Research Note

The pathogenic Asian fish tapeworm, *Bothriocephalus acheilognathi* Yamaguti, 1934 (Cestoda) in the Red discus (*Symphysodon discus*)

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Summary

The Asian fish tapeworm, *Bothriocephalus acheilognathi* Yamaguti, 1934, was detected for the first time in breeding of discus fish *Symphysodon discus* (Perciformes, Cichlidae), a popular South American cichlid. In examined samples, the adult and juvenile cestodes of *B. acheilognathi* with mean intensity of infection 30 (range 19 – 47) individuals per fish were found. The infected fish displayed acute behavioral symptoms. Mortality was significant; it reached almost 80 % in youngest age categories. As treatment, praziquantel immersions in dose 2 mg per liter were well tolerated and efficacious. The risk of spreading pathogens via imported fish is actual menace for ornamental breeding fish, therefore, thorough quarantine and prophylactic measures needs to be done by all fish imports and introductions. Import and subsequent release of infected ornamental fish into freshwater ecosystems may represent serious risk for spread and establishment of the parasite in native fish species. This case study illustrates that ornamental fish play also a key role in national and international movements of parasites in freshwater habitats.

Keywords: *Bothriocephalus*; new host; mortality; aquarium breeding; intestinal obstruction

Introduction

The invasive Asian fish tapeworm *Bothriocephalus acheilognathi* Yamaguti, 1934 (Cestoda, Bothriocephalidea) was originally described from a small cyprinid fish, *Acheilognathus rhombeus* (Cypriniformes, Cyprinidae), from Lake Ogura in Japan (Yamaguti, 1934). Nowadays, it is worldwide distributed and parasitic in more than two hundred species from around 14 orders of freshwater fish (Salgado-Maldonado & Pineda-Lopez, 2003; Scholz *et al.*, 2012). Due to its fast colonization to almost all continents and its pathogenicity, *B. acheilognathi* caused worldwide concern as an important pathogenic and most dangerous fish tapeworm, which causes bothriocephalosis of feral but especially of cultured fish (Scholz *et al.*, 2012). There are no records of *B. acheilognathi* published up to date from native fishes of South America but only two findings from introduced common carps: from Brazil, most probably as a result of the import of carp from Europe (Rego *et al.*, 1999) and from Argentina, found in introduced wild population of *Cyprinus carpio* (Waicheim *et al.*, 2014).

The Red discus *Symphysodon discus* (Perciformes, Cichlidae), is one of the most popular South American cichlids cultured worldwide. Up to date, several cases of parasite infestation by helminths from this frequently bred fish were reported as oxyuroid nematode *Ichthyouris bursata* (Moravec & Prouza, 1995; Moravec & Laoprasert, 2008; Sobecka *et al.*, 2012) and monogeneans *Dactylogyrus* sp. (Mohammadi *et al.*, 2012) and *Sciadicleithrum variabilum* (Aquaro *et al.*, 2012). Besides helminths, protozoan parasites such as ciliates *Chilodonella hexasticha* (Imai *et al.*, 1984), *Ichthyophthirius multifiliis* (Sobecka *et al.*, 2012), *Tetrahymena piriformis* (Mohammadi *et al.*, 2012), *Trichodina* sp. and *Vorticella* sp. (Mohammadi *et al.*, 2012); diplomonads *Hexamita symphysodonis* (Sobecka *et al.*, 2012), *Spironucleus vortens* (Paull & Matthews, 2001) and amoeba *Entamoeba* sp. (Guz & Szczepaniak, 2009) have been recorded from this fish species under aquarium conditions. In wild populations of *S. discus*, isopoda *Artystone trysibia* (Huizinga, 1974), cestode *Proteocephalus macrophallus* (Vidal-Martinez & Kennedy, 2000) and monogenean *Sciadicleithrum variabilum* (Thatcher, 2006) were reported. The parasites list of genus *Symphysodon*

Table 1. Parasites of genus *Symphysodon* indicating locality of host origin and references

