PARASITE THREATS FROM THE ORNAMENTAL FISH TRADE

Thesis submitted by

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To mom and dad who echo in my career

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Cover image: Ornamental fish (Drawn by Eden Cartwritght for the Marine Parasitology Laboratory, James Cook University. Drawing arrangement by the Author of this thesis.

COLLABORATION OF OTHERS

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ABSTRACT

The ornamental fish trade is an important commodity sector that involves the capture or farming of fish species for their aesthetic value. Since the 1960s, technological advances have enabled multiple countries to trade numerous ornamental fish species globally. As such, the ornamental fish trade is a pathway for the introduction of exotic fish species and their associated parasites and pathogens into endemic environments, with the potential for detrimental effects on biodiversity, ecosystems, industries, and their dependent local communities.

Governments can establish quarantine measures to detect, prevent and mitigate the risks of introducing exotic parasites and pathogens. For example, Australia has established import requirements for ornamental fish species based on risk assessments undertaken by the Australian government Department of Agriculture and Water Resources (DAWR). However, Australian risk assessments largely focus on parasites and pathogens of global significance in food fish production (i.e., salmonids and prawns). As such, established biosecurity requirements for the import of ornamental fish to Australia (DAWR 2018), focus on pathogenic bacteria (e.g., *Aeromonas salmonicida* (Lehmann and Neumann, 1896)) and viruses (e.g., spring viraemia of carp virus (SVCV)) known to impact aquaculture, while a much broader parasite fauna of ornamental fishes remains to be assessed. The aim of this thesis was to address three specific gaps of knowledge of the ornamental trade. First, I examined limitations in data collation of t ornamental fish imported to Australia (Chapter 2). Second, I examined the diversity of parasite fauna infecting traded marine and freshwater ornamental fish species (Chapter 3 and 4), and; third, I evaluated the validity of cutting-edge

molecular methods to detect parasites infecting imported ornamental fishes at border control (Chapters 5 and 6).

Accurate data that describes the supply and demand of the global ornamental trade is essential for the development of comprehensive biosecurity protocols to protect endemic ecosystems and natural resources from introduced pathogens and parasites. To quantify the species diversity and volume of ornamental fishes imported to Australia, I examined publicly available data of aquarium fish imports to Australia between 2010-2016, collated and curated by DAWR (Chapter 2). I found that DAWR provides publicly available records of imported ornamental fish species ascribed to categories that offered limited resolution regarding the specific species identity. Taxonomically sound evaluation of Australian aquarium imports would be useful to understand the importance of the Australian aquarium trade in the translocation of potentially hazardous parasites and pathogens, and aid international conservation policies.

Following, I surveyed freshwater and marine ornamental fish populations imported from Asia (i.e., Singapore, Malaysia, Thailand and Sri Lanka) to Australia for the presence of protozoan (Chapter 3) and metazoan parasites (Chapter 4). Fish were received following veterinary certification by exporting countries declaring no clinical signs of pests or diseases, and visual inspection by Australian Quarantine Services. Fish necropsies revealed a diverse array of parasite species, including 18 putative types of myxozoans (e.g. *Ceratomyxa*, *Kudoa* and *Myxobolus* spp.), and 14 parasitic monogenean species (e.g. *Dactylogyrus*, *Gyrodactylus*, *Urocleidoides*, and *Trianchoratus* spp.). One of the major findings was that goldfish, *Carassius auratus* Linnaeus, 1758, which are the most frequently traded freshwater fish

species world-wide, exhibited high parasite diversity (Chapter 3 and 4). Subsequently, I conducted an exhaustive review of the history of the goldfish trade and parasite richness to provide insight into how the international trade of this species may have facilitated parasite co-introduction and co-invasion (Chapter 5). I found that more than 113 parasite species infect goldfish in their native range, of which 26 species were likely co-introduced with the international trade of goldfish (or other cyprinids). These included harmful, generalist parasite species in freshwater aquaculture fishes such as *Ichthyophthirius multifiliis* Fouquet, 1876, *Lernaea cyprinacea* Linnaeus, 1758, and *Schyzocotyle acheilognathi* (Yamaguti, 1934). It is concluded that the goldfish trade likely continues to facilitate the introduction and invasion of exotic parasites on a global scale.

It is clear that pre-export health requirements for the importation of ornamental fish species into Australia are not being met (Chapters 3-5), and that cryptic parasites are not detected during visual inspections at border control. Thus, inspection prior to exportation and at border control must account for the highly cryptic nature of parasites and pathogens and consider alternatives to current pre-export conditions and visual inspections at border control. For this reason, I proposed screening fish transport water for the presence of parasite environmental DNA (eDNA) as a detection method for enhanced biosecurity (Chapter 6). I examined water samples from 11 target populations (cyprinids susceptible to *Dactylogyrus* spp. infections) and seven non-target fish populations (non-cyprinids, not susceptible to *Dactylogyrus* spp. infections) imported from southeast Asia to Australia for the presence of eDNA from five *Dactylogyrus* species (Monogenea: Dactylogyridae) using novel species-specific quantitative PCR (qPCR) assays. *Dactylogyrus* spp. eDNA was detected in all targeted fish populations, showing that eDNA presents a considerable advantage over visual inspections and parasitological necropsies. However, *Dactylogyrus* spp. eDNA was also

detected in water from non-cyprinid fish populations that are not susceptible to and were not infected by *Dactylogyrus* parasites, highlighting the risk of false positive detections associated with contaminated water sources used to transport ornamental fish species. Environmental DNA screening for parasite DNA offers a highly sensitive and non-invasive detection tool during *pre-export* monitoring of ornamental species and could aid quarantine officers to triage high-risk ornamental fish exports based on eDNA detection of parasite DNA in the exporting country. Nonetheless, quarantine officers should be vigilant in the limitations posed by contaminated water sources if eDNA screening methods are used at border control.

Parasite eDNA detection in water samples from non-cyprinid fish populations in Chapter 5 suggested the possibility of false positive detections by eDNA screening. For this reason, I tested the reliability of eDNA screening methods by qPCR for biosecurity purposes in an experimental system simulating the export process (Chapter 7). Experimentally infected live fish (i.e., the monogenean *Neobenedenia girellae* (Hargis, 1955) infecting *Lates calcarifer* (Bloch, 1790)) were used to detect parasite eDNA in water samples, simulating the export process from packaging to delivery over a 48 h period. The consignments included 'infected fish', 'treated fish', and 'contaminated water' (containing dead parasites) delivered by 'exporting companies'. Quantitative PCR tests were inaccurate when detecting eDNA collected from low parasite intensities (mean intensity ± S.D. = 6.80 ± 4.78 parasites/fish). Quantitative PCR tests detected parasite eDNA in 50% of infected fish indicating a high plausibility of false negative detections because of low eDNA concentrations in water samples. Furthermore, parasite eDNA was detected in70% of non-infected fish in contaminated water samples, indicating the possibility of false positive detection of DNA from dead parasites present in the water. Environmental DNA screening methods, while more sensitive than current biosecurity protocols, are limited for accurate and reliable use where

differentiation between live parasite infections and dead, non-viable parasites in the water is paramount.

This thesis highlights the limitations of the DAWR current data collation framework to accurately examine aquarium fish import data and determined that a large diversity of protozoan and metazoan parasites are not detected at border control. Import conditions for ornamental species are not being met by exporting companies. While eDNA screening methods offer a potential tool for the detection of cryptic pathogens, the limitations of this technique need to be considered for development as a detection tool to demonstrate freedom from parasite infection in the ornamental fish trade.

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CHAPTER 1

GENERAL INTRODUCTION

1.1. History and growth of the global ornamental fish trade

The ornamental fish trade involves the capture or culture of fish species for their aesthetic value. Keeping fishes as commodities goes as far back to the T'ang Dynasty in China (AD 265-420) where wild fish were captured for consumption, but coloured varieties were kept as highly valued "red scaled" fish as bartering commodities (Chen 1956). Aesthetic fish varieties may have been kept in rudimentary, accessible bodies of water (Smartt 2001), followed by the use of more permanent ponds and basic forms of aquaculture, giving way to larger numbers of fish kept at any given time (Chen 1956; Smartt 2001). These events of semi-domestication made human communities less dependent on wild "red-scaled" fish stocks. Fish in-breeding events in permanent ponds, resulted in multiple fish varieties and species cultured for their aesthetic value (Smartt 2001). For example, "ponds of mercy" were constructed ~1000 years ago by Buddhists in China as a symbolic gesture to save "food fish" from being killed, and consequently, is one of the first records of domestication of fishes (Chen 1956).

The trade of ornamental fish gradually increased in volume, diversity and range. Historic records are mostly associated with the goldfish, *Carassius auratus* (Linnaeus, 1758) and Koi carp, *Cyprinus carpio* Linnaeus, 1758, traded first between China and Japan as early as 1502 and 1620 (Balon 2004), and between China and Europe as early as 1611 and 1691 (Kottelat 1997). In Europe, fish stocks may have been introduced by the Portuguese from Java to South Africa and from there to Lisbon (Balon 2004). Following these events, trade of

goldfish between Portugal, England and France may have occurred around 1691 and 1755, respectively (Balon, 2004). After their establishment in Europe, fish stocks may have been traded between Europe and North America around 1846 (Mulertt 1896). There are no available records suggesting that live fish were traded between Asian countries and America via Japan, Oceania, and North America (Balon 2004). However, this trade route was common for other commodities, and it is possible that some fish may have been traded directly between China and America (Balon 2004).

Although ornamental fishes have been traded for almost 2,000 years, it was only until the 1960s that the ornamental trade flourished (Balon 2004). Advances in technology and aviation facilitated the transport of large volumes of live animals between countries in short periods of time, increasing the number of trade connections available. By the early 2000s, the ornamental fish trade was a multimillion-dollar global industry, with over 90% of marine specimens sourced from wild coral reefs in the Pacific (Green 2003; Olivier 2003; Wabnitz et al. 2003; Rhyne et al. 2012a; 2017) and over 90% of freshwater species reared in semi-intensive aquaculture systems predominantly in southeast Asia (Wabnitz et al. 2003; Monticini 2010). The global ornamental trade now involves more than 100 countries either as exporters or importers, creating countless trade connections and fish translocations, with over 1 billion ornamental fish traded in 2005 (Whittington and Chong 2007).

1.2. Estimating the value and diversity of the ornamental fish trade

In the year 2000, the value of the industry inclusive of retail sales, associated materials, wages and non-exported product was estimated to be approximately US$15 Billion (Whittington and Chong 2007; Bartley 2000). A previous report by the Food and Agriculture

Organisation of the United Nations (FAO) estimated that the ornamental fish industry produced an average annual growth rate of 14% since 1985 to 1996, increasing from approximately US$ 24 million to an approximate global export value of US$ 206 million (Bartley, 2000). Recent estimates from accessioned records of the United Nations suggest that approximately 10 million net kilograms (weight of boxes containing bags with water and fish) with a value of US$ 320 million were traded in 2014 (United Nations Comtrade division of official international trade statistics (Comtrade) 2014). The six largest exporters of ornamental fish in 2014 (based on total numbers of live ornamental fish) were the Philippines, Singapore, Indonesia, Thailand, Malaysia, Japan, and the largest six importers were the United States, China, the United Kingdom, Germany, Netherlands, and Belgium (Figure 1, Comtrade 2014). The ornamental fish industry had an approximate average annual growth rate of 8.7% since 1996 (Comtrade 2014).

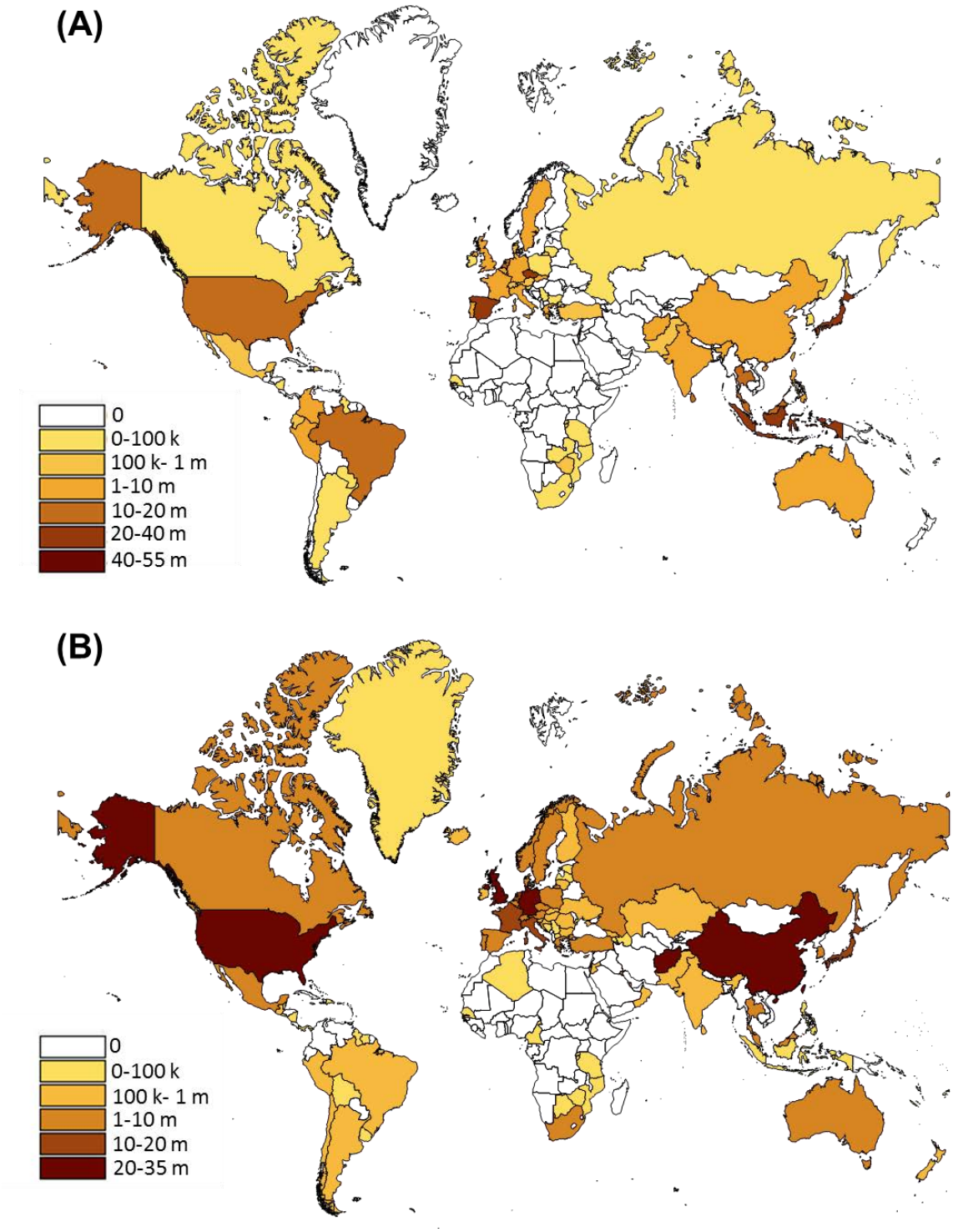

Figure 1. Total number of exported (A) and imported (B) fish globally in 2014. Data were analysed from publicly available records collated by the United Nations Comtrade division of

official international trade statistics (Comtrade 2014). Only data categorised as "030110-Fish; live, Ornamental" were considered in this analysis (Comtrade 2014).

Trade records from the United Nations are based on non-mandatory accessions and were not intended for the specific monitoring of the wildlife trade (Rhyne et al. 2012a). Compulsory data are maintained for species listed by the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) (Militz and Foale 2017), however, previous studies and have found CITES data to be inaccurate, incomplete, or insufficient (Blundell and Mascia 2005; Bickford et al. 2011; Rhyne et al. 2012b). Lack of detail and inaccuracies of trade records have so far prevented any accurate global studies on the ornamental fish trade (Blundell and Mascia 2005; Rhyne et al. 2012a; 2017).

Effective monitoring of ornamental fishes is essential to examine the sustainability and connectivity of the aquarium fish trade (Smith et al. 2009; Rhyne et al. 2012a; 2017; Biondo 2017; 2018). Exploratory research has shown the overarching impacts of over-extraction of animals and habitat destruction in source countries (Andrews 1990; Kolm and Berglund 2003; reviewed by Thornhill 2012; Raghavan et al. 2018), as well as the potential negative impacts of species translocation (Padilla and Williams 2004; García-Berthou 2007; Schofield 2010; Chucholl 2013; Holmberg et al. 2015). Species-specific import information is valuable to understand the risks of exotic disease incursions (Rimmer et al. 2015), invasive species (Holmberg et al. 2015), and source habitat concerns (Biondo 2017; Rhyne et al. 2012a; 2017). As such, several countries have made progress towards real time monitoring of aquarium fish imports at the species level (Rhyne et al. 2012a; 2017; Biondo 2018) and

improving local understanding of trends in supply and demand of ornamental fish species (Wabnitz et al. 2003, Rhyne et al. 2012a).

1.3. Environmental impacts of the ornamental fish trade

More than 100 countries are known to engage in supplying the international market, with fish originating from both aquaculture and wild fisheries (Monticini 2010; Rhyne et al. 2017). Approximately 90% of freshwater ornamental fishes are farmed, while only 10 % of marine ornamental species are reliably cultured, either because their reproduction in captivity is difficult or growth and ecological requirements are not fully understood (Green 2003; Wabnitz et al. 2003; Olivotto et al. 2011). Sustainably managed extraction from wild fisheries can incentivize conservation of marine ecosystems by increasing the perceived value of source habitats to local inhabitants and provide alternatives to destructive livelihood opportunities (Wabnitz et al. 2003; Foale et al. 2016). However, inadequate enforcement of laws managing the harvest of ornamental fishes allows for the persistence of destructive fishing practices (Barber and Pratt 1998), and poor aquaculture management can have negative impacts on the traded fishes and the broader environment (Tlusty 2002; Burke et al. 2011).

Most fish in the global aquarium trade originate from source countries in the Asia-Pacific region (Monticini 2010; Rhyne et al. 2017), where weak local and national governance capacity, combined with high international demand for aquarium fishes, have resulted in limited and ineffective management of the trade (Monticini 2010; Dee et al. 2014). Indeed, destructive extraction methods as well as uncontrolled extraction have negative impacts in wild ecosystems and reduce local species richness and abundance (Barber and

Pratt 1998; Rubec et al. 2001; Bruckner and Roberts 2008), with instances where the aquarium trade has threatened the existence of wild fish species (e.g., Banggai cardinal, *Pterapogon kauderni* Koumans, 1933) (Kolm and Berglund 2003; Lunn and Moreau 2004). Understanding the supply and demand of the aquarium trade can inform governing entities to implement effective management decisions (Militz and Foale 2017; Rhyne et al. 2017). Such understanding can be achieved by collating detailed import data of ornamental fish species in the aquarium trade, which can provide valuable accurate information on the location, volume and richness of species being extracted form wild ecosystems (Rhyne et al. 2012a; 2017; Biondo 2018), however, few countries have surveillance methods that accurately collect these data (Rhyne et al. 2012a).

1.4. Parasite translocation and introduction from the ornamental trade

Human population growth, increased transport capacity and economic globalisation have facilitated the trade of live animals and their associated parasite infections and diseases (Whittington and Chong 2007; Lymbery et al. 2014; Amaral-Zettler et al. 2018). Animals prepared for transit are commonly subject to chronic stress associated with animal handling, housing and method of transport (Dickens et al. 2010), which increase their susceptibility to infections (Smith et al. 2012; Amaral-Zettler et al. 2018). As such, translocated farmed and wild species have been directly associated to disease outbreaks in aquaculture (Whittington and Chong 2007) and wild ecosystems (Smith et al. 2009; Rosen and Smith 2010).

Traded ornamental species be introduced into non-native habitats and become invasive (Lymbery et al. 2014). Invasive fish species can be introduced into areas outsides of

their natural range, establish self-sustaining populations, and spread beyond their initial point of introduction (Kolar and Lodge 2001). Invasive fish can affect endemic species directly, either through competition (Lookwood et al. 2013) or predation (Doherty et al. 2016), with deleterious impacts on the environment and the economy (Early et al. 2016). Most importantly, invasive fish species may be infected with exotic parasites and pathogens, which can establish self sustaining populations in endemic environments by infecting introduced exotic hosts (Lymbery et al. 2014).

Co-introduced parasites can become co-invasive if they are able to infect endemic host species in the new environment (Lymbery et al. 2014). Evidence suggests that co-introduced parasites with complex, indirect life cycles are no less likely to infect endemic hosts and become co-invasive than parasites with direct life cycles, given similarities in host diversity and environmental factors between exotic and endemic localities (Bauer 1991; Kennedy 1993; Lymbery et al. 2014). As such, co-invasive parasites with either direct or complex life cycles influence the composition and structure of animal communities by regulating the abundance of their host population (Mouritsen and Poulin 2002; Mouritsen and Poulin 2010), affect the functioning of ecosystems (Thomas et al. 2005), and cause cascading effects on other endemic fauna (Mouritsen & Poulin, 2010). Although it is not always straightforward to identify exotic species in endemic ecosystems (Lymbery et al. 2014), monitoring parasite fauna and host populations is necessary to assess the risks associated with established co-introduced and co-invasive parasites.

There is a distinct lack of consistent baseline monitoring for the detection and identification of invasive aquatic parasites and pathogens (Lymbery et al. 2014; Rosen and Smith 2010; Amaral-Zettler et al. 2018). Few studies provide substantial evidence showing that parasites can become or became established in native environments directly linked to the

ornamental trade, mostly because human-mediated translocation of infected ornamental fish began long before wildlife monitoring and surveillance programs (Lymbery et al. 2014). Furthermore, a majority of historic records of exotic parasites are based on unverifiable descriptions of organisms found infecting imported fish species, with few researchers accessioning voucher specimens (International Commission on Zoological Nomenclature (ICZN), article 73, 1999). Lack of accessioned material, as well as limited molecular sequences for parasite species (Gómez 2014; Palesse et al. 2011) have resulted in multiple ambiguous parasite descriptions without reliable information on their origin and true identity (Carlton 1996; Lymbery et al. 2014). Understanding the origin of parasites infecting imported ornamental fishes is important to analyse the risks of co-introduced and co-invasive parasites to endemic environments and resources. Therefore, future surveys should consider parasite richness of endemic fish species, to determine which parasites infecting imported ornamental fishes might be considered exotic (Smit et al. 2017). However, projects rarely have the opportunity to do exhaustive surveys because of time limitations and cost of sampling.

1.5. Australian biosecurity and parasites from the ornamental trade

Biosecurity can be defined as an approach designed to prevent or decrease the transmission of naturally occurring infectious diseases and pests in crops and livestock (Koblentz 2010). This definition has been expanded to include invasive exotic species and their associated threats to the economy and the environment (Meyerson and Reaser 2002). Depending on the context, the definition of biosecurity has been modified to suit the aims and requirements of independent organisations. For example, the Food and Agriculture Organization of the United Nations (FAO) defines biosecurity as “a strategic and integrated approach that encompasses the policy and regulatory frameworks (including instruments and

activities) for analysing and managing relevant risks to human, animal and plant life and health, and associated risks to the environment" (FAO 2007). For the purpose of this thesis, biosecurity is defined as:

'A set of measures or procedures designed to protect countries against the risks that may arise from exotic pests entering, establishing and spreading in local ecosystems, thereby threatening the economy and endemic environments'

This definition, modified from the Australian Government Department of Agriculture and Water Resources (DAWR), aims to prevent, respond to and recover from pests and diseases that threaten the Australian economy and environment (DAWR 2014). As a signatory country of the World Trade Organization (WTO) and the Agreement on the Application of Sanitary and Phytosanitary (SPS) Measures, the Australian government can set risk levels of given hazards based on science-based risk analyses (Doyle et al. 1996; Kahn et al. 1999; Whittington and Chong 2007; Moore et al. 2010). Science-based risk analyses consider the level of biosecurity risks associated with the importation of a good and identify appropriate ways to manage these risks (DAWR 2016a). As such, the DAWR undertakes Biosecurity Import Risk Analyses (BIRA) in response to requests to import goods into Australia, where goods have not been imported before, or have not been imported into Australia from a particular country or region (DAWR 2016a). Currently, Australia has established biosecurity protocols to detect, prevent or mitigate the impact of 23 reportable finfish diseases (DAWR 2016b, Supplementary S1), and provides guidelines for researchers, officials, and the public to recognise diseases of significance to aquaculture and fisheries in Australia (DAWR 2012).

In the specific case of the ornamental fish trade, the DAWR has completed two separate BIRA for ornamental finfish imported to Australia since 1999. The first BIRA in 1999 reported that five viral diseases and five parasite species known to infect imported ornamental fish did not meet Australian Appropriate Level of Protection (ALOP) and were considered as high risks for Australian biosecurity (Kahn et al. 1999). Following, the DAWR improved its biosecurity protocols and established mandatory documentation and quarantine requirements for the importation of both freshwater and marine ornamental finfish (DAWR 1999a, b). The second BIRA in 2014, considered the risks associated with the importation of ornamental fishes and iridovirus infections. It found that imported ornamental gouramis, cichlids and poeciliids could be infected with megalocytiviruses, which were subsequently considered to be high risks for Australian biosecurity (DAWR 2014). Following this BIRA, import requirements of freshwater ornamental fishes included mandatory health requirements to certify that imported fish were free of megalocytivirus and iridovirus infections by the exporting country (DAWR 2014). Freedom from these viral diseases must be certified by approved health specialists in the exporting country using molecular diagnostics (i.e., Polymerase Chain Reaction, PCR) and mandatory sampling guidelines provided by DAWR, modified from freedom from disease surveillance standards of the World Organisation for Animal Health (OIE; DAWR 2014). Australian Biosecurity import conditions can be separated into: pre-export, border control, and post-export requirements (Table 1), aimed at detecting, preventing and managing specific parasitic and viral infections with high risks to Australia, which remain enforced with regular revisions and audits to maintain stringent biosecurity (DAWR 2018).

The last BIRA conducted by DAWR did not survey the parasite diversity infecting ornamental fishes imported into Australia (DAWR 2014). Multiple fish species from diverse sources involved in the trade remain to be assessed, and their risks to Australian fauna or

industries remain unknown (Whittington and Chong 2007). This limits a comprehensive understanding of what potential new parasite threats are likely to be translocated into Australia with ornamental fish. Most importantly, visual inspections at border control, which aim to determine if imported fish present obvious signs of infection or disease, do not account for infected fish that are asymptomatic or are infected with parasites that are not possible to detect with the naked eye (Chapter 3). For this reason, a cross sectional survey is required to determine: 1) the parasite fauna infecting ornamental fish imported to Australia, and; 2) if current import conditions for ornamental fish species are being met. This research was considered to be high priority by DAWR and the Fisheries Research and Development Corporation (FRDC). Subsequently, the research conducted in this thesis comprised a component of a research grant awarded to the University of Sydney and James Cook University in 2014 *"Strategic approaches to identifying pathogens of quarantine concern associated with the importation of ornamental fish"*.

Table 1. The Australian Department of Agriculture and Water Resources (DAWR) import conditions for live freshwater and marine finfish to Australia (DAWR 2018).

Stage	Level	Requirement	
		Freshwater	**Marine**
Pre-export	Export premises	Valid import permit issued by the Department of Agriculture and Water Resources.	
	Source population	Fish species must be eligible for importation into Australian territory from approved countries. All fish being held at the export premises exhibit no clinical signs of significant infectious disease or pests and are sourced from populations not associated with any significant disease or pests within the six months prior to certification. The fish originate from a country, zone or export premises determined to be free from megalocytiviruses. Goldfish originate from a country, zone or export premises (the population) determined to be free from spring viraemia of carp virus (SVCV) and *Aeromonas salmonicid*a (other than goldfish ulcer disease strains). The fish originate from a country, zone or export premises determined by the Competent Authority to be free from megalocytiviruses. The fish have not been kept in water in common with farmed food fish (fish farmed for human consumption including recreational fishing) or koi carp.	Fish species must be eligible for importation into Australian territory from approved countries. Fish must be collected at least 5 Km from any finfish aquaculture operation and the fish in the consignment have not come into contact with water, equipment or fish associated with farmed food fish (fish farmed for human consumption including recreational fishing). The fish are not sourced from a population associated with any significant infectious disease or pests and there have not been any outbreaks of infectious fish disease or pests in the areas from which the fish have been collected during the six months prior to collection. The fish are wild caught and have not been bred or hatched on a farm or other premises.

	Health inspection	The fish in the consignment have been inspected within seven days prior to export and show no clinical signs of infectious disease or pests. The batch of consigned fish have been tested and found negative for megalocytiviruses. All goldfish must be certified free from spring viraemia of carp virus (SVCV) and *Aeromonas salmonicida* (other than goldfish ulcer disease strains), and treated with an effective parasiticide (e.g. trichlorfon, formaldehyde, sodium chloride) during the seven days prior to export to Australia to eliminate infestation by the gill flukes *Dactylogyrus vastator* and *D. extensus*.	Visual inspection certificate of the consignment by a competent authority within seven days prior to export, showing no clinical signs of infectious disease or pests.
Border Control	Documentation	Each exported fish consignment must have a DAWR import permit number, the exporters name, address, phone number, fax number and e-mail address, shipping invoice number, valid health certificate including name of the species, number of fish and boxes.	
	Visual inspection	All ornamental fish consignments are visually inspected by the DAWR on arrival to ensure that fish are healthy, documentation is in order, and fish do not contain non-permitted material or material of biosecurity concern. Fish not meeting these criteria and non-permitted material will be seized, exported or disposed of at the importers expense.	
Post-export	Approved Arrangement Site	Fish inspected by the department on arrival and found to satisfy all import conditions, are to be transported to an Approved Arrangement site (AA site) named on the import permit and quarantined for 21 (goldfish) or seven days (all other freshwater species).	Fish inspected by the department on arrival and found to satisfy all import conditions, are to be transported to an Approved Arrangement site (AA site) named on the import permit and quarantined for seven days.
	Health inspection	Based on fish species, country of origin, historical factors or any other relevant information, the department may test samples of imported fish during quarantine to determine their health status. The cost of testing will be at the exporter's expense. In the event of any imported fish showing clinical signs of an infectious disease or producing a positive result to any tests indicating the presence of an infectious disease agent or pest, the department may cause any or all the fish in the premises to be either detained in quarantine for further observation, tested and treated, or to be disposed of. Costs of any such action will be borne by the person in charge of the goods. If any fish are destroyed during any period of quarantine, compensation will not be paid by the Government.	
	Final inspection	Following the post-export quarantine period, fish will be inspected by the department and must be found free from clinical signs of pest and disease before they are released from biosecurity control.	

1.6. Thesis objectives and aims

The overall aim of this thesis was to gain a comprehensive understanding of the ornamental fish trade including the primary traded species in Australia, their associated parasite fauna and molecular mechanisms to facilitate the detection of parasites at border control. This broad aim was tackled though three major research questions, presented as five discrete research studies or data Chapters in this thesis (Chapters 2-6). First; I sought publically available data to determine the diversity, volume and international connectivity of the Australian ornamental fish trade (Chapter 2). Second, I examined live fish imports from southeast Asia to determine whether imported live ornamental fish meet import conditions as determined by the Australian Government Department of Agriculture and Water Resources (DAWR) (Chapter 3). Following, I examined parasite richness and plausible spread from the international trade of goldfish, *Carassius auratus* (Linnaeus, 1758) (Chapter 4). Third, I critically evaluated the application of environmental DNA as a detection method for aquatic parasites in biosecurity (Chapters 5 and 6). The following data chapters presented in this thesis comprise original scientific research that determined the limitations of current record keeping and assessment of parasite risks to Australia from the ornamental fish trade and sought to resolve limitations with current and alternative detection tools.

CHAPTER 2 PUBLICATION STATEMENT

Chapter 2 was accepted for publication on the 12th of November 2018 in *Wildlife Research*:

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Chapter 2 includes changes made following comments from three separate reviewers.

CHAPTER 2

DATA MANAGEMENT LIMITS BIODIVERSITY DATA: A CASE STUDY IN AUSTRALIA

Abstract

More than 10,000 shipments encompassing more than 78 million live fishes were imported to Australia between 2010 and 2016 for the aquarium trade. Imported fishes generate revenue both within the country and abroad, but consequently add pressure to wild source populations of ornamental species. Australia has a global responsibility to ensure its own consumption of aquarium trade organisms is not undermining conservation agendas in neighbouring source countries. This chapter examines publicly available data of aquarium fish imports to Australia during 2010-2016, collated and curated by the Australian Government Department of Agriculture and Water Resources (DAWR), and reviews the present framework for recording aquarium fish imports. Records were provided by DAWR as an administrative release of the collated depersonalised data following a Freedom of Information Act request. Records were compared to checklists from the International Union for Conservation of Nature (IUCN) Red List status and the Convention on International Trade in Endangered Species (CITES) to address whether the Australian aquarium industry is potentially importing threatened species. The provided records were apportioned by DAWR into categories of "marine", "cichlid", "goldfish", "gourami", "poeciliid" and "other freshwater species". A total of 10,320 consignments encompassing more than 78.6 million aquarium fishes were imported to Australia between 2010 and 2016. A total of 4628 species of fishes were permitted import to Australia for the aquarium trade with 73 of the marine species (2.0 %) and 81 of the freshwater species (7.5 %) found to be threatened with some degree of extinction risk. The

data reporting framework for aquarium fish imports offered limited capacity to taxonomically differentiate imports and only 12.5 % of all aquarium fishes imported could be identified to species.

2.1. Introduction

An objective of the United Nations' 2030 Agenda is the sustainable use of ecosystems to halt the loss of biodiversity (United Nations 2015). Reducing biodiversity loss and achieving environmental stewardship goals requires understanding what threatens biodiversity, how fast threats change in type and intensity, and establishing appropriate management actions to avert risks (Joppa et al. 2016; Cawthorn and Mariani 2017). Among the myriad of human-mediated threats, biological resource use (i.e., the consumptive use of "wild" biological resources) represents the most common direct threat to biodiversity (Salafsky et al. 2008; Henderson et al. 2011; Maxwell et al. 2016; Vall-Ilosera and Cassey 2017; Latombe et al. 2017; García-Díaz et al. 2018). Indeed, the combination of large-scale monitoring schemes and advances in information technology provide unprecedented insight into global threats to biodiversity (Pimm et al. 2015), however, global and regional data of biological resource use are limited (Rhyne et al. 2017), inaccurate (Rhyne et al. 2012; Janssen and Shepherd *in press*), or of little value to accurately analyse spatial and temporal distribution of anthropogenic threats to biodiversity (Joppa et al. 2016).

Globalisation and improved shipping technology have inherently increased the supply of live organisms for the global aquarium trade extracted from remote environments (Wabnitz et al. 2003). The aquarium fish trade now encompasses millions of individual

marine (Rhyne et al. 2017) and freshwater (Monticini 2010) fishes traded on an annual basis. More than 100 countries are known to engage in supplying the global aquarium trade, with fish originating from both wild fisheries and aquaculture (Monticini 2010, Rhyne et al. 2017). While source countries continue to understand and manage threats to local biodiversity due to the aquarium trade (e.g., Kolm and Berglund 2003; Moreau and Coomes 2006, 2007; Raghavan et al. 2013; Madduppa et al. 2018), importing countries face threats from introducing exotic (i.e., non-native; Lymbery et al. 2014) fishes and diseases (e.g., Lintermans 2004; Whittington and Chong 2007; Albins and Hixon 2008; Rimmer et al. 2015).

Appropriate management of the aquarium fish trade requires accurate accounts of the source, production method, quantity, and diversity of fishes traded between countries (Smith et al. 2009; Dee et al. 2014; Rhyne et al. 2012, 2017; Biondo et al. 2017; 2018; Hood et al. *in press*). However, comprehensive and overarching data relating to the global aquarium trade (Rhyne et al. 2017) as well as reporting frameworks designed to record species-specific data of live aquarium fish imports remain deficient (Biondo 2017). It is unclear how source and importing countries can monitor the aquarium trade effectively and, as consequence, how mitigation of the potential threats from the aquarium trade are adequately achieved given the lack of accessible trade data. The development of specific data systems for recording detailed information where fish are exported or imported to replace or enhance existing data reporting frameworks is seen as a possible solution to monitoring the biodiversity in the aquarium trade (Rhyne et al. 2012, 2017; Biondo et al. 2017, 2018).

An evaluation of the data reporting frameworks presently employed by countries engaged in the aquarium trade is merited to better understand the means by which

comprehensive data on the aquarium trade can be made more accessible. To this end, we examine the data reporting framework for aquarium imports to Australia and the capacity for existing data to contribute to an improved understanding of threats to biodiversity loss from the aquarium trade both within Australia and among the source countries supplying Australia. A case study on Australia is justified on the basis of the country (i) participating in the global aquarium trade as a consumer of aquarium fishes, importing millions of fishes annually over a time span of several decades (McKay 1984; Kahn et al. 1999; O'Sullivan et al. 2008), (ii) being a leader of environmental conservation in the Asia-Pacific region (Kingsford et al. 2009) from where a large percentage of the global trade in aquarium fishes are sourced (Monticini 2010, Rhyne et al. 2017), (iii) having strict import biosecurity measures which are presently undergoing reform (Hood et al. *in press*).

2.2. Methods

Data reporting framework

The importation of fishes to Australia is regulated by the *Environment Protection and Biodiversity Conservation Act 1999* (EPBC Act) administered by The Australian Government Department of Environment and Energy (DEE), and by the *Biosecurity Act 2015* administered by The Australian Government Department of Agriculture and Water Resources (DAWR). Species permitted import to Australia for the aquarium trade must be listed on both the *List of Specimens taken to be Suitable for Live Import* established by DEE and the *List of Permitted Live Freshwater/Marine Fish Suitable for Import* (hereafter Permitted Fish List) established by DAWR (DAWR 2018). Live fishes may only be imported from the DAWR list of approved countries, specified separately for freshwater and marine species (DAWR

2017). Marine fishes may only be imported if wild-caught and sourced from an area more than 5 km from aquaculture operations. On the other hand, no restriction on production method is placed for freshwater fishes, but freshwater fish stocks must exhibit no clinical signs of significant infectious disease or pests and must be sourced from populations not associated with any significant disease or pests within the 6 months prior to health certification (DAWR 2018).

Biosecurity officers inspect all imported consignments of aquarium fishes at border control for compliance with import conditions and retain copies of the accompanying documents. The current DAWR record keeping policy (current since 2015) requires that all records must be managed in digital format. Incoming paper documents are scanned, and digital copies stored in systems that have approved record keeping functionality. Paper documents received prior to 2015 are stored at off-site storage facilities and are registered in the DAWR's record keeping system. Additionally, the DAWR is currently undertaking bulk scanning of these paper documents to meet the digitising standards set by the National Archives of Australia. Paper documents that have been digitised are kept for approximately 12 months (allowing time for quality assurance of the digital copies). As the documents accompanying consignments contain personal and commercial-in-confidence information, access to the paper and digital copies is restricted by a Dissemination Limiting Marker (a security classification prescribed under the Australian Government Information Security Management Protocol) (DAWR *pers. comm*).

The DAWR collates depersonalised consignment-specific information from the digitised invoices and health certificates into a verification surveillance system used for data

reporting (Hood et al. *in press*). The information captured from consignments includes country of export, region/State of import, quantity of fishes, and non-compliance information. The quantity of fishes are apportioned by their particular biosecurity *risk group*, which groups species based on their susceptibility to specific biosecurity threats (Hood et al. *in press*). The groups are (i) all marine species, (ii) cichlid, (iii) goldfish, (iv) gourami, (v) poeciliid, and (vi) other freshwater species. The poeciliid group was only included in the reporting framework commencing 2015, where prior to 2015 poeciliids were reported as other freshwater species. Although DAWR has access to import documents detailing the quantity of fishes by species imported, this data is not transposed due to the associated administrative burden being excessive and unreasonable (DAWR *pers. comm.*). Information pertaining to the quantity of fishes per container or the production method (i.e., cultured or wild-caught) of imported fishes is also not transposed.

Data analysis

Aquarium fish import records for the period 2010 to 2016 were provided free of charge by DAWR as an administrative release of the collated depersonalised data following a Freedom of Information Act request. The obtained records included information on the date of consignment arrival to Australia, the country of export, and the number of fishes by risk group within the consignment. The DAWR data represents shipments importers have declared as aquarium fish, and consignments improperly declared, mislabelled, or smuggled into the country may affect reporting accuracy (Natural Resource Management Ministerial Council (NRMMC) 2006).

Aquarium fish import records provided by DAWR were used to determine the absolute quantity of consignments and individual fishes imported to Australia between 2010 and 2016. To determine if there were any general trends in the number of fish imports over time, Kendall's correlation tests (function: *cor.test*, package: *stats*) were conducted for the total number of individual fishes and consignments against year using the R statistical software (version 3.3.3). For categorical comparisons, data was summarised as the total percentage of individuals for each year.

The maximum potential biodiversity of imports and the taxonomic resolution at which aquarium fish imports to Australia were reported in the DAWR database was determined from the Permitted Fish List (BICON 2018). Listed species permitted import were assigned based on their taxonomic identification in FishBase (Froese and Pauly 2017) to the risk groups utilised in the reporting structure of the DAWR database. Where import of species at a genus or family level was permitted, all valid species identified by FishBase within the listed taxonomic group were considered. All data enquires to FishBase were managed using the *rfishbase* package (Boettiger et al. 2012) in the R statistical software.

To determine if Australia is potentially importing species threatened with extinction, the International Union for Conservation of Nature (IUCN) Red List status was assessed for all species permitted import. A review of the IUCN Threat Classification Scheme was undertaken for each threatened species (i.e., those critically endangered, endangered, vulnerable, near threatened, and conservation dependent) through the IUCN web portal

(www.iucnredlist.org) to identify species known to be threatened by biological resource use. Additionally, all species permitted import were queried against the Checklist of CITES Species (checklist.cites.org) for listing by the Convention on International Trade in Endangered Species.

2.3. Results

Between 2010 and 2016, DAWR aquarium fish import records indicated 10,320 consignments encompassing more than 78.6 million aquarium fishes were imported to Australia (Fig. 2; [Dataset] Trujillo-González 2018). On average (mean ± 95 % Clopper-Pearson exact Confidence Interval), 1474 ± 208 consignments and 11.2 ± 1.6 million aquarium fishes were imported to Australia each year. There was no significant trend in the number of individual fishes (τ = -0.24, P = 0.56) or consignments (τ = -0.14, P = 0.77) imported to Australia during the study period (Fig. 2). Most imports comprised freshwater species exported from the Asia-Pacific region (97.7 % of individual fishes, Fig. 3). A small percentage of imports were marine species (2.3 % of individual fishes, Table 2) originating primarily from Indonesia (68.4 % of marine fishes, Fig. 2). In total, Australia imported freshwater species from 11 countries and marine species from 13 countries, resulting in 14 unique countries supplying aquarium fishes to Australia during 2010-2016 (Fig. 2).

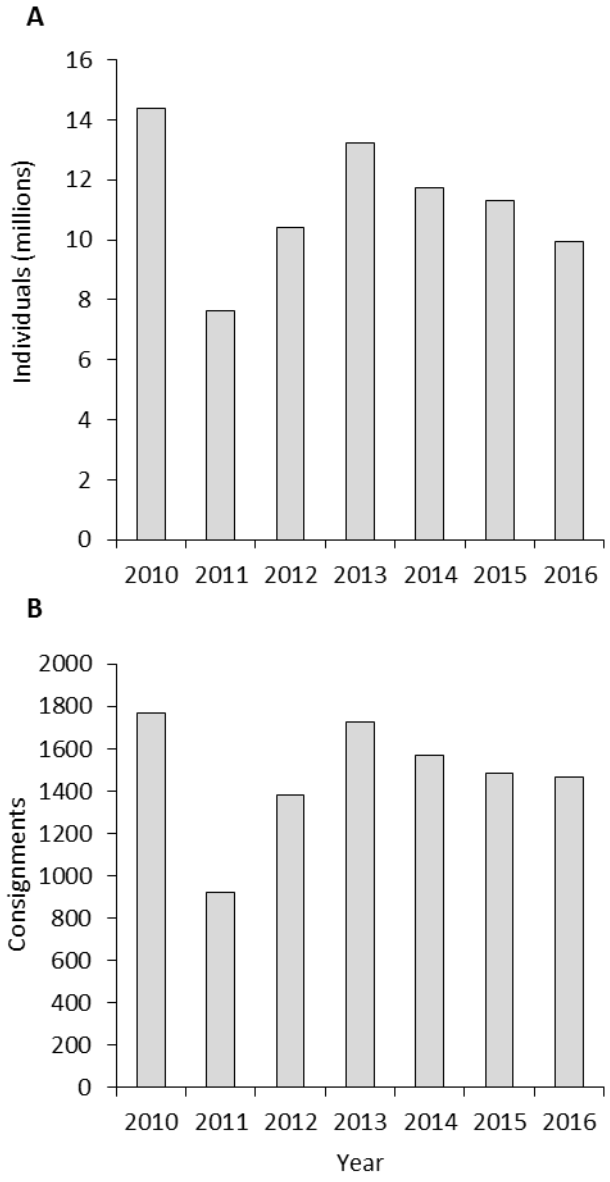

Figure 2. Number of individual fishes imported (A) and number of consignments (B) imported to Australia for the aquarium fish trade during 2010-2016. Neither individuals nor consignments imported exhibited a significant change in quantity between 2010 and 2016 (individuals: $R^2 = -0.17$, $F_{1, 5} = 0.14$, $P = 0.73$; consignments: $R^2 = -0.19$, $F_{1, 5} = 0.06$, $P = 0.82$).

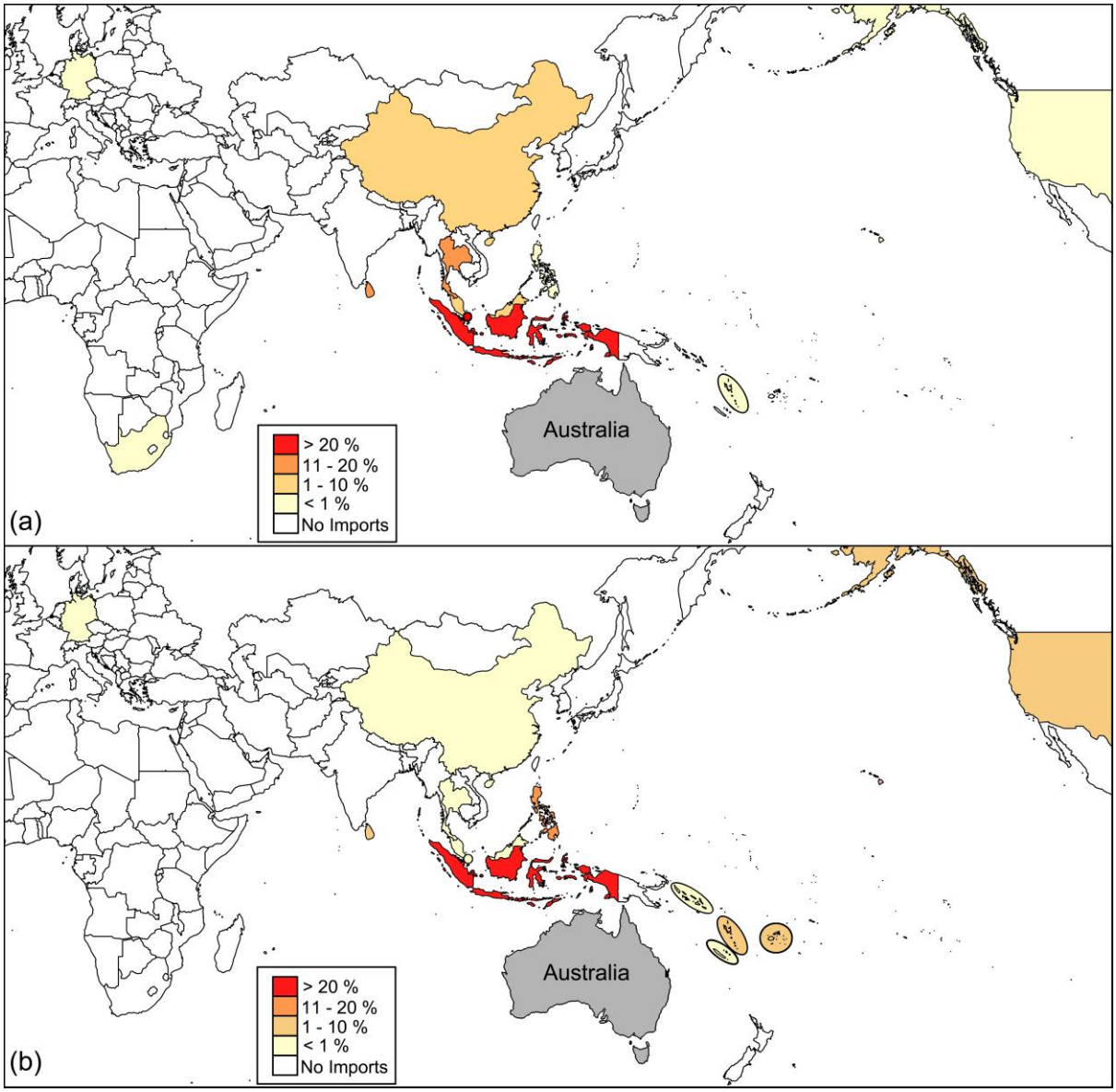

Figure 3. Origin of freshwater (A) and marine (B) aquarium fish species imported to Australia during 2010-2016. Percentages are number of fish in each 'species group'/ Total number of fishes imported to Australia. Data presented does not account for the 0.7 % of consignments with no country of origin information.

The Permitted Fish List encompasses 4628 species of fishes permitted for import to Australia specifically for the aquarium trade (DAWR, 2017), however, data provided by DAWR had limited capacity to taxonomically differentiate imports. The 'marine species' risk group encompassed 3553 permitted species (Table 3) while 'cichlid', 'goldfish', 'gourami', 'poeciliid', and 'other freshwater species' risk groups accounted for the remaining 1075 permitted species, of which only the 'goldfish' risk group identified imports as a single

species (*Carassius auratus* (Linnaeus, 1758)) (Table 3). Therefore, only goldfish could be taxonomically identified to species, which accounted for 12.5 % of all aquarium fishes imported to Australia during 2010 - 2016 (Table 3). The DAWR data reporting framework was also limited in its capacity to taxonomically differentiate exports from the 14 source countries. Countries exporting aquarium fishes to Australia varied in the maximum potential biodiversity of exports, ranging from 811 to 4628 species (Table 4). China was the only source country for which the majority (79.7 %) of exported fishes could be identified to species (i.e., *C. auratus*) using the DAWR aquarium fish import records (Table 4).

Table 2. Number of individual fishes collectively imported to Australia and the categorical composition of aquarium fish imports between 2010 and 2016.

Year	Total fishes	% Marine	% Poeciliid	% Cichlid	% Gourami	% Goldfish	% Other freshwater species
2010	14,380,798	1.9 %	NA	5.2 %	2.3 %	15.2 %	75.3 %
2011	7,653,085	2.0 %	NA	3.5 %	1.8 %	12.3 %	80.4 %
2012	10,428,451	2.5 %	NA	3.6 %	2.3 %	11.5 %	80.1 %
2013	13,251,003	2.4 %	NA	3.4 %	2.2 %	12.9 %	79.1 %
2014	11,719,815	2.5 %	NA	3.7 %	2.4 %	10.9 %	80.5 %
2015	11,324,049	2.2 %	2.8 %	3.6 %	2.4 %	11.3 %	77.6 %
2016	9,934,080	2.7 %	15.7 %	3.9 %	4.6 %	12.9 %	60.2 %
TOTAL	**78,691,281**	**2.3 %**	**2.4 %**	**3.9 %**	**2.6 %**	**12.5 %**	**76.3 %**

Table 3. Assignment of the List of Specimens taken to be Suitable for Live Import aquarium fish species by import category to their International Union for Conservation of Nature (IUCN) Red List status.

Status	Marine	Poeciliid	Cichlid	Gourami	Goldfish	Other freshwater species
Critically endangered	3	0	0	4	0	4
Endangered	6	0	0	1	0	20
Vulnerable	58	0	13	8	0	23
Near threatened	18	0	1	1	0	2
Conservation dependent	0	0	0	0	0	4
Least Concern	946	1	46	14	1	147
Data deficient	180	1	3	6	0	22
Not evaluated	2397	5	107	52	0	589
Total species in category	**3608**	**7**	**170**	**86**	**1**	**811**

Seventy-three of the marine species (2.0 %) and 81 of the freshwater species (7.5 %) permitted import for the aquarium trade were found to be threatened with some degree of

extinction risk (Table 3). Of these, 33.8 % are known to be directly threatened from biological resource use, while 67.0 % of marine species and 70.0 % of freshwater species permitted import to Australia have not been evaluated by the IUCN (Table 3). None of the permitted species were listed by CITES.

Table 4. Maximum potential number of species, quantity of individual fishes, and percentage of individual fishes taxonomically identified exported to Australia from each source country. Data presented does not account for the 0.7 % of consignments with no country of origin information.

Country	Potential number of species	Total fishes	Identified (%)
Indonesia	4683	25,373,939	1.3
Singapore	4683	19,294,962	7.9
Thailand	2058	9,232,029	6.8
Sri Lanka	4683	9,030,561	0.9
China	3946	8,259,547	79.7
Malaysia	4501	5,822,836	9.8
Philippines	4676	560,614	13.0
Vanuatu	4676	404,660	10.3
Germany	1497	223,800	0
United States	4676	132,786	2.9
Fiji	3608	29,051	0
Solomon Islands	3608	12,066	0
South Africa	811	10,300	0
New Caledonia	1544	1544	0

2.4. Discussion

Aquarium fish import records provided by DAWR had little taxonomic resolution and limited capacity for researchers/personnel outside DAWR to assess the biodiversity of

aquarium fishes imported to Australia. Imports quantified by species were available for only one of the 4628 species permitted import to Australia, raising concern on the quantity of individual fishes and production method of the remaining 4627 fish species permitted for import to Australia. The lack of taxonomic resolution in aquarium fish import records is not unique to the DAWR reporting framework. Assessment of the biodiversity in the aquarium trade has been hindered by the inaccuracies of documents accompanying imports (Allen *et al.* 2017; Biondo 2017) and the taxonomic resolution at which data reporting frameworks collate information (Smith *et al*. 2008; Rhyne *et al*. 2012, 2017; Biondo 2018). For example, taxonomic resolution of data collated by the UN Comtrade Database (comtrade.un.org) is limited to "ornamental fish, freshwater" (H.S. code 030111) and "ornamental fish, other than freshwater" (H.S. code 030119). Similarly, data collated by the Food and Agriculture Organization of the UN accessible through FishStatJ are limited to "freshwater" or "saltwater" ornamental species descriptors that are tabulated by weight of imports (FAO 2018). The Global Marine Aquarium Database (GMAD) encouraged industry to improve data reporting through voluntary submissions of detailed export and import records (Wabnitz *et al.* 2003); however, these records offer little insight into the biodiversity of imports to Australia given the focus on marine species, limited temporal scope, and the voluntary nature of submissions (Wabnitz *et al.* 2003; Morrisey *et al.* 2011, Murray *et al*. 2012). Similarly, compulsory data maintained for CITES-listed species accounted for none of the species permitted import to Australia for the aquarium trade.

In Australia, detailed information accompanying imports is required by DAWR import conditions. Physical and digitised documents accompanying consignments are retained at border control and verified by biosecurity officers (Hood *et al.* in press). While detailed species-level information for live fish imports is available in these documents, its

public availability is constrained by laws protecting personal or commercial-in-confidence information, and documents are depersonalised by DAWR before data is made publicly available. Most importantly, The DAWR data reporting framework was designed to support biosecurity risk analysis by grouping species into risk groups based on their susceptibility to certain biosecurity hazards (Hood *et al*. in press) and provides limited insight on the aquarium trade beyond the scope of biosecurity risks to Australia. While improving the taxonomic resolution of aquarium fish import records will not have any bearing on the rigor of biosecurity or environmental risk assessments undertaken by DAWR, making more comprehensive data accessible offers opportunity for research beyond the scope of these assessments. Recognising this value, countries have made progress towards real time monitoring of aquarium fish imports at the species level (Rhyne *et al*. 2012, 2017; Biondo 2018). Access to species-specific aquarium import data has allowed research to explore exotic disease incursion (Hood *et al.* in press), invasive species (Holmberg *et al.* 2015), and source habitat threats (Rhyne *et al*. 2012, 2017; Biondo 2017, 2018) from the aquarium trade. The value of species-specific import data is expanded below:

2.4.1. Exotic disease research

The DAWR is presently developing innovative real-time, responsive risk-based surveillance capabilities to manage biosecurity risks associated with aquarium fish imports (Hood and Perera 2016; Hood *et al*. in press). The surveillance and pathway analysis system collects species-specific consignment, epidemiological, and histopathological data for consignments showing clinical signs of non-compliance with health certificates to identify emergent exotic disease patterns of biosecurity concern (Hood *et al.* in press). While capturing species-specific consignment information is presently not implemented for compliant consignments permitted import (Hood *et al*. in press), such information would

better allow DAWR to identify which species are most representative of a particular risk group and trade pathway when selecting specimens for testing from compliant consignments.

A database of historical aquarium fish imports to Australia with a high degree of taxonomic resolution would be of benefit where an emergent biosecurity concern is identified among species of a risk group (e.g., Becker *et al.* 2014; Rimmer *et al.* 2015; Trujillo-González *et al.* 2018). This would allow DAWR to retrospectively determine the total quantity of consignments imported to Australia containing the species from the pathway of concern. Such information could aid in the allocation of resources (e.g., to a specific port/importer) to detect the possibly of disease incursion having occurred prior to the surveillance and pathway analysis system identifying the biosecurity concern.

2.4.2. Invasive species research

There has been a steady increase in the number of exotic freshwater fishes that have become established in waterways of Australia over the past decades (McNee 2002; Lintermans 2004; Corfield *et al.* 2008; García-Díaz *et al.* 2018). At least 30 species are thought to have come into the country via the aquarium fish trade (Lintermans 2004; Corfield *et al.* 2008; García-Díaz *et al.* 2018) and, presently, nine of these species are still permitted import to Australia (DAWR 2019). Removal of established species from the Permitted Fish List has been suggested if the risk of becoming a pest is high, its value to the industry is low, and preventing importation would reduce the risk of further establishment (Corfield *et al.* 2008). However, an evaluation of a species' value to industry and the significance of preventing importation requires data on the quantity of individuals imported. This information is not captured in the present data reporting framework, and past studies have had to rely on proxy

indicators and qualitative assessment when making policy recommendations (Corfield *et al*. 2008). The extent to which imported ornamental species can become invasive is influenced by the species' availability to consumers, propagule pressure, and the number of pathways by which species can be spread to the wild (Kolar and Lodge 2001; Semmens *et al.* 2004; Corfield *et al.* 2008; Gertzen *et al.* 2008; Simberloff 2009; García-Díaz *et al.* 2018). Detailed species-specific import data could inform on the likelihood of exotic species being introduced to waterways (Holmberg *et al.* 2015; Groom *et al.* 2017) and potential methods to mitigate the spread and establishment of introduced invasive species (Groom *et al.* 2017).

2.4.3. Conservation and sustainability research

Biosecurity import risk assessments (BIRAs) are undertaken by DAWR to assess risks associated with the importation of live ornamental fish species (Kahn *et al.* 1999; NRMMC 2006; DAWR 2014). However, BIRAs do not prioritise conservation-related concerns for traded species (NRMMC 2006). As such, it is unclear to what extent the current taxonomic resolution of the DAWR data reporting framework informs on the impact of importing any of the 154 permitted fish species threatened with some degree of extinction risk. Species-level taxonomic resolution of aquarium fish imports is critical for identifying to what extent the aquarium trade is threatening biodiversity and for which species risks occur (Biondo 2017, 2018; Rhyne *et al.* 2017).

Increasing the sustainability of the aquarium fish trade should be considered a primary initiative for all participants along the supply-chain and not solely a burden of source countries (Tlusty *et al.* 2013; Militz and Foale 2017). Many of the countries found to export aquarium fishes to Australia do not possess a legal framework that regulates or monitors the

harvest of threatened fishes for the aquarium trade (Dee *et al.* 2014). Weak local and national governance, limited management resources, and corruption undermine the capacity for many source countries to adequately capture trade data necessary to inform effective policy (Moreau and Coomes 2007; Raghavan *et al.* 2013; Dee *et al.* 2014). For example, *Pterapogon kauderni*, Koumans, 1933, is a popular, endangered marine ornamental fish endemic to Indonesian that is permitted import to Australia for the aquarium trade (Allen and Donaldson 2007; DAWR 2017; 2019). Harvest of *P. kauderni* for the global aquarium trade has been identified as a direct threat to the species' survival (Kolm and Berglund 2003; Lunn and Moreau 2004; Allen and Donaldson 2007). Nonetheless, wild-caught *P. kauderni* can be imported to Australia in accordance with DAWR import conditions for marine species (DAWR 2018; FRL 2018), and the present data reporting framework for aquarium fish imports combines *P. kauderni* imports with 3607 other species in the marine risk group (DAWR 2018). Thus, a database of aquarium fish imports tabulated by species would both allow Australia to monitor its own consumption and assist source countries in monitoring exploitation.

2.4.4. Possible solutions

Avenues by which aquarium fish import data can be collated at greater taxonomic resolution by regulatory agencies has been explored in several previous studies (Wabnitz *et al.* 2003; Rhyne *et al.* 2012, 2017; Biondo 2017, 2018). In the context of Australia, depersonalising and transposing information from documents arriving with consignments is the primary challenge in facilitating accessibility to more comprehensive data on aquarium fish imports. The use of automated optical character recognition software to retrieve information from digital copies of import documents has been shown to address similar

issues in monitoring aquarium fish imports to the United States (Rhyne *et al.* 2012, 2017). Application of this technology to capture data from the digitised import documents curated by DAWR should be explored for feasibility. Alternatively, amending import conditions to require the electric submission of select consignment information through a purpose-built web portal would eliminate the need for government agencies to manually transpose data and would place the cost-burden of data entry on stakeholders financially benefiting from trade (e.g., exporters/importers). The Trade Control and Expert System (TRACES) of the European Union is one example of such a web portal by which data on the quantity and diversity of aquarium imports is captured (Biondo 2017, 2018). By adapting the Australian Biosecurity Import Conditions (BICON) web portal through which live specimen import permits are processed, data on aquarium fish imports could be delivered direct to a database following submission of data into a semi-automated template. Either approach offers potential for trade data to be monitored in real time, which is a necessary consideration for the full value of the resulting dataset to be obtained (Rhyne *et al.* 2012, 2017).

2.4.5. Conclusions

Accessible, detailed information on aquarium fish imports is necessary to support research capable of addressing threats to biodiversity loss (Joppa *et al.* 2016). Data reporting systems employed by regulatory agencies have been limited in the extent to which the collated data can be used to monitor the biodiversity of the aquarium trade (Smith *et al.* 2008; Morrisey *et al.* 2011; Murray *et al.* 2012; Raghavan *et al.* 2018; Rhyne *et al.* 2012, 2013; Biondo 2017, 2018). In Australia, the data reporting framework for aquarium fish imports collated data with respect to risk groups of specific biosecurity hazards, but by doing so obscured the taxonomic resolution of imports. Developing solutions to capture more detailed information from import documents will be necessary to obtain an improved understanding of

the biodiversity imported for the Australian aquarium trade and capitalise on the value such knowledge can bring to Australia and partner trading countries.

2.5. Acknowledgements

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CHAPTER 3 PUBLICATION STATEMENT

Chapter three presents data collected during 2015-2016 as part of the Fisheries Research and Development Corporation (FRDC) project 2014/001: *Aquatic Animal Health Subprogram: Strategic approaches to identifying pathogens of quarantine concern associated with the importation of ornamental fish.* Chapter three shows data collected for myxozoan and monogenean parasites detected during this cross-sectional survey. The sections on myxozoans and monogeneans have been prepared separately for publication and are presented in this thesis in two separate sections within the single Chapter. Section one on myxozoans is currently in preparation for publication and section two on monogeneans has been published in *Parasitology Research* as follows:

Trujillo-González, A., Becker, J.A., Vaughan, D.B., and Hutson, K.S. 2018. Monogenean parasites infect ornamental fish imported to Australia. Parasitol. Res. 117, 995–1011. doi: 10.1007/s00436-018-5776-z

Furthermore, a peer-reviewed published report containing additional data pursuant to this Chapter was also prepared by the author with colleagues, but is not presented for examination as part of this thesis. It is mentioned here as an additional resource for potentially interested parties:

Becker, J. A., Hick, P., Hutson, K. S., **Trujillo-González, A.**, Tweedie, A., Miller, T., Whittington R., and Robinson A. 2016. Aquatic Animal Health Subprogram: Strategic approaches to identifying pathogens of quarantine concern associated with the importation of ornamental fish. Fisheries Research and Development Corporation, Project No 2014/001, pp. 83. ISBN 978-1-74210-399-0

CHAPTER 3

SURVEY OF PARASITES IN THE AUSTRALIAN ORNAMENTAL FISH TRADE

Abstract

The ornamental fish trade provides a pathway for the global translocation of aquatic parasites. I examined a total of 1,020 fish imported from southeast Asia, including freshwater and marine fish species, for myxozoan and monogenean parasites. Fish were received following veterinary certification that they showed no clinical signs of pests and diseases from the exporting country and visual inspection at Australian border control. Myxozoan parasites infected 8 of 13 freshwater populations and 8 of 12 marine populations. 18 putative types of myxozoan parasites and 14 putative types of monogenean were identified using a combined morphological and molecular approach. A total of 12 morphologically distinct *Myxobolus* spores were detected amongst all *Carassius auratus* Linnaeus, 1758 populations. *Myxidium* spores were detected in *Helostoma temminckii* Cuvier, 1829, and four putative *Ceratomyxa* sp. spores were detected in *Cheilodipterus quinquelineatus* Cuvier, 1828, *Pterapogon kauderni* Koumans, 1933, and *Zoramia leptocantha* (Bleeker, 1856). Monogenean diversity included seven *Dactylogyrus* spp. (including *Dactylogyrus vastator* Nybelin, 1924), and three *Gyrodactylus* spp. infecting goldfish, *C. auratus*. *Dactylogyrus ostraviensis* Řehulka, 1988, infected rosy barb, *Pethia conchonius* Hamilton, 1822, while two *Trianchoratus* spp. infected three spot gourami, *Trichopodus trichopterus* Pallas, 1970 and pearl gourami *Trichopodus leerii* Bleeker, 1852. *Urocleidoides reticulatus* Mizelle *et* Price, 1964, infected guppy, *Poecilia reticulata* Peters, 1859. Australian import conditions require mandatory treatment for goldfish with parasiticide (e.g. trichlorfon, formaldehyde, sodium chloride) for the

presence of gill flukes (*D. vastator* and *Dactylogyrus extensus* Mueller et Van Cleave, 1932) prior to export. The discovery of myxozoan and monogenean infections, including *D. vastator* in goldfish, show that pre-export health requirements and visual inspection did not reliably prevent parasite infections. Inspection prior to exportation and at border control must account for the highly cryptic nature of parasites and consider alternatives to current pre-export conditions and visual inspection at border control.

SECTION 1: MYXOZOA

3.1. Introduction

The live ornamental fish trade is a growing commodity sector, with millions of fish from multiple species traded by over 100 countries globally (Whittington and Chong 2007). Globalization, advances in technology and transport capability have facilitated the translocation of fish from remote locations and increased the number and volume of species traded. Trade of live animals at this scale presents the potential for introduction of invasive fish species and their associated pathogens to endemic ecosystems (Whittington and Chong 2007; Knight 2010; Mendoza et al. 2015). The spread of exotic pathogens can have impacts on endemic wildlife, farmed fish species and natural resources (Kolar and Lodge 2001; Lymbery et al. 2014). For this reason, governments may establish biosecurity protocols to detect and prevent the translocation of hazardous parasites and pathogens though the live ornamental trade (Whittington and Chong 2007).

Australia has strict import conditions to manage risks associated with the importation of ornamental fish species (Australian Government Department of Agriculture and Water

Resources (DAWR) 2018). Specifically, Australia has mandatory pre-export health requirements, visual inspections at border control, and post-export quarantine periods to detect parasites and pathogens imported with ornamental fish species (DAWR 2018). Nonetheless, many parasites and pathogens can be impossible to detect by visual inspections and microscopic life stages may be present in the transport water. Indeed, ectoparasitic monogenean parasite species infecting imported ornamental fishes have gone undetected during pre-export quarantine periods and border control inspections in Australia (Kahn et al. 1999; Chapter 3). Disease caused by endoparasites, such as myxozoans, is impossible to detect through visual inspection if fish are asymptomatic.

Myxozoans are ubiquitous metazoan endoparasites of importance to aquaculture (Kent et al. 2001). For example, *Ceratonova shasta* (Noble, 1950) (Hallett et al. 2012), *Enteromyxum leei* (Diamant, Lom & Dyková, 1994) (Sekiya et al. 2016), multiple *Kudoa* species (Moran et al. 1999; Kristmundsson and Freeman 2014; Marshall et al. 2015) and *Myxobolus cerebralis* Hofer, 1903 (Fetherman et al. 2011) are known to cause mortalities and economic loses in food fish aquaculture. Indeed, whirling disease caused by infection with *Myxobolus cerebralis*, is a notifyable aquatic disease in Australia (DAWR 2016b) Some *Myxobolus* spp. form plasmodia on the body surface of fish hosts and can cause severe disfigurement in farmed ornamental goldfish *Carassius auratus* (see Caffara et al. 2009; Zhang et al. 2010). The species richness and regularity with which myxozoan infections occur in imported fish in the ornamental fish trade remain largely unexplored, with few studies reporting myxozoan infections in imported ornamental species (Caffara et al. 2009; Baska et al. 2009). As such, the relative risk of myxozoan parasites being co-introduced through the ornamental trade remains poorly assessed.

Considering that there are no import risk analyses for myxozoan parasites infecting imported ornamental fish to Australia, The aim of this study was to determine myxozoan parasite species richness in ornamental fish species imported to Australia using a combined morphological and molecular approach. This study provides the first survey of myxozoan infections in the Australian ornamental fish trade, needed to assess the risks associated with myxozoan infections in ornamental fish species. Fish populations imported from southeast Asia complied with Australian Biosecurity Import Conditions (BICON) and were visually inspected at border control by quarantine inspection officers prior to release to an Approved Arrangement site (AA site) for examination by necropsy.

3.2. Methods

3.2.1. Fish importation and collection

A repeated cross-sectional survey was conducted to examine imported ornamental fish under quarantine for the presence of nationally listed aquatic pathogens that are associated with at least one ornamental fish host imported to Australia. All fish collected were considered pre-import and under quarantine at the time of testing. A total of 37 fish populations representing 11 species of freshwater fishes and seven species of marine fishes from 11 consignments were commissioned from Sri Lanka, Singapore, Indonesia and Thailand for examination of parasite fauna. A consignment was defined as all the ornamental fishes received from an exporter on a specific day. A population was defined as a single fish species received from an exporter on a specific day. Fish species were prioritized based on

prior knowledge of potential for infection with nationally listed pathogens in consultation with Department of Agriculture and Water Resources (DAWR).

On arrival to Australia, all fish were subjected to quarantine practices, which involved clearance from Australian customs and visual inspection and approval from Australian Quarantine Services. Following release by Quarantine Services (DAWR), fish were transported by road to an Approved Arrangement Laboratory at the Sydney School of Veterinary Sciences, University of Sydney, Camden, Australia, for necropsy. It is important to note that all fish populations were still under quarantine authority, as Australian import conditions require all fish released by Quarantine Services to undergo a final quarantine period for 21 days in the case of goldfish, and seven days for all other fish in an Approved Arrangement site (BICON 2018). Thirty fish were randomly selected for sampling from each population to enable 95% confidence of detecting parasite prevalence ≥ 10%, assuming 100% sampling sensitivity (Post and Millest 1991; Sergeant 2018). Apparent prevalence was presented with 95% confidence intervals using the exact binomial approximation. When there were no observed parasites, the proportion was calculated with a one-sided upper 97.5% confidence limit. Fish were euthanized inside the original transport container used by the exporters for delivery (i.e., 20 L plastic bag for freshwater fish populations and individual 5 L plastic bags for marine fish) using benzocaine (100 mg/L) within 12 hours of receipt from the DAWR, as per import conditions. To maximize the diversity of fish species available for examination within the 12-hour time limit, three large sampling events were completed in January, May and October of 2015. At each sampling event, eleven staff (including four parasitologists and one virologist) assisted with specimen preparation and dissection. Each sampling event was approximately two weeks in duration. Animal ethics approval was obtained from the University of Sydney Animal Ethics Committee (approval number: 720).

3.2.2. Tissue sampling and necropsy

Imported fish species were examined for protozoan and metazoan parasites via external macroscopic examination and necropsies of internal organs using wet mount microscopy preparations. Immediately following euthanasia, individual fish were placed in a disposable Petri dish to be photographed, weighed and measured. Then, tissue samples from each fish were sequentially dissected, placed on glass slides under a large coverslip (with either saltwater or freshwater according to the origin of the fish, or saline solution for internal organs) and examined for the presence of parasites using a compound microscope (Olympus BX41). First, skin scrapes were collected from the left dorsal area of the fish. Then the gill basket was carefully removed and placed in a cavity block with either saltwater or freshwater according to the origin of the fish, and each gill arch carefully dissected. Tissue samples were collected in order from the brain, muscle tissue in the left dorsal muscle area, liver, spleen and kidney. The gall bladder was removed onto a glass slide, immersed in saline solution under a large coverslip and carefully depressed, allowing bile to fill the glass slide. Lastly, the digestive tract was separated between the stomach and intestines, chopped with a scalpel blade and immersed in saline on a glass slide under a coverslip, followed separately by the heart in the same manner. If microspores or plasmodia were detected, microphotographs were taken using an Olympus UC50 digital camera and NISElements Basic Research 3.0 software (Nikon Corporation, Japan). Samples from each organ were placed in individual Eppendorf tubes with 70 % ethanol, labelled and stored for DNA extraction. Glass slides were left to air dry. Once dried, slides were held in slide holders with 70% ethanol for further examination.

Due to time constraints associated with the 12 h time limit of DAWR to euthanise fish, some freshwater fish were not able to be examined fresh following euthanasia. Fish not examined fresh were all photographed, measured and weighed, and underwent external examinations. Subsequently, they were preserved whole with an incision across the ventral surface to permit more rapid fixation in 70% ethanol. These fish were shipped to the Marine Parasitology Laboratory (James Cook University, Australia), and dissected following the methods described above. Parasites were detected using a compound microscope (Olympus BX53) fitted with direct interference contrast, and a dissecting microscope (Leica M60). Microphotographs were taken using an Olympus UC50 mounted camera and Labsense image analysis software (Olympus v. 1). All marine fish were examined fresh in this survey.

3.2.3. Myxozoan morphological analysis

Measurements of myxozoan parasites were made from photomicrographs using the image analysis package Fiji from Image J (Schindelin et al. 2012). Measurements were only collected from spores positioned ventrally, with the aim of obtaining a minimum of 20 spores from each putative myxozoan species from each host/parasite combination. Measurements for *Myxobolus* spore ventral length and width were collected from each spore, as well as the length, width and number of ridges of each polar capsule within each spore, following protocols by Lom and Arthur (1989) and Burger and Adlard (2011). Measurements for *Ceratomyxa* spore length and thickness, and polar capsule length and width were collected following protocols by Heiniger et al. (2008). Principal component analyses were used to create Euclidean plots and differentiate myxozoan spores by a correlation matrix using S-PLUS (v 8.0). Factors were rotated to improve data interpretation when principal factors were equally loaded (Supplementary S2).

3.2.4. DNA extraction, PCR amplification and sequencing

DNA was extracted from myxozoan spores isolated from samples stored in 70 % ethanol using a Qiagen DNeasy blood and tissue kit (QIAGEN Pty Ltd, Australia) according to the manufacturer's instructions. Ribosomal DNA for myxozoan parasites was amplified using several primer combinations (Table 5). Polymerase Chain Reaction (PCR) assays (25 µL) contained 2 µL DNA, 1 µL of each corresponding primer combination (10 nM), 12.5 µL Qiagen Hotstart Taq Master Mix (Qiagen, Australia), 4.5 or 7.25 µL MilliQ® water, and 2 or 1.25 µL of Dimethyl Sulfoxide (DMSO, Sigma-Aldrich, Australia). Reactions were performed in a Veriti® Thermal Cycler (Sigma-Aldrich, Australia) under the following cycling conditions: initial denaturation at 95°C for 15 min, followed by 40 cycles of 95°C denaturation for 30 sec then primer-specific annealing temperature for 30 sec (Table 5), 65 °C extension for 30 sec, and a final extension at 65 °C for 5 min. Amplicons were visualised on agarose gels and selected amplicons were Sanger sequenced by the Australian Genome Sequencing Facility (Brisbane, Australia). Sequences were aligned using Geneious (v10.0.9) and identity confirmed by BLAST (Johnson et al. 2008). Selected ribosomal DNA sequences were downloaded from GenBank and included sequences from recent myxosporean phylogenies (Heiniger et al. 2011). Consensus phylogenetic trees were created by Bayesian analysis (500 iterations) using Mr Bayes (v3.2.6), and nodal support was analysed by parsimony analysis in MEGA (v7).

