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# First report of metacercariae of *Cyathocotyle prussica* parasitising a fish host in the Czech Republic, Central Europe

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Article info	Summary
Received January 7, 2016	Examination of western tubenose goby ( <i>Proterorhinus semilunaris</i> ) from the River Dyje (Czech Republic, Danube basin) for metazoan parasites revealed the presence of metacercariae of <i>Cyathocotyle prussica</i> Mühling, 1896 (Digenea: Cyathocotylidae) in both muscle tissue and the peritoneal cavity. This is the first time that tubenose gobies have been reported as an intermediate host for <i>C. prussica</i> , and the first time that <i>C. prussica</i> metacercariae have been registered parasitising fish hosts in the Czech Republic. Here, we describe the morphology of metacercariae and five-day preadults, cultivated <i>in vitro</i> , and discuss the importance of recently established, non-native species as suitable hosts for this parasite.
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#### Introduction

The digenean trematode *Cyathocotyle prussica* Mühling, 1896 (Digenea: Cyathocotylidae) is an intestinal parasite of aquatic birds such as the mallard (*Anas platyrhynchos* L., 1758), Eurasian coot (*Fulica atra* L., 1758), common pochard (*Aythya ferina* L., 1758), ferruginous pochard (*Aythya nyroca* L., 1758) and common moorhen (*Gallinula chloropus* L., 1758) (Vojtek, 1981; Sulgostowska 2007). Adults are frequent intestinal parasites of ducks in the Palearctic region and have also been recorded in Central Europe, e.g. Poland (Sulgostowska 2007; Rząd *et al.*, 2013), the Czech Republic (Sitko *et al.*, 2006) and Hungary (Sey, 1966).

While faucet snails (*Bithynia tentaculata* L., 1758) are known to serve as the first intermediate host of at least two species of *Cyathocotyle*, i.e. *C. prussica* (Mathias, 1935; Kanev, 1984) and *C. bushiensis* (Karateyev et al., 2012), there has been far less attention paid to possible secondary intermediate hosts. The range of potential host species comprises either rare occurrences or exper-

imental infections. For example, C. prussica metacercariae have been reported in adult frogs (Pelophylax esculenta (L., 1758)) from southern Slovakia (Vojtková, 1962) and in small fishes, such as stone loach (Barbatula barbatula L., 1758), European bitterling (Rhodeus amarus (Bloch, 1782), gudgeon (Gobio gobio L., 1758), common dace (Leuciscus leuciscus L., 1758) and Chinese sleeper (Perccottus glenii Dybowski, 1877), from Russia (Razmashkin, 1975; Rubanova, 2010). In Eastern Europe, metacercariae naturally infecting fish are known from Bulgaria, where they have been found in gudgeon (Gobio sp.), common carp (Cyprinus carpio L., 1758), European chub (Squalius cephalus (L., 1758)) and European bitterling (Kanev, 1984), and Poland, where they were found in roach (Rutilus rutilus L., 1758), though the actual region was not mentioned (Niewiadomska, 2003; Pojmańska et al., 2007). Metacercariae of unidentified cyathocotylids have also been found in the round goby (Neogobius melanostomus Pallas, 1814) along the Bulgarian stretch of the River Danube, and the monkey goby (Neogobius fluviatilis Pallas, 1814) and racer goby (Babka gymnotrachelus Kessler, 1857) in the River Vistula basin in Poland (Ondračková et al., 2012).

In this study, we describe the taxonomic status of cyathocotylid metacercariae collected from western tubenose gobies (*Proter-orhinus semilunaris*) in the Czech Republic.

### **Material and Methods**

Twenty tubenose gobies (mean standard length 59 mm  $\pm$  13 (S.D.)) were sampled from the River Dyje (German – Thaya; Danube river basin, Czech Republic) near the town of Břeclav (N48.737616, E16.889691) in May 2011, and a further 20 from the Mušov Reservoir (River Dyje drainage) near the village of Pasohlávky (N48.901321, E16.538270), in February 2015. All fish were dissected under a binocular microscope.

