ISTANBUL UNIVERSITY PRESS

EISSN 2602-473X

AQUATIC SCIENCES AND ENGINEERING

Aquat Sci Eng 2020; 35(1): 1-5 • DOI: https://doi.org/10.26650/ASE2019566509

Short Comminication

First Record of *Contracaecum rudolphii* Hartwich, 1964 in *Carassius gibelio* (Bloch, 1782) From Turkey

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Cite this article as: İnnal, D., Stavrescu-Bedivan, M., Öztürk, M. O., Özmen, Ö. (2020). First record of Contracaecum rudolphii Hartwich, 1964 in Carassius gibelio (Bloch, 1782) from Turkey. *Aquatic Sciences and Engineering, 35*(1), 1–5.

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Submitted:
16.05.2019

Revision Requested:
09.07.2019

Last Revision Received:
19.09.2019

Accepted:
28.09.2019

Online published:
30.10.2019

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ABSTRACT

Thirty-eight individuals of Prussian carp, *Carassius gibelio* (Bloch, 1782) were collected from Karataş Lake, Burdur-Turkey and analyzed for parasite fauna. We found the nematode larvae of *Contracaecum rudolphii* Hartwich, 1964 in one sample (prevalence 2.63%, mean intensity of infestation 27 parasites per fish). The individual parasite was found around the pancreas, fibrous connective tissue and its mesentery. To our knowledge, this is the first record of anisakid nematode, *Contracaecum rudolphii* in Turkey. Therefore, a new locality has been added to the geographical distribution of the parasite species. Furthermore, slight to severe inflammatory cells were seen on the infected tissue. Granulomatous reaction characterized by mononuclear cells and fibrous tissue proliferations were also seen around the parasite located areas.

Keywords: Nematoda, Anisakidae, first record, histopathology

INTRODUCTION

The nematodes of the genus *Contracaecum* belonging to the family Anisakidae are able to infect both terrestrial and aquatic animals (Shamsi, 2019). The cosmopolitan *Contracaecum rudolphii* Hartwich, 1964 has a complex life cycle, involving invertebrates such as copepods, ostracods or gammarids as first intermediate hosts, teleost fish as second intermediate or paratenic host and piscivorous birds e.g. cormorants as final hosts (Bartlett, 1996; Dziekońska-Rynko, Rokicki, Mierzejewska, Wziątek, & Bielecki, 2013).

As far as we know, the first-stage larva formed in the embryonated eggs released with the faeces of definitive host moult to the second-stage, a variety of organisms including mollusks and crustaceans being able to eat eggs or larvae of *Contracaecum* parasites (Shamsi, 2019). Fish play the role of common hosts for the third larval stage after ingesting the already infested invertebrates (Dziekońska-Rynko & Rokicki, 2007). Infections of cyprinids under laboratory conditions have been proved by various authors that found nematode three-stage nematode larvae encysted in the intestinal wall or located in the internal organs of the fish (Moravec, 2009; Dziekońska-Rynko et al., 2013).

At the adult stage, *C. rudolphii sensu lato (s.l.)* is a parasite common in fish-eating birds such as great cormorant, *Phalacrocorax carbo* (Szostakowska & Fagerholm, 2007; Al-Moussawi, 2017). Dziekońska-Rynko, Rokicki, & Wziątek B (2008) suggested that nematodes prefer to develop in warm water bodies when cormorants become infected during their wintering season.

