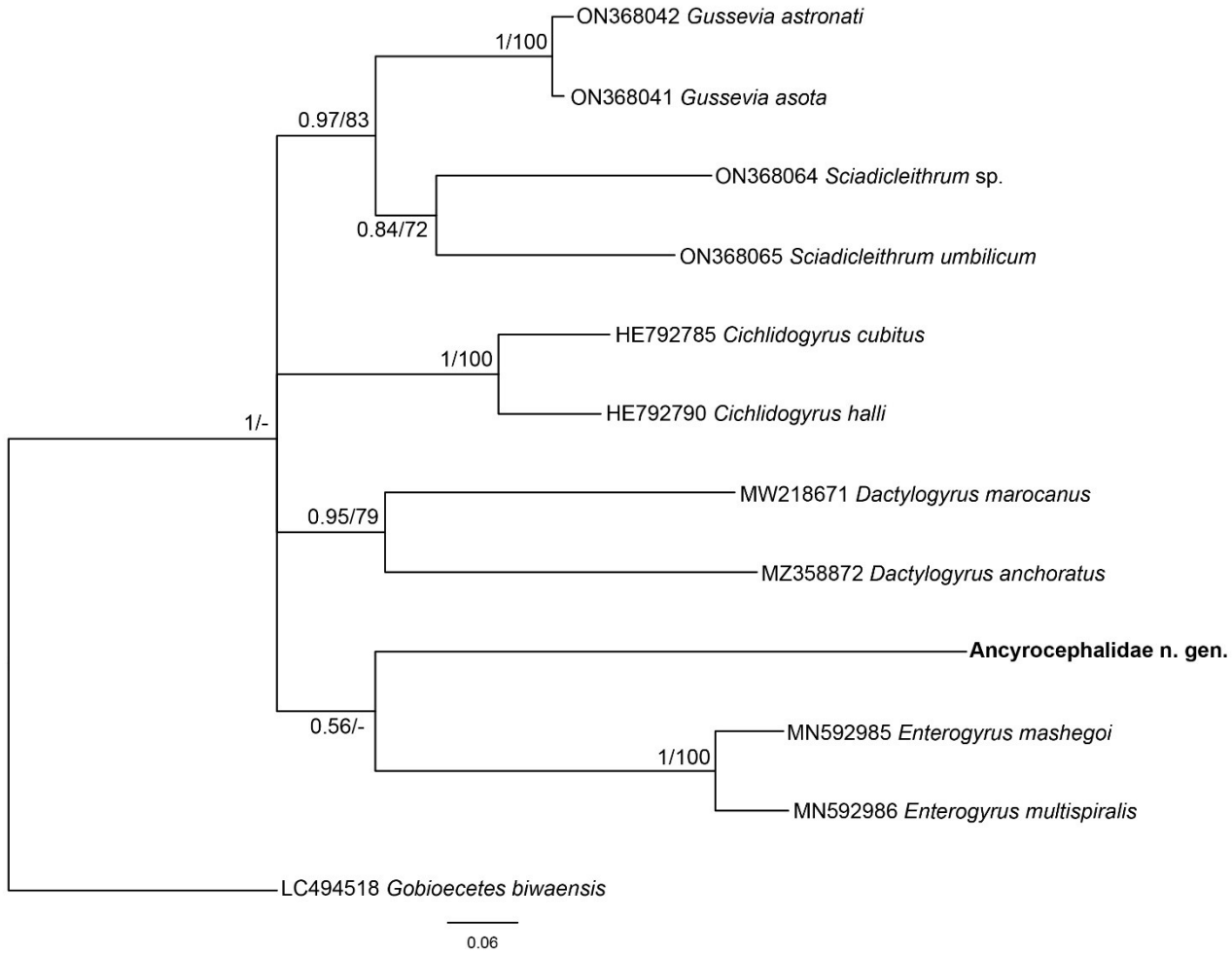

Figure 5.4: Bayesian inference consensus phylogram of Ancyrocephalidae n. gen. and taxa selected from GenBank. Consensus phylogram is generated based on 748 bp long 18S rDNA, ITS1 sequences. Posterior probabilities and bootstrap values for Bayesian inference and Maximum likelihood are respectively displayed behind branches. Ancyrocephalidae n. gen. is in bold. *Gobiocetes biwaensis* was selected as an outgroup.

