CSIRO PUBLISHING

RESEARCH PAPER
https://doi.org/10.1071/MF23095

MARINE & FRESHWATER RESEARCH

Characterisation of nematode larvae found in a vulnerable native Australian fish, the southern pygmy perch, *Nannoperca australis* Günther

Shokoofeh Shamsi[A,*], Luke Pearce[B] and Xiaocheng Zhu[C,D]

For full list of author affiliations and declarations see end of paper

***Correspondence to:**
Shokoofeh Shamsi
Gulbali Institute and School of Agricultural, Environmental and Veterinary Sciences, Charles Sturt University, Wagga Wagga, NSW 2678, Australia
Email: sshamsi@csu.edu.au

Handling Editor:
Max Finlayson

ABSTRACT

Context. The southern pygmy perch (*Nannoperca australis*) is an endemic freshwater fish in Australia that is facing population decline and is listed as endangered or vulnerable in several states. **Aims.** The aim of this study was to investigate the presence of parasites in the southern pygmy perch population and provide insights into their effect on the health and conservation of the species. **Methods.** In total, 81 southern pygmy perch specimens were examined for parasite infections, followed by characterisation of the parasites. **Key results.** The postmortem examination of the fish specimens did not show any visible parasites. However, through the incubation method, nematode larvae were discovered in 14 fish (mean intensity 1.6, mean abundance 0.28). **Conclusions.** This study represents the first report of nematode larvae belonging to the genus *Spiroxys* in Australia, specifically in the southern pygmy perch. These findings highlighted the presence of parasite infections in the endangered southern pygmy perch and underscored the importance of conducting further research on parasites and their potential effect on the health and conservation of this species. **Implications.** The discovery of nematode larvae in the southern pygmy perch raises concerns about the potential effects of parasites on the population.

Keywords: detection method, endangered species, Gnathostomatidae, life cycle, native fish, Nematoda, parasites, wildlife.

Introduction

Southern pygmy perch (*Nannoperca australis*) is a small endemic freshwater fish species found in Australia (Allen 1989). It exhibits a maximum length of up to 85 mm, although it rarely exceeds 65 mm (FishBase, ver. 4.0, see https://www.fishbase.org.au/v4/summary/14920). Southern pygmy perch populations are primarily distributed in the south-eastern region of Australia, occupying various well-vegetated slow-flowing aquatic environments, such as gently flowing streams, lakes, billabongs, drains, dams, swamps, ephemeral creeks and wetlands. Their diet comprises a range of small aquatic crustaceans, insects and their larvae (Annonymous 2012).

In recent years, the southern pygmy perch has experienced significant population declines, particularly in New South Wales (Bray and Thompson 2022). These declines have resulted in severe fragmentation and range reductions, primarily within the Murray–Darling Basin. Consequently, the species has been classified as endangered, protected or vulnerable in several states because of various threats (Pearce *et al.* 2019). These threats include habitat degradation resulting from the loss of riparian and instream vegetation, increased sedimentation, poor water quality, river regulation reducing permanent floodplain habitats and dispersal opportunities, drought, flooding, predation and competition from introduced fish species such as redfin, brown trout, rainbow trout, eastern gambusia and common carp.

Despite the southern pygmy perch's threatened status and declining population, limited information is available regarding the infectious agents that may cause diseases in this fish species. To the best of our knowledge, there is currently no existing report or study specifically addressing the parasites that infect southern pygmy perch. Therefore, the

Received: 18 May 2023
Accepted: 30 June 2023
Published: 27 July 2023

Cite this:
Shamsi S *et al.* (2023)
Marine and Freshwater Research, **74**(12), 1095–1101.
doi:10.1071/MF23095

objective of this study was to report the occurrence of parasite infections in southern pygmy perch collected during a mortality event in February 2019.

Understanding the parasitic infections in endangered species such as the southern pygmy perch is crucial for effective conservation and management strategies. Parasites can exert significant impacts on the health, survival and reproductive success of host populations (Habibi and Shamsi 2018; Shamsi *et al.* 2021*a*). Additionally, parasites can serve as bioindicators of ecosystem health and environmental quality (Mascarenhas *et al.* 2021; Shamsi *et al.* 2023). By investigating parasite infections in the southern pygmy perch, valuable insights can be gained into the overall health status and ecological dynamics of this endangered fish species, and important baseline data on the prevalence, intensity and abundance of parasites infecting the southern pygmy perch will be provided.

Materials and methods

Study site and sample collection

The study was conducted at a farm dam located at 35.862745°S, 147.252098°E. This particular population of southern pygmy perch is a translocated one, originating from fish rescued from a drying lake in the catchment and relocated to the farm dam in September 2014. The fish population has since established a self-sustaining population. On 5 February 2019, a mortality event occurred during a routine monitoring event using bait traps. Bait traps consist of a small collapsible mesh 'box' with external dimensions of 245 × 245 × 400 mm, with a single funnel entrance at each end. The mesh size used is ~2.5 mm and the funnel entrances are ~35 mm in diameter. Traps were unbaited and left overnight (set late afternoon, retrieved the following morning). The bait traps were set overnight and retrieved the following morning, with a majority of the trapped fish found dead. The cause of death was likely attributed to low water oxygen concentration resulting from diurnal turnover or water movement within the dam, preventing the fish from accessing suitable oxygen concentrations. No other mortalities were observed elsewhere within the dam.