In Turkey, the first case of intense infection with adult *Contracaecum* sp. in a piscivorous bird was reported by Girişgin, Alasonyalilar-Demirer, & Girişgin (2012) during the necropsy of the Dalmatian pelican, *Pelecanus crispus*. So far, larval forms of *Contracaecum* sp. were confirmed in the helminthofauna of several fish species from Turkey: *Alburnus alburnus*, *Barbus* *lacerta*, *B. plebejus escherichi*, *Carassius auratus*, *C. carassius*, *Capoeta tinca*, *Scardinius erythrophthalmus*, *Rutilus rutilus* and *Vimba vimba* (Koyun & Altunel, 2007; Selver, Aydogdu, & Cirak 2009; Koyun, Ulupınar, & Gül, 2015). Moreover, larvae of *Contracaecum* sp. were mentioned for the first time in 2016 in the intestine of *Carassius gibelio* from Marmara Lake, western Turkey (Demir & Karakişi, 2016). Nevertheless, there is still no information upon larvae of *Contracaecum rudolphii* parasitizing fish species in Turkey. This study describes the first case of identified *C. rudolphii* Hartwich, 1964 in a freshwater fish host in Turkey.

MATERIALS AND METHODS

Overall, 38 Prussian carp (Figure 1) were caught using nets by local commercial fishermen in November 2018 in Karataş Lake, Burdur. Fish speciemens were identified to species level according to Kottelat and Freyhof (2007). The individuals were transported alive to the research laboratory of Biology, Burdur Mehmet Akif Ersoy University.

Figure 1. *Carassius gibelio* from Karataş Lake.

The total body length (cm) and weight (g) of each *Carassius gibelio* individuals were recorded. After all fish were sacrificed, their skin, fins, gills, oesophagus, liver, gall-bladder, stomach and intestine of the samples were dissected out and placed in petri dishes with a physiological solution. To determine the presence of parasite specimens, all parts were thoroughly examined under a binocular microscope. Parasite individuals found in the host fishes were removed using a preparation needle. The parasites were fixed in formaldehyde stained with Mayer's haematoxylin and identified using the reference keys (Pritchard & Kruse, 1982; Anderson, 1992). The percentage of hosts infected with the parasites (prevalence, %) and intensity were computed according to Bush, Lafferty, Lotz, & Shostak (1997).

The gut and mesenteries samples collected from the fish during necropsy were fixed in 10% neutral formalin. The samples were then routinely prepared by automatic tissue processor equipment (Leica ASP300S, Wetzlar, Germany) and embedded in paraffin wax. Tissue sections were cut into 5-μm-thickness by a rotary microtome (Leica RM2155, Leica Microsystems, Wetzlar, Germany). Then, samples were stained with hematoxylin-eosin (HE), placed on a coverslip with mounting media, and examined under a light microscope.

RESULTS AND DISCUSSION

The total length of Prussian carp individuals varied between 20 and 33 cm, while the weight values ranged between 153 and 560 grams. Among the total number of 38 specimens of *Carassius gibelio* examined for parasite presence, only one individual fish was infected with nematode *Contracaecum rudolphii* (prevalence 2.63%, mean intensity of infestation 27 parasites per fish) (Figure 2).

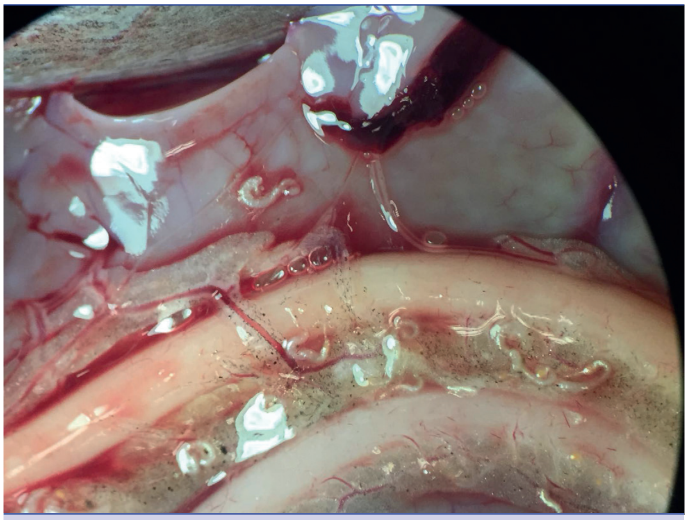

Figure 2. *Contracaecum rudolphii* in Carassius gibelio.

