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The Asian fish tapeworm (*Schyzocotyle acheilognathi*) discovered in Western Australia may pose a threat to the health of endemic native fishes

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Research Paper

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Abstract

The Asian fish tapeworm (*Schyzocotyle acheilognathi*) is an important fish parasite with a wide host range that infects over 300 species of fish worldwide. *Schyzocotyle acheilognathi* has been reported from eastern coastal areas of Australia, but has not been previously reported in Western Australia (WA). During a control program for invasive freshwater fishes in south-western WA, a region with a unique and highly endangered freshwater fish fauna, tapeworms identified as *S. acheilognathi* from their distinctive scolex morphology were found at a prevalence of 3.3% in goldfish (*Carassius auratus*), 37.0% in koi carp (*Cyprinus carpio haematopterus*) and 65.0% in eastern gambusia (*Gambusia holbrooki*) in a small suburban lake to the north of Perth. For molecular confirmation, the 18S ribosomal RNA gene was targeted at hypervariable V4 region. Koi carp isolates were 100% identical to *S. acheilognathi* isolated from varying hosts, including the red shiner (*Cyprinella lutrensis*) and a human sample. Sequences obtained from two eastern gambusia were identified as *S. acheilognathi*, but formed a discrete cluster and may represent a novel genotype. Isolates from two other eastern gambusia and two goldfish formed a distinct clade with only 91.9% similarity to previously sequenced isolates of *S. acheilognathi*. This emphasizes the importance of molecular identification methods in addition to morphological identification. The presence and potential for transmission of these parasites in south-western WA may threaten the health of native fishes, which are immunologically naïve to this introduced parasite. Immediate control or containment measures should be implemented to halt the spread of these parasites.

Introduction

The Asian fish tapeworm (*Schyzocotyle* (formerly *Bothriocephalus*) *acheilognathi*) is an intestinal cestode parasite of fishes native to East Asia, and was originally described in 1934 as *Bothriocephalus acheilognathi* from a single small cyprinid fish (*Acheilognathus rhombeus*) in Japan (Yamaguti, 1934; Kuchta *et al.*, 2018). Unusually for a tapeworm, *S. acheilognathi* has a very broad host range and has been found in at least 312 freshwater fish species, belonging to 38 families and 14 orders, as a result of worldwide introductions (Kuchta *et al.*, 2018). The species has also been identified in non-piscine vertebrate hosts including a human, amphibians, reptiles and birds (Prigli, 1975; García-Prieto & Osorio-Sarabia, 1991; Scholz, 1999; Yera *et al.*, 2013; de León *et al.*, 2018; Kuchta *et al.*, 2018), although these are considered accidental hosts (Kuchta *et al.*, 2018). Infection with *S. acheilognathi* can cause bothriocephalosis in its host, with clinical signs including blockage of the gastrointestinal tract, destruction of intestinal mucosa, intestinal rupturing, distended abdomen, weight loss, protein depletion, intestinal inflammation, anaemia, diminished swimming ability and eventual mortality (Davydov, 1978; Scott & Grizzle, 1979; Brouder, 1999; Hansen *et al.*, 2006; Matey *et al.*, 2015; Cole & Choudhury, 2016). The clinical signs can also be subtle, leading to reduced growth in the host, which can result in higher predation rates (Hansen *et al.*, 2006; Choudhury *et al.*, 2013). The global spread of invasive fish species and their parasites, such as *S. acheilognathi*, is likely attributable to the exotic fish trade (Košuthová *et al.*, 2015; Kuchta *et al.*, 2018).

During the 1960s and 1970s, *S. acheilognathi* was imported from East Asia to Europe and North America in grass carp, which was introduced to reduce the growth of vegetation in freshwater ecosystems (Choudhury *et al.*, 2006, 2013; Matey *et al.*, 2015; de León *et al.*, 2018). The parasite has since spread globally (Cole & Choudhury, 2016; Kuchta *et al.*, 2018) and has