Fish examination

In total, 81 dead fish were examined for parasite infections. All fish were examined on 6 and 7 February 2019. Postmortem examination was initially performed, followed by the incubation method as described by Shamsi and Suthar (2016) to enhance parasite recovery. The study had approval of the Charles Sturt University's Animal Ethics Committee (Approval number A19024).

Morphological examination

Parasites collected from the fish were subjected to morphological examination by using light microscopy to capture their characteristic features, as previously described (Shamsi *et al.* 2019). Identification of the nematode larvae was based on the taxonomic descriptions of nematode larvae provided by Moravec (1998), Moravec (1994) and (Shamsi *et al.* 2018*a*). All parasite specimens found in this study were deposited in the South Australian Natural History Museum.

Molecular examination

Genomic DNA (gDNA) was extracted from the collected parasites by using a modified protocol based on the manufacturer's instructions (Qiagen) (Shamsi *et al.* 2018*b*). Polymerase chain-reaction (PCR) amplification of the internal transcribed spacer (*ITS*) region of the ribosomal DNA (rDNA) was performed using the primer sets NC16 and NC2, following the conditions described previously (Shamsi *et al.* 2021*b*). The PCR products were sent to the Australian Genome Research Facility (AGRF) for sequencing by using the same primers as for the PCR. Sequence chromatograms were quality checked using SeqMan (ver. 8.1.0, DNASTAR, Inc.), and primer sequences were removed for downstream analysis.

Sequence analysis

The obtained *ITS-1* sequences from the nematode larvae (GenBank accession: OR186188) were compared with known sequences available in GenBank for identification purposes (Table 1). Pairwise genetic distances between the sequences were calculated using MEGA (ver. 11.0.10, see https://www.megasoftware.net; Kumar *et al.* 2018), excluding gaps. Phylogenetic analysis was performed using MrBayes (ver. 3.2, see http://nbisweden.github.io/MrBayes/index.html; Ronquist and Huelsenbeck 2003) with the HKY + G model, as determined by jModelTest2 (ver. 2.0, see https://github.com/ddarriba/jmodeltest2; Darriba *et al.* 2012). The analysis was run for 2 000 000 generations until the standard deviation of split frequencies reached below 0.05. *Habronema muscae* (KX868082; Jian *et al.* 2017), another member of suborder Spirurina other than Habronematidae family, was used as an outgroup.

Data analysis

The prevalence of parasite infection was calculated as the percentage of infected fish of the total examined. Mean intensity represents the average number of parasites per infected fish, and mean abundance represents the average number of parasites per examined fish. These parameters were calculated following the methods described by Bush *et al.* (1997).

Results

Post-mortem examination alone did not show any parasites. However, employing the incubation method resulted in the recovery of nematode larvae in 14 fish, indicating an overall

Table 1. Details of the taxa the sequences of which were used to build phylogenetic tree in the present study.

Taxon (developmental stage)	GenBank accession number	Host (common name followed by scientific name)	Locality	Reference
Spiroxys sp. (larva)	OR186188	Southern pygmy perch, *Nannoperca australis*	Australia	Present study
Spiroxys ankarafantsika (adult)	MW550279	*Pelusios castanoides* *P. sinuatus* *P. subniger*	Mozambique, South Africa	Nel *et al.* (2021)
Spiroxys sp. (larva)	MH843727	Killifish, family: Nothobranchiidae	Mozambique	Unpublished
Spiroxys japonica	KF530321	*Pelophylax nigromaculatus*	China	Li *et al.* (2014)
Spiroxys japonica	KF530322	*Pelophylax nigromaculatus*	China	Li *et al.* (2014)
Spiroxys japonica	KF530323	*Pelophylax nigromaculatus*	China	Li *et al.* (2014)
Spiroxys japonica	KF530324	*Pelophylax nigromaculatus*	Japan	Li *et al.* (2014)
Spiroxys japonica	KF530325	*Lithobates catesbeianus*	Japan	Li *et al.* (2014)
Spiroxys hanzaki	KF530326	*Andrias japonicus*	Japan	Li *et al.* (2014)
Spiroxys hanzaki, (adults and larva)	LC605542	Hybrid *Andrias* between *A. japonicus* and Chinese *Andrias* sp.	Japan	Tsuchida *et al.* (2021)
Gnathostoma nipponicum (adult)	AB181157	Weasel, *Mustela sibirica*	Japan	Ando *et al.* (2006)
Gnathostoma spinigerum	AB181155	Swamp eel, *Fluta alba*	Thailand	Ando *et al.* (2006)
Gnathostoma binucleatum (larva)	AB181159	Fish, *Rhamdia cinerascens*	Ecuador	Ando *et al.* (2006)
Gnathostoma doloresi (adult)	AB181156	Wild boar, *Sus scrofa*	Japan	Ando *et al.* (2006)
Gnathostoma hispidum (adult)	AB181158	Pig stomach from larvae in loaches imported from China	Japan	Ando *et al.* (2006)
Echinocephalus sp. (larva)	MW136167	*Rhabdosargus sarba*	Australia	Shamsi *et al.* (2021*b*)
Echinocephalus sp. (larva)	MW136169	*Acanthopagrus australis*	Australia	Shamsi *et al.* (2021*b*)
Echinocephalus sp. (larva)	MW136168	*Rhabdosargus sarba*	Australia	Shamsi *et al.* (2021*b*)
Habronema muscae	KX868082	Donkey	China	Jian *et al.* (2017)