Species	Taxon	¹ Hosts	² Locality	³ Comment	References
<i>Chilodonella hexasticha</i> (Kiernik, 1909)	Ciliophora	Sy di	JP	AQ	Imai <i>et al.</i> , 1984
<i>Trichodina</i> sp.	Ciliophora	Sy di	IR	AQ	Mohammadi <i>et al.</i> , 2012
<i>Ichthyophthirius multifiliis</i> Fouquet, 1876	Ciliophora	Sy di	TH	FF	Sobecka <i>et al.</i> , 2012
<i>Tetrahymena piriformis</i> (Ehrenberg, 1830)	Ciliophora	Sy aeq, Sy di, Sy sp.	IR, PL	AQ, FF, IM	Mohammadi <i>et al.</i> , 2012; Sobecka <i>et al.</i> , 2012
<i>Hexamita symphysodonis</i> Tonguthai & Chinabut, 1989	Diplomonadida	Sy aeq, Sy di, Sy sp.	PL, TH	FF, IM	Sobecka <i>et al.</i> , 2012
<i>Spironucleus</i> sp.	Diplomonadida	Sy aeq, Sy sp.	EG, US	AQ, FF	El-Ghany <i>et al.</i> , 2014; Yanong <i>et al.</i> , 2004
<i>Spironucleus vortens</i> Poynton <i>et al.</i> , 1995	Diplomonadida	Sy di	GB	AQ	Paull & Matthews, 2001
<i>Ichthyobodo necator</i> (Henneguy, 1884)	Kinetoplastida	Sy aeq, Sy sp.	PL	FF, IM	Sobecka <i>et al.</i> , 2012
<i>Cryptobia iubilans</i> Nohýnková, 1984	Kinetoplastida	Sy aeq	US	FF	Yanong <i>et al.</i> , 2004
<i>Erlamoeba</i> sp.	Sarcodina	Sy aeq, Sy sp.	PL	FF, IM	Guz & Szczepaniak, 2009; Sobecka <i>et al.</i> , 2012
<i>Dadayius puruensis</i> Lopes <i>et al.</i> , 2011	Digenea	Sy aeq	BR	WS	Lopes <i>et al.</i> , 2011
<i>Dactylogyrus</i> sp.	Monogenea	Sy di	IR,	AQ	Mohammadi <i>et al.</i> , 2012
<i>Soiadicleithrum variabilum</i> (Mizelle & Kritsky, 1969)	Monogenea	Sy aeq, Sy di, Sy sp.	BR, CZ IT, PL, TR	AQ, FF, IM, WS	Ergens & Prouza, 1984; Kritsky <i>et al.</i> , 1989; Onal <i>et al.</i> , 2011; Aquaro <i>et al.</i> , 2012; Sobecka <i>et al.</i> , 2012; Thatcher, 2006
<i>Proteocephalus macrophallus</i> (Diesing, 1850)	Cestoda	Sy di	BR	WS	Vidal-Martinez & Kennedy, 2000
<i>Eustrongylides</i> sp.	Nematoda	Sy aeq	PL	IM	Sobecka <i>et al.</i> , 2012
<i>Procamlanus</i> sp.	Nematoda	Sy aeq	BR, US	FF, WS	Vidal-Martinez & Kennedy, 2000; Yanong <i>et al.</i> , 2004
<i>Neocapillaria pterophylli</i> Heinze, 1933	Nematoda	Sy aeq	CZ, TH	AQ	Moravec & Gut, 1982
<i>Capillaria</i> sp.	Nematoda	Sy aeq	IR, PL, US	FF, IM	Yanong <i>et al.</i> , 2004; Rahmati-holasoo <i>et al.</i> , 2010; Sobecka <i>et al.</i> , 2012
<i>Ichthyouris bursata</i> (Moravec & Prouza, 1995)	Nematoda	Sy di, Sy sp.	CZ, TH	FF, IM	Moravec & Prouza, 1995; Moravec & Laoprasert, 2008;
<i>Myxobolus</i> sp.	Myxosporea	Sy aeq	PL	IM	Sobecka <i>et al.</i> , 2012
<i>Ichthyophonus hoferi</i> Plehn & Mulsow, 1911	Ichthyosporea	Sy sp	PL	FF	Sobecka <i>et al.</i> , 2012
<i>Philodina</i> sp.	Rotifera	Sy aeq	PL	IM	Sobecka <i>et al.</i> , 2012
<i>Artystone trysibia</i> Schioedte, 1866	Isopoda	Sy di	BR	WS	Huizinga, 1974

¹*Symphysodon aequifasciatus* – Sy aeq; *Symphysodon discus* – Sy di; *Symphysodon* sp. – Sy sp²BR – Brazil; CZ – Czech Republic; EG – Egypt; GB – Great Britain; IR – Iran; IT – Italy; JP – Japan; PL – Poland; TH – Thailand; TR – Turkey; US – United States of America³AQ – fish from private aquariums; FF – fish from commercial farm; IM – fish imported from Amazon River basin to Europe; WS – wild specimens examined

contains only 5 reports of parasite found in wild population, but 12 reported from commercial farms. Ten parasites originated in fish imports from Amazon River basin to Europe (Table 1).