Table 5.8: Uncorrected pairwise distances (p-distances) between Ancyrocephalidae n. gen. and sequences included in the phylogeny inferred based on a 748 bp long 18S rDNA, ITS1 sequence alignment. P-distances are in percentage. Ancyrocephalidae n. gen. is in bold.

Species	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
1. **Ancyrocephalidae n. gen.**											
2. *G. astronoti*	27.1										
3. *G. asota*	27.5	2.5									
4. *Sciadicleithrum* sp.	27.9	22.8	22.7								
5. *S. umbilicum*	26.9	21.3	21.0	21.8							
6. *C. cubitus*	27.9	24.7	24.2	27.4	26.1						
7. *D. marocanus*	30.9	26.5	27.0	28.6	27.4	26.4					
8. *Cichlidogyrus halli*	27.5	23.8	23.8	25.0	26.5	12.7	27.9				
9. *E. mashegoi*	27.2	26.1	25.5	28.4	28.8	26.1	29.0	27.0			
10. *E. multispiralis*	27.0	27.1	26.5	28.2	28.7	26.5	28.8	27.9	9.0		
11. *D. anchoratus*	29.9	24.9	25.3	27.3	25.1	26.5	25.0	24.9	28.3	28.4	
12. *G. biwaensis*	28.6	26.9	28.0	29.7	29.1	27.8	27.6	28.7	27.2	29.1	27.4

Similar to the BLAST results for the 18S, ITS1 segment, only about 50% of the 28S segment for Ancyrocephalodae n. gen. could be compared with the selected species from GenBank (Table 5.9). Eleven most similar species could be found and included in the phylogenetic analysis based on a 748 bp long sequence alignment. These species consisted of *Ligophorus* spp., *Scutogyrus* spp., *Cichlidogyru*s spp., and *Gobiocetes biwaensis* Ogawa and Itoh, 2017. None of the selected parasite sequences were collected from the same host and country as Ancyrophelidae n. gen. All the selected *Ligophorus* spp. were collected from one host, *Moolgarda buchanani* (Bleeker, 1853), and from the same country, Malaysia. All *Cichlidogyrus* spp. were collected from host species of the genus *Oreochromis* Günther, 1889, but sourced from different countries. *Scutogyrus* spp. were collected from different species. The phylogeny inferred using the selected sequences is shown in Figure 5.5.

The phylogeny revealed two lineages, lineage A consisting of *Ligophorus* spp. clustered with Ancyrocephalidae n. gen. and lineage B consisting of a cluster of *Cichlidogyrus* spp. (cluster b1), and a cluster of *Scutogyrus* spp. (cluster b2). *Ligophorus* spp. were clustered together with all these species infecting *M. buchanani.* All *Cichlidogyrus* spp. infected *Oreochromis* spp. and *Scutogyrus* spp. were clustered together with these specimens having being sourced from African cichlids, with *Scutogyrus vanhovei* Pariselle, Nyom and Bilong, 2013 collected from Cameroon and *S. minus* collected from Côte d'Ivoire. The p-distances between the 28S rDNA sequences are shown in Table 5.10.

Ancyrocephalidae n. gen. was found to have highly diverged from the selected taxa. The p-distance between Ancyrocephalidae n. gen. and *Ligophorus* spp. ranged from 26.5% to 28.4%. A p-distance of 28.7% was observed between Ancyrocephalidae n. gen. and *Cichlidogyrus* spp., and a p-distance range of 29.3 – 29.5% from *Scutogyrus* spp. All the selected *Cichlidogyrus mbirizei* Muterezi Bukinga, Vanhove, Van Steenberge and Pariselle, 2012 specimens were identical to *Cichlidogyrus* sp. (accession DQ537367).