During the study, at the gross examination, hyperemias at the mesenteric vessels of infected host samples were observed. Parasites were found around the pancreas, fibrous connective tissue and its mesentery. Slight to severe inflammatory cells were seen on the infected tissue. Granulomatous reaction characterized by mononuclear cells and fibrous tissue proliferations were also seen around the located parasite areas. Some granulomas contained more than one parasite. Some granulomatous became necrotic when effected with the parasite. Numerous melano macrophages were found around the necrotic granulomas (Figure 3).

Based on genetic data evidence, Li et al. (2005) identified the existence of two-strains of the parasite, *C. rudolphii*-A and *C. rudolphii*-B. From these strains, *C. rudolphii*-B was determined as parasite of freshwater fishes (Szostakowska & Fagerholm, 2007; Moravec, 2009). In the present study, the larval parasite species, *C. rudolphii* was identified using morphologic and anatomic features detailed by Anderson (1992), in *Carassius gibelio* from Karataş Lake. According to the above knowledge, the larval parasite species *C. rudolphii* determined in the present study might belong to *C.rudolphii*-B. In further research, DNA sequencing is obviously required in order to establish that the taxon identified in this study is *C.rudolphii*-B.

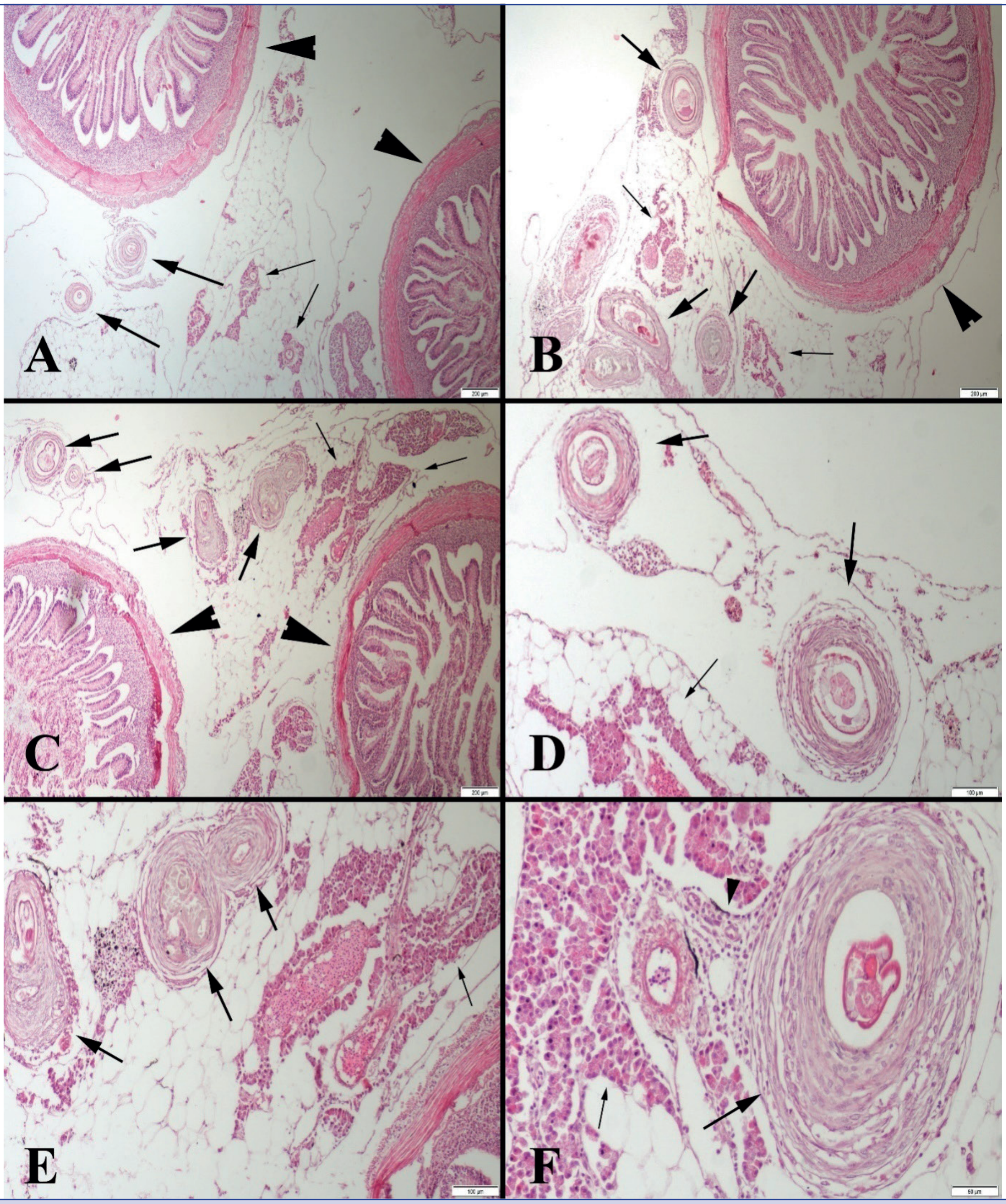

Figure 3. (A) Numerous parasites (thick arrows) localized at mesentery of the intestine (arrow heads) near the pancreas (thin arrows), HE, Bar= 200µm. (B) Another area and numerous parasite (thick arrows) around the intestine (arrow heads) and the pancreas (thin arrows), HE, Bar= 200µm. (C) Parasites (thick arrows), near the gut (arrow heads) and pancreas (thin arrows), HE, Bar= 200µm. (D-E) Higher magnification of the parasites (thick arrows), near the pancreas (thin arrows), HE, Bar= 100µm. (F) Fibrous connective tissue (arrow) around the parasite and inflammatory cell infiltrations (arrow head), HE, Bar= 50µm.

