

Research Note

Metacercariae of *Centrocestus formosanus* in goldfish (*Carassius auratus* L.) imported into Croatia

E. GJURČEVIĆ¹, Z. PETRINEC¹, Z. KOZARIĆ², S. KUŽIR², V. GJURČEVIĆ KANTURA², M. VUČEMILO³, P. DŽAJA⁴

¹Department of Biology and Pathology of Fishes and Bees; ²Department of Anatomy, Histology and Embryology; ³Department of Animal Hygiene, Environment & Ethology; ⁴Department of Forensic and Juridical Veterinary Medicine, Faculty of Veterinary Medicine, University of Zagreb, Heinzelova 55, Zagreb 10 000, Croatia, E-mail: egjurcevic@vef.hr

Summary

The presence of metacercariae of *Centrocestus formosanus* was detected on the gills of goldfish (*Carassius auratus* L.) imported from Singapore and obtained from two pet shops. This is the first report of the parasite in Croatia. The histopathological changes observed lead to the conclusion that the infection could be one of the reasons for the death of goldfish.

Key words: *Centrocestus formosanus*; goldfish; histopathological changes

Introduction

Centrocestus formosanus (Nishigori, 1924) (Trematoda: Heterophyidae) has a complex life cycle, during which it uses a snail as the first intermediate host and several fish species as the second. The cycle is completed when a fish infected with metacercariae is eaten by a definitive host, piscivorous birds or fish-eating mammals, in whose digestive tract the adult trematode develops (Premvati & Pande, 1974; Paperna, 1996; Scholz & Salgado-Maldonado, 2000; Scholz *et al.*, 2001; Hernández *et al.*, 2003; Mitchell *et al.*, 2005). The parasite has been found in the gills of many fish species from numerous Asian countries (Yanohara & Kagei, 1983; Madhavi, 1986), United States (Mitchell *et al.*, 2000) and other parts of the world (Vélez-Hernández *et al.*, 1998; Hernández *et al.*, 2003; Salgado-Maldonado *et al.*, 2005). In Turkey, Yildiz (2005) first recovered the metacercariae of *C. formosanus* from the gills of ornamental fish imported from Singapore.

Material and Methods

The study involved 50 goldfish imported from Singapore and obtained from two pet shops in Zagreb, Croatia. Wet mounts of gill tissue were observed under an optical microscope, Olympus BX41 at 40 – 1000X. The gills of 10 infected goldfish were removed and placed in small Petri dishes. A 2.5 % solution of Trypsin (pH = 6,2) was added to the encysted metacercariae. Excysted metacercariae were identified as wet mounts under a microscope. Cysts and excysted metacercariae were measured with an Olympus DP 12 digital camera and Cell B software (Soft Imaging System). The mean values of all measurements are presented in μm , and ranges are provided in parentheses. For histopathological examination, the gills of each fish were fixed in 10 % buffered formalin and Bouin's solution. The fixed material was embedded in paraffin wax and serially sectioned. The 6 μm thick sections were stained with hematoxylin and eosin (H&E), Mallory's aniline blue and Azan (Sheehan & Hrapchak, 1980).

Results and Discussion

Metacercariae of *Centrocestus formosanus* were found in 20 (40 %) of the 50 goldfish. Cysts, randomly distributed along the entire length of the gill filaments, were oval in shape, measuring 186 (165 – 205) x 138 (116 – 158). Excysted metacercariae were elongate and pyriform in shape, measuring 240 (185 – 323) x 114 (94 – 130) and completely covered with tegumental spines. The oral sucker was located at the anterior end, 30 (22 – 38) long, 41 (33 – 49) wide, with 32 circumoral spines in two rows around it.

The pharynx was well developed, measuring 30 (20 – 39) x 21 (16 – 28). The oesophagus was short, anterior to the ventral sucker, the intestine bifurcated into two caeca extending towards the posterior end to reach the anterior level of the excretory vesicle. The ventral sucker was oval, measuring 33 (27 – 41) x 40 (32 – 45), and posterior to the middle of the body. The testes were oval, and symmetrical in the posterior third of the body. The left testis measured 27 (23 – 34) x 32 (31 – 35), and the right testis 28 (26 – 34) x 33 (30 – 40). The ovary was oval, situated to the right of the median line, anterior to the excretory vesicle. The excretory vesicle was X-shaped, in the posterior third of the body, between the testes (Fig. 1).