During the parasitological examination of juvenile discus fish originating from Slovakian monocultural commercial farm, massive infection by the Asian fish tapeworm was detected. Examined cichlids came not from direct import but were the progeny of brood stock imported recently from the Czech Republic. The goal of this work was to document the occurrence and pathogenicity of *B. acheilognathi* in *S. discus* which represents a new host record.

Material and Methods

In May 2014, 6 fish of *Symphysodon discus* displaying not specific behavioral changes were sent by breeder to our laboratory for parasitological examination. The mean standard length of fish was 12.50 ± 0.10 cm and mean weight of 17.08 ± 0.86 g. Subsequently, other juvenile individuals of all cultured colour forms (red spotted, blue diamond and red melon) have been dissected on site by breeder. Examined fish were selected from different cohorts displaying and non-displaying any visible symptoms. We visited the farm and dissected more fish but with negative results because the fish were treated already.

Tapeworms were isolated from the intestine of freshly anaesthetized and killed fish hosts and rinsed in saline solution. For morphological studies, live worms found were fixed with hot (almost boiling) 4 % formaldehyde solution, subsequently stained with acetocarmine, dehydrated in an ethanol series, clarified in clove oil and mounted in Canada balsam as permanent preparations (Oros *et al.*, 2010). Microphotographs were digitally captured with Leica DFC 450C camera mounted on Leica DM 5000B light microscope with Differential Interference Contrast (DIC). Specimens selected for scanning electron microphotograph (SEM) observations, after fixation with hot 4 % formaldehyde solution, were dehydrated through a graded ethanol series, followed by a graded amylacetate series, dried by HMDS (hexamethyldisilazane), sputter-coated with 20 – 25 nm of gold and examined with a Jeol JSM 6510LA microscope (see Oros *et al.*, 2010). Planktonic copepods, apparent intermediate hosts of *B. acheilognathi*, were collected from all aquariums by using the plankton nets. More than 50 individuals were transported to laboratory and determined.

Results and Discussion

Current case report provides the presence and pathogenicity of the Asian fish tapeworm, *Bothriocephalus acheilognathi* in the popular Red discus, *Symphysodon discus*, which represents a new definitive host of this pathogenic parasite. It is of great significance that *B. acheilognathi* is not widely distributed in South America and this infection is caused by different origin of pathogen, definitive host and intermediate host.

The tapeworms found shows typical characteristics of the species *Bothriocephalus acheilognathi*, especially the heart shaped scolex with a pair of deep, slit-like openings positioned dorso-ventrally (Fig. 1B, C), first proglotids immediately posterior to scolex, neck absent, immature segments wider than they are long (Fig. 1A),

whereas more developed gravid segments (fully developed genital organs) are quadrate, longer than they are wide.

In examined samples, the adult and juvenile cestodes of *B. acheilognathi* with mean intensity of infection 30 (range 19 – 47) individuals per fish were found. Parasites found occupied anterior part of the intestine (Fig. 1D), where intestine obstruction was observed. The fish being typically darker than normal, displayed acute behavioral symptoms such as lethargy, segregation at the tank bottom and showing decreased activity or excitation, and appetite loss. In some of the affected fish groups also growth retardation, abdominal distension and white stringy faeces were present. Mortality was significant in fish displaying acute symptoms; it reached almost 80 % in the youngest age categories. We proved the presence of intermediate hosts in the farm, belonging to species *Microcyclops varicans* and *Acanthocyclops vernalis*. Due to the central filtration, intermediate hosts have spread to all breeding aquariums. As reported by owner, this farm represents a closed type of intensive aquaculture using only tap water and frozen or dry food. Based on anamnesis, when morbidity and mortality started, introduction of infected fish or copepods was related to the recent brood stock import from the Czech farm.