Table 5.9: List of parasite sequences, along with their accession numbers and hosts, included in infering the phylogenetic relationship of Ancyrophelidae n. gen. to similar species based on 28S rDNA regions.

Host	Parasite sequence and accession number	Query cover (%)	Similarity(%)	Country
Glossolepis incisus	Ancyrocephalidae n. gen.			Indonesia
Moolgarda buchanani (Bleeker, 1853)	KM221920.1. *Ligophorus navjotsodhii*	52	86.82	Malaysia
	KM221912.1. *Ligophorus chelatus*	52	86.82	
	KM221910.1. *Ligophorus belanaki*	52	86.57	
	KM221914.1. *Ligophorus funnelus*	52	87.06	
Oreochromis aureus (Steindachner, 1864)	MW580331.1. *Cichlidogyrus cirratus*	53	86.95	Belgium
Oreochromis mossambicus (Peters, 1852)	MT994741.1. *Cichlidogyrus mbirizei*	53	86.95	China
Oreochromis niloticus (Linnaeus, 1758)	MN078042.1. *Cichlidogyrus mbirizei*	52	87.15	
	DQ537367.1. *Cichlidogyrus* sp.	53	86.95	
Pelmatolapia mariae (Boulenger, 1899)	MW580366.1. *Scutogyrus vanhovei*	53	86.24	Cameroon
Sarotherodon melanotheron (Rüppell, 1852)	MW580365.1. *Scutogyrus minus*	53	86.27	Côte d'Ivoire
Limnothrissa miodon (Boulenger, 1906)	MH071783. *Kapentagyrus tanganicanus*			Democratic Republic of the Congo