prevalence of 17.3%. The number of nematode larvae per infected fish ranged from one to four, with the majority of fish (n = 9) harbouring only one larva. One fish was found to be infected with four larvae. The mean intensity, representing the average number of parasites per infected fish, was 1.6, whereas the mean abundance, representing the average number of parasites per examined fish, was 0.28.

Morphological examination of the nematode larvae confirmed their identification as *Spiroxys* larvae. The larvae exhibited a finely striated cuticle and distinct triangular lateral pseudolabia (Fig. 1*c, f*). The oesophagus was composed of muscular (Fig. 1*e*) and glandular regions. Biometric measurements of the larvae were as follows (average followed by range and number examined): total body length 1.61 mm (0.77–2.18 mm; n = 19), maximum body width 1.61 mm (0.77–2.18 mm; n = 19), nerve ring to the anterior end 0.12 mm (0.09–0.15 mm; n = 3), deirids 0.20 mm (0.16–0.25 mm; n = 5), excretory pore 0.15 mm (0.15–0.15 mm; n = 2) from the anterior end, tail 0.07 mm (0.04–0.13 mm; n = 19), and genital pore (Fig. 1*d*) 0.05 mm (0.04–0.06 mm; n = 10) from the tip of the tail (Fig. 1*d, g, h*).

Molecular analysis targeting the *ITS-1* region of the rDNA successfully obtained sequences from two nematode larva specimens (Specimen numbers: 50-1-1, 49-1-1). However, no identical sequences were found in GenBank for comparison (Table 2). Phylogenetic analysis (Fig. 2) confirmed the assignment of the specimens to the family Gnathostomatidae and the genus *Spiroxys*.

The phylogenetic analysis showed that the nematode larvae found in this study represent a unique genetic lineage within the *Spiroxys* genus. Owing to the absence of identical sequences from well-identified adult specimens, the specific identity of these larvae remains unknown at this stage.

Discussion

The present study has provided important insights into the occurrence and identification of *Spiroxys* nematode larvae in southern pygmy perch (*N. australis*) from a farm dam in south-eastern Australia. The mortality event that occurred in February 2019 prompted the investigation of potential parasitic infections in the affected fish population. During the comprehensive examination of the fish, it was determined that only nematode larvae were present in the examined

