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THE FIRST RECORD OF *ASPIDOGASTER CONCHICOLA* (TREMATODA: ASPIDOGASTRIDAE) IN THE FRESHWATER MUSSEL *UNIO PICTORUM* IN CROATIA

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Although *Aspidogaster conchicola* is a common and widespread parasite of freshwater molluscs, knowledge about its distribution and hosts in Croatia is still insufficient. The aim of our research was to investigate the presence of *A. conchicola* in the freshwater mussel *Unio pictorum,* which is widely distributed in the rivers of Europe. The study was conducted in the Mrežnica River, where *U. pictorum* is a common species. Five specimens of *U. pictorum* were collected by scuba diving in October 2021. The dissection of mussels was carried out in the laboratory, and all tissues were inspected under a stereo microscope. Determination of the parasite was performed based on the number and arrangement of alveoli. The results showed that the prevalence of infection was 40% (two out of five mussels were infected), and the intensity of infection was 2 to 3 specimens per mussel. All parasites were detected in the mussels' pericardial cavity. In conclusion, this is the first record of *A. conchicola* in *U. pictorum* in Croatia.

Keywords: trematodes, prevalence and intensity of infection, Aspidogastrea, pericardial cavity, Unionidae

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Aspidogaster conchicola je čest i široko rasprostranjen parazit slatkovodnih mekušaca. Ipak, saznanja o njegovoj rasprostranjenosti i domaćinima u Hrvatskoj još su nedostatna. Cilj našeg istraživanja bio je istražiti prisutnost *A. conchicola* u slatkovodnom školjkašu *Unio pictorum* koji je široko rasprostranjen u europskim rijekama. Istraživanje je provedeno u rijeci Mrežnici u kojoj je *U. pictorum* česta vrsta. Pet jedinki *U. pictorum* prikupljeno je ronjenjem u listopadu 2021. godine. Disekcija školjkaša je obavljena u laboratoriju, a sva tkiva su pregledana pod stereo mikroskopom. Determinacija metilja je obavljena na temelju broja i rasporeda alveola. Rezultati su pokazali da je prevalencija infekcije bila 40% (dvije od pet jedinki bile su zaražene), a intenzitet infekcije 2 do 3 jedinke metilja po školjkašu. Svi paraziti su otkriveni u perikardijalnoj šupljini školjkaša. Zaključno, ovo je prvi nalaz metilja *A. conchicola* u školjkašu *U. pictorum* u Hrvatskoj.

Ključne riječi: metilji, prevalencija i intenzitet infekcije, Aspidogastrea, perikardijalna šupljina, Unionidae

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INTRODUCTION

The painter's mussel, *Unio pictorum* (Linnaeus, 1758), is one of the most abundant and widespread freshwater mussel species in Europe, not including the Iberian and Apennine peninsulas (Lopes-Lima *et al.*, 2017). The species is found from Great Britain to the Ural River in Russia, and is also present in Asia in the upper tributaries of the Ob River basin, in Russia and Kazakhstan (Lopes-Lima *et al.*, 2017; Vinarski *et al.*, 2020). The habitats of this species include rivers, streams, lakes, flood plains, and even reservoirs and fishponds (Lopes-Lima *et al.*, 2017). It has recently been assessed as Least Concern (Van Damme, 2011). In recent decades, however, there has been a worrying decline in populations throughout its range, with many local populations considered threatened and several countries having very strict conservation regulations for the species (Lopes-Lima *et al.*, 2017; Beran, 2019). It is also important to highlight that *U. pictorum* has had whole genome assembled as the only representative of the order Unionida (Gomes-dos-Santos *et al.*, 2023).

The parasitic worm *Aspidogaster conchicola* Baer, 1827 belongs to the subclass Aspidogastrea characterized by a simple developmental cycle that involves a mollusc as an obligate host and a vertebrate (fish or turtle) as a facultative or obligate final host (Rohde, 2002). *Aspidogaster conchicola* has a circumpolar Holarctic area of distribution and was found in several species of freshwater bivalves, most often from the Unionidae family (Skryabin, 1952; Dvodryadkin, 1967; Fuller, 1974; Alves *et al.*, 2015). This species has also been found in snails of the family Viviparidae (Skryabin, 1952; Dvodryadkin, 1967; Michelson, 1970) and in the intestines of some molluscivorous fish (Dvodryadkin, 1967; Gao *et al.*, 2003; Alves *et al.*, 2015). *Aspidogaster conchicola* specimens are typically found in the pericardial or renal cavities of bivalve molluscs where, according to Gentner (1971), they feed on blood cells and hemolymph.

Huehner & Etges (1981) found that mussels have the ability to encapsulate *A. conchicola*. They reported that capsule contents included living or moribund adult worms, viable eggs or empty egg shells, juveniles hatched from eggs deposited by encapsulated adults, and host cells of which "brown cells" were most abundant. Because of the high frequency of encapsulated moribund worms observed in that study (more than 60%), the authors infer that this host reaction probably contributed to the parasites' death.

