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Characterisation of Nematoda and Digenea in selected Australian freshwater snails

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ABSTRACT

Freshwater snails are integral to local ecosystems as a primary food source for various vertebrate species, thereby contributing significantly to ecological food webs. However, their role as intermediate hosts also makes them pivotal in the transmission of parasites. In Australia, research on freshwater snails has predominantly focused on their role as intermediate hosts for livestock parasites, while there has been limited exploration of the impact of these parasites on snail health and population dynamics. The aim of this study was to determine parasitic infection in freshwater snails. This study was conducted in the south-eastern region of Australia, in 2022. A total of 163 freshwater snails from four different species were collected and examined in the Murrumbidgee catchment area in the southeastern part of Australia during the Southern Hemisphere summer and autumn months (February to May). The species included *Isidorella hainesii, Glyptophysa novaehollandica, Bullastra lessoni* (endemic species), and *Physella acuta* (an introduced species). Through the analysis of sequence data from the various regions of the nuclear ribosomal DNA, we determined that the Digenea species in this study belonged to three distinct species, including *Choanocotyle hobbsi, Petasiger* sp. and an unidentified species belonging to Plagiorchioidea. Additionally, analysis of the sequences from Nematoda found in this study, revealed they could be categorized into two separate taxa, including *Krefftascaris* sp. and an unidentified nematode closely associated with plant and soil nematodes. This research holds significant implications for the future understanding and conservation of Australian freshwater ecosystems. Most parasites found in the present study complete their life cycle in snails and turtles. As many of freshwater snail and turtle species in Australia are endemic and face population threats, exploring the potential adverse impacts of parasitic infections on snail and turtle health, is crucial for advancing our understanding of these ecosystems and also paving the way for future research and conservation efforts. While none of the native snail species in the present study have been listed as endangered or threatened, this may simply be attributed to the absence of regular population surveys.

1. Introduction

Freshwater snails serve crucial ecological roles by acting as decomposers of organic matter, recyclers of nutrients, and filters of particles, particularly in regions with abundant populations of these snails (Dillon, 2000). They contribute significantly to water quality by regulating algae growth and providing clean surfaces for benthic organisms (Johnson, 2005). In aquatic ecosystems, freshwater snails hold a vital position within food webs, serving as essential nourishment for a variety of invertebrate and vertebrate species, such as crayfish, ducks, fish, and turtles. This interconnection establishes pivotal links within the ecosystem's dynamics (Johnson et al., 2013). Despite their ecological significance and the amplified risk of extinction due to climate change and human-driven pressures, freshwater snails are frequently neglected in biodiversity research and studies (Wood and Johnson, 2015).

Of notable importance is the role of freshwater snails in the life cycle of numerous parasites. For example, in the life cycle of digenean parasites, the snail intermediate host typically facilitates asexual

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reproduction of the larval phases, leading to the production of cercarial stages. The majority of known digenean species heavily rely on snails as obligatory first intermediate hosts, signifying the crucial influence snails have on the community structure of digenean parasites. In fact, they are often referred to as "keystone species" for parasitic digeneans, as their absence would result in the cessation of digenean populations (Esch et al., 2001). Despite, like many other countries, in Australia, the focus of research on freshwater snails is primarily directed towards their role as intermediate hosts for parasites affecting introduced livestock, particularly justified due to the significant economic impact of parasites like *Fasciola hepatica* on the Australian sheep industry (Love, 2017). Therefore, the lack of understanding regarding the role of snails in the life cycles of parasites affecting native wildlife, as well as the adverse impact of parasites on snails' health are concerning.

The study area Murray-Darling River system, encompassing the Murrumbidgee River catchment area where this study was conducted, stands as Australia's largest river system, spanning over 1 million km^2 and spanning three states. This expansive region harbours a multitude of freshwater snails, making it challenging to pinpoint the exact count. Ponder (1997) estimated that Australia boasts approximately 1020 named species-group taxa of non-marine molluscan fauna, with a significant 25 % of them listed as threatened by the IUCN in Australia. Limited studies have been undertaken concerning freshwater snails and their parasites outside the context of production animals (Barton et al., 2022; Johnston and Angel, 1941a; Koch, 2003; Mitchell and Leung, 2016; Shamsi et al., 2021b, 2023). This study presents findings that detail and characterize digenean and nematode parasites found within four species of freshwater snails within the Murrumbidgee Catchment area in south-eastern Australia.

2. Materials and methods

Freshwater snails were collected from various locations within the Murrumbidgee Catchment area of the Murray Darling Basin in New South Wales (NSW) during the austral summer and autumn months of 2022 (Fig. 1). Snail identification was conducted using the Illustrative Key for Freshwater and Estuarine Mollusks in New South Wales (Ponder et al., 2020). To assess the presence of parasites, the examination methods described in previous studies were followed (Barton et al., 2022; Caron et al., 2008; Shamsi et al., 2021b, 2023). In summary, the snails were placed in a water-filled petri dish, euthanized with clove oil overdose, and gently squashed to make the parasites visible. Using a pipette, the parasites were then collected and sorted into nematodes and digeneans. Each of these was further categorized into morphotypes based on specific morphological features. For digeneans, this included aspects like the anatomy of their tail and the ratio of tail length to body length, while for nematodes, it involved examining the morphology of the digestive system, tail, and reproductive organs. Additionally, factors such as the specific snail host and the location of collection contributed to their grouping (Barton et al., 2022; Caron et al., 2008; Shamsi et al., 2021b, 2023). Representatives from each group were then individually transferred to Eppendorf tubes and stored at −20 degrees Celsius for DNA sequencing purposes. A subset of each morphotype was also placed on slides and mounted in glycerine jelly for detailed morphological examination. The remaining parasites from each group were carefully labelled, preserved in 70 % ethanol, and stored for further analysis.

Each snail specimen was assigned a level of infection score based on the number of parasites present: light infections (1–4 parasites), medium infections (5–15 parasites), and heavy infections (over 15 parasites per host specimen). Parasite prevalence (P) was calculated following the methodology outlined by Bush et al. (1997).

