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SHORT COMMUNICATION

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Emergence of *Discocotyle sagittata* (Monogenea: Polyopisthocotylea) in rainbow trout (*Oncorhynchus mykiss*) and brown trout (*Salmo trutta*) in an Austrian aquarium

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The Museum Niederösterreich, Haus für Natur (St. Pölten, Lower Austria), features a large fish exhibition. In one of the tanks, the main attraction was a large huchen (*Hucho hucho*). The tank was holding 22,000 L of recirculated water with a temperature between 10°C and 14°C. Every 2 weeks, 10% of the water was replaced. In addition to several cyprinid fish species, rainbow trout (*Oncorhynchus mykiss*) were introduced occasionally to the tank to serve as food fish for the huchen. These trout were acquired from a distributor in Germany. In March 2020, a number of brown trout (*Salmo trutta*) from an Austrian breeder were also added to the tank. During the following months, a portion of the rainbow trout died with no further investigations performed. In December 2020, three rainbow trout were observed to be lethargic and subsequently died a few days later. One of them was submitted to the Clinical Division of Fish Medicine of the University of Veterinary Medicine, Vienna, within 24 hr. During the following weeks, a new set-up of the tank was planned and the remaining trout were thus also submitted for examination.

In total, two rainbow trout (29 cm and 32 cm, 213 g and 272 g, respectively) and four brown trout (21–28 cm, 107–185 g) were collected. Fish that arrived alive at the clinic (three out of four brown trout) were killed (buffered MS 222, 1 g/L; Sigma-Aldrich). Necropsy of these three brown trout showed no pathological changes. In contrast, the two deceased rainbow trout and the deceased brown trout showed extremely pale gills and internal organs, which could not be attributed to decaying processes and, instead, were indicative of severe generalized anaemia (Figure 1). Attached to their gill filaments, parasitic worms were observed with the naked eye. The gill arches were removed, placed in Petri dishes with distilled water and examined with a stereo microscope (Olympus SZX 10, Olympus Austria). Fifty to >100 worms were observed on each of the four gill arches. Most of them were attached to the peripheral region of the filaments, whereas fewer parasites were observed in close proximity to the gill skeleton. The first gill arches carried the highest parasitic burden (70 to >100 specimens). The small number of examined fish did not allow for statistical analysis of these findings. For further morphological identification, the worms were examined with stereo- and bright-field microscopy (Olympus BX 53, Olympus Austria). Two buccal suckers and four pairs of characteristic clamps at the posterior end allowed a preliminary identification as the monogenean *Discocotyle sagittata* (Leuckart 1842) based on the description provided by Pugachev et al., (2010). The size of the parasites varied from 2 to 10 mm. The developmental stages were differentiated according to Rubio-Godoy and Tinsley (2002) based on the number of clamp pairs and sexual maturity. In the 170 investigated parasite specimens, only individuals with four clamp pairs were found – of which 25 (14.7%) were producing one (22/25) or two (3/25) eggs (Figure 2). For molecular specification, the DNA of six individual specimens was extracted using a DNeasy Blood and Tissue kit (Qiagen) and analysed using nested PCR targeting of the 650-bp section of the 18S rRNA gene (*18S*) of metazoan (Metazoa18S_F: GGATAACTGTGGTAATTCTAGAGC, Metazoa18S_R: CCTCACTAAATCATTCAATCGGTAG; Metazoa18S_intF: 5′-GTAATTCCAGCTCCAATAGCGT-3′, Metazoa18S_intR: 5′-CCGTGTTGAGTCAAATTAAGCC-3′; Lewisch et al., 2021). The

reaction volume (25 µl) contained 1 µl of genomic DNA template, 14.675 µl of nuclease-free water, 5.0 µl of 5X Green GoTaq® Reaction Buffer (Promega), 2.0 µl of 25 mM $MgCl_2$, 0.2 µl of 25 mM dNTP mix, 0.125 µl GoTaq G2® Polymerase (5 U/µl, Promega) and 1.0 µl each of 10 mM oligonucleotide primers. The cycling protocol for both reactions included an initial cycle of 94°C for 2 min, followed by 20 (nest 1)/35 (nest 2) cycles of 94°C for 30 s, 54°C for 30 s, 72°C for 60 s and a final extension of 72°C for 10 min. Three of the obtained amplification products were sequenced. The obtained sequences showed 100% identity to the *18S* gene sequence AJ287504 of *Discocotyle sagittata* deposited in GenBank. The new sequences were deposited in NCBI GenBank under the accession numbers MZ127791–93.