In Croatia this parasite species was found for the first time in 2020 in the unionid mussels *Anodonta anatina* (Linnaeus, 1758) and *Unio tumidus* Philipsson, 1788 sampled in the Korana River (Karlovac town) (Runje, 2021; Matanović *et al.*, 2023). In order to increase knowledge about the distribution and hosts of this species, we conducted research in the Mrežnica River, a main tributary of the Korana River. The aim of our study was to check for the presence of this species in the mussel *U. pictorum*, which is a common species in the Mrežnica River.

MATERIALS AND METHODS

The freshwater mussel *Unio pictorum* (Fig. 1) was sampled from the Mrežnica River, Croatia. The River Mrežnica belongs to the Sava River Basin and is 64 km long. In its upper part, the Mrežnica is a typical karst river with canyons, numerous travertine waterfalls and lacustrine areas, whereas in its lower part it enters the lowland region and becomes wider and shallower. The sampling site (Figs. 2 and 3), was located abo-

Fig. 1. Shells of the painter's mussel, *Unio pictorum*, from the Mrežnica River in the pericardial cavity of which three adult specimens of *Aspidogaster conchicola* were found (shell length = 8.51 cm) (Photograph by I. Lajtner).

ut 2 km upstream from the town of Duga Resa, in the lower section of the Mrežnica River and represented the site with low anthropogenic influence (45° 26′ 28.40″ N, 15° 30′ 15.39″ E). Detailed information on the geographical characteristics of the section of the Mrežnica River studied, as well as hydrological and physico-chemical data referring to the time of mussel sampling were presented in paper of Dragun *et al.* (2022). Most importantly, the measurement of several physico-chemical parameters and nutrients in the surface water along the lower course of the Mrežnica River in 2021 indicated its rather good quality (Dragun *et al.*, 2022).

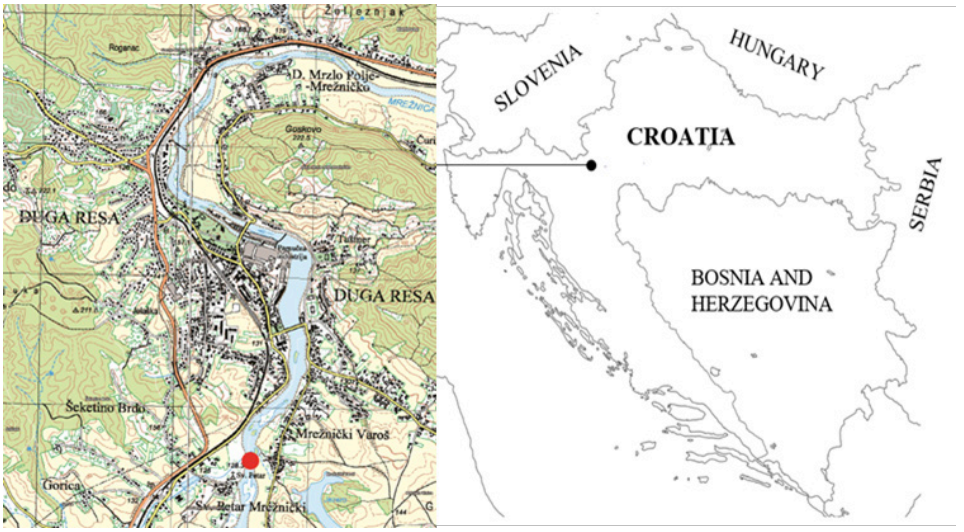

Fig. 2. Map showing the sampling site (red circle) in the Mrežnica River near the town of Duga Resa.

Fig. 3. Researched location in the Mrežnica River (Photograph by M. Cvitanović).

The use of mussels for this study was in accordance with the Ordinance on the protection of animals used for scientific purposes (NN 55, 2013). The samples of *U. pictorum* were collected by scuba-diving in early October 2021. Altogether five mussels were transported to the laboratory in dark and cool containers where they were depurated 24 h prior to dissection. The dissection of mussels was carried out in the laboratory of the Department of Zoology (Faculty of Science, University of Zagreb) and all tissues were inspected under a stereo microscope Olympus SZ61. Determination of the parasite *A. conchicola* was performed based on the number and arrangement of alveoli (Michelson, 1970).

RESULTS AND DISCUSSION

The results of our research confirmed the hypothesis that *A. conchicola* lived in the mussel *U. pictorum* in the river Mrežnica (Fig. 4). Two out of five mussels were infected, which means that the prevalence of infection was 40%. As mentioned in the Introduction, the first finding of *A. conchicola* in Croatia was in 2020 in the unionid mussels *A. anatina* and *U. tumidus* from the Korana River (central part of Croatia) (Runje, 2021; Matanović *et al.*, 2023). That research included four unionid species. The prevalence of infection in *U. tumidus* was 14.3% while only one specimen of *A. anatina* was examined, from which one specimen of *A. conchicola* was isolated. During that investigation no aspidogastreans were found in *U. pictorum* and *Sinanodonta woodiana* (I. Lea, 1834), which were sampled at the same location.