Table 5. Primers for Myxozoa ribosomal DNA.

Primer combination	Primer	5' -Sequence- 3'	Amplicon (Bp)	Annealing temperature (˚C)	Reference
1	Act1f	GGC AGC AGG CGC GCA AAT TAC CCA A	1900	55	Hallett and Diamant 2001
	Myx4r	CTG ACA GAT CAC TCC ACG AAC			
2	Kt28S1F	CAA GAC TAC CTG CTG AAC	850	50	Whipps et al. 2004
	28S1R	GTG TTT CAA GAC GGG TCG			
3	18E	CTG GTT GAT CCT GCC AGT	1483	56	Hillis and Dixon 1991
	Mbseq1R	CAA TCC TAT CAA TGT CTG GAC CTG			Burger et al. 2008
4	Kud6F	TCA CTA TCG GAA TGA ACG	866	56	Whipps et al. 2003a
	18R	CTA CGG AAA CCT TGT TAC G			Whipps et al. 2003b
5	NLF184	ACC CGC TGA AYT TAA GCA TAT	1400	56	Heiniger and Adlard 2013
	NLR1270	TTC ATC CCG CAT CGC CAG TTC			
6	MyxospecF	TTC TGC CCT ATC AAC TWG TTG	1100	46	Heiniger et al. 2008
	18R	CTA CGG AAA CCT TGT TAC G			

3.3. Results

3.3.1. Myxozoa infecting imported fish species

Myxozoan parasites were detected in 62% (8 of 13) of freshwater populations and 66% (8 of 12) of marine populations (Table 6). A total of 12 morphologically distinct *Myxobolus* spores were detected amongst all *C. auratus* populations (Figure 4), and *Myxidium* spp. spores where found in kissing gourami, *Helostoma temminckii* Cuvier, 1829, (population 11 imported from Singapore; Table 5). Four morphologically distinct *Ceratomyxa* sp. spores were detected in five-lined cardinal fish *Cheilodipterus quinquelineatus* Cuvier, 1828, Banggai cardinal fish, *Pterapogon kauderni* Koumans, 1933, and threadfin cardinal fish. *Zoramia leptocantha* Bleeker, 1856, imported from Indonesia (Table 6, Figure 5). *Kudoa* sp. spores were detected in *C. quinquelineatus* and *Z. leptocantha* imported from Indonesia (Table 6), and *Myxidium* spores were detected in *P. kauderni* and *Z. leptocantha* (Table 6). Populations 1, 21 and 25 were seized at border control because of irregularities in their documentation and were excluded from the survey (i.e., Siamese fighting fish, *Betta splendens* Regan, 1910, *T. trichopterus* and *X. maculatus*, respectively, Table 6). Fish from populations 12-20 were degraded despite ample fixation and deemed inadequate for recovery of optimal myxozoan parasites. These populations were excluded from this study (Table 6).

Table 6. Apparent prevalence of myxozoan parasites infecting imported ornamental fish. All freshwater species were farmed in their country of origin, while all marine species were wild caught. Thirty fish were examined from each population unless stated otherwise. Populations 12-20 were excluded from this study. *=populations were sacrificed at border control by quarantine officers and were not sampled during this study.

Population	Fish Species	Environment	Sample date	Exporter I.D.	Parasite species	infected fish	Apparent Prevalence % (95% CI)
1*	*Beta splendens*	Freshwater	29/10/2015	Sri Lanka	Not sampled		
2	*Beta splendens*	Freshwater	30/10/2015	Malaysia	Not detected		
3	*Carassius auratus*	Freshwater	3/06/2015	Singapore	*Myxobolus sp.*	10	33.3 (17.3-52.8)
4	*Carassius auratus*	Freshwater	5/06/2015	Singapore	*Myxobolus* sp.	10	33.3 (17.3-52.8)
5	*Carassius auratus*	Freshwater	5/06/2015	Thailand	*Myxobolus* sp.	16	53.3 (34.3-71.7)
6	*Carassius auratus*	Freshwater	28/10/2015	Thailand 1	*Myxobolus* sp.	6	20 (7.7-38.6)
7	*Carassius auratus*	Freshwater	28/10/2015	Thailand 2	*Myxobolus* sp.	5	16.7 (5.6-34.7)
8	*Carassius auratus*	Freshwater	30/10/2015	Malaysia	*Myxobolus* sp.	15	50 (31.3-68.7)
9	*Carassius auratus*	Freshwater	30/10/2015	Malaysia	*Myxobolus* sp.	12	40 (22.7-59.4)
10	*Danio rerio*	Freshwater	6/01/2015	Sri Lanka	Not detected		
11	*Helostoma temminckii*	Freshwater	27/05/2015	Singapore	*Myxidium* sp.	3	10 (2.1-26.5)
21*	*Trichopodus trichopterus*	Freshwater	29/10/2015	Sri Lanka	Not sampled		
22	*Xiphophorus hellerii*	Freshwater	29/10/2015	Sri Lanka	Not detected		
23	*Xiphophorus maculatus*	Freshwater	6/05/2015	Thailand	Not detected		
24	*Xiphophorus maculatus*	Freshwater	3/06/2015	Singapore	Not detected		
25*	*Xiphophorus maculatus*	Freshwater	29/10/2015	Sri Lanka	Not sampled		
26	*Amphiprion bicintus*	Marine	22/10/2015	Indonesia	Not detected		
27	*Amphiprion ocellaris*	Marine	28/05/2015	Indonesia	Not detected		
28	*Amphiprion ocellaris*	Marine	23/10/2015	Indonesia	Not detected		
29	*Amphiprion sebae*	Marine	27/05/2015	Singapore	*Coccomyxa* sp.	1	3.3 (0.1-17.2)
30	*Cheilodipterus quinquelineatus*	Marine	23/01/2015	Indonesia	*Kudoa* sp.	7	23.3 (9.9-42.3)
31	*Cheilodipterus quinquelineatus*	Marine	28/05/2015	Indonesia	*Ceratomyxa* sp.	4	13.3 (3.8-30.7)
32	*Pterapogon kauderni*	Marine	16/01/2015	Singapore	*Ceratomyxa* sp. *Myxidium* sp.	15 3	50 (31.3-68.7) 10 (2.1-26.5)
33	*Pterapogon kauderni*	Marine	20/01/2015	Singapore	*Ceratomyxa* sp.	18	60 (40.6-77.3)
34	*Pterapogon kauderni*	Marine	22/01/2015	Indonesia	*Ceratomyxa* sp.	18	60 (40.6-77.3)
35	*Sphaeramia nematoptera*	Marine	22/10/2015	Indonesia	Not detected		
36	*Zoramia leptocantha*	Marine	28/05/2015	Indonesia	*Kudoa* sp.	6	20 (7.7-38.6)
37	*Zoramia leptocantha*	Marine	23/10/2015	Indonesia	*Myxidium* sp.	3	10 (2.1-26.5)

Populations 3 and 11 had one mortality at the time of sampling. Mortalities were excluded, and examinations were done from a total of 29 examined fish for each population

Not detected = apparent prevalence = 0% (95% CI 0–11.4%)

3.3.2. Morphometric analysis

Principal component analysis (PCA) supported the morphological separation of *Myxobolus* spores detected infecting imported goldfish populations (Figure 4). Spore length contributed the most to differences in the morphometric analysis (Figure 4). *Myxobolus* spores 1, 2, 7, 9, 10, 11 and 12 were distinct in spore length and width while *Myxobolus* spores 3, 4 and 5, and 6 and 8 grouped within two similar clusters, respectively (Figure 4). All 12 *Myxobolus* spores displayed similar shape and capsule morphology (Figure 4, Table 7), consistent with over 48 *Myxobolus* species reported for *C. auratus* in southeast Asia (Eiras et al. 2005; Eiras et al. 2014). Within these species, *Myxobolus diversus* Nie and Li, 1973, *Myxobolus turpisrotundus* Zhang, Wang, Gong 2010, and *Myxobolus lentisuturalis* Dyková, Fiala and Nie, 2002, have been reported infecting farmed ornamental *C. auratus* (see Chapter 4) and have consistent measurements and myxosporean morphology as the spores reported in this study.

The PCA for *Ceratomyxa* spores indicated that spore length contributed the most to differences in the morphometric analysis (Figure 5). However, it is important to consider that all samples for marine fishes were examined using fresh mounts, and spore length may have been affected by positioning of *Ceratomyxa* polar extensions (Figure 5). *Ceratomyxa* spores 1, 3 and 4 were detected infecting the gall bladder of *P. kauderni* imported from Singapore (population 32, Figure 5, Table 8), while spore 2 was detected infecting *C. quinquelineatus* (population 31), and *P. kauderni* (populations 32 and 34) (Figure 5, Table 8). This study provides the first record of *Ceratomyxa* species infecting wild caught *C. quinquelineatus* and *P. kauderni*. Spore measurements and spore morphology reported by this study were

consistent with previous records of *Ceratomyxa cardinalis* Heiniger & Adlard, 2013, *Ceratomyxa talboti* Gunter & Adlard, 2008, and *Ceratomyxa ireneae* Heiniger & Adlard, 2013 (Heiniger and Adlard 2013). However, it was not possible to confirm the identity of *Ceratomyxa* spores in this study because no sequences were amplified using selected primers (Table 5). *Kudoa* spp. spores were detected infecting *C. quinquelineatus* and *P. kauderni* imported from Indonesia (Table 6, Table 8), however, staining was inadequate for morphological diagnosis of *Kudoa* spores found infecting populations 32, 33, 35, 36 and 37.

Table 7. Morphometric comparison of *Myxobolus* spores found infecting imported *Carassius auratus* populations and similar *Myxobolus* species. LPC: Large Polar Capsule, SPC: Small Polar Capsule, PC: Polar Coils. Mean measurements are provided in micrometres (range), taken from microphotographs of preserved material. It was not possible to count polar coils. *Myxobolus turpisrotundus*, and *Myxobolus kingchowensis* have been reported in multiple tissues and are provided for comparison with *Myxobolus* spores in this study. Measurements for *M. kingchowensis* are provided from two separate studies to highlight phenotypic plasticity (i.e., Eiras et al. 2005; Zhao et al. 2008).

Population/ Reference	*Myxobolus* spore/species	Host location	Locality	n	Spore length	Spore width	LPC length	LPC width	SPC length	SPC width	PC
3	6	Brain	Singapore	1	13.5	7.44	6.48	2.09	5.49	1.44	-
7	6		Thailand 2	1	14.29	7.59	6.78	2.71	6.2	2.87	-
7	3	Digestive tract	Thailand 2	1	11.62	7.01	6.35	2.54	6.17	2.33	-
8	6		Malaysia	2	13.21 (12.82—13.60)	8.07 (7.92—8.22)	6.99 (6.80—7.17)	2.98 (2.93—3.02)	5.90(5.83—5.98)	2.54 (2.51—2.58)	-
Eiras et al. 2005	*Myxobolus wushingensis*	Intestines	China	-	11.0 (10.8–12)	8.7 (8.2–9.6)	6.8 (6.0–8.2)	3.2 (2.6–3.6)	-	-	5–7
3	6	gall bladder	Singapore	2	13.61 (13.33—13.89)	7.311 (7.12—7.5)	6.26 (6.04—6.47)	2.58 (2.17—2.99)	5.66 (4.98—6.34)	2.53 (2.41—2.64)	-
4	6		Singapore	1	13.57	8.01	7.92	3.82	5.92	2.99	3
8	6		Malaysia	11	13.47 (12.17—14.39)	7.88 (6.97—8.36)	6.63 (5.62—8.11)	2.75 (2.15—3.09)	5.57 (4.42—6.28)	2.32 (1.74—2.74)	3
9	6		Malaysia	4	13.78 (13.34—14.49)	7.84 (7.57—8.11)	6.81 (6.53—7)	2.95 (2.66—3.32)	6.38 (5.92—6.65)	2.99 (2.88—3.10)	3
Eiras et al. 2005	*Myxobolus changkiangensis*		China	-	12.2 (10.8–13.4)	8.8 (8.4–9.6)	6.7 (6.0–7.2)	3.4 (3.1–3.6)	6.7 (6.0–7.2)	3.4 (3.1–3.6)	5—6
3	10	Gills	Singapore	16	16.5 (14.85—18.08)	8.44 (7.28—9.24)	7.68 (6.95—8.98)	2.89 (2.60—3.37)	6.90 (5.66—7.45)	2.78 (2.01— 3.37)	-
Zhao et al. 2008	*Myxobolus ampullicapsulatus*		China	-	18 (16.5—19.5)	9.3 (8.5—10.0)	8.5 (7.0—10.0)	3 (2.5— 4.0)	8.5 (7.0—10.0	3 (2.5— 4.0)	9—10
Eiras et al. 2005	*Myxobolus tanakai*		Japan	40	17.2 (15.4–18.6)	6.8 (6.3–8.4)	8.7 (7.6–9.4)	2.4 (2.0–2.7)			8–10
3	5	Heart	Singapore	1	11.428	7.296	6.114	2.491	4.927	2.19	-
3	11		Singapore	1	14.13	12.53	6.38	3.38	6.19	2.88	-
4	6		Singapore	1	12.37	7.69	6.73	2.91	4.69	2.44	-
9	6		Malaysia	1	13.46	7.38	7.29	3.08	6.29	2.44	-
Eiras et al. 2005	*Myxobolus hearti*	Heart	China	-	14.8 (13.2–15.8)	11.2 (10.4–12)	7.0 (6.6–7.2)	3.4 (3.0–3.6)	7.0 (6.6–7.2)	3.4 (3.0–3.6)	7–8
3	9	Kidney	Singapore	1	17.11	9.42	8.16	2.902	7.37	3.20	-
4	2		Singapore	1	9.10	6.71	5.25	2.56	4.41	2.36	-
4	5		Singapore	1	10.89	6.309	5.397	2.414	4.691	2.632	
4	6		Singapore	2	13.94 (13.46—14.42)	7.70 (6.93—8.47)	6.58 (6.12—7.03)	2.60 (2.44—2.76)	5.55 (4.68—6.42)	2.62 (2.59—2.66)	-

5	5		Thailand	1	11.43	6.98	5.72	2.39	5.68	2.25	-
8	4		Malaysia	2	11.62 (10.78 — 12.46)	7.28 (6.84—7.72)	6.00 (5.87—6.14)	2.72 (2.46—2.99)	4.81 (4.02—5.61)	2.10 (1.80—2.41)	-
8	12		Malaysia	1	10.02	6.27	4.14	5.42	3.562=	2.88	-
9	7		Malaysia	6	14.73 (13.85—15.90)	7.19 (6.58—7.75)	7.43 (6.73—8.11)	2.83 (2.48—3.13)	6.28 (4.97—7.22)	2.38 (1.98—2.5)	3
Eiras et al. 2005	*Myxobolus auratus*	Kidney	China	-	15.6 (15–16.2)	14 (13.8–14.4)	8.3 (7.8–8.6)	5.5 (4.8–6)	8.3 (7.8–8.6)	5.5 (4.8–6)	6—8
Eiras et al. 2005	*Myxobolus echengensis*	Kidney	China	-	14.4 (13.2–15.6)	9.4 (9.0–10.2)	7.3 (6.6–8.4)	3.5 (3.0–3.6)	7.3 (6.6–8.4)	3.5 (3.0–3.6)	6—7
8	1	Liver	Malaysia	1	9.53	6.47	5.05	2.19	4.29	2.3	-
9	11		Malaysia	7	14.71 (14.1—15.04)	10.3 (9.90—10.76)	6.81 (6.21—7.23)	3.57 (3.04—4.21)	6.72 (5.89—7.52)	3.47 (2.98—3.83)	-
Eiras et al. 2005	*Myxobolus pekingensis*	Liver	China	-	14.3 (13.2–15.6)	10.6 (8.4–13)	6.1 (6.0–6.6)	3.5 (3.0–3.6)	6.1 (6.0–6.6)	3.5 (3.0–3.6)	6—7
3	5	Muscle	Singapore	1	11.55	6.72	6.08	2.35	5.3	2.03	-
4	8		Singapore	13	14.72 (13.56—15.85)	8.75 (7.82—9.41)	5.97 (4.74—6.73)	2.78 (2.22—3.30)	5.96 (5.17—6.47)	2.72 (2.32— 3.04)	-
9	8		Malaysia	9	14.06 (13.41—14.80)	7.12 (6.41—7.56)	6.84 (6.11—7.58)	2.75 (2.26—3.13)	5.76 (5.25—6.55)	2.44 (1.80—2.87)	3
Caffara et al. 2009	*Myxobolus lentisuturalis*	Muscle	China	-	11.8 (11.2–12.4)	7.6 (7.2–8.4)	4.2 (4.0–4.4)	2.5 (2.0–2.8)	4.2 (4.0–4.4)	2.5 (2.0–2.8)	4
3	8	Spleen	Singapore	3	13.57 (11.64—14.95)	7.43 (7.27—7.69)	6.37 (5.52—8.03)	2.42 (2.02—2.66)	5.51 (4.98—6.38)	2.11 (1.68—2.61)	-
4	8		Singapore	3	13.57 (12.91— 14.89)	7.25 (6.36—8.14)	6.77 (6.21—7.89)	2.78 (2.27—3.64)	6.01 (5.06—6.74)	2.5 (2.17—2.84)	-
7	8		Thailand	1	13.29	8.40	6.93	3.43	7.05	3.12	-
Zhang et al. 2010	*Myxobolus turpisrotundus*	Subepidermal tissues of skin, Intestinal cavity	China		8.6–10.0	8.2–10.0	4.1–5.1	2.5–3.1	4.1–5.1	2.5–3.1	5—6
Eiras et al. 2005	*Myxobolus kingchowensis*	Almost all organs	China	-	10.7 (9.6–12)	8.3 (7.2–8.4)	7.2 (6.2–8.4)	3.4 (2.6–3.6)	-	-	3—4
Zhang et al. 2018	*Myxobolus kingchowensis*	kidney, muscle	China	-	11.21 (9.63–12.20)	8.43 (7.83–9.14)	7.38 (6.01–8.14)	3.54 (3.01–3.93)	5.98 (5.05–6.81)	2.93 (2.14–3.32)	3—5

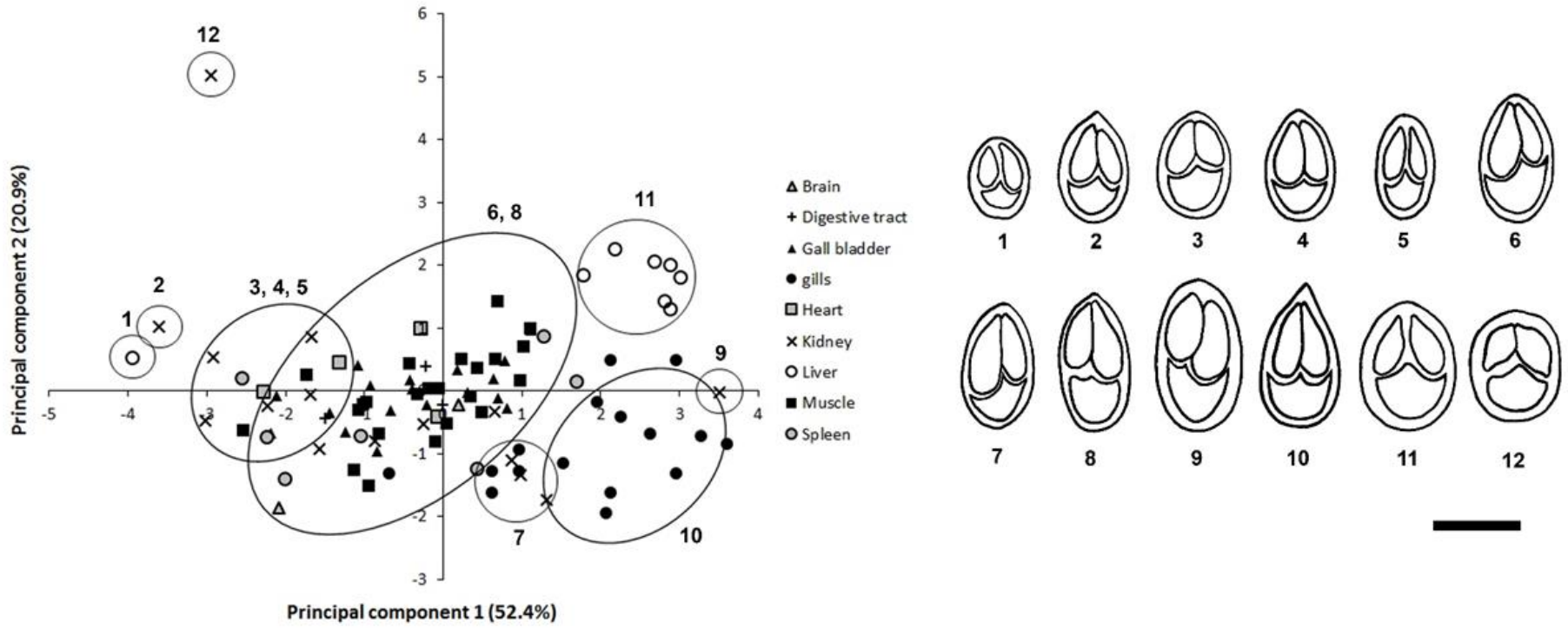

Figure 4. Principal Component analysis (PCA) of *Myxobolus* mature spores found in tissue samples from *Carassius auratus*. Drawings for 12 morphologically distinct spores are provided in congruence with the PCA analysis. Principal component 1 (spore length) explained 52.4% of variation, while Principal Component 2 (spore width) explained 20.9% of variation. Scale bar = 10 µm.

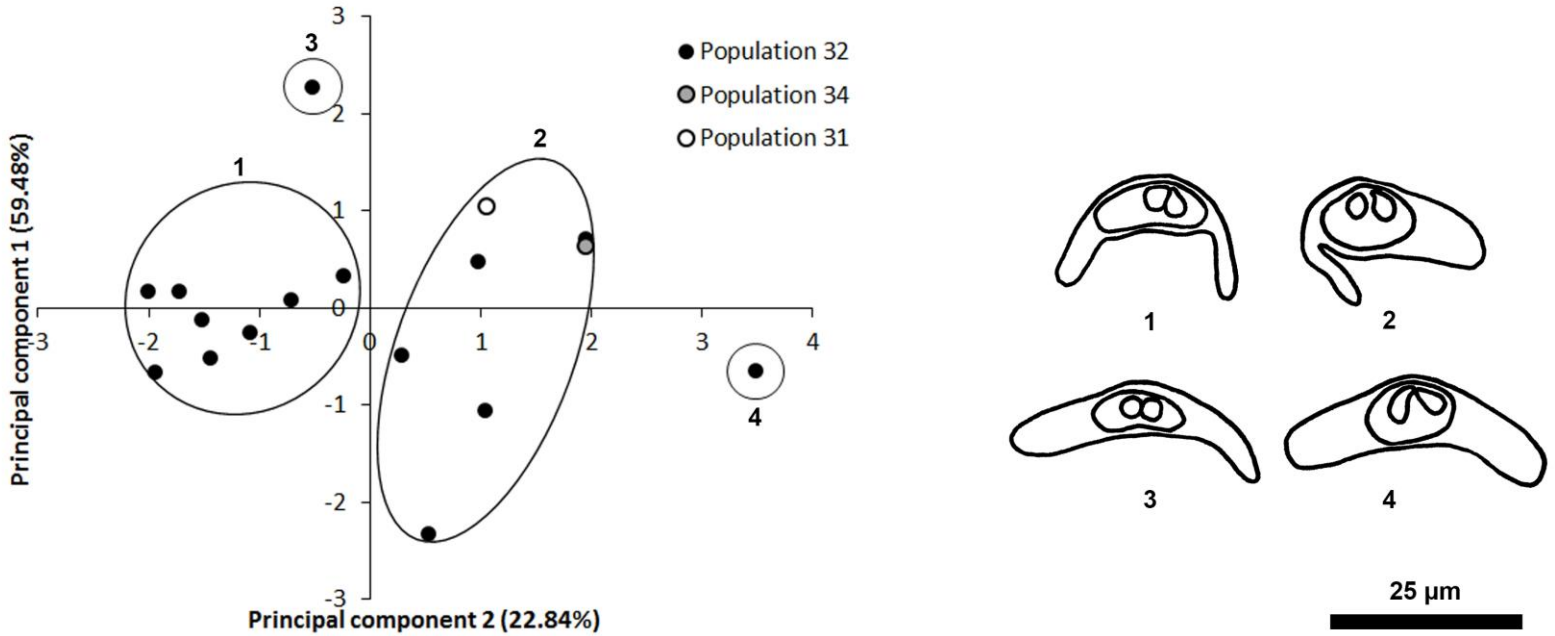

Figure 5. Principal Component Analysis (PCA) of *Ceratomyxa* spores found in marine fish populations. Drawings for four morphologically distinct *Cetatomyxa* spores are provided in congruence with the PCA analysis. Principal component 1 (spore length) explained 59.48% of variation, while Principal Component 2 (polar capsule width) explained 22.84% of variation. Scale bar = 25 µm

Table 8. Measurements from *Ceratomyxa* spores found infecting the gall bladder of marine fish imported to Australia and comparison to similar *Ceratomyxa* species. LPC: Large Polar Capsule, SPC: Small Polar Capsule, PC: Polar Coils. Mean measurements are provided in micrometres (range). Mean measurements are provided in micrometres (range) taken from microphotographs of freshly mounted material.

Population/ reference	**Fish Species**	**Exporter I.D.**	***Ceratomyxa* spore/species**	**n**	**Spore length**	**Spore thickness**	**PC length**	**PC width**
31	*Cheilodipterus quinquelineatus*	Indonesia	2	1	6.04	20.1	2.78	2.68
32	*Pterapogon kauderni*	Singapore	1	8	4.88 (3.54—5.76)	12.7 (10.17—16.44)	2.43 (2.21—2.83)	1.94 (1.69—2.29)
			2	5	6.03 (4.23—7.34)	22.00 (19.64—24.12)	2.99 (2.59—3.53)	2.01 (1.48—2.53)
			3	1	4.573	18.403	2.105	2.909
			4	1	8.001	31.33	3.267	2.377
34	*Pterapogon kauderni*	Indonesia	2	1	6.392	26.602	2.894	2.601
Sanil et al. 2017	*Chaetodon collare*	India	*Ceratomyxa collarae*	30	5.20 (4.54–5.92)	16.32 (15.2–19.76)	2.23 (1.98–2.53)	2.24 (1.94–2.53)
Gunter et al. 2009	*Epinephelus quoyanus*	Australia	*Ceratomyxa hooperi*		4.9 (4.0–5.5)	12.9 (10.0–15.5)	1.5 (1.0–2.0)	1.4 (1.0–2.0)
Heiniger et al. 2008	*Thalassoma lunare*	Australia	*Ceratomyxa thalassoma*		5.0 (3.3–6.4)	18.9 (16.4–22.2)	2.9 (2.2–3.3)	2.8 (2.2–3.0)
Heiniger and Adlard 2013	*Archania funcata*	Australia	*Ceratomyxa ireneae*	30	5.2 (4.5–6.2)	14.5 (12.2–17.3)	2.0 (1.6–2.3)	1.7 (1.5–2)
Heiniger et al. 2008	*Oxycheilinus digramma*	Australia	*Ceratomyxa oxycheilinae*	30	9.4 (8.3–10)	29.8 (22.8–33.9)	3.0 (2.5–3.6)	2.8 (2.4–3.3)

3.3.3. Molecular analysis

A total of four separate 1225 bp fragments from the 18s gene region were generated from *Myxobolus* isolates infecting goldfish populations 7-9 (Figure 6). All fragments were 98.3-99 % homologous to *Myxobolus kingchowensis* Chen & Ma, 1998 (KP400625), clustering together in a single clade (Figure 6). No sequences were recovered for any *Myxidium* species infecting *H. temminckii*.

A total of two 680 bp fragments in the 28s gene region and one 835 bp fragment in the 18s region were amplified from *Kudoa* isolates infecting *C. quinquelineatus* and *Z. leptocantha* (populations 31 and 36, respectively, table 6) using primer pair 5 for 28s primer pair 6 for 18s (Table 5). *Kudoa* sp. fragments for the 28s and 18s gene regions strongly supported single clades with *Kudoa cheilodipteri* Heiniger, Cribb & Adlard, 2013, collected from *C. quinquelineatus* in Australia (Figure 7). The *Coccomyxa* sp. fragment amplified in this study was 845 bp in length within the 28s gene region and formed a single clade with all other five *Coccomyxa* sequences used in this study (Figure 8). This sequence, amplified from the gall bladder of *A. sebae* imported from Singapore, formed a weakly supported clade with *Coccomyxa* sp. (DQ323043) infecting *Istiblennius edentulus* (Forster & Schneider, 1801) from Israel in the Red Sea (Figure 8).

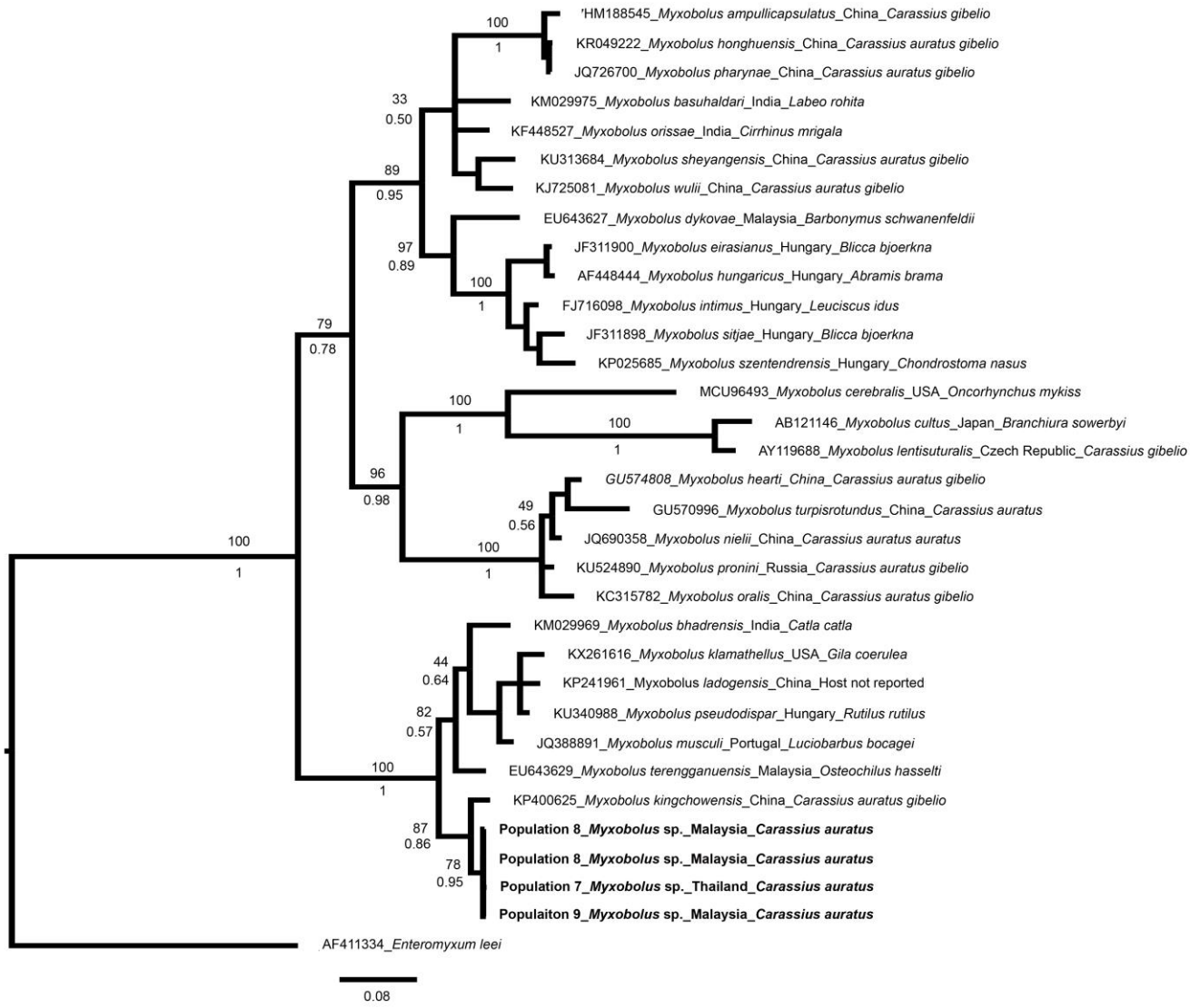

Figure 6. Consensus phylogenetic tree of *Myxobolus* sp. gene region 28s estimated by Bayesian analysis. *Enteromyxum leei* (Genbank No. AF411334) was used as the outgroup sequence. Best-fit evolutionary model was *Kimura 2-parameter model + Gamma distribution*. Nodal support is shown by bootstrap percentages from the parsimony analysis (above, 100 bootstrap iterations) and Bayesian posterior probabilities (below, 500 iterations). Sequences in bold are from this study.

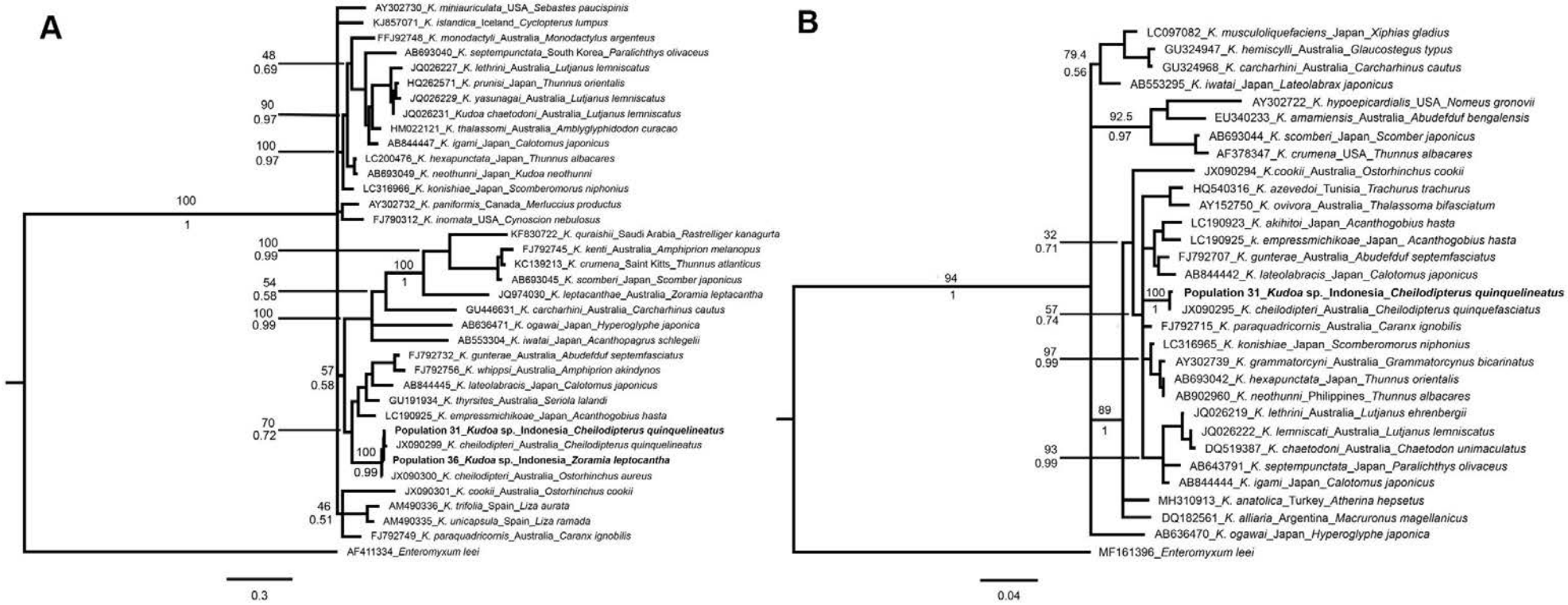

Figure 7. Consensus phylogenetic tree of *Kudoa* spp. gene regions 28s (A) and 18s (B) estimated by Bayesian analysis. *Enteromyxum leei* Genbank No. AF411334 (A) and MF161396 (B) were used as outgroup sequences. Best-fit evolutionary models were *General Time Reversible + Invariable Gamma distribution* for 28s, and *Tamura 3-parameter model + Invariable Gamma distribution* for 18s. Nodal support is shown by bootstrap percentages from the parsimony analysis (above, 100 bootstrap iterations) and Bayesian posterior probabilities (below, 500 iterations). Sequences in bold are from this study.

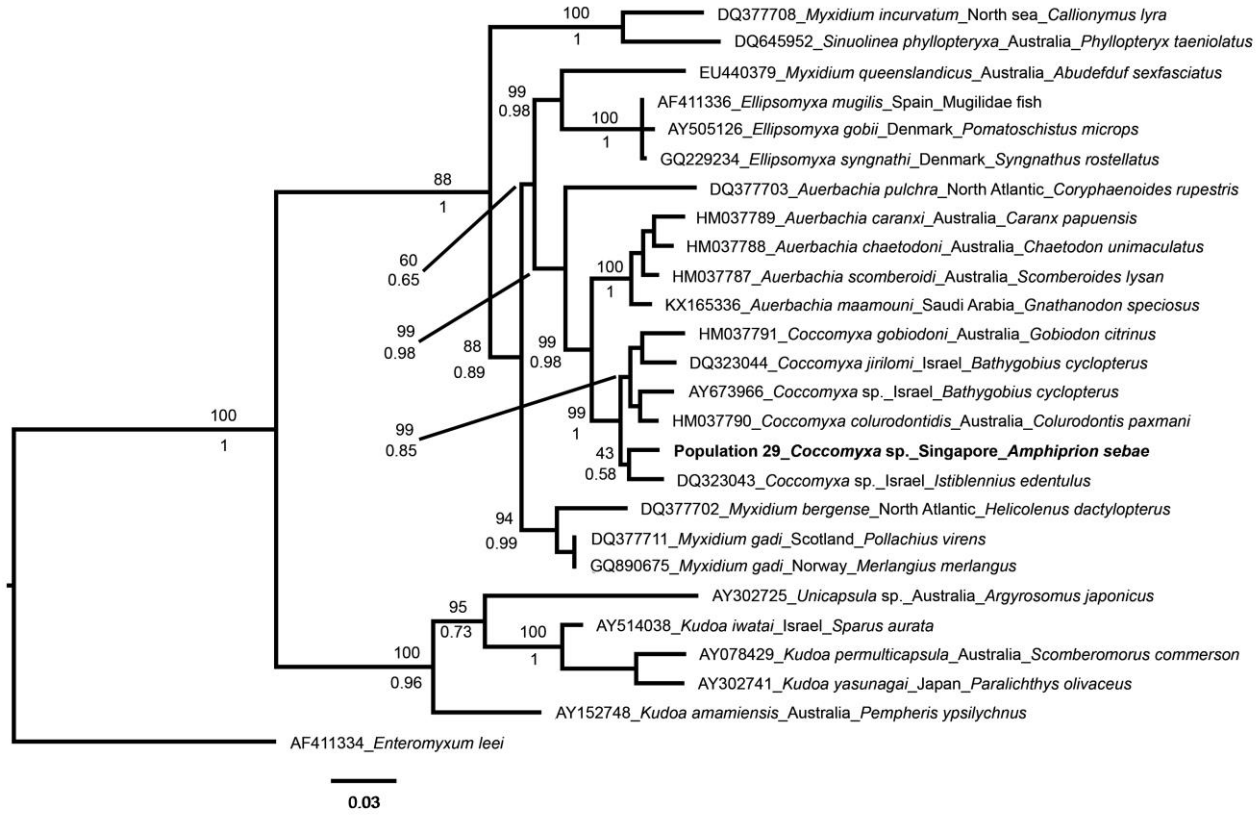

Figure 8. Consensus phylogenetic tree of *Coccomyxa* sp. gene region 28s estimated by Bayesian analysis. *Enteromyxum leei* (Genbank No. AF411334) was used as the outgroup sequence. Best-fit evolutionary model was *Tamura 3-parameter model + Invariable Gamma distribution*. Nodal support is shown by bootstrap percentages from the parsimony analysis (above, 100 bootstrap iterations) and Bayesian posterior probabilities (below, evolutionary model= T92+G+I, 500 iterations). Sequence in bold is from this study.

3.4. Discussion

Myxozoan infections were detected in 64% (16 of 25) of ornamental fish populations imported to Australia following veterinary certification from the exporting countries and inspection at the Australian border control. *Myxobolus* spp. were detected in all goldfish, *C. auratus* populations, while *Ceratomyxa*, *Kudoa* and *Myxidium* spp. infections were detected

in 66% of marine populations. *Myxobolus kingchowensis* was confirmed by Sanger sequencing in 3/7 *C. auratus* populations imported from Malaysia (populations 8-9) and Thailand (population 7; Figure 6). *Kudoa cheilodipteri* was confirmed infecting *C. quinquelineatus* (population 31) and *Z. leptocantha* (population 31) imported from Indonesia, and one unidentified *Coccomyxa* species was confirmed by Sanger sequencing infecting *A. sebae* (population 29) imported from Singapore (Table 6). This study provides the first record of *Ceratomyxa*, *Kudoa* and *Myxidium* species infecting wild caught *C. quinquelineatus*, *P. kauderni* and *Z. leptocantha* from southeast Asia. Detecting myxozoan infections in such a high number of fish populations was unsurprising given they are endoparasitic and that there are currently no import requirements for myxozoan parasites infecting ornamental fish species imported to Australia (DAWR, 2018).

Severe myxozoan infections can cause significant tissue hyperplasia, macroscopic cysts and erosive necrotic lesions on the host fish (Kent et al. 2001; Morsy et al. 2012; Saha and Bandyopadhyay 2017). Detections of myxozoan infections in this study however, were mostly of mature spores present in internal organs with no instances of obvious external hyperplasia or superficial plasmodia. Myxozoan spores are impossible to detect with the naked eye and were consequently undetected by visual inspection at border control. Myxozoan infections are considered emerging threats to ornamental aquaculture development and have been associated with significant fish mortalities and economic losses (Saha and Bandyopadhyay 2017). Most importantly, an increasing number of myxozoan species are commercially important pathogens of fish, with multiple myxozoan species associated with economic losses in aquaculture (Shinn et al. 2015). Considering the risk myxozoan infections present to the ornamental trade and aquaculture production, it is imperative to review current biosecurity measures used to detect parasites infecting imported ornamental fish species,

given that visual inspections fail to detect subclinical infections and assess the effectiveness of alternative detection methods.

Adequate treatments against myxozoan infections have not been assessed for Australian biosecurity. Current treatments for myxozoan infections are poorly studied beyond the scope of aquaculture, nonetheless, infection intensities of the myxosporean stage of *Kudoa neurophila* (Grossel, 2003) in hatchery reared *Latris lineata* (Bloch & Schneider 1801) were reduced to 0% when treating source water with dose-controlled ultraviolet irradiation ≥ 44 mJ cm^{-2} UV (Cobcroft and Battaglene 2013). Ozonating source water with > 700 mV Oxidation-Reduction Potential for 10 min prevented *K. neurophila* infections (Cobcroft and Battaglene 2013). Sand and cartridge filtration of seawater (filtration< 5 μm), followed by ultraviolet (UV) irradiation at a dose of 46 mJ/cm^2 was shown to prevent *Kudoa septempunctata* Matsukane, Sato, Tanaka, Kamata, and Sugita-Konishi, 2010, from infecting farmed *Paralichthys olivaceus* (Temminck & Schlegel, 1846) (see Nishioka et al. 2016). Considering that DAWR places emphasis on managing biosecurity risks off-shore at exporting countries (Hood and Perera 2016), future risk analyses should assess treatment conditions of water sources by exporting companies and consider the inclusion of source water treatment or rearing requirements prior to export to Australia.

Species delineation of myxozoans is challenging solely through morphological features alone because of varation in measurements following preparation, few distinct morphological features and phenotypic plasticity. Similarities in spore measurements, size and morphology prevented delineation of *Ceratomyxa* and *Myxobolus* types to species level. Diagnostic features for Myxozoa have been recently questioned given the possibility of intra-

specific phenotypic plasticity and the lack of genetic sequences available for comparison (Smothers et al. 1994; Sanil et al. 2017; Zhang et al. 2018). As such, studies have questioned the validity of many myxozoan species because of their incomplete and purely morphological descriptions, insufficient comparison with other known species, and recent revision of taxonomic criteria for myxozoans (Zhang et al. 2010). Future research should complement myxozoan morphological descriptions with accessioned sequences, which would greatly improve current knowledge of myxozoan parasite diversity (Zhang et al. 2018).

This study was limited by the amount of available genetic sequences for myxozoan species in public databases. Myxozoa are a relatively novel group of organisms that have received minimal attention on their genetic diversity, life histories and host specificity (Zhang et al. 2018). As such, molecular data for gene regions of myxozoan species is currently lacking, limiting previous research examining myxozoan diversity (Shahar et al. 2017). Traditionally, genetic sequences for Myxozoa have been the result of phylogenetic studies using Small Subunit RNA (SSU RNA) as a basis of evolutionary inference (e.g. 18S, 16S; Heiniger et al. 2011; Hallett and Diamant 2001; Whipps et al. 2004; Hillis and Dixon 1991; Burger et al. 2007, Whipps et al. 2003a, Whipps et al. 2003b; Heiniger and Adlard 2013). Such sequences, which are beneficial for evolutionary studies of Myxozoa, lack the genetic variability of less conserved gene regions that can offer species-level resolution (e.g. Internal Transcribed Spacer regions; Trujillo-González et al. 2018b). Future research should consider targeting variable gene regions with species-level resolution and accessioning comprehensive nucleotide sequence data on myxozoan species and corresponding morphological taxonomy to improve current knowledge on myxozoan diversity infecting fish species in the aquarium trade.

In conclusion, this study showed that despite stringent pre-export quarantine and border control requirements, myxozoan infections were undetected by visual inspections at border control. Myxozoan parasites are emerging threats that can cause considerable economic losses to ornamental and food fish aquaculture and should be assessed as potential risks for biosecurity in the ornamental fish trade. Future research should explore adequate treatments of source water to prevent myxozoan infections in farmed fish species, and biosecurity measures should analyse the risks of exotic myxozoan parasites with imported ornamental species. This study highlights the need for comprehensive genetic databases for the improvement of parasitological research and understanding of emerging parasite threats for biosecurity.

3.5. Acknowledgements

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SECTION 2: MONOGENEA

3.6. Introduction

The ornamental fish trade is characterised by the aggregation of numerous species from multiple source countries. It comprises wild and cultured fishes, invertebrates and plants, from which Asia accounts for 51% of exports, Europe for 29%, North America 4% and South America 6% (Monticini 2010). Animals are frequently sourced from developing countries in the tropics into high value-added wholesale markets such as Singapore and Spain with on-sale to developed countries (Whittington and Chong 2007; Monticini 2010). More than one billion ornamental fish were traded throughout the world in 2005 comprising over 5000 fish species (Hensen et al. 2010).

The aquarium trade has been associated with the introduction of exotic fish, plant, and invertebrate species globally (Lintermans 2004; Padilla and Williams 2004; Rixon et al. 2005; Cohen et al. 2007; Cobo et al. 2010; Duggan 2010). Ornamental fishes present a high risk for introducing exotic parasites into non-native environments following the release of exotic fishes into the wild (Lintermans 2004; Freyhof and Korte 2005; Whittington and Chong 2007; Corfield et al. 2008). To minimize transboundary disease spread, government authorities follow the agreements of the World Trade Organisation (WTO), including the agreement on the Application of Sanitary and Phytosanitary (SPS) measures to set an acceptable risk level for their authority (Whittington and Chong 2007). However, despite strict quarantine practices, there have been incidents of parasites infecting ornamental fish remaining undetected at quarantine (Evans and Lester 2001), and of parasites being co-

introduced into ecosystems via translocation of live ornamental fish (Dove and Ernst 1998; Kahn et al. 1999; Hassan et al. 2008).

Monogenean flukes are a class of important helminth parasites of wild and farmed fish. Monogeneans have direct life cycles and multiple reproductive strategies ranging from sexual reproduction to reproduction in isolation, enabling rapid proliferation in closed environments (Whittington 1996; Whittington and Chisholm 2008; Dinh-Hoai and Hutson 2014; Kearn and Whittington 2015). Monogeneans are well known to infect imported ornamental fish species (Di Cave et al. 2000; Mousavi et al. 2009; Iqbal and Haroon 2014). Specifically, there are cases of monogeneans infecting introduced exotic fish in wild environments, such as *Dactylogyrus anchoratus* (Dujardin, 1845) and *Gyrodactylus kobayashii* Hukuda, 1940, infecting invasive goldfish *Carassius auratus* Linnaeus, 1758, and koi carp *Cyprinus carpio* Linnaeus, 1758, respectively, in Australia (Dove and Ernst 1998). There is also evidence for exotic monogeneans infecting native fish populations following their initial co-introduction with an infected exotic host (i.e., co-invasion; Lymbery et al. 2014). For example, native *Cichlasoma callolepis* (Regan, 1904) and *Cichlasoma fenestratum* (Regan, 1904) became infected with exotic *Cichlidogyrus longicornis* Paperna and Thurston, 1969, *Cichlidogyrus sclerosus* Paperna and Thurston, 1969, *Cichlidogyrus tilapiae* Paperna, 1960, and *Enterogyrus malmbergi* Bilong, 1998, following the release of African cichlids *Oreochromis aureus* (Steindachner, 1864) and *Oreochromis niloticus* (Linnaeus, 1758) in Mexico (Jiménez-García et al. 2001).

The ornamental fish supply to Australia is largely dominated by imports of Asian origin (Australian Bureau of Agricultural and Resource Economics and Sciences (ABARES) 2016). Australian Biosecurity import conditions (BICON) require all imported fish to be

inspected and certified to show no clinical signs of pests and diseases seven days prior to importation by an approved veterinarian in the exporting country (DAWR 2018). Additional conditions apply for 'gouramis', 'bettas', 'paradise fish', 'cichlids' and 'poeciliids', which must be tested for megalocytiviruses (categories as per DAWR 2018). Furthermore, goldfish, *Carassius auratus* must be free from spring viraemia of carp (SVC) virus and *Aeromonas salmonicida* (Lehmann and Neumann, 1896), and must be specifically treated with a parasiticide (e.g. trichlorfon, formaldehyde, sodium chloride) during the seven days prior to export to Australia to eliminate infestations by gill monogeneans *Dactylogyrus vastator* Nybelin, 1924 and *Dactylogyrus extensus* Mueller *et* Van Cleave, 1932 (DAWR 2018). On arrival to Australia, fish are visually inspected by the Australian Quarantine Service for signs of infection and disease. Nonetheless, visual inspections do not account for hidden, microscopic pests, with cases of exotic viruses going undetected at quarantine and entering Australia via the ornamental fish trade (Becker et al. 2014; Rimmer et al. 2015). For this reason, the Department of Agriculture and Water Resources, Australia, is reforming current biosecurity protocols by placing greater emphasis on managing biosecurity risks off-shore at exporting countries (Hood and Perera 2016). However, monogeneans are nearly impossible to detect with the naked eye (Whittington 1996), and may be undetected, despite preventative measures to prohibit their movement. The aim of this study was to determine whether monogenean parasites enter Australia with live ornamental fish populations imported from south-east Asia.

3.7. Methods

3.7.1. Fish importation and collection

As previously described in section 3.2.1.

3.7.2. Parasite collection and preparation

Monogenean parasites were recovered from the skin and the gills of individual fish. Immediately following euthanasia, skin scrapes were taken from each individual fish with the blunt edge of a scalpel blade and placed on glass slides (with either saltwater or freshwater according to the origin of the fish) under a large coverslip and examined for the presence of monogeneans using a compound microscope (Olympus BX41). Following, the gill basket was removed, and gill arches were separated individually onto a glass slide and immersed in salt or freshwater under a large coverslip and microscopically examined. Skin and gill monogeneans were carefully collected with a micropipette and placed in individual Eppendorf tubes with 70% ethanol, labelled and stored for further identification. Due to time constraints, some fish were not examined fresh following euthanasia, and were preserved whole in 70% ethanol for later inspection. In this case, skin scrapes, gill baskets and sediment in the container were taken from each preserved fish, placed into separate cavity blocks with 70% ethanol and examined for the presence of monogeneans using a dissecting microscope (Leica M60).

Parasites were identified using a combined morphological and molecular approach. Preserved parasites were initially hydrated in distilled water for dissection. The body of each parasite was then carefully separated into two parts using a 30 G gauge needle, one containing the posterior sclerotized structures for morphology, including the male copulatory organ, and the other retained for DNA analysis. The posterior portion was placed on a microscope slide for proteolytic digestion to liberate the male copulatory organ and haptoral armature (as per Vaughan and Christison 2012). In brief, tissue was digested using 5 µL of Proteinase-K (1 mg/L) with ATL buffer added directly to the haptor on a microscope slide using a micro-pipette. The digestion process was monitored and controlled by adding additional Proteinase-K solution heated to 55°C or cool distilled water to inhibit the process and re-hydrate crystals during the procedure. Excess crystals were re-hydrated and removed using paper towelling until only the sclerotized structures remained. Thereafter, a small drop of molten glycerine jelly was placed quickly onto an inverted coverslip and slowly lowered onto the liberated sclerotized structures. Once the glycerine jelly had hardened the edge of the coverslip was sealed with clear nail varnish. The anterior portion of each parasite was placed in an individual Eppendorf tube in 70% ethanol for DNA analysis. Hamuli mounted on glass slides for each species were accessioned to the Australian Helminth Collection (AHC) at the South Australian Museum, Adelaide (SAMA).

3.7.3. Hamulus measurements

One hamulus per pair from each individual monogenean was measured to facilitate species identification and to avoid pseudo-replication. Hamulus measurements are described in Figure 8. Prior to performing individual measurements, each hamulus was photographed and orientated into a superimposed rectangle to eliminate excessive measurement error by using a quadrangular grid reference to repeatedly return the same measurement points of

origin between specimens (as per Vaughan and Christison 2012). *Gyrodactylus* spp. hamulus measurements were obtained based on the methodology of Shinn et al. (2004). Monogenean marginal hooklets are very small structures and their measurements are known to reflect a high degree of variance, considered in part the result of limitations of measurement hardware and software (see Shinn et al. 2004; Vaughan and Christison 2012). As such, we did not include these structures in the species identifications.

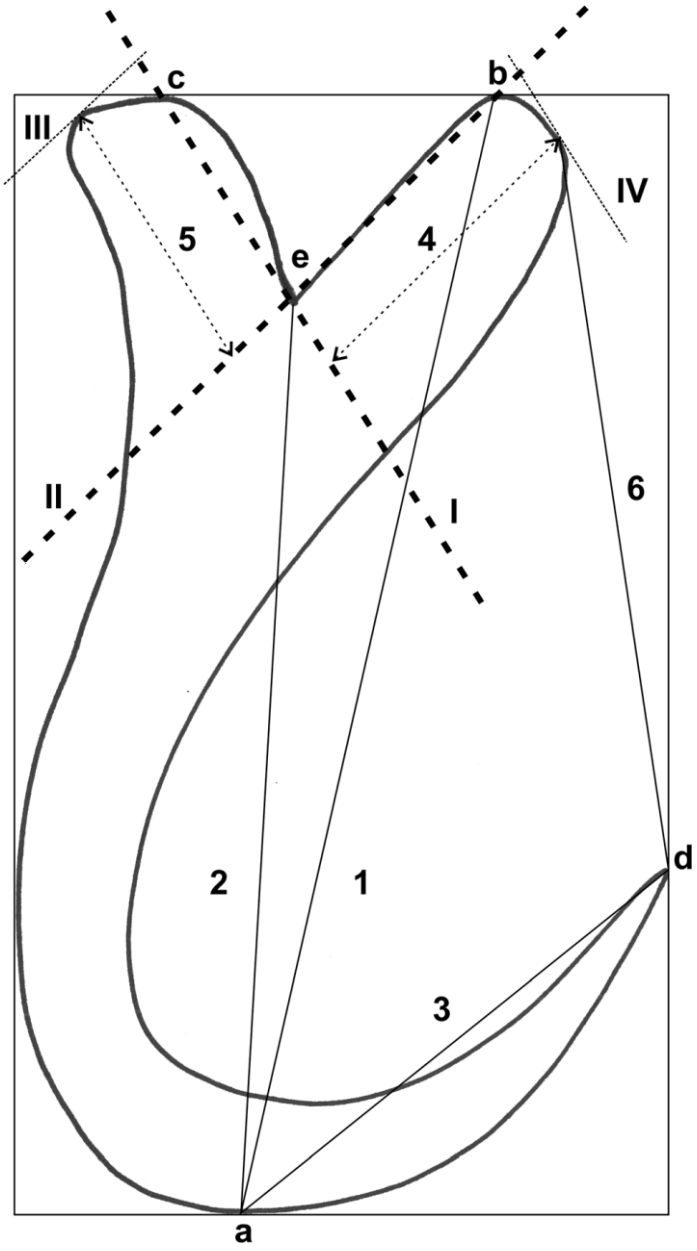

Figure 9. Representative measurements of *Dactylogyrus* spp. hamuli. Points a–d describe the location where the superimposed rectangle touches the hamulus. Point e describes the lowest point of the root saddle. Thick dotted lines (I and II) are drawn between points b–e and c–e, respectively; lines III and IV are drawn perpendicular to lines II and I, respectively, touching the highest point of each root (see lines III and IV). 1 = total hamulus length; 2 = basal hamulus length; 3 = total blade length; 4 = inner root length; 5 = outer root length; 6 = total gap length; 7 = gap ratio (gap length/blade length).

3.7.4. DNA extraction, PCR amplification and sequencing

The anterior portion of the parasite samples were centrifuged at 10,000 rpm for 5 min and excess ethanol was carefully removed. Total genomic DNA from each specimen was then extracted using a DNeasy Blood and Tissue Kit (Qiagen) as per manufacturer's specifications. Primer pairs specific to Monogenea, Worm A (5'-GCGAATGGCTCATTAAATCAG-3') and Worm B (5'- CTTGTTACGACTTTTACTTCC-3'), were used to amplify the 18S gene region in a primary PCR, followed by a nested PCR using primers 1270R (5'-CCGTCAATTCCTTTAAGT-3') and 930F (5'-GCATGGAATAATGGAATAGG-3') using combinations WormA + 1270R and 930F + WormB (Plaisance et al. 2005). Primary PCRs were done with primer pairs Dact A (5'-AGGTGAACCTGCGGAAGGATC -3') and Dact B (5'- AGCCGAGTGATCCAGCAC -3') to amplify the partial 18S region and complete ITS1 region of the *Dactylogyrus* genus, and using primers ITS2 (5'-TCCTCCGCTTAGTGATA-3') and ITS4.5 (5'-CATCGGTCTCTCGAACG-3') to amplify a fragment spanning ITS2 for *Gyrodactylus* parasites (as per Matejusová et al. 2001). Primer pairs Dact A and Dact B were created based on sequences accessioned in Genbank for *Dactylogyrus*: KJ854364, KJ854363, KM525669,

KC876018, KC876016, KM487695, AJ564159, AJ564139, AJ564135, AJ564111, and AJ490161.

Primary PCR amplifications were performed with 5 µL of DNA extract, 0.25 µL of each PCR primer, 5 µL of 5X MyTaq Buffer solution, 1 µL of MyTaq Polymerase and 13.75 µL of High Purity Water. For the nested PCR, all the conditions were the same as the primary PCR except that 1 µL of primary PCR amplicon and 17.75 µL of High Purity Water were used. For primers Worm A, Worm B and Dact A, Dact B, thermal cycling was performed with an initial denaturation for 3 min at 94 °C, followed by 40 cycles for primary PCRs and 35 cycles for nested PCR (30 s at 94 °C, 30 s at a gene specific-annealing temperature, 2 min at 72 °C, with a final extension of 10 min at 72 °C). Annealing temperatures were as follows: 55 °C for primary 28S rDNA and 18S rDNA; and 58 °C for 18S rDNA (Plaisance et al. 2005). For primer pair ITS2 and ITS4.5, thermal conditions were 5 min at 95 °C (hot start), then 25 cycles of 1 min at 92 °C, 30 s at 50 °C and 30 s at 72 °C, with a final extension of 4 min at 72 °C (modified from Matejusová et al. 2001). Amplicons were sent for sequencing to the Australian Genome Sequencing Facility (Brisbane, Australia).

Sequences obtained from the Australian Genome Sequencing Facility for each parasite species were selected if forward and reverse sequences had >95% base similarity using Geneious (v10.0.9). Selected sequences were then aligned with accessioned records in Genbank using Geneious (v10.0.9) for molecular identification. Genbank sequences were selected if they included gene region ITS1 and if the accessioned sequences had corresponding voucher specimens. Best-fit partitioning schemes and models of molecular evolution were selected using the program PartitionFinder (v2) using the concatenated

alignment created in Geneious. A consensus phylogenetic tree was created with Geneious (v8.0) using a Bayesian analysis (partitions= 1, Evolutionary model= TVM+G, iterations= 100,000) and a Parsimony analysis (100 bootstrap iterations) to provide nodal support for the phylogenetic tree.

3.8. Results

Forty percent (15/34) of imported ornamental fish populations examined were positive for monogenean infections (Table 9). Monogeneans commonly infected 60% freshwater fish populations (13/22), while none were detected in marine fishes (0/14) (Table 9). Fourteen parasite species were found infecting five freshwater fishes (*Carassius auratus*, *Pethia conchonius* Hamilton, 1822, *Poecilia reticulata* Peters, 1859, *Trichopodus leerii* Bleeker, 1852, and *Trichopodus trichopterus* Pallas, 1770; Table 9). Four parasites species infected *P. conchonius*, *P. reticulata*, *T. leerii* and *T. trichopterus* from Sri Lanka, seven parasites infected *C. auratus* from Malaysia, four parasites infected *C. auratus* and *P. conchonius* from Singapore, and eight parasites infected *C. auratus* and *P. conchonius* from Thailand (Table 9). In total, 950 individual monogenean parasites were collected from 1,020 imported ornamental fishes.

Table 9. Apparent prevalence and mean intensity of monogenean parasites infecting imported ornamental fish. All freshwater species were farmed in their country of origin, while all marine species were wild caught. Thirty fish were examined from each population unless stated otherwise.

Population No.	Fish Species	Environment	Sample date	Exporter I.D.	Parasite species	Number of infected fish	Apparent Prevalence % (95% CI)	Mean Intensity ± S.D.
1	*Beta splendens*	Freshwater	29/10/2015	Sri Lanka 2	Not sampled*	-	-	-
2	*Beta splendens*	Freshwater	30/10/2015	Malaysia 1	Not detected	0	-	-
3	*Carassius auratus*	Freshwater	3/06/2015	Singapore 2	*Dactylogyrus baueri*	1	3.4 (0.1−17.2)	1
					Dactylogyrus sp. 2	1	3.4 (0.1−17.2)	14
					Gyrodactylus sp.	1	3.4 (0.1−17.2)	1
4	*Carassius auratus*	Freshwater	5/06/2015	Singapore 2	Not detected	0	-	-
5	*Carassius auratus*	Freshwater	5/06/2015	Thailand 1	*Dactylogyrus intermedius*	6	20 (8−39)	1.83 ± 1.21
					Dactylogyrus vastator	4	13.3 (2−27)	1
					Dactylogyrus sp. 2	4	13.3 (2−27)	1.33 ± 0.58
					Gyrodactylus gurleyi	1	3.3 (0.1−17.2)	1
6	*Carassius auratus*	Freshwater	28/10/2015	Thailand 1	*Dactylogyrus anchoratus*	8	26.6 (12−46)	2.75 ± 2.53
					Dactylogyrus baueri	6	20 (8−39)	1.83 ± 0.98
					Dactylogyrus formosus	2	6.7 (0.82−22)	1.5 ± 0.71
					Dactylogyrus intermedius	13	43.3 (25−63)	2.31 ± 1.44
					Dactylogyrus vastator	12	40 (23−59)	1.42 ± 0.67
					Dactylogyrus sp. 2	4	13.3 (2−27)	2 ± 1.89
7	*Carassius auratus*	Freshwater	28/10/2015	Thailand 1	*Dactylogyrus anchoratus*	1	3.3 (0.1−17.2)	1
					Dactylogyrus baueri	18	60 (40−77)	1.61 ± 0.96

					Dactylogyrus formosus	13	43.3 (25−63)	2.46 ± 2.02
					Dactylogyrus intermedius	9	30 (15−49)	2 ± 2
					Dactylogyrus vastator	5	16.6 (2−29)	1.6 ± 0.89
					Dactylogyrus sp. 2	8	26.6 (12−46)	1.63 ± 0.74
8	*Carassius auratus*	Freshwater	30/10/2015	Malaysia 1	*Dactylogyrus* baueri	1	3.3 (0.1−17.2)	2
					Dactylogyrus formosus	1	3.3 (0.1−17.2)	1
					Dactylogyrus intermedius	1	3.3 (0.1−17.2)	1
					Dactylogyrus vastator	1	3.3 (0.1−17.2)	1
					Dactylogyrus sp. 1	1	3.3 (0.1−17.2)	1
					Gyrodactylus kobayashii	4	13.3 (2−27)	1
9	*Carassius auratus*	Freshwater	30/10/2015	Malaysia 1	*Dactylogyrus formosus*	2	6.6 (0.82−22)	2 ± 1.41
					Dactylogyrus intermedius	1	3.3 (0.1−17.2)	1
					Dactylogyrus sp. 2	1	3.3 (0.1−17.2)	6
					Gyrodactylus gurleyi	1	3.3 (0.1−17.2)	1
10	*Danio rerio*	Freshwater	6/01/2015	Sri Lanka 1	Not detected	0	-	-
11	*Helostoma temminckii*	Freshwater	27/05/2015	Singapore 1	Not detected	0	-	-
12	*Pethia conchonius*	Freshwater	6/01/2015	Sri Lanka 1	*Dactylogyrus ostraviensis*	8	26.6 (12−46)	2 ± 1.07
13	*Pethia conchonius*	Freshwater	6/03/2015	Singapore 2	*Dactylogyrus ostraviensis*	8	26.6 (12−46)	0.44 ± 0.5
14	*Pethia conchonius*	Freshwater	5/06/2015	Thailand 1	*Dactylogyrus ostraviensis*	10	33.3 (17−53)	1.6 ± 0.7
15	*Pethia conchonius*	Freshwater	28/10/2015	Thailand 1	*Dactylogyrus ostraviensis*	22	73.3 (54−88)	6.05 ± 3.48
16	*Pethia conchonius*	Freshwater	30/10/2015	Malaysia 1	Not detected	0	-	-
17	*Poecilia reticulata*	Freshwater	29/10/2015	Sri Lanka 2	*Urocleidoides reticulatus*	26	86.6 (69−96)	18.30 ± 18.22
18	*Trichopodus leerii*	Freshwater	1/06/2015	Sri Lanka 1	*Trianchoratus leerium*	11	38 (20−58)	2.27 ± 1.14

19	*Trichopodus trichopterus*	Freshwater	6/05/2015	Thailand 1	Not detected	0	-	-
20	*Trichopodus trichopterus*	Freshwater	1/06/2015	Sri Lanka 1	*Trianchoratus* sp.	8	26.6 (12–46)	0.875 ± 1.13
21	*Trichopodus trichopterus*	Freshwater	29/10/2015	Sri Lanka 2	Not sampled*	-	-	-
22	*Xiphophorus hellerii*	Freshwater	29/10/2015	Sri Lanka 2	Not detected	0	-	-
23	*Xiphophorus maculatus*	Freshwater	6/05/2015	Thailand 1	Not detected	0	-	-
24	*Xiphophorus maculatus*	Freshwater	3/06/2015	Singapore 2	Not detected	0	-	-
25	*Xiphophorus maculatus*	Freshwater	29/10/2015	Sri Lanka 2	Not sampled*	-	-	-
26	*Amphiprion bicintus*	Marine	22/10/2015	Indonesia 3	Not detected	0	-	-
27	*Amphiprion ocellaris*	Marine	28/05/2015	Indonesia 2	Not detected	0	-	-
28	*Amphiprion ocellaris*	Marine	23/10/2015	Indonesia 3	Not detected	0	-	-
29	*Amphiprion sebae*	Marine	27/05/2015	Singapore 1	Not detected	0	-	-
30	*Cheilodipterus quinquelineatus*	Marine	23/01/2015	Indonesia 2	Not detected	0	-	-
31	*Cheilodipterus quinquelineatus*	Marine	28/05/2015	Indonesia 2	Not detected	0	-	-
32	*Pterapogon kauderni*	Marine	16/01/2015	Singapore 1	Not detected	0	-	-
33	*Pterapogon kauderni*	Marine	20/01/2015	Indonesia 1	Not detected	0	-	-
34	*Pterapogon kauderni*	Marine	22/01/2015	Indonesia 2	Not detected	0	-	-
35	*Sphaeramia nematoptera*	Marine	22/10/2015	Indonesia 3	Not detected	0	-	-
36	*Zoramia leptocantha*	Marine	28/05/2015	Indonesia 2	Not detected	0	-	-
37	*Zoramia leptocantha*	Marine	23/10/2015	Indonesia 3	Not detected	0	-	-

*These populations were seized by Australian Quarantine Services and euthanized. We received the dead fish in a plastic bag with no water. Fish were not sampled

Not detected= Apparent Prevalence = 0% (95% CI 0–11.4%)

Population 3 and 11 had one mortality at the time of sampling. Mortalities were excluded, and examinations were done from a total of 29 examined fish for each population

3.8.1. Goldfish, Carassius auratus

Goldfish, *Carassius auratus*, exhibited the highest parasite diversity of all the fishes examined. Ten monogenean parasite species were found in six out of the seven goldfish populations examined including seven *Dactylogyrus* spp. and three *Gyrodactylus* spp.. Dactylogyrids were identified from Thailand, Singapore and Malaysian populations, and included *Dactylogyrus anchoratus* Dujardin, 1845, *Dactylogyrus baueri* Gussev, 1955, *Dactylogyrus formosus* Kulwiec, 1927, *Dactylogyrus intermedius* Wegener, 1909 and *Dactylogyrus vastator* Nybelin, 1924 (see Table 9 for parasite species and corresponding origin/s). Two morphologically distinct types were not able to be identified to species, *Dactylogyrus* sp. 1 and *Dactylogyrus* sp. 2 (Table 9). Gyrodactylids were identified from Malaysia, Thailand and Singapore populations (Table 9). Based on molecular analysis as well as hamuli morphology, *Gyrodactylus gurleyi* Price, 1937 infected fish in populations 5 and 9 (Genbank no. MF356250, Table 11), *Gyrodactylus kobayashii* Hukuda, 1940 infected population 8 (Genbank no. MF356251, Table 11), and an unidentified *Gyrodactylus* sp. infected population 3 (Table 11). Monogeneans were recovered with an apparent prevalence of 3.3% (95% CI= 0.1−17.2, Table 9) from one goldfish population from Singapore (population 3, Table 9). *Dactylogyrus anchoratus* and *G. kobayashii* have been reported previously infecting invasive cyprinids in Australia (Dove and Ernst 1998; Corfield et al. 2008).

Carassius auratus populations imported from Thailand exhibited the highest apparent prevalence and intensity of monogenean parasites amongst *C. auratus* populations (populations 5-7; Table 9). *Dactylogyrus intermedius* and *D. vastator* had an apparent prevalence of 43% (95% CI= 25−63) and 40% (95% CI= 23−59), respectively, in population

6 (Table 9), whilst *D. baueri* exhibited an apparent prevalence of 60% (95% CI= 40−77), followed by *D. formosus* with 43.3% (95% CI= 25−63), and *D. intermedius* with 30% (95% CI= 15−49) in population 7 (Table 9).

Dactylogyrus baueri and *Dactylogyrus* sp. 1 had similar hamulus morphology (Fig. 10A and B, respectively; Table 10), but the male copulatory organ morphology was considerably different (Fig. 14A and B, respectively). The male copulatory organ of *D. baueri* has a distinct arching curvature, and the accessory piece includes a prominent barb (*cf*. Ogawa and Egusa 1979). These features are lacking in the male copulatory organ of *Dactylogyrus* sp. 1, which is simple and straight, and is similar to *Dactylogyrus dulkeiti* Bychowsky, 1936 (*cf*. Ogawa and Egusa 1979). The overall shape of the *Dactylogyrus* sp. 2 hamulus (Fig. 10F) is similar to *D. intermedius* (Fig. 10E), but notably smaller in its measurements (Table 10). In addition, the male copulatory organ morphology (Fig. 14E) differed in structure with that of the published morphology for *D. intermedius* (see Ling et al. 2016). The male copulatory complex of *Dactylogyrus intermedius* consists of two roughly parallel parts extending out from a rounded basal sclerotised shield. A prominent perpendicular loop extends between both extended parts, folding completely around the thicker and slightly more curved extension (see Ling et al. 2016). This complete loop was not present in *Dactylogyrus* sp. 2, and the larger extension is not as curved as that of *D. intermedius*.

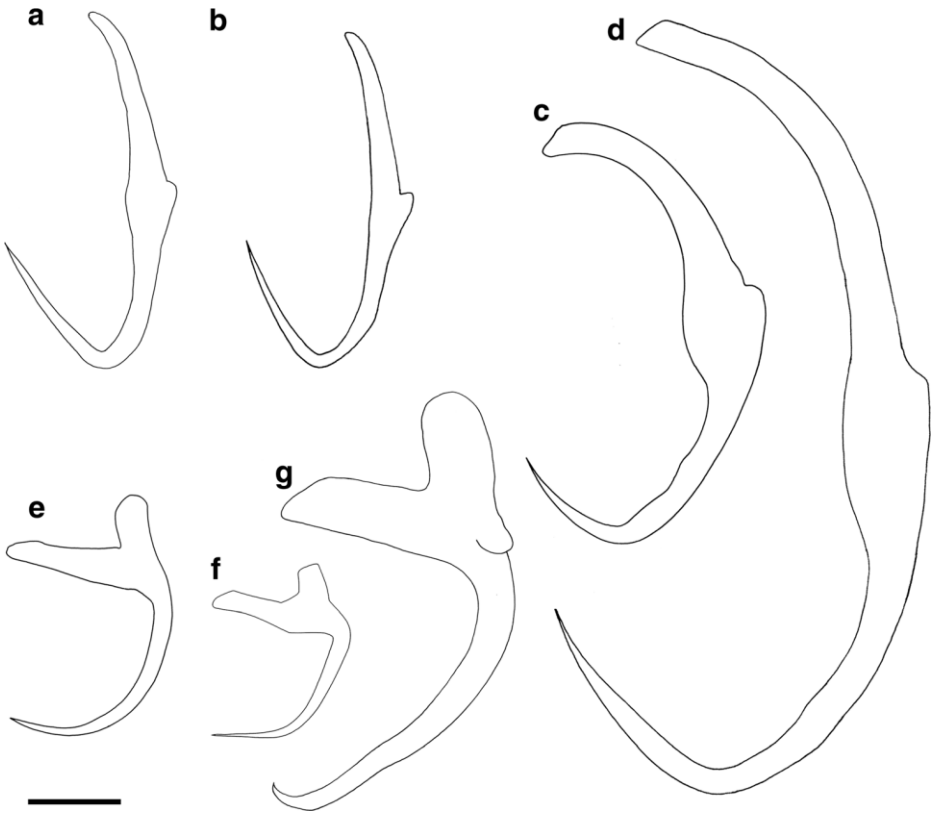

Figure 10. Hamulus morphology of *Dactylogyrus* species infecting goldfish, *Carassius auratus*. *Dactylogyrus baueri* (a), *Dactylogyrus* sp. 1 (b), *D. formosus* (c), *D. anchoratus* (d), *D. intermedius* (e), *Dactylogyrus* sp. 2 (f), and *D. vastator* (g). Scale bar = 10 μm

Gyrodactylus kobayashii, *G. gurleyi* and *Gyrodactylus* sp. displayed differences in the measurements of hamulus morphology (Table 11, Figure 11). *Gyrodactylus* sp. displayed hamulus morphology and measurements similar to *G. kobayashii* (Table 11, population 3 and population 8, respectively). However angular measurements from *Gyrodactylus* sp. suggests that the hamulus point curve angle and the inner hamulus aperture angle (HPCA and HIA, respectively, Table 11) are more obtuse than specimens from population 8 (Table 11). With only one specimen collected for *Gyrodactylus* sp., and no molecular identification, the identity of this parasite could not be confirmed.