DNA extraction was carried out using DNeasy Blood and Tissue Kits (Qiagen, Australia), with modifications to the manufacturer's instructions as described by Shamsi et al. (2019) . The amplification of different DNA regions followed the protocol outlined in Shamsi et al. (2023). PCR amplicons were sent to the Australian Genome Research Facility (AGRF) for sequencing, using the same primers employed in the PCR reactions. The resulting sequences were subjected to quality checks using SequenceScanner (Applied Biosystems/Thermo Fisher), and then aligned using the MUSCLE program in MEGA-X. Manual confirmation of

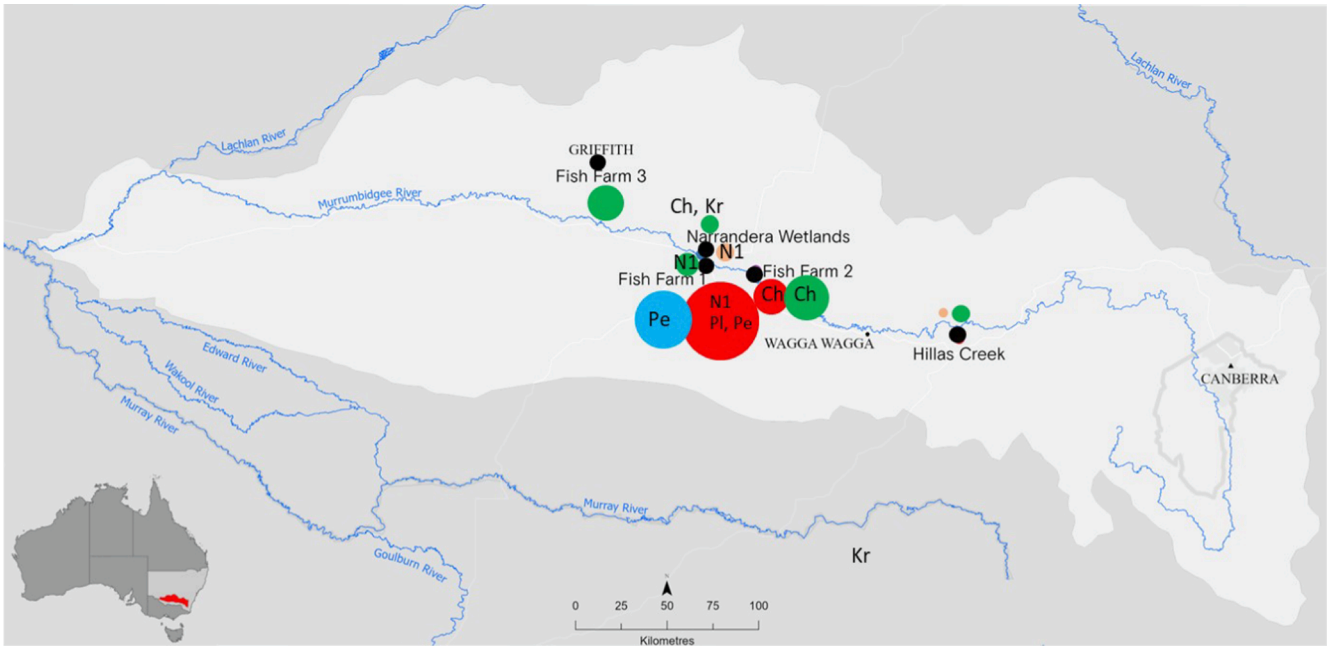

Fig. 1. Study area in the present study. A map of the Murrumbidgee catchment area with collection locations marked. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
Sourced from Office of Research Services and Graduate Studies, Spatial Data Analysis Network (SPAN), Charles Sturt University. Black circles refer to collection sites and correspond to Table 1. Green, red, brown and blue circles, represent snail hosts, *I. hainesii, G. novaehollandica, P. acuta* and *B. lessoni,* respectively. Abbreviations refer to 'Final ID' column in Table 1 and are as follow: N1: Nematode 1; Pe=Petasiger sp.; Pl: Plagiorchioidea; Ch: Choanocotyle hobbsi; Kr: Krefftascaris sp. Capitalised words are cities in the study area.

all variable sites in the original trace files was performed. To determine if the obtained sequences matched any known species, a BLAST search against the GenBank database was conducted. DNA pairwise distances were calculated using the Tamura-Nei substitution model in MEGA-X. Maximum likelihood (ML) analyses were performed. The ML tree was visualized using the tree explorer feature in MEGA-X.

3. Results

Four species of freshwater snails were collected: *Isidorella hainesii* (Tyron, 1866) (Family: Planorbidae) (n = 76), *Glyptophysa novaehollandica* (Bowdich, 1822) (Family: Planorbidae) (n = 51), *Bullastra lessoni* (Deshayes, 1831) (Family: Lymnaeidae) (n = 31) and *Physella acuta* (Draparnaud, 1805) (Family: Physidae) (n = 5) (Fig. 2). The collection location of snail species and their infection status at each location is provided in Table 1.

Overall, two types of parasites were observed in the snails: Digenea and Nematoda. Out of the 163 snails examined, 76 (46.6 %) were infected with at least one type of parasite. The majority of snails (n = 58) were infected with digeneans, while 14 snails were infected with nematodes. Among the four snail species, *Physella acuta* had the highest prevalence of infection, with all individuals (n = 5) of this species being infected. On the other hand, *Bullastra lessoni* had the lowest infection rate, with a prevalence of 3.2 % (1 out of 31). As depicted in Fig. 1, no infected snails were detected in the localities at the two ends of the study area, namely Hillas Creek and Fish Farm 3. The majority of infected snails were concentrated in the central part of the study area, particularly in the Narrandera Wetland and adjacent areas encompassing fish farms 1 and 2.

3.1. Parasites

3.1.1. Parasites found in the present study belonged to two distinct helminths: Digeanea and Nematoda

Digenea – All Digenea were in the larval stage. Two morphotypes of cercariae were found; i) those that resembled morphotype b of Barton et al., (2022), with the presence of a stylet protruding from the oral sucker, a slightly larger oral sucker than ventral sucker, a short tail relative to body length and small tegmental spines covering the body of the cercaria (Fig. 3a,b), collected from *Isidorella hainesii* and *Glyptophysa novaehollandica,* and ii) another morphotype collected from *Isidorella hainesii* and *Bullastra lessoni*, only from aquaculture facilities (Fish Farm 1), which resembled cercariae belonging to the family Echinostomatidae with a small body relative to tail length, a slightly larger ventral sucker than oral sucker and the presence of collar spines (Fig. 3d-g).

Analyses of the sequence data from specimens identified as Morphotype b of Barton et al., (2022) revealed two distinct genotypes. The majority grouped with sequences identified as *Choanocotyle hobbsi* for each of the ITS, 28S and 18S analyses (Fig. 4) with 100 % identical sequence for 28S and 18S regions with previously reported *C. hobbsi* collected from the same study area, and ITS showing a high level of similarity. The second genotype of morphotype b of Barton et al., (2022), represented by accession number ON866951 in Fig. 4 made a clade with species of the superfamily Plagiorchioidea.