Bakker & Davids (1973) described the finding of *A. conchicola* in the mussels *U. pictorum* (prevalence ranging from 9 to 10%) and *A. anatina* (prevalence ranging from 0 to 55%), ascribing the observed differences in frequency to different sampling locations. Several studies have been conducted on the prevalence of *A. conchicola* in the invasive freshwater mussel *S. woodiana*. Pavlyuchenko (2005) found *A. conchicola* in *S. woodiana*

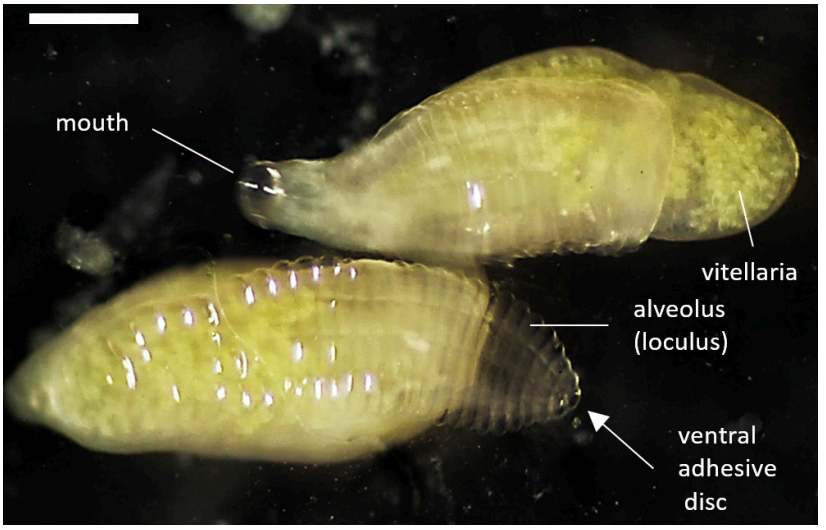

Fig. 4. Two adult live specimens of parasite *Aspidogaster conchicola* isolated from the pericardial cavity of the mussel *Unio pictorum*. Scale bar = 0.5 mm (Photograph by J. Lajtner).

in Kugurluy Lake (Danube Basin, Ukraine) with the prevalence of infection 27.3%. Yuryshynets (2004) stated that in the Danube-Sasyk Channel (Danube Basin) the prevalence of *A. conchicola* in *S. woodiana* was 13.3%. Yuryshynets & Krasutska (2009) found *A. conchicola* in *S. woodiana* from some freshwater bodies in Poland (Konin Lakes System) and Ukraine (Danube Basin), with the prevalence of infection ranging from 5 to 30%.

Marszewska & Cichy (2015) investigated the diversity and the prevalence of trematodes from the subclasses Digenea and Aspidogastrea in three native unionid mussels and in dreissenid mussels residing on the surface of their shells. The authors stated that the most infected unionid species was *A. anatina* and that a smaller percentage of infections was recorded for *U. tumidus*, while *U. pictorum* carried no trematodes. Among them only *U. tumidus* was infected by *A. conchicola* with a prevalence of 1.4%. The results also indicated that unionids in most cases played the role of the first intermediate host for digenetic trematodes or final hosts for aspidogastrean trematodes, while dreissenids were mainly the second intermediate hosts.

In our study the intensity of infection was 2 to 3 specimens per mussel, which is higher than the values known so far. For example, in *S. woodiana*, Yuryshynets (2004) reported an intensity of 1 parasite specimen per mussel. Yuryshynets & Krasutska (2009) found that the intensity of infection was 1 to 2 parasite specimens per mussel. Pavlyuchenko (2005) found *A. conchicola* in *S. woodiana* with an average intensity of 1.5 specimens per mussel.

All parasites in our study were detected in the mussels' pericardial cavity, which is in accordance with other studies conducted on mussels from the family Unionidae (Yuryshynets, 2004; Pavlyuchenko, 2005; Yuryshynets & Krasutska, 2009). According to Gentner (1971), these parasites feed on blood cells and haemolymph. In this paper, we did not investigate histopathology, so histopathological changes on the tissue, if present, were not recorded, and will be studied in future.

Another aspidogastrean species recorded in freshwater molluscs in Croatia was *Aspidogaster limacoides* Diesing, 1834. This species was found in the zebra mussel, *Dreissena polymorpha* (Pallas, 1771), from the Drava River (Lajtner, 2012). A single specimen was found in the mussel's stomach cavity and the results based on histopathology and image analysing system demonstrated that infection by *A. limacoides* resulted in a significant reduction of all the organs examined. In the meantime, this species was also found in the snail *Esperiana esperi* (Férussac, 1823) from the Korana River (Runje, 2021; Matanović *et al.*, 2023).

In conclusion, these preliminary results have confirmed that *A. conchicola* indeed exists in the mussel *U. pictorum* from the Mrežnica River, with comparatively high prevalence and intensity. Thus, additional ecological and histopathological studies are needed.

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