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been the cause of numerous devastating infections and high mortalities of naïve hosts in fish hatcheries, with serious economic consequences for the aquaculture industry (Han *et al.*, 2010; Kilian *et al.*, 2012; Oros *et al.*, 2015; Xi *et al.*, 2016; Boonthai *et al.*, 2017). Fish are a major source of global income (Shelton & Rothbard, 2006; Olsen & Hasan, 2012; Tacon & Metian, 2013), particularly for developing countries (Mulokozi *et al.*, 2020), where koi carp (*Cyprinus carpio haematopterus*) are often bred for consumption or aesthetic purposes (Rahaman *et al.*, 2012). In addition, the invasion of waterways outside of its natural range by *S. acheilognathi* may threaten native fishes (Choudhury *et al.*, 2006; de León *et al.*, 2018). Brouder & Hoffnagle (1997) studied fishes from the Colorado River, USA, and found that three of the rivers' four endangered fish species host *S. acheilognathi* (Brouder & Hoffnagle, 1997). A subsequent study found *S. acheilognathi* parasitizing all fish species in the Little Colorado River (Choudhury *et al.*, 2004). *Schyzocotyle acheilognathi* has contributed to the decline of 40–60% of the humpback chub (*Gila cypha*) in the Grand Canyon National Park, Arizona, USA, since the 1990s and is being monitored in the desert of the south-west USA (Choudhury & Cole, 2012).

In Australia, *S. acheilognathi* has been previously reported from the eastern coastal states including New South Wales (NSW), Queensland and Victoria (Dove & Fletcher, 2000). Only three studies have molecularly confirmed the species identity of the parasite in Australia, and all are from NSW (Luo *et al.*, 2002; Xi *et al.*, 2016; Kuchta *et al.*, 2018; Rochat *et al.*, 2020). With the advent of molecular technology, there has been an increasing need for genus-specific primers that amplify hypervariable regions to enable species identification. Hadziavdic *et al.* (2014) compared all of the regions within the 18S ribosomal RNA (rRNA) gene of *S. acheilognathi* and found that the hypervariable region V4 had the longest variable region with the greatest length of polymorphisms, and also included a 70 bp conserved region for primer annealing. Primers targeting the 18S rRNA V4 region in *S. acheilognathi* have been successful in identifying the species (Nickrent & Sargent, 1991; Bean *et al.*, 2007; Hadziavdic *et al.*, 2014). In the present study, we report the first evidence of *S. acheilognathi* from Western Australia (WA), within the South-Western Ichthyological Province, a region of extreme endemism, using morphological and molecular identification at the 18S rRNA V4 locus.

Materials and methods

Study site and sample collection

Between February and May 2018, 134 introduced fish from three species were collected from Blue Lake, a modified wetland in the City of Joondalup, WA (S 31° 44.700′, E 115° 45.9668′) as part of an approved feral fish control program. The fish consisted of 91 goldfish (*Carassius auratus*), 26 koi carp (*C. carpio haematopterus*) and introduced eastern gambusia or mosquitofish (*Gambusia holbrooki*).

Freshly euthanized fishes were dissected at Murdoch University's Fish Health Unit, Perth, WA, and dead tapeworms were removed from the intestine. Half of the tapeworms from each sample were placed in 10% formalin for microscopy and the remainder placed in 100% ethanol for molecular analysis.

Statistical analysis

Prevalence [percentage (%) of hosts infected with *S. acheilognathi*, with 95% confidence intervals (CIs)] and intensity of infection (number of *S. acheilognathi* found in each host) (Bush *et al.*, 1997) were calculated using the Program QPweb 3.0 (Reiczigel *et al.*, 2019).

Morphological analysis

For morphological analysis on samples from goldfish and koi carp, tapeworms were first relaxed in sterile water overnight and then stained using a dilute Semichon's acetocarmine stain, dehydrated using a graded alcohol series, cleared in methyl salicylate, mounted whole in Canada balsam and viewed in brightfield on an Olympus BX50 compound microscope [10×–40× (Olympus Corporation, Shinjuku, Tokyo, Japan)] with an Olympus DP71 universal camera (Olympus Corporation, Shinjuku, Tokyo, Japan), using cellSens software (Olympus Corporation, Shinjuku, Tokyo, Japan). The eggs were viewed on the same microscope with a DP1 universal camera but using a Nomarski filter. Infection intensities were viewed on an Olympus stereo microscope SZX7 with an Olympus DP27 camera, using cellSens software.