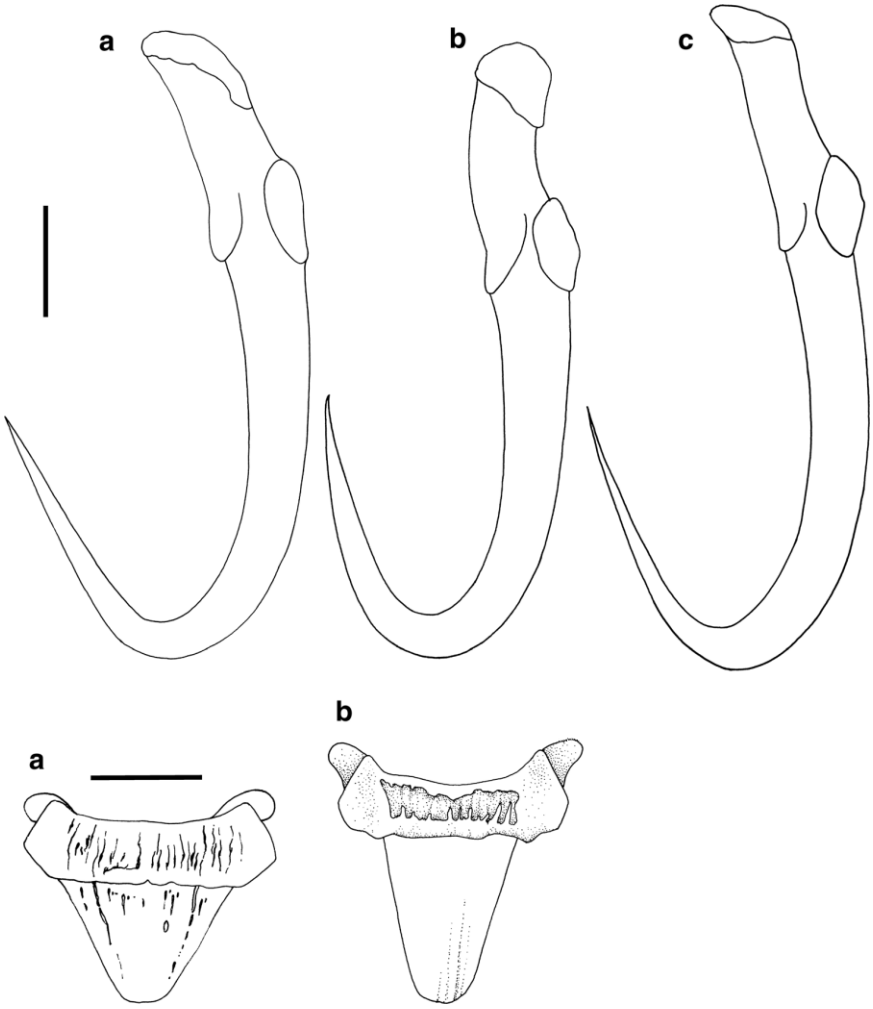

Figure 11. Hamulus and ventral bar representations of *Gyrodactylus* spp. on *Carassius auratus*. *Gyrodactylus kobayashii* (a), *Gyrodactylus gurleyi* (b), and *Gyrodactylus* sp. Scale bar = 10 μm.

3.8.2. Rosy barb, Pethia conchonius *and guppy,* Poecilia reticulata

Four out of five rosy barb, *Pethia conchonius*, populations were infected with *Dactylogyrus ostraviensis* Řehulka, 1988 (Table 9; Fig. 12A). Fish from Thailand were the most infected with apparent prevalence of 33.3% (95% CI=17−53) (population 14) and 73.3% (95% CI=54−88) (population 15; Table 9). This is the first record of *Dactylogyrus ostraviensis* infecting *P. conchonius* imported to Australia.

Poecilia reticulata, were infected with *Urocleidoides reticulatus* Mizelle and Price, 1964 (Fig. 12B-C). Fish imported from Sri Lanka were heavily infected, with an apparent prevalence of 86% (95% CI= 69−96) of fish infected with a mean intensity ± S.D. of 18.30 ± 18.22 (Table 9). *Urocleidoides reticulatus* has been previously reported infecting imported *P. reticulata* in Australia (Evans and Lester 2001).

3.8.3. Gourami, Trichopodus *spp.*

Two out of three *Trichopodus* spp. populations exhibited monogenean infections. *Trianchoratus leerium* Lim, 1986 (Fig. 13C-D) infected *Trichopodus leerii* Bleeker, 1852 (syn. *Trichogaster leerii* Bleeker, 1852), from Sri Lanka (population 18) with 36% apparent prevalence (95% CI= 20−58, Table 9). Hamulus morphology and measurements for *Trianchoratus* sp. infecting *Trichopodus trichopterus* Pallas, 1770 (syn. *Trichogaster trichopterus* Pallas, 1970), from Sri Lanka (population 20, Fig. 13A-B) was consistent with the hamulus morphology of *Trianchoratus aecleithrium* Price and Berry, 1966 (see Lim 1986). However, we could not confirm this diagnosis morphologically because we were

unsuccessful in recovering the male copulatory organ. This provides the first record of *Trianchoratus* spp. infecting imported *T. leerii* and *T. trichopterus* in Australia.

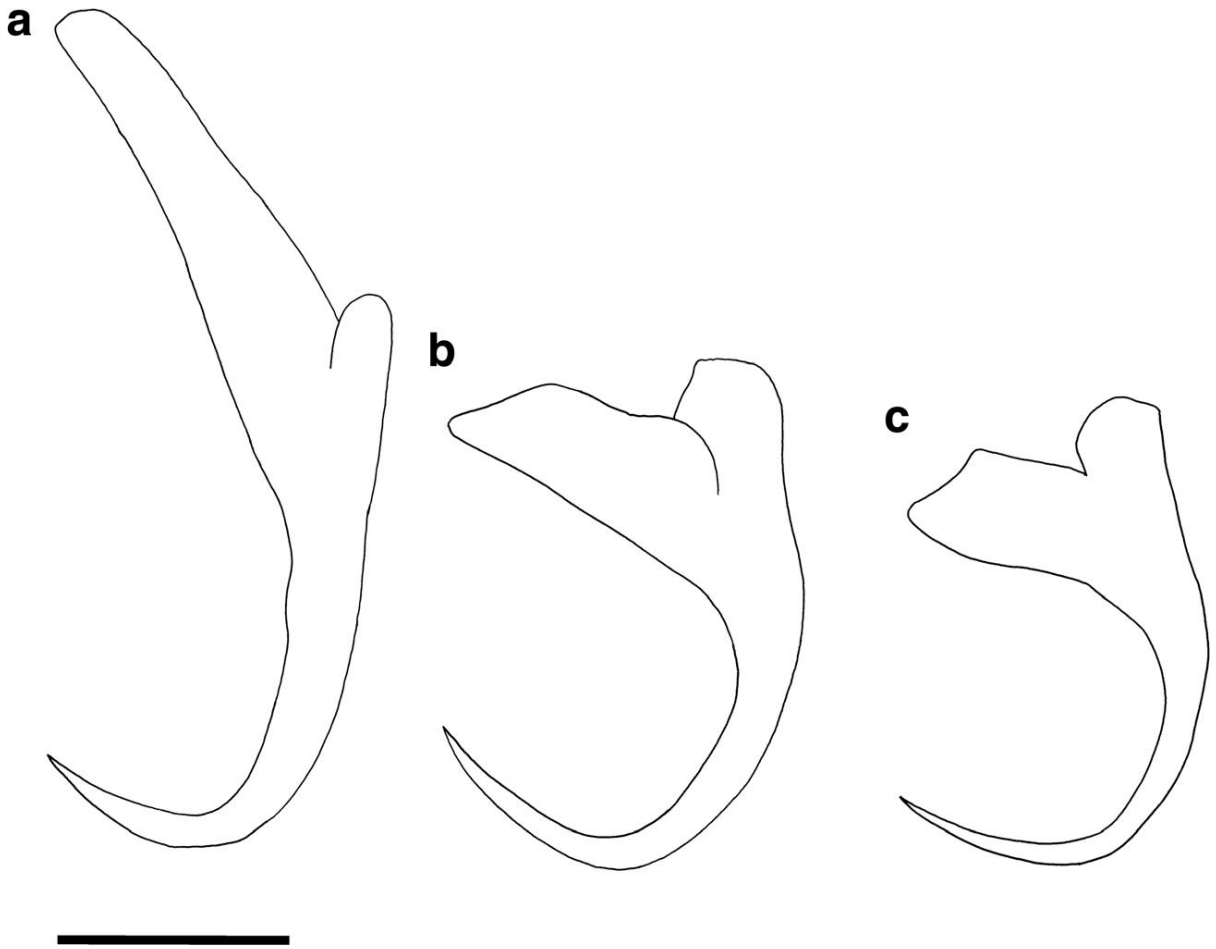

Figure 12. Hamulus morphology of *Dactylogyrus ostraviensis* (a) infecting *Pethia conchonius*, and *Urocleidoides reticulatus* (ventral hamulus = b, dorsal hamulus = c) infecting *Poecilia reticulata*. Scale bar = 10 μm.

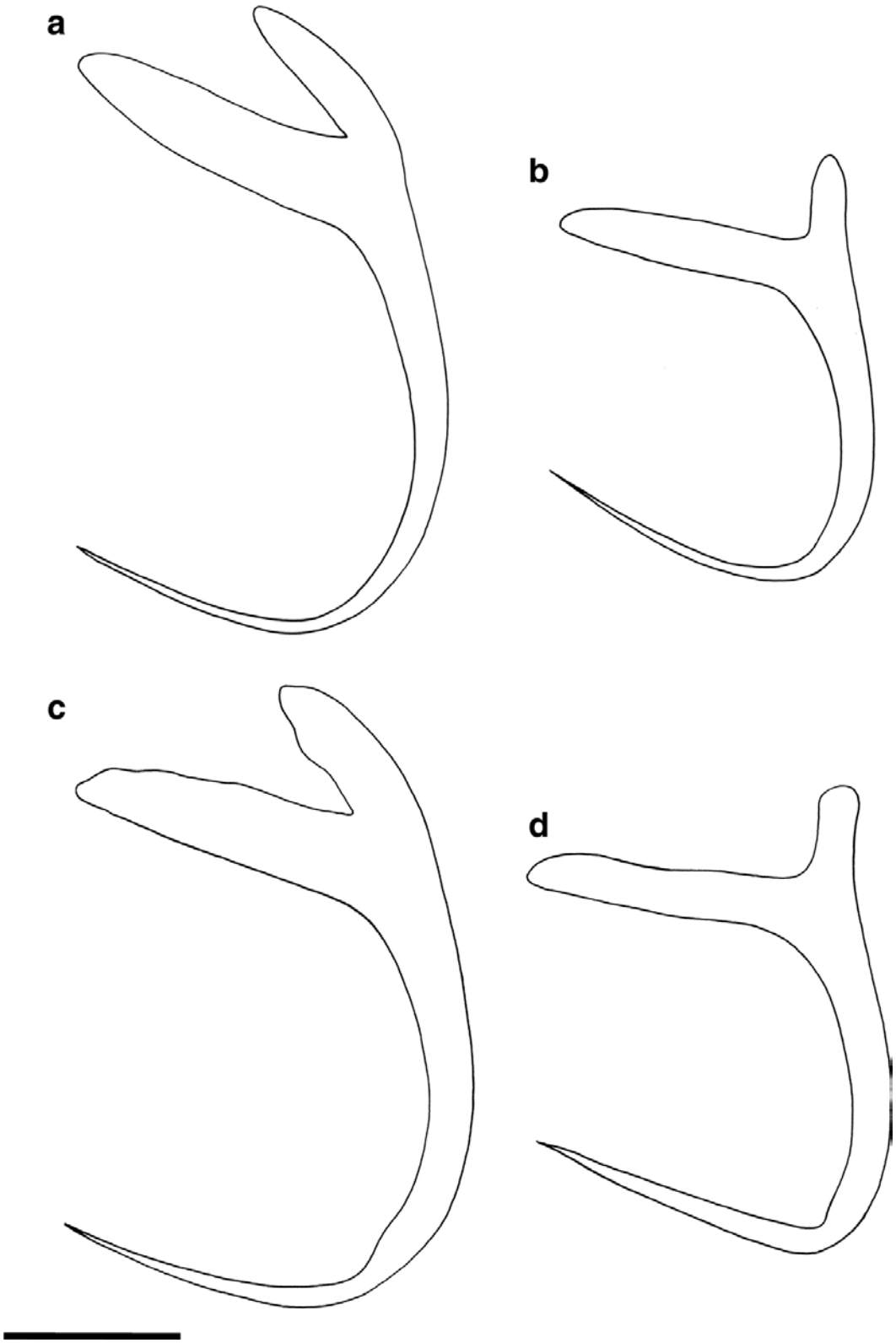

Figure 13. *Trianchoratus* sp. (ventral hamulus = a, dorsal hamulus = b) infecting *Trichopodus trichopterus* (population 20) and *Trianchoratus leerium* (ventral hamulus = c, dorsal hamulus = d) infecting *Trichopodus leerii* (population 18). Scale bar = 10 μm

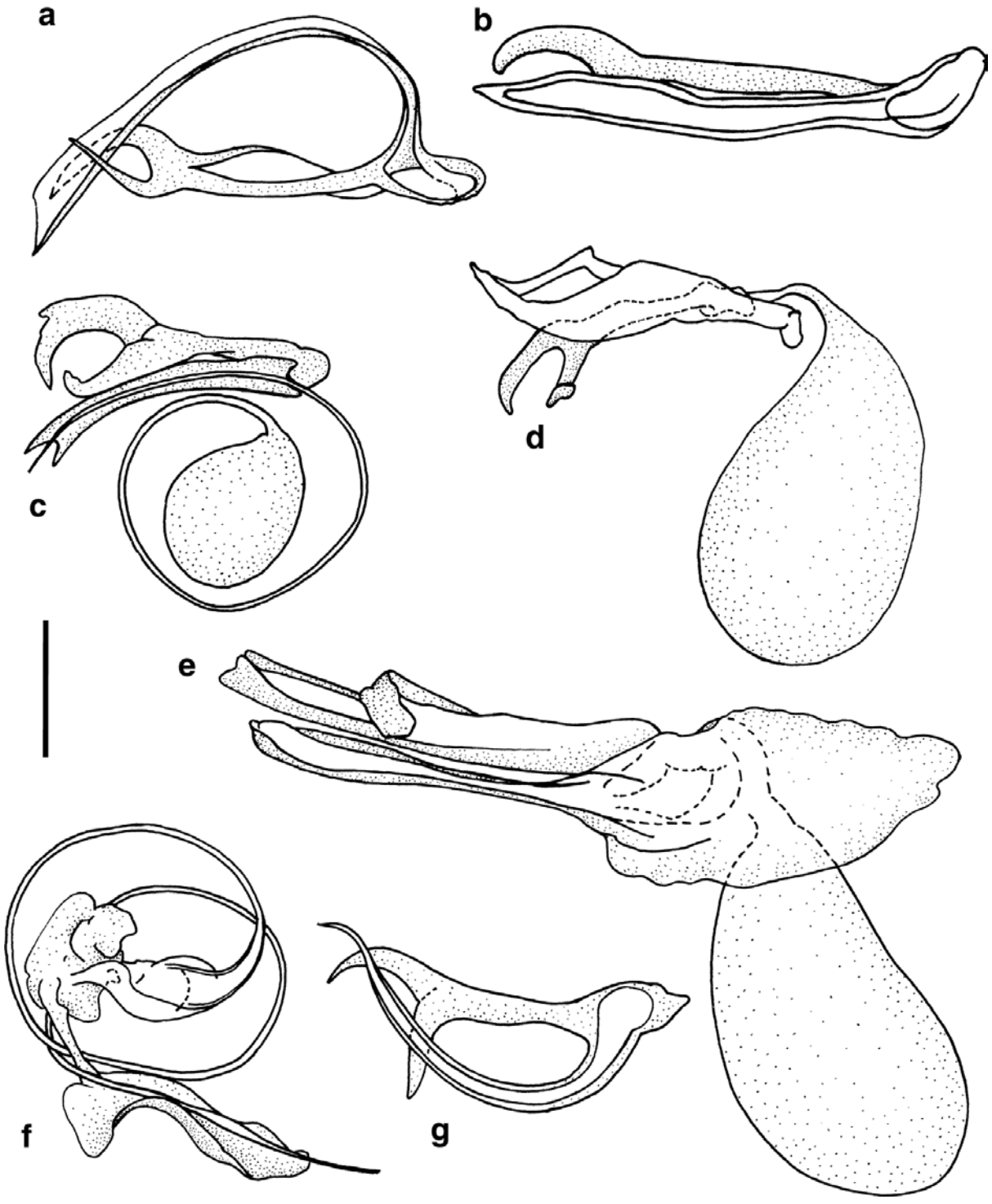

Figure 14. Male copulatory organ of *Dactylogyrus baueri* (a), *Dactylogyrus* sp. 1 (b), *Trianchoratus leerium* (c), *Dactylogyrus formosus* (d), *Dactylogyrus* sp. 2 (e), *Urocleidoides reticulatus* (f), and *Dactylogyrus ostraviensis* (g). We were unsuccessful in recovering the male copulatory organ for *Dactylogyrus anchoratus*, *Dactylogyrus intermedius*, *Dactylogyrus vastator*, and *Trianchoratus* sp. Scale bar = 10 μm.

3.8.4. Molecular characterisation and comparisons

Primer pairs Worm A and Worm B, followed by nested primers 1270R and 930F (Plaisance et al. 2005), amplified partial fragments (1035-1768 bp) spanning the 18S subunit for *Dactylogyrus*, *Gyrodactylus* and *Trianchoratus* species. However, fragments within the 18S subunit did not provide species-level identification and for this reason, fragments were not sequenced. It was not possible to amplify the 18S or ITS 1 region for *Trianchoratus* spp.. Sequences in Genbank for *Trianchoratus* species are only available for the 28S region, which can provide valuable information about the relationship of the *Trianchoratus* genus with other Ancyrocephalidae parasites, but because the region is highly conserved, it prevents further discrimination between *Trianchoratus* species (Tan et al. 2011). None of the primers used in this study amplified *U. reticulatus* samples.

Primers Dact A and Dact B amplified partial fragments within the ITS1 region (366-588 bp) of all dactylogyrid parasites in this study, with the exception of *Dactylogyrus* sp. 1 (Table 10). Similarly, primers ITS4.5 and ITS2 (Matejusová et al. 2001) amplified partial fragments (464-500 bp) spanning the ITS2 region for *Gyrodactylus* spp. from populations 5, 8 and 9. However, we could not amplify sequences for *Gyrodactylus* sp. in population 3. Based on molecular comparisons of the gene region ITS2, *Gyrodactylus gurleyi* identified in this study *(*Genbank no. MF356250) had a 100% base similarity alignment with *G. gurleyi* described by Li et al. (2014) (Genbank no. KC922453). Similarly, *G. kobayashii* from this study (Genbank no. MF356251) had 100% base similarity with *G. kobayashii* described by Li et al. (2014) (Genbank no. KC922452), Cable et al. (1999) (Genbank no. AJ132985) and Zietara and Lumme (2002) (Genbank no. AF484534).

The *Dactylogyrus* phylogenetic tree showed two distinct clades (Fig. 15). Clade 1 comprised *D. intermedius*, *D. vastator*, and *Dactylogyrus* sp. 2., and clade 2 comprised *D. baueri*, *D. dulkeiti*, *D. anchoratus*, *D. formosus*, and *D. ostraviensis*. These are the first ITS1 sequences for *D. baueri* and *D. ostraviensis* accessioned in Genbank. Within clade 1, the *D. intermedius* and *Dactylogyrus* sp. 2 clade was well supported (Bootstrap percentage/Bayesian posterior probability = 99/1, Fig. 15), but relations within the clade were not (Fig. 15). Interestingly, *D. vastator* showed a well-supported separation of two groups of samples, one sister to the *D. intermedius* + *Dactylogyrus* sp. 2 clade (90/1, Fig. 15) and the other outside but joint to the clade (88/1, Fig. 15). *Dactylogyrus baueri* formed a well-supported clade with *D. dulkeiti* within clade 2 (100/1, Fig. 15). Similarly, *D. anchoratus* samples grouped together in a single clade, joined to a separate group containing all sequences for *D. formosus* (Fig. 15). *Dactylogyrus ostraviensis* sequences were the most distinct sequences compared to all other *Dactylogyrus* sequences considering the number of base substitutions (100/1), grouping together with the *D. baueri*, *D. dulkeiti*, *D. anchoratus*, and *D. formosus* clade within clade 1 (Fig. 15).

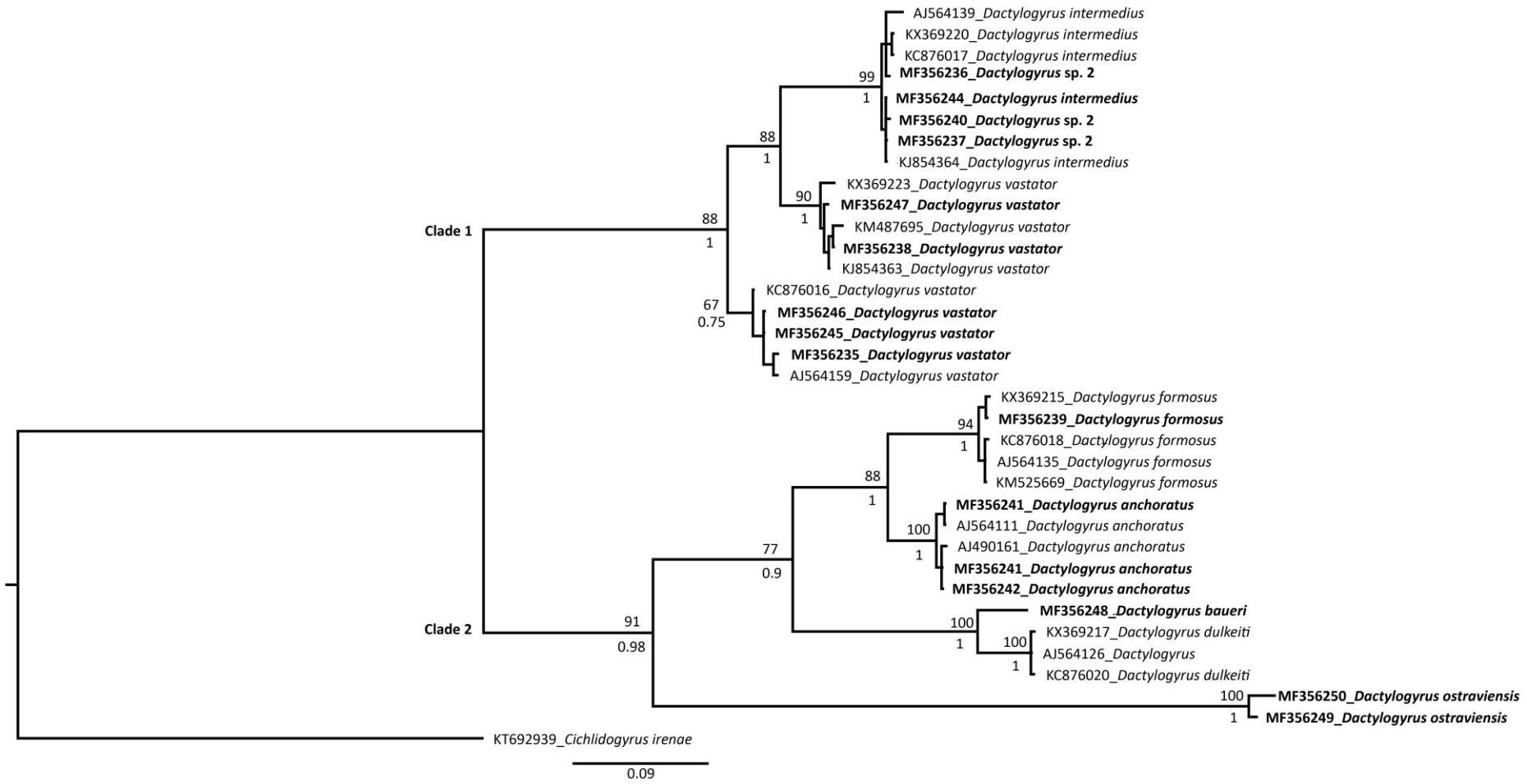

Figure 15. Consensus phylogenetic tree of *Dactylogyrus* spp. estimated by Bayesian analysis of gene sequence data of ITS1. *Cichlidogyrus irenae* Gillardin, Vanhove, Pariselle, Huyse and Volckaert, 2011 (Genbank No. KT692939) was used as the outgroup sequence. Nodal support is shown by bootstrap percentages from the parsimony analysis (above) and Bayesian posterior probabilities (below). Sequences in bold are accessions from this study.

Table 10. Hamuli measurements of monogenean parasites found infecting imported ornamental fish. *Measurements were taken only from hamuli mounted flat on the microscope slide. Measurements are provided in micrometres ± Standard Deviation. All mounted hamuli at an angle were excluded from the analysis.

Host species	Parasite species	n*	Mean total length		Mean basal length		Mean Blade length		Mean gap length		Mean inner root length		Mean outer root length		Mean Gap ratio	
Carassius auratus	*Dactylogyrus anchoratus*	20	97.37 ± 7.84		56.33 ± 5.01		30.36 ± 2.18		78 ± 7.86		51.84 ± 4.92		2.74 ± 0.59		2.57 ± 0.23	
	Dactylogyrus formosus	20	49.47 ± 2.43		30.93 ± 1.99		15.71 ± 1.36		39.57 ± 2.92		26.55 ± 2.00		1.68 ± 0.36		2.55 ± 0.42	
	Dactylogyrus intermedius	17	26.13 ± 1.58		22.98 ± 1.53		9.93 ± 1.39		22.38 ± 1.80		13.93 ± 1.17		5.89 ± 0.89		2.31 ± 0.47	
	Dactylogyrus vastator	20	33.97 ± 1.30		31.82 ± 1.51		18.55 ± 5.22		34.11 ± 1.53		18.84 ± 1.58		11.82 ± 1.68		1.92 ± 0.35	
	Dactylogyrus baueri	14	44.55 ± 3.19		25.47 ± 1.97		19.40 ± 2.39		30.79 ± 3.23		21.43 ± 2.85		2.10 ± 0.49		1.60 ± 0.65	
	Dactylogyrus sp. 1	11	39. 27 ± 1.31		22.85 ± 1.29		17.35 ± 1.51		27.96 ± 3.24		18. 40 ± 1.61		2.11 ± 0.48		1.62 ± 0.26	
	Dactylogyrus sp. 2	27	22.11 ± 2.53		19.49 ± 2.13		8.52 ± 0.92		19.25 ± 0.92		12.33 ± 2.42		4.65 ± 1.43		2.28 ± 0.99	
Pethia conchonius	*Dactylogyrus ostraviensis*	20	32.46 ± 3.20		21.18 ± 2.22		8.89 ± 0.55		27.48 ± 3.10		14.64 ± 2.61		1.87 ± 0.40		3.09 ± 0.28	
Poecilia reticulata	*Urocleidoides reticulatus*[a]	20	21.73 ± 0.78	19.53 ± 0.96	19.55 ± 0.71	17 ± 0.76	10.17 ± 0.70	9.75 ± 1.01	17.68 ± 0.97	16.53 ± 1.56	9.09 ± 0.70	8.79 ± 0.62	3.47 ± 0.56	4.35 ± 0.44	1.76 ± 0.21	1.71 ± 0.17
Trichopodus leerii	*Trianchoratus leerium*[b]	17	32.29 ± 1.34	23.1 ± 1.21	26.22 ± 0.91	18.67 ± 0.81	13.85 ± 0.93	14.3 ± 1.51	25.7 ± 1.50	15.12 ± 0.88	15.3 ± 1.15	12.74 ± 0.93	9.25 ± 1.31	4.3 ± 0.86	1.87 ± 0.22	1.07 ± 0.15
Trichopodus trichopterus	*Trianchoratus* sp.[b]	4	36.23 ± 1.65	29.95 ± 3.33	31.17 ± 1.16	25.56 ± 3.90	15.55 ± 1.40	17.49 ± 3.81	29.1 ± 1.52	21.81 ± 2.51	16.12 ± 1.59	12.33 ± 1.52	9.52 ±0.86	6.43 ± 1.72	1.89 ± 0.22	1.27 ± 0.18

[a] Mean measurements are provided for ventral (left) and dorsal hamuli (right)

[b] Mean measurements are provided for ventral (left) and developed dorsal hamuli (right). Measurements for non-diagnostic rudimentary dorsal hamuli were excluded

Table 11. Hamuli measurements of *Gyrodactylus* spp. infecting *Carassius auratus*. Hamulus aperture distance (HAD), Hamulus proximal shaft width (HPSW), Hamulus point length (HPL), Hamulus distal shaft width (HDSW), Hamulus shaft length (HSL), Hamulus inner curve length (HICL), Hamulus root length (HRL), Hamulus total length (HTL), Hamulus aperture angle (HAA), Hamulus point curve angle (HPCA), Inner hamulus aperture angle (HIA) (Shinn et al. 2004). Measurements are provided in micrometres (HAD-HTL), and angles (HAA-HIA) in degrees ± Standard Deviation.

Population	Parasite species	n	HAD	HPSW	HPL	HDSW	HSL	HICL	HRL	HTL	HAA	HPCA	HIA
8	*Gyrodactylus kobayashii*	4	25.83 ± 1.87	6.83 ± 0.39	26.27 ± 0.99	3.52 ± 0.93	39.44 ± 2.93	0.80 ± 0.61	17.59 ± 1.53	53.70 ± 2.87	44.25 ± 2.50	3.63 ± 2.62	49.25 ± 3.95
5	*Gyrodactylus gurleyi*	1	15.76	8	25.41	4.71	31.52	4.47	15.76	49.41	31	14	38
9	*Gyrodactylus gurleyi*	1	17.14	7.14	26.28	4.28	33.14	4	18.57	55.42	32.50	13	38
3	*Gyrodactylus* sp.	1	23.72	5.18	28.15	2.96	38.5	2.22	18.51	57.78	47	8	40

3.9. Discussion

Fourteen monogenean parasite species were found infecting five imported ornamental freshwater fishes following veterinary certification from the exporting country and inspection by Quarantine Services at the Australian border (Table 9). Seven of these parasite species infected goldfish. Discovering such a high monogenean parasite diversity on goldfish is surprising, given that all imported ornamental goldfish must be treated with a parasiticide (e.g. trichlorfon, formaldehyde, sodium chloride) seven days prior to export to Australia for the presence of *D. vastator* and *D. extensus* (DAWR 2018). This is exacerbated by the

discovery of *Dactylogyrus vastator* in goldfish populations from Malaysia and Thailand, because it identifies that the requirements of import health certificates for goldfish populations prior to exportation to Australia are not being met (DAWR 2018). This is concerning given that *Dactylogyrus intermedius* and *D. vastator* have been associated with mortalities of farmed *C. auratus* in Asia (Ji et al. 2012; Jiang et al. 2013; Zhang et al. 2014a).

There is currently no mandatory requirement to treat freshwater ornamental fish species (other than goldfish) for the presence of parasites, although these fishes require veterinary inspection prior to export and testing for megalocytiviruses depending on species (DAWR 2018). The lack of parasiticide treatment is concerning because *Urocleidoides reticulatus* (which infected *Poecilia reticulata* from Sri Lanka) and *Dactylogyrus ostraviensis* (which infected *Pethia conchonius* from Thailand), displayed high apparent prevalence and mean infection intensities (Table 9). *Dactylogyrus ostraviensis* is exotic to Australian ecosystems, and *U. reticulatus* has been previously reported infecting imported *P. reticulata* in Australia (Evans and Lester 2001).

Import conditions require an 'effective parasite treatment' for goldfish and suggest the use of trichlorfon, formaldehyde or sodium chloride (DAWR 2018). However, import conditions fail to provide details on the dosage, or contact time with any of the three suggested chemicals (DAWR 2018). Trichlorfon, formaldehyde (e.g. formalin) and salinity bathing (i.e. sodium chloride) are traditional methods used to manage monogenean infestations on fish (Schmahl 1991). However, their efficacy varies with environmental factors, concentration, parasite resistance, parasite life stage, chemical residue and toxicity to the fish host (Goven and Amen 1982; Thoney and Hargis 1991; Schelkle et al. 2011). For example, 2.5 mg/L of trichlorfon caused 87.3% mortality of adult *D. vastator* infecting

goldfish *in vivo* but did not affect hatching success of *D. vastator* eggs under experimental conditions (Zhang et al. 2014a). Similarly, bathing *Seriola lalandi* Valenciennes, 1833, with 400 ppm of formalin for 1 h followed by a 5 min freshwater dip removed 100% of adult *Zeuxapta seriolae* (Yamaguti, 1963), but only 80% of adult *Benedenia seriolae* (Yamaguti, 1934) (see Sharp et al. 2004). Lastly, 15 min exposure to 25 g/L salinity bath removed 100% of *Gyrodactylus turnbulli* Harris, 1986, compared to 73% of *Gyrodactylus bullatarudis* Turnbull, 1956, infecting *P. reticulata* (see Schelkle et al. 2011). Stringent import conditions should provide detailed information on the use of required chemical treatments for imported fish, accounting for chemical concentration, time of treatment, and toxicity to the fish host, and consider the potential impact of parasite adaptive strategies on the efficacy of chemical treatments.

Monogenean parasites were not detected in any of the sampled marine fish populations with the true prevalence being between 0 and 11.6% (Table 9). This result could be associated with the method of euthanasia used in this study. Import conditions required all fish to be euthanized using benzocaine (100 mg/L) within 12 hours of receipt from the DAWR. Some methods of sedation and treatment of freshwater and marine fish are known to affect the attachment of monogenean parasites, causing dislodgement from the fish host (Pironet and Jones 2000). Specifically, 80 ppm of benzocaine is known to cause detachment of the monogenean *Entobdella hippoglossi* (Müller, 1776) infecting Atlantic halibut, *Hippoglossus hippoglossus* (Linnaeus, 1758) (Svendsen and Haug 1991), and an overdose of benzocaine administered via water bathing, killed the monogenean *Allencotyla mcintoshi* Price, 1962, infecting *Seriola dumerili* (Risso, 1810) (Montero et al. 2003). Using benzocaine which is dissolved in ethanol (70%) as a required method for fish euthanasia may have influenced the number of monogenean parasites recovered from marine fish in this study.

Despite pre-import and border conditions perceived to be stringent, the cryptic nature of the parasitic monogeneans found in this study suggests they would likely remain undetected in quarantine. All fish populations sampled for this study were within quarantine conditions as required by Quarantine Services, meaning that had the fish been destined for sale, all populations would still require a final quarantine period of a minimum of seven days (21 days for goldfish) in an approved facility provided by the importer (DAWR 2018). However, following this period, only visual inspection is required to release fish from quarantine (DAWR 2018) which would likely permit the distribution of fish infected with monogeneans into the broader retail industry, unless the infections had manifested, and fish exhibited clinical signs of disease. Therefore, it is imperative to review the efficacy of visual inspections at border control to detect parasite infections and consider alternative detections tools as effective preventive measures for Australian biosecurity.

The spread of monogenean parasites by ornamental fish from south-east Asia to other regions of the world may be much larger than expected. China, Indonesia, Malaysia, Singapore, Sri Lanka and Thailand are the largest exporters of ornamental fish globally (Monticini 2010). Many of these countries have been trading farmed ornamental fish species for hundreds of years (Balon 2004). Furthermore, countries like Singapore and Sri Lanka are considered as 'trade hubs' for other countries in south-east Asia, acting as wholesale markets with on-sale to developed countries, creating a much larger web of export-import interactions (Whittington and Chong 2007). For example, *Dactylogyrus ostraviensis*, which was first reported in India infecting *Pethia conchonius* in captive conditions (Řehulka 1988), has not been reported in Singapore, Sri Lanka or Thailand (Table 1). *Trianchoratus* spp., which have been reported infecting *T. leerii* and *T. trichopterus* in Malaysia (Lim 1986), were found

infecting both species imported from Sri Lanka (Table 9). Similarly, *U. reticulatus*, which has been reported infecting aquarium specimens of *P. reticulata* in Sacramento, California (Mizelle and Price 1964) and aquarium *P. reticulata* and *Poecilia sphenops* Valenciennes, 1846 (syn. *Mollienisia sphenops* Valenciennes, 1846) in the Czech Republic (Ergens and Moravec 1989), has not been reported in Sri Lanka (Table 9). Monogenean parasites may easily exploit continued human translocation of their hosts throughout south-east Asia.

Involuntary release of ornamental fish into wild waterways is a common occurrence in Australia (Dove and Ernst 1998; Lintermans 2004; Corfield et al. 2008). The co-invasion of exotic host-specific monogenean populations on Australian native fishes is considered less likely than for other parasitic groups due to the phylogenetic dissimilarity of native and exotic fishes (Fletcher and Whittington 1998). However, countries with native fauna phylogenetically similar to imported ornamental fish species, may be at a higher risk of co-introduced monogeneans invading native fishes. Exotic monogeneans could also be co-introduced in wild waterways by infecting invasive feral fish populations (Lymbery et al. 2014). This is the case of *Gyrodactylus bullatarudis* infecting feral *Poecilia reticulata* and *Xiphophorus hellerii*, *G. macracanthus* infecting feral *Misgurnus anguillicaudatus*, *Dactylogyrus extensus* infecting feral *Cyprinus carpio*, and *D. anchoratus* infecting feral *Carassius auratus* in Australia (Dove and Ernst 1998). The co-introduction of these parasites in Australia, as well as their feral hosts, has been directly associated with the import and release of ornamental species (Dove and Ernst 1998; Corfield et al. 2008).

The methodology and scope of this study prevented a detailed description of *Dactylogyrus* sp. 2, however, molecular identification suggests the species is closely related to *D. intermedius*. Differences in hamulus size, and morphology of hamuli and copulatory

organs, suggest that *Dactylogyrus* sp. 2 is a distinct species that is similar to *D. intermedius*. It is unlikely this is a case of phenotypic plasticity within *D. intermedius* as monogeneans are known to have distinct copulatory organ morphology between congeneric species as a reproductive barrier (Jarkovský et al. 2003). Similarly, this study shows no differences in hamulus morphology and measurements of *D. vastator* (Figure 10, Table 10), however molecular evidence suggests there are two molecularly distinct clades, and it could be a case of cryptic species (Fig. 15, clade 2). This is not surprising, as *Dactylogyrus* is one of the richest genera in the Monogenea (see Gibson et al. 1996), and its diversification has been explained by sympatric intra-host speciation, with multiple events of parasite duplications (Šimková et al. 2004). Further molecular analysis of other conserved genes could provide greater resolution on *D. vastator* (see Šimková et al. 2004).

Whittington and Chong (2007) assessed the import conditions/quarantine for Australia and other countries and considered Australia is perceived as one of the most stringent countries globally. Considering that over 950 monogeneans were undetected at border control in Australia during this study, Australia and other countries with less stringent biosecurity and import conditions could be at a high risk of introducing invasive monogeneans and must consider the adequacy of their import and quarantine conditions to account for microscopic pathogens and parasites. Although the new approach proposed by DAWR aims to ensure off-shore biosecurity in exporting countries (Hood and Perera 2016), treatment for monogenean parasites, both prior to exportation and during quarantine following border control, must be effective to maintain healthy stock for continued ornamental trade and to limit biosecurity risks to wild fisheries and the aquaculture industry. In addition to effective treatment of all fish populations for monogeneans, pre-export and import inspections should consider that visual examination does not provide reliable information on the presence or absence of

monogeneans on the fish host. If undetected, parasites could present a threat to the profitability and sustainability of the ornamental trade and wild environments. Assuming pre-export treatment of fish populations was done effectively, lethal sampling of subsampled fish could offer a reliable examination of pre-exported fish. However, sensitive, and time-efficient detection methods must be explored as alternatives to visual inspections at border control.

3.10. Acknowledgements

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CHAPTER 4 PUBLICATION STATEMENT

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Table 1 from this published review is provided as Supplementary S3.

CHAPTER 4

PARASITE DISPERSAL FROM THE ORNAMENTAL GOLDFISH TRADE

Abstract

Goldfish, *Carassius auratus* Linneaus, 1758, are immensely popular ornamental cyprinid fish, traded in more than 100 countries. For more than five hundred years, human translocation has facilitated the spread of goldfish globally, which has enabled numerous and repeated introductions of parasite taxa that infect them. The parasite fauna assemblage of goldfish is generally well documented, but few studies provide evidence of parasite co-invasion following the release of goldfish. This review provides a comprehensive synopsis of parasites that infect goldfish in farmed, aquarium-held, native, and invasive populations globally and summarises evidence for the co-introduction and co-invasion of goldfish parasites. More than 113 species infect goldfish in their native range, of which 26 species have probably co-invaded with the international trade of goldfish. Of these, *Schyzocotyle acheilognathi* (Cestoda: Bothriocephalidae), *Ichthyophthirius multifiliis* (Ciliophora: Ichthyophthiriidae), *Argulus japonicus* (Crustacea: Argulidae), *Lernaea cyprinacea* (Crustacea: Ergasilidae), *Dactylogyrus anchoratus*, *Dactylogyrus vastator* and *Dactylogyrus formosus* (Monogenea: Dactylogyridae) are common to invasive goldfish populations in more than four countries and are considered a high-risk of continued spread. Co-invasive parasites include species with direct and complex life cycles, which have successfully colonised new environments either through utilisation of new native hosts and/or invasive suitable hosts. Specifically, *I. multifiliis*, *A. japonicus* and *L. cyprinacea* can cause harm to farmed freshwater fish species and are important parasites to consider for biosecurity. These species may threaten other aquatic animal industries given their low host-specificity and adaptable life histories. Future attention to biosecurity, management and border detection methods could limit the continued spread of exotic parasites from the ornamental trade of goldfish.

4.1. Introduction

The risks posed by invasive species associated with the trade of live fish are a growing concern globally (Whittington and Chong 2007; Peeler et al. 2011). This includes non-native ornamental fish species introductions which can threaten biodiversity, the integrity of ecosystems, economically important industries, and can establish self-sustaining populations which can spread beyond their initial point of introduction (Kolar and Lodge 2001; Lymbery et al. 2014). Indeed, an increasing incidence of exotic ornamental fish being introduced into native environments has been documented in Australia (Lintermans 2004), Canada (Gertzen et al. 2008), England (Copp et al. 2005), and Mexico (Jiménez-Garcia et al. 2001). As such, the ornamental fish trade is considered an important pathway through which exotic parasites can be translocated between countries (e.g. Kahn et al. 1999; Whittington and Chong 2007; Corfield et al. 2008; Chang et al. 2009).

Parasite co-introductions can occur with the release of infected ornamental fish species into natural environments, use of infected ornamental species as live bait, or by disposal of water carrying viable life stages of parasite species (Bunkley-Williams and Williams 1994; Lintermans 2004; Corfield et al. 2008). As such, parasites can become co-introduced (transported with an exotic host to a new locality, outside of their natural range) or co-invasive (co-introduced and then spread to new, native hosts) (Lymbery et al. 2014). Host-switching, or the accidental colonization of a new host species by parasite individuals that establish a viable population, is most likely to occur in parasite species that display low host specificity, high tolerance to variable abiotic factors, direct life cycles and multiple reproductive strategies (Littlewood 2005). Exotic fishes may also acquire local parasites and become reservoirs that sustain endemic parasite populations with the ability to reinfect native

host species (e.g. *Neogobius melanostomus* Pallas, 1814 in the Danube River; Francová et al. 2011).

Goldfish, *Carassius auratus* Linnaeus, 1758 (Cypriniformes: Cyprinidae) is one of the most traded ornamental fish species worldwide (Kahn et al. 1999; Gertzen et al. 2008; Andras 2012; Maceda-Veiga et al. 2013). Native to rivers and lakes of Asia (Podlesnykh et al. 2015), goldfish were probably introduced from China to Japan between 1502–1620*,* and to Europe and elsewhere from China as early as 1611 (Kottelat 1997; Balon 2004). It is speculated that goldfish stocks were introduced by the Portuguese from Java to South Africa and from there to Lisbon, Portugal, as early as 1611 or 1691 (Balon 2004). Introduction to England and France likely occurred between 1691–1755 (Balon 2004). Following its establishment in Europe, *C. auratus* may have been introduced to America in 1846 after escapees became established in natural water ways in North America (Mulertt 1896). Goldfish are currently farmed globally, and invasive populations are known to occur in America (Bunkley-Williams and Williams 1994; Guzman-Cornejo and Garcia-Prieto 1999; Kuperman et al. 2002), Africa (Basson and Van As 1993; Mahmoud et al. 2009), Europe (Macchioni et al. 2015), the middle East (Molnar and Jalali 1992; Gussev et al. 1993) and Oceania (Arthur and Lumanlan-Mayo 1997; Arthur and Ahmed 2002; Arthur and Te 2006).

The spread of goldfish globally has enabled numerous introductions of invasive parasite taxa (e.g. Hudson and Bowen 2002; Dove and O'Donoghue 2005; Hassan et al. 2008). Parasites have been detected infecting imported ornamental goldfish in more than 14 countries including Australia (Evans and Lester 2001), Brazil (Piazza et al. 2006), Bulgaria (Borisov 2013), Croatia (Gjurčević et al. 2007), Germany (Moravec et al. 1999), Iran (Mousavi et al. 2009), Italy (Di Cave et al. 2000), Korea (Kim et al. 2002), Sri Lanka

(Thilakaratne et al. 2003), Norway (Levsen 1995), Spain and Portugal (Maceda-Veiga et al. 2013), the United States of America (USA) (Elliot and Shotts 1980; Rixon et al. 2005) and Turkey (Yildiz 2005). Research on goldfish and its associated parasite fauna is substantial and, given the popularity of goldfish in the ornamental trade, requires collation to examine the impact of goldfish in the translocation of parasite species. Here, we collated parasite records for goldfish, identified which were the most widely distributed in invasive goldfish populations, and provided evidence for specific parasite species that have been repeatedly co-introduced with goldfish, with comments on which parasite species could have become co-invasive because of goldfish introductions. We reviewed the biological attributes and life history traits of the most widely distributed goldfish parasite species and discussed emerging parasite risks enhanced by the goldfish trade.

4.2. Data collation

A detailed compilation of parasites infecting goldfish was conducted to generate a database of known protozoan and metazoan parasite fauna of goldfish documented between 1912 and 2017. For the purpose of this review, goldfish varieties *Carassius auratus burgeri*, *Carassius auratus gibelio*, *Carassius auratus grandoculis*, and *Carassius auratus langsdorfii* were excluded due to the genetic variability within the *C. auratus* species complex, and because several studies consider that these varieties are independent species or subspecies (see Takada et al. 2010). Only *Carassius auratus* Linnaeus, 1758 and the ornamental variety *Carassius auratus auratus* were considered in this review. Endemicity of *C. auratus* as well as *C. auratus auratus* within the *C. auratus* complex has been generally placed in mainland China, however human mediated translocation historically makes it difficult to discern

endemicity in historical records (Gao et al. 2012). To avoid this issue, goldfish (here on used to discuss both *C. auratus* and *C. auratus auratus*) were considered native if collected from mainland China and the islands of Japan (see Gao et al. 2012).

The major electronic search engines used to compile parasite-host records included the bibliographic database Web of Science (http://apps.webofknowledge.com), the library catalogue of James Cook University, Australia (https://www.jcu.edu.au/library) and the online search engine Google Scholar, using the search criteria '*Carassius auratus'*, 'goldfish', 'parasite' and 'infection'. The parasite-host database of the Natural History Museum (http://www.nhm.ac.uk/research-curation/scientific-resources/taxonomy-systematics/host-parasites; accessed in February 2017) was examined for additional records of platyhelminth parasites infecting goldfish.

Parasite records were organised by geographical location of the report (i.e. country) and non-discrete categories accounting for fish origin (i.e. native, invasive, farmed, import/export, aquarium-held). Fish were considered 'native' if they were sampled from natural environments within their natural range (see above), 'invasive' if collected from wild populations outside their natural range, 'farmed' if they were cultured fish, 'imported' or 'exported' if fish were sampled from a consignment of fish (a shipment of fish identified by an invoice containing details of the numbers and species of fish, the date of shipment, the origin and destination) or 'aquarium-held' for aquarium or experimental fish with no indication of origin. Parasites were considered 'co-introduced' if records indicated that they had established populations in non-native environments without records of infecting native fish species, and 'co-invasive' if records showed that the co-introduced parasite infected

native fish species in addition to the introduced exotic host in non-native environments (Lymbery et al. 2014). Parasite taxonomy was confirmed using the World Register of Marine Species (WORMS; www.marinespecies.org).

The compiled dataset and associated literature was scrutinised to identify parasite species which: 1) have been co-introduced with goldfish and have subsequently become co-invasive; 2) have impacted native environments, aquaculture, the aquarium industry, and human health, and; 3) present an emerging threat and warrant consideration in biosecurity and quarantine agendas. Parasite species that were determined to infect invasive or farmed goldfish common to more than four countries were emphasized with regard to their potential to become co-invasive based on host-specificity and life history traits. Furthermore, the threat of these species to freshwater aquaculture industries was assessed with respect to the five most harvested freshwater fish species (volume (tonnes)/year) per region (i.e. Africa, Americas, Asia, Europe, Oceania, as per the Food and Agriculture Organization; FAO 2017).

4.3. Goldfish parasite diversity and distribution

A total 197 parasite species infect goldfish, based on 556 parasite records from 195 published journal papers, books, museum records, reports, and communications (Supplementary S3). Validation of parasite identifications could not be made from preserved

material because few authors deposited accessioned parasite specimens into museum collections.

Probably the first published record of parasites infecting goldfish was by Robertson (1912). Robertson surveyed the species composition of an enclosed pond in the gardens of the Lister Institute at Elstree, London, and discussed the transmission of the trypanosome, *Trypanoplasma cyprini* Plehn, 1903 (Kinetoplastida: Cryptobiidae) and *Hemiclepsis marginata* Müller, 1774 (Hirudinea: Glossiphoniidae) infecting aquarium-held goldfish. Thereafter, Muto (1917) examined the role of the freshwater snail (*Semisulcospira* sp.) as the first intermediate host of the human intestinal trematode *Metagonimus yokogawai* Katsurada, 1912, and showed that the cercariae of this parasite could infect *C. auratus* in experimental conditions.

Since the 1930s, more than 152 parasite records for 79 parasite species have been reported to infect invasive goldfish, while 141 parasite records were made for 113 parasite species from native goldfish. Seventy-three parasite records for 41 parasite species have been reported in aquarium-held goldfish since 1912, 39 records for 21 parasite species have been reported infecting traded fish since 1947, and 66 records were made for 33 parasite species infecting farmed goldfish since the 1970s (Figure 16; Supplementary S3).

Parasite records were documented from 41 countries (Supplementary S3). A total of 173 species have been reported in Asia, 91 in Europe, 31 in the Middle East, 23 in North America, ten in Oceania, four in Africa, four in South America, and one in the Caribbean

(Figure 17). It is important to consider that these records may not necessarily represent true parasite diversity, but the relative research effort in these regions. There are 87 parasites species that have been only been reported within the goldfish native range (Figure 18), while 21 parasite species infecting native goldfish have also been reported in aquarium-held, farmed, invasive, or traded goldfish (Figure 18). Of the 87 parasite species reported in native goldfish (mainland China and islands of Japan), 31 are *Myxobolus* species reported by Chen and Ma (1998) (Supplementary S3). Chen and Ma's (1998) publication is part of the series Fauna Sinica in China and reports 269 *Myxobolus* species (including 129 new species) parasitising freshwater fishes in China. In total, 76 *Myxosoma*/*Myxobolus* species are included in the list as occurring in three host species of the genus *Carassius* (Bloch) (Chen and Ma 1998) (see also Dyková et al. 2002). Although the authors accessioned holotypes in the Institute of Hydrobiology in the Chinese Institute of Science (Wuhan, Hubei Province), other studies have questioned the validity of some of these myxozoan species, and suggest their re-evaluation because of their incomplete morphological descriptions, insufficient comparison with other known species, and recent revision of taxonomic criteria for myxozoans (see Zhang et al. 2010). We provide records for 31 species infecting goldfish (specifically reported on *C. auratus* or *C. auratus auratus*) described by Chen and Ma (1998) in Supplementary S3, but consider these to be *species inquerenda* until further analysis and comparison provide sufficient evidence for validation.

The collated parasite database indicated that at least 26 parasite species that infect native goldfish have been translocated outside their native range. A further 48 parasite species have probably been acquired by invasive goldfish in their new environment, with no records of these species infecting native, aquarium-held, farmed, or traded (i.e. imported and exported) goldfish (Figure 18). This suggests that invasive goldfish may be potential

reservoirs of infection by sustaining endemic parasite populations with the ability to reinfect native host species (see Francová et al. 2011).

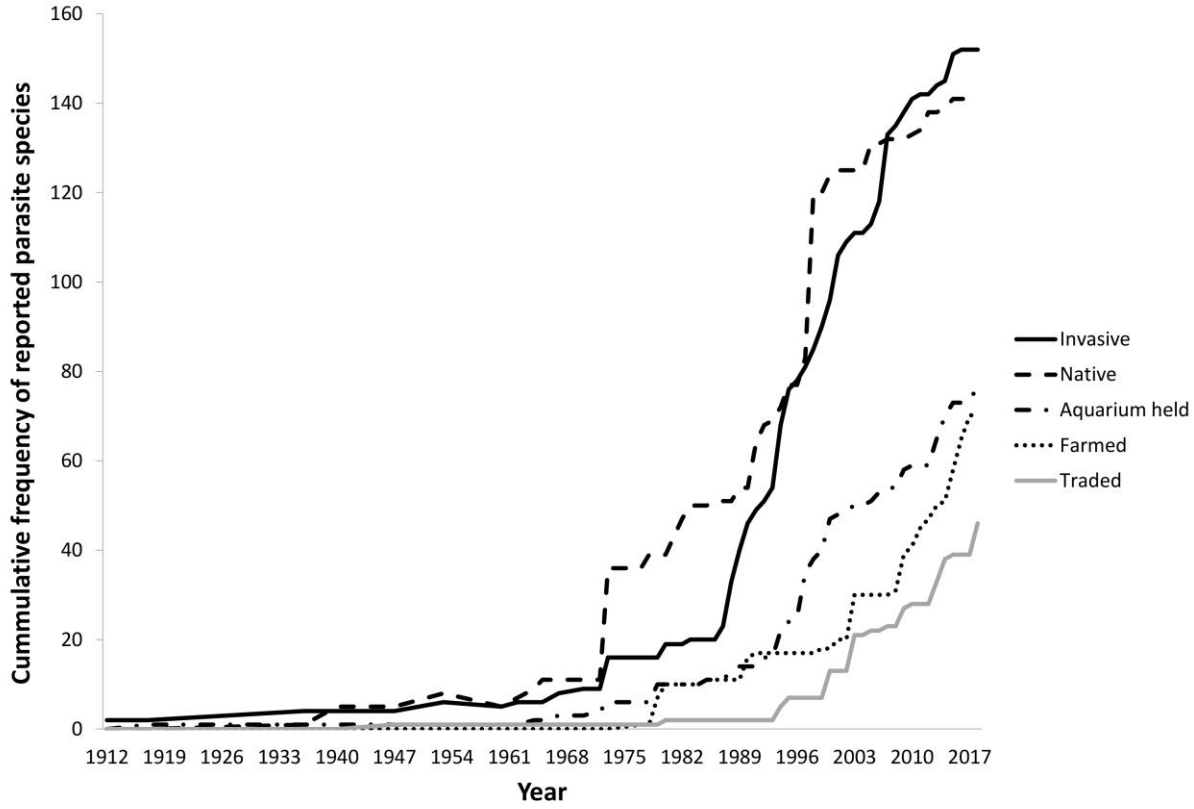

Figure 16. Cumulative number of parasite records infecting goldfish from 1900 to present. Reports are categorised based on parasites reported from invasive, native, farmed, traded (i.e. imported and exported) fish, and aquarium-held fish. Parasites not identified to species in published literature (a total of 119 occurrences) were excluded.

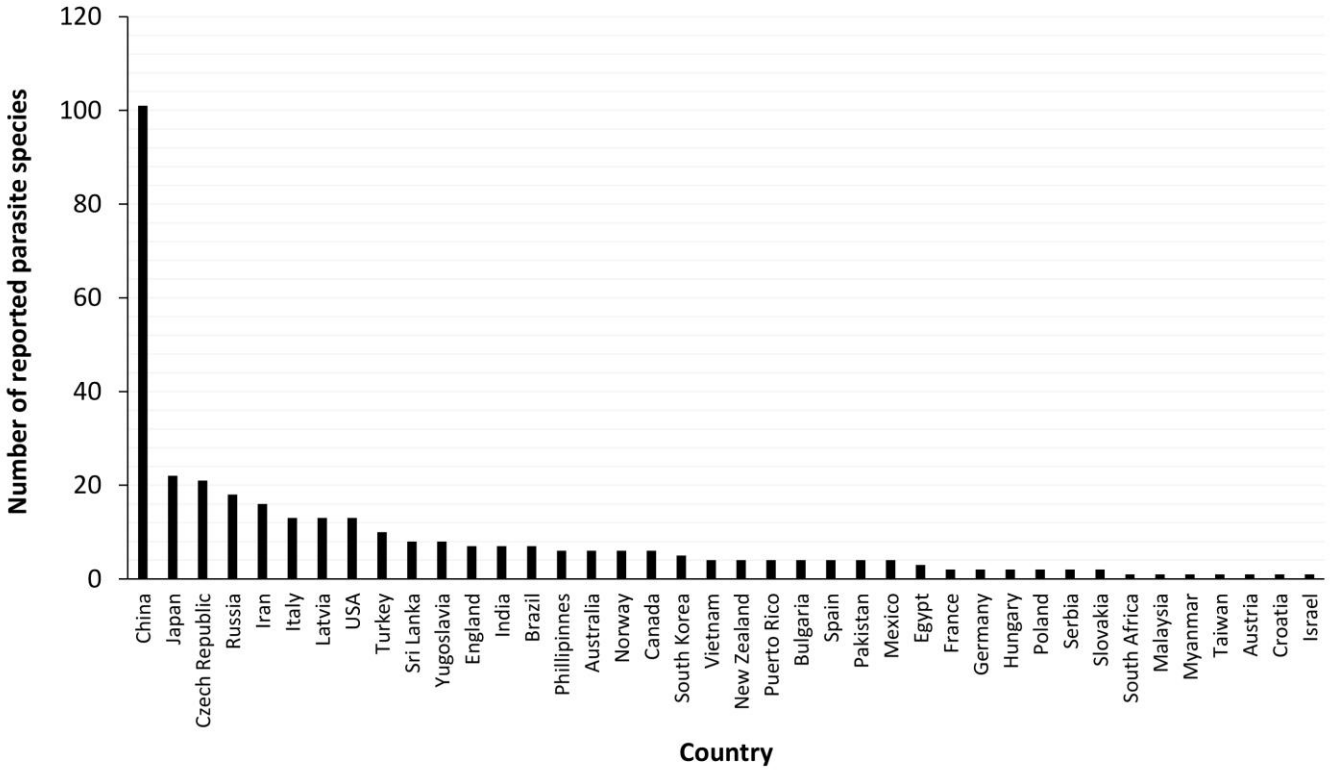

Figure 17. Number of parasite species reported to infect *Carassius auratus* in forty-one countries. Records with unspecified origin and location (Langdon 1990; Harris et al. 2004), and one record from the Caspian Sea (Ataev 1969) were not included.

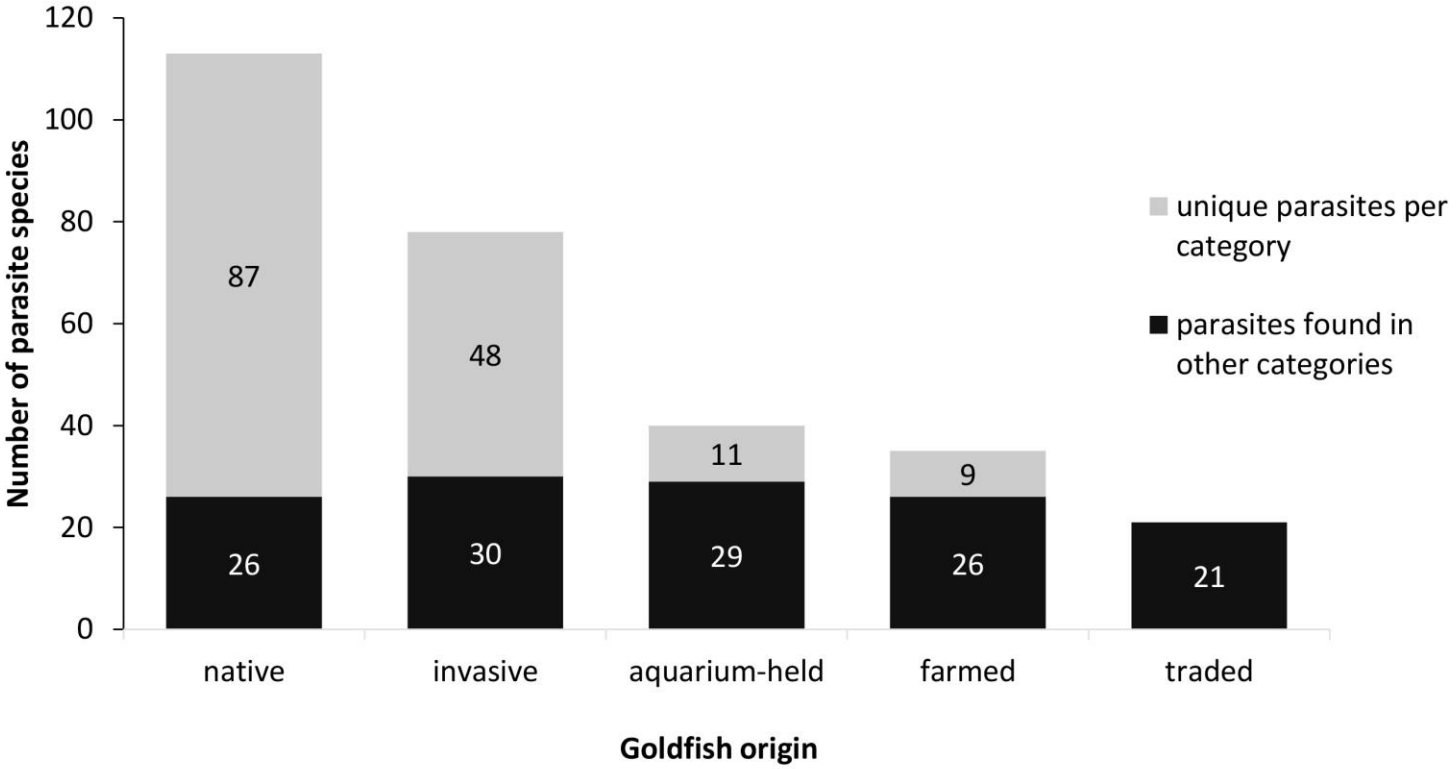

Figure 18. Number of parasite species infecting goldfish from varied sampling origins. Parasite species unique to each category are highlighted in grey, and parasite species found infecting fish in multiple categories are in black. Thirteen records with unspecified origin (Moravec 1995; Moravec 1995; Sicard et al. 2001; Harris et al. 2004), one record from the Caspian Sea (Ataev 1969) and 119 records for unidentified parasite species were not included.

4.4. Parasites translocated through the trade of goldfish

Countries with established biosecurity protocols follow the guidelines of the Sanitary and Phytosanitary Measures agreement (SPS) from the World Trade Organization (WTO) (Whittington and Chong 2007). Import conditions for live ornamental fish vary in stringency between countries, but requirements normally entail health certificates, treatment for pests and quarantine periods (i.e. pre-import conditions), inspection (of the exported fish by government representatives, i.e. border control and customs), and quarantine periods prior to co-habitation with other ornamental species (post-import conditions) (Whittington and Chong 2007; Tripathi 2015). Exclusion or sacrifice of imported live fish relies purely on the ability to detect parasites and diseases. Although parasites in the ornamental fish trade have been documented for decades, a handful of studies have addressed issues with biosecurity and detection methods for quarantine and border control (e.g. Kahn et al. 1999; Whittington and Chong 2007; Tripathi 2015). This lack of research limits the scope of current biosecurity protocols and detection methods used by quarantine divisions from countries around the world (Whittington and Chong 2007).

A total of 39 parasite species and 28 unidentified parasites have been reported infecting traded goldfish (Supplementary S3). Amongts these species, *Argulus foliaceus* (Walker et al. 2008), *Centrocestus formosanus* (Scholz and Salgado-Maldonado 2000), *Chilodonella piscicola* (syn. *C. cyprini*, see Kayis et al. 2013), *Dactylogyrus anchoratus* (Mueller 1936), *Ichthyophthirius multifiliis* (Butcher 1947), and *Learnea cyprinacea* (Hassan et al. 2008), have been reported previously as co-invasive parasites linked to the ornamental

trade. Interestingly, these parasite species are either skin or gill-dwelling, most species are microscopic or highly cryptic in nature and can easily go undetected if the fish host is not carefully examined. Inspection at border control can be highly limited by time availability to process the volume of imported live ornamental fishes received daily. Officers have a limited time to inspect all imports, which increases the possibility of parasites remaining undetected at border control.

4.5. Co-introduction, establishment and co-invasion

It is not always straightforward to determine whether a parasite is exotic or native to a region. This is because human mediated translocation of organisms began long before taxonomic surveys and species monitoring programs, and because many species, particularly parasites, are difficult to identify or have ambiguous taxonomies (Lymbery et al. 2014). It is usually inferred that exotic parasites can be co-introduced with exotic fish, even if the host species and the event are unknown. Nonetheless, suggestions of specific fish species linked to specific co-invasive parasites must be approached with caution.

Exotic parasites can be co-introduced into native environments with translocated exotic or native hosts (see Lymbery et al. 2014), and occasionally without any host (e.g. free living parasite stages of the isopod *Orthione griffenis* Markham, 2004, transported with ballast water to North America (Chapman et al. 2012), and translocation of eggs and juvenile parasitic stages of the nematode *Anguillicoloides crassus* (Kuwahara, Niimi et Itagaki, 1974) through aquaculture transport to the United Kingdom (Kirk, 2003). The establishment of

exotic parasite life cycles in non-native environments has been discussed in detail by Lymbery et al. (2014). Parasite establishment (and possible co-invasion) depends greatly on the specificity of parasite founding populations, and the availability and density of suitable hosts in non-native environments (Lymbery et al. 2014). As such, exotic parasites could initially be co-introduced in non-native environments, but lack the capacity to become established, and subsequently co-invasive. Parasites most likely to become co-invasive have been usually considered to display low host specificity and simple, direct life cycles (Dobson and May 1986; Bauer 1991; Torchin and Mitchell 2004). However, these are not exclusive characteristics of invasive parasites, and parasites with complex life cycles can also become co-invasive if susceptible intermediate hosts are available (Lymbery et al. 2014). Intermediate and definitive hosts may be available in areas where there is shared ancestry between invasive and native hosts, and co-introduced parasites are able to infect new native hosts (Lymbery et al. 2014; Poulin 2016), or areas where invasive susceptible hosts have already been established (Torchin and Mitchell 2004; Lymbery et al. 2014). Pathogenicity and host-specificity differs between parasite species (Lom and Dyková 1992; Kearn 2011) and importing countries should consider the risk of co-invasive parasites considering the susceptibility of native, invasive and farmed fauna. Herein, the life history and potential impact of parasites species infecting invasive goldfish and reported in multiple countries (more than four) are examined.

Five parasites species are common to invasive populations of goldfish in more than four different countries (Figure 19A). These include the crustacean parasite *L. cyprinacea,* the cestode *S. acheilognathi* (reviewed in detail by Kuchta et al. 2018), and the monogeneans *D. anchoratus*, *D. formosus*, and *D. vastator* (Figure 19A). Two parasite species, *A. japonicus* and *I. multifiliis*, have been reported in four countries infecting farmed goldfish (Figure 19B). These parasite species can spread easily because they can infect a range of host

fishes: *L. cyprinacea* has been reported to infect more than 60 fish species representing 25 families, *S. acheilognathi* has been reported infecting more than 141 fish species representing 21 fish families, *D. anchoratus* in 20 species from two families, *D. formosus* in four different cyprinid species and *D. vastator* in 15 species from three families (Figure 20). In the case of parasites infecting farmed goldfish, *A. japonicus* has been reported in 29 fish species from 10 families and *I. multifiliis* has been recorded to infect more than 79 fish species representing 25 fish families.

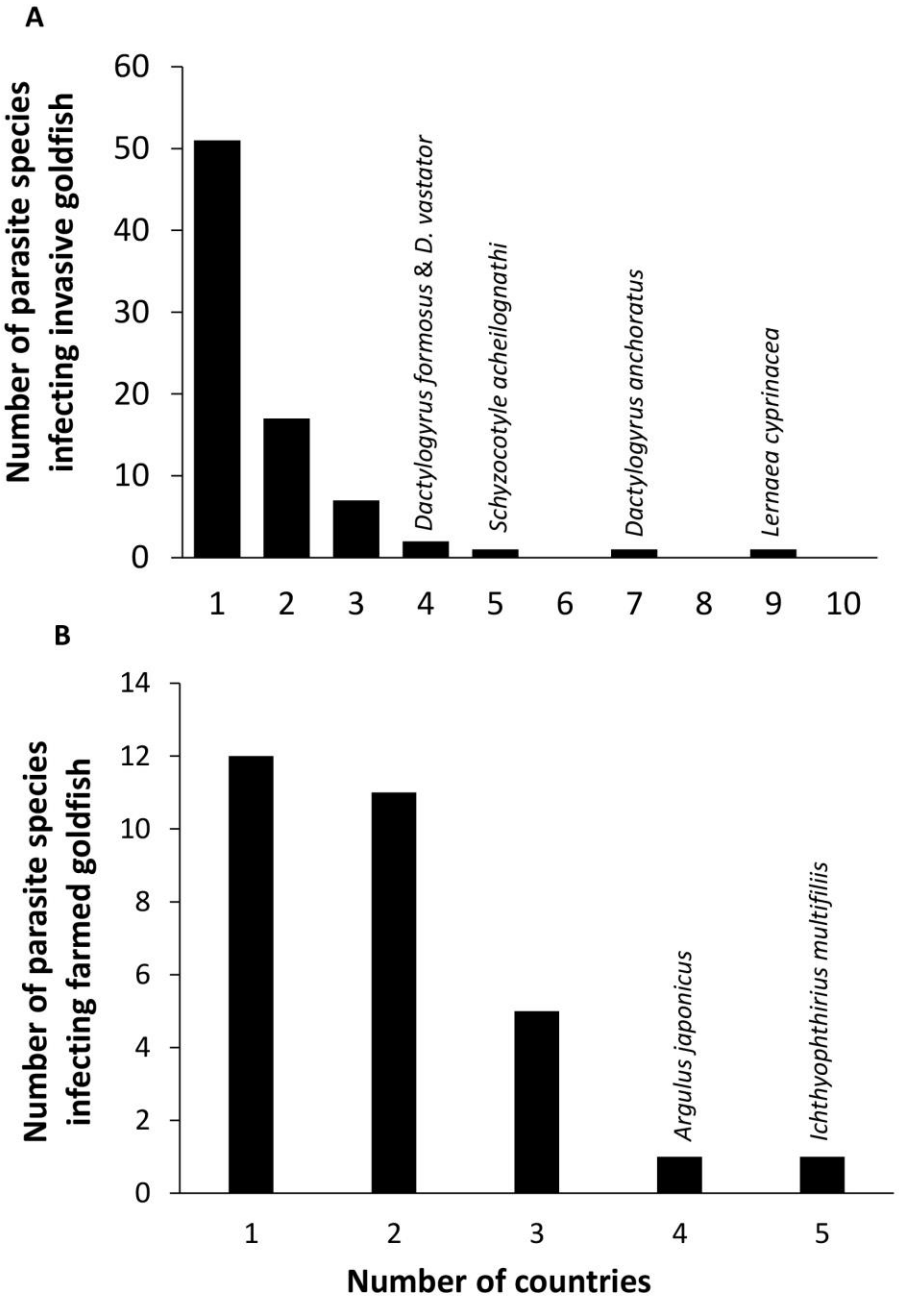

Figure 19. Parasite species reported in multiple countries infecting (A) invasive goldfish and, (B) farmed goldfish. A total of 119 parasite records not verified to species were excluded. Parasite species names are indicated above columns for species reported in four or more countries; the complete list of parasite species records can be sourced from Supplementary S3.

4.5.1. Protozoa

Protozoans exhibit rapid and exponential reproductive strategies (e.g. *Chilodonella* spp. in Basson and Van As 2006), and versatile, resilient life stages (e.g. *Ichthyophthirius multifiliis* in Dickerson 2012), which have allowed parasitic protozoa to colonize aquatic environments globally. Amongst fish protozoa, *Ichthyophthirius* and *Trichodina* are two of the most predominant genera globally (Lom and Dyková 1992). *Ichthyophthirius multifiliis* is one of the most contagious ciliophoran parasites of fishes (Matthews 2005; Dickerson 2006). This parasite accounts for significant economic losses in aquaculture, the ornamental fish trade, and epidemics in wild fish populations, resulting in mass mortalities (Matthews 2005).

It is likely that the goldfish trade has played a role in the spread of *I. multifiliis* internationally. It has been suggested that *I. multifiliis* was originally endemic to Asia and introduced to Europe in the middle ages with the development of carp culture (Hoffman 1970a) and to other countries, including the USA, through the importation of goldfish (Hoffman 1970b; Hoffman 1978). *Ichthyophthirius multifiliis* is now widespread globally, with a geographical range extending from the tropics to temperate regions and northwards in Europe to the Arctic Circle (Matthews 2005), facilitated by human trade and translocation between countries (Nigrelli et al. 1976).

It is unclear how many host fish species are susceptible to *I. multifiliis*. This review indicates that it has been reported in over 79 fish species from 25 families (Figure 20), but it has been suggested that it can infect all freshwater fishes, with infections reported from

virtually all regions where fishes are cultured, as well as in invasive fish populations in the tropics and sub-arctic (Dickerson 2006). Moreover, studies have reported epidemics in Australia, Bolivia, Canada, South Africa, and Uganda in areas naïve to the parasite following introduction of exotic fishes infected with *I. multifiliis* (see Butcher 1947, Wurtsbaugh and Tapia 1988; Traxler et al. 1998; Bragg 1991; Paperna 1972, respectively).

Trichodina species are opportunistic ciliophoran parasites that display low host specificity and can infect a wide range of fish hosts within the same environment (Dove and O'Donoghue 2005). Few species are as widely distributed as *T. acuta, T. heterodentata*, *T. mutabilis and T. nigra* (Basson and Van As 2006; Islas-Ortega and Aguilar-Aguilar 2014). The global distribution of *T. mutabilis* has been associated with transcontinental introductions of exotic cyprinids carrying the parasite (Basson and Van As 2006). For example, *T. mutabilis* and *T. reticulata* have been suggested to be co-introduced in Australia following the release of imported cyprinids (i.e. *Cyprinus carpio* Linnaeus, 1758, and *Carassius auratus*, respectively; see Dove and O'Donoghue, 2005).