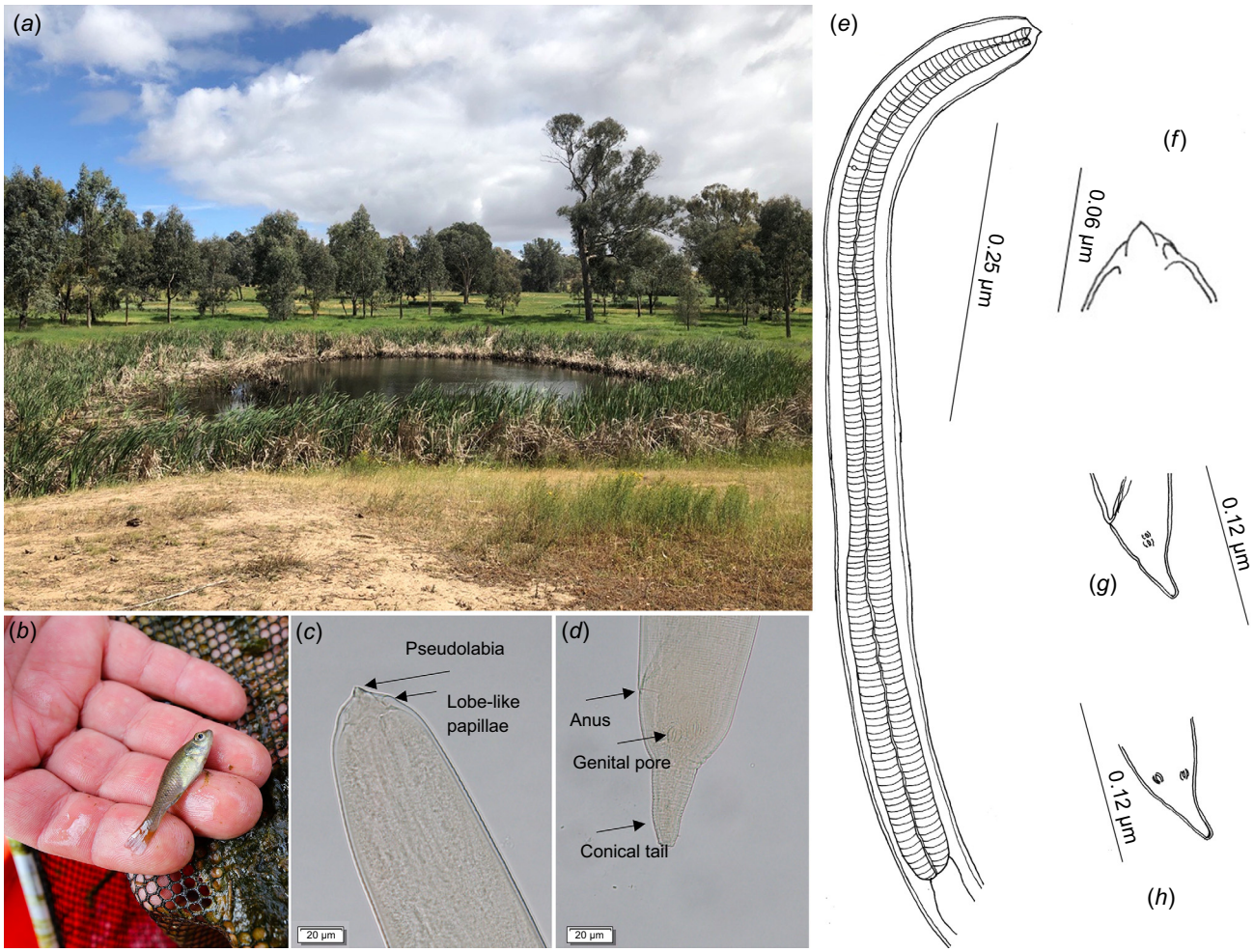

Fig. 1. Showing (*a*) the habitat, (*b*) the host and (*c–h*) the parasite, *Spiroxys* sp. larva in the present study. (*c*) and (*d*) are light-microscopy images of the anterior and posterior ends of the larval nematode respectively; (*e–h*) are illustrations of taxonomically important features of the larval nematode, including, anterior section and eosophagus (e), mouth part (*f*), lateral view of the posterior end (*g*), and ventral view of the tails region (*h*). Terminology follows Truong and Bullard (2021). Note, image (*f*) corresponds with image (*c*), and image (*g*) with image (*d*).

specimens. Through a combination of morphological and molecular techniques, the nematode larvae were identified as belonging to the genus *Spiroxys*, representing a unique genetic lineage within the genus.

The values for prevalence, mean intensity and mean abundance of *Spiroxys* larvae indicated a low infection rate and parasite load in the studied population. However, considering the potential negative effects of parasitic infections on host fitness and population dynamics, further investigations are warranted to assess the potential consequences of *Spiroxys* infection on the southern pygmy perch population. Furthermore, it is well-documented that larval parasites have the ability to alter the behaviour of their intermediate fish hosts so as to facilitate their transmission to definitive hosts and complete their life cycle as adults (Shamsi *et al.* 2021*c*; Freire *et al.* 2022). Consequently, an important avenue for future research would involve investigating the effect of *Spiroxys* larval infection on fish behaviour. Understanding how these parasites influence the behaviour of their hosts will provide valuable insights into the ecological dynamics of the parasite–host relationship and contribute to our overall understanding of the complex interactions between parasites and their hosts.

The morphological characteristics observed in the nematode larvae, including the finely striated cuticle, triangular lateral pseudolabia, and the presence of muscular and glandular regions in the oesophagus, align with the description of *Spiroxys* larvae provided by previous studies (Moravec 1994; Moravec 1998). The biometric measurements of the larvae also corresponded to the reported size ranges for *Spiroxys* larvae. However, the precise species identification of the larvae could not be established because of the lack of identical sequences in GenBank for comparison. Further investigations involving the identification of adult *Spiroxys* specimens from the same location are required to confirm the species identity of the larvae.

Table 2. Pairwise genetic distance among *ITS* sequences of our sample and other *Spiroxys* species in GenBank, shown as *P*-difference (%, below the diagonal) and number of differences (above the diagonal).

