The Discovery of Asian Fish Tapeworm (*Schyzocotyle Acheilognathi*) and *Schyzocotyle* Sp. In Western Australia May Pose a Threat to the Health of Endemic Native Fishes

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Abstract

Purpose

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).

Methods

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 mosquitofish (*Gambusia holbrooki*). For molecular confirmation, the 18S rRNA gene was targeted at hypervariable region V4 using conventional PCR and Sanger sequencing.

Results

Koi carp isolates were 100.0% identical to *S. acheilognathi* isolated from varying hosts including the red shiner (*Cyprinella lutrensis*) and a human sample. Sequences from two eastern mosquitofish were identified as *S. acheilognathi*, but formed a discrete subclass and may represent a novel genotype. Isolates from two other eastern mosquitofish and two goldfish formed a distinct clade with only 91.9% similarity to previously sequenced isolates of *S. acheilognathi*, indicating a potentially novel *Schyzocotyle* species.

Conclusion

This emphasises the importance of molecular identification methods in addition to morphological identification. The presence and potential for spread 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 (AFT) (*Schyzocotyle* (formerly *Bothriocephalus*) *acheilognathi*) is an intestinal cestode parasite of fishes native to East Asia. The species was originally described by Yamaguti (1934) as *Bothriocephalus acheilognathi* from a single small cyprinid fish (*Acheilognathus rhombeus*) in Lake Ogura, Japan, despite the most common host being grass carp (*Ctenopharyngodon idella*) (Yamaguti 1934; Kuchta et al. 2018). Since then, despite the morphologically distinct heart-shaped scolex with deep bothria, the taxonomy remains contentious with more than 10 different synonyms described (e.g. *Bothriocephalus opsariichthydis*, *Bothriocephalus gowkongensis*, *Bothriocephalus sinensis*, *Bothriocephalus phoxini*, *Bothriocephalus aegyptiacus*, *Bothriocephalus kivuensis*, and *Coelobothrium gambusiense*) on the basis of scolex shape and proglottid dimension, typically caused by differences in specimen preservation or host-specific adaptations (Brabec et al. 2016; Xi et al. 2016).

Unusually for a tapeworm, *S.acheilognathi* has a very broad definitive host range and has been found in at least 312 freshwater fish species, belonging to 38 families and 14 orders, worldwide (Kuchta et al. 2018). The species has also been identified in non-piscine vertebrate hosts, including amphibians, reptiles, and birds (García-Prieto and Osorio-Sarabia 1991; Scholz 1999; Kuchta et al. 2018), although these are considered accidental hosts

(Kuchta et al. 2018). In a study conducted by Prigli (1975), *S. acheilognathi* was transmitted to ducks when they fed on young infected carp. Viable eggs were found in the faeces 3 h after ingestion, suggesting that birds could act as potential vectors of AFT (Prigli 1975). *Schyzocotyle acheilognathi* can also infect humans, a male patient from France, who regularly ate raw fish, had persistent abdominal pain and AFT eggs were found in his stool (Yera 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 (Kořuthová et al. 2015; Kuchta et al. 2018). Transmission of *S. acheilognathi* is facilitated by its simple lifecycle which can be completed in 14 days at 25 °C. The definitive host is a fish, the eggs are shed from the host and hatch into free-swimming coracidia, which are consumed by the intermediate host, cyclopoid copepods (Arthropoda, Crustacea). Infected copepods are then consumed by the fish and larval stages progress into mature tapeworms (Kuchta et al. 2018).

During the 1960's and 1970's, *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 (Matey et al. 2015). The parasite has since spread to all continents, except Antarctica, and has 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; Xi et al. 2016). In addition, the invasion of waterways outside of its natural range by *S. acheilognathi* may threaten native fishes. Brouder and Hoffnagle (1997), for example, studied fishes from the Colorado River, USA, and found that three of the rivers' four endangered fish species host *S. acheilognathi*, with an introduced alien fish species in the same river having a prevalence of 22.5% (Brouder and Hoffnagle 1997). A subsequent study in 2004 found *S. acheilognathi* parasitising all fish species in the Little Colorado River (Choudhury et al. 2004).