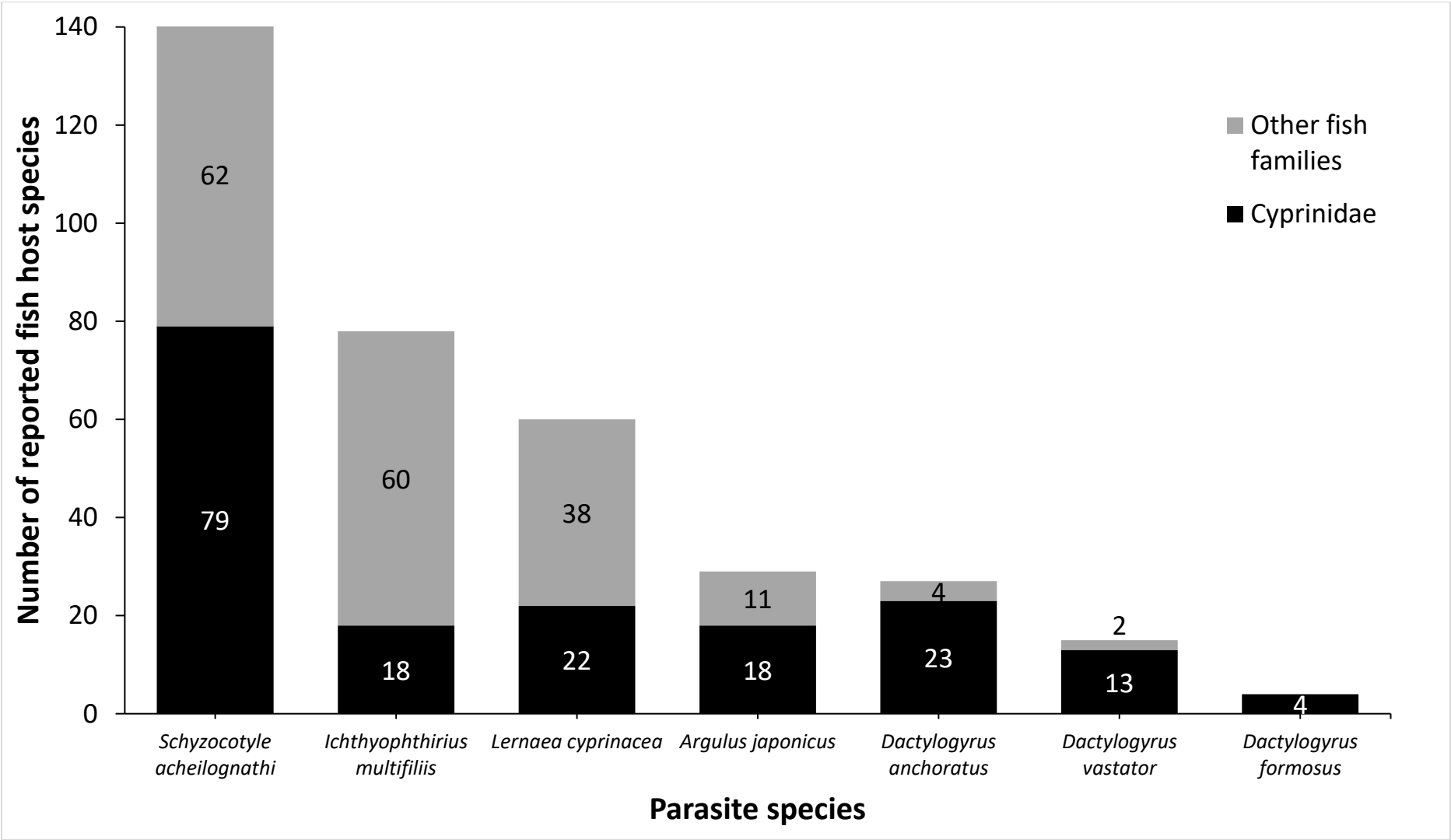
Number of reported fish host species
140
120
100
80
60
40
20
0
62
79
60
18
38
22
11
18
4
23
2
13
4
Other fish families
Cyprinidae
Schyzocotyle acheilognathi
Ichthyophthirius multifiliis
Lernaea cyprinacea
Argulus japonicus
Dactylogyrus anchoratus
Dactylogyrus vastator
Dactylogyrus formosus
Parasite species

Figure 20. Number of fish host species reported for parasites infecting invasive and farmed goldfish in over four different countries. Other fish families for *Schyzocotyle acheilognathi* include species from Acipenseridae, Atherinopsidae, Centrarchidae, Characidae, Cichlidae, Clariidae, Eleotridae, Esocidae, Fundulidae, Gobiidae, Goodeidae, Ictaluridae, Moronidae, Nemacheilidae, Notopteridae, Percidae, Pimelodidae, Poeciliidae, Profundulidae, Retropinnidae, Siluridae and Terapontidae; for *Ichthyophthirius multifiliis* include species from Acestrorhynchidae, Arapaimidae, Cyprinodontidae, Erythrinidae, Esocidae, Gasteropelecidae, Heptapteridae, Ictaluridae, Lebiasinidae, Loricariidae, Mastacembelidae, Moronidae, Osteoglossidae, Percidae, Pimelodidae, Serrasalmidae, Sisoridae, Terapontidae and Triportheidae; for *Lernaea cyprinacea* include species from Amiidae, Anguillidae, Catostomidae, Channidae, Cichlidae, Clupeidae, Cobitidae, Cottidae, Cyprinodontidae, Esocidae, Fundulidae, Gasterosteidae, Ictaluridae, Lotidae, Mastacembelidae, Stromateidae, and Umbridae; for *Argulus japonicus* include species from Salmonidae, Cichlidae, Clariidae, Clupeidae, Gasterosteidae, Ictaluridae, Percichthyidae, Percidae and Siluridae; for *Dactylogyrus anchoratus* include species from Esocidae, Gasterosteidae, Percidae and Salmonidae; and for *D. vastator* include Esocidae and Cobitidae. References for host records are available in Supplementary S4.

4.5.2. Cestoda

Schyzocotyle acheilognathi, is a notorious and highly successful invasive cestode reported in a wide spectrum of freshwater fishes (Kuchta et al. 2018). First recorded infecting *Ctenopharyngodon idellus* (Cyprinoformes: Cyprinidae) in the Amur river, China (Yamaguti 1934), *S. acheilognathi* now displays a global distribution and is considered one of the most invasive parasite species globally (Kuchta et al. 2018). Co-invasion of *S. acheilognathi* in Europe has been directly associated to the co-introduction of grass carp, *Ctenopharyngodon idella* Valenciennes and Valenciennes, 1844 (Cypriniformes: Cyprinidae) for culture in the 1970s (Hoffman and Shubert, 1984), and although it is unclear when *S. acheilognathi* was co-introduced into the American continent, it is a co-invasive parasite infecting native fish species and feral populations of grass carp and goldfish in Canada and the United States(Brouder and Hoffnagle 1997; Choudhury et al. 2006). The presence of *S. acheilognathi* in North America has been directly linked to the ornamental fish trade (Choudhury et al. 2006). Most importantly, S. *acheilognathi* is known to cause serious damage in fry and small fish (Salgado-Maldonado and Pineda-Lopez 2003), cause significant fish mortalities in farmed fish, has the potential to regulate fish populations (Clarkson *et al.* 1997), and is considered an emerging threat to aquaculture and ecosystems given its unusually low host specificity (Kuchta et al. 2018).

4.5.3. Monogenea

Monogeneans are notorious parasites in aquaculture with diverse life history traits that ensure their survival (Thoney and Hargis 1991; Kearn and Whittington 2015). Traits include

multiple reproductive mechanisms including oviparity (Whittington and Chisholm 2008), viviparity (Harris and Tinsley 1987), reproduction in isolation (Dinh-Hoai and Hutson 2014), camouflage (Whittington 1996), and behavioural responses to host and environmental cues that favour enhanced infection success (Whittington and Ernst 2002). There are over 246 described monogenean species infecting fishes in south-east Asia, of which 69 have been reported from cyprinids, with *Ancyrocephalus*, *Dactylogyrus*, *Gyrodactylus*, and *Paradiplozoon* being the most dominant genera (Lim 1998).

Dactylogyrus species are common parasites of cultured cyprinid fishes throughout south-east Asia (Thilakaratne et al. 2003; Řehulková and Gelnar 2006; Wang et al. 2011) and have been associated with economic losses in aquaculture (Lio-Po and Lim 2002; Ji et al. 2012; Ling et al. 2016). *Dactylogyrus anchoratus, D. intermedius and D. vastator* are considered dominant species infecting *C. auratus* and *Cyprinus carpio,* with records of imported goldfish from south-east Asia infected by either one or all three dactylogyrid species (Di Cave et al. 2000; Mousavi et al. 2009). Records suggest that *D. anchoratus*, *D. formosus* and *D. vastator* can infect multiple host fish families. Specifically, *D. anchoratus* has been reported to infect hosts from Esocidae, Gasterosteidae, Percidae and Salmonidae, and *D. vastator* infects species in Esocidae and Cobitidae (Figure 20; Gibson et al. 2005). However, *Dactylogyrus* spp. commonly display high host-specificity, with the majority reported from a single host in the Cyprinidae (Lim 1998; Bakke et al. 2002). The Esocidae, Gasterosteidae, Percidae and Salmonidae are not closely related to the Cyprinidae and are not part of the Cypriniformes (Nelson et al. 2016). Records of *D. anchoratus* and *D. vastator* infecting host species from other families apart from Cyprinidae need to be verified as they imply that *D. anchoratus* and *D. vastator* display lower host specificity, or that parasites were incorrectly identified. Nonetheless, *D. anchoratus*, *D. extensus*, *D. intermedius*, and *D.*

vastator have been reported as co-introduced parasites in natural ecosystems associated with the trade of cyprinids in the USA and Puerto Rico, Australia, Iran, and Italy respectively (Mueller 1936; Molnar and Jalali 1992; Bunkley-Williams and Williams 1994; Dove and Ernst 1998; Macchioni et al. 2015).

Gyrodactylus spp. have been detected on invasive goldfish in Australia, Canada, Czech Republic, England, Italy, Japan, Latvia, Russia, Spain, the USA, and former Yugoslavia (Supplementary S3). *Gyrodactylus* spp. range from being highly host specific (71% of 409 described *Gyrodactylus* species infect a single host) to displaying low host specificity (*Gyrodactylus alviga* recorded from 16 hosts; Bakke et al. 2002). Although *Gyrodactylus* species are potentially highly pathogenic (Bauer 1988; Bakke et al. 2002; Jalali et al. 2005), the pathogenicity of *Gyrodactylus* species is variable and parasite-induced host death is dependent on host species, size and parasite intensity and other environmental factors (Bakke et al. 2007). The richness of *Gyrodactylus* species has been explained by their predominantly viviparous life history, direct life cycle and no specialised transmission stage (Kearn 1994; Huyse and Volckaert 2005). Most importantly, the presence of *Gyrodactylus* spp. in exotic environments has been linked to the trade of live fish species (Johnsen and Jensen 1991; Fletcher and Whittington 1998; Macchioni et al. 2015). Specifically, the co-introduction of *Gyrodactylus elegans*, *G. gurleyi*, *G. kobayashii* and *G. longoacuminatus*, have been directly linked to the trade of live cyprinids, including goldfish and carp, in Italy, the USA, Canada, and England (Macchioni et al. 2015; Mueller 1936; McDonald and Margolis 1995; Shinn et al. 1997; Cable et al. 1999, respectively).

Nevertheless, *Gyrodactylus* remains a poorly studied genus beyond the scope of aquaculture (Bakke et al. 2002; Huyse and Volckaert 2005), and little is known of their capacity to host switch in non-native environments. It is possible that *Gyrodactylus* spp. continuously face opportunities to infect different host individuals because of their viviparous life style (e.g. facilitated by mixing of fish strains; Bakke et al. 2002), in contrast to the highly specialised larvae (oncomiracidia) of other monogeneans (Kearn 1994; Bakke et al. 2002; Kearn 2011). *Gyrodactylus* spp. readily infect native fish fauna in cases where invasive and native hosts are closely related (Johnsen and Jensen 1991; Huyse and Volckaert 2005). On the other hand, some studies do not provide evidence of host switching in *Gyrodactylus* spp. infecting exotic ornamental fishes in non-native environments (Dove and Ernst 1998; Rubio-Godoy et al. 2016; García-Vásquez et al. 2017). Further research is needed on host switching habits of *Gyrodactylus* spp. to fully understand their potential impact in native environments following co-introduction through the ornamental trade.

4.5.4. Crustacea

Lernaea cyprinacea has been detected on invasive goldfish in Australia (Hassan et al. 2008), Egypt (Mahmoud et al. 2009), India (Kalita et al. 2010), Iran (Raissy et al. 2013), Italy (Macchioni et al. 2015), Japan (Yoshimine et al. 2015), New Zealand (Hine et al. 2000), The USA (Kuperman et al. 2002), Uruguay (Carnevia and Speranza 2003), and Vietnam (Arthur and Te 2006), and is considered one of the most invasive parasite species globally. *Lernaea cyprinacea* can infect over 60 different fish species from 24 families (Figure 20) including amphibians (Nagasawa et al. 2007; Kupferberg et al. 2009) and aquatic insects (McAllister et al. 2011). *Lernaea cyprinacea* has a multi-stage direct life cycle that includes three free-

living nauplii, and five parasitic copepodid stages (Lester and Haywood 2006), and is known to cause high mortalities of small farmed fish and economic loss in aquaculture (e.g. *Oncorhynchus mykiss* Walbaum, 1792; Berry et al. 1991; Avenant-Oldewage 2012). *Lernaea cyprinacea* is native to Asia (Robinson and Avenant-Oldewage 1996), but has invaded America, Africa, Asia, Europe, and Oceania, which may be the result of trade in ornamental cyprinid hosts such as *C. auratus* and *Cyprinus carpio* (see Amin et al. 1973; Amin 1981; Robinson and Avenant-Oldewage 1996; Corfield et al. 2008; Oscoz et al. 2010). Recently, *L. cyprinacea* was reported infecting *Ambystoma mexicanum* Shaw, 1789 (native to Uruguay) which was linked to the release of imported goldfish (Carnevia and Speranza 2003). Similarly, *L. cyprinacea* was considered co-invasive after infections were found in four fish species native to Western Australia (*Bostockia porosa* Castelnau, 1873, *Nannoperca vittata* Castelnau, 1873 (syn. *Edelia vittata* Castelnau, 1873)*, Galaxias occidentalis* Ogilby, 1899, and *Tandanus bostocki* Whitley, 1944 (syn. *Plotosus bostocki* Whitley, 1944); Hassan et al. 2008). Co-invasion of *L. cyprinacea* has been associated with the trade and release of imported *C. auratus* and *Cyprinus carpio* (Hassan et al. 2008).

The branchiurid crustacean, *Argulus japonicus*, has been detected on invasive goldfish in Australia and Japan (Heegaard 1962; Tokioka 1936, respectively) and farmed goldfish in China, India, Iran, Turkey and the USA (Alsarakibi et al. 2014; Chanda et al. 2011; Mousavi et al. 2011; Koyuncu 2009; Wafer et al. 2015, respectively). *Argulus japonicus* is considered highly pathogenic and is known to parasitise over 28 fish species from 10 families (Figure 20) (Avenant-Oldewage 2001). *Argulus japonicus* is native to Asia where it infects *C. auratus* and *Cyprinus carpio.* The parasite has a direct life cycle, is cryptic in nature and infected fish may not display obvious signs of disease (Møller 2012; Wafer et al. 2015). The species is known to cause significant morbidity and mortality in farmed fish populations

(Wafer et al. 2015). Furthermore, *Argulus* species are known to be the vehicle for other fish pathogens, including *Rhabdovirus carpio*, larval nematodes, and water mould *Saprolegnia* (see Avenant-Oldewage 2001). Co-introduction and subsequent co-invasion of *A. japonicus* in Africa, Israel, and the USA has been directly linked to the trade in Asian cyprinids, with specific mention of *C. auratus* and *Cyprinus carpio* as initial sources of infection (Kruger et al. 1983).

4.6. Goldfish parasites infecting farmed fish

Events of live animal translocation, be it for aquaculture, through ballast water, or the aquarium trade, are always at risk of exotic hosts and their parasites being co-introduced (Ruiz et al. 1997; Minchin et al. 2009; Lymbery et al. 2014). In the case of aquaculture, co-introduced and subsequently co-invasive fish parasites have had detrimental impacts on fish production, causing significant fish mortalities and morbidity (see Butcher 1947; Johnsen and Jensen 1991; Deveney et al. 2001; Whittington and Chong 2007).

Few records directly associate the trade of *C. auratus* with the co-introduction and/or co-invasion of parasites with impacts in aquaculture. Associations are mostly speculative or anecdotal observations (see Mueller 1936; Butcher 1947). Nonetheless, co-invasive parasites species associated with the spread of goldfish or other cyprinids (Figure 18) have been reported to infect some of the most globally important aquaculture fishes (Figure 21).

In the case of parasites displaying low host specificity, co-invasive *I. multifiliis*, *L. cyprinacea* and *S. acheilognathi* have been reported to infect some of the most farmed freshwater fishes in five global regions (Figure 21, FAO 2017). Farmed *C. carpio* for example, has been infected with invasive *I. multifiliis* and *S. acheilognathi* in every region (Figure 21), as have *Oreochromis* spp. farmed in Africa, America, and Oceania (Figure 21). In the case of Europe, invasive *L. cyprinacea* has been recorded from all five most harvested freshwater fishes (tonnes/year) (Figure 21, FAO 2017). Invasive *Argulus japonicus* has been reported to infect farmed fishes in Africa, Asia, and Europe, but no records were found for *A. japonicus* infecting the five most farmed freshwater fishes in America and Oceania (Figure 21). Compared to parasites with low host-specificity, *Dactylogyrus* species only infect cyprinids (see Figure 20). Co-introduced *Dactylogyrus anchoratus* and *D. formosus* have been reported to infect only farmed *C. carpio* in Africa, America, Asia and Europe, and *D. vastator* has been reported infecting farmed *C. carpio* and *Hypophthalmichthys molitrix* in Asia, and *C. carpio* and *Ctenopharyngodon idellus* in Europe (Figure 6). No records were found for *D. anchoratus* and *D. formosus* or *D. vastator* infecting farmed fishes from other families apart from the Cyprinidae.

Without appropriate historical data, linking parasites infecting farmed fish species to specific species co-introduction events is not possible. It is plausible that multiple ornamental fish species, particularly other cyprinid species, have facilitated the spread of 'goldfish' parasites. Importantly, crustaceans *A. japonicus* and *L. cyprinacea,* monogeneans *D. anchoratus*, *D. formosus* and *D. vastator*, the cestode *S. acheilognathi* and the protozoan *I. multifiliis* are native to Asia (Supplementary S3), and records of such parasites infecting farmed fish in African, America, Europe, and Oceania (Figure 21) indicate that these

parasites were translocated with live fish or other contaminated sources through human mediation.

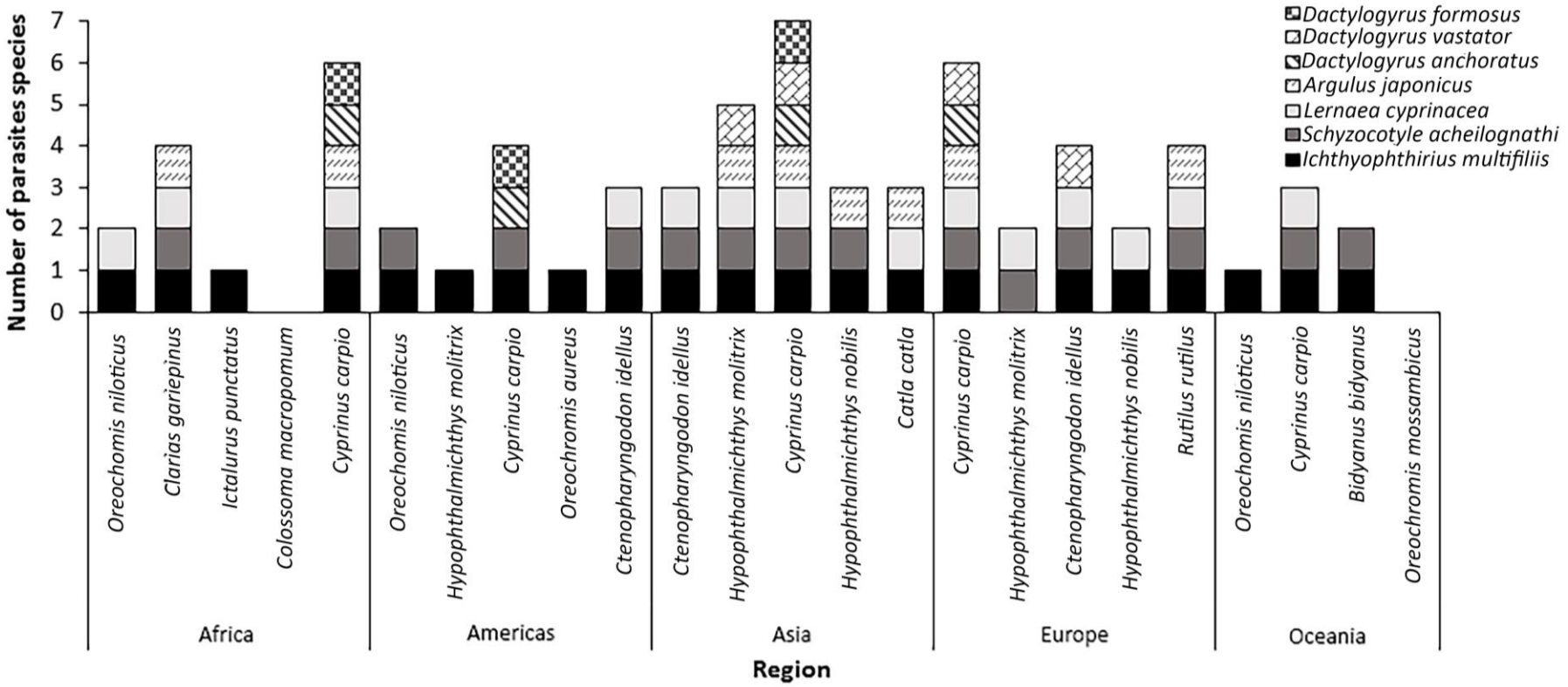

Figure 21. Parasite species shared between goldfish (*Carassius auratus*) and most farmed freshwater fish in five major regions of production. Farmed freshwater fish species were selected based on the top five species produced in 2016 (total volume in tonnes) reported to the Food and Agriculture Organization of the United Nations by global regions (i.e. Africa, Americas, Asia, Europe, and Oceania) (FAO 2017). Fish were organised per continent from most harvested (left) to least harvested (right). References for infection records are available in Supplementary S5.

4.7. Zoonotic parasites infecting goldfish

The goldfish trade could facilitate the translocation of zoonotic parasites. Of the 12 zoonotic parasites reported infecting goldfish (Supplementary S3), three species (i.e. *Centrocestus formosanus*, *Metagonimus yokogawai* (Katsurada, 1912), and *Pseudamphistomum truncatum* (Rudolphi, 1819), have been reported from invasive goldfish populations (Supplementary S3). *Centrocestus formosanus* has been reported infecting goldfish imported from Singapore, infecting goldfish collected from commercial suppliers in Croatia (Gjurčević et al. 2007), Turkey (Yildiz 2005), and from aquarium-held goldfish sampled from five commercial suppliers in Iran (Mood et al. 2010). *Centrocestus formosanus* (Digenea: Heterophyidae) is a food-borne intestinal trematode inhabiting the small intestine of birds and mammals, including chickens, ducklings, mice, rats, rabbits, dogs, cats, and foxes (Han et al. 2008), and human infections have been reported in the Lao People's Democratic Republic (Chai et al. 2013) as well as experimental human infections in Taiwan (Nishigori 1924; Han et al. 2008). Similarly, *Metagonimus yokogawai* is an important food-borne trematode that causes metagonimiasis in China, Japan, Korea, and Taiwan (Chai and Lee 2002). It can infect a wide range of fish species from different fish families, which serve as second intermediate hosts (Li et al. 2013) and was reported infecting invasive goldfish in Spain (Cordero Del Campillo et al. 1980).

Pseudamphistomum truncatum (Digenea: Opisthorchiidae) is a food-borne trematode native to eastern Europe, and one of several *Pseudamphistomum* species known to cause pseudamphistomosis in commercially important ruminants (Sanabria and Romero 2008; Skov et al. 2008; Mason et al. 2012), with one case of zoonosis in Russia (Khamidullin et al. 1991).

Pseudamphistomum truncatum has an indirect life cycle, which includes gastropod snails (mainly, but not exclusively *Bithynia* species, see Schuster et al. (2001)), cyprinid species as second intermediate hosts, and a broad range of mammals as definitive hosts (Skov et al. 2008; Neimanis et al. 2016). Following reports of *P. truncatum* zoonosis (see Khamidullin et al. 1991), studies reported infections of the parasite in *Rutilus rutilus* in Germany (Schuster et al. 2001), Denmark (Skov et al. 2008) and Ireland (Hawkins et al. 2010), as well as multiple mammals in Europe, including foxes, otters, minks, wolves, stoats, and weasels (see Skov et al. 2008). *Pseudamphistomum truncatum* is now considered an emerging parasite of grey seals (*Halichoerus grypus*, Nilsson, 1820) in the Baltic sea (see Neimanis et al. 2016), and mustelids in the United Kingdom and Ireland, where the introductions of imported ornamental sunbleak (*Leucaspius delineatus* Heckel, 1843) and topmouth gudgeon (*Pseudorasbora parva* Temminck and Schlegel, 1846) are considered as the possible sources of infection (Simpson et al. 2005).

The diversity of co-introduced and co-invasive zoonotic parasites may be underestimated and misrepresented. Traditional identification techniques of zoonotic parasites have depended largely on the morphological identification of parasite eggs in human faecal examinations. However, multiple studies have raised issues with this approach because parasite eggs have been proven to be highly similar between zoonotic species (see Chai and Lee 2002; Chai et al. 2005; Yera et al. 2013). For example, egg morphology of *Metagonimus* spp. and other heterophyid species can be undistinguishable (Chai and Lee 2002), and in some cases can be confused for liver fluke eggs (Chai et al. 2005). Recently, Yera et al. (2013) reported a case of possible human accidental infection (i.e. parasite passage through human intestine after the consumption of an infected fish host) with *S. acheilognathi* in French Guiana. In this study, morphological criteria wrongly suggested that the eggs observed in the patient stool were

those of *Diphyllobothrium pacificum* reported from South America (Scholz et al. 2009). However, molecular identification showed the eggs were those of *S. acheilognathi,* the most important pathogenic cestode of cyprinid fish (Scholz et al. 2012) which has been reported infecting invasive goldfish in Australia (Langdon 1990; Dove and Fletcher 2000), Czech Republic (Scholz 1989), Mexico (Prieto and Sarabia 1991; Salgado-Maldonado and Pineda-Lopez 2003), Slovakia (Macko et al. 1993), and the USA (Kuperman et al. 2002).

The diversity of zoonotic parasites in the ornamental trade is poorly known. This obstacle prevents further understanding of the current distribution and clinical relevance of zoonotic parasites infecting ornamental species, including goldfish. For example, of the four zoonotic parasites found infecting invasive goldfish populations, records only exist for *C. formosanus* infecting traded goldfish. Similarly, *Clonorchis sinensis*, the most common human liver fluke in East Asia (with over 200 million people vulnerable to infection in China, Korea, Russia, Taiwan, and Vietnam; Hong and Fang 2012) has been reported infecting wild goldfish populations in China in five separate studies (Supplementary S3). However, there is little knowledge of the occurrence of this parasite in the ornamental trade and it is unclear if *C. sinensis* infects other farmed and traded ornamental cyprinids in China or other Asian countries.

4.8. Emerging threats of translocated goldfish parasites

Several parasite species that infect goldfish are well-known threats in the ornamental trade (e.g. *I. multifiliis*, *A. japonicus* and *L. cyprinacea*). These parasite species have been translocated with multiple ornamental fish species for decades, with substantial evidence showing their detrimental impact in native ecosystems and food production industries (Supplementary S3). Nonetheless, there are other parasite species that could be a major threat for aquarium shops without appropriate quarantine measures. For example, myxozoan parasites *Myxobolus lentisuturalis* Dyková, Fiala and Nie, 2002, and *Myxobolus turpisrotundus* Zhang, Wang and Gong, 2010, form plasmodia on the body surface of the host, causing severe disfigurement of the host tissue (Caffara et al. 2009; Zhang et al. 2010). Similarly, monogeneans *D. anchoratus*, *D. intermedius*, *D. formosus*, and *D. vastator* increase the morbidity of aquarium-held and traded goldfish, and may cause significant mortalities if undetected (Ling et al. 2016). Parasites that affect the aesthetic value of popular ornamental cyprinids (e.g. *Carassius* spp., *C. carpio*) could cause significant economic losses to aquarium shops if undetected.

Zoonotic parasites may be exacerbated by the ornamental trade. Co-invasive *C. formosanus* and *M. yokogawai* found infecting invasive goldfish (Supplementary S3) have established in non-native ecosystems as they can infect native and co-introduced hosts to complete their life cycles (see *M. yokogawai* in Cordero Del Campillo et al. 1980, *C. formosanus* in Scholz and Salgado-Maldonado 2000). *Centrocestus formosanus* for example, is now widely distributed in Mexico, due to various factors including the introduction of its intermediate host freshwater snail, *Melanoides tuberculata* (Müller, 1774), an ornamental mollusc in the aquarium trade (see Scholz and Salgado-Maldonado 2000). Similarly, *M. yokogawai*, a common endemic zoonotic parasite in Asia (see Yu and Mott 1994; Chai et al. 2005), has been reported in Russia (Besprozvannykh et al. 1987) and Spain (Cordero del

Campillo, 1980). Future surveys should consider the presence of zoonotic parasites infecting native fish fauna and appropriate precautions to avoid possible zoonosis.

Goldfish parasites such as *Pseudamphistomum truncatum* could present problems for the health of native fauna and ruminant industries. Simpson et al. (2005) discussed how *P. truncatum* was co-introduced into the United Kingdom in the 1980s with imported ornamental cyprinids. Now a co-invasive parasite, *P. truncatum* has been associated with cattle and sheep mortalities in England (Foster et al. 2008) and Scotland (Mason et al. 2012), as well as infecting multiple native mammal species in western and eastern Europe (Skov et al. 2008). However, *P. truncatum* remains poorly studied as an emerging parasite (Simpson et al. 2005; Neimanis et al. 2016), and the potential of translocation, co-invasion, and possible zoonosis through the ornamental trade is not well understood.

4.9. Future directions and biosecurity

Goldfish and other ornamental aquatic species have probably facilitated parasite co-invasions (Taraschewski 2006; Hassan et al. 2008; Davis et al. 2011; Adel et al. 2015). However, linking specific host species to parasite co-invasions should be made cautiously, especially when there is poor knowledge of native and exotic biodiversity. Human mediated translocation of organisms began long before taxonomic surveys and species monitoring programs and this limitation may prevent accurate identification of which fish species was the original culprit associated with a specific parasite co-invasion (Lymbery et al. 2014).

In many countries, there are no regular surveillance programs to fully understand the diversity and economic value of native fish species. Hence, there is a poor understanding of the long-term impacts of invasive parasite species. Regular surveillance of native fauna would provide valuable insight on the vulnerability of native ecosystems to events of parasite co-introductions and colonization of the exotic hosts.

To prevent further parasite incursions, it is important to quantify the impact of the ornamental trade as a route of translocation for exotic parasites. Countries rely on the stringency of their biosecurity protocols to prevent undetected parasite threats from entering the country. Nonetheless, the capacity to efficiently review each imported consignment of fish is greatly limited by the size and volume of fish traded between countries. Biosecurity protocols should reflect priorities to protect native fauna, industries, and resources, thus providing a framework in which quarantine acts as an effective defence.

Quarantine protocols should also account for the life history traits of high risk parasites infecting imported ornamental species. Most importing countries require fish consignments to be quarantined for specific periods of time following inspection at border control (Whittington and Chong 2007). Fish could be infected with different life stages of multiple parasites that are impossible to detect when initially inspected. Containment quarantine periods should comprise periods of time that aim to break parasite life cycles and treat imported fish for possible parasite infections before fish are held in aquaria with other fish or sold.

Molecular techniques have the potential to provide rapid and efficient detection for quarantine inspection and border control. Molecular techniques have been used to detect viral infections in ornamental species, with highly sensitive and accurate results (see Becker et al. 2014; Rimmer et al. 2015) and may prove to be highly efficient tools in parasite detection and parasitology research for biosecurity (Bass et al. 2015). Environmental DNA (eDNA) for example, offers non-invasive and comprehensive methods for assessing parasite diversity in imported fish consignments by testing the water used to transport the fish (Collins et al. 2013). However, translating this information into assessment of disease risk or its use as diagnostic evidence, remains challenging and requires extensive validation before its use in notification procedures or detection programs (Bass et al. 2015).

4.10. Conclusions

This study showed that at least 197 parasites have been reported infecting goldfish since the 1900s. However, centuries of goldfish translocation have left a myriad of undocumented events where parasites were co-introduced and became co-invasive. Considering the extent of historical events of ornamental fish translocations, the availability of historical documents detailing events of parasite infections in traded live fish species may be quite rare. Indeed, parasite surveys began long after parasites were moved between countries, and although there is significant evidence showing the ornamental trade is an important route for parasite translocations, linking specific parasite co-invasions to ornamental species introductions may not be possible without prior surveys of local parasite fauna. Nonetheless, at least 26 parasite species have been reported infecting invasive goldfish

outside their natural range, and over 48 parasite species, not known to occur in native goldfish, have been reported infecting invasive goldfish populations globally.

Parasite species that cause harmful impacts in aquaculture have been translocated through the goldfish trade. This is the case for *I. multifiliis*, *A. japonicus* and *L. cyprinacea,* which are highly invasive and important parasites to consider for biosecurity. Other emerging parasites to consider in the aquarium industry are myxozoans and monogeneans, which are highly cryptic in nature and may have significant impacts on the aesthetic value of ornamental fish.

Timely detection of parasites and pathogens is a critical priority for biosecurity and border control. However, inspection of imported ornamental fish can be time consuming, rendering the use of molecular techniques as a last resort and relying purely on visual inspections and documentation. Although extensive validation is needed before molecular techniques are used at border control, the use of molecular techniques in biosecurity, such as environmental DNA, should be considered as it presents a non-invasive and potentially more accurate alternative to visual inspection of imported fish. Future research efforts could enable highly sensitive and time efficient molecular techniques to detect high priority parasites at border control.

CHAPTER 5 PUBLICATION STATEMENT

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CHAPTER 5

PARASITE DETECTION IN THE ORNAMENTAL FISH TRADE USING ENVIRONMENTAL DNA

Abstract

Effective border control relies on stringent biosecurity protocols to detect and prevent introductions of exotic pests and diseases. Detection of pathogens and parasites in the live ornamental fish trade using environmental DNA (eDNA) techniques has the potential to improve current biosecurity practices. We examined water samples from 11 target consignments (cyprinids susceptible to *Dactylogyrus* spp. infections) and seven non-target fish consignments (non-cyprinids, not susceptible to *Dactylogyrus* spp. infections) imported from Southeast Asia to Australia for the presence of eDNA from five *Dactylogyrus* species (Monogenea: Dactylogyridae). A four-step predictive framework was used to predict putative positive and putative negative detections from quantitative PCR assays. Both target and non-target consignments were positive for *Dactylogyrus* spp. eDNA as confirmed by Sanger sequencing. Positive detections for *Dactylogyrus* spp. eDNA in non-target fish consignments demonstrates the possibility of source water contamination, limiting the applicability of eDNA screening methods at border control. This study suggests eDNA screening should be tested during pre-export quarantine periods to avoid false positive detections at border control, highlights the utility of a predictive framework to avoid both false positive and negative detections, and discusses the potential for eDNA to advance ornamental fish trade biosecurity.

5.1. Introduction

The ornamental fish trade is a known route of exotic pathogen translocations globally (Chapter 3-4; Kahn et al. 1999; Whittington and Chong 2007; Corfield et al. 2008; Chang et al. 2009). Parasites and their infected hosts have been co-introduced to non-native environments with detrimental effects on biodiversity, ecosystems, industries, and dependent local communities (Lymbery et al. 2014). To minimize pathogen translocation through the ornamental fish trade, governments can establish quarantine measures based on scientific risk analyses that consider the origin and history of fish stocks, parasite life cycles, host susceptibility to infection, risk of transmission to native species, and the reliability of detection methods (Hine 2001; Whittington and Chong 2007). Australia for example, has stringent mandatory pre-export quarantine requirements, biosecurity protocols at border control, and post arrival mandatory quarantine requirements following strict biosecurity import risk assessments of ornamental fish imports(Whittington and Chong 2007; Becker et al. 2016). Despite current biosecurity protocols, recent surveys of ornamental fish species imported to Australia have shown that a high diversity of parasites were not detected during inspection at border control, highlighting the need for more detection sensitivity (Chapter 3). Considering the limitation of visual inspection under current biosecurity protocols it is important to explore new and complimentary methods to increase biosecurity rigor and the possible integration of molecular genetic techniques.

Environmental DNA (eDNA) refers to the DNA that is naturally shed by organisms such as epidermal sloughing, metabolic waste excretions or post-mortem decay into their local environment[11]. In the case of microscopic parasites , life stages like eggs, spores, cysts, active larvae, juveniles and adults can be present in the water column, in sediment, or in extracellular DNA disassociated from host organisms (Bass et al. 2015). As such, parasite genomic (gDNA) and nucleic (nDNA) can be captured with eDNA samples (Bass et al.

2015), extracted, and screened for target species using standard molecular genetic techniques like quantitative real-time polymerase chain reaction (qPCR) (Thomsen & Willerslev 2015; Barnes and Turner 2016;Goldberg et al. 2016). Environmental DNA could enable species-level detection and monitoring in aquatic parasitology with important benefits to human health, animal welfare, freshwater fisheries, coastal aquaculture, conservation, and ecosystem health (Bass et al. 2015). Indeed, captured and extracted eDNA from water samples has been shown to accurately detect pathogenic trematodes infecting wild amphibians (Huver et al. 2015) and to monitor parasite populations infecting farmed (Hallett et al. 2012; Agawa et al. 2016; Bastos-Gomes et al. 2017;) and wild fish species (Rusch et al. 2018). Environmental DNA was recently proposed to be a non-destructive and sensitive detection tool for biosecurity, and was used to determine the presence of ornamental fish species present at low densities within high risk mixed imports (Collins et al. 2013). Screening water used to import ornamental fish consignments for the presence of parasites has the potential for biosecurity monitoring advancement; however, there are no studies to date that have specifically tested this utility of eDNA.

False positive and false negative errors are commonly encountered in qPCR analyses (Schmidt et al. 2013; Lahoz-Monfort et al. 2016). From a biosecurity perspective, misinterpreting qPCR data could lead to pathogen-free consignments being considered hazards during quarantine inspection (i.e., false positive error), or high-risk pathogens going undetected in infected consignments (i.e., false negative error). As such, preventative measures must be developed to ensure accurate interpretation of qPCR data (Lahoz-Monfort et al. 2016) and reduce the possibility of false positive and negative results.

The aim of this study was to determine if eDNA screening by qPCR is an applicable detection tool for biosecurity. A four-step predictive framework was designed to minimize the possibility of false positive and false negative qPCR detections and used to determine the presence or absence of five ectoparasitic monogenean flukes (*Dactylogyrus anchoratus*, *D. formosus*, *D. intermedius*, *D. vastator* and *D. ostraviensis*) previously detected by necropsies infecting ornamental cyprinid fishes (*Carassius auratus* and *Pethia conchonius*) imported from Southeast Asia to Australia (Chapter 3).

5.2. Methods

5.2.1. Dactylogyrus *spp. eDNA collection*

All water samples analysed for the presence of eDNA from *Dactylogyrus* species in this study were collected during a cross-sectional survey for the presence of nationally listed aquatic pathogens associated with at least one ornamental fish host (Becker et al. 2016). Briefly, 37 ornamental fish consignments representing 11 freshwater and seven marine fish species were imported from Southeast Asia to Australia in 2015 following Australian Biosecurity Import Conditions (BICON) and subjected to Australian quarantine protocols, which involved gross visual inspection and clearance by Australian Quarantine Services. A 'consignment' of fish was defined as a unique fish species within a shipment of fish, identified by an invoice containing details of the numbers and species of fish, date of shipment, origin and destination, accompanied by health certification (Whittington and Chong 2007). Following release from quarantine inspection, all consignments were

transported by road to an Approved Arrangement Site (AA Site) at the University of Sydney (Camden, Australia).

Freshwater consignments arrived at the AA Site in either one large plastic bag or several medium plastic bags, containing 40 to 200 individuals depending on species and size (Becker et al. 2016). Each plastic bag contained approximately 1 - 5 L of freshwater and was sealed with either rubber bands or metal clasps. All consignments were housed inside large Styrofoam boxes during transit (12 - 48 hours including export, delivery, inspection, and release to the importer) before water samples were collected from each consignment and preserved. Negative controls (distilled water) were collected prior to collecting triplicate 15 mL samples from each fish consignment. To minimize the risk of eDNA cross contamination, each 15 mL replicate was collected from all plastic bags holding each consignment using a new disposable 20 mL sterile glass pipette attached to an automatic pipette controller (EasyPet, Eppendorf). Water samples were dispensed directly into individual pre-labelled DNA-free 50mL centrifuge tubes, each with 33.5 mL absolute ethanol and 1.5 mL 3M sodium acetate for preservation and then stored at room temperature (Bastos-Gomes et al. 2017). Following water sample collection, 30 fish from each consignment were randomly selected, euthanized, and examined for the presence of monogenean parasites by necropsy, as described in a separate study (Chapter 3). In brief, all 30 fish were sequentially surveyed for external parasites by an experienced parasitologist using a compound microscope to carefully examine gill samples from each fish for the presence or absence of parasites (Chapter 3). A sample size of 30 fish per consignment was selected to achieve a minimum detection prevalence of 10% with 95% confidence limits determined by using exact binomial approximation[8]. As such, samples where no parasites were detected by necropsy were considered to have an apparent prevalence of 0%, with a 95% confidence interval (CI) of 0 -

11.4%, assuming a perfect test (Becker et al. 2016). Environmental DNA was extracted using cetyl trimethylammonium bromide (CTAB), which included phenol-chloroform isolation and terminal isopropanol precipiation (Bastos-Gomes et al. 2017). All DNA was resuspended in 60 µL 1x Tris-EDTA (TE) buffer and stored at -20°C until screening for *Dactylogyrus* spp. eDNA by qPCR. Animal ethics, method and sampling approval was obtained from the University of Sydney Animal Ethics Committee (approval number: 720) and all methods were performed in accordance with guidelines and regulations of the University of Sydney Animal Ethics Committee.

5.2.2. Design of species-specific Dactylogyrus primers and assay validation

Novel species-specific oligonucleotide primers were design to detect and discriminate between five *Dactylogyrus* species (Monogenea: Capsalidae): *Dactylogyrus anchoratus* (Dujardin, 1845), *Dactylogyrus formosus* Kulwiec, 1927, *Dactylogyrus intermedius* Wegener, 1909, *Dactylogyrus ostraviensis* Řehulka, 1988, and *Dactylogyrus vastator* Nybelin, 1924. All five *Dactylogyrus* spp. are highly specific to cyprinid fish hosts (Whittington et al. 2000; Cribb et al. 2002). All qPCR assays targeted the internal transcribed spacer 1 (*ITS1*) between base pair 366 and 588. The *ITS1* is a high abundance nuclear gene known to be detectable in eDNA extracted from water samples (Minamoto et al. 2017) and to provide species-level resolution for *Dactylogyrus* (Chapter 3) and other helminths given its low intraspecific yet high interspecific variability (Van Herwerden et al. 1999). Each *Dactylogyrus*-specific primer was designed to target the *ITS1* region that contained the most mismatches (≥ 1) between target and all non-target *Dactylogyrus* species (Table 12). To achieve this, previously accessioned *Dactylogyrus* spp. *ITS1* nucleotide sequences (Chapter

3) were downloaded from GenBank (NCBI) and aligned using ClustalW (www.genome.jp/tools/clustalw, version 1.81).

All qPCR assays were tested for specificity *in silico* using the National Center for Biotechnology Information (NCBI) Primer BLAST (Johnson et al. 2008), Amplify4 (engels.genetics.wisc.edu/amplify), and Amplifx 1.7.0 (Nicolas Jullien; CNRS, Aix-Marseille Université: crn2m.univ-mrs.fr/pub/amplifx-dist). For Amplify4 and Amplifx 1.7.0 *in silico* tests, virtual PCRs were run against *ITS1* nucleotide sequences for all five target *Dactylogyrus* species. All assays demonstrated specificity to the targeted *Dactylogyrus* species across all three *in silico* tests. Primers were synthesized (standard desalting; Sigma-Aldrich, Australia), resuspended in 1x TE at 100 µM, and stored at -20 ˚C. Lastly, all qPCR assays were tested for species-specificity *in vitro* using both end-point PCR and qPCR with previously extracted genomic DNA (gDNA) from each target *Dactylogyrus* species (Chapter 3). All assays demonstrated specificity to the targeted *Dactylogyrus* species across all *in vitro* tests (Table 12; Supplementary S6), produced 120 – 210 bp amplicons and performed optimally at assay-specific annealing temperatures (60˚C or 65˚C; Table 12).

Quantitative PCR assays (10 µL or 20 µL) contained 3 or 6 µL gDNA, 0.5 or 1 µL each PCR primer (400 nM), 5 or 10 µL PowerUP® SYBR GreenER qPCR Master Mix (Life Technologies, Australia) and 1 or 2 µL MilliQ® water, respectively, and were performed under the following fast cycling conditions (ramp rate = 2.70 °C/sec): UDG incubation at 50 °C for 2 min, initial denaturation at 95 °C for 2 min, 40 cycles of 95°C denaturation for 15 sec then 60 or 65°C primer-specific annealing for 60 sec (Table 1), and terminal dissociation curve generation (60 – 95 °C at 0.15 °C/sec). Previously extracted *Dactylogyrus* spp. gDNA (Chapter 3) was quantified on a NanoDrop™ spectrophotometer (Invitrogen Inc.) and then

each species-specific gDNA sample was serially diluted 1:10 to generate a five-point standard curve for each target *Dactylogyrus* species (1×10^{-2} - 1×10^{-6} ng/µL). Species-specific gDNA standards were used as template to determine assay amplification efficiency (E; i.e., increase in amplicon per cycle (Ruijter et al. 2009)) and limit of detection (LOD; i.e., lowest gDNA standard detected across all technical qPCR replicates) for each corresponding species-specific qPCR assay. All qPCR assays were run on a QuantStudio3™ Real-Time PCR System (ThermoFisher Scientific Inc., Brisbane), and threshold cycle value (C_t) based on a common fluorescence threshold of 0.2. Melting temperature (T_m) values were determined for each amplicon using QuantStudio™ Design and Analysis Software (version 1.4.2). All data was exported to Microsoft Excel for comparative analyses.

Table 12. Primers for *Dactylogyrus* spp. ITS1 eDNA assay. The efficiency, R^2 and limit of detection for each quantitative PCR assay is provided. Primer cross-reactivity tests are provided in Supplementary S6.

Parasite species	Primer	Amplicon (bp)	Annealing (°C)	Primer sequence (5' – 3')	qPCR efficiency (%)	R^2	Limit of detection (ng/µL)
Dactylogyrus anchoratus	D. anchoratus F	185	60	5'- GCCATCCTTGAGGGAATATGCCCA -3'	75.12	0.981	0.00065
	D. anchoratus R			5'- GAGTTTACGTTGACCGCCCGACAT -3'			
Dactylogyrus formosus	D. formosus F	184	65	5'- ATCATCCTTGTGGGAATCTGCCCG -3'	119.55	0.984	0.0079
	D. formosus R			5'- AAGTGTACGTTGACCGCCAGCAG -3'			
Dactylogyrus ostraviensis	D. ostraviensis F	120	65	5'- TCGTCGTGACGACCTTGG -3'	97.3	0.98	0.00092
	D. ostraviensis R			5'- CACATACTGCAGTGACCCT -3'			
Dactylogyrus vastator	D. vastator F	210	60	5'- GTTGCGGAACTGAACCCTAGCCA -3'	98.99	0.95	0.00009
	D. vastator R			5'- AGACTGCACGACACGTTACCAA -3'			
Dactylogyrus intermedius	D. intermedius F	210	60	5'- TCAGAATCTGAACCCTATCCAATAC -3'	104.6	0.982	1.32E-07
	D. intermedius R			5'- TGCCGCACGACACGTTA -3'			

5.2.3. *Stepwise criteria for eDNA detection and samples tested for* Dactylogyrus *spp.*

A four-step conservative predictive framework was developed to minimise the risk false positive and false negative results in qPCR T_m analysis (Schmidt et al. 2013; Davidson et al. 2014; Lahoz-Monfort et al. 2017). These criteria were selected considering the need to accurately determine absence from disease in biosecurity (World Organization for Animal Health (OIE) 2019) and future applications of T_m analysis to ensure accurate and reliable detection. For each qPCR assay the T_m of each amplicon was compared to the mean T_m of the corresponding species-specific gDNA, which was calculated from all technical qPCR replicates across the entire standard curve ± 99.7% CI (Ririe et al. 1997). The absolute difference between the mean T_m of the species-specific gDNA standard curve and each individual qPCR technical replicate amplicon within a corresponding species-specific assay ($|\Delta T_m|$) was calculated by subtracting the T_m of each technical replicate amplicon from the mean T_m of the corresponding species-specific gDNA standard. Calculated $|\Delta T_m|$ values were then used to categorise each putative positive detection (i.e., amplicon) into one of three confidence levels: CL 1 = high (amplicon expected to be positive for *Dactylogyrus* spp. detection), CL 2 = medium (amplicon suspected to be positive for *Dactylogyrus* spp. detection), and CL 3 = low (amplicon predicted to not be positive for *Dactylogyrus* spp. detection, i.e., false positive) (Figure 1).

Amplicons were categorized as CL 1 if: 1) amplification curves crossed the common threshold fluorescence within 40-cycles (Criterion 1.1, Figure 22), 2) T_m values were within 99.7% CI of the corresponding species-specific mean gDNA standard T_m (Criterion 2: CL 1, Figure 22), and 3) agarose gel visualization confirmed length to match that observed and

expected for corresponding species-specific gDNA standard (Criterion 3, Figure 22). Amplicons were categorized as CL 2 if they matched CL 1 criteria (see above) but exhibited a $|\Delta T_m|$ outside 99.7% CI and ≤ 1˚C from mean T_m of corresponding species-specific standards (Criterion 2: CL 2, Figure 22). Amplicons were categorized as CL 3 if they matched CL 1 criteria but exhibited $|\Delta T_m|$ outside 99.7% CI and > 1˚C from mean T_m of corresponding species-specific standard (Criterion 2: CL 3, Figure 22). Putative positive CL 1, CL 2, and CL 3 amplicons were Sanger sequenced (Australian Genome Research Facility, Brisbane) for *Dactylogyrus* spp. level confirmation (NCBI BLAST; Criterion 4, Figure 22). If any given *Dactylogyrus* spp. eDNA assay had ≥ 2 putative positive amplicons categorized as CL 1 or CL 2 then two representatives for each CL were chosen for Sanger sequencing (one with lowest and one with highest $|\Delta T_m|$ value), otherwise one or both putative positive amplicons were sequenced. If any *Dactylogyrus* spp. eDNA assay had ≥ 2 putative positive amplicons categorized as CL 3 then the amplicons with the lowest and highest $|\Delta T_m|$ values (i.e., most and least likely to be confirmed as positive detections) were sequenced, otherwise both putative positive amplicons were sequenced.

Amplicons were considered to be putative false negative detections if no amplification curves were produced or failed to cross the common fluorescence threshold within 40 cycles (Criterion 1.2) but exhibited $|\Delta T_m|$ values within 99.7% CI of mean T_m of corresponding species-specific standards (false negative, Figure 22). Amplicons categorized as putative false negatives were re-amplified by qPCR to determine if a $|\Delta T_m|$ value within 99.7% CI of mean T_m of corresponding species-specific standards and expected amplicon length were produced when amplified using 1 µL of PCR product from initial amplification. Putative false negative amplicons were re-amplified using six replicate 20µL qPCRs containing 1 µL of post-PCR product, 1 µL of each PCR primer (400 nM), 10 µL PowerUP® SYBR GreenER qPCR Master Mix (Life Technologies, Australia) and 8 µL MilliQ® water,

and were run under the same cycling conditions described above. Any amplicons produced from qPCR re-amplification that met Criteria 1, 2, and 3 (see above; Figure 22) was Sanger sequenced for confirmation.

If an entire assay did not produce any amplicons that crossed common fluorescence threshold within 40 cycles (Criterion 1.2, Figure 22) and no amplicons exhibited a discernible T_m then the entire assay was repeated. An assay was considered negative if neither initial or subsequent qPCR runs produced amplicons that crossed common fluorescence threshold within 40 cycles (Criterion 1.2, Figure 22) and neither initial or subsequent qPCR runs produced amplicons with detectable T_m (Criterion 2, Figure 22).

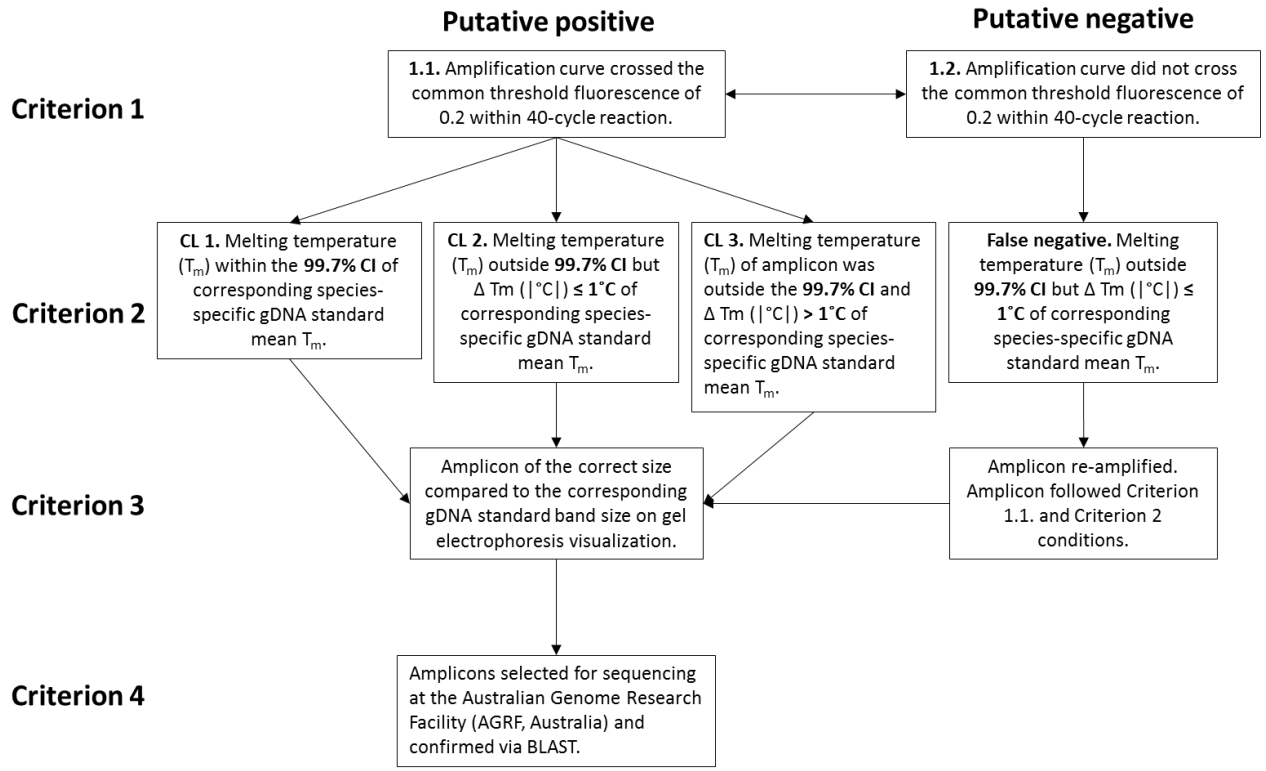

Figure 22. Predictive framework designed to interpret qPCR amplicon data for eDNA detection determination.

Species-specific qPCR assays were used to test extracted DNA in water samples from target and non-target fish consignments for the presence of *Dactylogyrus* spp. eDNA (Table 13). Imported consignments were considered 'target' or 'non-target' fish consignments based on published records of infection for any of the *Dactylogyrus* spp. targeted in this study (n = 5) (Chapter 3-4, Řehulka 1988; Whittington et al. 2000; Cribb et al. 2002). Based on this criteria, seven goldfish (*Carassius auratus* (Linnaeus, 1758)) consignments were considered targets for *D. anchoratus*, *D. formosus*, *D. intermedius,* and *D. vastator* whereas four rosy barb (*Pethia conchonius* (Hamilton, 1822)) consignments were considered targets for *D. ostraviensis* (Table 13). Based on the same criteria, one guppy (*Poecilia reticulata*, Peters 1859), one pearl gourami (*Trichopodus leerii* (Bleeker, 1852)), one three-spot gourami (*Trichopodus trichopterus* (Pallas, 1770)), one green swordtail (*Xiphophorus hellerii* Heckel, 1848), and three platyfish (*Xiphophorus maculatus* (Günther, 1866)) consignments were considered non-target hosts for all five *Dactylogyrus* species. All target and non-target host fish consignments were screened for the presence of eDNA from all five *Dactylogyrus* species using species-specific qPCR assays (Table 13) followed by assessment of each produced amplicon based the selection criteria described above (Figure 22).

5.3. Results

5.3.1. Positive Dactylogyrus *spp. eDNA detection in target fish populations*

Dactylogyrus spp. eDNA was detected in all consignments where *Dactylogyrus* spp. were detected by standard necropsies. Specifically, eDNA from *D. formosus* and *D. vastator* was detected in water samples from all *C. auratus* consignments, and eDNA from *D. anchoratus* and *D. intermedius* was detected in all consignments except for consignments 4 and 6, respectively (Table 13). *Dactylogyrus anchoratus* was detected by both approaches (eDNA and necropsy) in consignments 6 and 7 while neither approach detected parasites in consignment 4. *Dactylogyrus ostraviensis* eDNA was detected in all target *P. conchonius* consignments, while necropsies did not detect *D. ostraviensis* in consignment 12 (Table 13). *Dactylogyrus* spp. eDNA was detected in five *C. auratus* and one *P. conchonius* consignments considered to have *Dactylogyrus* spp. apparent prevalence of 0% (95% CI 0–11.4%) by necropsy (Chapter 3, Table 13). No eDNA was detected in negative controls.

5.3.2. Positive Dactylogyrus *spp. eDNA detections in non-target fish populations*

A total of 39 amplicons produced across all 58 qPCR tests of non-target fish consignments were confirmed positive for *Dactylogyrus* spp. eDNA (Table 13). *Dactylogyrus formosus*, *D. intermedius,* and *D. vastator* eDNA was detected in *P. conchonius* consignment 13 (Singapore 2; Table 13). *Dactylogyrus intermedius* and *D. ostraviensis* eDNA was detected in *X. maculatus* consignment 24 (Singapore 2, Table 13) while *D. vastator* and *D.*

intermedius eDNA was detected in *X. maculatus* consignment 23 (Thailand 1; Table 13). Similarly, *D. ostraviensis* eDNA was detected in *C. auratus* consignments 3 and 4 as well as *X. maculatus* consignment 24 (Singapore 2; Table 13). Lastly, *D. formosus*, *D. intermedius*, *D. vastator,* and *D. ostraviensis* eDNA was detected by qPCR in *P. reticulata* consignment 17, *T. leeri* consignment 18, and *X. maculatus* consignment 25 (Sri Lanka; Table 13). No target *Dactylogyrus* spp. were detected on non-target fish consignments from fish necropsies.

5.3.3. Accuracy of predictive framework

All amplicons categorized as high confidence of *Dactylogyrus* detection (CL 1) from all *Dactylogyrus* spp. qPCR assays were confirmed positive by Sanger sequencing (Figure 22 Criterion 4). All amplicons categorized as moderate confidence (CL 2) from *D. anchoratus*, *D. formosus*, and *D. intermedius* qPCR assays were also confirmed positive by Sanger sequencing (Figure 22 Criterion 4). Of the amplicons categorized as CL 2 from *D. ostraviensis* and *D. vastator* qPCR assays, 80% and 87.5% (n = 4/5 and 7/8)) were confirmed positive by Sanger sequencing, respectively. These two CL 2 amplicons were unable to be confirmed as positive detections due to poor sequencing quality (i.e., not due to non-target amplification; see Figure 23D for *D. ostraviensis* and Figure 24 for *D. vastator*).

No low confidence (CL 3) categorized amplicons from *D. anchoratus*, *D. formosus*, *D. intermedius,* or *D. ostraviensis* qPCR assays were confirmed positive by Sanger sequencing. However, 81.25% (n = 13/16) of CL 3 categorized amplicons from *D. vastator* qPCR assays were confirmed positive by Sanger sequencing (Figure 1, Criterion 4). One *D.*

vastator qPCR assay amplicon from *T. tricopterus* consignment 14 was initially considered a putative false negative (Figure 22 Criterion 2) but was subsequently categorized as CL 1 following qPCR reamplification (Figure 1) and confirmed positive by Sanger sequencing (Figure 22 Criterion 4, Figure 24 “amplicon 19_4”). All other putative false negative amplicons produced during *Dactylogyrus* spp. eDNA assays were confirmed negative following the selective framework (Figure 22, Supplementary S7).

Table 13. Comparison between necropsies and environmental DNA (eDNA) detection of *Dactylogyrus* species in imported ornamental fish populations. Detections by necropsy presented as mean apparent prevalence % (95% Confidence Interval, CI; Chapter 3) and eDNA detections as confirmed positive amplicons/total number of amplicons. Grey areas indicate assays of species-specific target populations, and asterisks (*) indicate populations where *Dactylogyrus* spp. were not detected by necropsies but were detected by eDNA assays. Negative symbols (-) indicate that no parasites were detected by necropsy in a total of 30 fish and had an apparent prevalence = 0% (95% CI = 0 – 11.4%; Chapter 3), and that no parasite eDNA was detected from a total of six eDNA sample replicates.

Fish pop.	Fish species	Exporter	*Dactylogyrus anchoratus*		*Dactylogyrus formosus*		*Dactylogyrus intermedius*		*Dactylogyrus vastator*		*Dactylogyrus ostraviensis*	
			Necropsy	eDNA	Necropsy	eDNA	Necropsy	eDNA	Necropsy	eDNA	Necropsy	eDNA
3	*Carassius auratus*	Singapore 2	-	4/6*	-	4/6*	-	4/6*	-	4/6*	-	6/6
4	*Carassius auratus*	Singapore 2	-	0/12	-	4/6*	-	6/6*	-	6/6*	-	5/6
5	*Carassius auratus*	Thailand 1	-	4/6*	-	6/6*	20 (8–39)	5/6	13.3 (2–27)	6/6	-	-
6	*Carassius auratus*	Thailand 1	26.6 (12–46)	6/6	6.7 (0.82–22)	5/6	43.3 (25–63)	0/12	40 (23–59)	4/6	-	-
7	*Carassius auratus*	Thailand 1	3.3 (0.1–17.2)	4/6	43.3 (25–63)	4/6	30 (15–49)	6/6	16.6 (2–29)	6/6	-	-
8	*Carassius auratus*	Malaysia 1	-	1/6*	3.3 (0.1–17.2)	3/3	3.3 (0.1–17.2)	3/3	3.3 (0.1–17.2)	3/3	-	-
9	*Carassius auratus*	Malaysia 1	-	5/6*	6.6 (0.82–22)	4/6	3.3 (0.1–17.2)	4/6	-	4/6*	-	-
13	*Pethia conchonius*	Singapore 2	-	-	-	1/6	-	1/6	-	2/6	26.6 (12–46)	6/12
14	*Pethia conchonius*	Thailand 1	-	-	-	-	-	-	-	-	33.3 (17–53)	4/6
15	*Pethia conchonius*	Thailand 2	-	-	-	-	-	-	-	1/6	73.3 (54–88)	4/6
16	*Pethia conchonius*	Malaysia 1	-	-	-	-	-	-	-	1/6	-	2/12*
17	*Poecilia reticulata*	Sri Lanka 2	-	-	-	2/6	-	1/6	-	-	-	-
18	*Trichopodus leerii*	Sri Lanka 1	-	-	-	-	-	-	-	1/6	-	5/6
19	*Trichopodus trichopterus*	Thailand 1	-	-	-	-	-	-	-	-	-	-
22	*Xiphophorus hellerii*	Sri Lanka 2	-	-	-	-	-	-	-	-	-	-
23	*Xiphophorus maculatus*	Thailand 1	-	-	-	-	-	1/6	-	2/6	-	-
24	*Xiphophorus maculatus*	Singapore 2	-	-	-	-	-	1/6	-	-	-	5/6
25	*Xiphophorus maculatus*	Sri Lanka 2	-	-	-	4/6	-	-	-	-	-	-

5.3.4. Amplicon sequence confirmation

All confirmed positive *D. anchoratus* amplicons were 100% homologous to *D. anchoratus ITS1* GenBank sequences (AJ564111, AJ490161, MF356241, KY859795, MF662103, MF356243, and MF356242). All confirmed positive *D. formosus* amplicons were 100% homologous to *D. formosus ITS1* GenBank sequences (AJ564135, MF356239, KM525669, KX369215, and KC876018). All confirmed positive *D. intermedius* amplicons were 100% homologous to *D. intermedius* ITS1 GenBank sequences (KC876017, KX369220, MF356236, MF356244, KJ854364, MF356237, and MF356240). All confirmed positive *D. ostraviensis* amplicons were 100% homologous to *D. ostraviensis ITS1* GenBank sequences (MF356250 and MF356249; which are the only two sequences available; Chapter 3).

Confirmed positive *D. vastator* amplicons, unlike all other *Dactylogyrus* spp. amplicons, separated into two distinct groups (Figure 24). *Dactylogyrus vastator* Group 1 amplicons exhibited an average $T_m \pm$ SD of 86.64˚C ± 0.59 with average $|\Delta T_m|$ being ± 0.6°C away from T_m of gDNA standards ($|\Delta T_m|$; Figure 24), while amplicons in Group 2 exhibited an average $T_m \pm$ SD of 85.37˚C ± 0.47 with average $|\Delta T_m|$ being ± 1.97°C away from T_m of gDNA standards (Figure 24). The six confirmed positive *D. vastator* amplicons that fell within the 99.7% CI of *D. vastator* gDNA standards (Group 1) were 98-100% homologous to the following *D. vastator ITS1* GenBank sequences: MF356235 (Thailand), KY207446 (Croatia), AJ564159 (Czech Republic), MF806586 (Iran), MF356246 (Thailand), KY201104 (Italy), and KY201092 (Bosnia and Herzegovina). The 11 positive *D. vastator* amplicons that fell outside the 99.7% CI of the same *D. vastator* gDNA standards (Group 2) were 96-100%

homologous to the following *D. vastator ITS1* GenBank sequences: KX369223 (China), MF356247 (Thailand), KY201103 (Czech Republic), and KM487695 (China). Groups 1 and 2 *D. vastator* amplicons differed by a total of 16 fixed nucleotide differences (Supplementary S8).

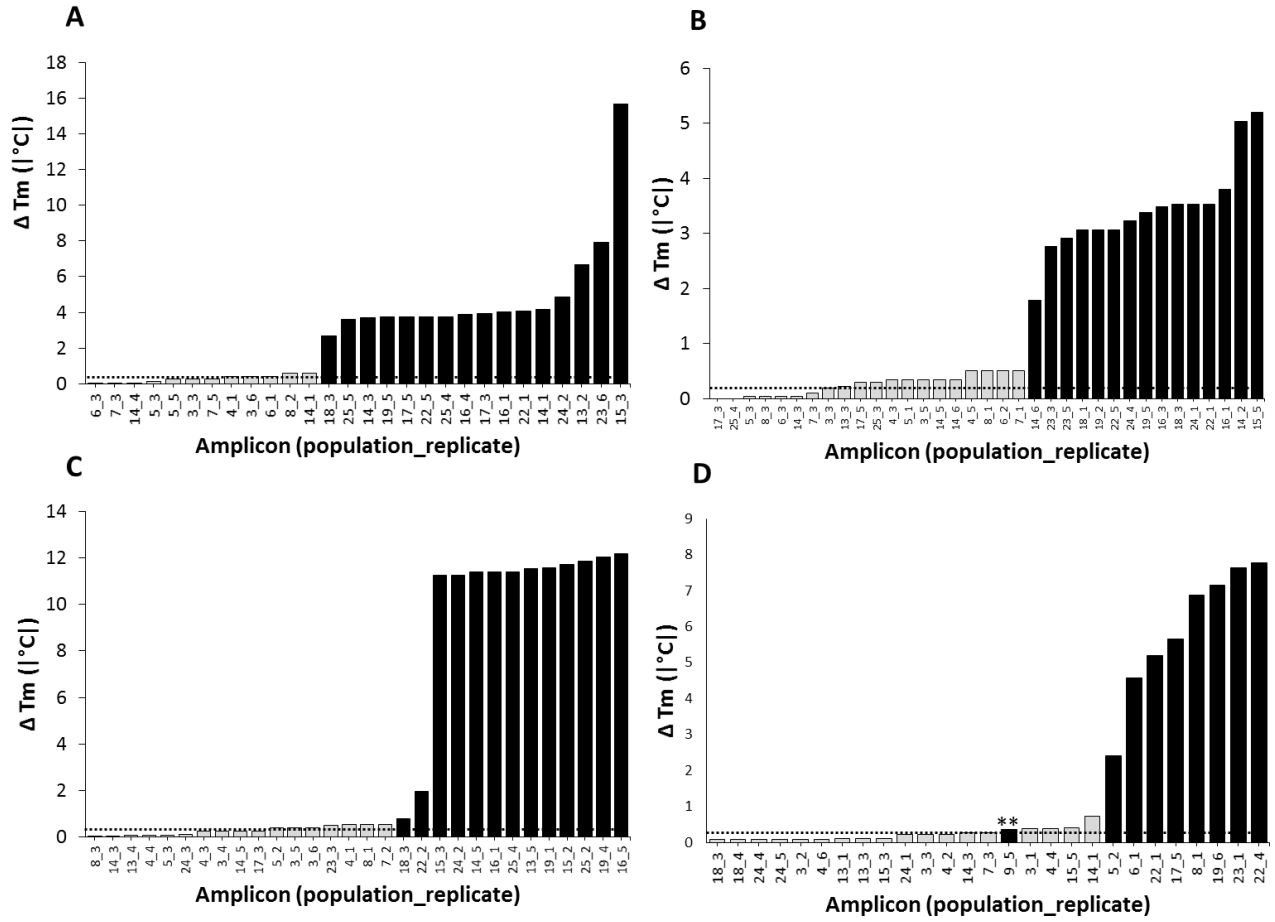

Figure 23. Absolute difference in melting temperature (|ΔTm|) between sequenced amplicons and their corresponding genomic DNA standards for *Dactylogyrus anchoratus* (A), *Dactylogyrus formosus* (B), *Dactylogyrus intermedius* (C) and *Dactylogyrus ostraviensis* (D). Grey and black bars in Panels A-D represent confirmed positive and confirmed negative amplicons, respectively. Horizontal dotted lines in Panels A-D represent the upper 99.7% Confidence interval for T_m of species-specific standards. ** Forward and reverse sequences were low in quality; however, a 72 bp fragment of consensus alignment was found to be 100% similar to *Cyprinus carpio* GenBank sequence LN599613 (i.e. considered as confirmed negative).

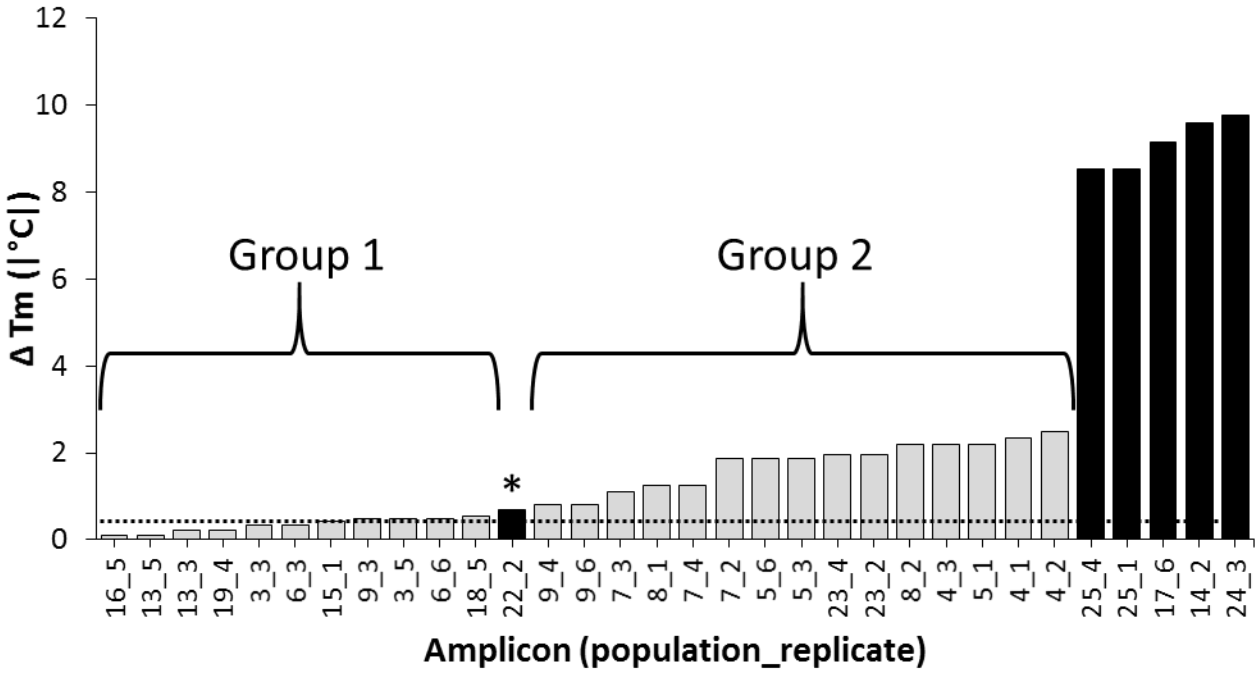

Figure 24. Absolute difference in melting temperature (|ΔTm|) between *Dactylogyrus vastator* amplicons derived from environmental DNA (eDNA) assays and genomic DNA (gDNA) standards confirmed by Sanger sequencing. Grey and black bars represent confirmed positive and confirmed negative amplicons, respectively. Horizontal dotted lines represent the upper 99.7% CI for T_m of serially diluted *D. vastator* gDNA standard. Group 1 amplicons had 1 - 2 base pair differences between sequences obtained compared to *D. vastator* gDNA standard, while Group 2 amplicons had 2 - 18 base pair differences between sequences obtained compared to *D. vastator* gDNA. Asterisk (*): consensus sequence could not be determined for this amplicon because reverse sequence failed; however, forward sequence had 93.8% similarity to *Contraceacum* sp. [GenBank accession KM463761] and 91% similarity to *Contracaecum rudolphii* Hartwich, 1964 [GenBank accession JQ071409] and thus this amplicon was considered as a confirmed negative detection. ClustalW alignment of all *D. vastator ITS1* amplicon sequences provided in supplementary Information (Supplementary S8).

5.4. Discussion

The developed qPCR assays detected *Dactylogyrus* spp. eDNA in all consignments where necropsies detected *Dactylogyrus* spp. (Chapter 3). Species-specific qPCR assays were able to detect *Dactylogyrus* spp. eDNA in six target fish consignments where necropsies considered *Dactylogyrus* spp. to have an apparent prevalence of 0% (95% CI 0 - 11.4; Table 13). As such, qPCR-based eDNA detection had higher surveillance sensitivity than necropsies, detecting *Dactylogyrus* spp. DNA in triplicate 15 mL water samples and confirming amplicons by Sanger sequencing.

However, *D. intermedius,* which was reported to infect *C. auratus* in consignment 6 by necropsy (Chapter 3) was not detected by eDNA screening in any qPCR technical replicates (n = 12; Table 13). Consequently, this was the only false negative eDNA detection observed in this study (1/90 tests; Table 13). It is possible that *D. intermedius* present in consignment 6 were genetically distinct from *D. intermedius* infecting consignments 5, 7, 8 and 9 (Table 13). The possibility of unique ITS1 genotypes in D. *intermedius* is supported by sequenced data of *D. vastator*, which displayed two *ITS1* genotypes observed across screened goldfish consignments (Figure 24; Supplementary S8). Unlike the *D. vastator* assay, the *D. intermedius* assay appears to target an *ITS1* region that is sufficiently hypervariable to prevent primer binding (Van Herwerden et al. 1999; Van Herwerden et al. 2003; Warberg et al. 2005); however, this was unknown at the time of assay development due to limited nucleotide sequence information available for *D. intermedius* populations. Such a lack of comprehensive nucleotide sequence information has also limited other molecular genetic studies aimed at investigating parasite diversity (Van Herwerden et al. 2003; Gómez 2014).

As such, Successful implementation of the four-step predictive framework relied on the comprehensiveness of species-specific gDNA standards, suggesting $|\Delta T_m|$ analysis requires careful interpretation given the inherent dependence on sequence homology between amplicons and standards for targeted gene(s) that may or may not be known. This study highlights the need for more comprehensive nucleotide sequence data, parasite populations, the possibility of *Dactylogyrus* species complexes, and the need for robust corresponding morphological taxonomy to ensure accuracy of designed qPCR assays and corresponding standards for $|\Delta T_m|$ analyses.

A total of 39 amplicons from non-target fish consignments were confirmed positive for *Dactylogyrus* spp. eDNA (Table 13). Considering that all *Dactylogyrus* spp. in this study are highly specific to cyprinid species (Řehulka 1988; Whittington et al. 2000; Cribb et al. 2002), positive detections in water samples from non-target consignments suggest that detected eDNA was not present due to active shedding from live infesting *Dactylogyrus* parasites. This interpretation is further supported by the absence of infection records for the selected *Dactylogyrus* specimens in non-target host fish species (Whittington et al. 2000; Cribb et al. 2002) and non-detection by necropsies (Chapter 3; Table 13). *Dactylogyrus* spp. occur naturally in southeast Asia (Chapter 4) and their environmental stages could be present in recirculating aquaculture systems, raceways, or ponds used to rear freshwater species by exporting companies. As such, it is possible that exporters could have used a water source contaminated with *Dactylogyrus* spp. environmental life stages (Bass et al. 2015) or degraded eDNA to transport exported fish consignments. If exporters do not use clean (e.g. filtered or UV treated) water to export ornamental fish consignments, then the accuracy and interpretability of eDNA assays at border control is limited, given that their applicability would depend greatly in differentiating between live, active infections and dead or inactive

environmental parasite stages in the water column. Furthermore, and considering that Australian quarantine officers have limited time to process imported consignments, eDNA-based detection by qPCR may not be applicable or reliable at border control using T_m analysis to carefully interpret qPCR results within an acceptable timeframe and biosecurity standard.