Sequences from the Echinostomatidae morphotype were closely related to *Petasiger* sp. for ITS analysis, however, was grouped in a single clade.

Nematoda – Two different groups of nematodes were found, herein named as morphotypes 1 (adult specimens) and 2 (larval specimens) (Fig. 4). Measurements of the body size of the nematodes belonging to morphotype are presented in Table 2.

Sequencing of the ITS region from 11 nematodes from morphotype 1 (accession number of the representative specimen: ON866952), and 1 specimen belonging to morphotype 2, represented by accession number ON866953 were obtained. In addition, sequence of the 18S region was successfully obtained for morphotype 2 (GenBank accession number: ON775557). There was no identical sequence available for ITS and 18 S regions of these nematodes in the GenBank. As Fig. 5 shows, morphotype 1 grouped closely with free living/plant pathogen nematodes, whereas morphotype 2 grouped with *Krefftascaris* spp.

4. Discussion

This study has shown that like other countries (e.g., in Germany (Selbach et al., 2020)) Australian freshwater snails may serve as hosts for a variety of parasites, indicating their potential as indicators of parasite fauna in other animals within the ecosystem they inhabit.

The possibility that these parasites can also adversely impact snail health is a critical aspect that has not been investigated yet. Many of Australia's endemic freshwater snail species face threats to their

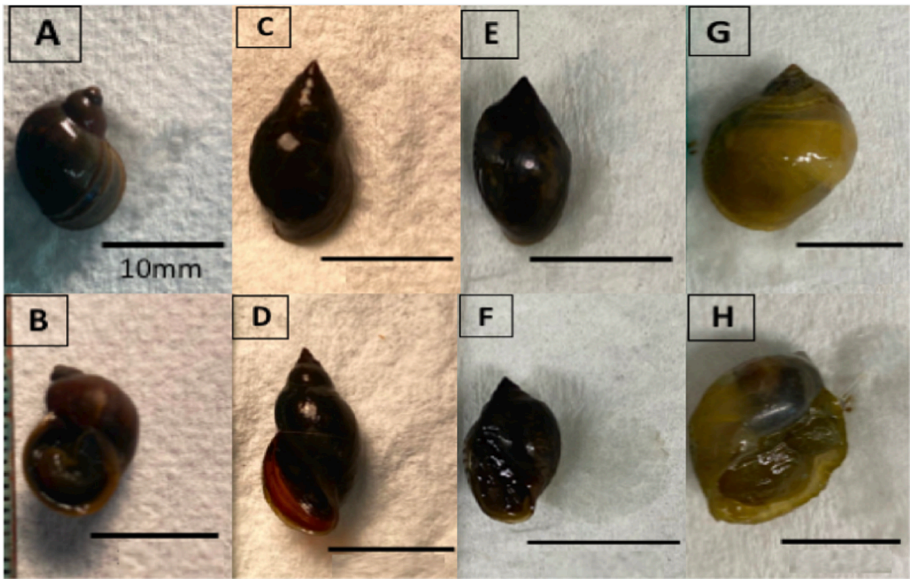

Fig. 2. Photographs of snail specimens (scale bars: 10 mm). *Isidorella hainesii*: A, dorsal view, B, ventral view. *Physella acuta*: C, dorsal view, D, ventral view. *Glyptophysa novaehollandica*: E, dorsal view, F, ventral view. *Bullastra lessoni*: G, dorsal view, H, ventral view. Note the lines on the dorsal side of the shell and rounded whorls of *I. hainessi*, the tall, pointed spiral of *P. acuta,* the mottled appearance of the shell of *G. novaehollandica* and the left-sided operculum of *B. lessoni.*

Table 1
Details of the snails, corresponding locations, prevalence (P) and infection level found in the present study. Locality of collection are the aquaculture facilities (Fish Farm 1 (FF1), Fish Farm 2 (FF2) and Fish Farm 3 (FF3)) and natural waterways (Hillas Creek (HC) and Narrandera Wetlands (NW)). The infection levels were defined as light digenean (LD) or nematode (LN) infection, medium digenean (MD) or nematode (MN) infection, heavy digenean (HD) or nematode (HN) infection and mixed infection (Mixed). The infection level is indicated by the number of snails with that level of infection and the prevalence (P, expressed as a percentage). The exact number of parasites per snail specimen could not be determined. Asterisk denotes introduced snail species. See Results section for Provisional ID of parasite. N/A, Not Applicable.

Snail Species	Locality	Pond No.	No. Snails	**No infected, P**	LD	MD	HD	LN	MN	HN	Mixed	Provisional ID of parasite	Final ID
Planorbidae													
Isidorella hainesii	FF1	25	10	0, 0 %	–	–	–	–	–	–	–	–	–
	FF1	29	10	6, 60 %	–	1, 17 %	–	4, 50 %	–	1, 12 %	–	Nematode 1 Echinostomatidae	Nematode 1 *Petasiger* sp.
	FF1	39	35	14, 40 %	11, 79 %	1, 7 %	1, 7 %	1, 7 %	–	–	–	Nematode 1 Morphotype b	Nematode 1 Plagiorchioidea
	FF1	42	7	7, 100 %	2, 29 %	1, 14 %	–	–	3, 43 %	1, 14 %	–	Nematode 1	Nematode 1
	FF1	3	1	0, 0 %	–	–	–	–	–	–	–	–	–
	FF2	NA	13	8, 61 %	7, 88 %	2, 12 %	–	–	–	–	–	Morphotype b	*Choanocotyle hobbsi*
Glyptophysa novaehollandica	HC	NA	5	5, 100 %	5, 100 %	–	–	–	–	–	–	–	–
	NW	NA	4	4, 100 %	2, 50 %	–	–	1, 25 %	–	–	1, 25 %	Nematode 2 Morphotype b	*Krefftascaris* sp. *Choanocotyle hobbsi*
	FF1	3	7	4, 57 %	1, 25 %	–	–	1, 25 %	1, 25 %	1, 25 %	–	Nematode 1	Nematode 1
	FF2	NA	22	22, 100 %	22, 100 %	–	–	–	–	–	–	Morphotype b	*Choanocotyle hobbsi*
	FF3	NA	13	0	–	–	–	–	–	–	–	–	–
Physidae													
*Physella acuta**	NW	NA	4	4, 100 %	–	–	–	–	4, 100 %	–	–	Nematode 1	Nematode 1
	HC	NA	1	1, 100 %	1, 100 %	–	–	–	–	–	–	–	–
Lymnaeidae													
Bullastra lessoni	FF1	27	31	1, 3.2 %	–	1, 100 %	–	–	–	–	–	Echinostomatidae	*Petasiger* sp.

populations (Ponder, 1997). While none of the native species in the present study have been listed as endangered or threatened, this may simply be attributed to the absence of regular population surveys.