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1 OR186188[A]		47	47	44	44	43	43	44	69	69	150	145	146	145	148	143	143	143	141
2 MW550279	14.87		0	47	46	45	45	46	65	65	142	144	142	140	144	146	146	146	148
3 MH843727	14.87	0.00		47	46	45	45	46	65	65	142	144	142	140	144	146	146	146	148
4 KF530321	13.92	14.87	14.87		2	2	2	1	74	74	143	140	140	143	143	144	144	144	146
5 KF530322	13.92	14.56	14.56	0.63		2	2	1	75	75	142	139	139	144	142	142	142	142	147
6 KF530324	13.61	14.24	14.24	0.63	0.63		0	1	73	73	144	141	141	144	144	144	144	144	145
7 KF530325	13.61	14.24	14.24	0.63	0.63	0.00		1	73	73	144	141	141	144	144	144	144	144	145
8 KF530323	13.92	14.56	14.56	0.32	0.32	0.32	0.32		74	74	143	140	140	143	143	143	143	143	146
9 LC605542	21.84	20.57	20.57	23.42	23.73	23.10	23.10	23.42		0	159	156	158	153	159	153	153	153	142
10 KF530326	21.84	20.57	20.57	23.42	23.73	23.10	23.10	23.42	0.00		159	156	158	153	159	153	153	153	142
11 AB181157	47.47	44.94	44.94	45.25	44.94	45.57	45.57	45.25	50.32	50.32		21	12	27	17	173	173	173	151
12 AB181155	45.89	45.57	45.57	44.30	43.99	44.62	44.62	44.30	49.37	49.37	6.65		17	31	22	171	171	171	149
13 AB181159	46.20	44.94	44.94	44.30	43.99	44.62	44.62	44.30	50.00	50.00	3.80	5.38		33	19	169	169	169	152
14 AB181156	45.89	44.30	44.30	45.25	45.57	45.57	45.57	45.25	48.42	48.42	8.54	9.81	10.44		28	173	173	173	151
15 AB181158	46.84	45.57	45.57	45.25	44.94	45.57	45.57	45.25	50.32	50.32	5.38	6.96	6.01	8.86		174	174	174	154
16 MW136167	45.25	46.20	46.20	45.57	44.94	45.57	45.57	45.25	48.42	48.42	54.75	54.11	53.48	54.75	55.06		0	0	175
17 MW136169	45.25	46.20	46.20	45.57	44.94	45.57	45.57	45.25	48.42	48.42	54.75	54.11	53.48	54.75	55.06	0.00		0	175
18 MW136168	45.25	46.20	46.20	45.57	44.94	45.57	45.57	45.25	48.42	48.42	54.75	54.11	53.48	54.75	55.06	0.00	0.00		175
19 KX868082	44.62	46.84	46.84	46.20	46.52	45.89	45.89	46.20	44.94	44.94	47.78	47.15	48.10	47.78	48.73	55.38	55.38	55.38	

[A]Specimen obtained in the present study.

The phylogenetic analysis showed that the *Spiroxys* larva in this study clustered within the family Gnathostomatidae and formed a distinct genetic lineage within the *Spiroxys* genus.

This finding may suggest the existence of potential cryptic diversity within *Spiroxys* species. It also could be a geographical variation for the parasite, given that there are no other sequences from an Australian representative in the GenBank. Nevertheless, the findings highlighted the need for comprehensive taxonomic and genetic studies to elucidate the species boundaries and evolutionary relationships within the genus. Additional sampling efforts and the inclusion of adult *Spiroxys* specimens from different geographic locations will contribute to a better understanding of the diversity and distribution of *Spiroxys* species in Australia.

Nematodes belonging to the genus *Spiroxys* Schneider are mainly parasites of freshwater chelonians, frogs, salamander, and snakes (Berry 1985) in adult stage, some of which are also commonly found in the locality where these fish were collected in the present study. Of the 18 species assigned to the genus *Spiroxys* (Baker 1987; Hasegawa *et al.* 1998; Moravec and Vargas-Vazquez 1998; Roca *et al.* 2007; Nel *et al.* 2021), one has been reported in Australia (Berry 1985). The Australian *Spiroxys* species, *S. chelodinae*, in its adult form, occurs in three species of freshwater chelonian turtles, namely *Chelodina longicollis, C. expansa* and *C. oblonga*, and has been reported from Armidale (New South Wales), Fraser Island (Queensland), Milang, Tailem Bend, Mannum and Lake Alexandrina (South Australia). Australian freshwater turtles eat insects, tadpoles, small freshwater fish, fresh and saltwater prawns and yabbies, snails and mussels and worms. *Chelodina longicollis* has been sighted in the area of the fish collection in the present study. The presence of *Spiroxys* larva in the farm dam, where southern pygmy perch was collected, raises the possibility of an association between the fish and freshwater chelonians. Therefore, it is plausible that the *Spiroxys* larvae found in the southern pygmy perch may represent the larval stage of *S. chelodinae*, suggesting a potential transmission pathway involving chelonians as intermediate hosts. Further investigations, including the examination of chelonians in the vicinity and the molecular confirmation of the adult *Spiroxys* species, are required to confirm this hypothesis.

