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New cases of pathogens imported with ornamental fish

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Abstract: The global trade in live ornamental fish carries with it the potential threat of unwanted movement of pathogens. The live ornamental fish, when released into the natural environment of their destination countries, may disseminate their parasites, threatening local fish and causing economic loss in fish aquaculture. This study was aimed to explain the reasons for deterioration of health and premature death of ornamental fish: *Nannostomus* spp. from Colombia and *Symphysodon* spp. Some specimens of *Symphysodon aequifasciatus* Pellegrin, 1904 were captured from their natural habitats in the Amazon River basin and shipped to Poland by air mail. The hybrids of *Symphysodon* spp. originated from an aquarium in Poland (shared with *S. aequifasciatus*). The third discus fish species, *Symphysodon discus* Heckel, 1840 came from a fish farm in Thailand and represents an Asian breeding line. All the studied fish were infected with pathogens. *Nannostomus* spp. housed a single parasite species on its skin, namely *Artystone minima.* This isopod species was not recorded in Poland and probably in Europe before. The examined fish of the genus *Symphysodon* yielded 13 pathogens. Five of them *Philodina* sp., *Ichthyobodo necator*, *Hexamita symphysodonis*, *Sciadicleithrum variabilum* and *Eustrongylides* sp. were not earlier recorded in this host species in Poland. The findings of *Ichthyophonus hoferi* and *Myxobolus* sp. are new host records for *Symphysodon* spp. fish. Hybrid fish and *S. aequifasciatus* are reported here as hosts of *Entamoeba* sp. for the first time.

Keywords: ornamental fish, parasite migration, *Sciadicleithrum variabilum, Artystone minima, Philodina* sp.

INTRODUCTION

Symphysodon aequifasciatus Pellegrin, 1904 and *Symphysodon discus,* Heckel, 1840 (Cichlidae: Perciformes) (Froese & Pauly 2010) (discus fish) are ornamental cichlid fish that occur in floodplain lakes and flooded forests of the lowland Amazon Basin (Crampton 2008). Their aquaculture in several Asian countries is well developed (Sands 1988). Increasingly often, hybrids of these 2 species are bred. *Nannostomus* spp. (Lebiasinidae: Characiformes) (pencil fish) are widely distributed: from the rivers of Colombia, Venezuela and the Guyanas in the north to the southern

Amazon basin in the south, and from Peru in the west and Brazil in the east (Froese & Pauly 2010). *Nannostomus* spp. and *Symphysodon* spp. are popular as aquarium fish. They are imported to many countries. Those fish, when released into the natural environment in their destination countries, may disseminate their parasites, threatening local fish.

This study was undertaken to find the reasons for the poor condition and death of the *Symphysodon* spp. and *Nannostomus* spp. fish imported to Poland, and those grown in experimental aquaria.

MATERIALS AND METHODS

Fish origin

Discus fish (*Symphysodon* spp.) were obtained from various sources. One source was their natural site of occurrence (Rio and Lago Téfe, Amazon River basin). *Symphysodon aequifasciatus*, Pellegrin, 1904 (wild discus – 3 specimens) were captured and sent by air mail to Poland and, after quarantine, transferred to the experimental aquarium. The second source of discus fish was Polish aquaculture from the Asiatic line of *S. aequifasciatus* crossed with wild discus (hybrids – 35 specimens); they lived in the same experimental aquarium (to broaden the gene pool). The third source of *Symphysodon discus* Heckel, 1840 (13 specimens), was an Asiatic (Thai) fish farm. This species was cultured separately. The 35 specimens of *Nannostomus unifasciatus* Steindachner, 1876 and *Nannostomus beckfordi* Günther, 1872 (pencil fish) were captured from their natural habitat in the Colombia River. They were placed in a third aquarium. The mean total length of the discus fish was 112.5±40.5 mm, and that of pencil fish was 37.5±3.2 mm.

Parasitological analysis

The examination of dead fish focused on the skin, vitreous humour, eye lens, mouth cavity, gills, liver, gastrointestinal tract, kidney, swim bladder, and peritoneum. The parasites found were identified from wet mounts or they were fixed and preserved in 75% alcohol, mounted in Hoyer's medium on microscopic slides, and identified. The slides of mucus and parenchyma of the internal organs were examined under an Olympus BX 50 microscope with differential interference contrast microscopy (DIC) Nomarski. The parasites were measured using a Nikon Eclipse TE 2000-S microscope. The stomach and intestine contents were studied by the decantation or wet slides methods. The parasites were preserved in Canada balsam. The species of parasites were determined using the key of Bauer (1987) and original articles on individual species.