The digenean cercariae found in the present study which were morphologically identified as 'morphotype b' from Barton et al. (2022) and genetically characterised as *Choanocotyle hobbsi,* have been previously reported (Barton et al., 2022; Shamsi et al., 2021b). However, this study shows that its host and geographical distribution is wider than previously thought, infecting another planorbid snail, *Glyptophysa novaehollandica*, from other locations (i.e., Narrandera wetlands) within the Murrumbidgee catchment area. To date, the only description of the adult parasite is from a freshwater turtle *Chelodina oblonga*, restricted to southwest Western Australia (Platt and Tkach, 2003). The definitive host for *Choanocotyle hobbsi* in the south eastern parts of Australia still remains unknown, but is most likely a freshwater turtle similar anatomically and physiologically to *Chelodina oblonga*, such as the eastern long-necked turtle *Chelodina longicollis*. Additionally, *Chelodina longicollis* is the most widespread species of freshwater turtle in New South Wales, increasing the likelihood it harbours the adult form of *Choanocotyle hobbsi* in the study area (NSW Government, 2018). Other freshwater turtle species of this genus in the area include *Chelodina expansa*, however due to the larger size of this turtle, its diet most likely consists of larger, more agile prey such as shrimp, rather than freshwater snails (Cann, 1998).

Interestingly, some specimens of digenean morphotype b found in *Isidorella hainesii* in the present study were also representing a different genotype, an unidentified plagiorchid digenean (GenBank accession number: ON866951). The phylogenetic tree showed that this genotype was placed close, but external, to the clade of sequenced genera that have been reported in freshwater turtles. Interestingly, the species *Aptorchis megapharynx* and *Aptorchis pearsoni* have been reported at localities in close proximity to the present study (Sue and Platt, 1999). Additionally, other species identified from freshwater turtles, such as *Thrinascotrema brisbanica*, have not yet been successfully sequenced (see Barton et al., 2022). Further study is required to examine the digenean fauna of freshwater turtles in south-eastern Australia to provide verification of the species present.

Molecular analysis of the Echinostomatidae cercariae further identified this group as belonging to the genus *Petasiger*, which was supported by a 100 % bootstrap value. We found at least 2.8 %-2.9 % sequence difference between *Petasiger* sp. in our study and those available in GenBank, for example *Petasiger phalacrocoracis,* which suggest they belong to different species. *Petasiger* is a genus that has not been well studied in Australia, with the last report being *Petasiger australis* over 80 years ago (Johnston and Angel, 1941b). Naturally, given the unavailability of molecular analysis in the early 20th century, the report of this species was morphological only. The cercaria they described as "*Cercaria gigantura*" was from the freshwater snail *Amerianna pyramidata* (now accepted as *Glytophysa gibbosa*) and was collected at the same time from the same locality as the adults of *Petasiger australis*. They

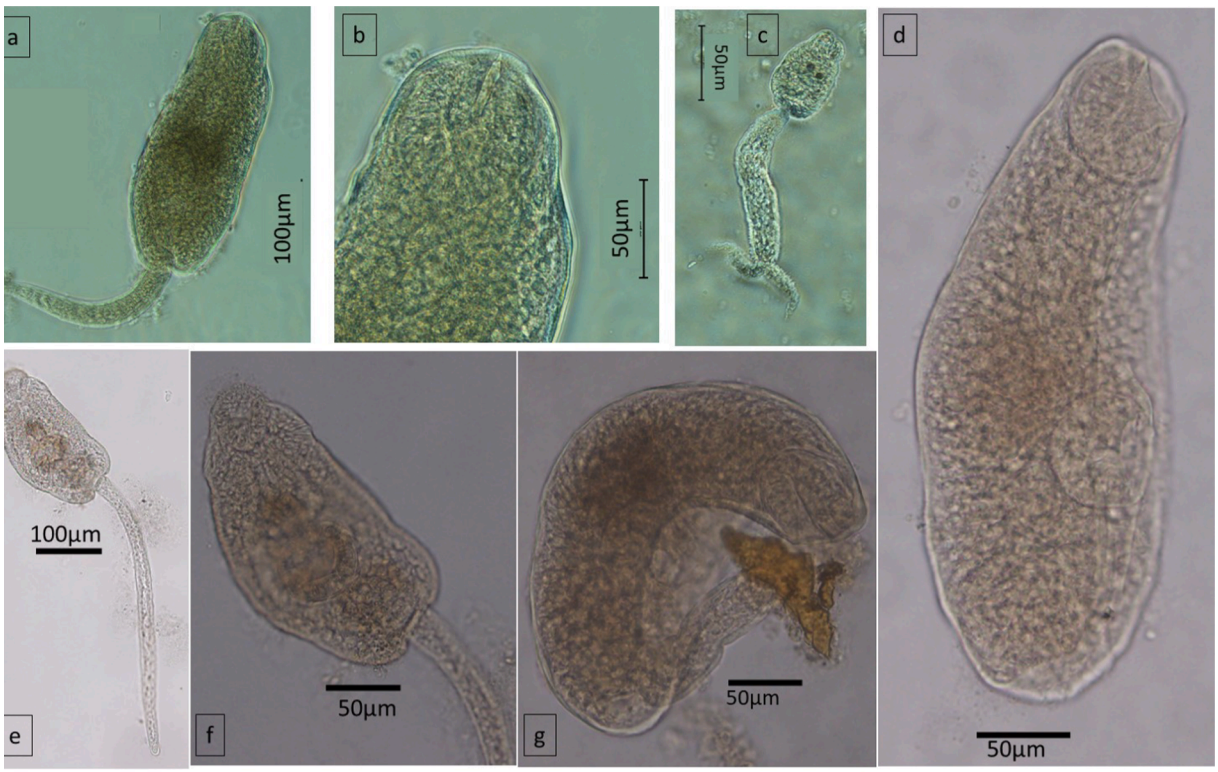

Fig. 3. Photographs of the digenean morphotypes: a &b) cercaria found in the presentent study, which morphologically resembled type b of Barton et al., 2022; c) *Clinostomum* cercaria previously found in *Bulastra lessoni,* d-g) cercaria belonging to the echinostomatid group found in the present study.

considered this cercaria to be the larval form of *Petasiger australis*, although no experimental proof was obtained. The description and drawings given by Johnston and Angel (1941a) are distinctly different to those in our study. Since Johnston and Angel (1941a), there have been no further investigations into the *Petasiger* genus in Australia, consequently no molecular data has been sequenced for an Australian species of *Petasiger*. The findings of this study therefore support the need for further analysis of the *Petasiger* genus in Australian freshwater systems. *Petasiger* spp. can infect fish as their second intermediate hosts, with reports from the lateral line scales of a freshwater roach fish (*Rutilus rutilus*) in Hungary (Molnar et al., 2015). Therefore, investigating the impact of parasite infection in Australian vulnerable fish species and their health and behaviour and welfare is an important are for future studies. In a previous study (Shamsi et al., 2023), *Bullastra lessoni* was found to be also host to *Clinostomum* sp., another Digenea that is found in freshwater fish in metacercarial stage in the strudy area (Shamsi et al., 2021a).