Farming and breeding fish is a major source of global income (Shelton and Rothbard 2006; Olsen and Hasan 2012; Tacon and Metian 2013), particularly for developing countries (Mulokozi et al. 2020). However, studies have shown *S. acheilognathi* can have devastating impacts on the industry. Koi carp (*Cyprinus carpio haematopterus*) are bred for consumption and aesthetic purposes (Rahaman et al. 2012). During a routine inspection, Oros et al. (2015) examined 10 market-sized koi carp from a breeding pond in Eastern Slovakia. *Schyzocotyle acheilognathi* was morphologically identified in 40.0% of the fish, representing a significant threat to traditional fisheries (Oros et al. 2015). In a more recent study, the live baitfish trade was emphasised as an important transmission vector for *S. acheilognathi*, to infect native fish communities (Boonthai et al. 2017). The use of live bait in the USA can compromise aquaculture facilities (Kilian et al. 2012) and AFT can spread to novel areas via bait-bucket introductions (e.g. minnows) with direct release of infected fish. Samples collected in a study from 78 retail stores in Michigan showed that bait fish had a prevalence of tapeworms as high as 58.0% (Boonthai et al. 2017).

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 and Grizzle 1979; Brouder 1999; Hansen et al. 2006; Matey et al. 2015). The clinical signs also lead to reduced growth in the host, which can result in higher predation rates (Choudhury et al. 2013). As a result, *S. acheilognathi* has been implicated in the decline of native fish species in many countries (Brouder 1999; Pérez-Ponce de León and Poulin 2018).

In Australia, *S. acheilognathi* has been previously reported from the eastern coastal states including New South Wales (NSW), Queensland, and Victoria (Dove and 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 universal genus-specific primers that amplify hypervariable regions to enable species identification. Hatziavdic et al. (2014) compared all of the regions within the 18S 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 and 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 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, 117 introduced fishes were collected from Blue Lake, a modified wetland in the City of Joondalup, WA (S 31.745001, E 115.766113) as part of an approved feral fish control program. The fish consisted of 91 goldfish (*Carassius auratus*) and 26 koi carp (*Cyprinus rubrofuscus*). Additionally, a small number ($n = 17$) of introduced eastern mosquitofish (*Gambusia holbrooki*) were also collected as part of the control program.

Fishes were dissected at Murdoch University's Fish Health Unit and tapeworms removed from the intestine. Half of the tapeworms from each sample were placed in formalin for microscopy and the remainder placed in 100% ethanol for molecular analysis.

Statistical analysis

Prevalence (% of infected fish, with 95% confidence intervals (CI) estimated assuming a binomial distribution) and mean intensity (number of parasites per infected fish, with 95% CI estimated by bootstrapping) were calculated using the program QP Web (Reiczigel et al. 2019).

Morphological analysis

For morphological analysis on samples from goldfish and koi carp, tapeworms were stained using a dilute Semichon's 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 (10x – 40x) using an Olympus DP71 universal camera. The eggs were viewed on the same microscope, but using a Nomarski filter. The tapeworms from *G. holbrooki* were examined using an Olympus microscope (10x – 40x).

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). The 18S rRNA V4 region was amplified using the forward primer Ces1 (5'-CCAGCAGCCGCGGTAAGTCCA-3') and reverse primer Ces2 (5'-CCCCGCCTGTCTCTTTTGAT-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 optimisation. Conventional PCRs were conducted using 25 µL reaction volumes, which consisted of 0.02 U/µL Taq DNA polymerase, 1x reaction buffer, 1 mM MgCl₂, 200 µM each of dNTP (dATP, dCTP, dGTP, and dTTP), 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, Australia) in Tris-Acetate (TAE) buffer (consisting of 40 mM Tris-HCl, 20 mM EDTA at pH 7.0). The DNA was stained with SYBR® safe DNA gel stain (Invitrogen, Oregon, USA). 5 µL of 100 bp ladder (Promega, Madison, USA) was used as a reference marker in the first well. PCR products were electrophoresed at 85 V for 30-40 min in a gel tank (BioRAD, USA). Gels were then visualised under a transilluminator (FisherBiotec, Australia). PCR products of the expected size were excised from the agarose gel with sterile blades, 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. Sequence chromatograms in the forward and reverse directions were aligned 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 sequences in GenBank® using the Basic Local Alignment and Search Tool (BLAST) and aligned with related sequences using the MUSCLE alignment tool (Edgar 2004). The 364 bp alignment (including gaps) was imported into the PhyML program (Guindon et al. 2010) to assess nucleotide substitution models based on Bayesian Information Criterion (BIC). The model GTR+G was used to construct a Bayesian phylogenetic tree using Geneious v8.1.9 and the MrBayes plugin v3.2.6 (Huelsenbeck and Ronquist 2001).