Molecular analysis

Total genomic DNA (gDNA) was extracted from ~25 mg of tapeworm tissue (one tapeworm per extraction) and fish intestinal tissue. The tapeworms and intestinal tissue were dissected in sterile petri dishes and extractions were conducted using a Qiagen DNeasy Blood and Tissue kit (Qiagen, St Louis, Missouri, USA). The 18S rRNA V4 region was amplified using the forward primer Ces1 (5′-CCAGCAGCCGCGGTAACTCCA-3′) and reverse primer Ces2 (5′-CCCCCGCCTGTCTCTTTTGAT-3′) producing a ~420 bp product as previously described (Scholz *et al.*, 2003; Bean *et al.*, 2007), except that the annealing temperature was adjusted to 64°C after optimization. Polymerase chain reaction (PCR) was conducted using 25 µl reaction volumes, which consisted of 0.02 U/µl Taq DNA polymerase, 1× reaction buffer, 1 mM $MgCl_2$, 200 µM of each dNTP, 1 µM of each primer, 2 µl template gDNA and PCR-grade water to the final volume. PCR cycling conditions for the Ces1/Ces2 primer set consisted of an initial denaturation at 95°C for 15 min, followed by 45 cycles of 94°C for 1 min, 64°C for 1 min and 72°C for 2 min, with a final elongation step at 68°C for 10 min. PCR products were separated by gel electrophoresis using a 1.0% (*w/v*) agarose gel (Fisher Biotec, Wembley, WA, Australia) in Tris-acetate buffer (consisting of 40 mM Tris-hydrochloride, 20 mM ethylenediamine tetraacetic acid at pH 7.0). PCR products of the expected size were excised from the agarose gel, purified using an in-house filter-tip-based method (Yang *et al.*, 2013) and sequenced using a Big Dye version 3.1 Terminator Cycle Sequencing Kit (Applied Biosystems, Massachusetts, USA) on a 96-capillary 3730xl DNA Analyzer (Thermo Fisher Scientific, Waltham, Massachusetts, USA) at Murdoch University and at the Australian Genome Research Facility (AGRF), Perth, WA. Sequences were assembled in the forward and reverse directions to produce a consensus sequence, assessed for quality and trimmed of primers using Geneious v10.2.2 (Kearse *et al.*, 2012). Consensus sequences were checked against nucleotide sequences in the GenBank database using the Basic Local Alignment Search Tool (BLAST) and aligned with related sequences using the MUSCLE alignment tool (Edgar, 2004) with Geneious software. The 364 bp alignment (including gaps) was imported into the PhyML program (Guindon *et al.*, 2010) to assess nucleotide substitution models based on

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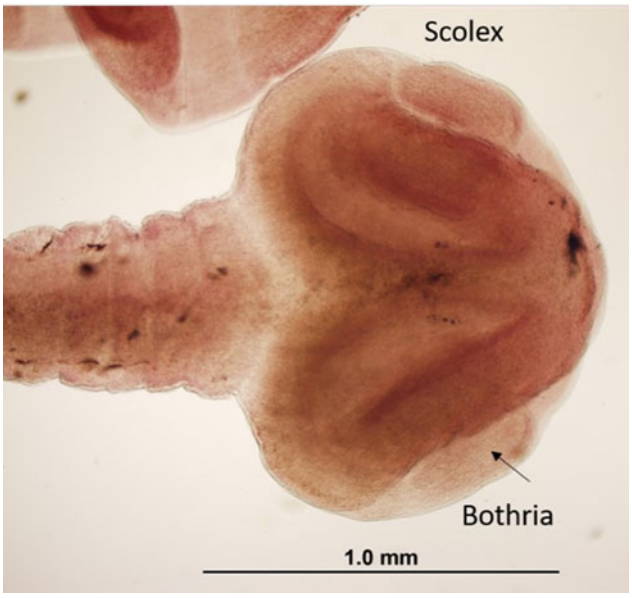

Fig. 1. Semichon's acetocarmine-stained heart-shaped scolex of *Schyzocotyle acheilognathi*, from the intestines of koi carp (*Cyprinus carpio haematopterus*), showing unarmed bothria and proglottids much narrower than the scolex with rounded edges (photo: Aileen Elliot).

Bayesian Information Criterion. The model general time reversible + gamma distribution (GTR + G) was used to construct a Bayesian phylogenetic tree using Geneious and the MrBayes plugin v3.2.6 (Huelsenbeck & Ronquist, 2001). The tree was built using the following parameters: Hasegawa-Kishino-Yano (HKY85) + GTR + G model; 1,100,000 Markov chain Monte Carlo (MCMC) length; 'burn-in' length of 10,000; subsampling frequency of 200. The tree was rooted with the outgroup sequence *Parabothriocephalus gracilis* (KR780945) (not shown).

Results

Morphological identification

The tapeworms from the koi carp and goldfish were identified as *S. acheilognathi* based on the following morphological characters: fleshy heart-shaped scolex with unarmed bothria that were short and deep; proglottids had rounded edges with an absent neck; the first proglottids were immediately posterior to the scolex and much narrower than the scolex; and in mature proglottids, genital pores and vitelline follicles were clearly visible (figs 1 and 2a, b). All tapeworms examined using microscopy had gravid proglottids (fig. 2c), with eggs being both operculated and unembryonated (fig. 2d).