Another group of parasites commonly found in snails belong to the Nematoda. Globally, snails are associated with approximately 50 species of Nematoda (Gordy et al., 2016; Grewal et al., 2003), although the majority are terrestrial with no records of nematodes requiring freshwater snails for the completion of their lifecycle (Morley, 2010). However, some nematodes have shown potential to utilise freshwater snails as an alternative host to its terrestrial counterpart (Grewal et al., 2003; Morley, 2010). In our study we reported two nematode species/morphotypes. Morphotype 1 were the dominant type of nematode in the present study and based on the ITS rRNA sequences, are most likely a free-living or plant-parasitic nematode. The majority of nematodes of this nature are without molecular description due to the fact they are generally harmless fungivores (Rybarczyk-Mydłowska et al., 2012). The presence of these nematodes in the snails in this study might be accidental and occurring through ingestion (Morley, 2010).

Nematodes belonging to morphotype 2 formed a close relationship with *Krefftascaris* spp., parasitic nematodes reported to infect freshwater turtles in Australia (Tkach et al., 2010). This study is the first report of the nematode *Krefftascaris* in south-eastern Australia and, to the best of our knowledge, the first report of a larval *Krefftascaris* from a freshwater snail anywhere in the world. There have been two species of *Krefftascaris* reported in Australia, *Krefftascaris sharpiloi* and *Krefftascaris parmenteri* (Tkach et al., 2010). *Krefftascaris parmenteri* was reported in freshwater turtles as far south as the Gara River in northern New South Wales (Sprent, 1980). Thus, this study extends the geographical distribution of this parasite to south-east New South Wales. The two species of *Krefftascaris* that have been previously reported are tightly grouped within their clades with 75 nucleotide differences between them (Tkach et al., 2010), thus molecular data strongly supports that nematode found in the present study is a new, undescribed species. The report of *Kreftasscaris parmenteri* in the Gara River was from *Chelodina longicollis*, a turtle commonly found in our study area. Therefore, the adult form of this nematode is most likely to be found in this, or a related, turtle species. The two freshwater snails that the nematodes were collected from came from the Narrandera Wetlands, a natural location home to many freshwater turtle species.

As it is evident, several parasites found in the present study complete their life cycle in freshwater turtles. In many places, these turtles are declining, which thought to be due to a combination of encountered hazards and their lengthy maturation period before reproduction begins (https://www.environment.nsw.gov.au/topics/animals-and-plants/native-animals/native-animal-facts/freshwater-turtles; sighted: 18/04/2024). Therefore, the possibility that infected snails may serve as an important disease reservoir in turtles warrants investigation.

Our findings indicate that parasite diversity and abundance are notably elevated in the Narrandera wetland area and the two adjacent fish farms. During the study period, which aligns with data from the Bureau of Meteorology (BoM; https://www.dpi.nsw.gov.au/climate-landing/ssu/nsw-state-seasonal-update-february-2022; sighted 17/04/2024), the region experienced average to above-average rainfall, with relatively consistent temperature and rainfall patterns observed across