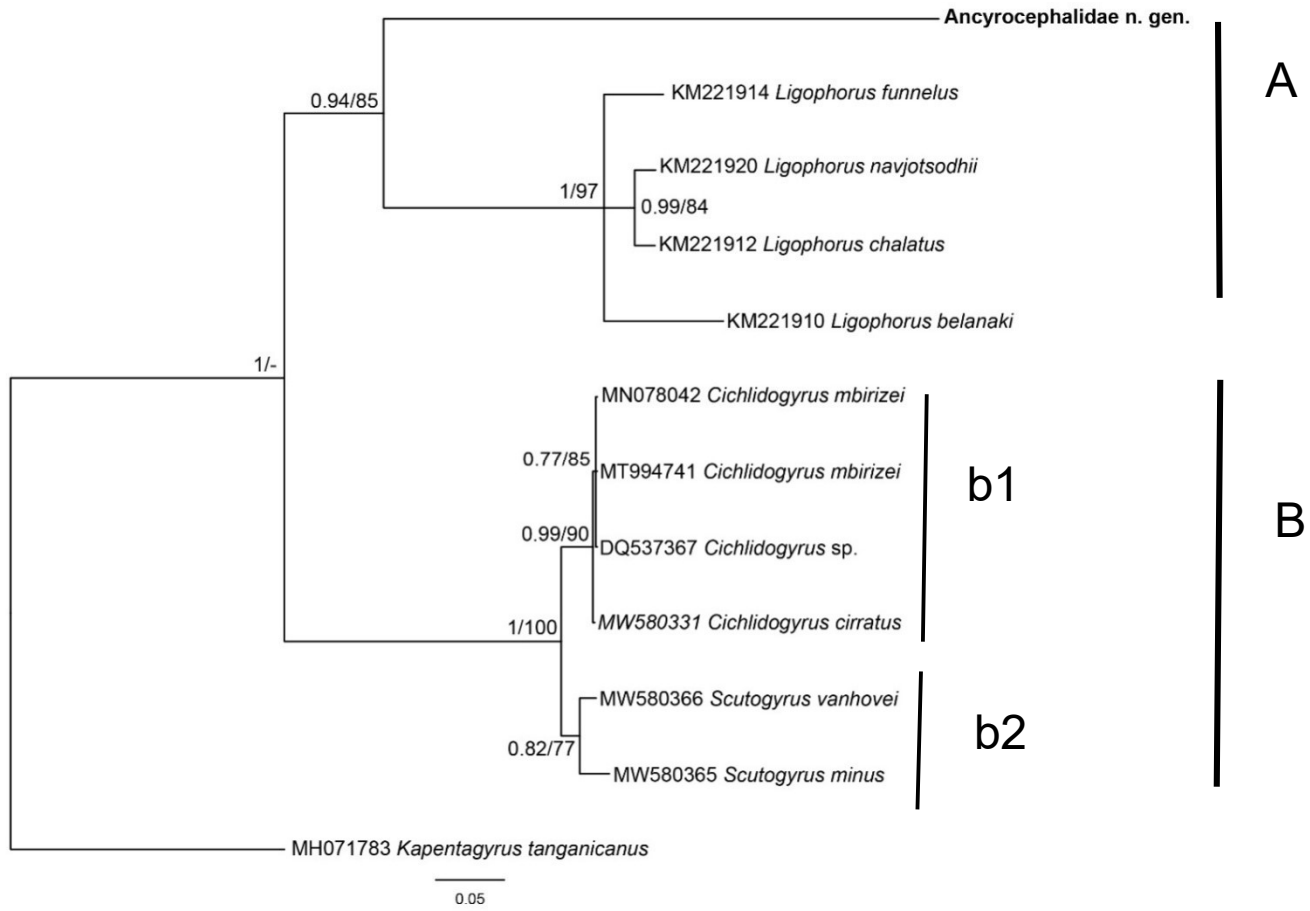

Figure 5.5: Bayesian inference consensus phylogram of Ancyrocephalidae n. gen. and taxa selected from GenBank. Consensus phylogram is generated based on 748 bp long 28S molecular sequencesr. Posterior probabilities and bootstrap values for Bayesian inference and Maximum likelihood respectively are displayed behind branches. Ancyrocephalidae n. gen. is in bold. *Kapentagyrus tanganicanus* was selected as an outgroup.

Table 5.10: Uncorrected pairwise distances (p-distances) between Ancyrocephalidae n. gen. and sequences included in phylogeny inferred based on a 748 bp long 28S rDNA sequence alignment. P-distances are in percentage. Ancyrocephalidae n. gen. is in bold.

Species	1	2	3	4	5	6	7	8	9	10	11
1. **Ancyrocephalidae n. gen.**											
2. *C. mbirizei*1	28.7										
3. *C. cirratus*	28.7	0.1									
4. *C. mbirizei 2*	28.7	0.0	0.1								
5. *Cichlidogyrus* sp.	28.7	0.0	0.1	0.0							
6. *L.. funnelus*	27.3	24.9	24.8	24.9	24.9						
7. *L.. navjotsodhii*	26.8	25.1	24.9	25.1	25.1	7.1					
8. *L.. chelatus*	26.5	24.7	24.5	24.7	24.7	6.8	2.6				
9. *L.. belanaki*	28.4	24.5	24.4	24.5	24.5	11.2	9.5	9.8			
10. *S. vanhovei*	29.5	4.1	4.1	4.1	4.1	24.7	24.5	24.1	25.6		
11. *S. minus*	29.3	4.8	4.8	4.8	4.8	24.6	25.0	24.6	25.4	3.0	
12. *K. tanganicanus*	33.1	27.2	27.1	27.2	27.2	28.2	28.6	29.1	29.7	26.9	27.7

5.4 Discussion

Monogenean species often share very similar morphological features, and therefore to confirm their identity, a molecular approach was utilised in the current study. The PCR was unsuccessful for *D. lampam*, and some specimens of *U. sinus.* All specimens for *Dactylogyrus* spp., Ancyrocephalidae n. gen., and *T. caecus* had positive PCR results, however, most of the generated segments were of poor quality (Figure 5.1). These unfavourable results could be due to PCR inhibitors. From the outset of utilising the PCR, PCR inhibitors have been a barrier to the completion or even the commencement of the reaction (Bessetti 2007). Well known PCR inhibitors include dust particles, cells, and tiny pieces of fabric (Tsai and Olson 1992; Shutler et al. 1999; Kim et al. 2001). In the current study, precautionary measures against PCR inhibitors were, however, put in place. Firstly, DNA extractions and amplifications were done in clean and sterilized lab conditions to avoid DNA contamination. Furthermore, using DNeasy® Blood and Tissue kit – QIAGEN, the DNA was purified from potential contaminants such as cells, blood, and bacteria. However, DNA purifying reagents have also been shown to act as PCR inhibitors themselves when in excess (Bessetti 2007), as these reagents include sodium chloride, sodium deoxycholate, and ethanol (Weyant et al. 1990; Loffert 1997).