Cysts with metacercariae were removed from tissue and the metacercariae mechanically excysted with pins. Three specimens were preserved in hot formalin (Chubb & Bray, 2010). Five of the excysted worms were cultivated at 41 °C on a medium consisting of 50 % chicken serum and 25 % NCTC-135 according to Stewart *et al.* (2003). After five days cultivation, one worm was preserved in hot formalin and the remaining four left to cultivate further. Preserved worms were stained with iron-acetocarmine according to Georgiev *et al.* (1986), following which they were dehydrated using ethanol at increasing concentrations. The samples were then mounted in Canada balsam on glass slides, examined under a light microscope and recorded photographically using a camera Lucida. In all, we measured three metacercariae and one pre-adult specimen.

#### Results

In 2011, one of 20 tubenose gobies was found to be infected with a single metacercaria, identified as *C. prussica*. By 2015, five gobies (of 20) were infected with *C. prussica* metacercariae, with an infection intensity of 1 - 2 specimens. The resilient, semi-transparent and off-white cysts containing *C. prussica* metacercariae were about 250 µm in diameter and located in both muscle tissue and the mesentery.

General description: Metacercariae of 'prohemistomulum' type (Fig. 1). Body massive, oval or pyriform, 262.8 (250.5 – 280.4) µm long and 196.5 (190.4 – 200.2) µm wide, without ventral concavity. Tegument and holdfast organ covered with small spines. Holdfast organ large, almost round, 114.9 (110.4 – 120.0) × 130.5 (114.4 – 140.2) µm, with aperture round or unshaped, elevated above ventral surface. Oral sucker and pharynx well developed: sub-terminal oral sucker 51.8 (51.7 – 51.9) × 51.9 (51.8 – 51.9) µm, pharynx 22.0 (21.7 – 22.5) × 21.2 (20.7 – 22.0) µm. Ventral sucker smaller than oral, 18.1 (16.2 – 19.6) × 18.3 (16.5 – 19.6) µm, located near intestinal bifurcation, covered by holdfast organ. Oesophagus very short. Primordia of gonads located in posterior part of body, distanced at 197.2 (181.1 – 210.4) µm from anterior end.



Fig. 1. Metacercaria of *Cyathocotyle prussica* from western tubenose gobies (*Proterorhinus semilunaris*), sourced from the Mušov Reservoir, River Dyje (Thaya) drainage. Scale bar: 50 μm

After five days cultivation, pre-adults larger (Fig. 2). Body pyriform, 527.27  $\times$  438.64  $\mu m$ . Gonads located in central part of the body. Testes round, diagonal,  $61.36-70.45\times50.00-56.82~\mu m$ , distanced at 208  $\mu m$  from anterior end of the body. Ovary round, small,  $56.82\times34.10~\mu m$ , anterior to testes, distanced at 165.9  $\mu m$  from anterior end of the body. Vitellarium in form of coarse follicles surrounding holdfast organ in peripheral part of body and overlying caeca. Further cultivation did not show results, metacercariae died at sixth day and were of no use for further morphological study.

#### Discussion

This is the first time that metacercariae of the trematode *C. prussica* have been registered as parasitising fish hosts in the Czech Republic. The life-cycle of *C. prussica* was first described by Mathias (1935), who successfully infected stone loaches and gudgeons with cercariae from naturally infected faucet snails. Further studies (see Introduction) have confirmed that the parasite is capable of utilising small-sized fishes (such as the tubenose goby) as an intermediate host. To date, however, naturally occurring cases of fish acting as intermediate hosts have been reported only rarely, with just a few cases in Russia (Razmashkin, 1975; Rubanova, 2010), Poland (Niewiadomska, 2003; Pojmańska *et al.*, 2007), and Bulgaria (Kanev, 1984). While leeches (Hirudinea) are known to act as second intermediate hosts for metacercariae of the related *C. opaca* (Vojtek *et al.*, 1967), occurrence of *C. prussica* metacer-



Fig. 2. Five-day pre-adult cultivated *Cyathocotyle prussica* from western tubenose gobies (*Proterorhinus semilunaris*), sourced from the Mušov Reservoir, River Dyje (Thaya) drainage. Scale bar: 100 μm

cariae is as yet unrecorded. At present, there is little information regarding the main second intermediate host of *C. prussica*; however, demersal fishes have been suggested as being potentially important in the *C. prussica* life-cycle as 72 % of gudgeon were found to be infected with up to 10 metacercariae in southern Siberia, Russia (Razmashkin, 1975). Note that, in each case, small fishes, frogs and tadpoles are all potential food items of ducks, the definitive host of *C. prussica* (del Hoyo *et al.*, 1992).