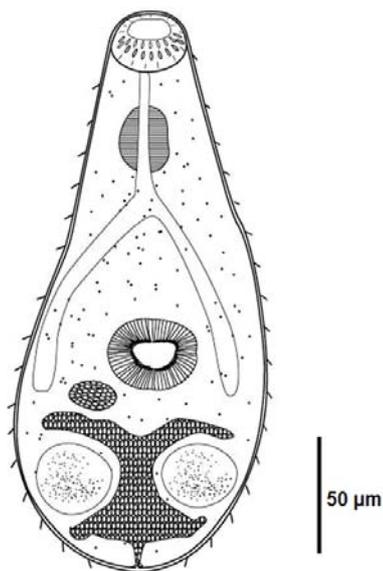


Fig. 1. Excysted metacercaria of *Centrocestus formosanus*, ventral view; free-hand drawing

Histopathologically, the changes were characterized by hyperplasia of the cartilage of the primary lamellae that envelops the metacercarial cysts. The metacercariae were surrounded by a 2 – 4 µm thick refractile capsule. The host response included encapsulation around the cyst. The host cyst wall consisted of chondrocytes which were generally elongate adjacent to the parasites. On the periphery of the reaction, chondrocytes were rounded and hypertrophied. The majority of the cysts were encapsulated separately, and in only in a few cases did the capsule enclose more than one cyst. The infected part of the lamella very often caused fusion of two primary lamellae (Fig. 2). The majority of the histopathological changes were accompanied by the thickening and distorting of the primary lamellae. Hyperplasia of the epithelial cells resulted in partial and in some parts, total fusion of the secondary lamellae. Gill hyperaemia was also observed. There were sporadic hypertrophies of epithelial cells. Moderate mononuclear infiltration was present in the peripheral area of the lesion. Whilst researching the presence of parasites in goldfish kept in an aquarium, we found that 40 % of the fish ex-

amined were infected with *C. formosanus*. The finding of metacercariae of *C. formosanus* on the gills of goldfish is also the first report of this parasite in the Republic of Croatia. Arizmendi (1992) and Hernández *et al.* (2003) gave a detailed description of the morphological characteristics of the metacercariae. The morphometrical values found by these authors differ from each other. In this study, the morphometrical values do not agree with the findings of Arizmendi (1992) but they are in line with the findings of Hernández *et al.* (2003) with the exception of small differences in the size of the excysted metacercariae.

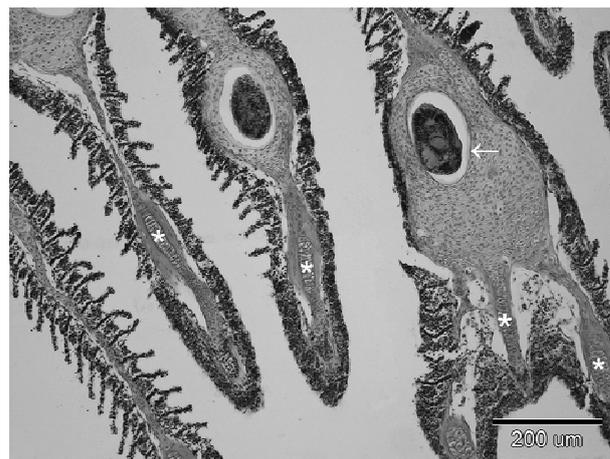


Fig. 2. Histological section of gills showing encysted metacercariae, fusion of gill filaments and hyperplasia of epithelial cells (HE)
* Original filament cartilage; → Refractile capsule

According to Hernández *et al.* (2003), the metacercariae were 244 – 322 µm x 83 – 86 µm and the oral sucker was 26 – 56 µm x 32 – 51 µm, whilst according to our research the metacercariae were 240 (185 – 323) µm x 114 (94 – 130) µm, and the oral sucker was 30 (22 – 38) µm x 41 (33 – 49) µm. Based on the number of circumoral spines and the characteristic shape of the excretory vesicle, we identified this parasite as *Centrocestus formosanus* (Nishigori, 1924).

The basic histopathological changes on the gills of the goldfish include hyperplasia of the cartilage of the primary lamellae that envelops the cysts, with the loss of respiratory surface. This histopathological finding is in line with the findings described by Blazer and Gratzek (1985), Vélez-Hernández *et al.* (1998), Mitchell *et al.* (2000) and Mitchell *et al.* (2002). We discovered hypertrophy of the epithelial cells of the secondary lamellae at the periphery of those changes, which had not been described by these authors. The hyperplasia of the gill cartilage discovered in our research was a defense reaction of the host tissue. Blazer and Gratzek (1985) made a similar observation. Our research, like that of Blazer and Gratzek (1985) and Mitchell *et al.* (2005), confirmed that histopathological changes cause reduction in the gill surface which leads to their decreased function. Since infection with *C. formosanus* metacercariae can cause fish death (Mitchell *et al.*, 2000), quarantine and constant health monitoring are essential during goldfish importation. Further research is

required to establish how great is the threat of introduction of these parasites into open waters in Croatia and of their further spread to indigenous fish species.

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