RESULTS

Despite the use of appropriate culture conditions, most of the discus fish died within the first month of the experiment. *Nannostomus* spp., which were reared in a separate aquarium also died.

Discus fish

All the studied fish were infected with pathogens. However, no pathogens were found on the skin, in the vitreous humour, eye lens, and mouth of all the individuals studied.

Wild *Symphysodon aequifasciatus* were the hosts of 9 pathogens. The gills of 2 individuals were congested. Semi-sessile rotifers *Philodina* sp. (Bdelloidea: Philodinidae) were living in the mucous of the gill filaments. There were 1 – 3 rotifers in the microscopic field of view. On the wet slides, *Saprolegnia parasitica* (Oomycetes) was found. From a single hypha to numerous hyphae were noted. We observed also living ciliated freshwater protozoans *Tetrahymena pyriformis* (Ehrenberg, 1830) Lwoff, 1947 (a few specimens in the microscopic field of view) and the flagellate *Ichthyobodo necator* (Henneguy, 1884) Pinto, 1928 (a few to 20 specimens in the field of view). On the gill filaments of all the fish, monogenean *Sciadicleithrum variabilum* (Mizelle and Kritsky, 1969) Kritsky, Thatcher and Boeger, 1989 (Kritsky et al. 1989) were attached. There were 1-4 specimens in the field of view (intensity of infection per fish = 26). Lengths of some skeletal elements, important for the taxonomy of Monogenea, are given in Table 1. In the peritoneum and beyond the serous membrane of the liver, cysts were found, including larvae of the nematode *Eustrongylides* sp. On the microscopic slides with mucus and mucous membrane from the terminal section of the intestine, we found eggs of the nematode *Capillaria* sp. at various developmental stages. No adult nematodes were observed. In the mucus of the empty intestine, we noticed 4 active trophozoites of *Entamoeba* sp. (Sarcodina) and living parasitic diplomonads *Hexamita symphysodonis* Tonguthai and Chinabut, 1989. In the field of view, there were a few to about 30 diplomonads. In one fish, on the external wall of the terminal section of the intestine and on the peritoneum near this site, 2 small cysts were found, of up to 0.5 mm in diameter. The cysts contained spores of myxosporeans *Myxobolus* sp.

Table 1. Measurements of the length (in μm) of some skeletal elements of *Sciadicleithrum variabilum*

Source	Ventral hamulus	Dorsal hamulus	Ventral bar	Dorsal bar	Marginal hook
Mizelle & Kritsky, 1969	29 (27–30)	31 (28–33)	35 (32–38)	34 (30–44)	13 (12–15)
Present study	30 (27–31)	32 (29–34)	34 (32–38)	34 (31–39)	13 (11–15)

The examination of the hybrid fish revealed the presence of 6 pathogens. On the gill filaments of all the fish, there were 4–7 specimens of *S. variabilum*. From one to about a ten *T. pyriformis* were also found in the field of view, and a few living *H.*

symphysodonis. A single specimen of *I. necator* was observed. In the liver and brain of one specimen, some single spores of *Ichthyophonus hoferi* Plehn and Mulsow, 1911 (Mesomycetozoa) were detected. In the alimentary tract of one fish, 2 active trophozoites of *Entamoeba* sp. were noted.

Symphysodon discus from the aquaculture in Thailand had only 3 species of parasites. On the gills, we found a single trophont of the ciliate *Ichthyophthirius multifiliis* Fouquet, 1876, while in the intestine, a single *H. symphysodonis*. In the terminal section of the intestine of one fish, there was one adult female nematode *Ichthyouris bursata* (Moravec and Prouza, 1995) (Moravec & Leoprasert 2008). The eggs of the nematode were oval, non-embryonated, with 1-2 filaments on each pole.

Pencil fish

All the studied dead *Nannostomus* spp. were infected only with one parasite species *Artystone minima* Thatcher and Carvalho, 1988 (Isopoda: Cymothoidae). It was noted on the skin and the gill chambers. There were 1–2 crustacean specimens per fish. *A. minima* was also found in the peritoneal cavity of one fish.