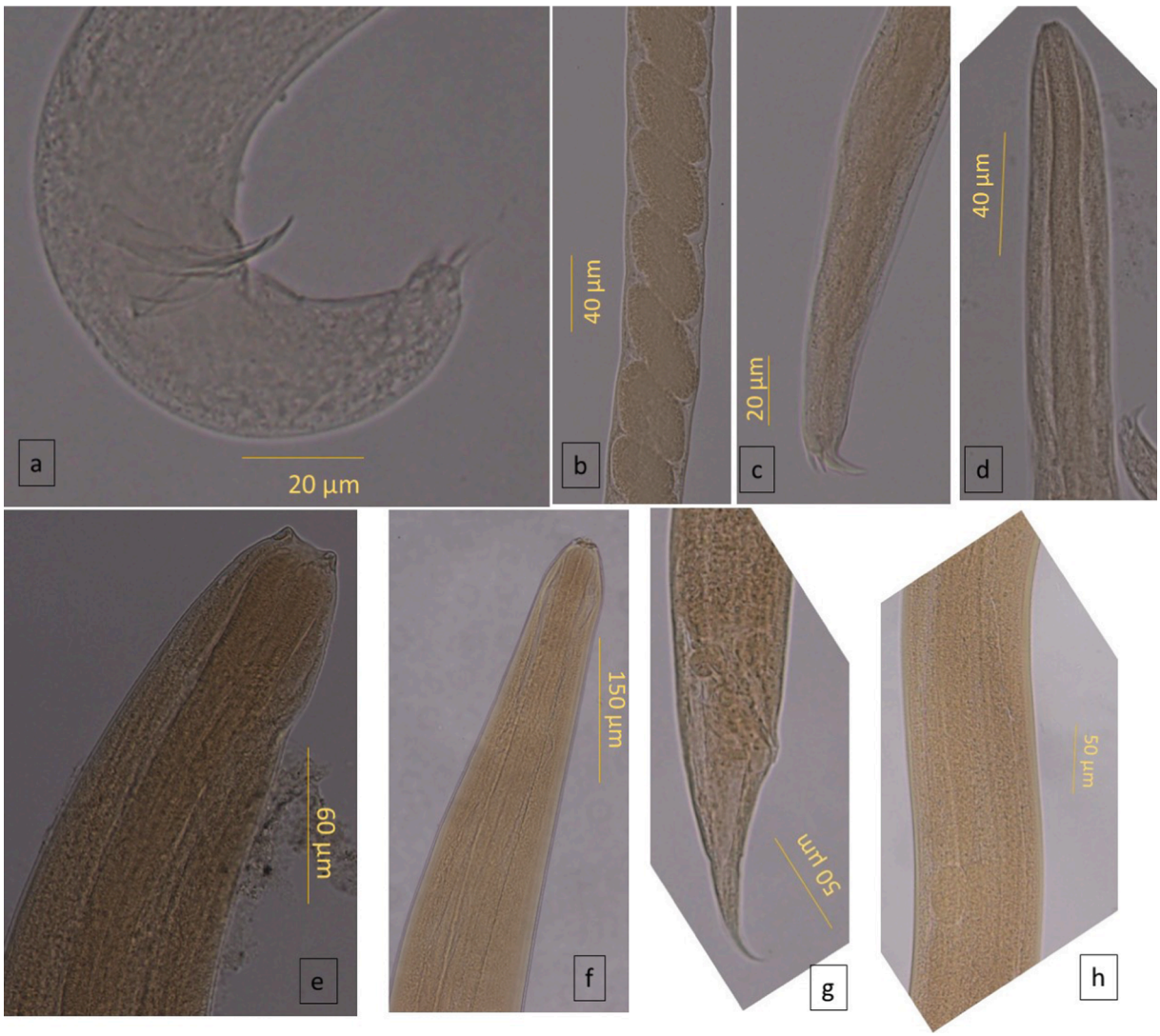

Fig. 4. Photographs of the nematode morphotypes: a to d morphotype 1, including a) posterior end of the male showing the spicule, b) mid body of the female showing the uterus, c) posterior end of the female nematode, d) anterior end of the nematode. e to d) morphotype 2, including e and f) anterior end of the type 2, g) posterior end of the type 2 and h) mid-body of the nematode showing the globular ventriculus and a short intestinal caecum.

Table 2
Measurements, presented as mean (range), of Nematoda found in this study, separated by host snail species.

	Location	Fish farm 1	Narrandera Wetlands	Fish farm 1
Morphotype 1	**Host Snail Species**	*Isidorella hainesii*	*Physella acuta* *Glyptophysa novaehollandica*	*Glyptophysa novaehollandica*
	Body Length	2505.4 (1960.2–2907.1; n = 6)	2658.7 (1867.3–3624.2; n = 5)	2555.2 (2331.6–2773.9; n = 7)
	Body Width	53.4 (39.2–59.2; n = 6)	53.8 (39.9–104.3; n = 5)	46.7 (34.2–57.3; n = 7)
Morphotype 2	**Host Snail Species**	–	*Physella acuta*	–
	Body Length	–	1510 (n = 1)	–
	Body Width	–	99 (n = 1)	–

all study sites. Throughout the study area, reports indicate high soil moisture levels, coupled with average rainfall and full water storages, contributing to the favourable conditions for parasite proliferation.

While mostly snails collected from outside aquaculture farms were infected with parasites, it is important to note that the number of snails collected between locations varied significantly, preventing reliable conclusions about differences in infections. The freshwater ecosystems present in the south-eastern part of Australia have been subjected to the introduction of exotic freshwater snail species, with the predominant exotic freshwater snail species being *Physella acuta* (Chessman and Hardwick, 2014). In the present study, the introduced *Physella acuta* showed the highest prevalence of infection, with 100 % of the snails

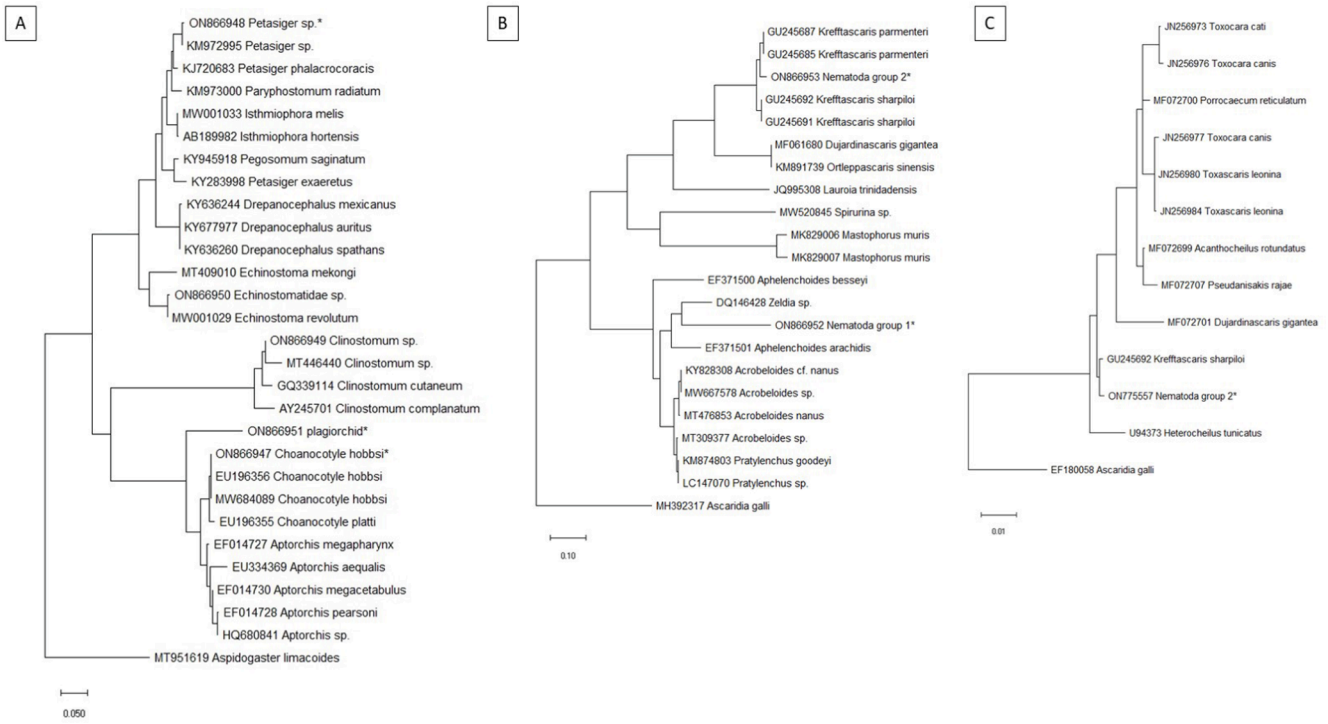

Fig. 5. Maximum likelihood trees of Digenea (A) and Nematoda (B and C) found in the present study. Tree A and B were made based on the ITS sequence data and C based on the 18S sequence data. Asterisks denote sequences generated in the present study.

infected, although only five snails were examined. Nonetheless according to Zukowski and Walker (2009) *Physella acuta* seems to be more adaptable and tolerant to the effects of climate change, potentially allowing them to survive and succeed over a native counterpart which may be the reason why they are more commonly used as an intermediate host. Host switching from a native gastropod to a phylogenetically similar exotic gastropod has been demonstrated by various digeneans (McCarthy, 1990; Mitchell and Leung, 2016a). For example, the introduction of *Pseudosuccinea columella* into New Zealand led to a rapid expansion of the range of liver fluke in that country (Pullan and Whitten, 1972). Therefore, the introduction of exotic freshwater snail species with the ability to host parasites has the potential to change the distribution of parasitic infection in the Australian's south-eastern freshwater ecosystems, making some parasites more widespread than before (Boray, 1978).