The current study successfully sequenced 18S, ITS1 and 28S rDNA regions of *T. caecus* collected from *P. hypophthalmus. Thaparocleidus* Jain, 1952 is one of the most species-rich genera in the family Dactylogyridae Bychowsky, 1933 (Lim et al. 2001; Wu et al. 2008; Verma et al. 2016) with over a 100 *Thaparocleidus* spp. having been described (Verma et al. 2017). Nonetheless, only 38 and 45 sequences for 18S, ITS and 28S rDNA sequences are available on GenBank respectively. To date, only the studies by Šimková et al. (2013) and Chaudhary et al. (2014) have included *T. caecus* in phylogenies of *Thaparocleidus* spp. using the 18S, ITS1 segment as a marker, *T. caecus* extracted from *P. nasutus* by Šimková et al. (2013) (FJ493153 *T. caecus*) and *T. caecus* from *P. hypophthalmus* by Taner et al. (2022) (MK464252 *T. caecus*) and in the current study were found to be identical. *Thaparocleidus caecus* extracted from *P. hypophthalmus* (FJ493152 *T. Caecus* cl 2) by Šimková et al. (2013) was, however, found to be genetically different from the other *T. caecus* individuals (p-distance = 9.6%). As a consequence, the 18S, ITS1 inferred phylogeny also revealed *T. caecus* from the current study, MK464252 *T. caecus,* and FJ493153 *T. caecus* to be the same

species, with FJ493152 *T. caecus* cl 2 representing another species. Cases are available whereby a cryptic monogenean species was discovered due to observed genetic variation between parasite individuals initially regarded as conspecifics (Razo-Mendivil et al. 2016; Baeza et al. 2019). Therefore, there is a need to re-evaluate the morphometry of the type-material of *T. caecus,* so as to establish which morphotype in connection to genotype represents the species *sensu stricto* and which would be a new species of high morphological similarity to *T. caecus*.

The current study also presents the first DNA sequencing of 18S, ITS and 28S rDNA segments of monogenean parasites collected from the host species *G. incisus.* The collected parasites represented new species of a new genus, herein referred to as Ancyrocephalidae n. gen. for convenience. The haptoral sclerites of Ancyrocephalidae n. gen. (see Chapter 4, Figure 4.5), hooks in particular, are morphologically similar to those of *Cichlidogyrus* spp. (Douëllou 1993; Pariselle and Euzet 1994; Geraerts et al. 2020). However, phylogenetic analysis using the 18S, ITS1 segment revealed the new monogenean genus to be a sister taxon to *Enterogyrus* spp., but this grouping was not well supported and the position of *Cichlidogyrus* spp. remained unresolved. Using the 28S rDNA marker, the inferred phylogram revealed Ancyrocephalidae n. gen. to be a sister taxon to *Ligophorus* spp., and this grouping is well-supported. The current study also confirms the identity of *Cichlidogyrus* sp. (listed as DQ537367.1. *Cichlidogyrus* sp. on GenBank) collected from *O. niloticus* by Ek-Huchim et al. (2012) to be *C. mbirizei* due to there being a 0% p-distance between sequences of *C. mbirizei* and *Cichlidogyrus* sp. as seen in Table 5.10. The p-distances calculated using 18S, ITS1 and 28S regions revealed the Ancyrocephalidae n. gen. to be genetically distant from sequences that could be found on the GenBank database. This could be due to undiscovered parasite species as fish have been shown to harbour new and undiscovered parasite species (Jogunoori et al. 2004; Benovics et al. 2017; Benovics et al. 2018; Benovics et al. 2020).