Beside the present case, *B. acheilognathi* was found in Slovak ornamental fish farm in Turčianske Teplice (20th February, 2003) in *Symphysodon* sp., *Pterophyllum scalare* and *Poecilia reticulata* (Dr. Kuchta, *pers. comm.*). There are at least three gateways of pathogen introduction to Slovakia; i) already infected fish were imported to Europe from Asia where the majority of cultured Red discus is bred and exported worldwide and the pathogen is widely distributed, ii) the brood stock were kept in polycultural aquarium and undiscovered presence of intermediate hosts enabled transmission from other farmed fish, or iii) only intermediate hosts harboring plerocercoids of pathogen has been introduced to the farm via water used for fish transport and fish got infected in Slovak farm. Occasionally, cestodes with low host specificity can enter aquariums harboured in live planktonic copepods caught from the wild used for feeding purposes (Piasecki *et al.*, 2004). The large number of cyclopoid copepods can serve as intermediate hosts for *B. acheilognathi* in both cultured and natural conditions, such as *Acanthocyclops*, *Cyclops*, *Macrocyclops*, *Megacyclops*, *Mesocyclops*, *Thermocyclops* and *Tropocyclops* (Marcogliese & Esch, 1989; Scholz *et al.*, 2012). Temperatures reaching 30 °C in culturing system of discus fish facilitate rapid development and maturity of *B. acheilognathi* larval stages (Pool, 1984). Larval development is completed in 21 – 23 days at 28 – 29 °C (Liao & Shih, 1956), but 1.5 – 2 months at 15 – 22 °C (Davydov, 1978). As treatment, praziquantel immersions in dose 2 mg per liter were well tolerated and efficacious. These baths has been widely used in a several fish parasite infections caused by adult cestodes (Moser *et al.*, 1986; Mitchell, 2004; Mitchell *et al.*, 2009). Few hours after application of the anthelmintic treatment, massive release of parasites from their hosts was observed. After 5 days, the treatment was repeated, which has resulted complete eradication of the infection with *B. acheilognathi*. To prevent reinfection and eradicate intermediate host, disinfection of all aquaria by Chloramine was provided.

The risk of spreading pathogens via imported fishes from exotic countries is actual menace for ornamental breeding fish (Moravec

Fig. 1. *Bothriocephalus acheilognathi* from the ornamental fish *Symphysodon discus*. (A) Whole-mount, total view; (B) SEM micrograph, anterior part of body with scolex, lateral view; (C) SEM micrograph, anterior part of body with scolex, dorso-ventral view; (D) Localization of the tapeworms in the anterior part of the intestine of *S. discus*, arrowhead indicates the massive infection. Abbreviations: b=bothria; i=intestine, so= slit-like openings.

et al., 1999; Whittington & Chong, 2007; Taylor *et al.*, 2010; Pretto *et al.*, 2013). On the other hand, exotic fish species in aquaria are threatened by freshwater parasites, as the case of *B. acheilognathi* found in aquarium-reared discus in the present study. This tapeworm is considered as predominant parasite of cyprinids, but other aquarium cultured ornamental fish species commonly available in pet stores, i.e. *Astyanax mexicanus* (Characidae), *Archocentrus nigrofasciatus*, *Cichlasoma urophthalmum*, *Herichthys cyanoguttatus*, *Thorichthys meeki* (Cichlidae), *Poecilia mexicana*,

P. sphenops, *Xiphophorus variatus* (Poeciliidae) were confirmed as suitable definitive hosts of *B. acheilognathi* (Salgado-Maldonado *et al.*, 1997; Evans & Lester, 2001; Salgado-Maldonado *et al.*, 2001; Vidal-Martínez *et al.*, 2001; Salgado-Maldonado & Pineda-López, 2003; Monks *et al.*, 2005; Salgado-Maldonado, 2006). Popular livebearers *Xiphophorus maculatus* and *P. reticulata* (Poeciliidae) infected with *B. acheilognathi* were imported to Australia from Asian farms where this parasite is widely distributed (Evans & Lester, 2001). If suitable intermediate hosts (copepods) inhabit breeding

tanks, tapeworms might permanently parasitize in the cultured fishes and thus, they might be spread with both fishes and infected copepods present in the transport water into new localities. Additionally, waste aquarium water released into an environment may also contain infected copepods, which represent potential source of infection in freshwater ecosystem.

Conclusion

The occurrence of cestodes in aquarium fishes is rare due to the absence of intermediate hosts in closed breeding systems in most cases, and quarantine measures. Occasionally, cestodes are transmitted into aquariums from nature by introduction of live planktonic copepods for feeding purposes. Nowadays, only a few breeders feed fish with plankton from ponds due this high risk potential. However, thorough quarantine and prophylactic measures needs to be done by all fish imports and introductions. Import and subsequent release of infected ornamental fish into freshwater ecosystems may represent serious risk for spread and establishment of the parasite in native fish species.

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