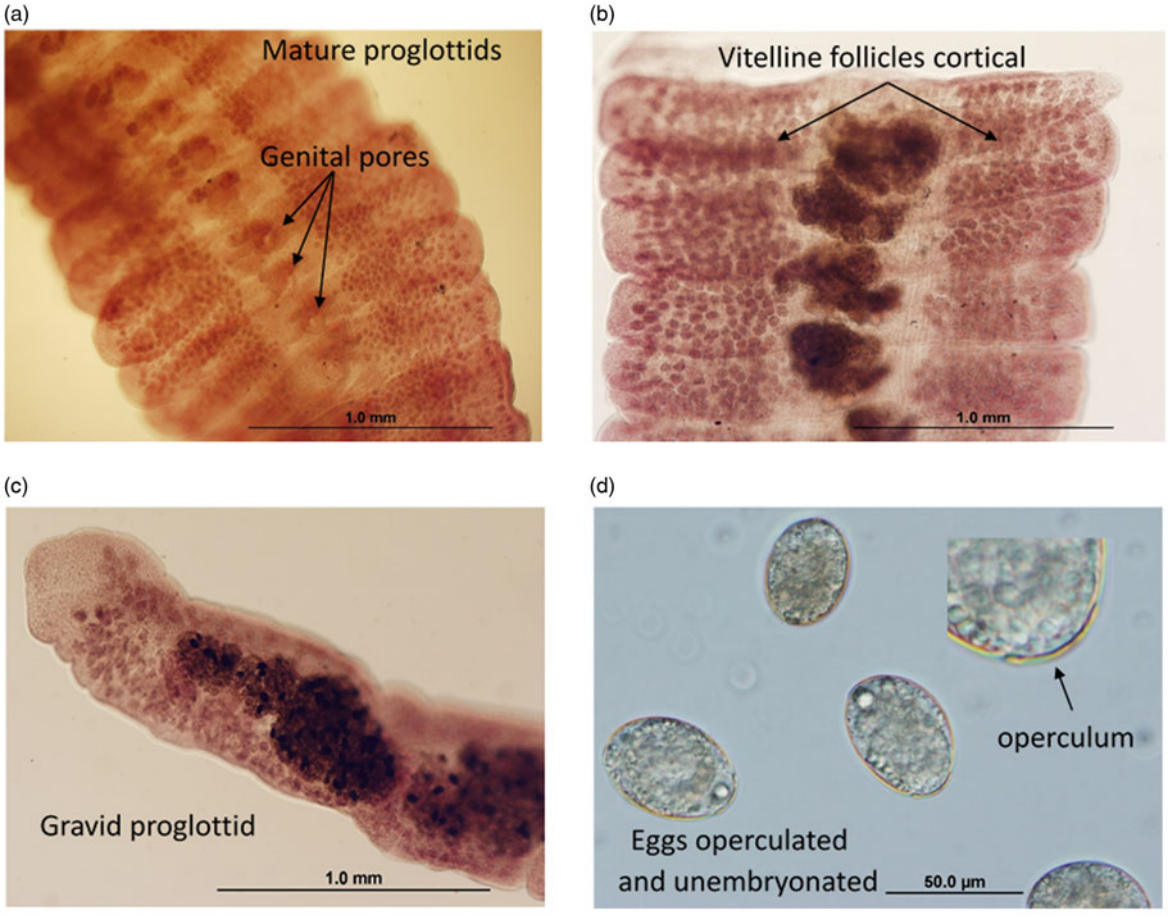

Fig. 2. Semichon's acetocarmine-stained mature and gravid proglottids of *Schyzocotyle acheilognathi*. (a) Arrows indicate genital pores and (b) vitelline follicles. (c) Gravid proglottids ready for dispersal and (d) operculated and unembryonated eggs, with arrow indicating operculum (photos: Aileen Elliot).

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Table 1. Length, weight and prevalence of fishes infected with *Schyzocotyle acheilognathi* from Blue Lake, Joondalup.