5.5 Conclusion

To confirm the identity of monogeneans collected from selected ornamental fish species, the current study successfully amplified and sequenced the 18S, ITS1 and 28S rDNA segments for *T. caecus* and Ancyrocephalidae n. gen. The current study found that there were genetic differences between monogenean specimens, that when using existing literature are both morphologically identified as *T. caecus.* The results

of the current study therefore revealed the need to re-evaluate morphological features that identify *T. caecus,* so as to distinguish between *T. caecus* and the species sharing high morphological similarities with *T. caecus.* This study, therefore, also infer morphometric identifications to not be sufficient to identity monogeneans to species level. Ancyrocephalidae n. gen. could not be assigned to any currently known genera and genetically it is very distant from any species uploaded on the GenBank database. With such findings, the current study confidently confirms this parasite to be a new species of a new genus. The current study therefore presents the first 18S, ITS1 and 28S rDNA sequences for this new species. There was also a lack of parasite sequences that had similarities to Ancyrocephalidae n. gen., either due to similar species not being yet discovered, or due to the sequences not being available on GenBank. The current study therefore acknowledges the importance of intensifying the availability of more 18S, ITS1 and 28S sequences on the GenBank database to allow for more accurate phylogenies. The current study also promotes the study of ornamental fish parasites, which can be a good source of discovering new parasite species, which will then be sequenced and uploaded to GenBank to assist in providing more accurate phylogenetic relationships between parasites.

Chapter 6: Final discussion and recommendations

To determine the effectiveness of Sri Lanka's pre-export parasite treatments, one of the aims of the current study was to identify and investigate the prevalence, mean intensity, and composition of parasites from various ornamental fish species imported into South Africa from fish suppliers in Sri Lanka. Of the ten ornamental fish species imported from Sri Lanka and examined in the current study only five were infected by parasites. These were *C. auratus, B. schwanenfeldii, P. hypophthalmus, M. hypsauchen* and *H. plecostomus*. The parasites collected from these fish species belonged to the groups Branchiura and Monogenea. Branchiura was represented by a single species and a single specimen, with Monogenea being represented by eight species belonging to five genera. Except for *C. auratus,* all the fish species examined from Sri Lanka were infected by a single species of monogenean, whilst *C. auratus* was infected by four monogenean species and a branchiuran.

For alien ichthyoparasite species to successfully invade a new habitat, the fish host must survive and overcome environmental barriers (Lymbery et al. 2014). In South Africa surface water temperatures fluctuate according to season and altitude with the pH of most freshwater systems ranging between 6 – 8 (Day et al. 1998; Dallas 2008). The fish imported and examined in this study were all tropical species and it is therefore unlikely that they would survive in local waters during colder periods if introduced. Moreover, to survive and propagate in a foreign environment alien parasites should have the ability to infect new fish hosts (Lymbery et al. 2014) with those parasites that have a low host specificity having an advantage over parasites with a high host specificity (Lymbery et al. 2014).

The branchiuran collected from *C. auratus* in the current study was identified to be *A. japonicus*, a parasite with low host specificity that has already been reported in South Africa and other countries worldwide (Kruger et al. 1983; Alsarakibi et al. 2014). In South Africa *A. japonicus* has been reported to infect indigenous fish species such as *Clarias gariepinus* Burchell, 1822 and *Labeo rosae* Steindachner, 1894 (Kruger et al. 1983; Avenant-Oldewage 1994). Also collected from *C. auratus* were the monogeneans *Dactylogyrus baueri* and *D. intermedius.* Gill monogeneans are generally specific to their hosts (Kritsky and Heckmann 2002) and thus, compared to other groups of parasites, they pose a lower risk of invasion in South Africa. However, gill infecting *Dactylogyrus* spp. such as *Dactylogyrus vastator* Nybelin, 1924,