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, 2A, B). All tapeworms examined had gravid proglottids (Fig. 2C), with eggs being both operculated and unembryonated (Fig. 2D).

Prevalence and intensity of infection

The goldfish ranged in total length between 106–498 mm and in weight from between 30.27–2350 g, whilst the koi carp ranged in total length between 448–768 mm and in weight ranged between 1466–4780 g, and the eastern

mosquitofish ranged in total length between 38-84 mm and in weight ranged between 0.67g and 4.03g. Tapeworms were found in 3 of the 91 goldfish (3.3%, 95% CI 3.0-4.3%) and 7 of the 26 koi carp (37.0%, 95% CI 36.0-38.0%), with an overall tapeworm prevalence of 8.5% (95% CI 7.5-9.5%). In addition, 11 of 17 eastern mosquitofish examined (65.0%, 95% CI 64.0-66.0%) were found to be infected.

The intensity of infection, measured in goldfish and koi carp was high, ranging from 203-607 parasites per infected fish (mean = 386, 95% CI 296-486) (Table 1), with evidence of intestinal perforation, blockage, and ischemia (Fig. 3). Several tapeworms exceeded 400 mm in length, which was almost equal to the length of the intestines in some fish. Eastern mosquitofish also had high parasite burdens, although intensity of infection was not measured (Fig. 4A-C).

Molecular characterisation

The 18S rRNA V4 region was amplified from two koi carp (CP1, CP4), two goldfish (CPAFT3, CPAFT4), and four eastern mosquitofish (CPAFT1, CPAFT2, CPAFT5, and CPAFT7) (Table 2). Sequencing confirmed that two of the koi carp isolates (CP1 and CP4, GenBank® accession numbers MT898660-MT898661) were 100.0% identical to each other (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 twelve *Schyzocotyle* isolates from other fishes including threadfin shad (*Dorosoma pretensense*) and *C. lutrensis* (DQ86690 and AY340106, respectively) (Škeriková et al. 2004; Bean et al. 2007). Two of the eastern mosquitofish isolates (CPAFT1 and CPAFT2) (GenBank® accession numbers MT898662-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 by this study from two goldfish (*C. auratus*) (CPAFT3 and CPAFT4) and two eastern mosquitofish (CPAFT5, CPAFT7) were 100.0% identical to each other (GenBank accession numbers MT898664 to MT898667), but only 98.7% similar to a single sequence named *S. acheilognathi* (AY340104) (Škeriková et al. 2004). These *Schyzocotyle* sp. genotype Blue Lake 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 2).

Phylogenetic analysis

All isolates were distinct from *Bothriocephalus* and *Senga* species and grouped within *Schyzocotyle* clades (Fig. 5). The koi carp sequences (CP4, MT898661 and CP1, MT898660) grouped with *S. acheilognathi* sequences (*Schyzocotyle* Group 2A), and two of the novel eastern mosquitofish 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) were most closely related to *S. acheilognathi*, but formed a sister group to *S. acheilognathi* (Group 3) with strong support (pp = 1.0), which included two goldfish (CPAFT3 and CPAFT4) and two eastern mosquitofish (CPAFT5 and CPAFT7) (Fig. 5).