Host species	Weight range	Total length range	% Hosts infected	# Hosts infected
Carassius auratus	30.27–23.50 g	106–498 mm	3.3% (CI 3.0–4.3)	3
Cyprinus carpio haematopterus	1466–4780 g	448–768 mm	37% (CI 36.0–38.0)	7
Gambusia holbroki	0.67 g and 4.03 g	38–84 mm	65% (CI 64.0–66.0)	11

Prevalence and intensity of infection

The length and weight range of the koi carp, goldfish and eastern gambusia as well as molecular confirmation of *S. acheilognathi* in each of the hosts was recorded with an overall tapeworm prevalence of 8.5% (95% CI 7.5–9.5) (table 1).

The intensity of infection measured in goldfish and koi carp was high, ranging from 203 to 607 parasites per infected fish (mean = 386, 95% CI 296–486) (table 2 and fig. 3), with evidence of intestinal perforation, blockage and ischemia. Several tapeworms exceeded 400 mm in length, which was almost equal to the length of the intestines in some fish. Eastern gambusia also had a high occurrence of parasite burdens, although intensity of infection was not measured.

Molecular characterization

The 18S rRNA V4 region was amplified from two koi carp (CP1, CP4), two goldfish (CPAFT3, CPAFT4) and four eastern gambusia (CPAFT1, CPAFT2, CPAFT5 and CPAFT7) (table 3). Sequencing confirmed that two of the koi carp isolates (CP1 and CP4, GenBank accession numbers MT898660 and MT898661) were identical to each other (supplementary appendix) and to *S. acheilognathi* (KX060604) isolated from red shiner (*Cyprinella lutrensis*) in the Czech Republic, and from a human sample (HM367066) (Yera *et al.*, 2013; Brabec *et al.*, 2016). Furthermore, isolates CP1 and CP4 were 99.0% similar to 18S rRNA V4 sequences from 12 *Schyzocotyle* isolates from other fishes, including threadfin shad (*Dorosoma pretensense*) and *C. lutrensis* (DQ86690 and AY340106, respectively) (Škerıková *et al.*, 2004; Bean *et al.*, 2007). Two of the eastern gambusia isolates (CPAFT1 and CPAFT2) (GenBank accession numbers MT898662 and MT898663) were identical and were 99.0% similar to nine BLAST hits, including *S. acheilognathi* from red shiner (*C. lutrensis*) (KX060604) and largemouth yellowfish (*Labeobarbus kimberleyensis*) (KX060602) (Brabec *et al.*, 2016). Four of the isolates obtained in this study from two goldfish (*C. auratus*) (CPAFT3 and CPAFT4) and two eastern gambusia (CPAFT5 and CPAFT7) were identical to each other (GenBank accession numbers (MT898664–MT898667), but only 98.7% similar to a single sequence named *S. acheilognathi* (AY340104) (Škerıková *et al.*, 2004). These *Schyzocotyle* sp. Blue Lake genotype isolates were then most similar to 12 *S. acheilognathi* isolated from Nazas chub (*Gila conspersa*) and a common carp (KX060601 and KX060600, respectively) (Brabec *et al.*, 2016). However, sequence similarity with these 12 isolates was only 91.0%, with 99.0% query cover (table 3) (see supplementary appendix table S1).

Phylogenetic analysis

All isolates were distinct from *Bothriocephalus* and *Senga* species and grouped within *Schyzocotyle* clades (fig. 4). The koi carp sequences (CP4, MT898661; and CP1, MT898660) grouped with *S. acheilognathi* sequences (*Schyzocotyle* Group 2A), and two of the novel eastern gambusia genotypes (CPAFT1, MT898662; and CPAFT2, MT898663) formed a discrete *S. acheilognathi* subgroup (*Schyzocotyle* Group 2B) with strong support (posterior probability (PP) = 0.9). Four sequences (MT898664–MT898667) formed a discrete subgroup to *S. acheilognathi* (Group 3) with strong support (PP = 1.0), which included two goldfish (CPAFT3 and CPAFT4) and two eastern gambusia (CPAFT5 and CPAFT7) (fig. 4).

Table 2. Number of tapeworm scolices found in the goldfish and koi carp from Blue Lake, Joondalup in Western Australia.

		Tapeworm scolices count[b]		
Host species	Sample ID[a]	Ethanol	Formalin	Total
Goldfish (*Carassius auratus*)	CPAFT3	261	256	**517**
	CPAFT4	158	161	**319**
	CPAFT9	111	96	**207**
Koi carp (*Cyprinus carpio haematopterus*)	CP1	240	367	**607**
	CP2	441	160	**601**
	CP3	206	182	**388**
	CP4	173	30	**203**
	CP5	79	130	**209**
	CP6	342	154	**496**
	CP7	186	129	**315**

[a]Sample identification code.
[b]Each scolex was counted as one cestode.