Dactylogyrus formosus Kulwiec, 1927 and *Dactylogyrus anchoratus* (Dujardin, 1845) and recorded in the current study *D. baueri* and *D. intermedius* have been reported to switch between different cyprinid hosts (Kritsky and Heckmann 2002; Trujillo-González et al. 2018a). *Dactylogyrus baueri* and *D. intermedius* have been reported to contribute to mass mortalities of *Cyprinus rubrofuscus* Lacépède, 1803 (Kritsky and Heckmann 2002), and thus could pose a threat to indigenous cyprinids of the genus *Labeo* Cuvier, 1816. Moreover, the gill monogenean *D. lampam* collected from *B. schwanenfeldii* in the current study has been recorded to infect different cyprinids in southern Asia (Lim 1992; Mohanta and Chandra 2000) and thus, has the potential to spill over to South African cyprinids. Conversely, monogeneans that generally infect the surface area of fish, especially the fins such as *Gyrodactylus* spp., tend to have a low host-specificity (Harris et al. 2004). As a consequence, *G. gurleyi* and *G. kobayashii* recorded in the current study pose a potential risk for invasion. However, these monogeneans have only been found infecting a few *Carassius* spp. (Daghigh Roohi et al. 2014).

Of concern is that the monogenean *T. caecus* has been reported to infect only two fish species, these being, *P. hypophthalmus* (Sauvage, 1878) and *Pangasius nasutus* (Bleeker, 1863) belonging to the family Pangasiidae in southern Asia (Šimková et al. 2013; Taner et al. 2022). With *T. caecus* displaying high host specificity, it can be assumed that the likelihood of this species spilling-over to infect South African indigenous fish species is low.

Fish such as *B. schwanenfeldii* are sensitive to environmental changes preferring temperatures of 22 – 25°C and a pH of 6.5 – 7.0 (Kottelat et al. 1993). As a consequence, this fish species along with its parasites are unlikely to survive should it be introduced under local conditions. Similarly, the same could be considered true for *P. hypophthalmus* which thrive under conditions of 22 – 26°C and a pH of 6.5 – 7.5 (Roberts and Vidthayanon 1991) and *M. hypsauchen* with a temperature preference of 24 – 28°C and a pH of 6.0 – 7.0 (Zarske and Géry 1999). In this study *M. hypsauchen* was infected by *U. sinus*, which has recently been described by Zago et al. (2020) and is a parasite that has also been recorded to infect *Leporinus tristriatus* Birindelli and Britski, 2013, *Schizodonnasutus* Kner, 1858, and *Schizodon intermedius* Garavello and Britski, 1990 belonging to the family Anostomidae (Zago et al. 2020). Hence, the current study presents the first report of *U. sinus* from *M. hypsauchen*

belonging to the family Serrasalmidae, which is a family indigenous to South America. With *U. sinus* reported to infect fish from two different families this species may potentially spill-over to infect indigenous fish species if introduced in local waters.

In this study the gill-infecting monogenean *H. heterotylus* was collected from *H. plecostomus,* a fish species that has a tolerance of 24 – 28°C and a pH of 6.2 – 8.2 (Baensch and Riehl 1985). To date *H. heterotylus* has only been found infecting South American catfish belonging to the genera *Hypostomus* Lacépède, 1803 and *Pterygoplichthys* Gill, 1858 from the family Loricariidae (Jogunoori et al. 2004; Nitta and Nagasawa 2013). Even though this species has been recorded to infect two fish genera, the current study assumes the likelihood of invasion by *H. heterotylus* to be low considering that this monogenean species has been found to only infect fishes belonging to the family Loricariidae, a family absent in local waters.