The health implications of parasites on Australian native freshwater snails remain an area with limited research. However, insights from studies on other species provide valuable understanding of the impact of parasitic infections on snail health. For instance, pulmonate snails such as *Hippeutis umbilicalis* and *Segmentina trochoideus* infected with *Fasciolopsis buski* exhibited histopathologic changes primarily in the ovotestis, including lytic lesions caused by mechanical damage, and complete loss of cellular elements. These infected snails ceased egg mass production and had a lifespan not exceeding 35 days (Graczyk et al., 2000). While our study's sample size is limited, hindering a robust statistical analysis, a notable trend emerged consistently: snails exhibiting low to moderate infections with nematodes and trematodes were more prevalent. Notably, highly infected snails were uncommon, as indicated by Table 1. This suggests a potential scenario where the parasites observed in our study may have fatal effects on snails, thereby shortening their lifespan and resulting in their reduced presence in our samples. This hypothesis warrants further investigation in future studies.

Competitive dynamics between different digeneans cohabiting the same body compartments of snails have been observed (Hourdin et al., 1993). Similarly, it is acknowledged that such antagonistic interactions may also occur between a trematode and a nematode parasite inhabiting distinct body compartments during their larval development, potentially resulting in heightened mortality rates in dually infected snails (Hourdin et al., 1993; Lim and Heyneman, 1972). In the present study only one snail, *Glyptophysa novaehollandica,* was found to have mixed infection (*Krefftascaris* sp. and *Choanocotyle hobbsi*) which makes drawing a conclusion difficult.

This study is subject to certain limitations, including the relatively small sample size available for analysis. Additionally, the unavailability of more comparable sequences in GenBank poses a challenge for the accurate identification of parasites identified in the present study. Access to a broader database of sequences would greatly enhance the precision and depth of parasite identification in future research endeavours. It is important to note that the findings may not necessarily be applicable to all snails in the area due to the limited scope of the study. Furthermore, the study only focused on the summer and autumn of 2022, potentially overlooking seasonal variations in parasite prevalence. Conducting the study over a longer period could provide valuable insights into how parasites fluctuate with changing seasons, thus improving our understanding of their dynamics in the ecosystem.

Considering that examining a relatively small sample size revealed high abundance and high diversity of parasites, clearly there is a bigger scope for future studies. A much larger sample size collected over a longer period of time and from a wider variety of locations in the study area should be included in the future studies. Examining freshwater snails for the larval stages of parasites that are known to infect a certain host could be key to understanding the population dynamics of that adult host, in a conservation context. As many species of freshwater turtles are endangered (Van Dyke et al., 2018), freshwater snails and the parasites they harbour could be used as a monitor for the presence of turtles.

In conclusion, this study sheds light on the presence and distribution of a number of parasitic infections on Australian native freshwater snails, albeit within the constraints of a limited sample size. While statistical analyses were hindered by the small sample, consistent observations revealed a prevalence of snails with low to moderate parasite

infections, with few instances of highly infected individuals. This pattern suggests a potential mortality impact of parasitic infections on snail populations, warranting further investigation to understand the mechanisms underlying this phenomenon. Moreover, insights from our study underscore the need for future research to explore the broader ecological consequences of parasitic infections on freshwater ecosystems and the potential implications for snail populations. By addressing these knowledge gaps, we can enhance our understanding of parasite-host interactions and contribute to the development of effective conservation and management strategies for freshwater ecosystems.

CRediT authorship contribution statement

Shokoofeh Shamsi: Conceptualization, Funding acquisition, Investigation, Methodology, Resources, Supervision, Validation, Writing – review & editing. **Alice Banfield:** Data curation, Formal analysis, Investigation, Methodology, Writing – original draft. **Nidhish Francis:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Supervision, Validation, Writing – review & editing. **Diane P. Barton:** Conceptualization, Resources, Supervision, Validation, Writing – review & editing. **Matthew McLellan:** Investigation, Methodology, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Barton, D.P., Zhu, X., Nuhoglu, A., Pearce, L., McLellan, M., Shamsi, S., 2022. Parasites of selected freshwater snails in the Eastern Murray Darling Basin Australia. Int. J. Environ. Public Health 19, 7236.

Boray, J.C., 1978. The potential impact of exotic *Lymnaea* spp. on fascioliasis in Australasia. Vet. Parasitol. 4, 127–141.

Bush, A.O., Lafferty, K.D., Lotz, J.M., Shostak, A.W., 1997. Parasitology meets ecology on its own terms: Margolis et al revisited. J. Parasitol. 83, 575–583.

Cann, J., 1998. Australian freshwater turtles. Beaumont Pub.

Caron, Y., Rondelaud, D., Losson, B., 2008. The detection and quantification of a digenean infection in the snail host with special emphasis on *Fasciola* sp. Parasitol. Res. 103, 735–744.

Chessman, B.C., Hardwick, L., 2014. Water regimes and macroinvertebrate assemblages in floodplain wetlands of the Murrumbidgee River, Australia. Wetlands 34, 661–672.

Dillon, R.T., 2000. The ecology of freshwater molluscs. Cambridge Univ. Press.

Esch, G., Curtis, L., Barger, M., 2001. A perspective on the ecology of trematode communities in snails. Parasitol 123, 57–75.

Gordy, M.A., Kish, L., Tarrabain, M., Hanington, P.C., 2016. A comprehensive survey of larval digenean trematodes and their snail hosts in central Alberta, Canada. Parasitol. Res. 115, 3867–3880.

Graczyk, T.K., Alam, K., Gilman, R.H., Mondal, G., Ali, S., 2000. Development of *Fasciolopsis buski* (Trematoda: Fasciolidae) in *Hippeutis umbilicalis* and *Segmentina trochoideus* (Gastropoda: Pulmonata). Parasitol. Res. 86, 324–326.

Grewal, P.S., Grewal, S.K., Tan, L., Adams, B.J., 2003. Parasitism of molluscs by nematodes: types of associations and evolutionary trends. J. Nematol. 35, 146–156.

Hourdin, P., Rondelaud, D., Cabaret, J., 1993. The development of *Fasciola hepatica parthenitae* in *Lymnaea truncatula* by modification of *Muellerius capillaris* infection. Int. J. Parasitol. 23, 235–243.