Fig. 3. Intensity of *Schyzocotyle acheilognathi* infection in one koi carp (photo: Aileen Elliot).

Discussion

AFT identification has historically been based on the unique morphology of the scolex, which is inadequate for detecting

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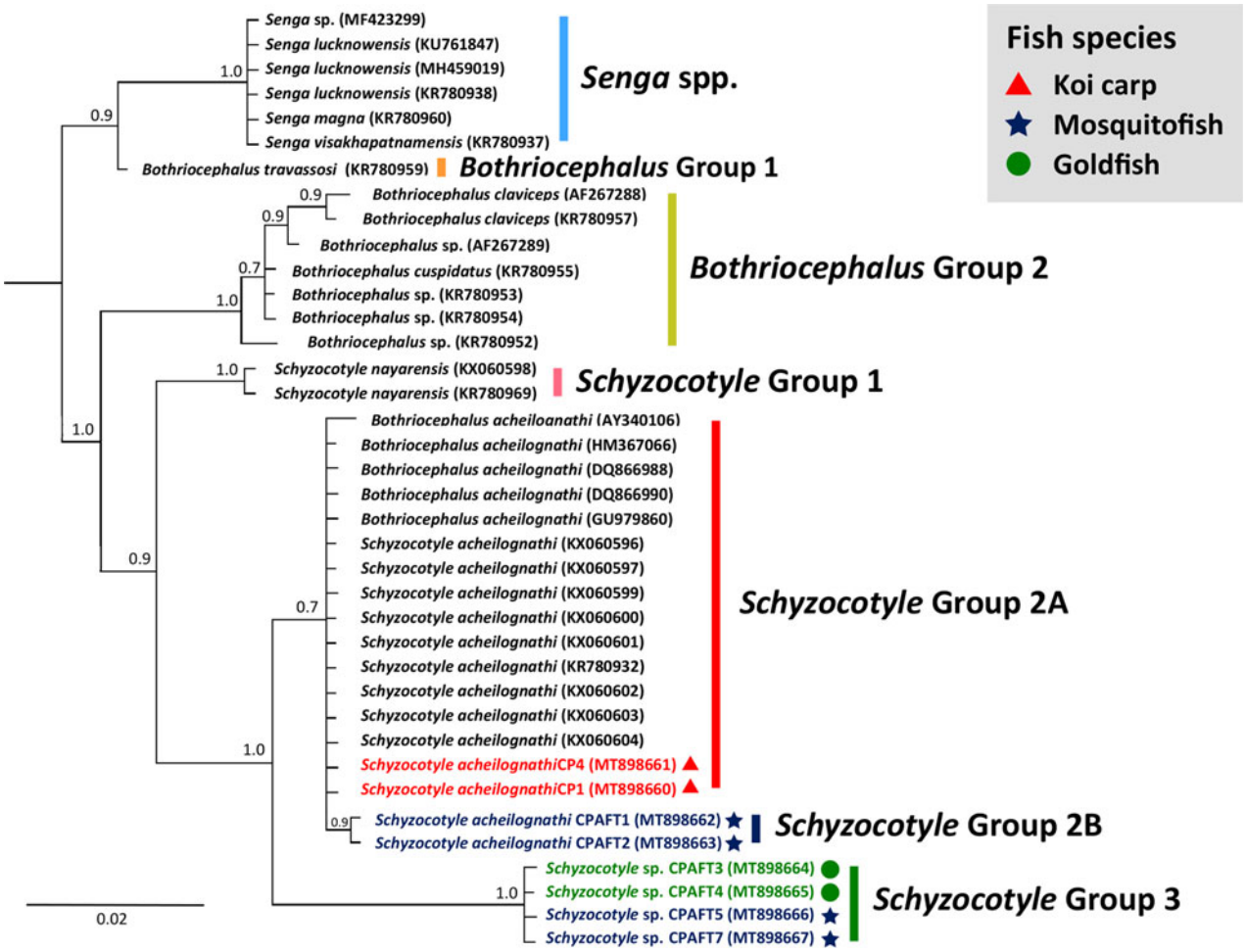

Fig. 4. Bayesian phylogenetic tree of a 354 bp alignment (including gaps) of 18S rRNA V4 sequences of known *Schyzocotyle acheilognathi* species and *Schyzocotyle* sequences derived from this study. Scale bar shows the number of nucleotide substitutions per site. Sequences from this study are indicated by colours and symbols in the figure's legend.