Globally, *Spiroxys* spp. larvae have been reported in various freshwater copepods, fishes and tadpoles (Moravec 1994). The present study is the first report of the larval stage of the genus in Australia, which shows the role of a native fish, southern pygmy perch, in its life cycle. According to Santos-Clapp *et al.* (2022), *Spiroxys* larvae have a higher incidence in smaller fish. Fish may acquire the infection by eating infected first intermediate hosts or paratenic hosts, such as copepods, aquatic insects and molluscs (Moravec 1998).

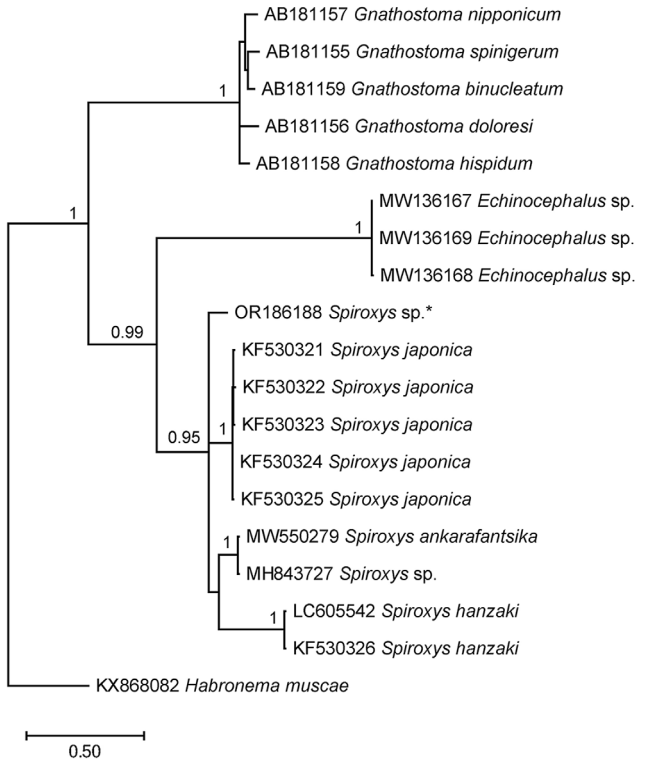

Fig. 2. Phylogenetic tree based on *ITS* sequences, showing the placement of our specimen with other *Spiroxys* spp. *Habronema muscae* was used as an outgroup. Bayesian posterior probability values of >90% are indicated above the branches (Ando *et al.* 2006; Li *et al.* 2014; Nel *et al.* 2021; Shamsi *et al.* 2021c; Tsuchida *et al.* 2021). The asterisk (*) indicates the specimen obtained from this study.

The detection of *Spiroxys* larvae in southern pygmy perch raises important ecological and conservation implications. As a native fish species, the southern pygmy perch plays a significant role in maintaining ecosystem balance and functioning in freshwater habitats. Parasitic infections, even at low prevalence and intensity, can influence the health and fitness of host individuals, potentially affecting their reproductive success, growth, and survival.

Understanding the factors influencing the occurrence and transmission of *Spiroxys* larvae in southern pygmy perch populations is crucial for effective conservation and management strategies. Factors such as habitat characteristics, water quality, host population dynamics, and the presence of intermediate hosts need to be further investigated to elucidate the epidemiology and transmission dynamics of *Spiroxys* infections in this system. Additionally, assessing the potential effects of *Spiroxys* infection on the fitness and population dynamics of southern pygmy perch, as well as its interaction with other stressors such as habitat degradation and climate change, is essential for guiding conservation efforts and maintaining the long-term viability of this native fish species.

Nematode larvae were the only parasite found in the present study, which could be due to way we examined the fish. External parasites, including Protozoa and Monogenea, usually leave the host after its death or after change in their environmental conditions and it is possible that we missed the opportunity to find more parasites in them.

Despite facing significant long-term threats, fish species that lack commercial or recreational value often are overlooked in conservation management efforts (Todd *et al.* 2017). Their exclusion from conservation focus poses a risk to their survival and undermines efforts to safeguard their future. Recognising the importance of these species and addressing the challenges they face is crucial for maintaining biodiversity and promoting effective conservation strategies. Effective management of threatened species requires a thorough understanding of their ecology, as well as the underlying factors that pose threats to their well-being and contribute to disease susceptibility.