Discussion

Asian fish tapeworm identification has historically been based on the unique morphology of the scolex, which is inadequate for detecting closely related subtypes (Brabec et al. 2016; Xi et al. 2016). Indeed, in this study, the gross morphology of the AFT and *Schyzocotyle* sp. genotype Blue Lake (GenBank® accession numbers MT898664-MT898667) was indistinguishable. *Schyzocotyle* sp. genotype Blue Lake was evident by the low sequence homology (91.9% sequence similarity) to AFT sequences in GenBank® (Table 2), and the phylogenetic grouping of this tapeworm was distinct from other *Schyzocotyle* species, with strong support from (pp = 1.0) (Fig. 5). This emphasises the need for molecular methods for species identification of *Schyzocotyle* sp. genotype Blue Lake and the AFT in future studies. As this study targeted a partial fragment of the 18S rRNA gene, characterisation of the complete or near full-length of this gene is required to confirm that *Schyzocotyle* sp. genotype Blue Lake represents a novel species from an unknown origin. Future studies should also aim to morphologically and genetically characterise *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 characterisation for the first time in WA, from introduced goldfish, koi carp and eastern mosquitofish 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 and Bonner 2010; Innal et al. 2016; Sara et al. 2016; McAllister et al. 2017; Güven and Öztürk 2018; Zhokhov et al. 2019), and has already threatened the populations of several endemic species in other countries (Salgado-Maldonado and Pineda-López 2003; Choudhury et al. 2013).

The inland waters in the south-west of WA have the highest proportion of endemic freshwater fish on the continent and is considered one of the world's diversity hotspots (Myers et al. 2000; Beatty and Morgan 2013; Morgan et al. 2014). Several species represent some of the earliest divergences of freshwater fish and are found nowhere else in the world. For example, the salamanderfish (*Lepidogalaxias salamandroides*) has an estimated divergence of up to 230 million years ago (Betancur-R et al. 2013; Morgan et al. 2014). Anthropogenic stressors, such as habitat loss, global warming, river regulation, secondary salinisation, decreased rainfall, and increased agricultural use has already threatened the survival of many of these endemic species. Further invasion of introduced species can lead to additional stressors, homogenisation, and possible extinction of native freshwater species (Olden et al. 2008; Beatty and Morgan 2013; Morgan et al. 2014). A recent study of the distribution of non-native fishes in the south-west of WA found that there has been an increase of up to 63.0% of introduced fishes since 1970, with an increase of 44.0% in the previous decade. Of the introduced species, up to 54.0% (seven species, including goldfish and koi carp) have established natural populations in the south-west of WA (Beatty and Morgan 2013).

Invasive fish species may predate or compete with native fishes, or alter the habitat to the detriment of native species. An additional threat posed by alien fishes, and one that is often under-appreciated, 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 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 goldfish or koi carp are likely to be the source as these are the natural hosts for this particular parasite (Dove and 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 eventually killing its host (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 2 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 endangered 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 and with the potential to impact native fishes further. *Gambusia holbrooki* has been previously identified as a host for *S. acheilognathi* (Dove et al. 1997; Innal 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 and 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 *Diphyllbothrium*, however, molecular analysis identified them as *S. acheilognathi*, highlighting the lack of morphological distinction in some parasites (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.

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Declarations

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Conflicts of interest/Competing interests

The authors declare that they have no conflicts of interest.

Ethics approval

The authors declare that the animals used in this research were euthanised in compliance with Animal Ethics Protocol ID. 401 Permit No. R2949/17.

Consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and material

All sequences generated by this study have been deposited in GenBank® under the Accessions: MT898660-MT898667.

Code availability

Not applicable

Authors' contributions

CJP, DLM, and SJB contributed to the conception and design of the study, and acquisition of data. CJP, SJB, and AE analysed and interpreted the morphological data. CJP and TLG analysed and interpreted the molecular data. CJP drafted the article, and DLM, SJB, AE, and TLG critically revised the manuscript. All authors approved the final version of the manuscript submitted for publication.

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Tables

Table 1 Scolices count of tapeworms found in the goldfish and koi carp from Blue Lake, Joondalup in Western Australia.