FIGURE 1 Rainbow trout infested with *Discocotyle sagittata*. Note the severe general anaemia. Inset: Gill arches in a Petri dish. Worms of various sizes are detached from the gill filaments

Discocotyle sagittata is a monogenean parasite of various salmonid fish species, including species of the families Coregonidae, Thymallidae and Salmonidae. Reports of non-salmonid fish remain to be confirmed (Pugachev et al., 2010). Like all monogeneans, they have a direct life cycle. Larvae, that is free-living ciliated oncomiracidium, hatch from the eggs. Depending on water temperature, the larvae can infest the gills of a suitable host and begin feeding on blood as soon as two hours after hatching (Gannicott and Tinsley, 1998b). Attached to the gills, they mature and develop to adult stages. According to Valtonen et al., (1990), only one generation of *D. sagittata* develops per year. During the life of *D. sagittata*, the temperature is the most important abiotic factor for successful reproduction. Water temperatures between 13 and 18°C favour egg production and development, whereas larval survival is longest at temperatures below 10°C (Gannicott and Tinsley, 1998a; Gannicott and Tinsley, 1998b). Rubio-Godoy and Tinsley (2002) described the distinct temperature-dependent pattern of the infestation cycle of farmed rainbow trout. At temperatures above 10°C, in early summer, eggs hatch *en masse*. Early developmental stages are found in the gills of the host together with the adult worms, which can be found in low numbers year-round. During summer, the juvenile worms mature and different development stages, including freshly hatched juveniles, can be found in a single fish. The development stages can be differentiated by the number of clamps, their size and the production of eggs. During winter, almost no transmission of the parasite occurs and only a few worms are present on the gills. Aside

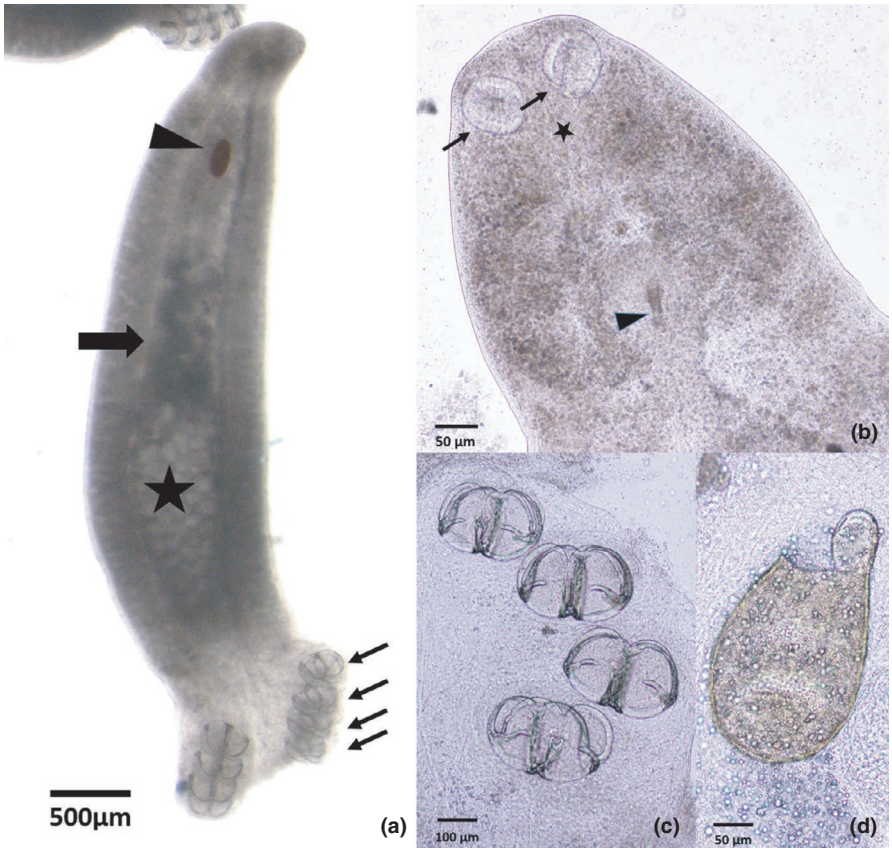

FIGURE 2 (a) Stereomicroscopic view *of Discocotyle sagittata*. Arrows: hooks; star: testis; bold arrow: ovarium and uterus; arrowhead: egg. (b) Detail of anterior end. Arrows: buccal suckers; star: pharynx; arrowhead: genital atrium. (c) Detailed view of hooks. (d) operculated egg. (b–d) light microscopy

from water temperature, the host–parasite co-evolution determines the prevalence and intensity of the parasite in its host.