References

Allen GR (1989) 'Freshwater fishes of Australia.' (TFH Publications: Brookvale, NSW, Australia)

Ando K, Tsunemori M, Akahane H, Tesana S, Hasegawa H, Chinzei Y (2006) Comparative study on DNA sequences of ribosomal DNA and cytochrome c oxidase subunit 1 of mitochondrial DNA among five species of gnathostomes. *Journal of Helminthology* **80**(1), 7–13. doi:10.1079/JOH2005315

Baker MR (1987) Superfamily Gnathostomatoidea. In 'Synopsis of the Nematoda parasitic in amphibians and reptiles. Occasional Papers in Biology. Vol. 11.' pp. 1–325. (Memorial University of Newfoundland)

Berry GN (1985) A new species of the genus *Spiroxys* (Nematoda; Spiruroidea) from Australian chelonians of the genus *Chelodina* (Chelidae). *Systematic Parasitology* **7**(1), 59–68. doi:10.1007/BF00010162

Bray DJ, Thompson VJ (2022) *Nannoperca australis* in Fishes of Australia. Available at https://fishesofaustralia.net.au/home/species/1828 [Verified 16 May 2023]

Bush AO, Lafferty KD, Lotz JM, Shostak AW (1997) Parasitology meets ecology on its own terms: Margolis *et al.* revisited. *The Journal of Parasitology* **83**(4), 575–583. doi:10.2307/3284227

Darriba D, Taboada GL, Doallo R, Posada D (2012) jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods* **9**(8), 772. doi:10.1038/nmeth.2109

Freire R, Rogers L, Creece D, Shamsi S (2022) Neophobic behavioural responses of parasitised fish to a potential predator and baited hook. *Applied Animal Behaviour Science* **254**, 105722. doi:10.1016/j.applanim.2022.105722

Habibi F, Shamsi S (2018) Preliminary report of occurrence of *Corynosoma* spp. (Acanthocephala: Polymorphidae) in Southern Caspian sprat (*Clupeonella grimmi*). *Parasitology Research* **117**(10), 3327–3331. doi:10.1007/s00436-018-6012-6

Hasegawa H, Miyata A, Doi T (1998) *Spiroxys hanzaki* n. sp. (Nematoda: Gnathostomatidae) collected from the giant salamander, *Andrias japonicus* (Caudata: Cryptobranchidae), in Japan. *The Journal of Parasitology* **84**(4), 831–834. doi:10.2307/3284598

Jian R, Wang S-W, Zhang W-X, Zhang L-P (2017) Morphological and molecular identification of *Habronema* spp. (Nematoda: Habronematidae) from donkeys in Xinjiang, China, and notes on the taxonomical status of *Habronema majus* (Creplin, 1849) and *H. microstoma* (Schneider, 1866). *Systematic Parasitology* **94**(4), 511–525. doi:10.1007/s11230-017-9714-8

Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution* **35**(6), 1547–1549. doi:10.1093/molbev/msy096

Li L, Hasegawa H, Roca V, Xu Z, Guo Y-N, Sato A, Zhang L-P (2014) Morphology, ultrastructure and molecular characterisation of

Spiroxys japonica Morishita, 1926 (Spirurida: Gnathostomatidae) from *Pelophylax nigromaculatus* (Hallowell) (Amphibia: Ranidae). *Parasitology Research* **113**(3), 893–901. doi:10.1007/s00436-013-3720-9

Mascarenhas CS, Silva RZ, Muller G (2021) Helminth's assemblage of *Trachemys dorbigni* (Testudines: Emydidae) in southern Brazil: implications of anthropogenic environments and host's genders. *Iheringia. Serie Zoologia* **111**, e2021011. doi:10.1590/1678-4766e2021011

Moravec F (1994) 'Parasitic nematodes of freshwater fishes of Europe.' (Academia: Prague, Czech Rebublic; and Kluwer Academic Publishers: Dordrecht, Netherlands)

Moravec F (1998) 'Nematodes of freshwater fishes of the Neotropical Region.' (Academia)

Moravec F, Vargas-Vazquez J (1998) Some endohelminths from the freshwater turtle *Trachemys scripta* from Yucatan, Mexico. *Journal of Natural History* **32**(3), 455–468. doi:10.1080/00222939800770241

Morris SA, Pollard DA, Gehrke PC, Pogonoski JJ (2001) Threatened and potentially threatened freshwater fishes of coastal New South Wales and the Murray–Darling Basin: report to Fisheries Action Program and World Wide Fund for Nature. Final report series number 33, Project Number AA 0959.98. (NSW Fisheries: Sydney, NSW, Australia) Available at https://www.dpi.nsw.gov.au/_data/assets/pdf_file/0010/545617/FFRS-33_Morris-et-al-2001.pdf

Nel T, du Preez L, Netherlands E, Syrota Y, Svitin R (2021) *Spiroxys ankarafantsika* Roca et Garcia, 2008 (Nematoda: Gnathostomatidae) and other nematodes parasitising *Pelusios* spp. *(Testudines: Pelomedusidae) from South Africa and Mozambique. Acta Parasitologica* **66**(3), 954–961.