Screening water samples for parasite eDNA by qPCR could be a valuable detection method during pre-export quarantine periods. Current risk analyses from the Australian Government Department of Agriculture and Water Resources aim to ensure off-shore biosecurity in exporting countries (Hood and Perera 2016) by enforcing strict regulations and health requirements prior to export (BICON 2018). For example, all imported goldfish consignments must be certified free of infection from gill flukes *Dactylogyrus extensus* and *D. vastator* prior to export (BICON 2018). Both species are reported to cause significant economic losses in Asian cyprinid aquaculture (Kahn et al. 1999; Zhang et al. 2014), and could pose significant risks to Australian aquarium shops if live parasite infections go undetected during quarantine (Kahn et al. 1999). Detection of eDNA by qPCR assays could be conducted on ornamental fish consignments during the mandatory quarantine period prior to export to support mandatory pre-export health certifications (BICON 2018). For instance, qPCR assays could be developed to assess the origin of parasite eDNA based on DNA decay rates by targeting various DNA fragment lengths (Pochon et al. 2017; Bylemans et al. 2018). Abundant long DNA fragments would indicate active shedding from live parasites while abundant short DNA fragments would indicate degrading DNA in the absence of live, shedding organisms (Bylemans et al. 2018). Similarly, qPCR assays could also assess cellular activity by targeting environmental RNA (eRNA) (Bass et al. 2015; Pochon et al. 2017; Zaiko et al. 2018). Environmental RNA is indicative of active gene transcription and is

proportionally less abundant in dormant stages than in metabolically active stages (Bass et al. 2015). Given that RNA is less able to persist extracellularly and degrades quickly in dead or sloughed-off cells (Bass et al. 2015), detection of eRNA by qPCR could be employed to determine the presence of metabolically active parasites infecting fish ready for export. Future research should consider designing qPCR assays to differentiate between active parasite infections and dead or non-active parasite stages and the applicability of eDNA detection during pre-export quarantine periods.

In conclusion, this first attempt at applying eDNA to ornamental fish parasite biosecurity highlights both the utility of incorporating molecular methods into biosecurity protocols as well as the limitations that need to be addressed if future applications and full integration are to be successful. We present a novel and comprehensive four-step predictive framework (Figure 22) for the accurate interpretation of species-specific eDNA data and reduce false positive and false negative detections generated by Sybr-based qPCR assays. The interpretability and reliability of eDNA detection at border control specifically is limited; however, eDNA screening could prove highly valuable if implemented following pre-export quarantine periods. Further research needs to address limitations encountered in this study and test the viability of eDNA-based detection methods in other stages of quarantine and biosecurity surveillance.

5.5. Acknowledgements

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CHAPTER 6 PUBLICATION STATEMENT

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CHAPTER 6

CAN ENVIRONMENTAL DNA BE USED FOR AQUATIC BIOSECURITY IN THE AQUARIUM FISH TRADE?

Abstract

The global ornamental fish trade enables translocation of exotic aquatic pathogens. In many countries, health certification and visual inspection of imported fish are key components of biosecurity to prevent the introduction of aquatic diseases. However, infected fish do not always exhibit clinical or behavioural signs of disease, and alternatives to visual inspection must be validated. This study examined the use of environmental DNA (eDNA) to detect sub-clinical parasite infections at border control. We simulated the export process of live ornamental fish in which uninfected fish, infected fish, treated fish, and non-infected fish held in contaminated water were packaged and delivered in 48 h. Quantitative PCR (qPCR) was used to detect eDNA of an ectoparasitic monogenean, *Neobenedenia girellae*, infecting barramundi, *Lates calcarifer*. The qPCR assay did not reliably detect parasite eDNA under 2 copies/μL from fish with sub-clinical infections (mean parasite intensity = 6.80 ± 4.78 S.D.), suggesting parasite eDNA shedding rates may be too low for reliable detection within the timeframe used to export live ornamental fish. Quantitative PCR tests detected parasite eDNA in 50% of infected fish and 70% of non-infected fish in contaminated transport water. This indicated a high plausibility of false negative detections because of low eDNA concentrations in transport water and false positive detections of DNA from dead parasites in the water. Environmental DNA screening has limited applicability for aquatic biosecurity where there may be low eDNA concentrations in the water and when differentiation between live parasite infections and dead, non-viable parasites is paramount.

6.1. Introduction

The global trade of exotic fish species can facilitate the introduction of fish pests and their parasites into new environments (Duggan 2010; Della-Venezia et al. 2018). Invasive species can have detrimental consequences on indigenous ecosystems (Sala et al., 2000; Doherty et al. 2016; Sandilyan 2016; Della-Venezia et al. 2018), because they can compete with (Lockwood et al. 2013) or predate on (Doherty et al. 2016) endemic species. Furthermore, invasive ornamental fish can harbour generalist pathogens that present a high likelihood of co-invasion with potential to impact endemic biodiversity (Gaither et al. 2013; Lymbery et al. 2014), and aquaculture industries (Whittington and Chong 2007).

To minimize the risk of exotic pathogen introductions through the ornamental fish trade, several countries have established quarantine measures based on scientific risk analyses (Whittington and Chong 2007). For example, Australian regulations require imported ornamental fishes to undergo quarantine periods, treatment and health certification prior to export, inspection at border control by Australian quarantine inspection services on arrival, and mandatory pre-import quarantine periods before they are sold in the aquarium market (Department of Agriculture and Water Resources (DAWR) 2018). Nonetheless, pathogens considered to present a risk to biosecurity can go undetected despite stringent biosecurity (Rimmer et al. 2016; Chapter 3), highlighting the need for more sensitive screening methods that can identify high risk shipments and subclinical infections.

Environmental DNA (eDNA) has emerged as a popular method for detection of DNA that is continuously shed by living organisms into the local environment (Barnes and Turner 2016). Environmental DNA can be captured and extracted from environmental samples (e.g., water or soil), and used to determine the presence or absence of target species using quantitative polymerase chain reaction (qPCR; see reviews by Barnes and Turner 2016; Goldberg et al.

2016; Thomsen and Willerslev 2015). Screening eDNA by qPCR as a non-lethal detection method has been shown to be highly sensitive and accurately detect parasites in wild aquatic ecosystems (Huver et al. 2015; Rusch et al. 2018) and aquaculture (Agawa et al. 2016; Bastos-Gomes et al. 2017; Hallett et al. 2012). Furthermore, detecting species-specific eDNA by qPCR has been suggested as a possible non-destructive method for biosecurity, and has been used as a sensitive species-level detection tool to target exotic fish species present at low densities within mixed imports of ornamental fish to the United States (Collins et al. 2013). Thus, eDNA could potentially be used to detect DNA of pathogens shed by infected fish in the shipment water of imported live fish.

Environmental DNA screening methods may be prone to both false positive and false negative errors (Lahoz-Monfort et al. 2016; Schmidt et al. 2013), which lead to the misinterpretation of qPCR data (Darling and Mahon 2011). Understanding and communicating this uncertainty has proven difficult when management decisions relating to trade and potential trade barriers are needed (Darling and Mahon 2011). From a biosecurity perspective, misinterpreting qPCR data could lead to pathogen-free consignments being considered hazards during quarantine inspection (i.e., false positive error), or high-risk pathogens going undetected in infected consignments (i.e., false negative error). As such, detection of pathogens using molecular techniques for biosecurity must be reliable.

The aim of this study was to determine the reliability of eDNA screening to detect parasite DNA for biosecurity and border control applications. We developed and applied an eDNA screening method for the obligate ectoparasite, *Neobenedenia girellae* (Hargis, 1953), in an experiment that simulated common handling practices used to export live ornamental fish. We examined the incidence of false negative and false positive results and suggested rigorous sampling and analytical criteria to avoid them.

6.2. Methods

6.2.1. Parasite-host model

Neobenedenia girellae was chosen as the model ectoparasite species because it is an obligate, generalist pathogen that commonly infects subtropical and tropical marine ornamental fishes and has been associated with outbreaks in the global aquarium trade (Brazenor et al. 2018). The parasite infects the external surfaces of fish and lays eggs that are shed directly into the water. Barramundi, *Lates calcarifer* (Bloch, 1790), were chosen as the model fish species because they are domesticated and susceptible to infection by *N. girellae*. One hundred and thirty hatchery reared *L. calcarifer* (110.3 ± 8.2 TL mm) were sourced from a local freshwater fish farm (Good Fortune Bay, Townsville, Australia) and maintained in a 5,000 L tank with dechlorinated freshwater (26 °C) with no circulation at the Marine and Aquaculture Research Facility Unit (James Cook University, Australia). Fish were fed to satiation every day with pellets formulated for *L. calcarifer* (Ridley Aqua-Feed™, Australia) until needed for experimentation. *Neobenedenia girellae* eggs were sourced from an experimental culture in the Marine Parasitology Laboratory (James Cook University, Australia), which was established using methods described previously (Hutson et al. 2018). Freshly laid *N. girellae* eggs were collected from the culture and egg clumps (containing approximately 50-300 individual eggs) were placed into Petri dishes with clean seawater (35 ppt, 27 °C). Water changes were performed daily until use as a source of freshly hatched larvae (= oncomiracidia; less than < 4 h post-hatch) for experiments.

6.2.2. Neobenedenia girellae eDNA and gDNA concentrations

Juvenile parasites were obtained from host fish to develop a standard for eDNA and gDNA concentrations. Live parasites were removed from host fish using 2-Phenoxyethanol as per Hutson et al. (2018). In brief, three fish were transferred to three individual 10 L aquaria with clean UV sterilised and filtered (1 μm) seawater and individually infected with 250 freshly laid *N. girellae* oncomiracidia (egg hatch < 4 h, 35 ppt, 25 ˚C). After two days, fish were transferred to individual containers with 1.5 mL of 2-Phenoxyethanol in 5 L of seawater until sedation was evident with mild opercula movement. Then, fish were gently massaged to dislodge all parasites and transferred to aerated aquaria for recovery. Parasites were not sexually mature to ensure that the source of eDNA was from the live parasite and not contamination from egg production. Juvenile parasites were 218.5 ± 0.40 SE μm in length. Live parasites left in the anaesthetic solution were collected using a disposable 2 mL pipette into a large sterile Petri dish under a dissecting microscope (Leica M60).

The number of DNA copies/mL of water detected by qPCR were compared between genomic and environmental DNA (gDNA and eDNA, respectively). To collect *N. girellae* gDNA, a total of 1, 2, 4, 8, 16, and 32 live *N. girellae* of the same age and size were gently collected using a micropipette with a 1 mL disposable tip into 1.5 mL Eppendorf tubes with 70% ethanol and stored at 4 ˚C until extraction. To collect *N. girellae* eDNA, live juvenile *N. girellae* were haphazardly allocated to six treatments, representing increasing parasites concentrations of 1, 2, 4, 8, 16, and 32 live parasites per 250 mL of seawater. Each treatment had five separate replicate sterile 250 mL plastic containers (Sarstedt, Brisbane) with clean UV-sterilized and filtered seawater, into which live parasites were carefully pipetted, and a negative control (i.e., clean UV sterilized-filtered seawater with no parasites). Containers were then sealed with a sterile lid and held in dark conditions at 25 ˚C for 48 h, representing

the maximum time-period for transport of fish in the aquarium trade, including packaging, export, delivery, inspection, and release to the importer. Following, treatment replicates were individually filtered (60 µm nylon mesh) into separate sterile containers to remove whole parasites from the water, and all water from each treatment replicate was filtered through a 0.22 mm Durapore membrane filter (Millipore) using 50 mL sterile syringes. To avoid contamination, samples were collected first from all negative controls in all treatments, followed by treatment replicates. Filter casings were placed inside small sterile plastic bags, kept on ice during sampling, and finally stored at -20 ˚C until extraction.

6.2.3. Live fish export experimental design

An experiment was devised to best represent the typical time frame taken to transport live fish in the ornamental trade and the subsequent application of eDNA methods for parasite detection at 'border control'. One hundred and twenty freshwater *Lates calcarifer* were acclimated to seawater in a 5,000 L tank by increasing salinity to 10, 20, 30 and 35 ppt over two days using UV-filtered (1 µm) seawater. Fish were then haphazardly allocated to three separate treatments representing three possible scenarios that would likely result in positive eDNA detections on arrival to border control. Treatment 1 contained infected fish that were not treated for parasitic infections and arrived with a viable infection at border control (Treatment 1, infected fish; Table 14). Treatment 2 contained infected fish treated for parasite infections with freshwater and arrived at border control following treatment (Treatment 2, treated fish; Table 14). Lastly, Treatment 3 contained uninfected fish, which were transported using water contaminated with dead parasites (Treatment 3, contaminated water; Table 14). Three controls were used including uninfected fish in clean seawater

(Control 1), clean seawater with no fish or parasites (Control 2), and filtered water previously used to hold fish with a viable *N. girellae* infection (Control 3; Table 14). All the equipment used to handle and maintain the fish (e.g., aquaria, airlines, air stones and fish nets) was decontaminated with 10% bleach 24 h prior to the experiment.

Fish allocated to Control 1 and Treatment 3 (Table 14) were held in individual large plastic bags (61 x 91 cm, J Blackwood & Son LTD, Australia) with 10 L of UV-filtered seawater. Plastic bags were held individually inside previously chlorinated 10 L plastic buckets for ease of handling, and sealed with duct tape, only allowing an airline and air stone inside the plastic bag and to prevent possible contamination between samples by splashing water. Fish allocated to Treatments 1 and 2 were allocated to bags in the same manner, but were individually infected with 50 freshly hatched *N. girellae* oncomiracidia, which were carefully pipetted into the water before sealing the bags with duct tape. Fish were kept in these conditions for 5 days to enable parasite attachment and growth, and simulate pre-export quarantine periods (DAWR 2018).

Following the infection period, treatments and controls were prepared in conditions representative of commonly used handling and shipment procedures for live ornamental fish. First, Control 2 was prepared by filling 30 aquarium bags (30 x 20 cm, A1 aquarium, Townsville) with 1 L of clean UV sterilised-filtered seawater, saturated with oxygen, sealed with two superimposed latex rings (Elastrator, Heiniger, Australia), and placed inside a Styrofoam box. Then, fish in Control 1 were removed from their plastic bags and placed in individual aquarium bags with 1 L of clean UV-filtered seawater. After Controls 1 and 2 were placed inside individual Styrofoam boxes, fish in Treatment 1 were carefully placed in individual aquarium bags. Following, infected fish in Treatment 2 were individually bathed with dechlorinated freshwater in plastic buckets for 10 min, which kills and detaches *N. girellae* (see Hutson et al. 2018). This was done to represent a typical parasite treatment by

the exporting country. The seawater was retained to be used for Control 3 (Table 14). Each fish was gently massaged by hand to dislodge any remaining parasites and placed in individual aquarium bags. Parasites from each water bath were counted in each bucket by naked eye with the aid of a flashlight and collected with a 2 mL disposable pipette into a sterile 250 mL to be used in Treatment 3 (Table 14). Following, seawater used to hold fish in Treatment 2 during infection was individually filtered (Polymesh, 60 µm) into sterile aquarium bags, saturated with oxygen, sealed with a rubber band and placed inside a Styrofoam box (Control 3; Table 14). Lastly, fish in Treatment 3 were placed in individual aquarium plastic bags and inoculated with 25 dead parasites each (collected from freshwater bathing fish in Treatment 2). Treatment 3 represented the possibility of residual DNA in transport water (Balasingham et al. 2017; Rusch et al. 2018) rather than live parasite infections. The experiment was carefully timed so that parasites had grown, but not reached sexual maturity which would re-contaminate the system with parasite eggs (Brazenor and Hutson 2015).

Treatments and controls were kept sealed inside Styrofoam boxes for 48 h, representing the maximum time period used to handle fish in the aquarium trade, including packaging, export, delivery, inspection, and release to the importer. After 48 h, 15 mL, 50 mL and 100 mL samples of the 'shipment' water were individually collected using 50 mL sterile syringes and filtered onto nitrocellulose Durapore membrane filters (0.22 µm HA; Merck Millipore). This method was used for its suitability and ease of handling, aiming to capture free-floating DNA inside each plastic bag and to be representative of how much eDNA may be accessible at border control without compromising fish well-being. Each filter casing was placed inside a small sterile plastic bag and kept on ice during sampling. Fish in each treatment (Treatments 1-3, Control 1) underwent a freshwater bath at the conclusion of the water sampling to determine parasite intensity. Specifically, this would determine; 1)

infection intensity in Treatment 1; 2) if parasites remained infecting fish in Treatment 2 following the freshwater bath treatment, and; 3) if there was any case of accidental infection in Treatment 3 or Control 1. After sampling, filter casings were stored at -20 ˚C for 24 h until DNA extraction.

Table 14. Experimental treatments for eDNA validation. Each Treatment and Control 1 had thirty replicate fish, while 10 replicate bags were made for Controls 2 and 3. Parasite inoculum= freshly hatched oncomiracidia (< 4 h) in Treatments 1 and 2, and 5 day old dead juveniles in Treatment 3. Parasite mean intensity and prevalence are shown following parasite recovery at the conclusion of the experiment, with the exception of Treatment 2 where [a]= Parasites recovered following initial fresh water treatment, and [b] = parasites recovered at the conclusion of the experiment. Confidence Intervals were calculated using the Clopper-Pearson exact method (Sergeant 2018).

Treatment	Parasite inoculum	Mean parasites intensity ± S.D.	Parasite prevalence (95% CI)
Treatment 1: Infected fish in clean seawater	50	6.80 ± 4.78	100 (88.43–100)
Treatment 2: Treated fish (previously infected) in clean seawater	50	9.33 ± 5.08[a]; 0[b]	100 (88.43–100)[a]; 0 (0–11.57)[b]
Treatment 3: Uninfected fish in contaminated seawater	25	0	0 (0–11.57)
Control 1: Uninfected fish in clean seawater	0	0	0 (0–11.57)
Control 2: Clean seawater (no fish or parasites)	0	-	-
Control 3: Filtered contaminated seawater (no fish or parasites)	0	-	-

6.2.4. DNA extraction protocol

Filter casings were separated underneath a laminar flow cabinet and filter papers were collected with sterile forceps, folded inwards and placed inside individual 2 mL tubes with 70% ethanol. To reduce the chance of contamination, filter casings were opened in the same order as they were collected. Filter paper from each sample was homogenised using sterile micro-scissors (i.e., sequentially dipped in 2% Vircon S solution (Lanxess Pty. Ltd., Australia), followed by a dip MilliQ® water, and a last dip in absolute ethanol). Homogenised samples were centrifuged at 3,000 rpm for 30 min at room temperature (RT= 25ºC), 70% ethanol was gently discarded from each sample and eDNA was extracted from all samples by use of the DNeasy Blood and Tissue Kit (Qiagen, USA) following the manufacturer's instructions, except the lysing step was done at 60 ºC for 1 h and DNA was eluted in 100 µL of AE buffer (10 mM Tris-Cl; 0.5 mM EDTA; pH 9.0). DNA extracts were transferred to 1.5 mL Eppendorf tubes samples and stored at -20ºC for qPCR analyses.

6.2.5. Primer design and qPCR protocol

Species-specific primers were developed to detect the cytochrome b gene (*cytb*) in mitochondrial DNA of *Neobenedenia* sp. (see Agawa et al. 2016). Primer pairs N.GirellaeMtF (5'- GTGTTTGCTGCTCATGTAATATTA-3') and N.GirellaeMtR (5'-CATCTAAAACCAAATCAGGAGAAG-3') (Agawa et al. 2016) were designed to target *Neobenedenia* sp., accounting for the lack of clarity surrounding the morphological identification of *N. girellae* and *N. melleni* (see Agawa et al. 2016; Brazenor et al. 2018).

Primer specificity was tested by Agawa et al. (2016) against *Benedenia epinepheli* (Yamaguti, 1937), *Benedenia hoshinai* Ogawa, 1984, *Benedenia sekii* (Yamaguti, 1937) and *Benedenia seriolae* (Yamaguti, 1934), and shown to have minor non-specific amplification for *B. seriolae*. In this study, primer specificity was tested against *N. girellae* and the host fish *L. calcarifer* by qPCR with previously extracted genomic DNA (gDNA).

DNA extracted from all samples in this study was amplified by qPCR with six technical replicates. Each technical replicate (20 µL) contained 6 µL of DNA, 1 µL of each PCR primer (10 nM), 10 µL PowerUP® SYBR GreenER qPCR Master Mix (Life Technologies, Australia) and 2 µL MilliQ® water. The following fast cycling conditions (ramp rate = 2.7 °C/sec) were used: UDG incubation at 50 °C for 2 min, initial denaturation at 95 °C for 2 min, 50 cycles of 95 °C denaturation for 15 sec then 60 °C annealing for 45 sec, and terminal dissociation curve generation (60 – 95 °C at 0.15 °C/sec). All qPCRs were run on a QuantStudio3™ Real-Time PCR System (ThermoFisher Scientific Inc., Brisbane) using QuantStudio™ Design and Analysis Software (version 1.4.2).

6.2.6. Estimation of eDNA copy number

Approximate copy numbers were estimated based on standard curves constructed using synthetic gBlocks® fragments (Integrated Gene Technologies (IGT), Australia). A 250 bp artificial standard was created in Geneious by aligning partial sequences of the *cytb* gene region for *N. girellae* and *N. melleni* according to Brazenor et al. (2018). Integrated Gene Technologies require sequences to have a complexity index < 10 to create all gBlocks® fragments. Artificial standards initially had a complexity higher than requirements of IGT

associated to a region of low GC content near the 3' end (Supplementary S9), as such, a total of 12 base-pair mismatches were created in the synthetic standard compared with *N. girellae* and *N. melleni* sequences to increase the GC content (Supplementary S9) and consequently, differentiate potential assay cross-contamination with artificial DNA (aDNA). Artificial DNA concentration was measured with a Quantus™ Fluorometer (Promega, Australia), using QuantiFluor® ONE dsDNA System reagents (Promega, Australia). Based on the aDNA fragment length of 250 bp, we estimated a molecular weight of 154465.9 g/mol for the fragment. A 1 x 10^{10} copies/µL aDNA stock solution was prepared by diluting the aDNA standard using TE buffer (10 mM TRIS-HCL, I mM EDTA, pH: 8.0). A standard curve of the *cytb* partial fragment was created by diluting the aDNA stock solution from 10^8 (200 million copies/µL) to 10^2 (2 copies/µL) using MilliQ® water. Artificial standards were used to determine qPCR efficiency (E) (increase in amplicon per cycle; Ruijter et al. 2009) and qPCR assay limit of detection (LOD) (lowest number of DNA copies/µL detected) using a baseline fluorescence threshold of 0.2 (minimum level of fluorescence measured before amplification can be detected).

6.2.7. Selection criteria for N. girellae *amplicons*

Melting temperatures (T_m) of qPCR replicate amplicons were directly compared to the mean melting temperature (T_m) of serially diluted *N. girellae* genomic standards (ΔT_m) (Ririe et al. 1997). Raw melt curve data of each assay was exported from the QuantStudio™ Design and Analysis Software (version 1.4.2) and analysed using Microsoft Office 365 ProPlus Excel (version 1804). The absolute difference between the mean T_m of the amplicons produced from each serial dilution of *N. girellae* gDNA standard and individual qPCR

technical replicate amplicons was calculated ($|\Delta T_m|$=T_m mean gDNA - T_m amplicon) and used to select putative positive amplicons (modified from Ririe et al. 1997).

Each amplicon was considered a putative positive if 1) the amplification curve crossed the common threshold fluorescence (0.2) within 50 cycles, and 2) $|\Delta T_m| \leq 1$ °C. Amplicons with amplification curves within 50 cycles, but $|\Delta T_m| > 1$ °C were considered putative false negatives. If an amplicon had no amplification crossing the common threshold within 50 cycles, but $|\Delta T_m|$ was within 1 °C, the amplicon was re-amplified by qPCR, in which case technical replicates (20 µL) contained 1 µL of qPCR product, 1 µL of each PCR primer (10 nM), 10 µL PowerUP® SYBR GreenER qPCR Master Mix (Life Technologies, Australia) and 7 µL MilliQ® water, and used the same cycling conditions as normal qPCRs. If an amplicon had no amplification crossing the common threshold within 50 cycles, and did not display T_m, the amplicon was considered negative. All amplicons considered putative positive, false negative, or re-amplified following the initial qPCR, were sent to the Australian Genome Research Facility, Brisbane, for Sanger sequencing to confirm identity.

6.2.8. Data analysis

Environmental DNA concentration was expressed as copy numbers/mL of bag water. Concentrations were calculated from eDNA copies per qPCR reaction (eDNA copies), the volume of template DNA used per qPCR reaction, the sample volume, and the total volume of water (Bylemans et al. 2018). Best-fit regression models were used to examine relationships between number of parasites and DNA concentrations. Best-fit regression analyses were done in SPSS v.25 (IBM). Assay diagnostic sensitivity was estimated using the

EpiTools epidemiological calculator for test evaluation (Ausvet, epitools.ausvet.com.au; Sergeant 2018). Point estimates of positive and negative likelihood ratios in each treatment are provided with Clopper-Pearson (exact) confidence limits.

6.3. Results

6.3.1. Neobenedenia girellae *qPCR specificity, LOD and efficiency*

The developed qPCR assay successfully amplified *N. girellae* DNA. The assay had an amplification efficiency of 99.7% (R^2=0.99), and standard curves displayed a LOD (lowest number of DNA copies/µL detected) of 2 copies/µL at a baseline threshold of 0.2, although variation in detection increased with aDNA ≤ 20 copies/µL (Figure 25A). All sequenced amplicons were 98.7-100% homologous to *N. melleni* (Genbank accession numbers: HQ684800, HQ684801, HQ684816, JQ038228) and *N. girellae* (Genbank accession numbers: MG193665-70; see Brazenor et al. (2018) for discussion on the plausible misidentification of *N. girellae* as *N. melleni*). The absolute difference in melting temperature (|ΔTm|) between sequenced amplicons and corresponding species-specific gDNA standards ranged between 0.055–2.41 °C (Figure 25B).

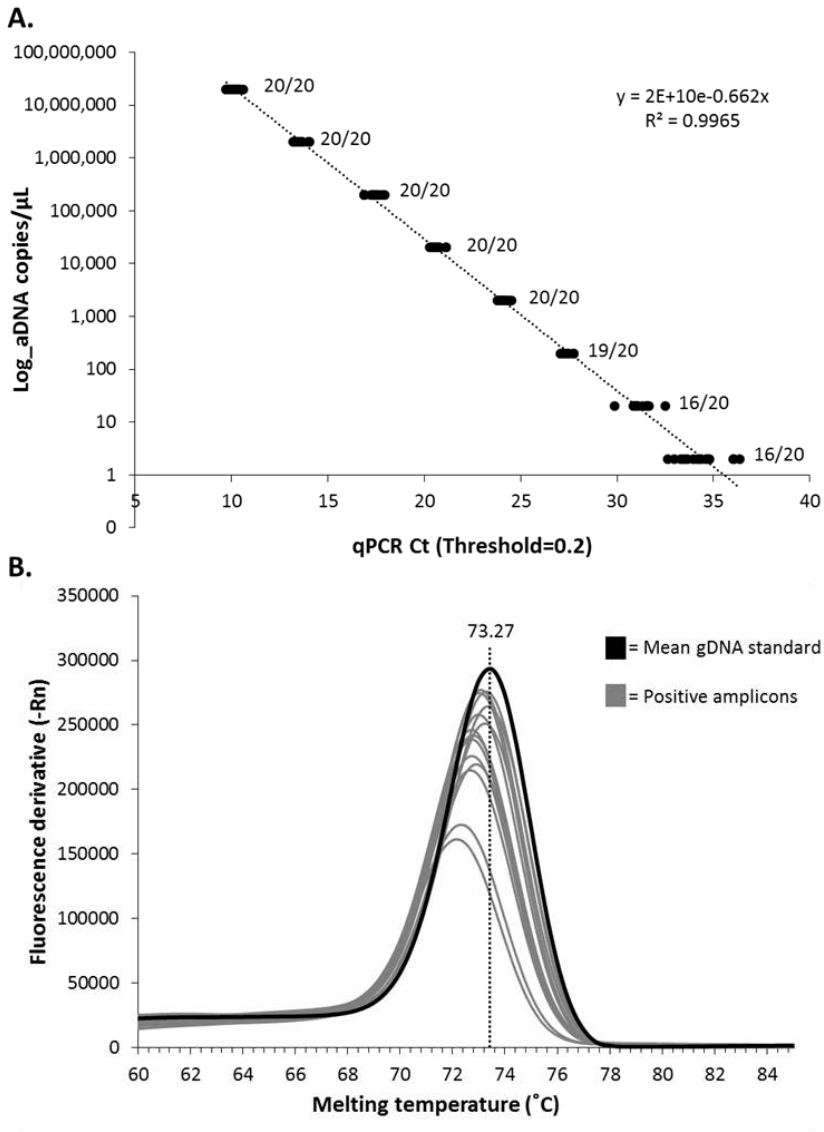

Figure 25. *Neobenedenia girellae* artificial DNA (aDNA) standard curve (A) and melting temperature (Tm) of the mean genomic DNA (gDNA) standard and amplicons confirmed positive for *N. girellae* (B).The number of amplified replicates/total replicates is provided for each aDNA standard (A), and the dotted line in (B) indicates the mean Tm of gDNA standards (73.27 ˚C).

6.3.2. *Neobenedenia girellae eDNA and gDNA concentrations detected by qPCR*

Real-time PCR assays amplified 0-0.15 copy numbers/mL of gDNA compared to 0.02-0.09 copies copy numbers/mL of eDNA (Figure 26). Mean DNA concentration was significantly higher in gDNA than eDNA in treatments with 16-32 parasites/250 mL (Figure 26), but there were no differences in mean DNA concentration between gDNA and eDNA in treatments with 1-8 parasites/250 mL (Figure 26). There was a significant relationship between detected *N. girellae* gDNA and number of parasites (best-fit$_{quadratic}$ model, $F_{1, 22}$=303.69, p<0.001), with 99% of variation explained by a polynomial regression (Figure 26A). Similarly, a significant relationship was also detected between *N. girellae* eDNA and number of parasites (best-fit$_{quadratic}$ model, $F_{1, 156}$=69.79, p<0.001); however, 73% of variation was explained by a polynomial regression, with high variation in detected eDNA copies/µL across all parasite concentrations (Figure 26B). The polynomial regression was significant and a reasonable fit to the data but the predicted decline in eDNA copies with increasing numbers of parasites above 16 is not likely a true representation but a reflection of the high variation of qPCR amplification and available eDNA volumes for detection (Figure 26B).

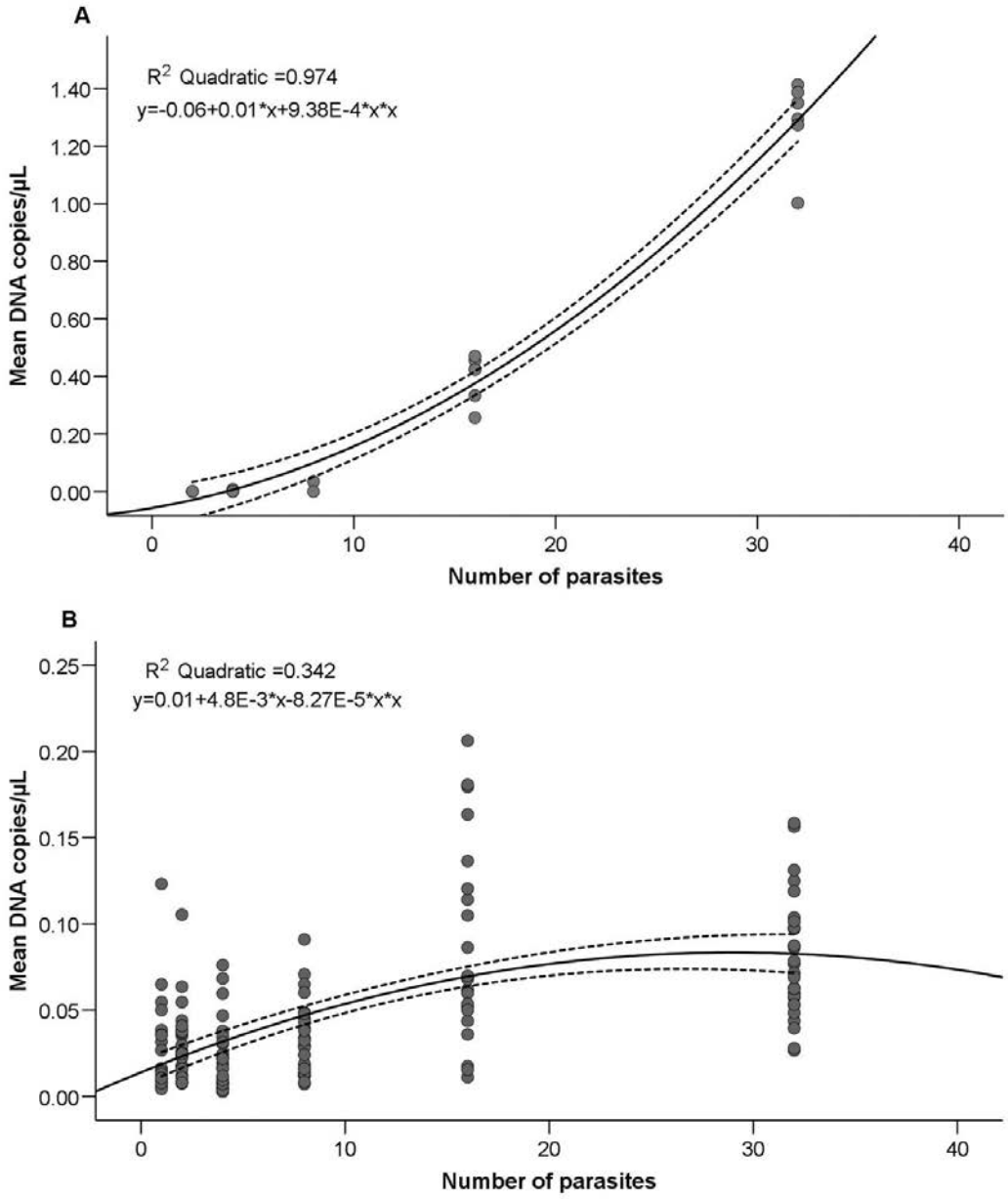

Figure 26. DNA concentration (eDNA copies/mL of bag water) of *Neobenedenia girellae* genomic (gDNA) (A) and environmental DNA (eDNA) (B). Quantitative PCR Efficiency = 101% (R^2 = 0.99); baseline threshold = 0.2. No significant differences were detected in samples with ≤ 8 parasites (Post hoc Tukey HDS test). There were significant relationships between detected copies/µL and number of parasites for gDNA (best-$fit_{quadratic}$ model, $F_{1, 22}$=303.69, p<0.001), and eDNA (best-$fit_{quadratic}$ model, $F_{1, 156}$=69.79, p<0.001). Dotted lines indicate upper and lower 95% confidence intervals.

6.3.3. *Environmental DNA detection in a border control scenario*

Neobenedenia girellae DNA was detected in 50% (15/30 replicates) of water samples collected from infected fish (Treatment 1), in 23% (7/30 replicates) of treated fish (Treatment 2), and in 70% (21/30 replicates) of contaminated water samples (Treatment 3) (Figure 3). Assay diagnostic sensitivity (95% CL) was 50% (31.3–68.7) for infected fish, 23.3% (9.9–42.3) for treated fish, and 70 (50.6–85.3) in contaminated water (Table 14). Infected fish exhibited a parasite prevalence (95% confidence interval) of 100% (88.43–100), with a mean infection intensity of 6.80 ± 4.78 ± S.D. parasites/fish (Table 14), and water samples had a mean DNA concentration of 0.098 ± 0.01 S.E. copies/mL (Figure 27). Treated fish, which had a parasite prevalence of 0% (0–11.57) post treatment (Table 14), had 0.042 ± 0.01 SE copies/mL in water samples. Contaminated water samples had the highest concentration of DNA = 0.4 ± 0.13 S.E. (Figure 27). No parasites were found in any of the controls (Table 14). There were three technical replicates from Control 1 that were considered putative false positive detections following the amplicon selection criteria that were confirmed negative following Sanger sequencing. As such, the qPCR assay designed in this study to detect *N. girellae* eDNA had a diagnostic specificity of 90% (95%CL 73.5– 97.9%) (Table 14). There were no other instances of putative positive or false negative detections in any of the controls. There was no correlation between amplified *N. girellae* eDNA concentration and parasite intensity in this study (Figure 28).

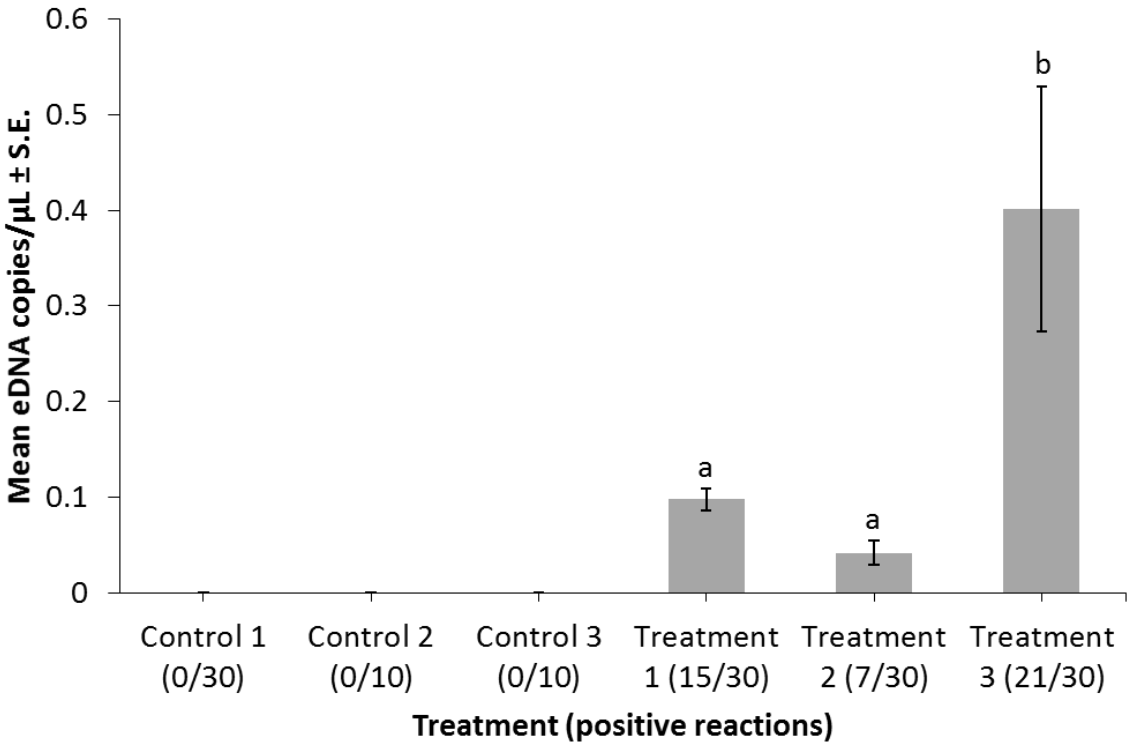

Figure 27. *Neobenedenia girellae* environmental DNA (eDNA copies/mL of bag water) amplified by qPCR in 50 cycles with a baseline threshold of 0.2. Amplified DNA in Treatment 3 (uninfected fish in contaminated seawater) was significantly higher than Treatments 1 (infected fish) and 2 (treated fish; one-way ANOVA, $F_{2, 136}=10.45$, $p<0.001$). 'a' and 'b' indicate differences between pairs of means determined using Tukey's HSD test.

Table 14. Environmental DNA assay sensitivity with a 95% Clopper-Pearson (exact) confidence limits (CL). Each treatment and Control 1 had thirty replicate water samples, while controls 2 and 3 had 10 replicate water samples. Each water sample had 6 qPCR technical replicates. Control 1 was considered the gold standard test for comparison, where 27/30 tests were correctly identified as negative detections by eDNA and 3/30 tests were incorrectly considered putative positive detections by eDNA, which were confirmed negative by Sanger sequencing. Assay specificity was 90 % (73.5–97.9 CL).

Treatment	**Sensitivity % (95% CL)**
Treatment 1: Infected fish in clean seawater	50 (31.3–68.7)
Treatment 2: Treated fish (previously infected) in clean seawater	23.3 (9.9–42.3)
Treatment 3: Uninfected fish in contaminated seawater	70 (50.6–85.3)
Control 1: Uninfected fish in clean seawater	90 (73.5–97.9)
Control 2: Clean seawater (no fish or parasites)	100 (69.2–100)
Control 3: Filtered contaminated seawater (no fish or parasites)	100 (69.2–100)

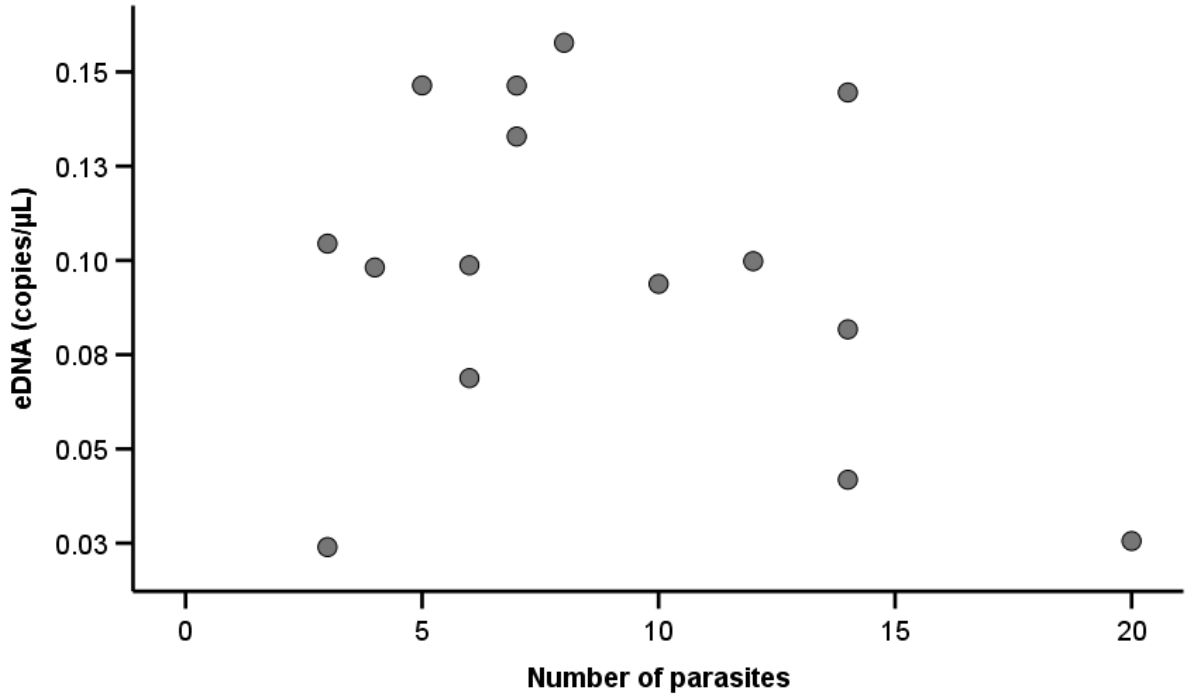

Figure 28. Relationship between *Neobenedenia girellae* environmental DNA (eDNA copies/mL of bag water) amplified in 50 cycles by qPCR and parasite intensities in Treatment 1 (infected fish in seawater). There was no significant relation between amplified DNA and parasite intensity (linear regression model, $F_{1, 14}$ =-1.11, R^2=0.0792, p=0.31).

6.4. Discussion

Environmental DNA tests of water samples collected using the syringe-filter extraction method in this study were unreliable in detecting subclinical *N. girellae* infections in *L. calcarifer*. Specifically, eDNA tests were 50% effective in the detection of ectoparasite DNA in water containing infected live fish in a controlled experiment representing standard import procedures of live fish. The qPCR assay in this study detected a mean ± S.E. eDNA concentration of 0.098 ± 0.01 mL from 100 mL of filtered samples collected from enclosed bags with mean ectoparasite infection intensity of 6.8 ± 4.78 S.D. parasites/fish. Most importantly, qPCR tests inconsistently detected DNA concentrations below the detection

threshold of 2 eDNA copies/µL (Figure 25-26, 28), and failed to detect *N. girellae* eDNA in the remaining 50% of infected fish, in 30% of contaminated water samples and in all filtered, contaminated water samples (Figure 27).

Quantitative PCR tests detected *N. girellae* DNA in 70% of replicates of contaminated water samples, suggesting a high probability of false positives at border control. Positive detection of *N. girellae* eDNA in contaminated water samples represents a situation where high quantities of residual eDNA from degrading, dead parasites, remains viable for extended periods of time in the water column (Corinaldesi et al. 2008; Pochon et al. 2017), or alternatively, a situation where parasite eggs or other life stages are present in the water. Environmental DNA studies, including this study, usually target short mitochondrial DNA (mtDNA) fragments because it is more abundant in environmental samples than nuclear DNA, is present in higher copy numbers per cell (Bylemans et al. 2018; Pietramellara et al. 2009) and persists longer in the environment (Foran et al. 2006; Pietramellara et al. 2009). While this approach improves sensitivity and detection, it prevents differentiation between residual DNA from dead parasites and DNA from viable parasite infections and life stages, a limitation highlighted in detecting live assemblages of invasive fish species (Pochon et al. 2017; Zaiko et al. 2018). This limits the application of eDNA assays at border control, as false positive qPCR detections could lead to consignments being mistakenly considered hazardous during inspection (Collins et al. 2013).

Australian import conditions require mandatory treatment for goldfish with parasiticides (e.g. trichlorfon, formaldehyde or sodium chloride) for the presence of gill flukes (*Dactylogyrus vastator* Nybelin, 1924 and *Dactylogyrus extensus* Mueller and Van Cleave, 1932) prior to export (The Australian Government Department of Agriculture and Water Resources (DAWR) 2018). However, treatment efficacy depends on environmental factors, the use of appropriate chemical concentration, parasite resistance, parasite life stages, and

toxicity to the fish host (Goven and Amen 1982; Thoney and Hargis 1991; Schelkle et al. 2011). In this study, qPCR tests detected *N. girellae* DNA in 23 % of water samples from fish previously given a fresh water bath treatment, which is generally considered to be 100% effective for eradication (Kaneko et al. 1988). Although no parasites were detected in treated fish at the conclusion of the experiment (Table 14), *Neobenedenia girellae* can attach underneath fish scales, which could prevent a freshwater treatment from killing the parasite (Trujillo-González et al. 2015). Residual eDNA from beneath the scales or trapped in fish mucus could explain why 23% of fish yielded positive results for *N. girellae* eDNA in this study. Considering that parasites can survive treatment during pre-export quarantine periods and the high possibility of false positive errors associated with dead parasites in this study, eDNA screening methods may not be sensitive enough to offer freedom of subclinical infection surveillance during pre- and post-export quarantine periods.

False negative detections indicated that qPCR tests did not reliably detect *N. girellae* eDNA in water samples used to hold fish during 48 h (Table 14). The inability to detect low amounts of eDNA by the qPCR assay (Figure 26) suggest that the *N. girellae* eDNA available for detection by qPCR was below the LOD and that parasite eDNA shedding rates may be too low within the timeframe used to export live ornamental fish. This is a considerable limitation in the reliability of eDNA screening by qPCR, as low parasite intensities could be present in imported fish populations (Trujillo-González et al. 2018). DNA shedding rates and therefore the amount of pathogen eDNA available for detection depends on multiple factors, including parasite intensity, host abundance (Rusch et al. 2018), the viability of pathogens in the absence of a suitable host (Hick et al. 2016) and environmental factors such as water temperature (Robson et al. 2016). The effect of these parameters in the availability of parasite eDNA are currently unknown and will have an impact on the reliability and performance of qPCR assays for biosecurity. Future studies should consider the impact of parasite DNA

shedding and decay rates on eDNA screening methods, as parasites may be available in small numbers and shed negligible amounts of eDNA in the water, having a negative impact on the performance and reliability of qPCR assays at border control.

The number of false negative detections may have been exacerbated by the sampling method chosen for this study. Filtration methods have been commonly used in ecological studies to monitor target species populations (Goldberg et al. 2016; Simpson et al. 2017; Rusch et al. 2018). Previous eDNA studies using filtration methods have commonly used much larger filters than those used in this study to account for filter blockage with debris from eDNA samples or use sequential filtering to remove large suspended particles before collecting eDNA samples for screening by qPCR (Goldberg et al. 2016; Robson et al. 2016). However, considerable volumetric sampling to monitor target species in ecosystems (Simpson et al. 2017) would be time-consuming and non-viable in the context of border control detection methods. In this study, eDNA collected by syringe and filtered through 0.22 µm filter casings resulted in inconsistent eDNA detections by qPCR (Figure 26B-27). Consequently, there was no significant correlation between detected eDNA concentrations (copies/mL) and parasite intensities in this study (Figure 28). This caveat has been highlighted previously and suggests that DNA capture and extraction methods must be improved to advance the applicability of eDNA methods to reliably measure the relative abundance and occurrence of targeted species (Ficetola et al. 2015; Fonseca 2018; Rice et al. 2018). As such, the extraction method tested in this study is limited in its approach to inform biosecurity and future studies should consider the applicability of other extraction methods aiming to increase DNA yield capture and their applicability in other stages of biosecurity monitoring and surveillance.

In conclusion, the qPCR assay developed in this study to detect *N. girellae* infecting *L. calcarifer* was not a reliable detection tool for biosecurity. This study highlighted three

important caveats of eDNA screening methods for border control. First, qPCR detections did not allow any differentiation between eDNA derived from live parasites infecting fish and eDNA derived from non-viable parasites or residual DNA in the water, resulting in a high number of false positive detections by qPCR. Second, low amounts of parasite eDNA affected the reliability of eDNA detection by qPCR, resulting in false negative detections. Third, the collection method used in this study provided inconsistent volumes of parasite eDNA, further exacerbating the number of false negative detections by qPCR. Future research would benefit from targeting variable DNA fragments in water samples, which could allow differentiation of eDNA derived from actively shedding parasite populations, or from contaminated samples with residual DNA (Bylemans et al. 2018). Alternatively, targeting environmental RNA (eRNA) rather than eDNA, could allow the detection of viable parasite infections for biosecurity. Environmental RNA detections would be indicative of active gene transcription, and would be less evident in dormant stages or dead parasites, compared to metabolically active cells (Poulsen et al. 1993; Bass et al. 2015; Pochon et al. 2017) and provide valuable insight for the advancement of eDNA techniques in biosecurity.

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CHAPTER 7

GENERAL DISCUSSION

The ornamental fish trade is a growing economic sector fuelled by the supply and demand of popular ornamental species. Accurate understanding of the volume and diversity of fish traded between countries not only provides industries with invaluable information to guide economic strategies, it allows governments to better gauge which fish species are commonly traded within their jurisdiction. Most importantly, governments can use accurate species-specific trade data to assess risks associated with the import of commonly traded ornamental fishes, their associated parasite fauna, and potential impacts of exotic parasites invading endemic ecosystems. In Australia, biosecurity is established to protect endemic ecosystems, natural resources and primary industries (DAWR 2018). Since 1999, two separate Import Risk Analyses have set the foundations of Australian biosecurity against risks associated with the importation of ornamental fish species. Currently, the Australian Government Department of Agriculture and Water Resources (DAWR) aims to ensure off-shore biosecurity in exporting countries, reviewing pre-export health conditions and export requirements to improve post-arrival biosecurity (Hood and Perera 2016). As such, imported ornamental fish species require stringent health certificates and veterinary inspections following specific requirements prior to delivery (DAWR 2018).

It has been 19 years since the risks associated with parasite infections of imported ornamental fish species were assessed. During this time the supply and demand of the Australian ornamental trade has changed dramatically, increasing its volume, import rate and number of fish species allowed for export to Australia. Consequently, the parasite diversity

being translocated to Australia through the ornamental fish trade has inherently changed and must be re-examined to determine the reliability of biosecurity measures against parasites considered hazards by Australia.

This thesis provided new collated information on import trade data, provided morphological and molecular identification of parasite species richness of ornamental fish species imported to Australia, and rigorously challenged the application of novel molecular detection techniques as a detection tool for Australian biosecurity. Collectively, the Chapters in this thesis represent considerable progress in our understanding of the ornamental fish trade and its associated parasite threats. The main findings of the thesis, implications of results, knowledge gaps filled, and possibilities for future research are discussed below.

There is a significant diversity of parasites that is currently going undetected at border control and is not being considered by Australian biosecurity import conditions. Myxozoans infections consistent with *Ceratomyxa*, *Kudoa* and *Myxobolus* spp., and 14 parasitic monogenean gill and skin fluke species (e.g. *Dactylogyrus*, *Gyrodactylus*, *Urocleidoides*, and *Trianchoratus* spp.) were detected by necropsies and molecular identification from 34 fish populations imported from southeast Asia (Chapter 3). Although parasites were found infecting multiple freshwater and marine species (Table 6 and 8) only *Carassius auratus* currently requires mandatory pre-export treatment for the presence of gill fluke infestations of *Dactylogyrus vastator* and *Dactylogyrus extensus* (DAWR 2018). No other parasites require mandatory health requirements prior to export (DAWR 2018). Considering the diversity of parasites found infecting not only goldfish but other freshwater and marine ornamental fish species, this research recommends the re-assessment of risks associated with parasite species

infecting ornamental fishes imported to Australia and provides baseline survey data for this purpose (Chapter 3).

Australian Biosecurity must consider emerging parasite threats of heavily traded ornamental fish species globally. Although 4,628 fish species are allowed for import to Australia, goldfish are by volume the most imported species to Australia and the world (Chapters 2 and 4). Over 197 parasite species are known to infect goldfish, of which 39 have been reported infecting traded goldfish (Chapter 4, Figure 19A). Of these several species, *Myxobolus lentisuturalis* and *Myxobolus turpisrotundus* form plasmodia on the body surface of the host and cause severe disfigurement of the host tissue (Caffara et al. 2009; Zhang et al. 2010). Similarly, monogeneans *D. anchoratus*, *D. intermedius*, *D. formosus*, and *D. vastator* increase the morbidity of aquarium-held and traded goldfish and may cause significant mortalities if undetected (Ling et al. 2016). There are currently no records of these parasites infecting endemic fauna in Australia, and their impact in native ecosystems remains to be evaluated. However, these parasites can affect the aesthetic value of popular ornamental cyprinids (e.g. *Carassius* spp., *C. carpio*), and could cause significant economic losses to aquarium shops if undetected and fish are not properly quarantined. Future assessment of goldfish imports to Australia should consider stringent regulations for exporting companies delivering infected fish to Australia, and analyse the risks of new emerging parasite threats infecting fish species commonly held by aquarium shops in Australia to mitigate the impact on local businesses.

Similarly, Australian biosecurity should target parasite species that could affect endemic Australian fauna. For example, the crustacean parasites *Argulus japonicus* and *Lernaea cyprinacea* are known invasive species globally with detrimental impact to

aquaculture and endemic ecosystems (Chapter 4). *Argulus japonicus* for example, was recently detected infecting imported goldfish populations from southeast Asia (Becker et al. 2016). Similarly, *Schyzocotyle acheilognathi* is the most important pathogenic cestode of cyprinid fish (Scholz et al. 2012), and one of the most invasive parasite species globally (Kuchta et al. 2018). Invasive *S. acheilognathi* has caused irreversible changes to endemic fish populations globally (Kuchta et al. 2018; Pérez-Ponce de Leon et al. 2018), and due to its adverse effect on fish health, has caused significant economic losses in cyprinid aquaculture (Choudhury and Cole 2012; Scholz et al. 2012). The current distribution of *S. acheilognathi* has been directly linked to decades of human mediated translocation of infected food fish as well as ornamental cyprinids (Kuchta et al. 2018). This species has been reported infecting fish species in Australian endemic ecosystems (Dove and Fletcher 2000), and is considered a neglected parasite species in global biosecurity (Brabec et al. 2018). These three invasive species could pose important risks to Australian native fauna and should be evaluated by future Biosecurity Import risks analyses.

This research challenged the reliability of visual inspections as a method to detect subclinical infections and cryptic, microscopic parasites at border control. Indeed, visual inspections should not be considered as a stand-alone method to detect parasites and pathogens of importance to Australian biosecurity and should be used to complement rigorous pre-export health requirements. Officers at border control should consider: 1) the accuracy and validity of health certifications and invoice information; 2) ensure imported species are approved for import to Australia; 3) ensure that populations do not contain non-permitted material or material of biosecurity concern, and; 4) visually inspect the health condition of imported fish, and ensure fish show no clinical signs of infectious disease or pests (DAWR 2018). Detection of disease and pests based on the presence of clinical signs

means that an infection must perpetuate in clear signs of behavioural distress or clinical disease. However, parasites may be present in low intensities and fish may appear asymptomatic, showing no obvious signs of infection (Chapter 3). Therefore, the efficacy of biosecurity measures at border control depends greatly on adequate pre-export treatment and how fish are certified to be free of disease and infections. Therefore, the way pre-export health requirements are enforced and monitored must be re-assessed to improve pre-export quarantine periods and the validity of health requirements prior to export.

This research examined the potential use of eDNA in biosecurity and border control. Although this study detected species-specific eDNA from five *Dactylogyrus* species infecting ornamental goldfish and rosy barb populations imported to Australia, results indicated the high possibility of false positive detections associated with contaminated source water (Chapter 5). The use communal source water by exporting companies to rear and export ornamental fishes would impede the use of eDNA at border control as a reliable detection tool. Furthermore, this research showed that eDNA screening was unreliable when used to detect low parasite intensities of *Neobenedenia girellae* in a simulated 'export scenario'. Lastly, the timeframe needed to collect, extract, and test water samples for parasite eDNA in this study was considerably time consuming. Therefore, this research shows that eDNA screening methods are not viable for aquatic parasites at border control, given the high possibility of false positive detections, lack of diagnostic sensitivity in detecting subclinical parasite intensities, and the considerable amount of time needed to test water samples for target eDNA, which is unfeasible within the limited timeframe of border control inspection. These results should not negate the consideration of eDNA approaches to other scenarios for detection of pathogens at border control (e.g. the detection of viral pathogens in animal feeds, Whittington and Chong 2007).

FUTURE RESEARCH DIRECTIONS

The research conducted in this thesis will contribute to the biosecurity objectives and goals of the Australian Government Department of Agriculture and Water Resources. Additionally, this thesis contributes to the published literature of parasite diversity in the aquarium trade, human-mediated translocation of exotic parasite species, and the novel application of eDNA molecular tools in biosecurity. There remain multiple topics to be addressed, which would greatly contribute to the study of exotic invasive parasite species and the use of eDNA as a viable diagnostic tool for biosecurity. Future research should consider:

1. Parasitology, which traditionally relied on morphological diagnosis for species identification, requires much needed genetic data. This thesis was limited by the availability of genetic sequences accessioned for gene regions that could discriminate between closely related parasite species (Chapter 3). Research must continue to increase molecular data in genetic databases, which would improve current understanding of intra-specific variation of parasite species, and the reliability of primer design for eDNA analysis. Reliable genetic data accessioned with corresponding specimens would allow researchers to examine the validity of parasite species descriptions, complement species records, and adequately examine parasite diversity and infer on possible host-switching events and speciation (see the case of *Cardicola* spp. in Nolan et al. 2014). Increasing the number of reliable genetic sequences in online repositories would allow future research in parasitology to detect possible parasite species complexes and monitor specific parasite species important to aquaculture and the ornamental trade.

2. The applicability of molecular techniques in biosecurity depends on their detection sensitivity of target DNA. In the case of biosecurity and freedom from disease surveillance, it is imperative to accurately determine the origin of eDNA to reduce the possibility of false positive errors. Determining if a positive detection is related to live, infecting parasites, or to dead, degrading parasite DNA in the water column is an imperative requirement for the development of eDNA based techniques in biosecurity. Future research should consider targeting RNA as an indication of active, live cells in water samples and therefore, the presence of live, infective parasites. Alternatively, future search targeting eDNA fragments in the water column could allow researchers to differentiate eDNA of live parasites from degraded, old eDNA in water samples.

3. The use of environmental DNA must be validated throughout the ornamental supply chain and stages of quarantine. This thesis demonstrates that eDNA screening methods are unreliable at border control because of time-consuming molecular workflows and sampling limitations which ultimately affected the reliability of detection for biosecurity. Nonetheless, eDNA screening methods could be highly beneficial at other stages of the ornamental supply chain and other industries. For example, eDNA could allow health specialist to monitor the presence of parasite DNA during quarantine periods, which have a minimum timeframe of seven days prior to export, and 14 days following border control inspection. Further research of eDNA screening methods during quarantine periods could offer valuable insight to the reliability of eDNA as a biosecurity monitoring tool, while considering the outcomes of this research (Chapters 5 and 6).

4. Improving the efficiency of collection and extraction methods is instrumental for the application of eDNA in biosecurity. Time-efficiency and reliability of detection will be important factors in the application of eDNA technologies for biosecurity. Therefore, molecular workflows must be improved and simplified. New extraction techniques that can remain sensitive while time-efficient should be explored and tested for biosecurity. Better extraction methods that could improve the sensitivity of detection for eDNA research would ultimately improve future studies for parasitology and aquatic animal health research.

5. Future studies should consider intra-specific genetic variation when developing primers for molecular techniques in biosecurity and standards of comparison. This research showed that selection criteria used to determine positive and negative detections depended on the quality of genomic standards, and how well standards encompassed the entirety of a species genotypic variation. Therefore, and for the future development of eDNA based methods in biosecurity, genomic standards and primers must use comprehensive genetic data and examine genotypic variation of parasites and diseases being translocated by animals in the ornamental trade.

POLICY RECOMMENDATIONS

The Department of Agriculture and Water Resources is currently undertaking a review of biosecurity practices in Australia (Hood et al. *in press*). Current priorities aim to improve on-arrival surveillance of high-risk species groups by monitoring unhealthy noncompliant fish consignments that are seized by Australian Biosecurity Inspectors at the border, random testing of fish consignments at border control (Hood et al.*in press*), and improved off-shore biosecurity requirements (Hood and Perera 2016). Indeed, the proposed syndromic surveillance for the unhealthy fish stream at Australian border control could improve monitoring of non-compliant exporting companies, however it is unclear what changes are currently being considered to improve detection at border control or to improve current surveillance measures of parasite infections in exporting countries. Future biosecurity assessments should consider:

1. Mandatory requirements for pre-export quarantine periods should include the use of clean, filtered and UV treated water to hold fish during quarantine. By holding fish in clean water, eDNA monitoring by qPCR or end-point PCR could determine the presence or absence of parasite DNA due to live shedding or the presence of parasite environmental stages. Such a requirement would allow the use of eDNA-based detection tools during pre-export quarantine periods as mandatory requirements to certify fish consignments free of infection.

2. Import permit application requirements should include stringent requirements to certify fish stocks free of infection and disease. Under the *Biosecurity Act 2015* and *Biosecurity Regulation 2016*, DAWR requires that permit applications include: 1. testing or expert review to assess the biosecurity risk associated with the goods as per

scientific advice, 2. Assessment of facilities purposed for the goods, and 3. Assessment of processes used in relation to the goods (BICON 2018). The DAWR should consider implementing regulations and requirements of aquaculture systems used to hold, grow and process ornamental fish species, with strict controls on how water is collected, cleaned and disposed from exporting facilities. The DAWR should consider the use of eDNA to monitor the presence of parasite DNA in water used to maintain fish stocks, which would improve biosecurity practices of exporting companies. Moreover, eDNA monitoring could also be a requirement for routine surveillance and assessment of companies with current import permits, complementary to off-shore certification of exporting companies following import and biosecurity requirements established by DAWR.

3. Pre-export health requirements, certification and biosecurity policies must be reviewed to prevent incursions of parasites from imported ornamental fish into domestic stocks. This thesis indicates that the health certification at exporting countries was insufficient to prevent fish parasite infections being exported to Australia. The DAWR should review requirements on chemical treatment of pre-export fish during quarantine to involve effective parasite treatment prior to export for all freshwater and marine ornamental fish. The health certificate requirements should include a description of the parasite treatment, including chemical name and manufacturer, chemical concentration, dosage rate and exposure time to chemical.

4. The Australian Government Department of Agriculture and Water Resources needs to re-assess the parasite fauna infecting ornamental fish imported to Australia with another Biosecurity Import Risk Analysis. The last BIRA for ornamental fish species

highlighted the risks associated with iridoviruses infecting imported fish to Australia and the possibility of subclinical infections going undetected during visual inspection at border control (DAWR 2014). This thesis provides ample evidence of myxozoan and monogenean parasites going undetected at border control, and highlights need to consider the emergence of myxozoan infections in southeast Asia as a possible risk to the Australian aquarium industry (Chapter 3). Most importantly, this thesis shows visual inspections at border control did not detect a high diversity of parasite species and alternative methods must be considered to detect to detect microscopic parasites during pre-export and post-import quarantine periods as well as border control.

CONCLUSION

Surveying parasite diversity of ornamental fish species imported to Australia by stringent necropsies provided a unique opportunity to elucidate current parasite diversity of imported ornamental fishes, limitations of current biosecurity protocols and the possibility of complementary eDNA screening detection methods for biosecurity. This research reports novel records of parasite species infecting wild caught marine fish and cultured freshwater species in the ornamental fish trade and provides detailed and accurate data for future biosecurity import risk analyses undertaken by the Australian Government Department of Agriculture and Water Resources. This research provides the first evaluation of eDNA screening methods for aquatic biosecurity, created five novel species-specific eDNA assays for *Dactylogyrus* species infecting ornamental goldfish imported to Australia, and validated the diagnostic sensitivity and specificity of eDNA screening methods to detect subclinical parasite infections at border control for biosecurity. The culmination of this research is a greater understanding of parasite diversity in the ornamental fish trade, and the importance of emerging parasite threats to global aquarium industries, endemic ecosystems and biosecurity.

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SUPPLEMENTARY INFORMATION

Supplementary S1

Supplementary material to:

Chapter 1: General introduction

Supplementary S1. Australia's National List of Reportable Diseases of Aquatic Animals: Finfish (DAWR 2016a).

Disease	Listed in the OIE Aquatic Animal Health Code (2015)	Listed regionally (OIE/NACA) (2015)	Exotic to Australia
1. Epizootic haematopoietic necrosis – EHN virus	Yes	Yes	-
2. European catfish virus / European sheatfish virus	-	-	Yes
3. Infectious haematopoietic necrosis	Yes	Yes	Yes
4. Spring viraemia of carp	Yes	Yes	Yes
5. Viral haemorrhagic septicaemia	Yes	Yes	Yes
6. Channel catfish virus disease	-	-	Yes
7. Viral encephalopathy and retinopathy	-	Yes	-
8. Infectious pancreatic necrosis	-	-	Yes
9. Infection with HPR-deleted or HPR0 infectious salmon anaemia virus	Yes	-	Yes
10. Infection with *Aphanomyces invadans* (epizootic ulcerative syndrome)	Yes	Yes	-
11. Bacterial kidney disease (*Renibacterium salmoninarum*)	-	-	Yes
12. Enteric septicaemia of catfish (*Edwardsiella ictaluri*)	-	Yes	-
13. Piscirickettsiosis (*Piscirickettsia salmonis*)	-	-	Yes
14. Gyrodactylosis (*Gyrodactylus salaris*)	Yes	-	Yes
15. Red sea bream iridoviral disease	Yes	Yes	Yes
16. Furunculosis (*Aeromonas salmonicida* subsp. *salmonicida*)	-	-	Yes
17. *Aeromonas salmonicida* - atypical strains	-	-	-
18. Whirling disease (*Myxobolus cerebralis*)	-	-	Yes
19. Enteric redmouth disease (*Yersinia ruckeri* – Hagerman strain)	-	-	Yes
20. Koi herpesvirus disease	Yes	Yes	Yes
21. Grouper iridoviral disease	-	Yes	Yes
22. Infectious spleen and kidney necrosis virus – like (ISKNV-like) viruses	-	-	Yes
23. Infection with salmonid alphavirus	Yes	-	Yes

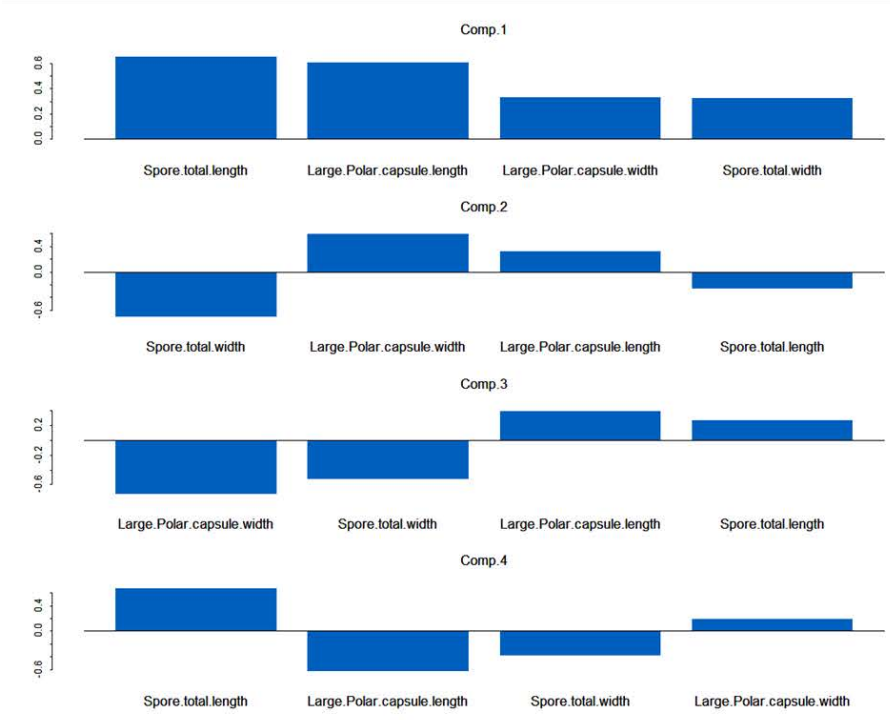
Supplementary S2

Supplementary material to:

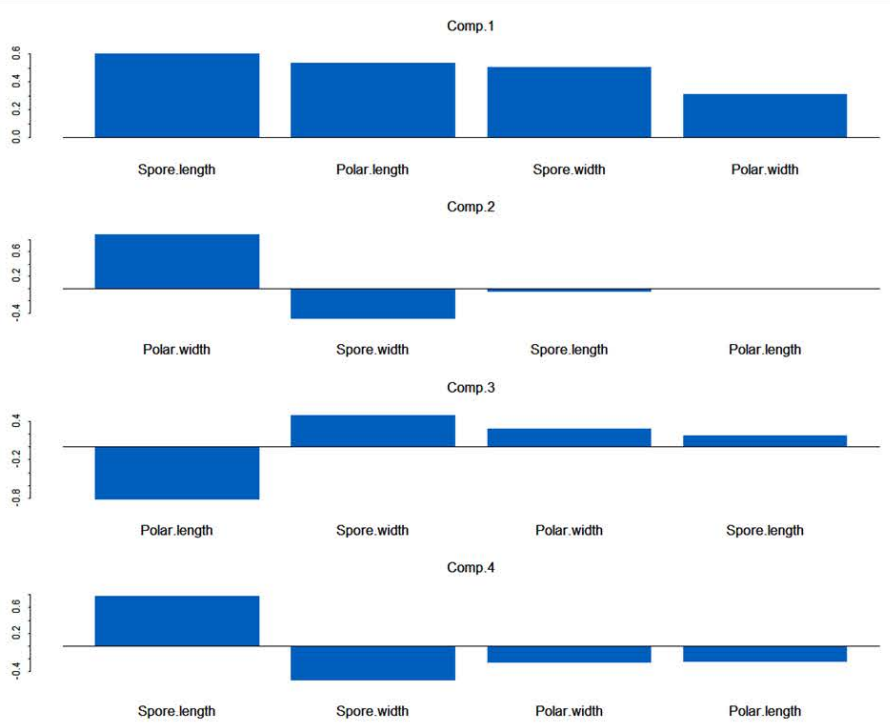
Chapter 3: Parasites in the Australian ornamental fish trade

Supplementary S2. *Myxobolus* and *Ceratomyxa* spp. Principal component analysis loading values.

Myxobolus sp. PCA loading values



Ceratomyxa sp. PCA loading values



Supplementary S3

Supplementary material to:

Chapter 4: Parasite dispersal in the goldfish trade

Trujillo-González A., Becker J. A., and Hutson K. S.

Supplementary S3. Parasite species records infecting *Carassius auratus*. Parasite species have been catalogued by phylum, class, and family.