closely related subtypes (Brabec *et al.*, 2016; Xi *et al.*, 2016). Indeed, in this study, the gross morphology of the AFT and *Schyzocotyle* sp. Blue Lake genotype (GenBank accession numbers MT898664–MT898667) was indistinguishable. *Schyzocotyle* sp. Blue Lake genotype was evident by the low sequence homology (91.9% sequence similarity) to AFT sequences in GenBank (table 3), and the phylogenetic grouping of this tapeworm was distinct from other *Schyzocotyle* species, with strong support (PP = 1.0) (fig. 4). This emphasizes the need for molecular methods for the subtype identification of *Schyzocotyle* sp. Blue Lake genotype and the AFT in future studies. As this study targeted a partial fragment of the 18S rRNA gene, characterization of the complete or near full-length of 18S rRNA is required to confirm that *Schyzocotyle* sp. Blue Lake genotype represents a novel species from an unknown origin. Future studies should also aim to morphologically and genetically characterize *Schyzocotyle* species identified by this study, which will also aid our understanding of the potential origins of the AFT in south-western WA (Luo *et al.*, 2002; Xi *et al.*, 2016).

In the present study, *S. acheilognathi* was identified by microscopy and molecular characterization for the first time in WA from introduced goldfish, koi carp and eastern gambusia in a modified urban wetland. If translocated from this locality (e.g. by humans, birds, floods, etc.) to adjacent lentic or lotic systems, this parasite would pose a serious threat to the unique freshwater (and possibly estuarine) fish fauna of south-western WA. Several studies have already shown that AFT can infect fish in environments with low to moderate salinity (Ozturk *et al.*, 2002; Bean, 2008; Bean & Bonner, 2010; İnnal *et al.*, 2016; Sara *et al.*, 2016; McAllister *et al.*, 2017; Güven & Öztürk, 2018; Zhokhov *et al.*, 2019), and has already threatened the populations of several endemic species in other countries (Salgado-Maldonado & Pineda-López, 2003; Choudhury *et al.*, 2006, 2013; de León *et al.*, 2018).

The inland waters in the south-west of WA have the highest proportion of endemic freshwater fish on the continent and are considered one of the world's biodiversity hotspots (Myers *et al.*, 2000; Beatty & Morgan, 2013; Morgan *et al.*, 2014). Invasive fish species may predate or compete with native fishes, or alter the habitat to the detriment of native species (Olden *et al.*, 2008; Beatty & Morgan, 2013; Morgan *et al.*, 2014). An additional threat posed by alien fishes, and one that is often underappreciated, is the introduction of new parasites and pathogens (co-invaders) (Lymbery *et al.*, 2014). Co-invading parasites can be more pathogenic to native fishes than to their natural hosts, possibly due to the lack of coevolutionary existence (Lymbery *et al.*, 2014) and, as such, can cause morbidity and mortality on

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Table 3. 18S rRNA V4 isolates from fishes with closest matches to sequences in GenBank.

Host species	Tapeworm species	Isolate name	GenBank accession no.	Top BLAST matches from GenBank	Top BLAST GenBank accession no.	Host species
Cyprinus carpio haematopterus	*Schyzocotyle acheilognathi*	CP1 CP4	MT898660 MT898661	*Schyzocotyle acheilognathi* 100.0% *Schyzocotyle acheilognathi* 100.0% *Schyzocotyle acheilognathi* 100.0% *Schyzocotyle acheilognathi* 99.6% *Schyzocotyle acheilognathi* 99.5%	KX060604 KR780932 HM367066 DQ866990 AY340106	*Cyprinella lutrensis* *Cyprinus carpio* *Homo sapiens* *Cyprinella lutrensis* *Dorosoma pretensense*
Gambusia holbrooki	*Schyzocotyle acheilognathi*	CPAFT1 CPAFT2	MT898662 MT898663	*Schyzocotyle acheilognathi* 99.0% *Schyzocotyle acheilognathi* 99.0% *Schyzocotyle acheilognathi* 99.0% *Bothriocephalus acheilognathi* 99.0%	KX060604 KX060603 KX060602 GU979860	*Cyprinella lutrensis* *Atherina boyeri* *Labeobarbus kimberleyensis* *Cyprinus rubrofuscus*
Carassius auratus *Gambusia holbrooki*	*Schyzocotyle* sp. Blue Lake genotype	CPAFT3 CPAFT4 CPAFT5 CPAFT7	MT898664 MT898665 MT898666 MT898667	*Bothriocephalus acheilognathi* 98.7% *Schyzocotyle acheilognathi* 90.7% *Schyzocotyle acheilognathi* 90.7% *Bothriocephalus acheilognathi* 90.7%	AY340104 KX060600 KX060597 DQ866988	*Dorosoma cepedianum* *Gila conspersa* *Labeobarbus nedgia* *Cyprinella lutrensis*