Pearce L, Bice C, Whiterod N, Raadik T (2019) Southern pygmy perch *Nannoperca australis*. In 'The IUCN Red List of Threatened Species 2019'. e.T123358579A123382811. (International Union for Conservation of Nature and Natural Resources) Available at https://www.iucnredlist.org/species/123358579/123382811

Roca V, Garcia G, Montesinos A (2007) Gastrointestinal helminths found in the three freshwater turtles (*Erymnochelys madagascariensis*, *Pelomedusa subrufa* and *Pelusios castanoides*) from Ankarafantsika National Park, Madagascar. *Helminthologia* **44**(4), 177–182. doi:10.2478/s11687-007-0028-2

Ronquist F, Huelsenbeck JP (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19**, 1572–1574. doi:10.1093/bioinformatics/btg180

Santos-Clapp MD, Duarte R, Albuquerque MC, Brasil-Sato MC (2022) Helminth endoparasites of endemic fish *Pygocentrus piraya* (Characiformes, Serrasalmidae) from Tres Marias reservoir, Minas Gerais, Brazil. *Anais da Academia Brasileira de Ciencias* **94**(4), e20201425. doi:10.1590/0001-3765202220201425

Shamsi S, Suthar J (2016) A revised method of examining fish for infection with zoonotic nematode larvae. *International Journal of Food Microbiology* **227**, 13–16. doi:10.1016/j.ijfoodmicro.2016.03.023

Shamsi S, Turner A, Wassens S (2018*a*) Description and genetic characterization of a new *Contracaecum* larval type (Nematoda: Anisakidae) from Australia. *Journal of Helminthology* **92**(2), 216–222. doi:10.1017/S0022149X17000360

Shamsi S, Steller E, Chen Y (2018*b*) New and known zoonotic nematode larvae within selected fish species from Queensland waters in Australia. *International Journal of Food Microbiology* **272**, 73–82. doi:10.1016/j.ijfoodmicro.2018.03.007

Shamsi S, Stoddart A, Smales L, Wassens S (2019) Occurrence of *Contracaecum bancrofti* larvae in fish in the Murray–Darling Basin. *Journal of Helminthology* **93**(5), 574–579. doi:10.1017/S0022149X1800055X

Shamsi S, Day S, Zhu X, McLellan M, Barton DP, Dang M, Nowak BF (2021*a*) Wild fish as reservoirs of parasites on Australian Murray Cod farms. *Aquaculture* **539**, 736584. doi:10.1016/j.aquaculture.2021.736584

Shamsi S, Steller E, Zhu X (2021*b*) The occurrence and clinical importance of infectious stage of *Echinocephalus* (Nematoda: Gnathostomidae) larvae in selected Australian edible fish. *Parasitology International* **83**, 102333. doi:10.1016/j.parint.2021.102333

Shamsi S, Rogers L, Sales E, Kopf RK, Freire R (2021*c*) Do parasites influence behavioural traits of wild and hatchery-reared Murray cod, *Maccullochella peelii*? *Parasitology Research* **120**(2), 515–523. doi:10.1007/s00436-020-07021-7

Shamsi S, Francis N, Masiga J, Barton DP, Zhu X, Pearce L, McLellan M (2023) Occurrence and characterisation of *Eustrongylides* species in Australian native birds and fish. *Food and Waterborne Parasitology* **30**, e00189. doi:10.1016/j.fawpar.2023.e00189

Todd CR, Koehn JD, Pearce L, Dodd L, Humphries P, Morrongiello JR (2017) Forgotten fishes: what is the future for small threatened freshwater fish? Population risk assessment for southern pygmy perch, *Nannoperca australis*. *Aquatic Conservation: Marine and Freshwater Ecosystems* **27**(6), 1290–1300. doi:10.1002/aqc.2808

Truong TN, Bullard SA (2021) Susceptibility of channel catfish (*Ictalurus punctatus*), blue catfish (*Ictalurus furcatus*), and their commercially cultured hybrid to metazoan parasite infection in earthen pond aquaculture. *Comparative Parasitology* **88**(1), 93–112. doi:10.1654/1525-2647-88.1.93

Tsuchida K, Urabe M, Nishikawa K (2021) The first survey for helminths parasitic in hybrid and introduced giant salamanders, genus *Andrias* (Amphibia: Caudata: Cryptobranchidae) in Kyoto, Japan. *Current Herpetology* **40**(2), 109–119. doi:10.5358/hsj.40.109

Data availability. Specimens were deposited in the South Australian Natural History Museum. Sequence data are publicly available through GenBank.

Conflicts of interest. The authors declare that they have no conflicts of interest.

Declaration of funding. This study was partially supported by Charles Sturt University through Fellowship (awarded to S. Shamsi).

Author affiliations

[A]Gulbali Institute and School of Agricultural, Environmental and Veterinary Sciences, Charles Sturt University, Wagga Wagga, NSW 2678, Australia.

[B]NSW Department of Primary Industries, Fisheries, Habitat and Threatened Species Unit, Freshwater Environment Branch, Albury, NSW 2640, Australia.

[C]School of Agricultural, Environmental and Veterinary Sciences, Charles Sturt University, Wagga Wagga, NSW 2678, Australia.

[D]NSW Department of Primary Industries, Wagga Wagga Agricultural Institute, Wagga Wagga, NSW 2650, Australia.