Phylum	Parasite group	Parasite	Environment	Locality	Reference
Acanthocephala	Palaeacanthocephala: Echinorhynchidae	*Acanthocephalus anguillae* (Müller, 1780)	Invasive, aquarium held	Germany	Taraschewski 1989
		Acanthocephalus dirus (Van Cleave, 1931)	Invasive	France	Golvan and De Buron 1988
	Palaeacanthocephala: Illiosentidae	*Brentisentis cyprini* Yin and Wu, 1984	Native	China	Yi and Huisheng 1989
	Palaeacanthocephala: Pomphorhynchidae	*Pomphorhynchus bulbocolli* Linkins and Van Cleave, 1919	Invasive	France	Golvan and De Buron 1988
		Pomphorhynchus laevis Müller, 1776	Invasive	Germany	Sures et al. 1997
		Pomphorhynchus laevis	Invasive	Turkey	Koyun 2001
		Pomphorhynchus laevis	Aquarium held	England	Sures and Sidall 2001
		Pomphorhynchus sp.	Invasive	Canada	Arai 1989
	Eoacanthocephala: Quadrigyridae	*Acanthogyrus pseudoholospinus* Wang, 1963	Native	China	Chen 1973

		Pallisentis ussuriense (Kostylew, 1941) (syn. *Acanthocephalorhynchoides ussuriensis* Kostylew, 1941)	Native	China	Chen 1973
Annelida	Hirudinea: Glossiphoniidae	*Hemiclepsis marginata* (Müller, 1774)	Invasive	England	Robertson 1912
	Hirudinea: Piscicolidae	*Piscicola geometra* (Linnaeus, 1761)	Invasive	Latvia	Kirjušina and Vismanis 2007
		Piscicola geometra	Invasive	Serbia	Cakic and Hristic 1987
Amoebozoa	Discosea: Vexilliferidae	*Vexillifera expectata* Dyková, Lom, Macháckova and Pecková, 1998	Invasive	Czech Republic	Dyková et al. 1998
Arthropoda	Crustacea: Argulidae	*Argulus coregoni* Thorell, 1864	Farmed	Iran	Mousavi et al. 2011
		Argulus foliaceus (Linnaeus, 1758)	Invasive	Italy	Macchioni et al. 2015
		Argulus foliaceus	Invasive	India	Kalita et al. 2010
		Argulus foliaceus	Invasive	Latvia	Kirjušina and Vismanis 2007
		Argulus foliaceus	Imported Aquarium held	Pakistan	Iqbal and Noreen 2014
		Argulus foliaceus	Farmed	Iran	Noaman et al. 2010
		Argulus foliaceus	Farmed	Turkey	Koyuncu 2009
		Argulus foliaceus	Export Farmed	Sri Lanka	Thilakaratne et al. 2003
		Argulus foliaceus	Aquarium held	Iran	Mirzaei and Khovand 2013
		Argulus foliaceus	Aquarium held	Turkey	Yildiz and Kumantas 2002
		Argulus japonicus Thiele, 1900	Invasive	Australia	Heegaard 1962
		Argulus japonicus	Native	Japan	Tokioka 1936
		Argulus japonicus	Aquarium held	New Zealand	Pilgrim 1967
		Argulus japonicus	Aquarium held	New Zealand	Hewitt and Hine 1972
		Argulus japonicus	Aquarium held	New Zealand	Hine et al. 2000

	Argulus japonicus	Aquarium held	Puerto Rico	Bunkley-Williams and Williams 1994
	Argulus japonicus	Farmed Invasive	China	Alsarakibi et al. 2014
	Argulus japonicus	Farmed Aquarium held	USA	Wafer et al. 2015
	Argulus japonicus	Farmed	Iran	Mousavi et al. 2011
	Argulus japonicus	Farmed	Turkey	Koyuncu 2009
	Argulus sp.	Farmed	India	Chanda et al. 2011
Crustacea: Cymothoidae	*Ichthyoxenus japonensis* Richardson, 1913	Native	China	Xu et al. 2007
Crustacea: Ergasilidae	*Abergasilus amplexus* Hewitt, 1978	Invasive	New Zealand	Hine et al. 2000
	Ergasilus ceylonensis Fernando and Hanek, 1973	Export farmed	Sri Lanka	Thilakaratne et al. 2003
	Neoergasilus japonicus (Harada, 1930) (syn. *Ergasilus japonicus* Harada, 1930)	Invasive	USA	Hudson and Bowen 2002
	Lernaea cyprinacea Linnaeus, 1758	Invasive	USA	Kuperman et al. 2002
	Lernaea cyprinacea	Invasive	Egypt	Mahmoud et al. 2009
	Lernaea cyprinacea	Invasive	Italy	Macchioni et al. 2015
	Lernaea cyprinacea	Invasive	Australia	Hassan et al. 2008
	Lernaea cyprinacea	Invasive	Uruguay	Carnevia and Speranza 2003
	Lernaea cyprinacea	Invasive	India	Kalita et al. 2010
	Lernaea cyprinacea	Invasive	Iran	Sayyadzadeh et al. 2016
	Lernaea cyprinacea	Invasive	Iran	Raissy et al. 2013
	Lernaea cyprinacea	Native	Japan	Yoshimine et al. 2015
	Lernaea cyprinacea	Invasive	New Zealand	Hine et al. 2000
	Lernaea cyprinacea	Invasive	Vietnam	Arthur and Te 2006
	Lernaea cyprinacea	Farmed	Iran	Adel et al. 2015
	Lernaea cyprinacea	Farmed	Turkey	Koyuncu 2009

		Lernaea cyprinacea	Export farmed	Sri Lanka	Thilakaratne et al. 2003
		Lernaea cyprinacea	Imported Aquarium held	Pakistan	Iqbal and Haroon 2014
		Lernaea cyprinacea	Aquarium held	Philippines	Arthur and Lumanlan-Mayo 1997
		Lernaea cyprinacea	Aquarium held	Puerto Rico	Bunkley-Williams and Williams 1994
		Lernaea sp.	Farmed	USA	Elliott and Shotts 1980
Choanozoa	Ichthyosporea: Dermocystidae	*Dermocystidium* sp.	Invasive	Egypt	Mahmoud et al. 2009
Ciliophora	Litostomatea: Balantiididae	*Balantidium* sp.	Invasive	Serbia	Andric 1984
	Phyllopharyngea: Chilodonellidae	*Chilodonella piscicola* (Zacharias, 1894) (syn. *C. cyprini* Moroff, 1902, *C. hexasticha* Kiernik, 1909)1	Invasive	Latvia	Kirjušina and Vismanis 2007
		Chilodonella piscicola	Native	China	Hu 2012
		Chilodonella piscicola	Imported Aquarium held	Turkey	Kayis et al. 2013
		Chilodonella sp.	Imported Farmed	England	Elliott and Shotts 1980
		Chilodonella sp.	Farmed	Turkey	Koyuncu 2009
		Chilodonella sp.	Farmed	USA	Elliott and Shotts 1980
	Oligohymenophorea: Epistylididae	*Apiosoma piscicola (*Blanchard, 1885) (*syn. Glossatella cylindriformis* Chen 1955*, Apiosoma magna* Banina 1968)	Invasive	Italy	Macchioni et al. 2015
		Apiosoma piscicola	Farmed	China	Li et al. 2008
		Apiosoma sp.	Farmed	Brazil	Moyses et al. 2015
		Apiosoma sp.	Aquarium held	Philippines	Arthur and Lumanlan-Mayo 1997
		Apiosoma sp.	Invasive	Latvia	Kirjušina and Vismanis 2007
		Epistylis sp.	Imported Aquarium held	Turkey	Kayis et al. 2013
		Epistylis sp.	Farmed	Brazil	Moyses et al. 2015

Oligohymenophorea: Ichthyophthiriidae	*Ichthyophthirius multifiliis* Fouquet, 1876	Invasive	Egypt	Mahmoud et al. 2009
	Ichthyophthirius multifiliis	Invasive	India	Kalita et al. 2010
	Ichthyophthirius multifiliis	Invasive	Latvia	Kirjušina and Vismanis 2007
	Ichthyophthirius multifiliis	Imported Farmed	Japan	Elliott and Shotts 1980
	Ichthyophthirius multifiliis	Imported Aquarium held	Pakistan	Iqbal and Noreen 2014
	Ichthyophthirius multifiliis	Imported Aquarium held	Pakistan	Iqbal and Haroon 2014
	Ichthyophthirius multifiliis	Imported Aquarium held	Turkey	Kayis et al. 2013
	Ichthyophthirius multifiliis	Farmed	Brazil	Moyses et al. 2015
	Ichthyophthirius multifiliis	Farmed	Iran	Roohi et al. 2016
	Ichthyophthirius multifiliis	Farmed	Iran	Mousavi et al. 2011
	Ichthyophthirius multifiliis	Farmed	Iran	Adel et al. 2015
	Ichthyophthirius multifiliis	Farmed	Turkey	Koyuncu 2009
	Ichthyophthirius multifiliis	Farmed	USA	Elliott and Shotts 1980
	Ichthyophthirius multifiliis	Imported	Australia	Butcher 1947
	Ichthyophthirius multifiliis	Aquarium held	Brazil	Piazza et al. 2006
	Ichthyophthirius multifiliis	Aquarium held	Croatia	Gjurčević et al. 2007
	Ichthyophthirius multifiliis	Aquarium held	Philippines	Arthur and Lumanlan-Mayo 1997
	Ichthyophthirius sp.	Farmed	India	Chanda et al. 2011
Oligohymenophorea: Tetrahymenidae	*Tetrahymena pyriformis* (Ehremberg, 1830)	Export farmed	Sri Lanka	Thilakaratne et al. 2003
	Tetrahymena sp.	Imported Aquarium held	Pakistan	Iqbal and Noreen 2014
	Tetrahymena sp.	Farmed	Brazil	Moyses et al. 2015
Oligohymenophorea: Trichodinidae	*Trichodina acuta* Lom, 1961	Invasive	England	Gaze and Wootten 1998
	Trichodina acuta	Aquarium held	Brazil	Piazza et al. 2006
	Trichodina borokensis Arthur and Lom 1984	Native	China	Tang and Zhao 2011

Trichodina centrostrigata Basson, Van as and Paperna, 1983	Native	China	Tang et al. 2005b
Trichodina domerguei Wallengren, 1897	Invasive	Latvia	Kirjušina and Vismanis 2007
Trichodina luzhoues Hu, 2012	Native	China	Hu 2012
Trichodina mutabilis Kazubski and Migala, 1968	Native	China	Hu 2012
Trichodina nigra Lom, 1961	Export farmed	Sri Lanka	Thilakaratne et al. 2003
Trichodina nigra	Invasive	Taiwan	Basson and Van As 1994
Trichodina nigra	Invasive	Latvia	Kirjušina and Vismanis 2007
Trichodina nobilis Chen, 1963	Farmed	Brazil	Martins et al. 2012
Trichodina pachyhamata Tang and Zhao, 2005	Native	China	Tang et al. 2005b
Trichodina paranigra Tang, Zhao and Chen, 2005	Native	China	Tang et al. 2005a
Trichodina pediculus Ehrenberg, 1838	Invasive	Vietnam	Arthur and Te 2006
Trichodina pediculus	Invasive	Latvia	Kirjušina and Vismanis 2007
Trichodina reticulata (Hirschmann and Partsch, 1955) (syn. *Trichodina domerguei f. megamicronucleus* Dogiel, 1940, *T. megamicronucleata* Dogiel, 1950)	Native	China	Hu 2012
Trichodina reticulata	Invasive	Egypt	Mahmoud et al. 2009
Trichodina reticulata	Invasive	Latvia	Kirjušina and Vismanis 2007
Trichodina reticulata	Invasive	South Africa	Basson and Van As 1993
Trichodina reticulata	Farmed	Brazil	Martins et al. 2012

		Trichodina reticulata	Farmed	Iran	Adel et al. 2015
		Trichodina reticulata	Farmed	Japan	Ahmed 1977
		Trichodina reticulata	Aquarium held	Philippines	Arthur and Lumanlan-Mayo 1997
		Trichodina reticulata	Aquarium held	Puerto Rico	Bunkley-Williams and Williams 1994
		Trichodina sp.	Invasive	Italy	Macchioni et al. 2015
		Trichodina sp.	Invasive	Serbia	Cakic and Hristic 1987
		Trichodina sp.	Imported farmed	Japan	Elliott and Shotts 1980
		Trichodina sp.	Imported Aquarium held	Pakistan	Iqbal and Noreen 2014
		Trichodina sp.	Imported Aquarium held	Pakistan	Iqbal and Haroon 2014
		Trichodina sp.	Imported Aquarium held	Turkey	Kayis et al. 2013
		Trichodina sp.	Farmed	Brazil	Moyses et al. 2015
		Trichodina sp.	Farmed	Iran	Roohi et al. 2016
		Trichodina sp.	Farmed	Iran	Mousavi et al. 2011
		Trichodina sp.	Farmed	Turkey	Koyuncu 2009
		Trichodina sp.	Farmed	USA	Elliott and Shotts 1980
		Trichodina sp.	Aquarium held	Croatia	Gjurčević et al. 2007
		Trichodina sp.	Aquarium held	Philippines	Arthur and Lumanlan-Mayo 1997
		Trichodinella epizootica (Raabe, 1950)	Aquarium held	Philippines	Arthur and Lumanlan-Mayo 1997
		Trichodinella carpi Duncan, 1977	Native	China	Tang et al. 2005b
		Trichodinella sp.	Invasive	Italy	Macchioni et al. 2015
	Oligohymenophorea: Vorticellidae	*Vorticella* sp.	Farmed	Brazil	Moyses et al. 2015
Cnidaria	Myxozoa: Chloromyxidae	*Chloromyxum auratum* Hallett, Atkinson, Holt, Banner and Bartholomew, 2006	Invasive	USA	Hallett et al. 2006
		Chloromyxum auratum	Invasive	USA	Atkinson et al. 2007

Myxozoa: Myxobolidae	*Myxobolus acinosus* Nie and Li, 1973	Native	China	Chen and Ma 1998
	Myxobolus acinosus Nie and Li, 1973	Native	China	Eiras et al. 2005
	Myxobolus aisanensis Chen in Chen and Ma, 1998[5]	Native	Off China	Eiras et al. 2005
	Myxobolus anomaliformis Chen in Chen and Ma, 1998[5]	Native	China	Eiras et al. 2005
	Myxobolus auratus nom. nov. for *Myxobolus orbiculatus* Chen in Chen and Ma, 1998[5]	Native	China	Eiras et al. 2005
	Myxobolus beibeiensis Zhang, 2001	Native	China	Zhang 2001
	Myxobolus bilis Akhmerov, 1960	Invasive	Russia	Akhmerov 1960; in Landsberg and Lom 1991
	Myxobolus bladderia Chen and Ma, 1998[5]	Native	China	Eiras et al. 2005
	Myxobolus cantonensis Chen in Chen and Ma, 1998[5]	Native	China	Eiras et al. 2005
	Myxobolus changkiangensis Chen in Chen and Ma, 1998[5]	Native	China	Eiras et al. 2005
	Myxobolus changshingensis Chen in Chen and Ma, 1998[5]	Native	China	Eiras et al. 2005
	Myxobolus chuchowensis Chen in Chen and Ma, 1998[5]	Native	China	Eiras et al. 2005
	Myxobolus cultus Yokoyama, Ogawa and Wakabayashi, 1995	Native	Japan	Yokoyama et al. 1995
	Myxobolus cultus	Native	Japan	Eiras et al. 2005
	Myxobolus diversus Nie and Li, 1973	Farmed	Hungary	Molnar and Szekely 2003

Myxobolus diversus	Native	China	Chen 1973; In Landsberg and Lom 1991
Myxobolus diversus	Native	China	Eiras et al. 2005
Myxobolus echengensis Chen in Chen and Ma, 1998[5]	Native	China	Eiras et al. 2005
Myxobolus egregius Nie and Li, 1973	Native	China	Chen 1973; In Landsberg and Lom 1991
Myxobolus egregius	Native	China	Chen and Ma 1998
Myxobolus hearti Chen in Chen and Ma, 1998[5]	Native	China	Eiras et al. 2005
Myxobolus hokiangensis Ma, 1998[5]	Native	China	Eiras et al. 2005
Myxobolus huananensis Chen in Chen and Ma, 1998[5]	Native	China	Eiras et al. 2005
Myxobolus huchowensis Chen in Chen and Ma, 1998[5]	Native	China	Eiras et al. 2005
Myxobolus hypseleotris Chen in Chen and Ma, 1998[5]	Native	Off China	Eiras et al. 2005
Myxobolus ichkeulensis Bahri and Marques, 1996	Farmed	India	Saha and Bandyopadhyay 2017
Myxobolus inflatus Chen in Chen and Ma, 1998[5]	Native	Off China	Eiras et al. 2005
Myxobolus kingchowensis Ma and Chen, 1998[5]	Native	China	Eiras et al. 2005
Myxobolus lentisuturalis Dyková, Fiala and Nie, 2002	Farmed	Italy	Caffara et al. 2009
Myxobolus liaoningensis Chen in Chen and Ma, 1998[5]	Native	China	Eiras et al. 2005
Myxobolus lieni (Nie and Li, 1973)	Native	China	Eiras et al. 2005

Myxobolus lokiaensis Chen in Chen and Ma, 1998[5]	Native	China	Eiras et al. 2005
Myxobolus microlatus Li and Nie, 1973	Native	China	Eiras et al. 2005
Myxobolus microsporus Li and Nie, 1973	Native	China	Chen and Ma 1998
Myxobolus microsporus	Native	China	Eiras et al. 2005
Myxobolus nanyangensis nom. nov. for *Myxosoma carassii* Hu, 1965	Native	China	Eiras et al. 2005
Myxobolus nanyuensis Chen in Chen and Ma, 1998[5]	Native	China	Eiras et al. 2005
Myxobolus nielii (Nie and Li, 1973)	Native	China	Eiras et al. 2005
Myxobolus paratoyamai Nie and Li, 1992	Native	China	Eiras et al. 2005
Myxobolus pavlovskii (Akhmerov, 1954)	Aquarium held	Philippines	Arthur and Lumanlan-Mayo 1997
Myxobolus pekingensis Chen in Chen and Ma, 1998[5]	Native	China	Eiras et al. 2005
Myxobolus pseudosquarae Chen in Chen and Ma, 1998[5]	Native	China	Eiras et al. 2005
Myxobolus pyramidis Chen in Chen and Ma, 1998[5]	Native	China	Eiras et al. 2005
Myxobolus qiankiangensis nom. nov. for *Myxosoma chungnanensis* Chen in Chen and Ma, 1998[5]	Native	China	Eiras et al. 2005
Myxobolus rutilus Nie and Li, 1973	Native	China	Eiras et al. 2005
Myxobolus shantungensis Hu, 1965	Native	China	Eiras et al. 2005
Myxobolus tuberculus Nie and Li, 1992	Native	China	Eiras et al. 2005
Myxobolus tunghuensis Chen in Chen and Ma, 1998[5]	Native	China	Eiras et al. 2005

Myxobolus turpisrotundus Zhang, Wang, Gong 2010	Farmed	China	Zhang et al. 2010
Myxobolus toyamai Kudo, 1917	Native	Japan	Landsberg and Lom 1991[4]
Myxobolus urinarybladderi nom. nov. for *Myxosoma tunghuensis* Chen in Chen and Ma, 1998[5]	Native	China	Eiras et al. 2005
Myxobolus velatus Li and Nie, 1973	Native	China	Chen 1973; In Landsberg and Lom 1991
Myxobolus wasjugani Bocharova and Donec, 1974	Invasive	Russia	Eiras et al. 2005
Myxobolus wuhanensis Chen in Chen and Ma, 1998[5]	Native	China	Eiras et al. 2005
Myxobolus wulii (Wu and Li, 1986)	Native	China	Eiras et al. 2005
Myxobolus wushingensis Chen in Chen and Ma, 1998[5]	Native	China	Eiras et al. 2005
Myxobolus sp.	Imported, farmed	Japan	Elliott and Shotts 1980
Myxobolus sp.	Aquarium held	Croatia	Gjurčević et al. 2007
Myxobolus sp.	Invasive	Vietnam	Arthur and Te 2006
Myxobolus sp.	Aquarium held	Philippines	Arthur and Lumanlan-Mayo 1997
Thelohanellus dipaki Saha and Bandyopadhyay 2017	Farmed	India	Saha and Bandyopadhyay 2017
Thelohanellus hoffmanni Lewisch, Soliman, Schmidt and El-Matbouli, 2015	Imported, Aquarium held	Austria	Lewisch et al. 2015
Thelohanellus hupehensis Nie and Li, 1992	native	China	Chen and Ma 1998; in Zhang et al. 2013
Thelohanellus liaohoensis Chen in Chen and Ma 1998[5]	native	China	Chen and Ma 1998; in Zhang et al. 2013

		Thelohanellus nanhaiensis Chen in Chen and Ma 1998[5]	native	China	Chen and Ma 1998; in Zhang et al. 2013
		Thelohanellus parasagittarius Chen and Ma 1998[5]	native	China	Chen and Ma 1998; in Zhang et al. 2013
		Thelohanellus relortus Chen in Chen and Ma 1998[5]	native	China	Chen and Ma 1998; in Zhang et al. 2013
	Myxozoa: Sphaerosporidae	*Sphaerospora angulata* Fujita, 1912	Invasive	USA	Holzer et al. 2013
		Sphaerospora angulata	Farmed	Czech Republic	Holzer et al. 2013
		Sphaerospora sp.	Farmed	Hungary	Eszterbauer and Székely 2004
Euglenozoa	Kinetoplastea	*Ichthyobodo necator* Henneguy, 1883 (syn. *Costia necatrix* Moroff, 1904, *Ichthyobodo necatrix* Henneguy, 1883)	Farmed	Brazil	Moyses et al. 2015
		Ichthyobodo necator	Farmed	USA	Elliott and Shotts 1980
		Ichthyobodo sp.	Farmed	Turkey	Koyuncu 2009
		Ichthyobodo sp.	Aquarium held	Philippines	Arthur and Lumanlan-Mayo 1997
	Kinetoplastea: Cryptobiidae	*Cryptobia* sp.	Aquarium held	Philippines	Arthur and Lumanlan-Mayo 1997
		Cryptobia sp.	Unspecified	Australia	Langdon 1990
		Trypanoplasma borelli Laveran and Mesnil, 1901	Aquarium held	Czech Republic	Dyková and Lom 1979
		Trypanoplasma cyprini Plehn, 1903	Invasive	England	Robertson 1912
		Trypanoplasma sp.	Aquarium held	Croatia	Gjurčević et al. 2007
		Trypanoplasma sp.	Unspecified	Australia	Langdon 1990
	Euglenozoa: Trypanosomatidae	*Trypanosoma danilewskyi* Laveran and Mesnil, 1904	Aquarium held	Czech Republic	Dyková and Lom 1979
Metamonada	Trichozoa: Hexamitidae	*Hexamita* sp.	Imported, Aquarium held	Turkey	Kayis et al. 2013
		Hexamita sp.	Farmed	USA	Elliott and Shotts 1980

Myzozoa	Conoidasida: Eimeriidae	*Goussia carpelli* (Leger and Stankovitch, 1921)	Farmed Aquarium held	USA	Kent and Hedrick 1985
		Goussia carpelli	Aquarium held	Philippines	Arthur and Lumanlan-Mayo 1997
	Dinoflagellata	*Piscinoodinium* spp.	Farmed	Brazil	Moyses et al. 2015
		Piscinoodinium spp.	Export farmed	Sri Lanka	Thilakaratne et al. 2003
Nematoda	Chromadorea: Anguillicolidae	*Anguillicoloides crassus (Kuwahara, Niimi and Itagaki, 1974) (syn. Anguillicola crassus Kuwahara, Niimi and Hagaki, 1974)*	Aquarium held	Hungary	Szekely 1996
	Chromadorea: Anisakidae	*Contracaecum* sp.	Native	China	Chen 1973
	Chromadorea: Camallanidae	*Procamallanus* sp.	Farmed	India	Chanda et al. 2011
	Chromadorea: Crenosomatidae	*Otostrongylus circumlitus* Railliet, 1899	Aquarium held	Canada	Bergeron et al. 1997
	Chromadorea: Philometridae	*Philometra carassii* (Ishii, 1934) (syn. *Filaria carassii* Ishii, 1931)	Native	China	Chen 1973
		Philometroides cyprini (Ishii, 1931) (syn. *Philometra lusiana* Vismanis, 1966)	Invasive	Serbia	Cakic et al. 2001
		Philometroides sanguinea (Rudolphi, 1819)	Invasive	Czech Republic	Gelnar et al. 1994
		Philometroides sanguinea	Invasive	Europe	Moravec 1995
		Philonema oncorhynchi Kuitunen-Ekbaum, 1933	Invasive	Canada	McDonald and Margolis 1995
	Chromadorea: Spiruridae	*Agamospirura* sp.	Native	China	Chen 1973
	Dorylaimea: Capillariidae	*Capillaria* sp.	Farmed	Turkey	Koyuncu 2009
		Capillaridae	Invasive	Italy	Macchioni et al. 2015
	Dorylaimea: Trichuridae	*Pseudocapillaria tomentosa* (Dujardin, 1843)	Invasive	Czech Republic	Gelnar et al. 1994
		Pseudocapillaria tomentosa	Invasive	Unspecified	Moravec 2001

		Schulmanella petruschewskii Shulman, 1948	Invasive	Czech Republic	Gelnar et al. 1994
		Schulmanella petruschewskii	Invasive	Europe	Moravec 2001
	Secernentea: Acuariidae	*Cosmocephalus obvelatus* Creplin, 1825	Invasive	Canada	McDonald and Margolis 1995
		Syncuaria squamata (Linstow, 1883)	Invasive	Canada	McDonald and Margolis 1995
		Paracuaria adunca (Creplin, 1846)	Invasive	Canada	McDonald and Margolis 1995
	Secernentea: Cucullanidae	*Cucullanus* sp.	Native	India	Chanda et al. 2011
	Secernentea: Gnathostomatidae	*Gnathostoma hispidum* Fedtschenko, 1872	Native	China	Chen and Lin 1991
Plathyhelminthes	Cestoda: Bothriocephalidae	*Schyzocotyle acheilognathi (Yamaguti, 1934) Brabec, Waeschenbach, Scholz, Littlewood and Kuchta, 2015 (syn. Bothriocephalus acheilognathi Yamaguti, 1934, Bothriocephalus opsariichthydis Yamaguti, 1934, Bothriocephalus gowkongensis Yeh, 1955)*	Invasive	Australia	Dove and Fletcher 2000
		Schyzocotyle acheilognathi	Unspecified	Australia	Langdon 1990
		Schyzocotyle acheilognathi	Invasive	Czech Republic	Scholz 1989
		Schyzocotyle acheilognathi	Invasive	Mexico	Salgado-Maldonado and Pineda-Lopez 2003
		Schyzocotyle acheilognathi	Invasive	Mexico	Prieto and Sarabia 1991
		Schyzocotyle acheilognathi	Invasive	Slovakia	Macko et al. 1993
		Schyzocotyle acheilognathi	Invasive	USA	Kuperman et al. 2002
		Schyzocotyle sp.	Invasive	Italy	Macchioni et al. 2015
		Senga sp.	Native	China	Smith 1997

Cestoda: Caryophyllaeidae	*Archigetes sieboldi* Leuckart, 1878	Invasive	Spain	Cordero Del Campillo et al. 1980
	Caryophyllaeus brachycollis Janiszewska, 1953	Native	China	Liu and Wang 1997
	Caryophyllaeus laticeps (Pallas, 1781)	Invasive	Czech Republic	Gelnar et al. 1994
	Khawia japonensis (Yamaguti, 1934)	Native	China	Chen 1973
	Khawia parva (Zmeev, 1936) (syn. *Caryophyllaeus parva* Zmeev, 1936)	Invasive	Slovakia	Oros and Hanzelova 2007
	Khawia sinensis Hsü, 1935	Invasive	Czech Republic	Scholz 1991
	Khawia sinensis	Invasive	Russia	Izyumova 1973
Cestoda: Dilepididae	*Dilepis unilateralis* Rudolphi, 1819	Invasive	Norway	Sterud 1999
	Gryporhynchus sp.	Native	China	Chen 1973
Cestoda: Diphyllobothriidae	*Digramma alternans* (Rudolphi, 1810) (syn. *Ligula alternans* Rudolphi, 1810, *Ligula interrupta* Rudolphi, 1810)	Native	Japan	Nagasawa 1989
	Digramma alternans	Invasive	Japan	Nagasawa et al. 1989
	Digramma sp.	Native	China	Luo et al. 2003
	Ligula intestinalis Linnaeus, 1758	Invasive	Russia	Izyumova 1973
	Ligula intestinalis	Invasive	Spain	Cordero Del Campillo et al. 1980.
Monogenea	Unidentified monogenean	Invasive	Australia	Fletcher and Whittington 1998
	Unidentified monogenean	Invasive	Austria	Gelnar et al. 2001
	Unidentified monogeneans	Aquarium held	Brazil	Piazza et al. 2006
Monogenea: Dactylogyridae	Dactylogyridae	Farmed	Brazil	Moyses et al. 2015
	Dactylogyridae gen. sp.	Invasive	Vietnam	Arthur and Te 2006
	Dactylogyrus anchoratus (Dujardin, 1845)	Invasive	USA	Mueller 1936

Dactylogyrus anchoratus	Native	China	Li and Zhang 1992[3]
Dactylogyrus anchoratus	Native	China	Ji et al. 1982[3]
Dactylogyrus anchoratus	Native	China	Chen 1973
Dactylogyrus anchoratus	Invasive	Czech Republic	Gelnar et al. 1994
Dactylogyrus anchoratus	Invasive	Latvia	Kirjušina and Vismanis 2007
Dactylogyrus anchoratus	Invasive	Puerto Rico	Bunkley-Williams and Williams 1994
Dactylogyrus anchoratus	Invasive	Russia	Izyumova 1973
Dactylogyrus anchoratus	Invasive	Russia	Izyumova 1987
Dactylogyrus anchoratus	Invasive	Turkey	Koyun 2001
Dactylogyrus anchoratus	Invasive	Turkey	Öztürk 2011
Dactylogyrus anchoratus	Invasive	Former Yugoslavia	Kiskaroly 1988
Dactylogyrus anchoratus	Farmed	Iran	Roohi et al. 2016
Dactylogyrus anchoratus	Imported Aquarium held	Italy	Di Cave et al. 2000
Dactylogyrus anchoratus	Imported Aquarium held	Norway	Levsen 1994
Dactylogyrus anchoratus	Farmed	Japan	Ogawa and Egusa 1979
Dactylogyrus anchoratus	Aquarium held	Czech Republic	Řehulková and Řehulka 1999
Dactylogyrus arcuatus Yamaguti, 1942	Native	China	Li and Zhang 1992[3]
Dactylogyrus arcuatus	Native	China	Chang and Ji 1978[3]
Dactylogyrus baueri Gussev, 1955	Native	China	Zhao and Ma 1995
Dactylogyrus baueri	Native	China	Wu et al. 1991[3]
Dactylogyrus baueri	Native	China	Wu et al. 2000
Dactylogyrus baueri	Native	China	Chen 1973
Dactylogyrus baueri	Native	China	Chang and Ji 1978[3]
Dactylogyrus baueri	Imported Aquarium held	Bulgaria	Borisov 2013
Dactylogyrus baueri	Imported Aquarium held	Italy	Di Cave et al. 2000

Dactylogyrus baueri	Imported Aquarium held	Iran	Mousavi et al. 2009
Dactylogyrus baueri	Farmed	Iran	Roohi et al. 2016
Dactylogyrus baueri	Farmed	Iran	Jalili and Molnar 1990
Dactylogyrus baueri	Farmed	Iran	Jalili and Molnar 1990
Dactylogyrus baueri	Farmed	Japan	Ogawa and Egusa 1979
Dactylogyrus crassus Kulviec, 1927	Invasive	Former Former Yugoslavia	Kiskaroly 1988
Dactylogyrus crucifer Wagener, 1857	Invasive	Czech Republic	Gelnar et al. 1994
Dactylogyrus dogieli Gussev, 1953	Native	China	Ji et al. 1982[3]
Dactylogyrus dulkeiti Bychowsky, 1936	Native	China	Ji et al. 1982[3]
Dactylogyrus dulkeiti	Native	China	Chen 1973
Dactylogyrus dulkeiti	Invasive	Russia	Lukyanzeva 1990[3]
Dactylogyrus dulkeiti	Invasive	Former Yugoslavia	Kiskaroly 1988
Dactylogyrus dulkeiti	Imported Aquarium held	Norway	Levsen 1995
Dactylogyrus dulkeiti	Farmed	Iran	Jalili and Molnar 1990
Dactylogyrus dulkeiti	Farmed	Japan	Ogawa and Egusa 1979
Dactylogyrus extensus Mueller and Van Cleave, 1932	Invasive	Australia	Dove and Ernst 1998
Dactylogyrus extensus	Invasive	Former Yugoslavia	Kiskaroly 1988
Dactylogyrus extensus	Imported Aquarium held	Pakistan	Iqbal and Haroon 2014
Dactylogyrus extensus	Farmed	Turkey	Koyuncu 2009
Dactylogyrus extensus	Export farmed	Sri Lanka	Thilakaratne et al. 2003
Dactylogyrus formosus Kulwiec, 1927	Native	China	Tu et al. 2015
Dactylogyrus formosus	Native	China	Ji et al. 1982[3]
Dactylogyrus formosus	Native	China	Chen 1973
Dactylogyrus formosus	Native	China	Li and Zhang 1992[3]

Dactylogyrus formosus	Invasive	Czech Republic	Gelnar et al. 1994
Dactylogyrus formosus	Invasive	Russia	Lukyanzeva 1990[3]
Dactylogyrus formosus	Invasive	Former Yugoslavia	Kiskaroly 1988
Dactylogyrus formosus	Invasive	Czech Republic	Lucky and Pidverbecka 1970
Dactylogyrus formosus	Imported Aquarium held	italy	Di Cave et al. 2000
Dactylogyrus formosus	Imported Aquarium held	Iran	Mousavi et al. 2009
Dactylogyrus formosus	Farmed	Iran	Roohi et al. 2016
Dactylogyrus formosus	Farmed	Iran	Jalili and Molnar 1990
Dactylogyrus formosus	Farmed	Japan	Ogawa and Egusa 1979
Dactylogyrus formosus	Aquarium held	Czech Republic	Řehulková and Řehulka 1999
Dactylogyrus inexpectatus Isjumova and Gussev, 1955	Native	China	Ji et al. 1982[3]
Dactylogyrus inexpectatus	Native	China	Chen 1973
Dactylogyrus inexpectatus	Native	China	Wu et al. 2000
Dactylogyrus inexpectatus	Native	China	Wu et al. 1991[3]
Dactylogyrus inexpectatus	Imported Aquarium held	Italy	Di Cave et al. 2000
Dactylogyrus intermedius Wegener, 1909	Invasive	Iran	Gussev et al. 1993
Dactylogyrus intermedius	Native	China	Ji et al. 1982[3]
Dactylogyrus intermedius	Native	China	Wu et al. 1991[3]
Dactylogyrus intermedius	Native	China	Chen 1973
Dactylogyrus intermedius	Native	China	Chang and Ji 1978[3]
Dactylogyrus intermedius	Invasive	Iran	Molnar and Jalali 1992
Dactylogyrus intermedius	Invasive	Vietnam	Arthur and Te 2006
Dactylogyrus intermedius	Imported Aquarium held	Bulgaria	Borisov 2013
Dactylogyrus intermedius	Imported Aquarium held	Italy	Di Cave et al. 2000
Dactylogyrus intermedius	Farmed	China	Wang et al. 2011

Dactylogyrus intermedius	Farmed	Iran	Roohi et al. 2016
Dactylogyrus intermedius	Farmed	Myanmar	Shinn and Tun 2013
Dactylogyrus intermedius	Farmed	Japan	Shinn and Tun 2013
Dactylogyrus intermedius	Farmed	Japan	Ogawa and Egusa 1979
Dactylogyrus magnihamatus (Akhmerov,1952)	Native	China	Chen 1973
Dactylogyrus spiralis Yamaguti, 1942	Native	China	Wu et al. 2000
Dactylogyrus vastator Nybelin, 1924	Invasive	Italy	Macchioni et al. 2015
Dactylogyrus vastator	Invasive	Czech Republic	Gelnar et al. 1994
Dactylogyrus vastator	Invasive	Russia	Lukyanzeva 1990[3]
Dactylogyrus vastator	Invasive	Russia	Izyumova 1987
Dactylogyrus vastator	Invasive	Former Yugoslavia	Kiskaroly 1988
Dactylogyrus vastator	Imported Aquarium held	Iran	Mousavi et al. 2009
Dactylogyrus vastator	Imported Aquarium held	Bulgaria	Borisov 2013
Dactylogyrus vastator	Imported Aquarium held	Italy	Di Cave et al. 2000
Dactylogyrus vastator	Imported Aquarium held	Norway	Levsen 1994
Dactylogyrus vastator	Export farmed	Sri Lanka	Thilakaratne et al. 2003
Dactylogyrus vastator	Farmed	Japan	Ogawa and Egusa 1979
Dactylogyrus vastator	Farmed	iran	Roohi et al. 2016
Dactylogyrus vastator	Farmed	iran	Jalili and Molnar 1990
Dactylogyrus wegeneri Kulwiec, 1927	Invasive	Russia	Lukyanzeva 1990[3]
Dactylogyrus wegeneri	Invasive	Former Yugoslavia	Kiskaroly 1988
Dactylogyrus sp.	Invasive	Australia	Rohde et al. 1989
Dactylogyrus sp.	Invasive	Australia	Fletcher and Whittington 1998

	Dactylogyrus sp.	Imported farmed	England	Elliott and Shotts 1980
	Dactylogyrus sp.	Imported farmed	Japan	Elliott and Shotts 1980
	Dactylogyrus sp.	Farmed	Italy	Marcer et al. 2001
	Dactylogyrus sp.	Farmed	Iran	Mousavi et al. 2011
	Dactylogyrus sp.	Farmed	USA	Elliott and Shotts 1980
	Dactylogyrus sp.	Farmed	India	Chanda et al. 2011
	Dactylogyrus sp.	Farmed	Iran	Adel et al. 2015
	Dactylogyrus sp.	Export farmed	Sri Lanka	Thilakaratne et al. 2003
	Dactylogyrus sp.	Imported Aquarium held	Iran	Mousavi et al. 2009
	Dactylogyrus sp.	Imported Aquarium held	Pakistan	Iqbal and Noreen 2014
	Dactylogyrus sp.	Imported Aquarium held	Turkey	Kayis et al. 2013
	Dactylogyrus sp.	Aquarium held	Croatia	Gjurčević et al. 2007
	Dactylogyrus sp.	Aquarium held	Philippines	Arthur and Lumanlan-Mayo 1997
	Unidentified Ancyrocephalinae	Invasive	Australia	Rohde et al. 1989
Monogenea: Diplozoidae	*Paradiplozoon doi* (Ky, 1971) (syn. *Diplozoon doi* Ky, 1971, *Sindiplozoon doi* Ky, 1968)	Invasive	India	Gussev 1973
	Paradiplozoon doi	Invasive	Malaysia	Lim 1989
	Paradiplozoon doi	Invasive	Vietnam	Lim 1998
	Paradiplozoon doi	Invasive	Vietnam	Arthur and Te 2006
	Eudiplozoon nipponicum (Goto, 1891)	Native	China	Jiang et al. 1989
	Eudiplozoon nipponicum	Native	Japan	Ogawa 1994
	Eudiplozoon nipponicum	Native	Japan	Nagasawa et al. 1989
	Eudiplozoon sp.	Invasive	Czech Republic	Gelnar et al. 1994
	Eudiplozoon sp.	Aquarium held	Europe	Sicard et al. 2001
	Paradiplozoon sp.	Invasive	Czech Republic	Gelnar et al. 1994

	Gyrodactylus baueri Ergens and Yukhimenko, 1975	Native	China	Ji et al. 1982[3]
	Gyrodactylus carassii Malmberg, 1957	Invasive	Czech Republic	Gelnar et al. 1994
	Gyrodactylus carassii	Unspecified	unspecified	Harris et al. 2004
	Gyrodactylus shulmani Ling, 1962 (syn. *Gyrodactylus chinensis* Ling, 1962)	Imported Aquarium held	Iran	Mousavi et al. 2009
	Gyrodactylus elegans Von Nordmann, 1832	Invasive	Italy	Macchioni et al. 2015
	Gyrodactylus elegans	Invasive	USA	Mueller 1936
	Gyrodactylus elegans	Native	China	Wu et al. 2000
	Gyrodactylus elegans	Invasive	Spain	Cordero del Campillo et al. 1994
	Gyrodactylus elegans	Imported Aquarium held	Norway	Levsen 1994
Monogenea: Gyrodactylidae	*Gyrodactylus gurleyi* Price, 1937	Invasive	USA	Cone and Wiles 1983
	Gyrodactylus gurleyi	Invasive	Canada	McDonald and Margolis 1995
	Gyrodactylus gurleyi	Invasive	Czech Republic	Matejusova et al. 2001
	Gyrodactylus gurleyi	Invasive	Czech Republic	Gelnar et al. 1994
	Gyrodactylus gurleyi	Unspecified	unspecified	Harris et al. 2004
	Gyrodactylus gurleyi	Farmed	England	Cable et al. 1999
	Gyrodactylus japonicus Kikuchi, 1929	native	Japan	Nagasawa 1989
	Gyrodactylus katherineri Malmberg, 1964	Unspecified	unspecified	Harris et al. 2004
	Gyrodactylus katherineri	Invasive	Latvia	Kirjušina and Vismanis 2007
	Gyrodactylus katherineri	Invasive	Turkey	Koyun 2001
	Gyrodactylus katherineri	Farmed	Turkey	Koyuncu 2009
	Gyrodactylus katherineri	Export farmed	Sri Lanka	Thilakaratne et al. 2003

Gyrodactylus kobayashii Hukuda, 1940	Invasive	Czech Republic	Matejusova et al. 2001
Gyrodactylus kobayashii	Invasive	England	Cable et al. 1999
Gyrodactylus kobayashii	Native	Japan	Ogawa 1994
Gyrodactylus kobayashii	Unspecified	unspecified	Harris et al. 2004
Gyrodactylus kobayashii	Farmed Aquarium held	Czech Republic	Fryzkova and Horak 2003
Gyrodactylus kobayashii	Farmed	China	Tu et al. 2015
Gyrodactylus kobayashii	Aquarium held	Australia	Jones et al. 1998
Gyrodactylus kobayashii	Aquarium held	Australia	Jones et al. 1997
Gyrodactylus kobayashii	Aquarium held	Australia	Fletcher and Whittington 1998
Gyrodactylus longoacuminatus Zitnan, 1964	Invasive	Czech Republic	Matejusova et al. 2001
Gyrodactylus longoacuminatus	Invasive	Latvia	Kirjušina and Vismanis 2007
Gyrodactylus longoacuminatus	Invasive	England	Shinn et al. 1997
Gyrodactylus longoacuminatus	Unspecified	unspecified	Harris et al. 2004
Gyrodactylus medius Kathariner, 1895	Native	China	Chen 1973
Gyrodactylus medius	Invasive	Former Yugoslavia	Kiskaroly 1988
Gyrodactylus medius	Invasive	Latvia	Kirjušina and Vismanis 2007
Gyrodactylus medius	Unspecified	unspecified	Harris et al. 2004
Gyrodactylus medius	Imported Aquarium held	Norway	Levsen 1995
Gyrodactylus medius	Farmed	israel	Paperna 1991
Gyrodactylus shulmani Ling, 1962	Native	China	Ling 1962
Gyrodactylus shulmani	Native	China	Chen 1973
Gyrodactylus shulmani	Unspecified	unspecified	Harris et al. 2004
Gyrodactylus sprostonae Ling, 1962	Native	China	Ling 1962

Gyrodactylus sprostonae	Native	China	Ji et al. 1982[3]
Gyrodactylus sprostonae	Native	China	Chen 1973
Gyrodactylus sprostonae	Invasive	Czech Republic	Gelnar et al. 1994
Gyrodactylus sprostonae	Invasive	Russia	Izyumova 1973
Gyrodactylus sprostonae	Unspecified	unspecified	Harris et al. 2004
Gyrodactylus vimbi (Shulman, 1954)	Invasive	Czech Republic	Moravec 2001
Gyrodactylus vimbi	Unspecified	unspecified	Harris et al. 2004
Gyrodactyloides sp.	Invasive	Spain	Cordero del Campillo et al. 1994
Gyrodactylus sp.	Invasive	Australia	Langdon 1988
Gyrodactylus sp.	Invasive	Australia	Fletcher and Whittington 1998
Gyrodactylus sp.	Invasive	Canada	McDonald and Margolis 1995
Gyrodactylus sp.	Imported Aquarium held	Italy	Di Cave et al. 2000
Gyrodactylus sp.	Imported Aquarium held	Italy from Hong Kong	Di Cave et al. 2000
Gyrodactylus sp.	Imported Aquarium held	Italy from Malaysia	Di Cave et al. 2000
Gyrodactylus sp.	Export farmed	Sri Lanka	Thilakaratne et al. 2003
Gyrodactylus sp.	Imported farmed	England	Elliott and Shotts 1980
Gyrodactylus sp.	Imported farmed	Japan	Elliott and Shotts 1980
Gyrodactylus sp.	Imported Aquarium held	Iran	Mousavi et al. 2009
Gyrodactylus sp.	Imported Aquarium held	Pakistan	Iqbal and Noreen 2014
Gyrodactylus sp.	Imported Aquarium held	Pakistan	Iqbal and Haroon 2014
Gyrodactylus sp.	Imported Aquarium held	Turkey	Kayis et al. 2013
Gyrodactylus sp.	Farmed	Iran	Mousavi et al. 2011
Gyrodactylus sp.	Farmed	India	Chanda et al. 2011
Gyrodactylus sp.	Farmed	Iran	Roohi et al. 2016
Gyrodactylus sp.	Farmed	Iran	Adel et al. 2015

	Gyrodactylus sp.	Farmed	USA	Elliott and Shotts 1980
	Gyrodactylus sp.	Farmed	Italy	Marcer et al. 2001
	Gyrodactylus sp.	Aquarium held	Croatia	Gjurčević et al. 2007
	Gyrodactylus sp.	Aquarium held	Philippines	Arthur and Lumanlan-Mayo 1997
	Gyrodactylus sp.	Aquarium held	Australia	Jones and Whittington 1992
Trematoda	Unidentified trematode	Invasive	South Korea	Kong et al. 1995
	Unidentified digenean metacercaria	Aquarium held	Philippines	Arthur and Lumanlan-Mayo 1997
Trematoda: Allocreadiidae	*Allocreadium isoporum* (Looss, 1894)	Invasive	Russia	Filimonova 1967[3]
	Allocreadium transversale (Rudolphi, 1802)	Invasive	Russia	Izyumova 1973
Trematoda: Aporocotylidae	*Sanguinicola inermis* Plehn, 1905	Invasive	Russia	Smith 1997
Trematoda: Bucephalidae	*Dollfustrema vaneyi* (Tseng, 1930)	Native	China	Long and Lee 1964
Trematoda: Cathaemasiidae	*Ribeiroia marini* (Faust and Hoffman, 1934)	Aquarium held	USA	Huizinga and Nadakavukaren 1997
Trematoda: Clinostomidae	*Clinostomum complanatum* Rudolphi, 1814	Invasive	Mexico	Guzman-Cornejo and Garcia-Prieto 1999
	Clinostomum complanatum	Invasive	South Korea	Chung et al. 1995
Trematoda: Cryptogonimidae	*Exorchis dongtinghuensis* Zhang, Zuo, Liu and Zhou, 1993	Native	China	Zhang et al. 1993
	Exorchis ovariolobularis Cao, 1990	Aquarium held	China	Tang and Wang 1997
	Exorchis oviformis Kobayashi, 1915 (syn. *Metadena oviformis* Kobayashi, 1915)	Native	Japan	Okabe 1940
	Pseudexorchis major (Hasegawa, 1927)	Native	Japan	Okabe 1940
Trematoda: Diplostomidae	*Diplostomum chromatophorum* (Brown, 1931)	Invasive	Russia	Tarmakhanov et al. 1990[3]

Diplostomum hupehensis (Pan and Wang, 1963)	Native	China	Chen 1973
Diplostomum niedashui (Pan and Wang, 1963)	Native	China	Chen 1973
Diplostomum pseudospathaceum Niewiadomska, 1984	Aquarium held	Poland	Graczyk 1992
Diplostomum pseudospathaceum	Aquarium held	Poland	Graczyk 1988
Diplostomum rutili Razmashkin, 1969	Invasive	Russia	Tarmakhanov et al. 1990[3]
Diplostomum spathaceum (Rudolphi, 1819)	Invasive	Italy	Macchioni et al. 2015
Diplostomum spathaceum	Farmed	Iran	Roohi et al. 2016
Diplostomum spathaceum	Invasive	Iran	Mokhayer 1989
Diplostomum spathaceum	Farmed	Italy	Marcer et al. 2001
Diplostomum spathaceum	Aquarium held	Poland	Graczyk 1988[3]
Posthodiplostomum cuticola (Von Nordmann, 1832)	Invasive	Czech Republic	Ondrackova et al. 1999
Posthodiplostomum minimum (Leidy, 1856)	Invasive	Mexico	Guzman-Cornejo and Garcia-Prieto 1999
Tylodelphys clavata (von Nordmann, 1832)	Invasive	Iran	Barzegar et al. 2008
Diplostomum sp.	Invasive	Mexico	Guzman-Cornejo and Garcia-Prieto 1999
Diplostomum sp. Metacercaria	Invasive	Poland	Niewiadomska 2003[3]
Diplostomum sp. *Metacercaria*	Invasive	Caspian Sea	Ataev 1969[3]
Diplostomum sp. Metacercaria	Invasive	Czech Republic	Gelnar et al. 1994
Diplostomum sp. Metacercaria	Invasive	Russia	Babyeva et al. 1989[3]
Diplostomum sp. Metacercaria	Invasive	Russia	Filimonova 1967[3]
Posthodiplostomum sp.	Native	Japan	Nagasawa et al. 1989

Trematoda: Echinochasmidae	*Echinochasmus fujianensis* Cheng, Lin, Chen, Fang, Guo, Xu and Wu, 1992	Native	China	Cheng et al. 1997
	Echinochasmus japonicus Tanabe, 1926	Native	China	Cheng et al. 1997
	Echinochasmus japonicus	Invasive	South Korea	Rim et al. 1996a
	Echinochasmus perfoliatus (Ratz, 1908)	Native	Japan	Okabe 1940
	Echinostomatidae metacercaria	Farmed	Italy	Marcer et al. 2001
	Petasiger grandivesicularis Ishii, 1935	Aquarium held	Bulgaria	Kostadinova and Chipev 1992
Trematoda: Gorgoderidae	*Phyllodistomum carassii Long and Wai, 1958 (syn. Phyllodistomum carassii Long and Wai, 1958)*	Native	China	Chen 1973
Trematoda: Haploporidae	*Carassotrema koreanum* Park, 1938 (syn. *Carassotrema mugilicola* Shireman, 1964)	Native	China	Wang et al. 1983
	Carassotrema koreanum	Native	China	Chen 1973
	Carassotrema koreanum	Aquarium held	China	Tang and Lin 1979[3]
	Carassotrema megapharyngus Wang, 1964 (syn. *Carassotrema heterosacca* Pan, 1965)	Native	China	Wu et al. 1991[3]
	Carassotrema schistorchis (Wang and Pan, 1984)	Native	China	Wu et al. 1991[3]
	Carassotrema wui Tang and Lin, 1979	Aquarium held	China	Tang and Lin 1979[3]
Trematoda: Heterophyidae	*Centrocestus formosanus* Nishigori, 1924	Native	Japan	Kagei and Yanohara 1995
	Centrocestus formosanus	Invasive	Mexico	Scholz and Salgado-Maldonado 2000
	Centrocestus formosanus	Imported Aquarium held	Turkey	Yildiz 2005
	Centrocestus formosanus	Imported Aquarium held	Iran	Mood et al. 2010

	Centrocestus formosanus	Imported	Croatia	Gjurcevic et al. 2007
	Centrocestus formosanus	Farmed	Italy	Marcer et al. 2001
	Centrocestus formosanus	Farmed	Mexico	Enríquez et al. 2009
	*Centrocestus taiwanense*2	Native	China	Cheng et al. 1997
	Centrocestus sp.	Export farmed	Sri Lanka	Thilakaratne et al. 2003
	Haplorchis pumilio (Looss, 1896)	Native	China	Cheng et al. 1997
	Haplorchis taichui (Nishigori, 1924) (syn. *Monorchotrema taichui* Nishigori, 1924, *Monorchotrema microrchia* Katsuda, 1932, *Haplorchis microrchis* Yamaguti, 1958)	Invasive	South Korea	World Health Organization (WHO) 1995
	Metagonimus sp.	Native	Japan	Hakoyama et al. 2001
	Metagonimus sp.	Invasive	South Korea	Rim et al. 1996a
	Metagonimus takahashii Takahashi, 1929	Invasive	South Korea	Chai et al. 2000
	Metagonimus takahashii	Invasive	Japan	Okabe 1940
	Metagonimus takahashii	Invasive	South Korea	Rim et al. 1996b
	Metagonimus takahashii	Aquarium held	Japan	Saito 1973
	Metagonimus yokogawai (Katsurada, 1912)	Aquarium held	Japan	Muto 1917
	Metagonimus yokogawai	Aquarium held	Japan	Shimazu and Kino 2015
	Metagonimus yokogawai	Aquarium held	Japan	Saito 1973
	Metagonimus yokogawai	Invasive	Spain	Cordero Del Campillo et al. 1980
Trematoda: Lissorchiidae	*Orientotrema japonicum* Tang, 1962	Native	China	Wang et al. 1983
	Orientotrema japonicum	Native	China	Chen 1973
	Asymphylodora japonica Yamaguti, 1938	Native	China	Wu et al. 1991[3]

		Asymphylodora markewitschi Kulakowskaja, 1947	Invasive	Russia	Izyumova 1973
		Asymphylodora sinensis Wang, 1983	Native	China	Qir and Wang 1995
		Asymphylodora tincae (Modeer, 1790)	Native	China	Qir and Wang 1995
		Asymphylodora tincae	Invasive	Russia	Filimonova 1967[3]
	Trematoda: Opecoelidae	*Coitocaecum parvum* Crowcroft, 1945	Invasive	New Zealand	Hine et al. 2000
	Trematoda: Opisthorchiidae	*Clonorchis sinensis* Looss, 1907	Native	China	Zhang et al. 2014b
		Clonorchis sinensis	Native	China	Chen et al. 2010
		Clonorchis sinensis	Native	China	Wu et al. 1991[3]
		Clonorchis sinensis	Native	China	Fang 1994
		Clonorchis sinensis	Native	China	Cheng et al. 1997
		Clonorchis sinensis	Aquarium held	South Korea	Chun 1964[3]
		Pseudamphistomum truncatum (Rudolphi, 1819)	Invasive	Russia	Coombs and Crompton 1991
		Amphimerus anatis Yamaguti 1933	Aquarium held	China	You and Min 1998
	Trematoda: Strigeidae	*Icthyocotylurus plathycephalus* (Creplin, 1825)	Invasive	Latvia	Kirjušina and Vismanis 2007
Protozoa	Protozoa	Unidentified Flagellate	Imported farmed	Japan	Elliott and Shotts 1980
		Unidentified Flagellate	Farmed	USA	Elliott and Shotts 1980

[1] Gomes et al. (2017) suggest that *Chilodonella hexasticha* (Kiernik, 1909) and *C. piscicola* (Zacharias, 1894; syn. *C. cyprini* (see Moroff, 1902) may be the same species based on molecular data.

[2] There was no verifiable source for the authority of *Centrocestus taiwanense* (Trematoda: Heterophyidae; see Cheng et al. 1997).

[3] Summaries and abstracts were located, but no translation was located for the full article. Records are provided in this table, but are excluded from all graphs.

[4] The original description of *Myxobolus toyamai* Kudo, 1917 (Kudo 1917) was reported in wild *Cyprinus carpio.* Landsberg and Lom (1991) attribute *M. toyamai* infections to both *C.auratus* and *C. carpio*.

[5] Species described by Chen and Ma (1998) are herein considered *species inquirenda.*

Supplementary S4

Supplementary material to:

Chapter 4: Parasite dispersal in the goldfish trade

Trujillo-González A., Becker J. A., and Hutson K. S.

Supplementary S4. Reference list for Figure 20. Number of fish host species reported for all parasites infecting invasive goldfish in over four different countries.

Parasite species	Host family	Host species	Reference
Argulus japonicus	Cyprinidae	*Abramis brama*	Walker PD, Velde GVD, Wendelaar–Bonga SE, Harris JE (2008) Differential host utilisation by different life history stages of the fish ectoparasite Argulus foliaceus (crustacea: Branchiura). Folia Parasitol 55: 141–149
Argulus japonicus	Cyprinidae	*Barbus holubi*	Shafir A, Oldewage WH (1992) Dynamics of a fish ectoparasite population: Opportunistic parasitism in Argulus japonicus (branchiura). Crustaceana 62: 50–64
Argulus japonicus	Cyprinidae	*Barbus kimberleyensis*	Shafir A, Oldewage WH (1992) Dynamics of a fish ectoparasite population: Opportunistic parasitism in Argulus japonicus (branchiura). Crustaceana 62: 50–64
Argulus japonicus	Cyprinidae	*Carassius carassius*	Walker PD, Velde GVD, Wendelaar–Bonga SE, Harris JE (2008) Differential host utilisation by different life history stages of the fish ectoparasite Argulus foliaceus (crustacea: Branchiura). Folia Parasitol 55: 141–149
Argulus japonicus	Cyprinidae	*Catla catla*	Sahoo PK, Kar B, Garnayak SK, Mohanty J (2012) Mixed infection of Argulus japonicus and Argulus siamensis (Branchiura, Argulidae) in carps (Pisces, Cyprinidae): loss estimation and a comparative invasive pattern study. Crustaceana 85: 1449–1462

Argulus japonicus	Cyprinidae	*Cirrhinus mrigala*	Sahoo PK, Hemaprasanth, Kar B, Garnayak SK, Mohanty J (2012) Mixed infection of Argulus japonicus and Argulus siamensis (Branchiura, Argulidae) in carps (Pisces, Cyprinidae): Loss estimation and a comparative invasive pattern study. Crustaceana 85: 1449–1462
Argulus japonicus	Clariidae	*Clarias gariepinus*	Kruger I, van As JG, Saayman JE (1983) Observations on the occurrence of the fish louse Argulus japonicus Thiele, 1900 in the western Transvaal. S Afr J Zool 18: 408–410
Argulus japonicus	Clariidae	*Clarias gariepinus*	Shafir A, Oldewage WH (1992) Dynamics of a fish ectoparasite population: Opportunistic parasitism in Argulus japonicus (branchiura). Crustaceana 62: 50–64
Argulus japonicus	Cyprinidae	*Ctenopharyngodon idella*	Lester RJG, Roubal FR (1995) Phylum Arthropoda.– In: Woo PTK (ed.) Fish diseases and disorders Vol 1. Protozoan and metazoan infections. CAB International, Wallingford, pp. 475–598
Argulus japonicus	Cyprinidae	*Cyprinus carpio*	Alsarakibi M, Wadeh H, Li G (2014) Parasitism of *Argulus japonicus* in cultured and wild fish of Guangdong, China with new record of three hosts. Parasitol Res 113: 769–775
Argulus japonicus	Clupeidae	*Dorosoma cepedianum*	Poly WJ (1998) New state, host, and distribution records of the fish ectoparasite, Argulus (branchiura), from illinois (U.S.A.). Crustaceana 71: 1–8
Argulus japonicus	Gasterosteidae	*Gasterosteus aculeatus*	Walker PD, Velde GVD, Wendelaar Bonga SE, Harris JE (2008) Differential host utilisation by different life history stages of the fish ectoparasite Argulus foliaceus (Crustacea: Branchiura). Folia Parasitol 55: 141–149
Argulus japonicus	Cyprinidae	*Gobio gobio*	Walker PD, Velde GVD, Wendelaar Bonga SE, Harris JE (2008) Differential host utilisation by different life history stages of the fish ectoparasite Argulus foliaceus (Crustacea: Branchiura). Folia Parasitol 55: 141–149
Argulus japonicus	Cyprinidae	*Hypophthalmichthys molitrix*	Alsarakibi M, Wadeh H, Li, G (2014) Parasitism of *Argulus japonicus* in cultured and wild fish of Guangdong, China with new record of three hosts. Parasitol Res 113: 769–775
Argulus japonicus	Cyprinidae	*Hypophthalmichthys nobilis*	Nagasawa K (2011) The biology of Argulus spp.(Branchiura, Argulidae) in Japan: a review. In: New Frontiers in Crustacean Biology, Vol 15 Crustaceana Monographs. Brill, pp 15–22. DOI: 10.1163/ej.9789004174252.i–354.13

Argulus japonicus	Ictaluridae	*Ictalurus punctatus*	Poly WJ (1998) New state, host, and distribution records of the fish ectoparasite, Argulus (branchiura), from illinois (U.S.A.). Crustaceana 71: 1–8
Argulus japonicus	Cyprinidae	*Labeo capensis*	Shafir A, Oldewage WH (1992) Dynamics of a fish ectoparasite population: Opportunistic parasitism in Argulus japonicus (branchiura). Crustaceana 62: 50–64
Argulus japonicus	Cyprinidae	*Labeo rohita*	Sahoo PK, Hemaprasanth, Kar B, Garnayak SK, Mohanty J (2012) Mixed infection of Argulus japonicus and Argulus siamensis (Branchiura, Argulidae) in carps (Pisces, Cyprinidae): Loss estimation and a comparative invasive pattern study. Crustaceana 85: 1449–1462
Argulus japonicus	Cyprinidae	*Labeo umbratus*	Shafir A, Oldewage WH (1992) Dynamics of a fish ectoparasite population: Opportunistic parasitism in Argulus japonicus (branchiura). Crustaceana 62: 50–64
Argulus japonicus	Cyprinidae	*Mylopharyngodon piceus*	Alsarakibi M, Wadeh H, Li G (2014) Parasitism of *Argulus japonicus* in cultured and wild fish of Guangdong, China with new record of three hosts. Parasitol Res 113: 769–775
Argulus japonicus	Salmonidae	*Oncorhynchus mykiss*	Alsarakibi M, Wadeh H, Li G (2014) Parasitism of *Argulus japonicus* in cultured and wild fish of Guangdong, China with new record of three hosts. Parasitol Res 113: 769–775
Argulus japonicus	Cichlidae	*Oreochromis niloticus*	Walker PD, Russon IJ, Duijf R, Velde GVD, Wendelaar Bonga SE (2011) The off–host survival and viability of a native and non–native fish louse (Argulus, Crustacea: Branchiura). Current Zool 57: 828–835
Argulus japonicus	Percidae	*Perca fluviatilis*	Alsarakibi M, Wadeh H, Li G (2014) Parasitism of *Argulus japonicus* in cultured and wild fish of Guangdong, China with new record of three hosts. Parasitol Res 113: 769–775
Argulus japonicus	Cyprinidae	*Pimephales promelas*	Lamarre E, Cochran P (1992) lack of host species selection by the exotic parasitic crustacean, Argulus japonicus. J Freshwater Ecol 7: 77–80
Argulus japonicus	Cyprinidae	*Rhodeus ocellatus*	Yamauchi T, Shimizu M (2013) New host and distribution records for the freshwater fish ectoparasite Argulus japonicus (Crustacea: Branchiura: Argulidae).Comp Parasitol 80: 136–137
Argulus japonicus	Cyprinidae	*Rutilus rutilus*	Walker PD, Velde GVD, Wendelaar Bonga SE, Harris JE (2008) Differential host utilisation by different life history stages of the fish ectoparasite Argulus foliaceus (Crustacea: Branchiura). Folia Parasitol 55: 141–149

Argulus japonicus	Salmonidae	*Salmo trutta*	Alsarakibi M, Wadeh H, Li G (2014) Parasitism of *Argulus japonicus* in cultured and wild fish of Guangdong, China with new record of three hosts. Parasitol Res 113: 769–775
Argulus japonicus	Cyprinidae	*Scardinius erythrophthalmus*	Walker PD, Velde GVD, Wendelaar Bonga SE, Harris JE (2008) Differential host utilisation by different life history stages of the fish ectoparasite Argulus foliaceus (Crustacea: Branchiura). Folia Parasitol 55: 141–149
Argulus japonicus	Siluridae	*Silurus asotus*	Nagasawa K , Katahira H, Mizuno K (2010) New Host and Locality of the Fish Ectoparasite Argulus japonicus (Crustacea, Branchiura, Argulidae) in Japan, with a Note on Its Heavy Infection. Biogeography 12: 17–20
Argulus japonicus	Percichthyidae	*Siniperca chuatsi*	Alsarakibi M, Wadeh H, Li G (2014) Parasitism of *Argulus japonicus* in cultured and wild fish of Guangdong, China with new record of three hosts. Parasitol Res 113: 769–775
Argulus japonicus	Cichlidae	*Tilapia sparrmanii*	Kruger I, van As JG, Saayman JE (1983) Observations on the occurrence of the fish louse Argulus japonicus Thiele, 1900 in the western Transvaal. S Afr J Zool 18: 408–410
Argulus japonicus	Cyprinidae	*Tinca tinca*	Walker PD, Velde GVD, Wendelaar Bonga SE, Harris JE (2008) Differential host utilisation by different life history stages of the fish ectoparasite Argulus foliaceus (Crustacea: Branchiura). Folia Parasitol 55: 141–149
Dactylogyrus anchoratus	Cyprinidae	*Abramis brama*	Gibson DI, Bray RA, Harris EA (Compilers) (2005) Host–Parasite Database of the Natural History Museum, London. URL. www.nhm.ac.uk/research–curation/scientific–resources/taxonomy–systematics/host–parasites
Dactylogyrus anchoratus	Cyprinidae	*Alburnoides*	Gibson DI, Timofeeva TA, Gerasev PI (1996) A catalogue of the nominal species of the monogenean genus Dactylogyrus Diesing, 1850 and their host genera. Syst Parasitol 35: 3–48
Dactylogyrus anchoratus	Cyprinidae	*Alburnus alburnus*	Koyun M (2011) Seasonal distribution and ecology of some Dactylogyrus species infecting Alburnus alburnus and Carassius carassius (Osteichthyes: Cyprinidae) from Porsuk river, Turkey. Afr J Biotechnol 10: 1154–1159

Dactylogyrus anchoratus	Cyprinidae	*Barbus brachycephalus*	Gibson DI, Bray RA, Harris EA (Compilers) (2005) Host–Parasite Database of the Natural History Museum, London. URL. www.nhm.ac.uk/research–curation/scientific–resources/taxonomy–systematics/host–parasites
Dactylogyrus anchoratus	Cyprinidae	*Barbus capito conocephalu*	Gibson DI, Bray RA, Harris EA (Compilers) (2005) Host–Parasite Database of the Natural History Museum, London. URL. www.nhm.ac.uk/research–curation/scientific–resources/taxonomy–systematics/host–parasites
Dactylogyrus anchoratus	Cyprinidae	*Capoeta*	Gibson DI, Timofeeva TA, Gerasev PI (1996) A catalogue of the nominal species of the monogenean genus Dactylogyrus Diesing, 1850 and their host genera. Syst Parasitol 35: 3–48
Dactylogyrus anchoratus	Cyprinidae	*Carassius carassius*	Gibson DI, Bray RA, Harris EA (Compilers) (2005) Host–Parasite Database of the Natural History Museum, London. URL. www.nhm.ac.uk/research–curation/scientific–resources/taxonomy–systematics/host–parasites
Dactylogyrus anchoratus	Cyprinidae	*Carassius gibelio*	Demir S, Karakisi H (2016) Metazoan parasite fauna of the prussian carp, Carassius gibelio (bloch, 1782) (cyprinidae), from Marmara lake, Turkey. Acta Zoologica Bulgarica 68: 265–268
Dactylogyrus anchoratus	Salmonidae	*Coregonus lavaretus*	Gibson DI, Bray RA, Harris EA (Compilers) (2005) Host–Parasite Database of the Natural History Museum, London. URL. www.nhm.ac.uk/research–curation/scientific–resources/taxonomy–systematics/host–parasites
Dactylogyrus anchoratus	Cyprinidae	*Ctenopharyngodon*	Gibson DI, Timofeeva TA, Gerasev PI (1996) A catalogue of the nominal species of the monogenean genus Dactylogyrus Diesing, 1850 and their host genera. Syst Parasitol 35: 3–48
Dactylogyrus anchoratus	Cyprinidae	*Cyprinus carpio*	Borji H, Naghibi A, Nasiri MR, Ahmadi A (2012) Identification of *Dactylogyrus* spp. and other parasites of common carp in northeast of Iran. J Para Dis: Official Organ of the Indian Society for Parasitology 36: 234–238
Dactylogyrus anchoratus	Cyprinidae	*Cyprinus carpio haematopterus*	Gibson DI, Bray RA, Harris EA (Compilers) (2005) Host–Parasite Database of the Natural History Museum, London. URL. www.nhm.ac.uk/research–curation/scientific–resources/taxonomy–systematics/host–parasites

Dactylogyrus anchoratus	Cyprinidae	*Cyprinus carpio*	Gibson DI, Bray RA, Harris EA (Compilers) (2005) Host–Parasite Database of the Natural History Museum, London. URL. www.nhm.ac.uk/research–curation/scientific–resources/taxonomy–systematics/host–parasites
Dactylogyrus anchoratus	Esocidae	*Esox*	Gibson DI, Timofeeva TA, Gerasev PI (1996) A catalogue of the nominal species of the monogenean genus Dactylogyrus Diesing, 1850 and their host genera. Syst Parasitol 35: 3–48
Dactylogyrus anchoratus	Gasterosteidae	*Gasterosteus*	Gibson DI, Timofeeva TA, Gerasev PI (1996) A catalogue of the nominal species of the monogenean genus Dactylogyrus Diesing, 1850 and their host genera. Syst Parasitol 35: 3–48
Dactylogyrus anchoratus	Cyprinidae	*Gobio gobio*	Gibson DI, Bray RA, Harris EA (Compilers) (2005) Host–Parasite Database of the Natural History Museum, London. URL. www.nhm.ac.uk/research–curation/scientific–resources/taxonomy–systematics/host–parasites
Dactylogyrus anchoratus	Cyprinidae	*Hemiculter leucisculus*	Gibson DI, Bray RA, Harris EA (Compilers) (2005) Host–Parasite Database of the Natural History Museum, London. URL. www.nhm.ac.uk/research–curation/scientific–resources/taxonomy–systematics/host–parasites
Dactylogyrus anchoratus	Cyprinidae	*Hypophthalmichthys molitrix*	Gibson DI, Bray RA, Harris EA (Compilers) (2005) Host–Parasite Database of the Natural History Museum, London. URL. www.nhm.ac.uk/research–curation/scientific–resources/taxonomy–systematics/host–parasites
Dactylogyrus anchoratus	Cyprinidae	*Labeo niloticus*	Gibson DI, Bray RA, Harris EA (Compilers) (2005) Host–Parasite Database of the Natural History Museum, London. URL. www.nhm.ac.uk/research–curation/scientific–resources/taxonomy–systematics/host–parasites
Dactylogyrus anchoratus	Cyprinidae	*Leucaspius delineatus*	Gibson DI, Bray RA, Harris EA (Compilers) (2005) Host–Parasite Database of the Natural History Museum, London. URL. www.nhm.ac.uk/research–curation/scientific–resources/taxonomy–systematics/host–parasites
Dactylogyrus anchoratus	Cyprinidae	*Leuciscus cephalus*	Gibson DI, Bray RA, Harris EA (Compilers) (2005) Host–Parasite Database of the Natural History Museum, London. URL. www.nhm.ac.uk/research–curation/scientific–resources/taxonomy–systematics/host–parasites

Dactylogyrus anchoratus	Cyprinidae	*Phoxinus*	Gibson DI, Timofeeva TA, Gerasev PI (1996) A catalogue of the nominal species of the monogenean genus Dactylogyrus Diesing, 1850 and their host genera. Syst Parasitol 35: 3–48
Dactylogyrus anchoratus	Cyprinidae	*Rutilus*	Gibson DI, Timofeeva TA, Gerasev PI (1996) A catalogue of the nominal species of the monogenean genus Dactylogyrus Diesing, 1850 and their host genera. Syst Parasitol 35: 3–48
Dactylogyrus anchoratus	Cyprinidae	*Schizothorax pseudaksaensis*	Gibson DI, Bray RA, Harris EA (Compilers) (2005) Host–Parasite Database of the Natural History Museum, London. URL. www.nhm.ac.uk/research–curation/scientific–resources/taxonomy–systematics/host–parasites
Dactylogyrus anchoratus	Cyprinidae	*Spinibarbichthys denticulatus*	Gibson DI, Bray RA, Harris EA (Compilers) (2005) Host–Parasite Database of the Natural History Museum, London. URL. www.nhm.ac.uk/research–curation/scientific–resources/taxonomy–systematics/host–parasites
Dactylogyrus anchoratus	Percidae	*Stizostedion lucioperca*	Gibson DI, Bray RA, Harris EA (Compilers) (2005) Host–Parasite Database of the Natural History Museum, London. URL. www.nhm.ac.uk/research–curation/scientific–resources/taxonomy–systematics/host–parasites
Dactylogyrus anchoratus	Cyprinidae	*Tinca tinca*	Gibson DI, Bray RA, Harris EA (Compilers) (2005) Host–Parasite Database of the Natural History Museum, London. URL. www.nhm.ac.uk/research–curation/scientific–resources/taxonomy–systematics/host–parasites
Dactylogyrus anchoratus	Cyprinidae	*Varicorhinus spp.*	Gibson DI, Timofeeva TA, Gerasev PI (1996) A catalogue of the nominal species of the monogenean genus Dactylogyrus Diesing, 1850 and their host genera. Syst Parasitol 35: 3–48
Dactylogyrus baueri	Cyprinidae	*carassius auratus*	Ogawa K, Egusa S (1979) Six species of Dactylogyrus (Monogenea: Dactylogyridae) collected from goldfish and carp cultured in Japan. Fish Pathol 14: 21–31
Dactylogyrus baueri	Cyprinidae	*Carassius gibelio*	Shamsi S, Jalali B, Aghazadeh Meshgi M (2009) Infection with Dactylogyrus spp. among introduced cyprinid fishes and their geographical distribution in Iran. Iranian J Vet Res 10: 70–74
Dactylogyrus baueri	Cyprinidae	*Cyprinus carpio*	Mousavi HE, Mood S, Omrani B, Mokhayer B, Ahmadi M, Soltani M, Mirzargar S, Masoumian M, Pazooki J (2009) Gill ectoparasites of goldfish (*Carassius auratus*, pearl scale variety) imported into Iran. Bull Eur Assn Fish P 29: 175–183

Dactylogyrus formosus	Cyprinidae	*Carassius carassius*	Bagge AM, Poulin R, Valtonen ET (2004) Fish population size, and not density, as the determining factor of parasite infection: A case study. Parasitol 128: 305–313
Dactylogyrus formosus	Cyprinidae	*Carassius gibelio*	Roohi J, Sattari M, Nezamabadi H, Ghorbanpour N (2014) Occurrence and intensity of parasites in prussian carp, Carassius gibelio from Anzali wetland, southwest Caspian sea. Iranian J Fisheries Sci 13 276–288
Dactylogyrus formosus	Cyprinidae	*Cyprinus carpio*	Kritsky DC, Heckmann R (2002) Species of *Dactylogyrus* (Monogenoidea: Dactylogyridae) and *Trichodina mutabilis* (Ciliata) infesting koi carp, *Cyprinus carpio*, during mass mortality at a commercial rearing facility in Utah, U.S.A. Comp Parasitol 69: 217–218
Dactylogyrus vastator	Cyprinidae	*Barbus barbus*	Gibson DI, Bray RA, Harris EA (Compilers) (2005) Host–Parasite Database of the Natural History Museum, London. URL. www.nhm.ac.uk/research–curation/scientific–resources/taxonomy–systematics/host–parasites
Dactylogyrus vastator	Cyprinidae	*Barbus brachycephalus*	Gibson DI, Bray RA, Harris EA (Compilers) (2005) Host–Parasite Database of the Natural History Museum, London. URL. www.nhm.ac.uk/research–curation/scientific–resources/taxonomy–systematics/host–parasites
Dactylogyrus vastator	Cyprinidae	*Barbus capito conocephalus*	Gibson DI, Bray RA, Harris EA (Compilers) (2005) Host–Parasite Database of the Natural History Museum, London. URL. www.nhm.ac.uk/research–curation/scientific–resources/taxonomy–systematics/host–parasites
Dactylogyrus vastator	Cyprinidae	*Carassius auratus gibelio*	Gibson DI, Bray RA, Harris EA (Compilers) (2005) Host–Parasite Database of the Natural History Museum, London. URL. www.nhm.ac.uk/research–curation/scientific–resources/taxonomy–systematics/host–parasites
Dactylogyrus vastator	Cyprinidae	*Carassius carassius*	Gibson DI, Bray RA, Harris EA (Compilers) (2005) Host–Parasite Database of the Natural History Museum, London. URL. www.nhm.ac.uk/research–curation/scientific–resources/taxonomy–systematics/host–parasites

Dactylogyrus vastator	Cyprinidae	*Cirrhinus molitorella*	Gibson DI, Bray RA, Harris EA (Compilers) (2005) Host–Parasite Database of the Natural History Museum, London. URL. www.nhm.ac.uk/research–curation/scientific–resources/taxonomy–systematics/host–parasites
Dactylogyrus vastator	Clariidae	*Clarias batrachus*	Arthur JR, Ahmed ATA (2002) Checklist of the parasites of fishes of Bangladesh. FAO Fisheries Technical Paper. No. 369/1. Rome, FAO. pp. 77
Dactylogyrus vastator	Cyprinidae	*Ctenopharyngodon idella*	Mhaisen FT, Al–Rubaie ARL (2016) Checklists of Parasites of Farm Fishes of Babylon Province, Iraq. J Parasitol Res 2016: 1–15
Dactylogyrus vastator	Cyprinidae	*Ctenopharyngodon spp.*	Gibson DI, Timofeeva TA, Gerasev PI (1996) A catalogue of the nominal species of the monogenean genus Dactylogyrus Diesing, 1850 and their host genera. Syst Parasitol 35: 3–48
Dactylogyrus vastator	Cyprinidae	*Cyprinion macrostomus*	Gibson DI, Bray RA, Harris EA (Compilers) (2005) Host–Parasite Database of the Natural History Museum, London. URL. www.nhm.ac.uk/research–curation/scientific–resources/taxonomy–systematics/host–parasites
Dactylogyrus vastator	Cyprinidae	*Cyprinus carpio*	Gibson DI, Bray RA, Harris EA (Compilers) (2005) Host–Parasite Database of the Natural History Museum, London. URL. www.nhm.ac.uk/research–curation/scientific–resources/taxonomy–systematics/host–parasites
Dactylogyrus vastator	Cyprinidae	*Cyprinus carpio haematopterus*	Gibson DI, Bray RA, Harris EA (Compilers) (2005) Host–Parasite Database of the Natural History Museum, London. URL. www.nhm.ac.uk/research–curation/scientific–resources/taxonomy–systematics/host–parasites
Dactylogyrus vastator	Esocidae	*Esox spp.*	Gibson DI, Timofeeva TA, Gerasev PI (1996) A catalogue of the nominal species of the monogenean genus Dactylogyrus Diesing, 1850 and their host genera. Syst Parasitol 35: 3–48
Dactylogyrus vastator	Cyprinidae	*Leuciscus cephalus*	Gibson DI, Bray RA, Harris EA (Compilers) (2005) Host–Parasite Database of the Natural History Museum, London. URL. www.nhm.ac.uk/research–curation/scientific–resources/taxonomy–systematics/host–parasites
Dactylogyrus vastator	Cobitidae	*Misgurnus spp.*	Gibson DI, Timofeeva TA, Gerasev PI (1996) A catalogue of the nominal species of the monogenean genus Dactylogyrus Diesing, 1850 and their host genera. Syst Parasitol 35: 3–48