Host species	Sample ID ^a	Tapeworm scolices count ^b		Total
		Ethanol	Formalin	
Goldfish (<i>Carassius auratus</i>)	CPAFT3	261	256	517
	CPAFT4	158	161	319
	CPAFT9	111	96	207
Koi carp (<i>Cyprinus carpio haematopterus</i>)	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

^aSample identification code

^bEach scolex was counted as one cestode

Table 2 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	GenBank® accession no.	Host species
<i>Cyprinus carpio haematopterus</i>	<i>Schyzocotyle acheilognathi</i>	CP1	MT898660	<i>Schyzocotyle acheilognathi</i> 100.0%	KX060604	<i>Cyprinella lutrensis</i>
		CP4	MT898661	<i>Schyzocotyle acheilognathi</i> 100.0%	KR780932	<i>Cyprinus carpio</i>
				<i>Schyzocotyle acheilognathi</i> 100.0%	HM367066	<i>Homo sapiens</i>
				<i>Schyzocotyle acheilognathi</i> 100.0%	DQ866990	<i>Cyprinella lutrensis</i>
				<i>Schyzocotyle acheilognathi</i> 99.6%	AY340106	<i>Dorosoma pretensense</i>
<i>Gambusia holbrooki</i>	<i>Schyzocotyle acheilognathi</i>	CPAFT1	MT898662	<i>Schyzocotyle acheilognathi</i> 99.0%		
		CPAFT2	MT898663	<i>Schyzocotyle acheilognathi</i> 99.0%	KX060604	<i>Cyprinella lutrensis</i>
				<i>Schyzocotyle acheilognathi</i> 99.0%	KX060603	<i>Atherina boyeri</i>
				<i>Schyzocotyle acheilognathi</i> 99.0%	KX060602	<i>Labeobarbus kimberleyensis</i>
				<i>Bothriocephalus acheilognathi</i> 99.0%	GU979860	<i>Cyprinus rubrofusculus</i>
<i>Carassius auratus</i> <i>Gambusia holbrooki</i>	<i>Schyzocotyle</i> sp. genotype Blue Lake	CPAFT3	MT898664	<i>Bothriocephalus acheilognathi</i> 98.7%	AY340104	<i>Dorosoma cepedianum</i>
		CPAFT4	MT898665	<i>Schyzocotyle acheilognathi</i> 90.7%	KX060600	<i>Gila conspersa</i>
		CPAFT5	MT898666	<i>Schyzocotyle acheilognathi</i> 90.7%	KX060597	<i>Labeobarbus nedgia</i>
		CPAFT7	MT898667	<i>Schyzocotyle acheilognathi</i> 90.7%	DQ866988	<i>Cyprinella lutrensis</i>
				<i>Bothriocephalus acheilognathi</i> 90.7%		

Figures

Figure 1

Semichon stained heart-shaped scolex of *Schyzocotyle acheilognathi* showing unarmed bothria and proglottids much narrower than the scolex with rounded edges (Photo: Aileen Elliot). Image taken at 40x magnification on an Olympus stereo microscope SZX7, at a magnification range of 0.8-5.6x, with an Olympus DP27 camera using cellSens software.

Figure 2

Semichon stained mature and gravid proglottids and of *Schyzocotyle acheilognathi*. A Arrows indicate genital pores and B vitelline follicles (Photos: Aileen Elliot). C Gravid proglottids ready for dispersal and D operculated and unembryonated eggs, with arrow indicating operculum (Photos: Aileen Elliot). Images A-C taken at 10-40x magnification, on an Olympus stereo microscope SZX7, at a magnification of 0.8-5.6x, with an Olympus DP27 camera using cellSens software. Image D taken at 10-40x magnification in Brightfield, on an Olympus BX50 compound microscope, using a DP1 universal camera and a Nomarski filter.

Figure 3

Intensity of the *Schyzocotyle acheilognathi* infection in carp (Photo: Aileen Elliot). Image taken on an Olympus stereo microscope SZX7, at a magnification of 0.8-5.6x , with an Olympus DP27 camera using cellSens software.

Figure 4

A-C Photos showing *Schyzocotyle* tapeworms (species unconfirmed) within the intestines of *Gambusia holbrooki* (Photos: Cindy Palermo). The scolex and bothria were morphologically similar to those found in the koi carp and

goldfish. Taken at 10-40x magnification in Brightfield, on an Olympus BX50 compound microscope, with a Nikon DS-L4 universal camera.

Figure 5

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. The tree was built using the following parameters: 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). Scale-bar indicates the number of nucleotide substitutions per site. Sequences from this study are indicated by bold typeface.