Even though the prevalence and mean intensity were more or less the same, the parasitic composition recorded from infected ornamental fishes examined in this study was lower than the findings in previous studies for the same fish species, an indication that fish suppliers from Sri Lanka treat fish against parasites. However, given that some of the fish examined were not completely free of parasites, the risk of their introduction and possible introduction and invasion into local systems still remains. Therefore, the current study recommends that custom authorities responsible for monitoring the import of live animals into the country should develop a protocol similar to that used by border control in other countries that inspect the health and condition of fish before admission. For example, South Africa could adopt and implement quarantine protocols similar to those implemented by the Australian government whereby, depending on the species, fish are quarantined for two to three weeks (Whittington and Chong 2007; Trujillo-González et al. 2019). A protocol proven to be effective at limiting the introduction of alien fish species and their ichthyoparasites (Whittington and Chong 2007; Trujillo-González et al. 2019). However, since fish euthanised post Australia's border control were found to have a high parasite composition in a study by Trujillo-González et al. (2018b), this shows that even with quarantine protocols in place, there is the possibility that parasites can enter undetected. As a consequence, it is recommended and encouraged that a pre-treatment against parasites for fish destined for South Africa be mandatory whereby on arrival a sample consisting of five fish specimens for a given species be examined

by a fish parasitologist. The number of five specimens is recommended due to the observations in the current study, whereby in most cases, it was observed that when the first five specimens were found without parasites, the rest of batch also had no parasites.

Given that physical examination requires the fish to be humanely killed, the use of environmental DNA (eDNA) is proposed as a non-invasive alternative to detecting parasites (Collins et al. 2013; Trujillo-González et al. 2019). For example, Trujillo-González et al. (2019) sampled water from a consignment of fish, and using eDNA analysis, detected *Dactylogyrus* spp. that evaded detection and protocols practiced by Australian authorities. A disadvantage of using eDNA, however, is that the procedure is costly and requires time for analyses.

With regard to host-parasite interactions, the red rainbowfish, *G. incisus*, was selected as a fish model based on the results recorded in a previous study by Lukhele (2021). In the current study the host-parasite interactions trial from initiation to the seventh week revealed that there were no significant changes in monogenean and nematode numbers. A drastic change was, however, observed in the final week (week nine) whereby monogenean numbers dwindled to zero and a mean intensity of 1.33 being recorded for nematodes. A reduction in parasite numbers was assumed to be due to a decrease in stocking density and water changes. Fish length was shown not to affect the parasite load of monogeneans and nematodes. In the wild, the nematode species recorded in the current study, i.e., *C. cotti* uses copepods as intermediate hosts to complete its life cycle, however, in aquarium conditions this species can directly infect its hosts but at a significantly lower fitness in terms of surviving to adulthood (Levsen and Jakobsen 2002) and may account for the downward trend in nematode numbers observed. It is suggested that a wide range of water parameters be measured in future studies to better explain possible interactions between water quality and fish host-parasite interactions. To limit fish mortalities, it is recommended that *G. incisus* be housed in spacious aquaria with water changes being made more frequently.

Given that monogenean species often share very similar features, molecular identification is thus needed to confirm their identification (Šimková and Morand 2008; Borisov 2013, Rahmouni et al. 2017). In the current study the 18S ITS1 and 28S ribosomal DNA segments of *T. caecus* and a new Ancyrocephalidae species referred

to as Ancyrocephalidae n. gen. were successfully sequenced. The *T. caecus* specimens collected in the current study had a genetic distance of 9.6% from *T. caecus* specimens collected by Šimková et al. (2013), moreover the 18S, ITS1 rDNA inferred phylogeny also revealed these *T. caecus* specimens to be in separate lineages. Based on the findings of this study it is recommended that the morphometry of the type-material of *T. caecus* be re-evaluated. This should be done to establish which species represents *T. caecus* and which would be a new species with high morphological similarity to *T. caecus*. Moreover, based on the findings in the current study the identification of monogeneans should always be confirmed genetically given that monogenean species can share nearly identical features. Ancyrocephalidae n. gen. was found to be genetically distant from the most similar DNA sequences of monogeneans that could be found on the GenBank database. This could be due to similar sequences not being uploaded to the GenBank database. However, this could also be due to monogeneans that are genetically similar to Ancyrocephalidae n. gen. being undiscovered given that ornamental fish have been shown to harbour parasite species yet to be identified (Benovics et al. 2017; Benovics et al. 2018; Benovics et al. 2020). From the findings of this study, it is recommended that more studies on ornamental fishes be conducted to establish the parasite composition and to fill in the evolutionary gaps between genetically distant parasite species.

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