Dactylogyrus vastator	Cyprinidae	*Rutilus rutilus*	Gibson DI, Bray RA, Harris EA (Compilers) (2005) Host–Parasite Database of the Natural History Museum, London. URL. www.nhm.ac.uk/research–curation/scientific–resources/taxonomy–systematics/host–parasites
Dactylogyrus vastator	Cyprinidae	*Varicorhinus spp.*	Gibson DI, Timofeeva TA, Gerasev PI (1996) A catalogue of the nominal species of the monogenean genus Dactylogyrus Diesing, 1850 and their host genera. Syst Parasitol 35: 3–48
Dactylogyrus vastator	Cyprinidae	*Vimba vimba*	Gibson DI, Bray RA, Harris EA (Compilers) (2005) Host–Parasite Database of the Natural History Museum, London. URL. www.nhm.ac.uk/research–curation/scientific–resources/taxonomy–systematics/host–parasites
Ichthyophthirius multifiliis	Acestrorhynchidae	*Acestrorhynchus falcatus*	Hoshino M, Neves L, Tavares–Dias M (2016) Parasite communities of the predatory fish, Acestrorhynchus falcatus and Acestrorhynchus falcirostris, living in sympatry in brazilian Amazon. Revista Brasileira De Parasitologia Veterinaria 25: 207–216
Ichthyophthirius multifiliis	Acestrorhynchidae	*Acestrorhynchus falcirostris*	Hoshino M, Neves L, Tavares–Dias M (2016) Parasite communities of the predatory fish, Acestrorhynchus falcatus and Acestrorhynchus falcirostris, living in sympatry in brazilian Amazon. Revista Brasileira De Parasitologia Veterinaria 25: 207–216
Ichthyophthirius multifiliis	cichlidae	*Aequidens diadema*	Aguinaga JY, Marcusso PF, Claudiano GDS, Lima BTM, Sebastião FDA, Fernandes JBK, Moraes JRE (2015) Parasitic infections in ornamental cichlid fish in the peruvian amazon. Revista Brasileira De Parasitologia Veterinária 24: 82–86
Ichthyophthirius multifiliis	cichlidae	*Aequidens tetramerus*	Tavares–Dias M, Oliveira MSB, Gonçalves RA, Silva LMA (2014) Ecology and seasonal variation of parasites in wild Aequidens tetramerus, a Cichlidae from the Amazon. Acta Parasitol 59: 158–164
Ichthyophthirius multifiliis	Loricariidae	*Ancistrus hoplogenys*	Tavares–Dias M, Lemos JRG, Martins ML (2010) Parasitic fauna of eight species of ornamental freshwater fish species from the middle negro river in the brazilian Amazon region. Revista Brasileira De Parasitologia Veterinária 19: 103–107
Ichthyophthirius multifiliis	Cyprinodontidae	*Aphanius sophiae*	Gholami Z, Youssefi MR, Marhaba Z, Alizadeh A, Rahimi MT (2016). Aphanius sophiae (Actinoptrygii, Cyprinodontidae), a new host for Ichthyophthirius multifiliis (Ciliophora) reported from Iran. J Para Dis 40: 1030–1032

Ichthyophthirius multifiliis	Cichlidae	*Apistogramma sp.*	Aguinaga JY, Marcusso PF, Claudiano GDS, Lima BTM, Sebastião FDA, Fernandes JBK, Moraes JRE (2015) Parasitic infections in ornamental cichlid fish in the peruvian amazon. Revista Brasileira De Parasitologia Veterinária 24: 82–86
Ichthyophthirius multifiliis	Gobiidae	*Apollonia melanostoma (syn. Neogobius melanostomus)*	Mühlegger JM, Jirsa F, Konecny R, Frank C (2010) Parasites of Apollonia melanostoma (Pallas 1814) and Neogobius kessleri (Guenther 1861) (Osteichthyes, Gobiidae) from the Danube river in Austria. J Helminthol 84: 87–92
Ichthyophthirius multifiliis	Arapaimidae	*Arapaima gigas*	Marinho RGB, Tavares–Dias M, Dias–Grigório MKR, Neves LR, Yoshioka ETO, Boijink CL, Takemoto RM (2013) Helminthes and protozoan of farmed pirarucu (Arapaima gigas) in eastern Amazon and host–parasite relationship. Arquivo Brasileiro De Medicina Veterinária e Zootecnia 65: 1192–1202
Ichthyophthirius multifiliis	cichlidae	*Astronotus ocellatus*	Tavares–Dias M, Sousa T, Neves L (2014) Parasitic infections in two benthopelagic fish from Amazon: the arowana Osteoglossum bicirrhosum (Osteoglossidae) and oscar Astronotus ocellatus (Cichlidae). Biosci J 30: 546–555
Ichthyophthirius multifiliis	Cichlidae	*Astronotus ocellatus*	WoRMS Editorial Board (2017). World Register of Marine Species. Available from http://www.marinespecies.org at VLIZ. Accessed 2017–08–22. doi:10.14284/170
Ichthyophthirius multifiliis	Cyprinidae	*Barbus barbulus*	Raissy M, Ansari M (2012) Parasites of some freshwater fish from armand river, Chaharmahal Va Bakhtyari province, Iran. Iranian J Parasitol 7: 73–79
Ichthyophthirius multifiliis	Cyprinidae	*Barbus grypus*	Raissy M, Ansari M (2012) Parasites of some freshwater fish from armand river, Chaharmahal Va Bakhtyari province, Iran. Iranian J Parasitol 7: 73–79
Ichthyophthirius multifiliis	Cyprinidae	*Barbus haasi*	Maceda–Veiga A, Salvadó H, Vinyoles D, De Sostoa A (2009) Outbreaks of Ichthyophthirius multifiliis in redtail barbs Barbus haasi in a mediterranean stream during drought. J Aquat Animal Health 21: 189–194
Ichthyophthirius multifiliis	Terapontidae	*Bidyanus bidyanus*	Mifsud C, Rowland SJ (2008) Use of salt to control ichthyophthiriosis and prevent saprolegniosis in silver perch, *Bidyanus bidyanus*. Aquacult Res 39: 1175–1180

Ichthyophthirius multifiliis	Cyprinidae	*Capoeta aculeata*	Raissy M, Ansari M (2011) Histopathological changes in the gills of naturally–infected Capoeta aculeata (Cuvier and Valenciennes, 1844) with parasites. Afr J Biotechnol 10: 15422–15425
Ichthyophthirius multifiliis	Cyprinidae	*Capoeta aculeata*	Raissy M, Ansari M (2012) Parasites of some freshwater fish from armand river, Chaharmahal Va Bakhtyari province, Iran. Iranian J Parasitol 7: 73–79
Ichthyophthirius multifiliis	Cyprinidae	*Capoeta capoeta*	Raissy M, Ansari M (2012) Parasites of some freshwater fish from armand river, Chaharmahal Va Bakhtyari province, Iran. Iranian J Parasitol 7: 73–79
Ichthyophthirius multifiliis	Cyprinidae	*Capoeta damascina*	Raissy M, Ansari M (2012) Parasites of some freshwater fish from armand river, Chaharmahal Va Bakhtyari province, Iran. Iranian J Parasitol 7: 73–79
Ichthyophthirius multifiliis	Gasteropelecidae	*Carnegiella martae*	Tavares–Dias M, Lemos JRG, Martins ML (2010) Parasitic fauna of eight species of ornamental freshwater fish species from the middle negro river in the brazilian Amazon region. Revista Brasileira De Parasitologia Veterinária 19: 103–107
Ichthyophthirius multifiliis	Gasteropelecidae	*Carnegiella strigata*	Tavares–Dias M, Lemos JRG, Martins ML (2010) Parasitic fauna of eight species of ornamental freshwater fish species from the middle negro river in the brazilian Amazon region. Revista Brasileira De Parasitologia Veterinária 19: 103–107
Ichthyophthirius multifiliis	Cyprinidae	*Catla catla*	Arthur JR, Ahmed ATA (2002) Checklist of the parasites of fishes of Bangladesh. FAO Fisheries Technical Paper. No. 369/1. Rome, FAO. pp. 77
Ichthyophthirius multifiliis	Cichlidae	*Cichlasoma sp.*	Aguinaga JY, Marcusso PF, Claudiano GDS, Lima BTM, Sebastião FDA, Fernandes JBK, Moraes JRE (2015) Parasitic infections in ornamental cichlid fish in the peruvian amazon. Revista Brasileira De Parasitologia Veterinária 24: 82–86
Ichthyophthirius multifiliis	Clariidae	*Clarias gariepinus*	Omeji S, Solomon SG, Idoga ES (2011) A Comparative Study of the Common Protozoan Parasites of Clarias gariepinus from the Wild and Cultured Environments in Benue State, Nigeria. J Parasitol Res 2011: 1–8
Ichthyophthirius multifiliis	Characidae	*Colossoma macropomum*	Dias M, Neves L, Marinho R, Pinheir D, Tavares–Dias M (2015) Parasitism in tambatinga (Colossoma macropomum x Piaractus brachypomus, Characidae) farmed in the Amazon, Brazil. Acta Amazonica 45: 231–238

Ichthyophthirius multifiliis	Characidae	*Colossoma macropomum*	Santos E, Tavares–Dias M, Pinheiro D, Neves L, Marinho R, Dias M (2013) Parasitic fauna of tambaqui Colossoma macropomum (Characidae) farmed in cages in the state of Amapa, eastern Amazon. Acta Amazonica 43: 105–111
Ichthyophthirius multifiliis	Cichlidae	*Crenicichla anthurus*	Aguinaga JY, Marcusso PF, Claudiano GDS, Lima BTM, Sebastião FDA, Fernandes JBK, Moraes JRE (2015) Parasitic infections in ornamental cichlid fish in the peruvian amazon. Revista Brasileira De Parasitologia Veterinária 24: 82–86
Ichthyophthirius multifiliis	Cyprinidae	*Ctenopharyngodon idellus*	WoRMS Editorial Board (2017). World Register of Marine Species. Available from http://www.marinespecies.org at VLIZ. Accessed 2017–08–22. doi:10.14284/170
Ichthyophthirius multifiliis	Cyprinidae	*Cyprinus carpio*	WoRMS Editorial Board (2017). World Register of Marine Species. Available from http://www.marinespecies.org at VLIZ. Accessed 2017–08–22. doi:10.14284/170
Ichthyophthirius multifiliis	Cyprinidae	*Danio rerio*	Coyne RS, Hannick L, Shanmugam D, Hostetler JB, Brami D, Joardar VS, Clark TG (2011) Comparative genomics of the pathogenic ciliate Ichthyophthirius multifiliis, its free–living relatives and a host species provide insights into adoption of a parasitic lifestyle and prospects for disease control. Genome Biol 12: R100–R100
Ichthyophthirius multifiliis	Esocidae	*Esox lucius*	WoRMS Editorial Board (2017). World Register of Marine Species. Available from http://www.marinespecies.org at VLIZ. Accessed 2017–08–22. doi:10.14284/170
Ichthyophthirius multifiliis	Cyprinidae	*Gila robusta*	Ward DL (2012) Salinity of the little Colorado river in grand canyon confers anti–parasitic properties on a native fish. West N American Nat 72: 334–338
Ichthyophthirius multifiliis	Sisoridae	*Glyptothorax silviae*	Raissy M, Ansari M (2012) Parasites of some freshwater fish from armand river, Chaharmahal Va Bakhtyari province, Iran. Iranian J Parasitol 7: 73–79
Ichthyophthirius multifiliis	Percidae	*Gymnocorymbus ternetzi*	Aydogan A, Avci H, Birincioglu S (2010) Ichthyophthirius multifiliis infection in a black tetra (Gymnocorymbus ternetzi). Kafkas Universitesi Veteriner Fakultesi Dergisi 16: 135–137
Ichthyophthirius multifiliis	Percidae	*Gymnocorymbus ternetzi*	WoRMS Editorial Board (2017). World Register of Marine Species. Available from http://www.marinespecies.org at VLIZ. Accessed 2017–08–22. doi:10.14284/170

Ichthyophthirius multifiliis	Characidae	*Hemibrycon surinamensis*	Guimarães MDF, Hoshino É.M, Tavares–Dias M (2014) First study on parasites of Hemibrycon surinamensis (characidae), a host from the eastern Amazon region. Revista Brasileira De Parasitologia Veterinária 23: 343–347
Ichthyophthirius multifiliis	Erythrinidae	*Hoplerythrinus unitaeniatus*	Alcântara NM, Tavares–Dias M (2015) Structure of the parasites communities in two erythrinidae fish from amazon river system (Brazil). Revista Brasileira De Parasitologia Veterinária 24: 183–190
Ichthyophthirius multifiliis	Erythrinidae	*Hoplias malabaricus*	Alcântara NM, Tavares–Dias M (2015) Structure of the parasites communities in two erythrinidae fish from amazon river system (Brazil). Revista Brasileira De Parasitologia Veterinária 24: 183–190
Ichthyophthirius multifiliis	Pimelodidae	*Hybrid Leiarius sp. (Leiarius marmoratus male x L. reticulatum female)*	Ventura A, Jeronimo G, Goncalves E, Tamporoski B, Martins M, Ishikawa M (2013) Parasitic fauna of the siluriform hybrids cachapinta and jundiara in the first stages of development. Pesquisa Agropecuaria Brasileira 48: 943–949
Ichthyophthirius multifiliis	Moronidae	*Hybrid Morone sp. (morone saxatilis × M. chrysops)*	Corrales J, Noga EJ (2011) Effects of feeding rate on the expression of antimicrobial polypeptides and on susceptibility to Ichthyophthirius multifiliis in hybrid striped (sunshine) bass (Morone saxatilis ♂ × M. chrysops ♀). Aquacult 318: 109–121
Ichthyophthirius multifiliis	Serrasalmidae	*Hybrid Piaractussp. (Piaractus mesopotamicus x P. brachypomus)*	Franceschini L, Zago AC, Schalch SHC, Garcia F, Romera DM, Silva RJD (2013) Parasitic infections of Piaractus mesopotamicus and hybrid (P. mesopotamicus x Piaractus brachypomus) cultured in Brazil. Revista Brasileira De Parasitologia Veterinária 22: 407–414
Ichthyophthirius multifiliis	Pimelodidae	*Hybrid Pseudoplatystoma sp. (Pseudoplatystoma corruscans male x P. reticulatum female)*	Ventura A, Jeronimo G, Goncalves E, Tamporoski B, Martins M, Ishikawa M (2013) Parasitic fauna of the siluriform hybrids cachapinta and jundiara in the first stages of development. Pesquisa Agropecuaria Brasileira 48: 943–949
Ichthyophthirius multifiliis	Characidae	*Hyphessobrycon copelandi*	Tavares–Dias M, Lemos JRG, Martins ML (2010) Parasitic fauna of eight species of ornamental freshwater fish species from the middle negro river in the brazilian Amazon region. Revista Brasileira De Parasitologia Veterinária 19: 103–107
Ichthyophthirius multifiliis	Cyprinidae	*Hypophthalmichthys molitrix*	Chih–leu C (1956) The protozoan parasites from four species of Chinese pond fishes: Ctenopharyngodon idellus, Mylopharyngodon piceus, Aristichthys nobilis and Hypophthalmichthys molitrix II. The protozoan parasites of Mylopharyngodon piceus. Acta Hydrobiologica Sinica 2: 296

Ichthyophthirius multifiliis	Cyprinidae	*Hypophthalmichthys nobilis*	Nematollahi A, Ahmadi A, Mohammadpour H, Ebrahimi M (2013) External parasite infection of common carp (Cyprinus carpio) and big head (Hypophthalmichthys nobilis) in fish farms of Mashhad, northeast of Iran. J Para Dis 37: 131–133
Ichthyophthirius multifiliis	Loricariidae	*Hypostomus plecostomus*	WoRMS Editorial Board (2017). World Register of Marine Species. Available from http://www.marinespecies.org at VLIZ. Accessed 2017–08–22. doi:10.14284/170
Ichthyophthirius multifiliis	Ictaluridae	*Ictalurus punctatus*	Xu D, Zhang D, Zhang Q, Shoemaker CA, Moreira GSA (2016) Molecular immune response of channel catfish immunized with live theronts of Ichthyophthirius multifiliis. Fish Shellfish Immunol 54: 86–92
Ichthyophthirius multifiliis	Cyprinidae	*Labeo rohita*	Upadhyay J, Jauhari RK, Pemola Devi N (2012) Parasitic incidence in a cyprinid fish Labeo rohita (Ham.) at river song in Doon valley (Uttarakhand). J Para Dis 36: 56–60
Ichthyophthirius multifiliis	Cyprinidae	*Leuciscus cephalus*	Abdel–Hafez G, Lahnsteiner F, Mansour N, Licek E (2014) Pathophysiology of Ichthyophthirius multifiliis Infection in Rainbow Trout (Oncorhynchus mykiss) and Chub (Leuciscus cephalus). J Comp Pathol 151: 394–399
Ichthyophthirius multifiliis	Cyprinidae	*Luciobarbus pectoralis*	WoRMS Editorial Board (2017). World Register of Marine Species. Available from http://www.marinespecies.org at VLIZ. Accessed 2017–08–22. doi:10.14284/170
Ichthyophthirius multifiliis	Mastacembelidae	*Mastacembelus mastacembelus*	Jalali B, Barzegar M, Nezamabadi H (2008) Parasitic fauna of the spiny eel, Mastacembelus mastacembelus Banks et Solander (Teleostei: Mastacembelidae) in Iran. Iranian J Vet Res 9: 158–161
Ichthyophthirius multifiliis	Cichlidae	*mesonauta acora*	Farias Pantoja WM, Vargas Flores L, Tavares-Dias M (2015) Parasites component community in wild population of Pterophyllum scalare Schultze, 1823 and Mesonauta acora Castelnau, 1855, cichlids from the brazilian Amazon. J Appl Ichthyol 31: 1043–1048
Ichthyophthirius multifiliis	Moronidae	*Morone chrysops*	Farmer BD, Fuller SA, Mitchell AJ, Straus DL, Bullard SA (2013) Efficacy of bath treatments of formalin and copper sulfate on cultured white bass, Morone chrysops, concurrently infected by Onchocleidus mimus and Ichthyophthirius multifiliis. J World Aquacult S 44: 305–310

Ichthyophthirius multifiliis	Lebiasinidae	*Nannostomus eques*	Tavares–Dias M, Lemos JRG, Martins ML (2010) Parasitic fauna of eight species of ornamental freshwater fish species from the middle negro river in the brazilian Amazon region. Revista Brasileira De Parasitologia Veterinária 19: 103–107
Ichthyophthirius multifiliis	Lebiasinidae	*Nannostomus unifasciatus*	Tavares–Dias M, Lemos JRG, Martins ML (2010) Parasitic fauna of eight species of ornamental freshwater fish species from the middle negro river in the brazilian Amazon region. Revista Brasileira De Parasitologia Veterinária 19: 103–107
Ichthyophthirius multifiliis	Gobiidae	*Neogobius fluviatilis*	WoRMS Editorial Board (2017). World Register of Marine Species. Available from http://www.marinespecies.org at VLIZ. Accessed 2017–08–22. doi:10.14284/170
Ichthyophthirius multifiliis	Gobiidae	*Neogobius kessleri*	Mühlegger JM, Jirsa F, Konecny R, Frank C (2010) Parasites of Apollonia melanostoma (Pallas 1814) and Neogobius kessleri (Guenther 1861) (Osteichthyes, Gobiidae) from the Danube river in Austria. J Helminthol 84: 87–92
Ichthyophthirius multifiliis	Salmonidae	*Oncorhynchus mykiss*	Forwood J, Harris J, Landos M, Deveney M (2015) Life cycle and settlement of an australian isolate of Ichthyophthirius multifiliis Fouquet, 1876 from rainbow trout. Folia Parasitol 62: 1–5
Ichthyophthirius multifiliis	Salmonidae	*Oncorhynchus mykiss*	WoRMS Editorial Board (2017). World Register of Marine Species. Available from http://www.marinespecies.org at VLIZ. Accessed 2017–08–22. doi:10.14284/170
Ichthyophthirius multifiliis	Cichlidae	*Oreochromis aureus*	Sin YM, Ling KH, Lam TJ (1994) Passive transfer of protective immunity against ichthyophthiriasis from vaccinated mother to fry in tilapias, Oreochromis aureus. Aquaculture 120: 229–237
Ichthyophthirius multifiliis	Cichlidae	*Oreochromis niloticus*	Xu D, Shoemaker CA, Klesius PH (2009) Enhanced mortality in nile tilapia Oreochromis niloticus following coinfections with ichthyophthiriasis and streptococcosis. Dis Aquat Org 85: 187–192
Ichthyophthirius multifiliis	osteoglossidae	*Osteoglossum bicirrhosum*	Tavares–Dias M, Sousa T, Neves L (2014) Parasitic infections in two benthopelagic fish from Amazon: the arowana Osteoglossum bicirrhosum (Osteoglossidae) and oscar Astronotus ocellatus (Cichlidae). Biosci J 30: 546–555
Ichthyophthirius multifiliis	Characidae	*Paracheirodon axelrodi*	Tavares–Dias M, Lemos JRG, Martins ML (2010) Parasitic fauna of eight species of ornamental freshwater fish species from the middle negro river in the brazilian Amazon region. Revista Brasileira De Parasitologia Veterinária 19: 103–107

Ichthyophthirius multifiliis	Characidae	*Piaractus brachypomus*	Dias M, Neves L, Marinho R, Pinheir D, Tavares–Dias M (2015) Parasitism in tambatinga (Colossoma macropomum x Piaractus brachypomus, Characidae) farmed in the Amazon, Brazil. Acta Amazonica 45: 231–238
Ichthyophthirius multifiliis	Characidae	*Piaractus mesopotamicus*	Franceschini L, Zago AC, Schalch SHC, Garcia F, Romera DM, Silva RJD (2013) Parasitic infections of Piaractus mesopotamicus and hybrid (P. mesopotamicus x Piaractus brachypomus) cultured in Brazil. Revista Brasileira De Parasitologia Veterinária 22: 407–414
Ichthyophthirius multifiliis	Poeciliidae	*Poecilia latipinna*	WoRMS Editorial Board (2017). World Register of Marine Species. Available from http://www.marinespecies.org at VLIZ. Accessed 2017–08–22. doi:10.14284/170
Ichthyophthirius multifiliis	Poeciliidae	*Poecilia reticulata*	WoRMS Editorial Board (2017). World Register of Marine Species. Available from http://www.marinespecies.org at VLIZ. Accessed 2017–08–22. doi:10.14284/170
Ichthyophthirius multifiliis	Poeciliidae	*Poecilia sphenops*	WoRMS Editorial Board (2017). World Register of Marine Species. Available from http://www.marinespecies.org at VLIZ. Accessed 2017–08–22. doi:10.14284/170
Ichthyophthirius multifiliis	Cichlidae	*Pterophyllum scalare*	Farias Pantoja WM, Vargas Flores L, Tavares-Dias M (2015) Parasites component community in wild population of Pterophyllum scalare Schultze, 1823 and Mesonauta acora Castelnau, 1855, cichlids from the brazilian Amazon. J Appl Ichthyol 31: 1043–1048
Ichthyophthirius multifiliis	Cichlidae	*Pterophyllum scalare*	Tavares–Dias M, Lemos JRG, Martins ML (2010) Parasitic fauna of eight species of ornamental freshwater fish species from the middle negro river in the brazilian Amazon region. Revista Brasileira De Parasitologia Veterinária 19: 103–107
Ichthyophthirius multifiliis	Heptapteridae	*Rhamdia quelen*	Tancredo KR, Gonçalves ELT, Brum A, Acchile M, Hashimoto GSO, Pereira SA, Martins ML (2015) Hemato–immunological and biochemical parameters of silver catfish Rhamdia quelen immunized with live theronts of Ichthyophthirius multifiliis. Fish Shellfish Immunol 45: 689–694
Ichthyophthirius multifiliis	Salmonidae	*Salmo trutta fario*	WoRMS Editorial Board (2017). World Register of Marine Species. Available from http://www.marinespecies.org at VLIZ. Accessed 2017–08–22. doi:10.14284/170

Ichthyophthirius multifiliis	Salmonidae	*Salmo trutta labrax*	WoRMS Editorial Board (2017). World Register of Marine Species. Available from http://www.marinespecies.org at VLIZ. Accessed 2017–08–22. doi:10.14284/170
Ichthyophthirius multifiliis	Salmonidae	*Salvelinus fontinalis*	WoRMS Editorial Board (2017). World Register of Marine Species. Available from http://www.marinespecies.org at VLIZ. Accessed 2017–08–22. doi:10.14284/170
Ichthyophthirius multifiliis	Cyprinidae	*Schizothorax niger*	Dar GH, Dar SA, Kaur H, Chishti MZ, Ahmad F, Tak IUR (2016) First record of protozoan parasites in cyprinid fish, Schizothorax niger Heckel, 1838 from Dal lake in Kashmir Himalayas with study on their pathogenesis. Microbial Pathogenesis 93: 100–104
Ichthyophthirius multifiliis	Cyprinidae	*schizothorax richardsonii*	Mallik S, Shahi N, Das P, Pandey N, Haldar R, Kumar B, Chandra S (2015) Occurrence of Ichthyophthirius multifiliis (white spot) infection in snow trout, Schizothorax richardsonii (gray) and its treatment trial in control condition. Indian J Animal Res 49: 227–230
Ichthyophthirius multifiliis	Cyprinidae	*Squaliobarbus curriculus*	Yao J, Zhou Z, Li X, Yin W, Ru H, Pan X, Shen J (2011). Antiparasitic efficacy of dihydrosanguinarine and dihydrochelerythrine from Macleaya microcarpa against Ichthyophthirius multifiliis in richadsin (Squaliobarbus curriculus). Vet Parasitol 183: 8–13
Ichthyophthirius multifiliis	Cichlidae	*Symphysodon aequifasciatus*	Aguinaga JY, Marcusso PF, Claudiano GDS, Lima BTM, Sebastião FDA, Fernandes JBK, Moraes JRE (2015) Parasitic infections in ornamental cichlid fish in the peruvian amazon. Revista Brasileira De Parasitologia Veterinária 24: 82–86
Ichthyophthirius multifiliis	Cichlidae	*Symphysodon discus*	WoRMS Editorial Board (2017). World Register of Marine Species. Available from http://www.marinespecies.org at VLIZ. Accessed 2017–08–22. doi:10.14284/170
Ichthyophthirius multifiliis	Cyprinidae	*Tinca tinca*	WoRMS Editorial Board (2017). World Register of Marine Species. Available from http://www.marinespecies.org at VLIZ. Accessed 2017–08–22. doi:10.14284/170
Ichthyophthirius multifiliis	Triportheidae	*Triportheus angulatus*	Oliveira MSB, Gonçalves RA, Tavares–Dias M (2016) Community of parasites in Triportheus curtus and Triportheus angulatus (Characidae) from a tributary of the Amazon river system (Brazil). Studies on Neotropical Fauna and Environment 51: 29–36

Ichthyophthirius multifiliis	Triportheidae	*Triportheus curtus*	Oliveira MSB, Gonçalves RA, Tavares–Dias M (2016) Community of parasites in Triportheus curtus and Triportheus angulatus (Characidae) from a tributary of the Amazon river system (Brazil). Studies on Neotropical Fauna and Environment 51: 29–36
Ichthyophthirius multifiliis	Poeciliidae	*Xiphophorus hellerii*	WoRMS Editorial Board (2017). World Register of Marine Species. Available from http://www.marinespecies.org at VLIZ. Accessed 2017–08–22. doi:10.14284/170
Ichthyophthirius multifiliis	Poeciliidae	*Xiphophorus maculatus*	WoRMS Editorial Board (2017). World Register of Marine Species. Available from http://www.marinespecies.org at VLIZ. Accessed 2017–08–22. doi:10.14284/170
Lernaea cyprinacea	Cyprinidae	*Acanthobrama terraesanctae*	WoRMS Editorial Board (2017). World Register of Marine Species. Available from http://www.marinespecies.org at VLIZ. Accessed 2017–08–22. doi:10.14284/170
Lernaea cyprinacea	Cyprinidae	*Alburnus mossulensis*	Sayyadzadeh G, Esmaeili HR, Ghasemian S, Mirghiyasi S, Parsi B, Zamanpoore M, Akhlaghi M (2016) Co–invasion of anchor worms Lernaea cyprinacea (Copepoda: Lernaeidae) in some freshwater fishes of the Kor River Basin, Southwest of Iran with some remarks on the ecological aspects of lernaeosis in the country. Iran J Fish Sci 15: 369–389
Lernaea cyprinacea	Clupeidae	*Alosa alosa*	WoRMS Editorial Board (2017). World Register of Marine Species. Available from http://www.marinespecies.org at VLIZ. Accessed 2017–08–22. doi:10.14284/170
Lernaea cyprinacea	Centrarchidae	*Ambloplites rupestris*	WoRMS Editorial Board (2017). World Register of Marine Species. Available from http://www.marinespecies.org at VLIZ. Accessed 2017–08–22. doi:10.14284/170
Lernaea cyprinacea	Ictaluridae	*Ameiurus melas*	WoRMS Editorial Board (2017). World Register of Marine Species. Available from http://www.marinespecies.org at VLIZ. Accessed 2017–08–22. doi:10.14284/170
Lernaea cyprinacea	Amiidae	*Amia calva*	WoRMS Editorial Board (2017). World Register of Marine Species. Available from http://www.marinespecies.org at VLIZ. Accessed 2017–08–22. doi:10.14284/170
Lernaea cyprinacea	Anguillidae	*Anguilla anguilla*	WoRMS Editorial Board (2017). World Register of Marine Species. Available from http://www.marinespecies.org at VLIZ. Accessed 2017–08–22. doi:10.14284/170

Lernaea cyprinacea	Cyprinodontidae	*Aphanius dispar*	WoRMS Editorial Board (2017). World Register of Marine Species. Available from http://www.marinespecies.org at VLIZ. Accessed 2017–08–22. doi:10.14284/170
Lernaea cyprinacea	Cyprinidae	*Aristichthys nobilis*	WoRMS Editorial Board (2017). World Register of Marine Species. Available from http://www.marinespecies.org at VLIZ. Accessed 2017–08–22. doi:10.14284/170
Lernaea cyprinacea	Cyprinidae	*Aspius aspius*	WoRMS Editorial Board (2017). World Register of Marine Species. Available from http://www.marinespecies.org at VLIZ. Accessed 2017–08–22. doi:10.14284/170
Lernaea cyprinacea	Cyprinidae	*Barbus barbulus*	Raissy M, Ansari M (2012) Parasites of some freshwater fish from armand river, Chaharmahal Va Bakhtyari province, Iran. Iranian J Parasitol 7: 73–79
Lernaea cyprinacea	Cyprinidae	*Barbus barbus*	WoRMS Editorial Board (2017). World Register of Marine Species. Available from http://www.marinespecies.org at VLIZ. Accessed 2017–08–22. doi:10.14284/170
Lernaea cyprinacea	Cyprinidae	*Capoeta aculeata*	Raissy M, Ansari M, Lashkari A, Jalali B (2010) Occurrence of parasites in selected fish species in Gandoman lagoon, Iran. Iranian J Fisheries Sci 9: 464–471
Lernaea cyprinacea	Cyprinidae	*Capoeta aculeata*	Sayyadzadeh G, Esmaeili HR, Ghasemian S, Mirghiyasi S, Parsi B, Zamanpoore M, Akhlaghi M (2016) Co–invasion of anchor worms Lernaea cyprinacea (Copepoda: Lernaeidae) in some freshwater fishes of the Kor River Basin, Southwest of Iran with some remarks on the ecological aspects of lernaeosis in the country. Iran J Fish Sci 15: 369–389
Lernaea cyprinacea	Cyprinidae	*Capoeta saadii*	Sayyadzadeh G, Esmaeili HR, Ghasemian S, Mirghiyasi S, Parsi B, Zamanpoore M, Akhlaghi M (2016) Co–invasion of anchor worms Lernaea cyprinacea (Copepoda: Lernaeidae) in some freshwater fishes of the Kor River Basin, Southwest of Iran with some remarks on the ecological aspects of lernaeosis in the country. Iran J Fish Sci 15: 369–389
Lernaea cyprinacea	Cyprinidae	*Carassius carassius*	WoRMS Editorial Board (2017). World Register of Marine Species. Available from http://www.marinespecies.org at VLIZ. Accessed 2017–08–22. doi:10.14284/170

Lernaea cyprinacea	Cyprinidae	*Catla catla*	Tasawar Z, Umer K, Hayat CS (2007) Observations on lernaeid parasites of *Catla catla* from a fish hatchery in Muzaffargarh, Pakistan. *Pakistan Vet J 27:* 17
Lernaea cyprinacea	Catostomidae	*Catostomus commersonii*	WoRMS Editorial Board (2017). World Register of Marine Species. Available from http://www.marinespecies.org at VLIZ. Accessed 2017–08–22. doi:10.14284/170
Lernaea cyprinacea	Channidae	*Channa punctata*	WoRMS Editorial Board (2017). World Register of Marine Species. Available from http://www.marinespecies.org at VLIZ. Accessed 2017–08–22. doi:10.14284/170
Lernaea cyprinacea	Cyprinidae	*Chondrostoma nasus*	WoRMS Editorial Board (2017). World Register of Marine Species. Available from http://www.marinespecies.org at VLIZ. Accessed 2017–08–22. doi:10.14284/170
Lernaea cyprinacea	Cobitidae	*Cobitis taenia*	WoRMS Editorial Board (2017). World Register of Marine Species. Available from http://www.marinespecies.org at VLIZ. Accessed 2017–08–22. doi:10.14284/170
Lernaea cyprinacea	Characidae	*Colossoma macropomum*	Luque JL, Vieira FM, Takemoto RM, Pavanelli GC, Eiras JC (2013) Checklist of Crustacea parasitizing fishes from Brazil. Check List 9: 1449–1470
Lernaea cyprinacea	Characidae	*Colossoma macropomum*	Borges–Bastos PAM, Carmona de São Clemente S, de Lima FC (1996) Aspectos anátomo–patológicos da parasitose por Lernaea cyprinacea (L.) (Crustacea: Copepoda) em Tambaqui (Colossoma macropomum Cuvier, 1818). Rev. bras. ciênc. vet. 3: 15–21
Lernaea cyprinacea	Cottidae	*Cottus gobio*	WoRMS Editorial Board (2017). World Register of Marine Species. Available from http://www.marinespecies.org at VLIZ. Accessed 2017–08–22. doi:10.14284/170
Lernaea cyprinacea	Cyprinidae	*Ctenopharyngodon idella*	WoRMS Editorial Board (2017). World Register of Marine Species. Available from http://www.marinespecies.org at VLIZ. Accessed 2017–08–22. doi:10.14284/170
Lernaea cyprinacea	Cyprinidae	*Cyprinus carpio*	WoRMS Editorial Board (2017). World Register of Marine Species. Available from http://www.marinespecies.org at VLIZ. Accessed 2017–08–22. doi:10.14284/170
Lernaea cyprinacea	Esocidae	*Esox lucius*	WoRMS Editorial Board (2017). World Register of Marine Species. Available from http://www.marinespecies.org at VLIZ. Accessed 2017–08–22. doi:10.14284/170

Lernaea cyprinacea	Fundulidae	*Fundulus heteroclitus heteroclitus*	WoRMS Editorial Board (2017). World Register of Marine Species. Available from http://www.marinespecies.org at VLIZ. Accessed 2017–08–22. doi:10.14284/170
Lernaea cyprinacea	Poeciliidae	*Gambusia affinis*	WoRMS Editorial Board (2017). World Register of Marine Species. Available from http://www.marinespecies.org at VLIZ. Accessed 2017–08–22. doi:10.14284/170
Lernaea cyprinacea	Gasterosteidae	*Gasterosteus aculeatus aculeatus*	WoRMS Editorial Board (2017). World Register of Marine Species. Available from http://www.marinespecies.org at VLIZ. Accessed 2017–08–22. doi:10.14284/170
Lernaea cyprinacea	Cyprinidae	*Gobio gobio*	WoRMS Editorial Board (2017). World Register of Marine Species. Available from http://www.marinespecies.org at VLIZ. Accessed 2017–08–22. doi:10.14284/170
Lernaea cyprinacea	Percidae	*Gymnocephalus cernuus*	WoRMS Editorial Board (2017). World Register of Marine Species. Available from http://www.marinespecies.org at VLIZ. Accessed 2017–08–22. doi:10.14284/170
Lernaea cyprinacea	Cyprinidae	*Hypophthalmichthys molitrix*	Barzegar M, Raeisi M, Bozorgnia A, Jalali B (2008) Parasites of the eyes of fresh and brackish water fishes in Iran. Iranian J Vet Res 9: 256–261
Lernaea cyprinacea	Cyprinidae	*Hypophthalmichthys molitrix*	WoRMS Editorial Board (2017). World Register of Marine Species. Available from http://www.marinespecies.org at VLIZ. Accessed 2017–08–22. doi:10.14284/170
Lernaea cyprinacea	Cyprinidae	*Hypophthalmichthys nobilis*	Goodwin AE (1999) Massive Lernaea cyprinacea infestations damaging the gills of channel catfish polycultured with bighead carp. J Aquat Animal Health 11: 406–408
Lernaea cyprinacea	Ictaluridae	*Ictalurus punctatus*	Goodwin AE (1999) Massive Lernaea cyprinacea infestations damaging the gills of channel catfish polycultured with bighead carp. J Aquat Animal Health 11: 406–408
Lernaea cyprinacea	Centrarchidae	*Lepomis gibbosus*	WoRMS Editorial Board (2017). World Register of Marine Species. Available from http://www.marinespecies.org at VLIZ. Accessed 2017–08–22. doi:10.14284/170
Lernaea cyprinacea	Centrarchidae	*Lepomis gibbosus*	Stavrescu–Bedivan M, Popa O, Popa L (2014) Infestation of Lernaea cyprinacea (copepoda: Lernaeidae) in two invasive fish species in Romania, Lepomis gibbosus and Pseudorasbora parva. Knowledge and Management of Aquatic Ecosystems 414: 12

Lernaea cyprinacea	Cyprinidae	*Leuciscus idus*	WoRMS Editorial Board (2017). World Register of Marine Species. Available from http://www.marinespecies.org at VLIZ. Accessed 2017–08–22. doi:10.14284/170
Lernaea cyprinacea	Mugilidae	*Liza abu*	WoRMS Editorial Board (2017). World Register of Marine Species. Available from http://www.marinespecies.org at VLIZ. Accessed 2017–08–22. doi:10.14284/170
Lernaea cyprinacea	Mugilidae	*Liza ramada*	WoRMS Editorial Board (2017). World Register of Marine Species. Available from http://www.marinespecies.org at VLIZ. Accessed 2017–08–22. doi:10.14284/170
Lernaea cyprinacea	Lotidae	*Lota lota*	WoRMS Editorial Board (2017). World Register of Marine Species. Available from http://www.marinespecies.org at VLIZ. Accessed 2017–08–22. doi:10.14284/170
Lernaea cyprinacea	Mastacembelidae	*Mastacembelus mastacembelus*	Jalali B, Barzegar M, Nezamabadi H (2008) Parasitic fauna of the spiny eel, Mastacembelus mastacembelus Banks et Solander (Teleostei: Mastacembelidae) in Iran. Iranian J Vet Res 9: 158–161
Lernaea cyprinacea	Centrarchidae	*Micropterus dolomieu*	WoRMS Editorial Board (2017). World Register of Marine Species. Available from http://www.marinespecies.org at VLIZ. Accessed 2017–08–22. doi:10.14284/170
Lernaea cyprinacea	Centrarchidae	*Micropterus salmoides*	WoRMS Editorial Board (2017). World Register of Marine Species. Available from http://www.marinespecies.org at VLIZ. Accessed 2017–08–22. doi:10.14284/170
Lernaea cyprinacea	Mugilidae	*Mugil cephalus*	WoRMS Editorial Board (2017). World Register of Marine Species. Available from http://www.marinespecies.org at VLIZ. Accessed 2017–08–22. doi:10.14284/170
Lernaea cyprinacea	Mugilidae	*Mugil platanus*	WoRMS Editorial Board (2017). World Register of Marine Species. Available from http://www.marinespecies.org at VLIZ. Accessed 2017–08–22. doi:10.14284/170
Lernaea cyprinacea	Gobiidae	*Neogobius fluviatilis*	WoRMS Editorial Board (2017). World Register of Marine Species. Available from http://www.marinespecies.org at VLIZ. Accessed 2017–08–22. doi:10.14284/170
Lernaea cyprinacea	Gobiidae	*Neogobius melanostomus*	WoRMS Editorial Board (2017). World Register of Marine Species. Available from http://www.marinespecies.org at VLIZ. Accessed 2017–08–22. doi:10.14284/170

Lernaea cyprinacea	Cyprinidae	*Notemigonus crysoleucas*	WoRMS Editorial Board (2017). World Register of Marine Species. Available from http://www.marinespecies.org at VLIZ. Accessed 2017–08–22. doi:10.14284/170
Lernaea cyprinacea	Salmonidae	*Oncorhynchus mykiss*	WoRMS Editorial Board (2017). World Register of Marine Species. Available from http://www.marinespecies.org at VLIZ. Accessed 2017–08–22. doi:10.14284/170
Lernaea cyprinacea	Salmonidae	*Oncorhynchus tshawytscha*	WoRMS Editorial Board (2017). World Register of Marine Species. Available from http://www.marinespecies.org at VLIZ. Accessed 2017–08–22. doi:10.14284/170
Lernaea cyprinacea	Cichlidae	*Oreochromis niloticus*	Ibrahim MM, Soliman MFM (2011) Parasite community of wild and cultured *Oreochromis niloticus* from lake Manzalah, Egypt. J Egypt S Parasitol 41: 685
Lernaea cyprinacea	Stromateidae	*Pampus argenteus*	WoRMS Editorial Board (2017). World Register of Marine Species. Available from http://www.marinespecies.org at VLIZ. Accessed 2017–08–22. doi:10.14284/170
Lernaea cyprinacea	Percidae	*Perca fluviatilis*	WoRMS Editorial Board (2017). World Register of Marine Species. Available from http://www.marinespecies.org at VLIZ. Accessed 2017–08–22. doi:10.14284/170
Lernaea cyprinacea	Poeciliidae	*Poecilia reticulata*	WoRMS Editorial Board (2017). World Register of Marine Species. Available from http://www.marinespecies.org at VLIZ. Accessed 2017–08–22. doi:10.14284/170
Lernaea cyprinacea	Poeciliidae	*Poecilia sphenops*	WoRMS Editorial Board (2017). World Register of Marine Species. Available from http://www.marinespecies.org at VLIZ. Accessed 2017–08–22. doi:10.14284/170
Lernaea cyprinacea	Cyprinidae	*Pseudorasbora parva*	Stavrescu–Bedivan M, Popa O, Popa L (2014) Infestation of Lernaea cyprinacea (copepoda: Lernaeidae) in two invasive fish species in Romania, Lepomis gibbosus and Pseudorasbora parva. Knowledge and Management of Aquatic Ecosystems 414: 12
Lernaea cyprinacea	Cyprinidae	*Pseudorasbora parva*	WoRMS Editorial Board (2017). World Register of Marine Species. Available from http://www.marinespecies.org at VLIZ. Accessed 2017–08–22. doi:10.14284/170
Lernaea cyprinacea	Cyprinidae	*Rhinichthys atratulus*	WoRMS Editorial Board (2017). World Register of Marine Species. Available from http://www.marinespecies.org at VLIZ. Accessed 2017–08–22. doi:10.14284/170

Lernaea cyprinacea	Cyprinidae	*Rutilus rutilus*	WoRMS Editorial Board (2017). World Register of Marine Species. Available from http://www.marinespecies.org at VLIZ. Accessed 2017–08–22. doi:10.14284/170
Lernaea cyprinacea	Salmonidae	*Salmo trutta trutta*	WoRMS Editorial Board (2017). World Register of Marine Species. Available from http://www.marinespecies.org at VLIZ. Accessed 2017–08–22. doi:10.14284/170
Lernaea cyprinacea	Salmonidae	*Salvelinus fontinalis*	WoRMS Editorial Board (2017). World Register of Marine Species. Available from http://www.marinespecies.org at VLIZ. Accessed 2017–08–22. doi:10.14284/170
Lernaea cyprinacea	Percidae	*Sander vitreus*	WoRMS Editorial Board (2017). World Register of Marine Species. Available from http://www.marinespecies.org at VLIZ. Accessed 2017–08–22. doi:10.14284/170
Lernaea cyprinacea	Cyprinidae	*Scardinius erythrophthalmus*	WoRMS Editorial Board (2017). World Register of Marine Species. Available from http://www.marinespecies.org at VLIZ. Accessed 2017–08–22. doi:10.14284/170
Lernaea cyprinacea	Cichlidae	*Tilapia zillii*	WoRMS Editorial Board (2017). World Register of Marine Species. Available from http://www.marinespecies.org at VLIZ. Accessed 2017–08–22. doi:10.14284/170
Lernaea cyprinacea	Cyprinidae	*Tinca tinca*	WoRMS Editorial Board (2017). World Register of Marine Species. Available from http://www.marinespecies.org at VLIZ. Accessed 2017–08–22. doi:10.14284/170
Lernaea cyprinacea	Umbridae	*Umbra pygmaea*	WoRMS Editorial Board (2017). World Register of Marine Species. Available from http://www.marinespecies.org at VLIZ. Accessed 2017–08–22. doi:10.14284/170
Schyzocotyle acheilognathi	Cyprinidae	*Abramis brama orientalis*	Gavrilova NG, Karimov SB (1989) On the changes in the parasite fauna of fishes of the Kairakkum water reservoir for many years. Parazitologiya. Akademiya Nauk SSSR. Leningrad 23(3): 250–256
Schyzocotyle acheilognathi	Cyprinidae	*Acheilognathus rhombea*	Dove ADM, Fletcher AS (2000) The distribution of the introduced tapeworm *Bothriocephalus acheilognathi* in Australian freshwater fishes. J Helminthol 74: 121–127
Schyzocotyle acheilognathi	cichlidae	*Aequidens caeruleopunctatus*	Choudhury A, García–Varela M, Pérez–Ponce de León G (2017) Parasites of freshwater fishes and the Great American Biotic Interchange: a bridge too far? J Helminthol 91: 174–196

Schyzocotyle acheilognathi	Cyprinidae	*Alburnoides bipunctatus*	Dove ADM, Fletcher AS (2000) The distribution of the introduced tapeworm *Bothriocephalus acheilognathi* in Australian freshwater fishes. J Helminthol 74: 121–127
Schyzocotyle acheilognathi	Cyprinidae	*Alburnus alburnus*	Dove ADM, Fletcher AS (2000) The distribution of the introduced tapeworm *Bothriocephalus acheilognathi* in Australian freshwater fishes. J Helminthol 74: 121–127
Schyzocotyle acheilognathi	Cyprinidae	*Algansea lacustris*	Salgado–Maldonado G, Pineda–López RE (2003) The Asian fish tapeworm Bothriocephalus acheilognathi: a potential threat to native freshwater fish species in Mexico. Biol Invasions 5: 261–268
Schyzocotyle acheilognathi	Cyprinidae	*Algansea rubescens*	Salgado–Maldonado G, Pineda–López RE (2003) The Asian fish tapeworm Bothriocephalus acheilognathi: a potential threat to native freshwater fish species in Mexico. Biol Invasions 5: 261–268
Schyzocotyle acheilognathi	Cyprinidae	*Algansea tincella*	Salgado–Maldonado G, Pineda–López RE (2003) The Asian fish tapeworm Bothriocephalus acheilognathi: a potential threat to native freshwater fish species in Mexico. Biol Invasions 5: 261–268
Schyzocotyle acheilognathi	Goodeidae	*Allophorus robustus*	Salgado–Maldonado G, Pineda–López RE (2003) The Asian fish tapeworm Bothriocephalus acheilognathi: a potential threat to native freshwater fish species in Mexico. Biol Invasions 5: 261–268
Schyzocotyle acheilognathi	Goodeidae	*Allotoca diazi*	Salgado–Maldonado G, Pineda–López RE (2003) The Asian fish tapeworm Bothriocephalus acheilognathi: a potential threat to native freshwater fish species in Mexico. Biol Invasions 5: 261–268
Schyzocotyle acheilognathi	Cyprinidae	*Aristichthys nobilis*	Arthur JR, Lumanlan–Mayo S (1997) Checklist of the parasites of fishes of the Philippines. FAO Fisheries Technical Paper 369. Rome, FAO. pp. 102
Schyzocotyle acheilognathi	Cyprinidae	*Aspius aspius*	Dove ADM, Fletcher AS (2000) The distribution of the introduced tapeworm *Bothriocephalus acheilognathi* in Australian freshwater fishes. J Helminthol 74: 121–127
Schyzocotyle acheilognathi	Characidae	*Astyanax fasciatus*	Salgado–Maldonado G, Pineda–López RE (2003) The Asian fish tapeworm Bothriocephalus acheilognathi: a potential threat to native freshwater fish species in Mexico. Biol Invasions 5: 261–268
Schyzocotyle acheilognathi	Atherinopsidae	*Atherinella crystallina*	Salgado–Maldonado G, Pineda–Lopez RE (2003) The Asian fish tapeworm Bothriocephalus acheilognathi: a potential threat to native freshwater fish species in Mexico. Biol Inv 5: 261–268

Schyzocotyle acheilognathi	Gobiidae	*Awaous guamensis*	Font WF, Tate DC (1994) Helminth parasites of native Hawaiian freshwater fishes: an example of extreme ecological isolation. J Parasitol 80: 682–688
Schyzocotyle acheilognathi	Cyprinidae	*Barbus altianalis*	Dove ADM, Fletcher AS (2000) The distribution of the introduced tapeworm *Bothriocephalus acheilognathi* in Australian freshwater fishes. J Helminthol 74: 121–127
Schyzocotyle acheilognathi	Cyprinidae	*Barbus barbus*	Dove ADM, Fletcher AS (2000) The distribution of the introduced tapeworm *Bothriocephalus acheilognathi* in Australian freshwater fishes. J Helminthol 74: 121–127
Schyzocotyle acheilognathi	Cyprinidae	*Barbus bynni*	Dove ADM, Fletcher AS (2000) The distribution of the introduced tapeworm *Bothriocephalus acheilognathi* in Australian freshwater fishes. J Helminthol 74: 121–127
Schyzocotyle acheilognathi	Cyprinidae	*Barbus callensis*	Dove ADM, Fletcher AS (2000) The distribution of the introduced tapeworm *Bothriocephalus acheilognathi* in Australian freshwater fishes. J Helminthol 74: 121–127
Schyzocotyle acheilognathi	Cyprinidae	*Barbus capito*	Dove ADM, Fletcher AS (2000) The distribution of the introduced tapeworm *Bothriocephalus acheilognathi* in Australian freshwater fishes. J Helminthol 74: 121–127
Schyzocotyle acheilognathi	Cyprinidae	*Barbus kimberleyensis*	Dove ADM, Fletcher AS (2000) The distribution of the introduced tapeworm *Bothriocephalus acheilognathi* in Australian freshwater fishes. J Helminthol 74: 121–127
Schyzocotyle acheilognathi	Cyprinidae	*Barbus mattozi*	Dove ADM, Fletcher AS (2000) The distribution of the introduced tapeworm *Bothriocephalus acheilognathi* in Australian freshwater fishes. J Helminthol 74: 121–127
Schyzocotyle acheilognathi	Cyprinidae	*Barbus sharpeyi*	Dove ADM, Fletcher AS (2000) The distribution of the introduced tapeworm *Bothriocephalus acheilognathi* in Australian freshwater fishes. J Helminthol 74: 121–127
Schyzocotyle acheilognathi	Cyprinidae	*Barbus trimaculatus*	Dove ADM, Fletcher AS (2000) The distribution of the introduced tapeworm *Bothriocephalus acheilognathi* in Australian freshwater fishes. J Helminthol 74: 121–127
Schyzocotyle acheilognathi	Terapontidae	*Bidyanus bidyanus*	Dove ADM, Fletcher AS (2000) The distribution of the introduced tapeworm *Bothriocephalus acheilognathi* in Australian freshwater fishes. J Helminthol 74: 121–127

Schyzocotyle acheilognathi	Pimelodidae	*Brachyplatystoma flavicans*	Rego AA, Chubb JC, Pavanelli GC (1999) Cestodes in South American freshwater teleost fishes: keys to genera and brief descriptions of species. Revista Brasileira de Zoologia 16: 299–367
Schyzocotyle acheilognathi	Cyprinidae	*Carassius auratus gibelio*	Gibson DI, Bray RA, Harris EA (Compilers) (2005) Host–Parasite Database of the Natural History Museum, London. URL. www.nhm.ac.uk/research–curation/scientific–resources/taxonomy–systematics/host–parasites
Schyzocotyle acheilognathi	Cyprinidae	*Carassius carassius*	Nedeva I (1988) To the biology of Bothriocephalus acheilognathi Yamaguti, 1934 (fam. Bothriocephalidae). Khelmintologiya 26: 32–38
Schyzocotyle acheilognathi	Atherinopsidae	*Chirostoma arge*	Salgado–Maldonado G, Pineda–Lopez RE (2003) The Asian fish tapeworm Bothriocephalus acheilognathi: a potential threat to native freshwater fish species in Mexico. Biol Inv 5: 261–268
Schyzocotyle acheilognathi	Atherinopsidae	*Chirostoma attenuatum*	Pérez–Ponce de Leon G, Mendoza BG, Pulido F (1994) Helminths of the charal prieto, Chirostoma attenuatum (Osteichthyes: Atherinidae), from Patzcuaro Lake, Michoacan, Mexico. J Helminthol S Washington 61: 139–141
Schyzocotyle acheilognathi	Atherinopsidae	*Chirostoma estor*	Hernandez SG, Prieto LG, Sarabia DO (1991) Revision historica de la taxonomia de Bothriocephalus acheilognathi (Cestoda: Pseudophyllidea). Anales del Instituto de Biologia, Universidad Nacional Autonoma de Mexico 62(3): 409–415
Schyzocotyle acheilognathi	Atherinopsidae	*Chirostoma grandocule*	Prieto LG, Sarabia DO (1991) Distribucion actual de Bothriocephalus acheilognathi en Mexico. Anales del Instituto de Biologia, Universidad Nacional Autonoma de Mexico 62: 523–526
Schyzocotyle acheilognathi	Atherinopsidae	*Chirostoma humboltianum*	Salgado–Maldonado G, Cabañas–Carranza G, Soto–Galera E, Aguilar–Aguilar R (2001) A checklist of the helminth parasites of freshwater fishes from the Lerma–Santiago River Basin, Mexico. Comp Parasitol 68: 204–218
Schyzocotyle acheilognathi	Atherinopsidae	*Chirostoma jordani*	Salgado–Maldonado G, Cabañas–Carranza G, Soto–Galera E, Aguilar–Aguilar R (2001) A checklist of the helminth parasites of freshwater fishes from the Lerma–Santiago River Basin, Mexico. Comp Parasitol 68: 204–218
Schyzocotyle acheilognathi	Atherinopsidae	*Chirostoma labarcae*	Salgado–Maldonado G, Cabañas–Carranza G, Soto–Galera E, Aguilar–Aguilar R (2001) A checklist of the helminth parasites of freshwater fishes from the Lerma–Santiago River Basin, Mexico. Comp Parasitol 68: 204–218

Schyzocotyle acheilognathi	Atherinopsidae	*Chirostoma ocotlanae*	Prieto LG, Sarabia DO (1991) Distribucion actual de Bothriocephalus acheilognathi en Mexico. Anales del Instituto de Biologia, Universidad Nacional Autonoma de Mexico 62: 523–526
Schyzocotyle acheilognathi	Atherinopsidae	*Chirostoma riojai*	Salgado–Maldonado G, Cabañas–Carranza G, Soto–Galera E, Aguilar–Aguilar R (2001) A checklist of the helminth parasites of freshwater fishes from the Lerma–Santiago River Basin, Mexico. Comp Parasitol 68: 204–218
Schyzocotyle acheilognathi	Cyprinidae	*Chondrostoma nasus*	Nedeva I (1988) To the biology of Bothriocephalus acheilognathi Yamaguti, 1934 (fam. Bothriocephalidae). Khelmintologiya 26: 32–38
Schyzocotyle acheilognathi	Cichlidae	*Cichlasoma cyanoguttatum*	Salgado–Maldonado G, Pineda–Lopez RE (2003) The Asian fish tapeworm Bothriocephalus acheilognathi: a potential threat to native freshwater fish species in Mexico. Biol Inv 5: 261–268
Schyzocotyle acheilognathi	Cichlidae	*Cichlasoma intermedium*	Gibson DI, Bray RA, Harris EA (Compilers) (2005) Host–Parasite Database of the Natural History Museum, London. URL. www.nhm.ac.uk/research–curation/scientific–resources/taxonomy–systematics/host–parasites
Schyzocotyle acheilognathi	Cichlidae	*Cichlasoma istlanum*	Salgado–Maldonado G, Pineda–Lopez RE (2003) The Asian fish tapeworm Bothriocephalus acheilognathi: a potential threat to native freshwater fish species in Mexico. Biol Inv 5: 261–268
Schyzocotyle acheilognathi	Cichlidae	*Cichlasoma labridens*	Salgado–Maldonado G, Pineda–Lopez RE (2003) The Asian fish tapeworm Bothriocephalus acheilognathi: a potential threat to native freshwater fish species in Mexico. Biol Inv 5: 261–268
Schyzocotyle acheilognathi	Cichlidae	*Cichlasoma meeki*	Salgado–Maldonado G, Pineda–López RE (2003) The Asian fish tapeworm Bothriocephalus acheilognathi: a potential threat to native freshwater fish species in Mexico. Biol Invasions 5: 261–268
Schyzocotyle acheilognathi	Cichlidae	*Cichlasoma nigrofasciatum*	Salgado–Maldonado G, Cabañas–Carranza G, Soto–Galera E, Aguilar–Aguilar R (2001) A checklist of the helminth parasites of freshwater fishes from the Lerma–Santiago River Basin, Mexico. Comp Parasitol 68: 204–218
Schyzocotyle acheilognathi	Cichlidae	*Cichlasoma urophthalmus*	Salgado–Maldonado G, Pineda–Lopez R, Vidal–Martinez VM, Kennedy CR (1997) A checklist of metazoan parasites of cichlid fish from Mexico. J Helminthol S Washington 64: 195–207
Schyzocotyle acheilognathi	Clariidae	*Clarias gariepinus*	Dove ADM, Fletcher AS (2000) The distribution of the introduced tapeworm *Bothriocephalus acheilognathi* in Australian freshwater fishes. J Helminthol 74: 121–127

Schyzocotyle acheilognathi	Characidae	*Colossoma macropomum*	Salgado–Maldonado G, Rubio–Godoy M (2014) Helmintos parásitos de peces agua dulce introducidos. México, Comisión Nacional para el Conocimiento y Uso de la Biodiversidad, pp 269–285
Schyzocotyle acheilognathi	Cyprinidae	*Coreius guichenoti*	Luo HY, Nie P, Zhang YA, Wang GT, Yao WJ (2002) Molecular variation of Bothriocephalus acheilognathi Yamaguti, 1934 (Cestoda: Pseudophyllidea) in different fish host speciesbased on ITS rDNA sequences. Syst Parasitol 52: 159–166
Schyzocotyle acheilognathi	Cichlidae	*Cryptoheros panamensi*	Choudhury A, García–Varela M, Pérez–Ponce de León G (2017) Parasites of freshwater fishes and the Great American Biotic Interchange: a bridge too far? J Helminthol 91: 174–196
Schyzocotyle acheilognathi	Cyprinidae	*Ctenopharyngodon idella*	Scholz T, Kuchta R, Williams C (2012) *Bothriocephalus acheilognathi.* In: Woo PTK, Buchmann K (ed) Fish Parasites: pathobiology and protection. CAB International, Wallingford, UK, pp 282–297
Schyzocotyle acheilognathi	Cyprinidae	*Culter alburnus*	Luo HY, Nie P, Zhang YA, Wang GT, Yao WJ (2002) Molecular variation of Bothriocephalus acheilognathi Yamaguti, 1934 (Cestoda: Pseudophyllidea) in different fish host speciesbased on ITS rDNA sequences. Syst Parasitol 52: 159–166
Schyzocotyle acheilognathi	Cyprinidae	*Culter dabryi*	Luo HY, Nie P, Zhang YA, Wang GT, Yao WJ (2003) Genetic differentiation in populations of the cestode Bothriocephalus acheilognathi (Cestoda, Pseudophyllidea) as revealed by eight microsatellite markers. Parasitol 126: 493–501
Schyzocotyle acheilognathi	Cyprinidae	*Culter erythropterus*	Nie P, Wang GT, Yao WJ, Zhang YA, Gao Q (2000) Occurrence of Bothriocephalus acheilognathi in cyprinid fish from three lakes in the flood plain of the Yangtze River, China. Dis Aquat Org 41: 81–82
Schyzocotyle acheilognathi	Cyprinidae	*Culterichthys erythropterus*	Luo HY, Nie P, Zhang YA, Wang GT, Yao WJ (2003) Genetic differentiation in populations of the cestode Bothriocephalus acheilognathi (Cestoda, Pseudophyllidea) as revealed by eight microsatellite markers. Parasitol 126: 493–501
Schyzocotyle acheilognathi	Cyprinidae	*Cyprinella lutrensis*	Heckmann RA, Greger PD, Furter RC (1993) The Asian fish tapeworm, Bothriocephalus acheilognathi in fishes from Nevada. J Helminthol S Washington 60: 127–128
Schyzocotyle acheilognathi	Cyprinidae	*Cyprinus carpio*	Han JE, Shin SP, Kim JH, Choresca Jr. CH, Jun JW, Gomez SC (2010) Park Mortality of cultured Koi *Cyprinus carpio* in Korea caused by *Bothriocephalus acheilognathi*. Afr J Microbiol Res 4: 543–546

Schyzocotyle acheilognathi	Cyprinidae	*Cyprinus carpio*	Kennedy CR (1993) Introductions, spread and colonization of new localities by fish helminth and crustacean parasites in the British Isles: a perspective and appraisal. J Fish Biol 43: 287–301
Schyzocotyle acheilognathi	Cyprinidae	*Dionda ipni*	Salgado–Maldonado G, Pineda–Lopez RE (2003) The Asian fish tapeworm Bothriocephalus acheilognathi: a potential threat to native freshwater fish species in Mexico. Biol Inv 5: 261–268
Schyzocotyle acheilognathi	Eleotridae	*Eleotris sandwicensis*	Font WF, Tate DC (1994) Helminth parasites of native Hawaiian freshwater fishes: an example of extreme ecological isolation. J Parasitol 80: 682–688
Schyzocotyle acheilognathi	Esocidae	*Esox lucius*	Scholz T (1989) Amphilinida and Cestoda, parasites of fish in Czechoslovakia. Prirodovedne Prace ustavu Ceskoslovenske Akademie Ved v Brne 23(4): 1–56
Schyzocotyle acheilognathi	Fundulidae	*Fundulus zebrinus*	Clarkson RW, Robinson AT, Hoffnagle L (1997) Asian tapeworm (Bothriocephalus acheilognathi) in native fishes from the Little Colorado River, Grand Canyon, Arizona. Great Basin Nat 57: 66–69
Schyzocotyle acheilognathi	Fundulidae	*Fundulus zebrinus*	Brouder MJ, Hoffnagle TL (1997) Distribution and prevalence of the Asian fish tapeworm, Bothriocephalus acheilognathi, in the Colorado River and tributaries, Grand Canyon, Arizona, including two new host records. J Helminthol S Washington 64: 219–226
Schyzocotyle acheilognathi	Poeciliidae	*Gambusia affinis*	Mars CL, Font WF (1993) Seasonal recruitment and maturation of Bothriocephalus acheilognathi in Louisiana mosquito fish Gambusia affinis. American J Trop Med Hyg 49: 136–137
Schyzocotyle acheilognathi	Poeciliidae	*Gambusia holbrooki*	Dove ADM, Fletcher AS (2000) The distribution of the introduced tapeworm *Bothriocephalus acheilognathi* in Australian freshwater fishes. J Helminthol 74: 121–127
Schyzocotyle acheilognathi	Poeciliidae	*Gambusia vittata*	Salgado–Maldonado G, Pineda–Lopez RE (2003) The Asian fish tapeworm Bothriocephalus acheilognathi: a potential threat to native freshwater fish species in Mexico. Biol Inv 5: 261–268
Schyzocotyle acheilognathi	Poeciliidae	*Gambusia yucatana*	(Scholz T, Vargas–Vázquez J, Moravec F, Vivas–Rodríguez C, Mendoza–Franco E (1996) Cestoda and Acanthocephala of fishes from cenotes (=sinkholes) of Yucatan, Mexico. Folia Parasitol 43: 141–152
Schyzocotyle acheilognathi	Cyprinidae	*Gila conspersa*	Aguilar–Aguilar R, Lagunas–Calvo O, Rivas G (2016) Endohelminths of Gila conspersa (actinopterygii: Cyprinidae) from the Aguanaval river basin, state of Zacatecas, central Mexico. Southwestern Naturalist 61: 269–273

Schyzocotyle acheilognathi	Cyprinidae	*Gila cypha*	Clarkson RW, Robinson AT, Hoffnagle L (1997) Asian tapeworm (Bothriocephalus acheilognathi) in native fishes from the Little Colorado River, Grand Canyon, Arizona. Great Basin Nat 57: 66–69
Schyzocotyle acheilognathi	Cyprinidae	*Gila cypha*	Cole RA (2002) What are parasitologists doing in the United States Geological Survey? Comp Parasitol 69(2): 132–134
Schyzocotyle acheilognathi	Cyprinidae	*Gila elegans*	Gibson DI, Bray RA, Harris EA (Compilers) (2005) Host–Parasite Database of the Natural History Museum, London. URL. www.nhm.ac.uk/research–curation/scientific–resources/taxonomy–systematics/host–parasites
Schyzocotyle acheilognathi	Cyprinidae	*Gila orcutti*	Gibson DI, Bray RA, Harris EA (Compilers) (2005) Host–Parasite Database of the Natural History Museum, London. URL. www.nhm.ac.uk/research–curation/scientific–resources/taxonomy–systematics/host–parasites
Schyzocotyle acheilognathi	Cyprinidae	*Gila robusta seminuda*	Scholz T (1997) A revision of the species of Bothriocephalus Rudolphi, 1808 (Cestoda: Pseudophyllidea) parasitic in American freshwater fishes. Syst Parasitol 36: 85–107
Schyzocotyle acheilognathi	Cyprinidae	*Gila robusta*	Heckmann RA, Greger PD, Furter RC (1993) The Asian fish tapeworm, Bothriocephalus acheilognathi in fishes from Nevada. J Helminthol S Washington 60: 127–128
Schyzocotyle acheilognathi	Goodeidae	*Girardinichthys multiradiatius*	Leon–Regagnon V (1992) Fauna helmintologica de algunos vertebrados acuaticos de la cienaga de Lerma, Mexico. Anales del Instituto de Biologia, Universidad Nacional Autonoma de Mexico 63(1): 151–153
Schyzocotyle acheilognathi	Cyprinidae	*Gnathopogon elongatus*	Dove ADM, Fletcher AS (2000) The distribution of the introduced tapeworm *Bothriocephalus acheilognathi* in Australian freshwater fishes. J Helminthol 74: 121–127
Schyzocotyle acheilognathi	Cyprinidae	*Gobio albipinnatus vladykovi*	Macko JK, Rysavy B, Spakulova M, Kralóva I (1993) Synopsis of cestodes in Slovakia: I. Cestodaria, Cestoidea: Caryophyllidea, Spathebothriidea, Pseudophyllidea, Proteocephalidea. Helminthologia 30: 85–91
Schyzocotyle acheilognathi	Cyprinidae	*Gobio albipinnatus*	Gibson DI, Bray RA, Harris EA (Compilers) (2005) Host–Parasite Database of the Natural History Museum, London. URL. www.nhm.ac.uk/research–curation/scientific–resources/taxonomy–systematics/host–parasites

Schyzocotyle acheilognathi	Cyprinidae	*Gobio gobio*	Nedeva I, Mutafova T (1988) To the morphology of Bothriocephalus acheilognathi Yamaguti, 1934 (fam. Bothriocephalidae). Khelmintologiya 26: 39–46
Schyzocotyle acheilognathi	Goodeidae	*Goodea atripinnis*	Salgado–Maldonado G, Pineda–Lopez RE (2003) The Asian fish tapeworm Bothriocephalus acheilognathi: a potential threat to native freshwater fish species in Mexico. Biol Inv 5: 261–268
Schyzocotyle acheilognathi	Cyprinidae	*Hemiculter bleekeri*	Luo HY, Nie P, Zhang YA, Wang GT, Yao WJ (2002) Molecular variation of Bothriocephalus acheilognathi Yamaguti, 1934 (Cestoda: Pseudophyllidea) in different fish host speciesbased on ITS rDNA sequences. Syst Parasitol 52: 159–166
Schyzocotyle acheilognathi	Cyprinidae	*Hemiculter leucisculus*	Luo HY, Nie P, Zhang YA, Wang GT, Yao WJ (2003) Genetic differentiation in populations of the cestode Bothriocephalus acheilognathi (Cestoda, Pseudophyllidea) as revealed by eight microsatellite markers. Parasitol 126: 493–501
Schyzocotyle acheilognathi	Poeciliidae	*Heterandria bimaculata*	Salgado–Maldonado G, Pineda–López RE (2003) The Asian fish tapeworm Bothriocephalus acheilognathi: a potential threat to native freshwater fish species in Mexico. Biol Invasions 5: 261–268
Schyzocotyle acheilognathi	Cyprinidae	*Hybopsis boucardi*	Salgado–Maldonado G, Cabañas–Carranza G, Soto–Galera E, Aguilar–Aguilar R (2001) A checklist of the helminth parasites of freshwater fishes from the Lerma–Santiago River Basin, Mexico. Comp Parasitol 68: 204–218
Schyzocotyle acheilognathi	Cyprinidae	*Hypophthalmichthys molitrix*	Hovhannissian RL (2000) The infection of fish in the carp farms of the Ararat plains. Acta Parasitologica 45(3): 263
Schyzocotyle acheilognathi	Cyprinidae	*Hypophthalmichthys nobilis*	Salgado–Maldonado G, Matamoros WA, Kreiser BR, Caspeta–Mandujano JM, Mendoza–Franco EF (2015) First record of the invasive Asian fish tapeworm Bothriocephalus acheilognathi in Honduras, Central America. Parasite 22: 5
Schyzocotyle acheilognathi	Eleotridae	*Hypseleotris klunzingeri*	Dove ADM, Fletcher AS (2000) The distribution of the introduced tapeworm *Bothriocephalus acheilognathi* in Australian freshwater fishes. J Helminthol 74: 121–127
Schyzocotyle acheilognathi	Ictaluridae	*Ictalurus punctatus*	Choudhury A, Hoffnagle TL, Cole RA (2004) parasites of native and nonnative fishes of the little Colorado river, Grand canyon, Arizona. J Parasitol 90: 1042–1053

Schyzocotyle acheilognathi	Cyprinidae	*Lepidomeda mollispinis*	Heckmann RA (2000) Asian tapeworm, Bothriocephalus acheilognathi (Yamaguti, 1934), a recent cestode introduction into the western United States of America; control methods and effect of endangered fish populations. Proc Parasitol 29: 1–24
Schyzocotyle acheilognathi	Centrarchidae	*Lepomis cyanellus*	Marcogliese DJ, Esch GW (1989) Experimental and natural infection of planktonic and benthic copepods by the Asian tapeworm, Bothriocephalus acheilognathi. Proc Helminthol S Washington 56: 151–155
Schyzocotyle acheilognathi	Centrarchidae	*Lepomis gibbosus*	Nedeva I (1988) To the biology of Bothriocephalus acheilognathi Yamaguti, 1934 (fam. Bothriocephalidae). Khelmintologiya 26: 32–38
Schyzocotyle acheilognathi	Cyprinidae	*Leuciscus cephalus*	Nedeva I (1988) To the biology of Bothriocephalus acheilognathi Yamaguti, 1934 (fam. Bothriocephalidae). Khelmintologiya 26: 32–38
Schyzocotyle acheilognathi	Cyprinidae	*Leuciscus idus*	Dove ADM, Fletcher AS (2000) The distribution of the introduced tapeworm *Bothriocephalus acheilognathi* in Australian freshwater fishes. J Helminthol 74: 121–127
Schyzocotyle acheilognathi	Cyprinidae	*Megalobrama amblycephala*	Salgado–Maldonado G, Pineda–Lopez RE (2003) The Asian fish tapeworm Bothriocephalus acheilognathi: a potential threat to native freshwater fish species in Mexico. Biol Inv 5: 261–268
Schyzocotyle acheilognathi	Atherinopsidae	*Melaniris balsanus*	Scholz T (1997) A revision of the species of Bothriocephalus Rudolphi, 1808 (Cestoda: Pseudophyllidea) parasitic in American freshwater fishes. Syst Parasitol 36: 85–107
Schyzocotyle acheilognathi	Centrarchidae	*Micropterus salmoides*	Salgado G, Sarabia DO (1987) Helmintis de algunos peces del lago de Patzcuaro. Ciencia y Desarrollo 74: 41–57
Schyzocotyle acheilognathi	Moronidae	*Morone chrysops*	Choudhury A, Charipar E, Nelson P, Hodgson JR, Bonar S, Cole RA (2006) Update on the distribution of the invasive asian fish tapeworm, Bothriocephalus acheilognathi, in the U.S. and canada. Comp Parasitol 73: 269–273
Schyzocotyle acheilognathi	Cyprinidae	*Mylocheilus caurinus*	Scholz T (1997) A revision of the species of Bothriocephalus Rudolphi, 1808 (Cestoda: Pseudophyllidea) parasitic in American freshwater fishes. Syst Parasitol 36: 85–107
Schyzocotyle acheilognathi	Cyprinidae	*Mylopharyngodon piceus*	Gibson DI, Bray RA, Harris EA (Compilers) (2005) Host–Parasite Database of the Natural History Museum, London. URL. www.nhm.ac.uk/research–curation/scientific–resources/taxonomy–systematics/host–parasites

Schyzocotyle acheilognathi	Cichlidae	*Nandopsis istlanum*	Salgado–Maldonado G, Mercado–Silva N, Cabañas–Carranza G, Caspeta–Mandujano JM, Aguilar–Aguilar R, Iñiguez–Dávalos LI (2004) Helminth parasites of freshwater fishes of the Ayuquila River, Sierra de Manantlan Biosphere Reserve, West Central Mexico. Comp Parasitol 71: 67–72
Schyzocotyle acheilognathi	Nemacheilidae	*Nemachilus angorae*	Dove ADM, Fletcher AS (2000) The distribution of the introduced tapeworm *Bothriocephalus acheilognathi* in Australian freshwater fishes. J Helminthol 74: 121–127
Schyzocotyle acheilognathi	Goodeidae	*Neophorus diazi*	Peresbarbosa–Rojas E, Pérez–Ponce de Leon G, Prieto LG (1994) Helmintos parasitos de tres especies de peces (Goodeidae) del lago de Patzcuaro, Michoacan. Anales del Instituto de Biologia, Universidad Nacional Autonoma de Mexico 65: 201–204
Schyzocotyle acheilognathi	Cyprinidae	*Notemigonus crysoleucas*	Heckmann RA, Greger PD, Furter RC (1993) The Asian fish tapeworm, Bothriocephalus acheilognathi in fishes from Nevada. J Helminthol S Washington 60: 127–128
Schyzocotyle acheilognathi	Notopteridae	*Notopterus lutrensis*	Heckmann RA, Deacon JE, Greger PD (1986) Parasites of the woundfin minnow Plagopterus argentissimus, and other endemic fishes from the Virgin River, Utah. Great Basin Nat 46: 662–676
Schyzocotyle acheilognathi	Cyprinidae	*Notropis atherinoides*	Marcogliese DJ, Gendron AD, Forest JJH, Li W, Boyce K, El–Shehabi F, Drake DAR, Mandrak NE, Sherry J, McLaughlin JD (2016) Range expansion and molecular confirmation of the Asian fish tapeworm in the lower great lakes and St. lawrence river with notes on infections in baitfish. J Great Lakes Res 42: 819–828
Schyzocotyle acheilognathi	Cyprinidae	*Notropis celayensis*	Salgado–Maldonado G, Pineda–López RE (2003) The Asian fish tapeworm Bothriocephalus acheilognathi: a potential threat to native freshwater fish species in Mexico. Biol Invasions 5: 261–268
Schyzocotyle acheilognathi	Cyprinidae	*Notropis lutrensis*	Scholz T (1997) A revision of the species of Bothriocephalus Rudolphi, 1808 (Cestoda: Pseudophyllidea) parasitic in American freshwater fishes. Syst Parasitol 36: 85–107
Schyzocotyle acheilognathi	Cyprinidae	*Notropis sallei*	Salgado–Maldonado G, Pineda–Lopez RE (2003) The Asian fish tapeworm Bothriocephalus acheilognathi: a potential threat to native freshwater fish species in Mexico. Biol Inv 5: 261–268
Schyzocotyle acheilognathi	Cyprinidae	*Opsariichthys uncirostris*	Dove ADM, Fletcher AS (2000) The distribution of the introduced tapeworm *Bothriocephalus acheilognathi* in Australian freshwater fishes. J Helminthol 74: 121–127

Schyzocotyle acheilognathi	Cichlidae	*Oreochromis niloticus*	Salgado–Maldonado G, Pineda–Lopez RE (2003) The Asian fish tapeworm Bothriocephalus acheilognathi: a potential threat to native freshwater fish species in Mexico. Biol Inv 5: 261–268
Schyzocotyle acheilognathi	Cyprinidae	*Pelecus cultratus*	Gavrilova NG, Karimov SB (1989) On the changes in the parasite fauna of fishes of the Kairakkum water reservoir for many years. Parazitologiya. Akademiya Nauk SSSR. Leningrad 23(3): 250–256
Schyzocotyle acheilognathi	Eleotridae	*Phylipnodon grandiceps*	Dove ADM, Fletcher AS (2000) The distribution of the introduced tapeworm *Bothriocephalus acheilognathi* in Australian freshwater fishes. J Helminthol 74: 121–127
Schyzocotyle acheilognathi	Cyprinidae	*Pimephales notatus*	Marcogliese DJ, Gendron AD, Forest JJH, Li W, Boyce K, El–Shehabi F, Drake DAR, Mandrak NE, Sherry J, McLaughlin JD (2016) Range expansion and molecular confirmation of the Asian fish tapeworm in the lower great lakes and St. lawrence river with notes on infections in baitfish. J Great Lakes Res 42: 819–828
Schyzocotyle acheilognathi	Cyprinidae	*Pimephales notatus*	Scholz T (1997) A revision of the species of Bothriocephalus Rudolphi, 1808 (Cestoda: Pseudophyllidea) parasitic in American freshwater fishes. Syst Parasitol 36: 85–107
Schyzocotyle acheilognathi	Cyprinidae	*Pimephales promelas*	Choudhury A, Charipar E, Nelson P, Hodgson JR, Bonar S, Cole RA (2006) Update on the distribution of the invasive asian fish tapeworm, Bothriocephalus acheilognathi, in the U.S. and canada. Comp Parasitol 73: 269–273
Schyzocotyle acheilognathi	Cyprinidae	*Pimephales promelas*	Clarkson RW, Robinson AT, Hoffnagle L (1997) Asian tapeworm (Bothriocephalus acheilognathi) in native fishes from the Little Colorado River, Grand Canyon, Arizona. Great Basin Nat 57: 66–69
Schyzocotyle acheilognathi	Cyprinidae	*Pimephales promelas*	Dove ADM, Fletcher AS (2000) The distribution of the introduced tapeworm *Bothriocephalus acheilognathi* in Australian freshwater fishes. J Helminthol 74: 121–127
Schyzocotyle acheilognathi	Cyprinidae	*Plagopterus argentissimus*	Heckmann RA (2000) Asian tapeworm, Bothriocephalus acheilognathi (Yamaguti, 1934), a recent cestode introduction into the western United States of America; control methods and effect of endangered fish populations. Proc Parasitol 29: 1–24

Schyzocotyle acheilognathi	Poeciliidae	*Poecilia butleri*	Salgado–Maldonado G, Mercado–Silva N, Cabañas–Carranza G, Caspeta–Mandujano JM, Aguilar–Aguilar R, Iñiguez–Dávalos LI (2004) Helminth parasites of freshwater fishes of the Ayuquila River, Sierra de Manantlan Biosphere Reserve, West Central Mexico. Comp Parasitol 71: 67–72
Schyzocotyle acheilognathi	Poeciliidae	*Poecilia mexicana*	Vincent AG Font WF (2003) Host specificity and population structure of two exotic helminths, Camallanus cotti (Nematoda) and Bothricephalus acheilognathi (Cestoda), parasitizing exotic fishes in Waianu Stream, Oahu, Hawaii. J Parasitol 89(3): 540–544
Schyzocotyle acheilognathi	Poeciliidae	*Poecilia reticulata*	Vincent AG Font WF (2003) Host specificity and population structure of two exotic helminths, Camallanus cotti (Nematoda) and Bothricephalus acheilognathi (Cestoda), parasitizing exotic fishes in Waianu Stream, Oahu, Hawaii. J Parasitol 89(3): 540–544
Schyzocotyle acheilognathi	Poeciliidae	*Poecilia sphenops*	Salgado–Maldonado G, Pineda–Lopez RE (2003) The Asian fish tapeworm Bothriocephalus acheilognathi: a potential threat to native freshwater fish species in Mexico. Biol Inv 5: 261–268
Schyzocotyle acheilognathi	Poeciliidae	*Poeciliopsis baenschi*	Salgado–Maldonado G, Mercado–Silva N, Cabañas–Carranza G, Caspeta–Mandujano JM, Aguilar–Aguilar R, Iñiguez–Dávalos LI (2004) Helminth parasites of freshwater fishes of the Ayuquila River, Sierra de Manantlan Biosphere Reserve, West Central Mexico. Comp Parasitol 71: 67–72
Schyzocotyle acheilognathi	Poeciliidae	*Poeciliopsis gracilis*	Salgado–Maldonado G, Pineda–López RE (2003) The Asian fish tapeworm Bothriocephalus acheilognathi: a potential threat to native freshwater fish species in Mexico. Biol Invasions 5: 261–268
Schyzocotyle acheilognathi	Profundulidae	*Profundulus portillorum*	Salgado–Maldonado G, Matamoros WA, Kreiser BR, Caspeta–Mandujano JM, Mendoza–Franco EF (2015) First record of the invasive Asian fish tapeworm Bothriocephalus acheilognathi in Honduras, Central America. Parasite 22: 5
Schyzocotyle acheilognathi	Acipenseridae	*Pseudoscaphirhynchus kaumanni*	Dove ADM, Fletcher AS (2000) The distribution of the introduced tapeworm *Bothriocephalus acheilognathi* in Australian freshwater fishes. J Helminthol 74: 121–127
Schyzocotyle acheilognathi	Cyprinidae	*Ptychocheilus lucius*	Dove ADM, Fletcher AS (2000) The distribution of the introduced tapeworm *Bothriocephalus acheilognathi* in Australian freshwater fishes. J Helminthol 74: 121–127

Schyzocotyle acheilognathi	Cyprinidae	*Ptychocheilus oregonensis*	Dove ADM, Fletcher AS (2000) The distribution of the introduced tapeworm *Bothriocephalus acheilognathi* in Australian freshwater fishes. J Helminthol 74: 121–127
Schyzocotyle acheilognathi	Cyprinidae	*Puntius binotatus*	Dove ADM, Fletcher AS (2000) The distribution of the introduced tapeworm *Bothriocephalus acheilognathi* in Australian freshwater fishes. J Helminthol 74: 121–127
Schyzocotyle acheilognathi		*Retropinna semoni*	Dove ADM, Fletcher AS (2000) The distribution of the introduced tapeworm *Bothriocephalus acheilognathi* in Australian freshwater fishes. J Helminthol 74: 121–127
Schyzocotyle acheilognathi	Cyprinidae	*Rhinichthys osculus*	Clarkson RW, Robinson AT, Hoffnagle L (1997) Asian tapeworm (Bothriocephalus acheilognathi) in native fishes from the Little Colorado River, Grand Canyon, Arizona. Great Basin Nat 57: 66–69
Schyzocotyle acheilognathi	Cyprinidae	*Rhinichthys osculus*	Clarkson RW, Robinson AT, Hoffnagle TL (1997) Asian tapeworm (*Bothriocephalus acheilognathi*) in native fishes from the Little Colorado River, Grand Canyon, Arizona. Great Basin nat 57: 66–69
Schyzocotyle acheilognathi	Cyprinidae	*Rutilus rutilus*	Dove ADM, Fletcher AS (2000) The distribution of the introduced tapeworm *Bothriocephalus acheilognathi* in Australian freshwater fishes. J Helminthol 74: 121–127
Schyzocotyle acheilognathi	Cyprinidae	*Schizothorax esocinus*	Al–Kalaq SN (1998) The nervous system of the cestode Bothriocephalus acheilognathi (Pseudophyllidea). Dirasat Med Biol Sci 25: 157–163
Schyzocotyle acheilognathi	Cyprinidae	*Schizothorax intermedius*	Bauer ON, Karimov SB (1990) Patterns of parasitic infections of fishes in a water body with constant temperature. J Fish Biol 36(1): 1–8
Schyzocotyle acheilognathi	Siluridae	*Silurus glanis*	Dove ADM, Fletcher AS (2000) The distribution of the introduced tapeworm *Bothriocephalus acheilognathi* in Australian freshwater fishes. J Helminthol 74: 121–127
Schyzocotyle acheilognathi	Cyprinidae	*Siphateles bicolor mohavensis*	Gibson DI, Bray RA, Harris EA (Compilers) (2005) Host–Parasite Database of the Natural History Museum, London. URL. www.nhm.ac.uk/research–curation/scientific–resources/taxonomy–systematics/host–parasites
Schyzocotyle acheilognathi	Percidae	*Stizostedion lucioperca*	Gavrilova NG, Karimov SB (1989) On the changes in the parasite fauna of fishes of the Kairakkum water reservoir for many years. Parazitologiya. Akademiya Nauk SSSR. Leningrad 23(3): 250–256

Schyzocotyle acheilognathi	Cyprinidae	*Symphysodon discus*	Košuthová L, Šmiga Ľ, Oros M, Barčák D, Košuth P (2015) The pathogenic Asian fish tapeworm, Bothriocephalus acheilognathi Yamaguti, 1934 (cestoda) in the red discus (Symphysodon discus). Helminthologia 52: 287–292
Schyzocotyle acheilognathi	Cyprinidae	*Tinca tinca*	Scholz T, Di Cave D (1992) Bothriocephalus acheilognathi (Cestoda: Pseudophyllidea) parasite of freshwater fish in Italy. Parassitologia 34: 155–158
Schyzocotyle acheilognathi	Cyprinidae	*Varicorhinus heratensis*	Dove ADM, Fletcher AS (2000) The distribution of the introduced tapeworm *Bothriocephalus acheilognathi* in Australian freshwater fishes. J Helminthol 74: 121–127
Schyzocotyle acheilognathi	Goodeidae	*Xenotoca variata*	Salgado–Maldonado G, Pineda–Lopez RE (2003) The Asian fish tapeworm Bothriocephalus acheilognathi: a potential threat to native freshwater fish species in Mexico. Biol Inv 5: 261–268
Schyzocotyle acheilognathi	Poeciliidae	*Xiphophorus helleri*	Vincent AG, Font WF (2003) Host specificity and population structure of two exotic helminths, Camallanus cotti (Nematoda) and Bothricephalus acheilognathi (Cestoda), parasitizing exotic fishes in Waianu Stream, Oahu, Hawaii. J Parasitology 89: 540–544
Schyzocotyle acheilognathi	Poeciliidae	*Xiphophorus hellerii*	Chaudhary A, Singh HS (2016) Molecular evidence of Bothriocephalus acheilognathi (cestoda: Bothriocephalidea) from India. Int J Infect Dis 45: 355–356
Schyzocotyle acheilognathi	Cyprinidae	*Yuriria alta*	Salgado–Maldonado G, Pineda–Lopez RE (2003) The Asian fish tapeworm Bothriocephalus acheilognathi: a potential threat to native freshwater fish species in Mexico. Biol Inv 5: 261–268
Schyzocotyle acheilognathi	Cyprinidae	*Zacco platypus*	Gibson DI, Bray RA, Harris EA (Compilers) (2005) Host–Parasite Database of the Natural History Museum, London. URL. www.nhm.ac.uk/research–curation/scientific–resources/taxonomy–systematics/host–parasites

Supplementary S5

Supplementary material to:

Chapter 4: Parasite dispersal in the goldfish trade

Trujillo-González A., Becker J. A., and Hutson K. S.