Tubenose gobies were first introduced into the Mušov Reservoir in 1994 and into the River Dyje two years later (Prášek & Jurajda, 2005; Lusk *et al.*, 2000). In the first study of parasites of tubenose gobies from the Mušov Reservoir in 1999 (Koubkova & Barus, 2000), cyathocotylid metacercariae were completely absent. Despite our study indicating a slight increase in the prevalence and intensity of infection between 2011 and 2015, low index values demonstrate that this fish is still of low importance in the dynamics of this parasite species.

We identified *C. prussica* in this study on the basis of its most important features. The typical characteristic of the genus *Cyathocotyle* is an absence of a ventral concavity and presence of a holdfast organ extending over the ventral surface of the body (Niewiadomska, 2002), which distinguishes *Cyathocotyle* from other cyathocotylids such as *Holostephanus*. Metacercariae of *Holostephanus* spp. are common in Ponto-Caspian gobiids (Ondračková *et al.*, 2009; 2010; Mierzejewska *et al.*, 2014; Kvach *et al.*, 2014; 2015). In *Holostephanus*, the holdfast organ is located within the ventral concavity (Niewiadomska, 2002). While the metacercariae of both genera have round or oval cysts with thick hyaline walls, the cysts of *Holostephalus* are much harder, making it almost impossible to isolate the metacercariae without chemical action. Although isolation of *Cyathocotyle* from cysts is difficult without chemicals, it is possible with the aid of pins.

Of the 17 species of the Cyathocotyle genus described (Lin et al., 2011), only three are known from Europe, i.e. C. prussica, C. opaca and C. orientalis Faust, 1921 (Vojtek, 1971; 1981; Vojtek et al., 1967; Sitko et al., 2006). Cyathocotyle orientalis is a parasite of sea-gulls from East Asia, but is also known from one occasional occurrence in the red-throated loon (Gavia stellata (Pontoppidan, 1763)) in the Czech Republic (Sitko et al., 2006; Sitko & Heneberg, 2015). The species is characterised by rather large metacercariae (400 – 500 µm diameter) located in the tissues of fishes, though parthenogenetic stages occur in the lesser edible snail (Sinotaia quadrata (Benson, 1842)) (Faust, 1921). These snails are only found in East Asia (Köhler & Richter, 2012). Cyathocotyle orientalis metacercariae are characterised by a thick cyst wall and a w-shaped excretory bladder (Sohn, 2009). Metacercariae of C. opaca are characterised by having parallel prolonged testes and the primordia of the reproductive organs located in the anterior part of the body (Vojtek et al., 1967).

In our *C. prussica* specimens, the location of the gonads differed between metacercariae and pre-adults (see also Vojtková, 1962), with the metacercariae having the gonadal primordia in the posterior part of the body, with the testes arranged diagonally and the ovarium posterior to the testes (Vojtková, 1962; current data; see Fig. 1). Adults, on the other hand, have the testicles positioned diagonally in the central part of the body and the ovarium anterior to the testes (Vojtková, 1962; Razmashkin, 1975). Five-day old pre-adults cultivated *in vitro* had gonads positioned typically for adults (see Fig. 2).

Despite intensive examination of fish parasites in the Czech Republic for more than 60 years (Moravec, 2001), metacercariae of *C. prussica* have remained unnoticed, probably due to the low importance of fish in this parasite's life-cycle or the absence of competent fish host species. As tubenose gobies become established in new locations around Europe (Roche *et al.*, 2013), the importance of this demersal fish as an intermediate host in the life-cycle of this parasite is likely to increase.

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