DISCUSSION AND CONCLUSIONS

The studied discus fish were the hosts to 13 pathogens. Eight of them were found in *S. aequifasciatus* from the Amazon River basin. The wild-caught specimens are prone to disease brought on by the stress of capture. Their food is also important, because the live worms used as food are usually infected with parasites, and therefore they can easily infect the discus fish.

Up till now, the myxosporean *Myxobolus* sp. was not noted in *Symphysodon* spp. (Eiras et al. 2005; Ousman et al. 2007). Békési et al. (2002), who studied the development of numerous known and unknown species of myxosporean fish parasites in Brazil, also did not report the presence of this parasite in discus fish.

The ectoparasitic flagellate *I. necator* is distributed worldwide, but not previously noted in discus fish in Poland. This flagellate feeds on gill epithelial cells, and the fish affected by it develop respiratory problems, in particular when also infected by monogeneans, mostly dactylogyrids. Such an infection was observed in many species of aquarium fish. Transmission is enhanced by overcrowding of fish or during manipulations by the aquarium owner. *Sciadicleithrum variabilum* has been reported in wild *S. discus* and occurs only in the fish from the Amazon River basin (Thatcher 2006). The Czech Republic is the only country in Europe where the presence of this species of parasite on *S. aequifasciatus* has been noted (Řehulková & Gelnar 2006). This parasite was never found on *Symphysodon* hybrids before. *Ichthyophthirius multifiliis* is a common parasitic ciliate on free-living and farmed fish. This parasite has been noted many times in Poland, also on discus fish (Stoskopf 1993; Antychowicz 2003).

Various species of *Tetrahymena* sp. have been reported in pet fish, e.g. *T. corlissi* and *T. pyriformis* in fish originating from Sri Lanka and Brazil. Besides, *T. corlissi* has been found in fish imported from Korea and South Africa (Bassleer 2003).

T. pyriformis is a free-living ciliate that occasionally becomes a parasite. The cichlids appear particularly susceptible, and *T. pyriformis* is considered to be a secondary or tertiary invader of lesions initiated by the other pathogens (Roberts et al. 2009). In Polish aquacultures, this parasite has already been noted (Antychowicz 2003).

Rotifers are generally not true parasites. They lodge on the skin or gills and may cause hyperactivity in affected fish (Stoskopf 1993). Most species of *Philodina* live in freshwater, but some dwell in moss and soil (Ricci & Melone 2000). This rotifer was not earlier found living on fish gills (May 1989). There is only one record of a rotifer that was either parasitic or epizooic on fish. It was described by Wiszniewski (1946), who found *Encentrum kozminskii* on the skin and gills of a carp from fish culture ponds. Wiszniewski described the species as a true parasite which fed on the gills' mucus and epithelium.

The presence of such a great number of pathogens on the gills of wild *Symphysodon equiasciatus* was most probably the reason why the pathogens were able to cause obstruction of the gills. The damage caused by the parasites resulted in a bacterial infection and permitted invasion of *Saprolegnia parasitica* as secondary invaders. The immunological system, weakened by the large number of parasites, permitted invasion of the organisms that only occasionally become parasites.

In the intestine of wild *Symphysodon aequifasciatus* and hybrid fish, active trophozoites of *Entamoeba* sp. were found. Probably it was a specific endocommensal, living only in the digestive tract of fish. Apart from the report about the intestinal amoebiasis in *S. discus* (Guz & Szczepaniak 2009), there is no other report on this parasite in *S. aequifasciatus* or in other tropical freshwater fish.

Wild *S. aequifasciatus* and hybrid fish reared in Poland were the hosts to flagellates *Hexamita symphysodonis,* previously not found on discus fish in Poland. However, *H. symphysodonis* was reported in other cichlids (*Pterophyllum* sp.), which are often put in the discus fish aquarium (Sieniawski 2006). If *H. symphysodonis* flagellates are in the intestine alone, they are not dangerous, but if they are accompanied by the nematodes *Capillaria* sp., they can cause death (Fenner 2010).

Ichthyophonus hoferi has been noted in free-living cichlid fish but rarely in aquarium fish (Stoskopf 1993). It was not earlier found in *Symphysodon* spp. *I. hoferi* varies in size and form, and is probably often misidentified.