In Europe, brown trout is considered a native host of *D. sagittata* (Llewellyn, 1956; Paling, 1965; Slinn, 1963), whereas the occurrence in the non-native, introduced rainbow trout has been observed in aquaculture (Gannicott, 1997) and experimental settings (Gannicott and Tinsley, 1997; Rubio-Godoy and Tinsley, 2002). The same authors demonstrated a different susceptibility of the two fish species based on innate and adaptive immune mechanisms (Rubio-Godoy et al., 2003, 2004). A higher susceptibility of rainbow trout to *D. sagittata*, paired with more intensive production conditions, consequently leads to a higher impact of the infestation for individual fish and rainbow trout production. The blood feeding of the parasite leads to severe anaemia in fish with high parasitic burdens. This may result in high mortality rates, as observed in certain fish farms in the Isle of Man (Gannicott, 1997).

To date, the occurrence of *D. sagittata* has been documented in the United Kingdom and the Isle of Man (Gannicott, 1997; Llewellyn, 1956; Owen, 1970; Paling, 1965), Finland (Valtonen et al., 1990), Canada (Hare & Burt, 1975) and Russia (Bauer, 1970; Pugachev et al., 2010).

Surprisingly, there are only a few publications on the occurrence and geographical distribution of the parasite; existing publications date back to the second half of the 20th century. More recent publications highlight special features of the parasite as well as interactions with the host or the environment. Documented reports of occurrence in addition to the above mentioned are not available. Thus, the observation of trout infested with *D. sagittata* in an Austrian aquarium raises some serious questions: (a) Has the presence of this parasite in rainbow trout aquaculture facilities been overlooked or neglected until now? Both assumptions seem unlikely, since the parasite can be seen with the naked eye and the common stocking densities would likely favour the development and transmission of the parasite, resulting in fish health problems or even high mortality rates. (b) Is the parasite prevalent in Austria's wild salmonid fish populations? Lacking a targeted survey, we cannot answer this question with absolute confidence. However, we have reason to think that it is not: since 2014, parasitological gill examinations have been performed by the Clinical Division of Fish Medicine on at least 1,260 salmonid fish from a variety of Austrian rivers and not a single specimen of *D. sagittata* was found. Nevertheless, the conditions in an artificial environment, such as the exhibition tank from which the infested trout originated, allowed for massive infestations of individual fish. Low water flow and exchange rates as well as a constant temperature of 10–14°C presumably led to a prolonged developmental cycle of the parasite. Unfortunately, with no information on stocking data and origin of the individual fish nor a year-round surveillance of infestation characteristics, this study can only present a snapshot observation. Still, the parasites were introduced to the tank with trout from aquaculture, where their prevalence is alarming. Water temperatures have been increasing in many regions with salmonid aquaculture throughout Central Europe during the last few years (Pletterbauer et al., 2018). This may favour the development and distribution of the parasite in covertly infested populations, since longer periods with water temperatures above 10°C promote an earlier and prolonged timeframe for the transmission of the parasite (Rubio-Godoy & Tinsley, 2008). Apart from favourable environmental conditions for parasites, fish movements for stocking farms and wild waters are a constant risk factor for the introduction and distribution of parasitic and other diseases. Another monogenean, *Gyrodactylus salaris*, has demonstrated this impressively with its destructive effect on Atlantic salmon (*Salmo salar*) in Norwegian rivers after translocation of infested fish stock to those rivers (Johnsen & Jensen, 1986; Mo, 1994).

The mere evidence of *D. sagittata* in an aquarium, by itself, might not be cause for concern. However, the fact that the parasite was most likely introduced to the aquarium with rainbow trout or brown trout from a German or Austrian aquaculture farm certainly deserves attention. Until now, there have been no documented reports of this parasite in Central Europe, but its spread to rainbow trout aqua farms might have disastrous effects.

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CONFLICTS OF INTEREST

The authors hereby declare that no conflicts of interests of any kind exist.

DATA AVAILABILITY STATEMENT

Specimens of the collected *Discocotyle sagittata* are stored in the Clinical Division of Fish Medicine under the accession numbers 20/152 and 20/176.

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