Johnson, P.D., Bogan, A.E., Brown, K.M., Burkhead, N.M., Cordeiro, J.R., Garner, J.T., Hartfield, P.D., Lepitzki, D.A., Mackie, G.L., Pip, E., 2013. Conservation status of freshwater gastropods of Canada and the United States. Fish 38, 247–282.

Johnson, P.D., 2005. Sustaining America's aquatic biodiversity. Freshwater Snail Biodiversity and Conservation. Virgina State University. 420-530 Digital Version (CNRE-76P).

Johnston, T.H., Angel, L.M., 1941a. Life cycle of the trematode. *Diplostomino murrayense* J & C Trans. R. Soc. S. Aust. 65, 140–144.

Johnston, T.H., Angel, L.M., 1941b. Life history of the trematode *Petasiger australis* n. sp. Trans. R. Soc. S. Aust. 65, 285–291.

Koch, M., 2003. The distribution of digenean trematodes within the New England Tablelands. Mem. Qld. Mus. 49, 659–664.

Lim, H.K., Heyneman, D., 1972. Intramolluscan inter-trematode antagonism: a review of factors influencing the host-parasite system and its possible role in biological control. Adv. Parasitol. 10, 191–268.

Love, S., 2017. Liver fluke-a review [Internet]. Primefact 813, NSW DPI.[Citado 2020 Marzo 14].

McCarthy, A.M., 1990. Speciation of echinostomes: evidence for the existence of two sympatric sibling species in the complex *Echinoparyphium recurvatum* (von Linstow 1873) (Digenea: Echinostomatidae). Parasitology 101, 35–42.

Mitchell, D., Leung, T., 2016. Sharing the load: a survey of parasitism in the invasive freshwater pulmonate, *Physa acuta* (Hygrophila: Physidae) and sympatric native snail populations. Hydrobiologia 766, 165–172.

Molnar, K., Gibson, D.I., Cech, G., Papp, M., Deak-Paulus, P., Juhasz, L., Toth, N., Szekely, C., 2015. The occurrence of metacercariae of Petasiger (Digenea: Echinostomatidae) in an unusual site, within the lateral line scales of cyprinid fishes. Folia Parasitol. 62.

Morley, N.J., 2010. Aquatic molluscs as auxiliary hosts for terrestrial nematode parasites: implications for pathogen transmission in a changing climate. Parasitology 137, 1041–1056.

Platt, T.R., Tkach, V.V., 2003. Two new species of *Choanocotyle* Jue Sue and Platt, 1998 (Digenea: Choanocotylidae) from an Australian freshwater turtle (Testudines: Pleurodira: Chelidae). J. Parasitol. 89, 145–150.

Ponder, W.F., 1997. Conservation status, threats and habitat requirements of Australian terrestrial and freshwater Mollusca. Mem Mus Vic 56, 421–430.

Ponder, W.F., Hallan, A., Shea, M.E., Clark, S.A., Richards, K., Klunzinger, M.W., Kessner, V., 2020. The snails and bivalves of Australian inland waters. Aust Mus.

Pullan, N.B., Whitten, L.K., 1972. Liver fluke, *Fasciola hepatica* in New Zealand: a spreading parasite in sheep and cattle. NZ Vet. J. 20, 69–72.

Rybarczyk-Mydłowska, K., Mooyman, P., van Megen, H., van den Elsen, S., Vervoort, M., Veenhuizen, P., van Doorn, J., Dees, R., Karssen, G., Bakker, J., Helder, J., 2012. Small subunit ribosomal DNA-based phylogenetic analysis of foliar nematodes (*Aphelenchoides* spp.) and their quantitative detection in complex DNA backgrounds. Phytopathology 102, 1153–1160.

Selbach, C., Soldánová, M., Feld, C.K., Kostadinova, A., Sures, B., 2020. Hidden parasite diversity in a European freshwater system. Sci. Rep. 10, 2694.

Shamsi, S., Barton, D.p., Zhu, X., 2019. Description and characterisation of *Terranova pectinolabiata* n. sp. (Nematoda: Anisakidae) in great hammerhead shark, *Sphyrna mokarran* (Rüppell, 1837), in great hammerhead shark, *Sphyrna mokarran* (Rüppell, 1837), in Australia. Parasitol Res 118, 2159–2168.

Shamsi, S., Day, S., Zhu, X., McLellan, M., Barton, D.P., Dang, M., Nowak, B.F., 2021a. Wild fish as reservoirs of parasites on Australian Murray Cod farms. Aquac 736584. https://doi.org/10.1016/j.aquaculture.2021.736584.

Shamsi, S., Banfield, A., Francis, N., Barton, D.P., McLellan, M., 2023. Occurrence of digenean parasites in freshwater snails in the Murrumbidgee catchment area. Australia, Food Waterborne Parasitol, p. e00202.

Shamsi, S., Nuhoglu, A., Zhu, X., Barton, D.P., 2021b. Genetic characterisation of cercarial stages of *Choanocotyle* Jue Sue and Platt, 1998 (Digenea: Choanocotylidae) in a native Australian freshwater snail, *Isidorella hainesii* (Tryon). Int. J .Parasitol. Parasites Wildl. 16, 48–51.

Sprent, J., 1980. Ascaridoid nematodes of amphibians and reptiles: *Angusticaecum* and *Krefftascaris* ng. J. Helminthol. 54, 55–73.

Sue, L.J., Platt, T.R., 1999. Description and life-cycle of three new species of *Dingularis* n. g. (Digena: Plagiorchiida), parasites of Australian freshwater turtles. Syst. Parasitol. 43, 175–207.

Tkach, V., Kuzmin, Y., Snyder, S., 2010b. *Krefftascaris* (Nematoda, Ascaridoidea) from Australian Side-Necked Turtles with description of *Krefftascaris Sharpiloi* sp. n. from *Chelodina rugosa*, Vestnik Zoologii, pp. e-1.

Van Dyke, J.U., Ferronato, B.d.O., Spencer, R.-J., 2018. Current conservation status of Australian freshwater turtles. Aust. J. Zool 66, 1–3.

Wood, C.L., Johnson, P.T., 2015. A world without parasites: exploring the hidden ecology of infection. Front. Ecol. Env. 13, 425–434.

Zukowski, S., Walker, K.F., 2009. Freshwater snails in competition: alien *Physa acuta* (Physidae) and native *Glytophysa gibbosa* (Planorbidae) in the River Murray, South Australia. Mar. Freshw. Res. 60, 999–1005.