already declining native populations. In south-western WA, many river systems have seasonally intermittent flow, and fish communities must survive the dry season in small, disconnected refuge pools, which could amplify the transmission and effects of co-invading parasites (Lymbery *et al.*, 2020). At least two other species of co-invading parasites, including *Ligula intestinalis* (Morgan, 2003; Chapman *et al.*, 2006) and *Lernaea cyprinacea*, have recently been identified in native fishes in south-western WA and they also infect diadromous and estuarine fishes that venture into freshwaters (Hassan *et al.*, 2008). *Lernaea cyprinacea* was introduced to WA's native waterways as a co-invader with goldfish and now infects at least six native freshwater species (Hassan *et al.*, 2008). The original source of the *S. acheilognathi* in WA is unknown, but it appears that either goldish or koi carp are likely to be the source as these are the natural hosts for this particular parasite (Dove & Fletcher, 2000; Košuthová *et al.*, 2015; Oros *et al.*, 2015; Salgado-Maldonado *et al.*, 2015; Kuchta *et al.*, 2018).

The histopathological signs of *S. acheilognathi* in fish hosts are frequently extreme, with the parasite often killing its host (Liao & Shih, 1956; Bauer *et al.*, 1969; Edwards & Hine, 1974; Scott & Grizzle, 1979; Pool *et al.*, 1984; Schäperclaus *et al.*, 1991; Brouder, 1999). In the present study, there was evidence of intestinal perforation and a large number (>600) of *S. acheilognathi* detected in each fish – far greater than previous studies, which have reported infection intensities of between two and 45 tapeworms (Scholz, 1997; Brouder, 1999; Košuthová *et al.*, 2015). In this study, the high intensity of tapeworms caused blockage and ischemia in parts of the intestines, resulting in intestinal perforations. The size of the tapeworms varied according to the size of the host and in one koi carp sample a single tapeworm measured over 400 mm in length. Over one quarter of the native fishes in south-western WA are listed as threatened with many very small in size (<100 mm total length) (Morgan *et al.*, 2014). As with many parasitic interactions, the pathogenicity of *S. acheilognathi* increases in smaller hosts, with the potential to impact native fishes further. *Gambusia holbrooki* has been previously identified as a host for *S. acheilognathi* (Dove *et al.*, 1997; İnnal *et al.*, 2016; McAllister *et al.*, 2017). *Gambusia holbrooki* is already widespread throughout Australia and, because of its very large population sizes, represents a potential vector for further spread of the parasite (Morgan & Buttemer, 1996; Reynolds, 2009). The discovery of *S. acheilognathi* in WA waters reinforces the importance of invasive fish control programs. Communication and education programs to the wider community are needed to help reduce the release of alien fishes into Australian waterways. It should also be noted that *S. acheilognathi* poses a potential health risk to humans. Although not generally considered zoonotic, there is one case study that identified a 32-year-old male who was initially diagnosed with *Diphyllobothrium*; however, molecular analysis of the DNA extracted from eggs in the patient's faeces identified them as *S. acheilognathi*, highlighting the importance of molecular identification techniques (Yera *et al.*, 2013). Future research investigations should also study other potential vectors, such as frogs, reptiles and birds (Kuchta *et al.*, 2018), for the transmission of *Schyzocotyle* spp. in south-western WA. It is imperative that the potential spread and impacts of the invasion of *S. acheilognathi* be identified in future research to effectively control and manage the transmission of the parasite.

Supplementary material. To view supplementary material for this article, please visit https://doi.org/10.1017/S0022149X21000365.

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Conflicts of interest. None.

Ethical standards. The authors declare that the animals used in this research were euthanized in compliance with Animal Ethics Protocol ID. 401 (permit number R2949/17).

Author contributions. C.J.P., D.L.M. and S.J.B. contributed to the conception and design of the study, and acquisition of data. C.J.P., S.J.B. and A.E. analysed and interpreted the morphological data. C.J.P. and T.L.G. analysed and interpreted the molecular data. C.J.P. drafted the article, and D.L.M., S.J.B., A.E. and T.L.G. critically revised the manuscript. All authors approved the final version of the manuscript submitted for publication.

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