To date, another taxon, *Contracaecum microcephalum* was recorded for the Prussian carp from Srebarna Nature Reserve, Bulgaria (Shukerova, 2005). Different pervalence values of *Contracaecum* infection were reported from fish species (Stoyanov, Mutafchiev, Pankov, & Georgiev, 2017; Oztürk & Yesil, 2018; Dziekońska-Rynko, Mierzejewska, Kubiak, Rydzewska, & Hliwa, 2018; Chunchukova & Kirin, 2018; Sokolova et al., 2018). Low values of prevalence for *Contracaecum* infection in fish host in the present research are consistent with other studies (Mancini et al., 2008; Roumbedakis et al., 2013; Stoyanov et al., 2017). This might be explained by reduced feeding activity of infested fish by copepods at low temperatures in the cold season (Barson, 2004).

Similar changes were noticed by Dezfuli et al. (2016) in intestinal walls of *Anguilla anguilla*. In gastrointestinal tract of great cormorant *Phalacrocorax carbo*, Rokicki, Sołtysiak, Dziekońska-Rynko, & Borucińska (2011) reported *C. rudolphii* causing lesions consisted of severe or diffuse gastritis. Larval stages of nematodes forming ulcerative eosinophilic granulomas have been found by Amato, Monteiro, & Amato (2006) in the proventriculus of *Phalacrocorax brasilianus*.

Li et al. (2005) have postulated that *C. rudolphii*-A and *C. rudolphii*-B were not related with human anisakidosis, although these two sibling species have shown a zoonotic potential due to larval development in numerous fishes.

In conclusion, *Contracaecum rudolphii* is recorded for the first time in *Carassius gibelio* from Turkey. Accordingly, a new locality has been added to the geographical distribution of the parasite species. Also, hyperemia and necrotic granulomas caused by the parasite species were determined.

Acknowledgement: The authors are grateful to students Enes Faruk Malpınar, Furkan İçinata, Fahrettin Burak Kazancı, Damla Çelikkaya from Biology Department of Mehmet Akif Ersoy University for their valuable technical assistance in the course of fieldwork and laboratory studies.

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