Larvae of the nematodes *Eustrongylides* sp. were earlier noted in *Symphysodon* spp. (Roberts et al. 2009) but not in Poland. Identification of species in the larval form is very difficult. Most records of these larvae are from fish from Neotropical regions, including Brazil (Moravec 1998).

Discus fish were highly susceptible to invasion of other nematodes *Capillaria* sp. The eggs found, most probably belong to *Capillaria pterophylli* (Heinze, 1933), earlier noted in *S. aequifasciatus* in the Czech Republic, Germany, and the United Kingdom. The eggs of nematodes can be introduced to the aquarium with food, or healthy fish can get infected when they are kept with the infected ones (Moravec 1998).

In the intestine of *S. discus* from Thai aquaculture, the nematode specimen *Ichthyouris bursata* was found. It has been noted in *S. discus* in other European countries. In Polish literature on *S. discus* parasites, the presence of pinworms is mentioned without the name of any genus or species (Antychowicz 2003).

Among the pathogens found in the discus fish in our study, *Protoopalina symphysodonis* Foissner, Schubert and Wilbert, 1974 was not found, although this parasite is often present in discus fish. In some cases the infection is severe, and the prevalence reaches 100% (Lom & Dykova 1992). The fish from aquacultures in general had fewer parasites than the wild discus, thanks to preventive and therapeutic procedures.

The cymothoid fauna of freshwater fish is rich and varied. Most of these isopods parasitize the mouth or gill chamber of their hosts. Only 2 genera, *Artystone* Schiödte, 1866 and *Riggia* Szidat, 1948, burrow into the peritoneal cavity. The small body size of these parasites permits them to infect smaller fish hosts. The parasite lies at right angles to the main axis of the fish and, although it does not penetrate the peritoneum, it damages the liver, stomach, and intestine by pressure atrophy (Thatcher & Carvalho 1988; Thatcher 2000). If the parasites penetrate near a pectoral fin, the fin is eventually lost. Just like *Sciadicleithrum variabilum, A. minima* is also a parasite with a high specificity and its hosts may only be a few species of fish from the same genus. This parasitic isopode was found and described for the first time in *N. beckfordi* in the Upper Negro River, Amazonas State, Brazil (Thatcher 2000). The specimens of *A. minima* found in the present study are significantly smaller (Table 2). Infrapopulation size can influence morphometry through crowding effects. *A. minima* was not previously recorded on fish in Europe.

Table 2. Measurements (in mm) of morphological characteristics of male and female *Artystone minima*

Source	Sex	Body length	Body width	Head length	Head width	Pleotelson length	Pleotelson width
Thatcher & Carvalho 1988	♂	3.8 (3.7–4.0)	1.9 (1.9–2.0)	0.6 (0.53–0.68)	1.0 (0.98–1.03)	0.68 (0.65–0.75)	1.19 (1.18–1.20)
	♀	6.2 (5.2–6.9)	3.3 (2.7–3.7)	0.75 (0.63–0.90)	1.19 (1.06–1.25)	1.24 (1.18–1.33)	1.61 (1.45–1.78)
Present study	♂	3.4 (3.3–3.5)	1.6 (1.4–1.7)	0.4 (0.35–0.46)	0.8 (0.78–0.82)	0.54 (0.50–0.57)	1.00 (0.96–1.1)
	♀	4.9 (4.7–5.1)	2.5 (2.4–2.7)	0.59 (0.53–0.64)	0.93 (0.88–1.00)	1.01 (0.91–1.11)	1.25 (1.18–1.33)

From the obtained results the following conclusions can be drawn:

1. Inter-regional trade in live fish usually as larvae or juveniles, is linked with the potential for parallel movements of pathogens (Boeger et al. 2002; Więcaszek et al. 2009). The imported fish can either get infected from the native fish or become the source of infection with parasites that were not previously found in the geographical region concerned.

2. On the basis of the parasite species listed in earlier publications, we identified 2 species new to the Polish fauna: *Artystone minima* Thatcher and Carvalho, 1988 and *Sciadicleithrum variabilum* (Mizelle and Kritsky 1969) Kritsky, Thatcher and Boeger, 1989 (Kritsky et al. 1989).

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