Supplementary S5. Reference list for Figure 21. Records of Parasites infecting the five most farmed freshwater fish species globally. Fish species were selected based on total volume (tonnes) produced in 2016 reported to the Food and Agriculture Organization of the United Nations by gobal regions (i.e. Africa, Americas, Asia, Europe, and Oceania) (FAO 2017).

Region	fish host species	Parasite species	Reference
Africa	*Clarias gariepinus*	*Argulus japonicus*	Shafir A, Oldewage WH (1992) Dynamics of a fish ectoparasite population: Opportunistic parasitism in Argulus japonicus (Branchiura). Crustaceana 62: 50–64
Africa	*Cyprinus carpio*	*Argulus japonicus*	Shafir A, Oldewage WH (1992) Dynamics of a fish ectoparasite population: Opportunistic parasitism in Argulus japonicus (Branchiura). Crustaceana 62: 50–64
Africa	*Oreochromis niloticus*	*Argulus japonicus*	No record found
Africa	*Ictalurus punctatus*	*Argulus japonicus*	No record found
Africa	*Colossoma macropomum*	*Argulus japonicus*	No record found
Africa	*Cyprinus carpio*	*Dactylogyrus anchoratus*	Paperna I (1980) Parasites, Infections and Diseases of Fish in Africa. FAO/CIFA Technical Paper No. 7. FAO Publications, Rome, pp 216
Africa	*Oreochromis niloticus*	*Dactylogyrus anchoratus*	No record found
Africa	*Clarias gariepinus*	*Dactylogyrus anchoratus*	No record found
Africa	*Ictalurus punctatus*	*Dactylogyrus anchoratus*	No record found
Africa	*Colossoma macropomum*	*Dactylogyrus anchoratus*	No record found
Africa	*Oreochromis niloticus*	*Dactylogyrus baueri*	No record found

Africa	*Clarias gariepinus*	*Dactylogyrus baueri*	No record found
Africa	*Ictalurus punctatus*	*Dactylogyrus baueri*	No record found
Africa	*Colossoma macropomum*	*Dactylogyrus baueri*	No record found
Africa	*Cyprinus carpio*	*Dactylogyrus baueri*	No record found
Africa	*Cyprinus carpio*	*Dactylogyrus formosus*	Paperna I (1980) Parasites, Infections and Diseases of Fish in Africa. FAO/CIFA Technical Paper No. 7. FAO Publications, Rome, pp 216
Africa	*Oreochromis niloticus*	*Dactylogyrus formosus*	No record found
Africa	*Clarias gariepinus*	*Dactylogyrus formosus*	No record found
Africa	*Ictalurus punctatus*	*Dactylogyrus formosus*	No record found
Africa	*Colossoma macropomum*	*Dactylogyrus formosus*	No record found
Africa	*Oreochromis niloticus*	*Dactylogyrus vastator*	No record found
Africa	*Clarias gariepinus*	*Dactylogyrus vastator*	No record found
Africa	*Ictalurus punctatus*	*Dactylogyrus vastator*	No record found
Africa	*Colossoma macropomum*	*Dactylogyrus vastator*	No record found
Africa	*Cyprinus carpio*	*Dactylogyrus vastator*	No record found
Africa	*Oreochromis niloticus*	*Ichthyophthirius multifiliis*	Bruton MN, Merron SV (1985) Alien and translocated aquatic animals in southern Africa: a general introduction, checklist and bibliography. S Afr Nat Sci Prog Rep 13: 1–71
Africa	*Ictalurus punctatus*	*Ichthyophthirius multifiliis*	Goven, B.A., Dawe, D.I. and Gratzeck, J.B., 1981. Protection of channel catfish (Ictalurus punctatus) against Ichthyophthirius multifiliis (Fouquet) by immunisation with varying doses of Tetrahymena pyriformis (Lwoff) cilia. Aquaculture, 23: 269–273.
Africa	*Cyprinus carpio*	*Ichthyophthirius multifiliis*	Hines RS, Spira DT (1973) Ichthyophthiriasis in the mirror carp *Cyprinus carpio* L. I. Course of infection. J Fish Biol 5: 385–392

Africa	*Clarias gariepinus*	*Ichthyophthirius multifiliis*	Hecht T, Endemann F (1998) The impact of parasites, infections and diseases on the development of aquaculture in sub–Saharan Africa. J Appl Icth 14: 213–221
Africa	*Colossoma macropomum*	*Ichthyophthirius multifiliis*	No record found
Africa	*Clarias gariepinus*	*Lernaea cyprinacea*	Barson M, Mulonga A, Nhiwatiwa T (2008) Investigation of a parasitic outbreak of Lernaea cyprinacea Linnaeus (Crustacea: Copepoda) in fish from Zimbabwe. Afr Zool 43: 175–183
Africa	*Cyprinus carpio*	*Lernaea cyprinacea*	Boane C, Cruz C, Saraiva A (2008) Metazoan parasites of Cyprinus carpio L. (Cyprinidae) from Mozambique. Aquacult 284: 59–61
Africa	*Oreochromis niloticus*	*Lernaea cyprinacea*	Ibrahim MM, Soliman MFM (2011) Parasite community of wild and cultured *Oreochromis niloticus* from lake Manzalah, Egypt. J Egypt S Parasitol 41: 685
Africa	*Ictalurus punctatus*	*Lernaea cyprinacea*	No record found
Africa	*Colossoma macropomum*	*Lernaea cyprinacea*	No record found
Africa	*Cyprinus carpio*	*Schyzocotyle acheilognathi*	Salgado–Maldonado G, Pineda–Lopez RE (2003) The Asian fish tapeworm Bothriocephalus acheilognathi: a potential threat to native freshwater fish species in Mexico. Biol Inv 5: 261–268
Africa	*Oreochromis niloticus*	*Schyzocotyle acheilognathi*	Bruton MN, Merron SV (1985) Alien and translocated aquatic animals in southern Africa: a general introduction, checklist and bibliography. S Afr Nat Sci Prog Rep 13: 1–71

Africa	*Colossoma macropomum*	*Schyzocotyle acheilognathi*	Kuchta R, Burianová A, Jirků M, Chambrier A,Oros M, Brabec J, Scholz T (2012) Bothriocephalidean tapeworms (Cestoda) of freshwater fish in Africa, including erection of Kirstenella n. gen. and description of Tetracampos martinae n. sp.. Zootaxa 3309: 1–35
Africa	*Clarias gariepinus*	*Schyzocotyle acheilognathi*	No record found
Africa	*Ictalurus punctatus*	*Schyzocotyle acheilognathi*	No record found
Americas	*Oreochromis niloticus*	*Argulus japonicus*	No record found
Americas	*Hypophthalmichthys molitrix*	*Argulus japonicus*	No record found
Americas	*Cyprinus carpio*	*Argulus japonicus*	No record found
Americas	*Oreochromis aureus*	*Argulus japonicus*	No record found
Americas	*Ctenopharyngodon idella*	*Argulus japonicus*	No record found
Americas	*Cyprinus carpio*	*Dactylogyrus anchoratus*	Kritsky DC, Heckmann R (2002) Species of Dactylogyrus (Monogenoidea: Dactylogyridae) and Trichodina mutabilis (Ciliata) infesting koi carp, Cyprinus carpio, during mass mortality at a commercial rearing facility in Utah, U.S.A. Comp Parasitol 69: 217–218
Americas	*Oreochromis niloticus*	*Dactylogyrus anchoratus*	No record found
Americas	*Hypophthalmichthys molitrix*	*Dactylogyrus anchoratus*	No record found
Americas	*Oreochromis aureus*	*Dactylogyrus anchoratus*	No record found
Americas	*Ctenopharyngodon idella*	*Dactylogyrus anchoratus*	No record found
Americas	*Oreochromis niloticus*	*Dactylogyrus baueri*	No record found
Americas	*Hypophthalmichthys molitrix*	*Dactylogyrus baueri*	No record found
Americas	*Cyprinus carpio*	*Dactylogyrus baueri*	No record found
Americas	*Oreochromis aureus*	*Dactylogyrus baueri*	No record found
Americas	*Ctenopharyngodon idella*	*Dactylogyrus baueri*	No record found

Americas	*Cyprinus carpio*	*Dactylogyrus formosus*	Kritsky DC, Heckmann R (2002) Species of Dactylogyrus (Monogenoidea: Dactylogyridae) and Trichodina mutabilis (Ciliata) infesting koi carp, Cyprinus carpio, during mass mortality at a commercial rearing facility in Utah, U.S.A. Comp Parasitol 69: 217–218
Americas	*Oreochromis niloticus*	*Dactylogyrus formosus*	No record found
Americas	*Hypophthalmichthys molitrix*	*Dactylogyrus formosus*	No record found
Americas	*Oreochromis aureus*	*Dactylogyrus formosus*	No record found
Americas	*Ctenopharyngodon idella*	*Dactylogyrus formosus*	No record found
Americas	*Oreochromis niloticus*	*Dactylogyrus vastator*	No record found
Americas	*Hypophthalmichthys molitrix*	*Dactylogyrus vastator*	No record found
Americas	*Cyprinus carpio*	*Dactylogyrus vastator*	No record found
Americas	*Oreochromis aureus*	*Dactylogyrus vastator*	No record found
Americas	*Ctenopharyngodon idella*	*Dactylogyrus vastator*	No record found
Americas	*Hypophthalmichthys molitrix*	*Ichthyophthirius multifiliis*	Dickerson HW, Dawe DL (2006) Ichthyophthirius multifiliis and Cryptocaryon irritans (phylum Ciliophora). Fish Dis Dis 1: 116–153
Americas	*Cyprinus carpio*	*Ichthyophthirius multifiliis*	Dickerson HW, Dawe DL (2006) Ichthyophthirius multifiliis and Cryptocaryon irritans (phylum Ciliophora). Fish Dis Dis 1: 116–153
Americas	*Oreochromis aureus*	*Ichthyophthirius multifiliis*	Dickerson HW, Dawe DL (2006) Ichthyophthirius multifiliis and Cryptocaryon irritans (phylum Ciliophora). Fish Dis Dis 1: 116–153
Americas	*Ctenopharyngodon idella*	*Ichthyophthirius multifiliis*	Riley DM (1978) Parasites of grass carp and native fishes in Florida. Trans Am Fish Soc 107(1), 207–212.
Americas	*Oreochromis niloticus*	*Ichthyophthirius multifiliis*	Xu DH, Klesius PH, Shoemaker CA (2008) Protective immunity of Nile tilapia against Ichthyophthirius multifiliis post–immunization with live theronts and sonicated trophonts. Fish shellfish immunol 25: 124–127

Americas	*Ctenopharyngodon idella*	*Lernaea cyprinacea*	Riley DM (1978) Parasites of grass carp and native fishes in Florida. Trans Am Fish Soc 107(1), 207–212.
Americas	*Oreochromis niloticus*	*Lernaea cyprinacea*	No record found
Americas	*Hypophthalmichthys molitrix*	*Lernaea cyprinacea*	No record found
Americas	*Cyprinus carpio*	*Lernaea cyprinacea*	No record found
Americas	*Oreochromis aureus*	*Lernaea cyprinacea*	No record found
Americas	*Oreochromis aureus*	*Schyzocotyle acheilognathi*	Mitchell AJ, Hobbs MS (2007) The acute toxicity of praziquantel to grass carp and golden shiners. N Amer J Aquacult 69: 203–206
Americas	*Hypophthalmichthys molitrix*	*Schyzocotyle acheilognathi*	Scholz T (1997) A revision of the species of *Bothriocephalus Rudolphi*, 1808 (Cestoda: Pseudophyllidea) parasitic in American freshwater fishes. Syst Parasitol 36: 85–107
Americas	*Ctenopharyngodon idella*	*Schyzocotyle acheilognathi*	Xi B, Wang G, Xie J (2011) Occurrence of *Bothriocephalus acheilognathi* (Cestoda, Bothriocephallidea) in grass carp *Ctenopharyngodon idella* in the Changjiang River drainage. Chinese J Oceanol Limnol 29: 564–567.
Americas	*Oreochromis niloticus*	*Schyzocotyle acheilognathi*	No record found
Americas	*Cyprinus carpio*	*Schyzocotyle acheilognathi*	No record found
Asia	*Hypophthalmichthys molitrix*	*Argulus japonicus*	Alsarakibi M, Wadeh H, Li G (2014) Parasitism of *Argulus japonicus* in cultured and wild fish of Guangdong, China with new record of three hosts. Parasitol Res 113: 769–775
Asia	*Hypophthalmichthys nobilis*	*Argulus japonicus*	Nagasawa K (2011) The biology of Argulus spp. (Branchiura, Argulidae) in Japan: a review. In: New Frontiers in Crustacean Biology. Brill, 15–22

Asia	*Catla catla*	*Argulus japonicus*	Sahoo PK, Hemaprasanth, Kar B, Garnayak SK, Mohanty J (2012) Mixed infection of Argulus japonicus and Argulus siamensis (Branchiura, Argulidae) in carps (Pisces, Cyprinidae): Loss estimation and a comparative invasive pattern study. Crustaceana 85: 1449–1462
Asia	*Cyprinus carpio*	*Argulus japonicus*	Sahoo PK, Mohanty J, Garnayak SK, Mohanty BR, Kar B, Jena J, Prasanth H (2013) Genetic diversity and species identification of *Argulus* parasites collected from major aquaculture regions of India using RAPD-PCR. Aquacult Res 44: 220–230
Asia	*Ctenopharyngodon idellus*	*Argulus japonicus*	No record found
Asia	*Cyprinus carpio*	*Dactylogyrus anchoratus*	Simkova A, Plaisance L, Matejusova I, Morand S, Verneau O (2004) Phylogenetic relationships of the Dactylogyridae Bychowsky, 1933 (Monogenea: Dactylogyridea): the need for the systematic revision of the Ancyrocephalinae Bychowsky, 1937. Syst Parasitol 54: 1–11
Asia	*Ctenopharyngodon idellus*	*Dactylogyrus anchoratus*	No record found
Asia	*Hypophthalmichthys molitrix*	*Dactylogyrus anchoratus*	No record found
Asia	*Hypophthalmichthys nobilis*	*Dactylogyrus anchoratus*	No record found
Asia	*Catla catla*	*Dactylogyrus anchoratus*	No record found
Asia	*Ctenopharyngodon idellus*	*Dactylogyrus baueri*	No record found
Asia	*Hypophthalmichthys molitrix*	*Dactylogyrus baueri*	No record found
Asia	*Cyprinus carpio*	*Dactylogyrus baueri*	No record found
Asia	*Hypophthalmichthys nobilis*	*Dactylogyrus baueri*	No record found
Asia	*Catla catla*	*Dactylogyrus baueri*	No record found

Asia	*Cyprinus carpio*	*Dactylogyrus formosus*	Simkova A, Plaisance L, Matejusova I, Morand S, Verneau O (2004) Phylogenetic relationships of the Dactylogyridae Bychowsky, 1933 (Monogenea: Dactylogyridea): the need for the systematic revision of the Ancyrocephalinae Bychowsky, 1937. Syst Parasitol 54: 1–11
Asia	*Ctenopharyngodon idellus*	*Dactylogyrus formosus*	No record found
Asia	*Hypophthalmichthys molitrix*	*Dactylogyrus formosus*	No record found
Asia	*Hypophthalmichthys nobilis*	*Dactylogyrus formosus*	No record found
Asia	*Catla catla*	*Dactylogyrus formosus*	No record found
Asia	*Hypophthalmichthys molitrix*	*Dactylogyrus vastator*	Alam MM, Khan MA, Hussain MA, Moumita D, Mazlan AG, Simon KD (2012) Intensity of parasitic infestation in silver carp, *Hypophthalmichthys molitrix*. Journal of Zhejiang University. Science. B, 13: 1024–1028
Asia	*Ctenopharyngodon idellus*	*Dactylogyrus vastator*	No record found
Asia	*Cyprinus carpio*	*Dactylogyrus vastator*	No record found
Asia	*Hypophthalmichthys nobilis*	*Dactylogyrus vastator*	No record found
Asia	*Catla catla*	*Dactylogyrus vastator*	No record found
Asia	*Hypophthalmichthys molitrix*	*Ichthyophthirius multifiliis*	Alam MM, Khan MA, Hussain MA, Moumita D, Mazlan AG, Simon KD (2012) Intensity of parasitic infestation in silver carp, *Hypophthalmichthys molitrix*. Journal of Zhejiang University. Science. B, 13: 1024–1028
Asia	*Catla catla*	*Ichthyophthirius multifiliis*	Arthur JR, Ahmed ATA (2002) Checklist of the parasites of fishes of Bangladesh. FAO Fisheries Technical Paper. No. 369/1. Rome, FAO. pp. 77

Asia	*Ctenopharyngodon idellus*	*Ichthyophthirius multifiliis*	Chih–leu C (1956) The protozoan parasites from four species of Chinese pond fishes: Ctenopharyngodon idellus, Mylopharyngodon piceus, Aristichthys nobilis and Hypophthalmichthys molitrix II. The protozoan parasites of Mylopharyngodon piceus. Acta Hydrobiologica Sinica 2: 296
Asia	*Hypophthalmichthys nobilis*	*Ichthyophthirius multifiliis*	Chih–leu C (1956) The protozoan parasites from four species of Chinese pond fishes: Ctenopharyngodon idellus, Mylopharyngodon piceus, Aristichthys nobilis and Hypophthalmichthys molitrix II. The protozoan parasites of Mylopharyngodon piceus. Acta Hydrobiologica Sinica 2: 296
Asia	*Cyprinus carpio*	*Ichthyophthirius multifiliis*	Lumanlan SC, Albaladejo MG, Bondad–Reantaso, Arthur JR (1992) Freshwater fish imported into the Philippines: their parasite faunas and role in the international spread of parasitic diseases. In: Shariff M, Subasinghe RP, Arthur JR (ed) Diseases in Asian Aquaculture, I. Fish Health Sector, Asian Fish Society, Manila, pp 323–335
Asia	*Cyprinus carpio*	*Lernaea cyprinacea*	Kabata Z (1985) Parasites and diseases of fish cultured in the tropics. Taylor and Francis Ltd., London, United Kingdom, pp 318
Asia	*Hypophthalmichthys molitrix*	*Lernaea cyprinacea*	Alam MM, Khan MA, Hussain MA, Moumita D, Mazlan AG, Simon KD (2012) Intensity of parasitic infestation in silver carp, *Hypophthalmichthys molitrix*. Journal of Zhejiang University. Science. B, 13: 1024–1028
Asia	*Catla catla*	*Lernaea cyprinacea*	Tamuli KK, Shanbhogue SL (1995) Biological control of Lernaea L. infection employing Oreochromis mossambica, Peters. J Assam Sci Soc 37: 123–128

Asia	*Ctenopharyngodon idellus*	*Lernaea cyprinacea*	Tasawar Z, Zafar S, Lashari MH, Hayat CS (2009) The prevalence of lernaeid ectoparasites in grass carp (Ctenopharyngodon idella). Pak Vet J 29: 95–96
Asia	*Hypophthalmichthys nobilis*	*Lernaea cyprinacea*	No record found
Asia	*Hypophthalmichthys molitrix*	*Schyzocotyle acheilognathi*	Han JE, Shin SP, Kim JH, Choresca CH, Jun JW, Gomez DK, Park SC (2010) Mortality of cultured Koi *Cyprinus carpio* in Korea caused by *Bothriocephalus acheilognathi*. Afr J Microbiol Res 4: 543–546
Asia	*Catla catla*	*Schyzocotyle acheilognathi*	Kennedy CR, Pojmanska T (1996) Richness and diversity of helminth parasite communities in the common carp and in three more recently introduced carp species. J Fish bio 48: 89–100
Asia	*Ctenopharyngodon idellus*	*Schyzocotyle acheilognathi*	Lumanlan SC, Albaladejo MG, Bondad–Reantaso, Arthur JR (1992) Freshwater fish imported into the Philippines: their parasite faunas and role in the international spread of parasitic diseases. In: Shariff M, Subasinghe RP, Arthur JR (ed) Diseases in Asian Aquaculture, I. Fish Health Sector, Asian Fish Society, Manila, pp 323–335
Asia	*Cyprinus carpio*	*Schyzocotyle acheilognathi*	Lumanlan SC, Albaladejo MG, Bondad–Reantaso, Arthur JR (1992) Freshwater fish imported into the Philippines: their parasite faunas and role in the international spread of parasitic diseases. In: Shariff M, Subasinghe RP, Arthur JR (ed) Diseases in Asian Aquaculture, I. Fish Health Sector, Asian Fish Society, Manila, pp 323–335
Asia	*Hypophthalmichthys nobilis*	*Schyzocotyle acheilognathi*	No record found
Europe	*Cyprinus carpio*	*Argulus japonicus*	Khan S, Ali W, Javid M, Ullah I, Hussain G, Shahnaz Z, Ullah I, Ullah I (2017). Prevalence of Argulus in Common Carp (Cyprinus carpio) From D.I. Khan (Khyber Pakhtunkhwa) Pakistan. J Entomol Zool S 5: 203–205

Europe	*Rutilus rutilus*	*Argulus japonicus*	Soes DM, Walker PD, Kruijt DB (2010) The Japanese fish louse Argulus japonicus new for The Netherlands. Lauterbornia 70: 11–17
Europe	*Hypophthalmichthys molitrix*	*Argulus japonicus*	No record found
Europe	*Ctenopharyngodon idella*	*Argulus japonicus*	No record found
Europe	*Hypophthalmichthys nobilis*	*Argulus japonicus*	No record found
Europe	*Cyprinus carpio*	*Dactylogyrus anchoratus*	Shamsi S, Jalali B, Aghazadeh Meshgi M (2009) Infection with Dactylogyrus spp. among introduced cyprinid fishes and their geographical distribution in Iran. Iranian J Vet Res 10: 70–74
Europe	*Hypophthalmichthys molitrix*	*Dactylogyrus anchoratus*	No record found
Europe	*Ctenopharyngodon idella*	*Dactylogyrus anchoratus*	No record found
Europe	*Hypophthalmichthys nobilis*	*Dactylogyrus anchoratus*	No record found
Europe	*Rutilus rutilus*	*Dactylogyrus anchoratus*	No record found
Europe	*Cyprinus carpio*	*Dactylogyrus baueri*	No record found
Europe	*Hypophthalmichthys molitrix*	*Dactylogyrus baueri*	No record found
Europe	*Ctenopharyngodon idella*	*Dactylogyrus baueri*	No record found
Europe	*Hypophthalmichthys nobilis*	*Dactylogyrus baueri*	No record found
Europe	*Rutilus rutilus*	*Dactylogyrus baueri*	No record found
Europe	*Cyprinus carpio*	*Dactylogyrus formosus*	No record found
Europe	*Hypophthalmichthys molitrix*	*Dactylogyrus formosus*	No record found
Europe	*Ctenopharyngodon idella*	*Dactylogyrus formosus*	No record found
Europe	*Hypophthalmichthys nobilis*	*Dactylogyrus formosus*	No record found
Europe	*Rutilus rutilus*	*Dactylogyrus formosus*	No record found
Europe	*Ctenopharyngodon idella*	*Dactylogyrus vastator*	Mhaisen FT, Al–Rubaie ARL (2016) Checklists of Parasites of Farm Fishes of Babylon Province, Iraq. J Parasitol Res 2016: 1–15

Europe	*Cyprinus carpio*	*Dactylogyrus vastator*	Shamsi S, Jalali B, Aghazadeh Meshgi M (2009) Infection with Dactylogyrus spp. among introduced cyprinid fishes and their geographical distribution in Iran. Iranian J Vet Res 10: 70–74
Europe	*Hypophthalmichthys molitrix*	*Dactylogyrus vastator*	No record found
Europe	*Hypophthalmichthys nobilis*	*Dactylogyrus vastator*	No record found
Europe	*Rutilus rutilus*	*Dactylogyrus vastator*	No record found
Europe	*Cyprinus carpio*	*Ichthyophthirius multifiliis*	Nematollahi A, Ahmadi A, Mohammadpour H, Ebrahimi M (2013) External parasite infection of common carp (Cyprinus carpio) and big head (Hypophthalmichthys nobilis) in fish farms of Mashhad, northeast of Iran. J Para Dis 37: 131–133
Europe	*Hypophthalmichthys nobilis*	*Ichthyophthirius multifiliis*	Nematollahi A, Ahmadi A, Mohammadpour H, Ebrahimi M (2013) External parasite infection of common carp (Cyprinus carpio) and big head (Hypophthalmichthys nobilis) in fish farms of Mashhad, northeast of Iran. J Para Dis 37: 131–133
Europe	*Ctenopharyngodon idella*	*Ichthyophthirius multifiliis*	Uzbilek MK, Yildiz HY (2002) A report on spontaneous diseases in the culture of grass carp (*Ctenopharyngodon idella* Val. 1844), Turkey. Turk J Vet Animal Sci, 26: 407–410
Europe	*Rutilus rutilus*	*Ichthyophthirius multifiliis*	Valtonen ET, Holmes JC, Koskivaara M (1997) Eutrophication, pollution and fragmentation: effects on parasite communities in roach (*Rutilus rutilus*) and perch (*Perca fluviatilis*) in four lakes in central Finland. Can J Fisheries Aq Sci 54: 572–585
Europe	*Hypophthalmichthys molitrix*	*Ichthyophthirius multifiliis*	No record found

Europe	*Hypophthalmichthys molitrix*	*Lernaea cyprinacea*	Barzegar M, Raeisi M, Bozorgnia A, Jalali B (2008) Parasites of the eyes of fresh and brackish water fishes in Iran. Iranian J Vet Res 9: 256–261
Europe	*Rutilus rutilus*	*Lernaea cyprinacea*	Fryer G (1968) The parasitic copepod Lernaea cyprinacea L. in Britain. J Nat Hist 2: 531–533
Europe	*Hypophthalmichthys nobilis*	*Lernaea cyprinacea*	Nematollahi A, Ahmadi A, Mohammadpour H, Ebrahimi M (2013) External parasite infection of common carp (Cyprinus carpio) and big head (Hypophthalmichthys nobilis) in fish farms of Mashhad, northeast of Iran. J Para Dis 37: 131–133
Europe	*Cyprinus carpio*	*Lernaea cyprinacea*	Perveen F, Ullah H (2013) Ectoparasites of indigenous and exotic fresh water carp fish (Cypriniformes: Cyprinidae) from Charbanda and Tarbela, Khyber Pakhtunkhwa, Pakistan. Amer J Res Comm 1: 255–269
Europe	*Ctenopharyngodon idella*	*Lernaea cyprinacea*	Tasawar Z, Zafar S, Lashari MH, Hayat CS (2009) The prevalence of lernaeid ectoparasites in grass carp (Ctenopharyngodon idella). Pakistan Vet J 29: 95–96
Europe	*Cyprinus carpio*	*Schyzocotyle acheilognathi*	Hovhannissian RL (2000) The infection of fish in the carp farms of the Ararat plains. Acta Parasitologica 45: 263
Europe	*Hypophthalmichthys molitrix*	*Schyzocotyle acheilognathi*	Kennedy CR, Pojmanska T (1996) Richness and diversity of helminth parasite communities in the common carp and in three more recently introduced carp species. J Fish bio 48: 89–100
Europe	*Hypophthalmichthys nobilis*	*Schyzocotyle acheilognathi*	Öktener A (2014) Revision of parasitic helminths reported in freshwater fish from Turkey with new records. Transyl Rev Syst Ecol Res 16: 1–56
Europe	*Ctenopharyngodon idella*	*Schyzocotyle acheilognathi*	No record found
Europe	*Rutilus rutilus*	*Schyzocotyle acheilognathi*	No record found
Oceania	*Oreochromis niloticus*	*Argulus japonicus*	No record found

Oceania	*Cyprinus carpio*	*Argulus japonicus*	No record found
Oceania	*Bidyanus bidyanus*	*Argulus japonicus*	No record found
Oceania	*Oreochromis mossambicus*	*Argulus japonicus*	No record found
Oceania	*Oreochromis niloticus*	*Dactylogyrus anchoratus*	No record found
Oceania	*Cyprinus carpio*	*Dactylogyrus anchoratus*	No record found
Oceania	*Bidyanus bidyanus*	*Dactylogyrus anchoratus*	No record found
Oceania	*Oreochromis mossambicus*	*Dactylogyrus anchoratus*	No record found
Oceania	*Oreochromis niloticus*	*Dactylogyrus baueri*	No record found
Oceania	*Cyprinus carpio*	*Dactylogyrus baueri*	No record found
Oceania	*Bidyanus bidyanus*	*Dactylogyrus baueri*	No record found
Oceania	*Oreochromis mossambicus*	*Dactylogyrus baueri*	No record found
Oceania	*Oreochromis niloticus*	*Dactylogyrus formosus*	No record found
Oceania	*Cyprinus carpio*	*Dactylogyrus formosus*	No record found
Oceania	*Bidyanus bidyanus*	*Dactylogyrus formosus*	No record found
Oceania	*Oreochromis mossambicus*	*Dactylogyrus formosus*	No record found
Oceania	*Oreochromis niloticus*	*Dactylogyrus vastator*	No record found
Oceania	*Cyprinus carpio*	*Dactylogyrus vastator*	No record found
Oceania	*Bidyanus bidyanus*	*Dactylogyrus vastator*	No record found
Oceania	*Oreochromis mossambicus*	*Dactylogyrus vastator*	No record found
Oceania	*Oreochromis niloticus*	*Ichthyophthirius multifiliis*	Arthur JR, Lumanlan–Mayo S (1997) Checklist of the parasites of fishes of the Philippines. FAO Fisheries Technical Paper 369. Rome, FAO. pp. 102
Oceania	*Cyprinus carpio*	*Ichthyophthirius multifiliis*	Arthur JR, Lumanlan–Mayo S (1997) Checklist of the parasites of fishes of the Philippines. FAO Fisheries Technical Paper 369. Rome, FAO. pp. 102
Oceania	*Bidyanus bidyanus*	*Ichthyophthirius multifiliis*	Mifsud C, Rowland SJ (2008) Use of salt to control ichthyophthiriosis and prevent saprolegniosis in silver perch, *Bidyanus bidyanus*. Aquacult Res 39: 1175–1180.

Oceania	*Oreochromis mossambicus*	*Ichthyophthirius multifiliis*	No record found
Oceania	*Cyprinus carpio*	*Lernaea cyprinacea*	Thilakaratne IDSIP, Rajapaksha G, Hewakopara A, Rajapakse RPVJ, Faizal ACM (2003) Parasitic infections in freshwater ornamental fish in Sri Lanka. Dis Aquat Org 54: 157–162
Oceania	*Oreochromis niloticus*	*Lernaea cyprinacea*	No record found
Oceania	*Bidyanus bidyanus*	*Lernaea cyprinacea*	No record found
Oceania	*Oreochromis mossambicus*	*Lernaea cyprinacea*	No record found
Oceania	*Oreochromis niloticus*	*Schyzocotyle acheilognathi*	Dove ADM, Fletcher AS (2000) The distribution of the introduced tapeworm *Bothriocephalus acheilognathi* in Australian freshwater fishes. J Helminthol 74: 121–127
Oceania	*Cyprinus carpio*	*Schyzocotyle acheilognathi*	Dove ADM, Fletcher AS (2000) The distribution of the introduced tapeworm *Bothriocephalus acheilognathi* in Australian freshwater fishes. J Helminthol 74: 121–127
Oceania	*Bidyanus bidyanus*	*Schyzocotyle acheilognathi*	No record found
Oceania	*Oreochromis mossambicus*	*Schyzocotyle acheilognathi*	No record found

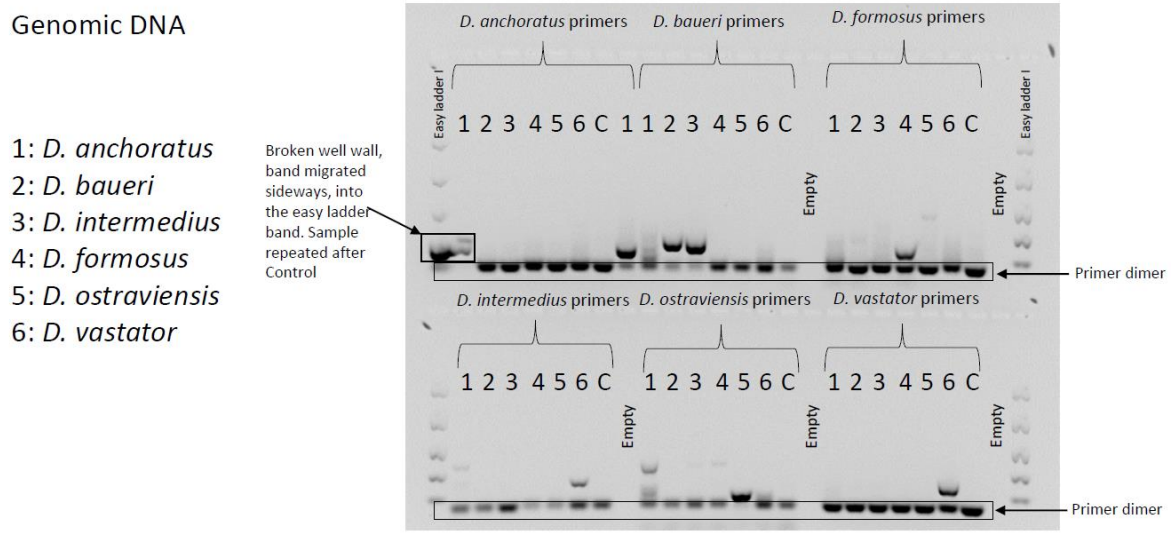
Supplementary S6

Supplementary material to:

Chapter 5: Parasite detection in the ornamental fish trade using environmental DNA

Trujillo-González, A., Edmunds, R.C., Becker, J.A., Hutson, K.S.

Supplementary S6. Primer cross-reactivity tests. Quantitative PCR tests were run at 60 and 65 °C to test cross-reactivity for all primers used in this study. Primers were initially tested for another species of *Dactylogyrus* (*D. baueri*), but cross-reactivity was not eliminated. As such, tests for *D. baueri* were removed from this study.



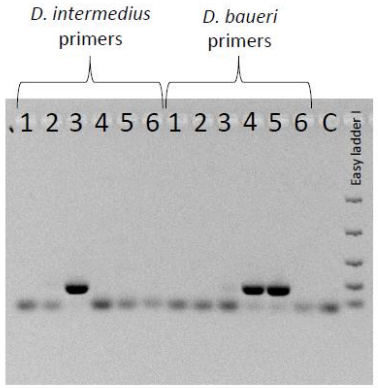
Cross-reactivity test 1

qPCR cycling conditions:
95/4min, (95/15s-60/30s-72/30s) 40 cycles, 72/7 min

Results:
D. anchoratus: Specific, test annealing at 65 °C to confirm
D. baueri: non-specific (intermedius). Develop new primer pair.
D. intermedius: Amplified wrong DNA, develop new primer.
D. formosus: Non-specific, test annealing at 65 °C.
D. ostraviensis: Non-specific, test , test annealing at 65 °C.
D. vastator: specific.

Genomic DNA

1: *D. anchoratus*
2: *D. baueri*
3: *D. intermedius*
4: *D. formosus*
5: *D. ostraviensis*
6: *D. vastator*



Cross-reactivity test 2

qPCR cycling conditions:
95/4min, (95/15s-60/30s-72/30s) 40 cycles, 72/7 min

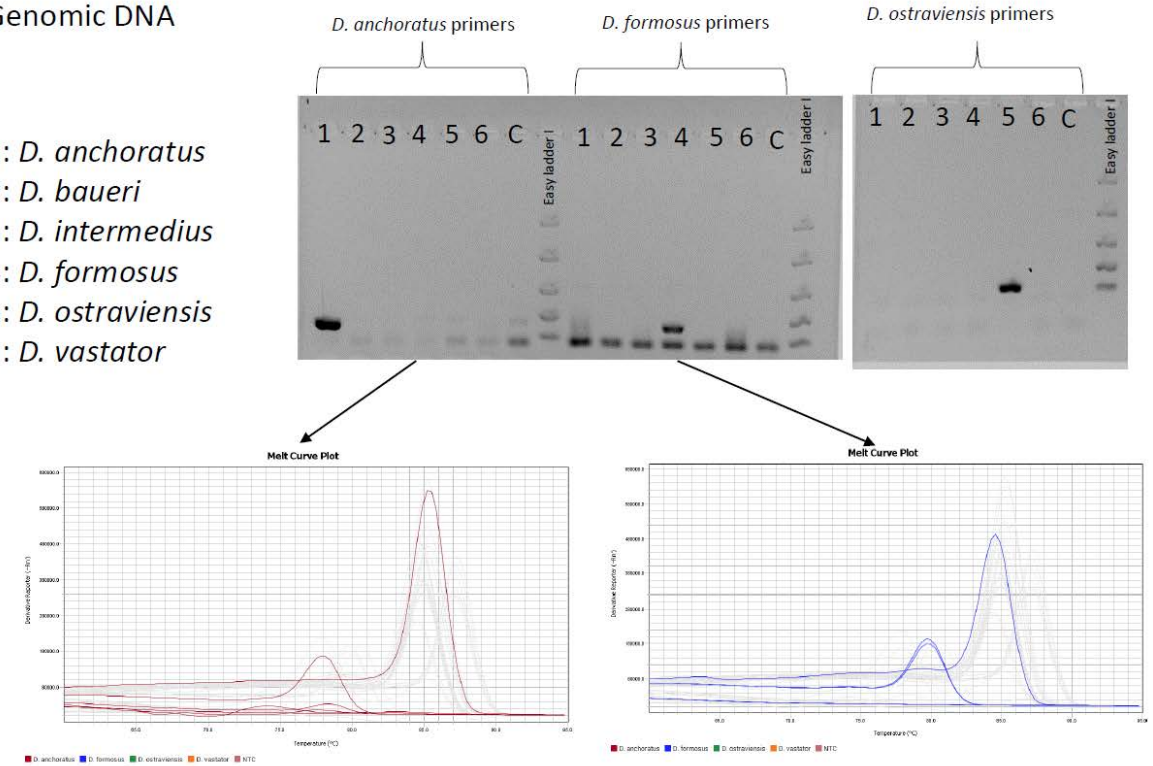
Primers:
Dactylogyrus intermedius primer pair 2

Dactylogyrus baueri primer pair 2

Results:
D. baueri: non-specific, and did not amplify *D. baueri* DNA. Remove altogether.
D. intermedius: Specific at 60 °C. Use this primer pair.

Genomic DNA

1: *D. anchoratus*
2: *D. baueri*
3: *D. intermedius*
4: *D. formosus*
5: *D. ostraviensis*
6: *D. vastator*



Cross-reactivity test 3

qPCR cycling conditions:
95/4min, (95/15s-65/30s-72/30s) 40 cycles, 72/7 min

Results:
D. anchoratus: Possible contamination, Tm of background bands is different from band in *D. anchoratus*. Specific at 65 °C.
D. formosus: Tm of background bands is different from band in *D. anchoratus*. Specific at 65 °C.
D. ostraviensis: Non-specific, test , test annealing at 65 °C.
D. vastator: specific.

Supplementary S7

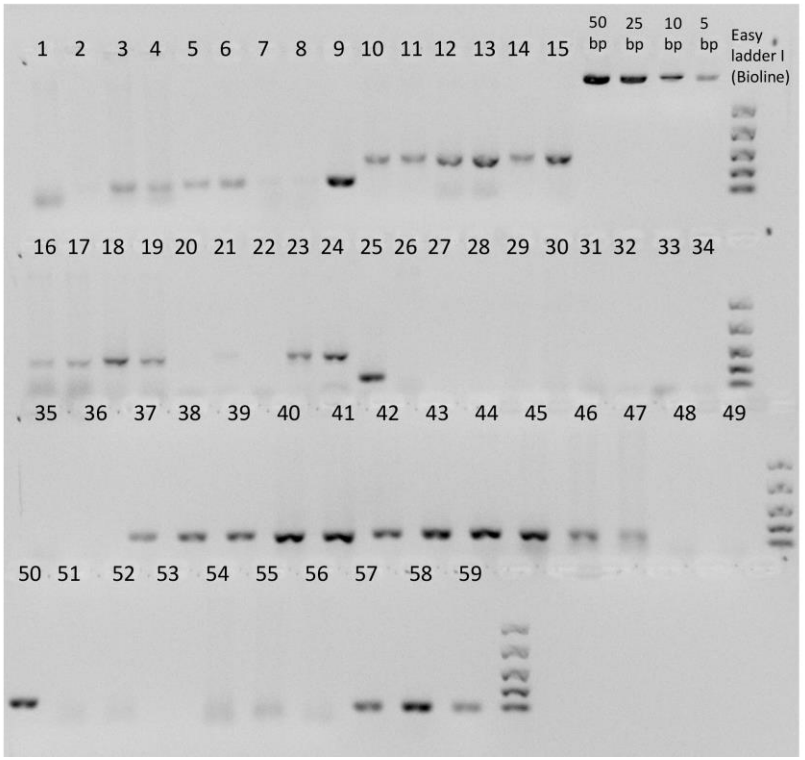
Supplementary material to:

Chapter 5: Parasite detection in the ornamental fish trade using environmental DNA

Trujillo-González, A., Edmunds, R.C., Becker, J.A., Hutson, K.S.

Supplementary S7. Band size comparison of CL 3 and putative negative amplicons on an agarose gel. Blue font indicates amplicons selected for sequencing and red font indicates samples considered negative.

1. D. anchoratus_24_2
2. D. anchoratus_19_5
3. D. anchoratus_22_1
4. D. anchoratus_22_5
5. D. anchoratus_14_1
6. D. anchoratus_14_3
7. D. anchoratus_16_1
8. D. anchoratus_16_4
9. D. anchoratus_std 1
10. D. formosus_18_1
11. D. formosus_18_3
12. D. formosus_24_1
13. D. formosus_24_4
14. D. formosus_19_2
15. D. formosus_19_5
16. D. formosus_23_3
17. D. formosus_23_5
18. D. formosus_22_1
19. D. formosus_22_5
20. D. formosus_14_2
21. D. formosus_14_6
22. D. formosus_15_5
23. D. formosus_16_1
24. D. formosus_16_3
25. D. formosus_std 1
26. D. intermedius_24_2
27. D. intermedius_19_1
28. D. intermedius_19_4
29. D. intermedius_25_2
30. D. intermedius_25_4
31. D. intermedius_13_5
32. D. intermedius_14_5
33. D. intermedius_15_2
34. D. intermedius_15_3
35. D. intermedius_16_1
36. D. intermedius_16_5
37. D. intermedius_std 1
38. D. vastator_4_2
39. D. vastator_4_3
40. D. vastator_5_1
41. D. vastator_5_6
42. D. vastator_8_2
43. D. vastator_7_2
44. D. vastator_7_4
45. D. vastator_9_6
46. D. vastator_23_2
47. D. vastator_23_4
48. D. vastator_25_1
49. D. vastator_25_4
50. D. vastator_std 1
51. D. ostraviensis_13 rerun_1
52. D. ostraviensis_13 rerun_3
53. D. ostraviensis_15_5
54. D. ostraviensis_17_5
55. D. ostraviensis_22_1
56. D. ostraviensis_22_4
57. D. ostraviensis_13_rerun_reamp_1
58. D. ostraviensis_13_rerun_reamp_3
59. D. ostraviensis_std 1

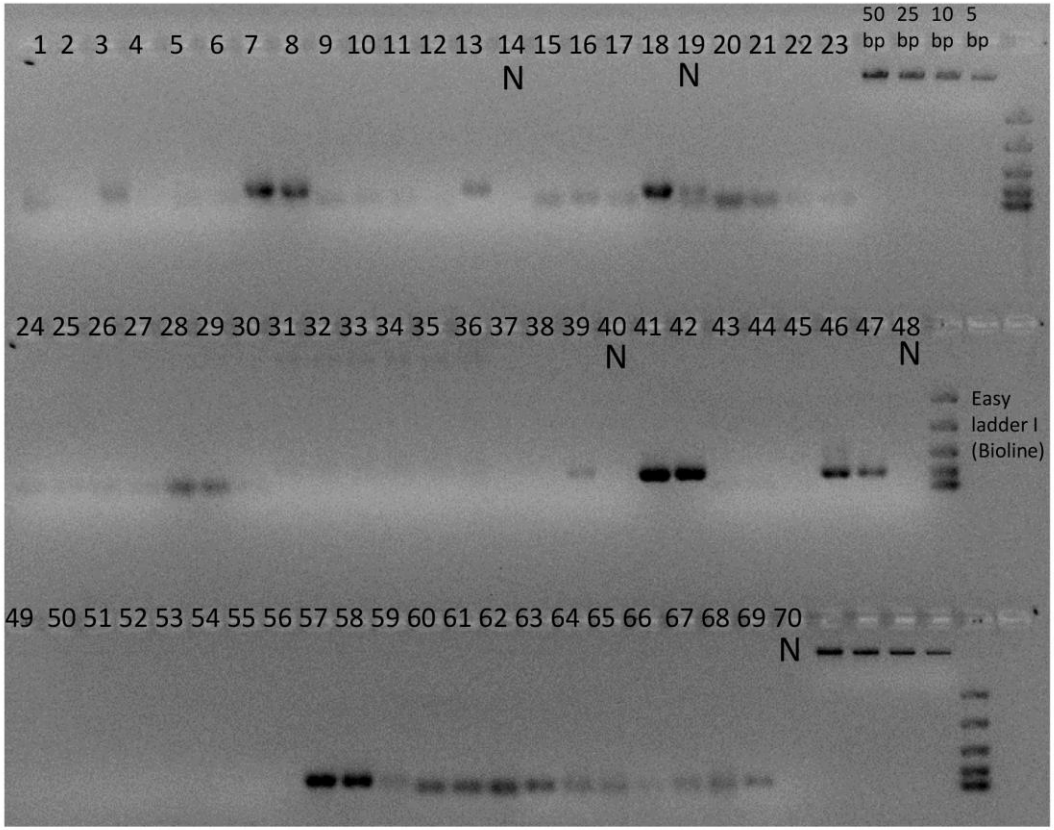


1. D. anchoratus_24_2
2. D. anchoratus_19_5
3. D. anchoratus_22_1
4. D. anchoratus_22_5
5. D. anchoratus_14_1
6. D. anchoratus_14_3
7. D. anchoratus_16_1
8. D. anchoratus_16_4
9. D. anchoratus_18_3
10. D. anchoratus_23_6
11. D. anchoratus_13_2
12. D. anchoratus_15_3
13. D. anchoratus_std 1
14. D. anchoratus_NTC

15. D. formosus_14_2
16. D. formosus_14_6
17. D. formosus_15_5
18. D. formosus_std 1
19. D. formosus_NTC

20. D. intermedius_24_2
21. D. intermedius_19_1
22. D. intermedius_19_4
23. D. intermedius_25_2
24. D. intermedius_25_4
25. D. intermedius_13_5
26. D. intermedius_14_5
27. D. intermedius_15_2
28. D. intermedius_15_3
29. D. intermedius_16_1
30. D. intermedius_16_5
31. D. intermedius_6_eDNA_1
32. D. intermedius_6_eDNA_2
33. D. intermedius_6_eDNA_3
34. D. intermedius_6_eDNA_4
35. D. intermedius_6_eDNA_5
36. D. intermedius_6_eDNA_6
37. D. intermedius_18_3
38. D. intermedius_22_2
39. D. intermedius_std 1
40. D. intermedius_NTC
41. D. vastator_23_2
42. D. vastator_23_4
43. D. vastator_25_1
44. D. vastator_25_4
45. D. vastator_14_2
46. D. vastator_8_1
47. D. vastator_std 1
48. D. vastator_NTC

49. D. ostraviensis_13_1
50. D. ostraviensis_13_3
51. D. ostraviensis_15_5
52. D. ostraviensis_17_5
53. D. ostraviensis_22_1
54. D. ostraviensis_22_4
55. D. ostraviensis_16_eDNA_1
56. D. ostraviensis_16_eDNA_2
57. D. ostraviensis_16_eDNA_3
58. D. ostraviensis_16_eDNA_4
59. D. ostraviensis_16_eDNA_5
60. D. ostraviensis_16_eDNA_6
61. D. ostraviensis_19_6
62. D. ostraviensis_23_1
63. D. ostraviensis_25_4
64. D. ostraviensis_5_2
65. D. ostraviensis_8_1
66. D. ostraviensis_6_1
67. D. ostraviensis_7_3
68. D. ostraviensis_9_5
69. D. ostraviensis_std 1
70. D. ostraviensis_NTC



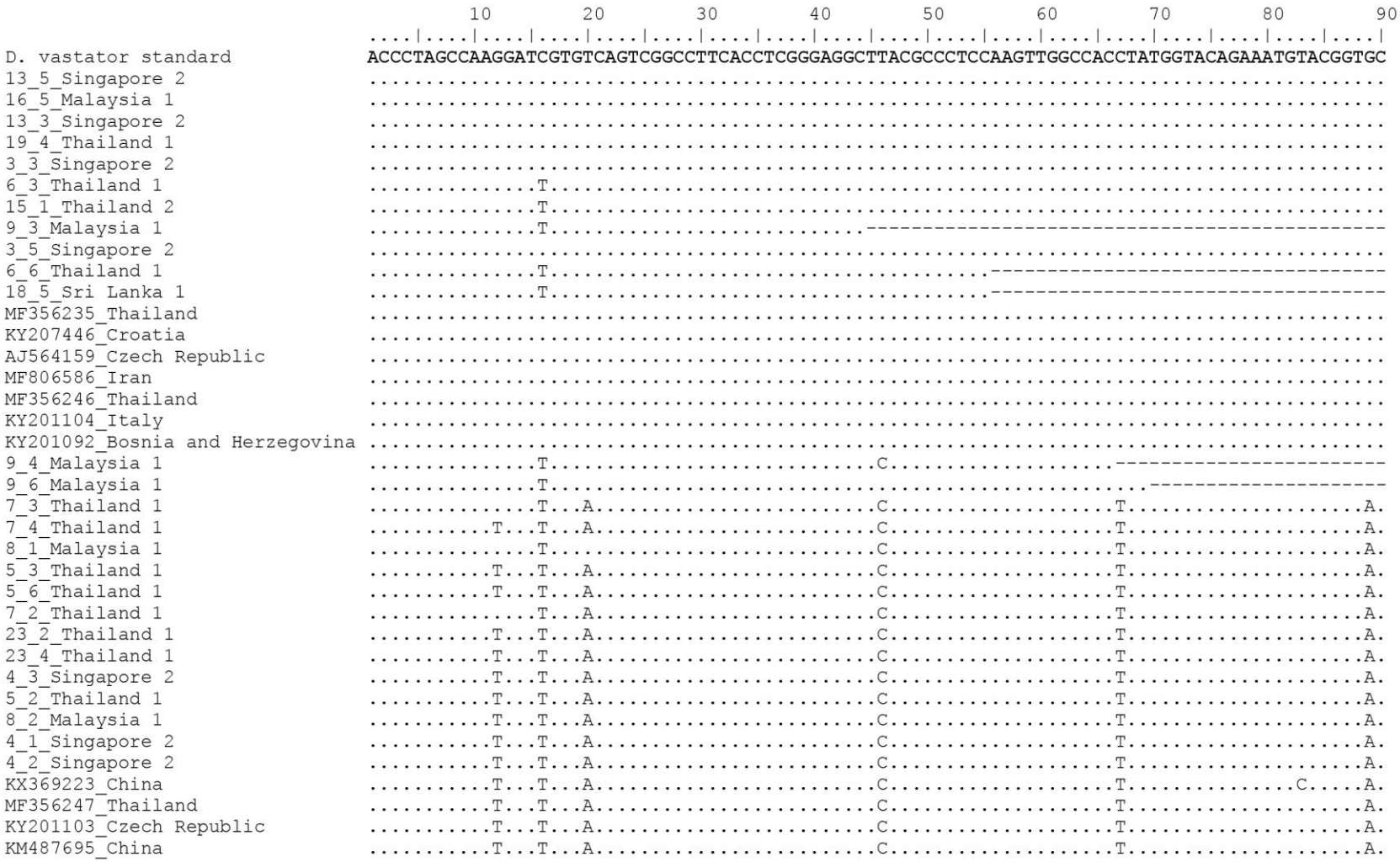
Supplementary S8

Supplementary material to:

Chapter 5: Parasite detection in the ornamental fish trade using environmental DNA

Trujillo-González, A., Edmunds, R.C., Becker, J.A., Hutson, K.S.

Supplementary S8. *Dactylogyrus vastator* alignment of sequenced Internal Transcribed Spacer 1 amplicons and accesioned sequences in Genbank from the National Center for Biotechnology Information.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13_5_Singapore 2
16_5_Malaysia 1
13_3_Singapore 2
19_4_Thailand 1
3_3_Singapore 2
6_3_Thailand 1
15_1_Thailand 2
9_3_Malaysia 1
3_5_Singapore 2
6_6_Thailand 1
18_5_Sri Lanka 1
MF356235_Thailand
KY207446_Croatia
AJ564159_Czech Republic
MF806586_Iran
MF356246_Thailand
KY201104_Italy
KY201092_Bosnia and Herzegovina
9_4_Malaysia 1
9_6_Malaysia 1
7_3_Thailand 1
7_4_Thailand 1
8_1_Malaysia 1
5_3_Thailand 1
5_6_Thailand 1
7_2_Thailand 1
23_2_Thailand 1
23_4_Thailand 1
4_3_Singapore 2
5_2_Thailand 1
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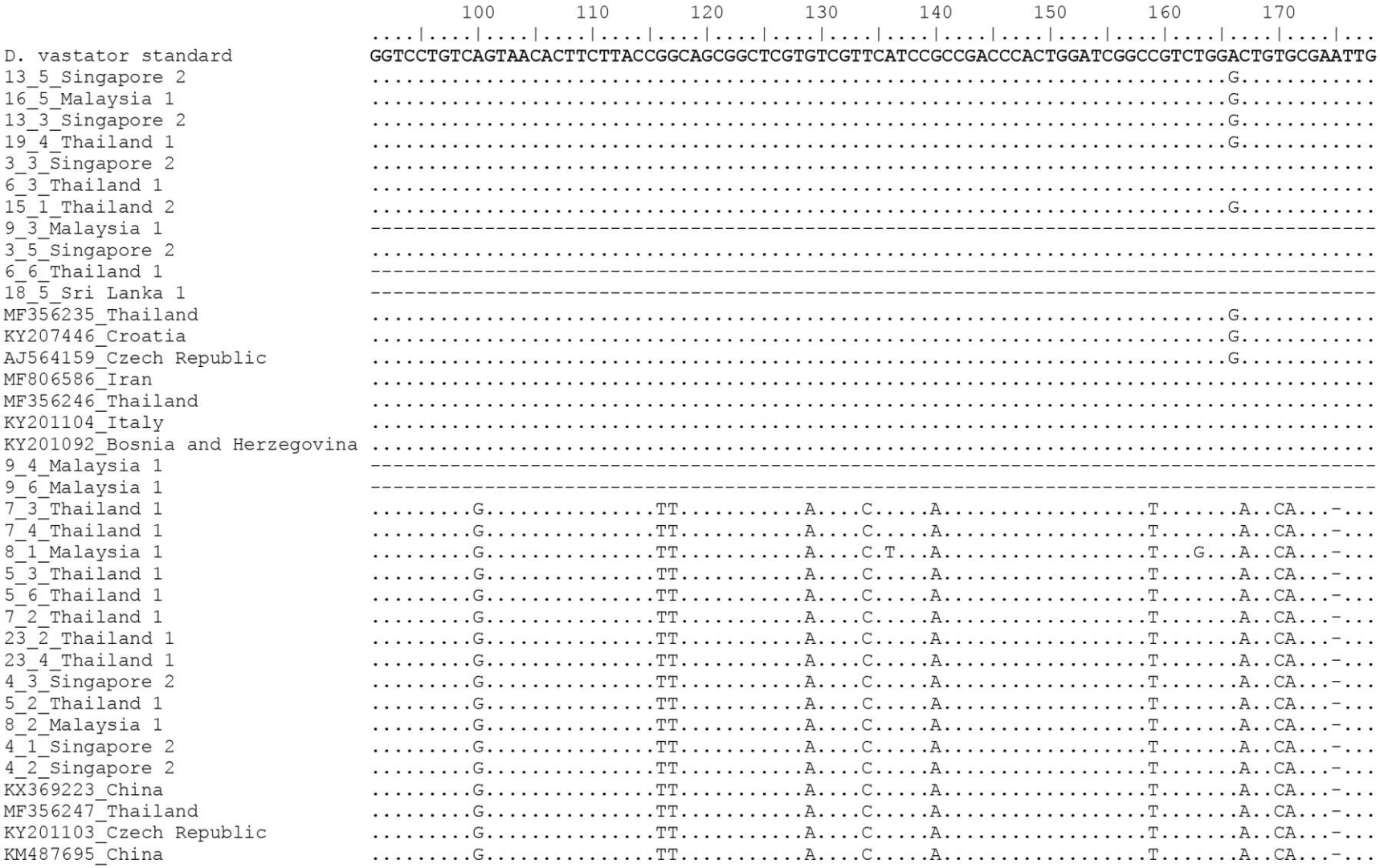
MF356247_Thailand
KY201103_Czech Republic
KM487695_China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13_5_Singapore 2
16_5_Malaysia 1
13_3_Singapore 2
19_4_Thailand 1
3_3_Singapore 2
6_3_Thailand 1
15_1_Thailand 2
9_3_Malaysia 1
3_5_Singapore 2
6_6_Thailand 1
18_5_Sri Lanka 1
MF356235_Thailand
KY207446_Croatia
AJ564159_Czech Republic
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MF356246_Thailand
KY201104_Italy
KY201092_Bosnia and Herzegovina
9_4_Malaysia 1
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8_1_Malaysia 1
5_3_Thailand 1
5_6_Thailand 1
7_2_Thailand 1
23_2_Thailand 1
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4_3_Singapore 2
5_2_Thailand 1
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MF356247_Thailand
KY201103_Czech Republic
KM487695_China

Supplementary S9

Supplementary material to:

Chapter 6: Can environmental DNA be used for aquatic biosecurity in the aquarium fish trade?

Trujillo-González, A., Becker, J.A., Saunders, R. and Hutson, K.S.

Supplementary S9. Base pair miss-matches used in synthetic standard. Gray areas indicate the forward and reverse primers used in this study

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                          10        20        30        40        50

                 ....|....|....|....|....|....|....|....|....|....|
Synthetic standard  GTGTTTGCTGCTCATGTAATATTAGCATTTGTTATTATTGGTTTAAGTGT
HQ684802            ..................................................
MG193668            ..................................................

                          60        70        80        90       100

                 ....|....|....|....|....|....|....|....|....|....|
Synthetic standard  TGTGCACTTAGTCTAGTTACAGAAGACAGGTTCAAAAAATCCATTATTTG
HQ684802            ...T..T...T....T.....T..........................
MG193668            ...T..T...T....T.....T..........................

                         110       120       130       140       150

                 ....|....|....|....|....|....|....|....|....|....|
Synthetic standard  CTCCTTCAGGTGATACCGATGCAGTCCATGTTCATAGATATGATTCTAAT
HQ684802            ...........T.............T...T...........T........
MG193668            ...........T.............T...T...........T........
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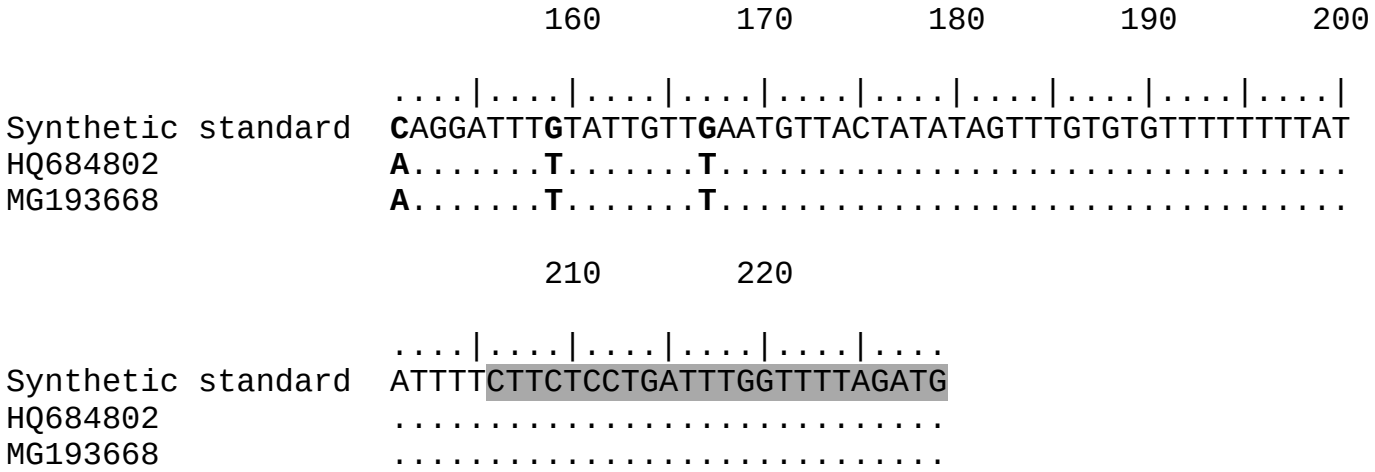
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HQ684802 A.....T.....T.....
MG193668 A.....T.....T.....
210 220
Synthetic standard ATTTTCTTCTCCTGATTTGGTTTTAGATG
HQ684802 .....
MG193668 .....

Glossary

word	Definition	Reference
Appropriate level of protection (ALOP)	The level of protection that a country considers appropriate to protect human, animal or plant life or health within its territory.	DAWR 2016a
Biosecurity	A set of measures or procedures designed to protect countries against the risks that may arise from exotic pests entering, establishing and spreading in local ecosystems, thereby threatening the economy and endemic environments.	DAWR 2014
Biosecurity Import Risk Analysis (BIRA)	A regulated scientific evaluation of the level of biosecurity risk associated with particular goods, or a class of goods, that may be imported into Australian territory. A BIRA can identify conditions that must be satisfied to manage the level of biosecurity risk to achieve Australia's ALOP.	DAWR 2016a
Co-introduced parasite	An exotic parasite species that has been transported into a new area with an alien host species.	Lymbery et al. 2014
Co-invasive parasite	A co-introduced parasite species that has infected native host species in the new range.	Lymbery et al. 2014
Cryptic parasite	Parasite camouflaged either by pigmentation and/or transparency, making it impossible to detect with the naked eye.	Whittington 1996
Endemic species	A species occurring within the range it occupies (or could occupy) naturally, independent of human activity.	Lymbery et al. 2014
Environmental DNA	DNA shed by organisms in the form of excreted cells or waste to the environment, which can then be sampled, extracted and analysed.	Modified from Thomsen and Willerslev 2015
Exotic species	A species that has been transported by human activity into an area outside its natural range. Synonymous to "alien", "non-indigenous".	Lymbery et al. 2014
feral species	Domesticated or captive species established in wild ecosystems following involuntary or voluntary release.	This thesis
Freedom from disease Surveillance	Risk-based surveillance for the purpose of demonstrating freedom from disease.	DAWR 2014
generalist parasite	Parasite able to infect a wide range of host species, either closely related or unrelated, and from different families. Generalist parasites display low host-specificity.	Combes 2001

Genomic DNA (gDNA)	DNA extracted directly from whole specimens or dissected tissue.	This thesis
Hamulus	Sclerotised hook-like structure in the anterior sucker of monogenean parasites, part of the haptoral armature.	Modified from Arya and Singh (2015)
Haptoral armature	Sclerotised structures that comprise the haptor of monogeneans. The haptoral armature usually includes Hamuli and marginal hooklets. Ventral transverse bar, together with the additional supporting dorsal bars can also be present in the haptoral armature.	Modified from Arya and Singh (2015)
Hazardous parasite/pathogen	Parasite or pathogen assessed by a Biosecurity Import Risk Analysis (BIRA) to have a non-acceptable level of protection (ALOP) and considered a hazard.	DAWR 2016a
host-specificity	See specialist and generalist parasite.	
Import Risk Analysis	Risk analysis undertaken by the DAWR in response to new information about biosecurity risks or to an import proposal.	DAWR 2016a
Introduced species	Exotic species that has been transported by humans into an area outside its natural range, but has not yet established self-sustaining populations in the wild.	Lymbery et al. 2014
Invasive species	Alien species that has been introduced, become established and is expanding its range, usually with deleterious consequences for native species.	Lymbery et al. 2014
Involuntary release	Accidental or un-planned release of organisms to wild ecosystems, including captive escapees. In parasitology, involuntary release may occur by disposing of contaminated water, infected organisms, or contaminated/infected biological material in wild ecosystems.	This thesis
Legacy DNA	DNA derived from decaying organic matter, rather than live active organisms.	This thesis
limit of Detection	Lowest gDNA standard detected across all technical qPCR replicates.	Ruijter et al. 2009
Melting temperature (Tm)	Temperature at which double-stranded DNA separates into single stranded DNA.	Ruijter et al. 2009
Monogenean	Class of parasitic Platyhelminthes.	
Non-lethal detection method	Method of detection that does not involve destructive sampling or sacrificing specimens for sample collection	This thesis
Ornamental fishes	Marine or freshwater fish species captured or cultured for their aesthetic value.	This thesis
Parasite	Symbiotic organism that derives its resources from another, unrelated living organism. Synonymous to pest.	This thesis

Parasite intensity	Number of individuals of a particular parasite species in a single infected host.	Bush et al. 1997
Parasite prevalence	Number of hosts infected with 1 or more individuals of a particular parasite species (or taxonomic group) divided by the number of hosts examined for that parasite species.	Bush et al. 1997
Pathogen	A pathogen is a biological agent that causes disease or illness to its host.	Modified from Thrusfield et al. 2018
PCR	Polymerase Chain Reaction. Molecular process by which a specific DNA fragment is exponentially amplified to generate thousands to millions of more copies through multiple cycles of increasing and decreasing temperature.	Modified from Ruijter et al. 2009
PCR amplicon	Amplified DNA fragment, product of PCR.	This thesis
qPCR	Quantitative Polymerase Chain Reaction. Molecular method by which a specific DNA fragment is exponentially amplified and monitored in real time, either using non-specific fluorescent dyes that intercalate with any double-stranded DNA, or sequence-specific DNA probes consisting of oligonucleotides labelled with a fluorescent dye. Synonymous to Real-Time PCR.	Modified from Ruijter et al. 2009
Specialist parasite	Parasite able to infect a single or a small number of closely related host species from the same family. Specialist parasites display high host-specificity.	Combes 2001
Species complex	Group of closely related species that are very similar phenotypically, but genotipically different and distinct.	This thesis
Species transboundary translocation	Human mediated movement of animals from one country or nation to another.	This thesis
Subclinical infection	An infection that has no symptoms or overt (noticeable) signs of disease.	Thrusfield et al. 2018
zoonotic disease/infection	Disease or infection transmissible in natural conditions between infected animals and humans.	Thrusfield et al. 2018