

Occurrence of parasites in *Cobitidae* from Croatian rivers draining into two different watersheds

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Summary

Members of the Cobitidae family are not economically important, thus few studies have been made on their pathology. A survey of parasitic fauna of some representatives of the loaches, such as *Cobitis elongata*, *C. elongatoides*, and the recently described *C. bilineata*, was carried out during the spring and summer of 2006. Fish samples were collected from four Croatian rivers: the Sava, Drava and Kupa draining into the Danubian system, and the Zrmanja draining into the Adriatic system. Specimens were of varying size, weight and age. Ectoparasitological examination revealed infections by the ciliate parasites *Trichodina nigra* (61; 29%), *Epistylis* sp. (12; 9%), *Chilodonella cyprinid* (22; 58%), *Ichthyophthyrus multifiliis* (81; 81%), dinoflagellate *Pisciodinium pillulare* (6; 45%) and monogenean *Gyrodactylus cobitis* (22; 22%). Histopathological examination of haematoxylin-eosin stained tissues showed the presence of *Sarcocystis* sp. in the muscle of one specimen. Dissections revealed an isolated finding of plerocercoid of *Ligula colymbi* in a Drava river specimen. A more comprehensive survey is necessary to determine the actual threat of the parasitoses to endangered species of the Cobitidae family.

Introduction

Species from the genus *Cobitis* are widespread in almost all European water systems, yet their distribution has been severely affected by anthropogenic factors. Loaches are also interesting model organisms for zoogeographic studies (Bohlen and Rab, 2001). Croatian rivers flow into two separate drainage systems: the Danubian and the Adriatic watersheds (Mrakovčić et al., 2000). Fish fauna in the Croatian section of the Danubian watershed are similar to those found in other parts of Europe and accommodate four species of the cobitid family: *Cobitis elongata*, *C. elongatoides*, *Misgurnus fossilis* and *Sabanejewia balcanica*. Rivers draining toward the Adriatic differ in length and either flow into lakes, the sea or disappear in karstic cavities; in this drainage system several loach species have been recognized: *Cobitis dalmatina*, *C. nerentana* and *C. bilineata*, the latter described for the first time by Buj et al. (2008).

Data on freshwater fish diseases are limited mostly to commercially important species. Loaches are hosts to numerous parasites and are affected by various diseases as well as acting as important secondary hosts for certain parasites (Koster et al., 2002). The presence of disease agents may influence the structure and size, feeding habits, growth rates and reproduction of loach populations. Few studies have been

published on the parasite fauna of representatives of the genus *Cobitis*, describing the digenean parasite *Allocreadium transversale* in the intestines of *C. taenia* (Robotham and Thomas, 1982). Bychovskij (1962) and Bauer (1984) listed parasites observed in representatives of the genus *Cobitis* comprising parasitic protozoa and metazoa. The high intensity of infection in *C. elongatoides* by plerocercoids of the tapeworm *Ligula colymbi* as well as metacercariae of the trematode *Posthodiplostomum cuticula* in hybrids of *C. elongatoides* x *C. sp.* in the Czech Republic was observed by Halačka et al. (2000). Parasitological examination of *C. elongatoides* and hybrids of *C. elongatoides* and *C. taenia* in Poland revealed infections by six trematode species (*Diplostomum* sp., *Echonostomatidae* gen. sp., *Tylodelphys clavata*, *Posthodiplostomum cuticula*, *Metorchis xanthosomus* and *Allocreadium transversale*) and the nematode *Rhabdochona ergensi* (Popiolek and Katusz, 2003, 2004; Popiolek et al., 2003). Scholz et al. (2004) reported the presence of the cestode *Neogryporhynchus cheilancristrosus* in *C. tenia* and *M. fossilis*.

Representatives of the genus *Cobitis*, with the goal of estimating their phylogenetic position and taxonomic status in both Croatian watersheds, were studied during the spring and summer of 2006. Samples of spined loach populations from several rivers were collected for investigation of their parasitofauna.

Materials and methods

Study area

Five sampling sites (Fig. 1) were included in the May, June and July 2006 study. From the Danubian watershed Site 1 (45°16'08"N; 16°55'17"E) and Site 2 (45°46'45"N; 16°01'00"E) were at the Sava river, Site 3 (45°26'49"N; 16°16'38"E) at the Kupa river, and Site 4 (45°56'22"N; 17°21'10"E) at the Drava river; Site 5 (44°11'59"N; 15°42'11"E) was at the Zrmanja river from the Adriatic watershed.

Fish samples

Representatives of the genus *Cobitis* were caught with nets at all sampling sites by electro fishing with a 7.5 kW generator during the spring and summer of 2006 (May through July). Live fish were placed in plastic containers with water from the sampling site and supplied with aeration during transport to the laboratory. Fish were killed by pithing the brain and immediately submitted to laboratory examination. Species determination was performed using colouration patterns and



Fig. 1. Sampling sites (ovals) for cobitid species during the study period (May–July 2006) in rivers of two watersheds in Croatia. Solid line = borderline of the watersheds

other morphological features according to determination keys of Vuković and Ivanović (1971), Povž and Sket (1990) and Maitland (2000). Total and standard lengths of each fish were measured in millimetres and the weight in grams. The Shapiro–Wilk test was used to test the null hypothesis as to whether data were normally distributed.

Parasitological study

Five representatives of the genus *Cobitis* were submitted for parasitological examination: two samples from the Sava river, and one each from the Kupa, Zrmanja and Drava rivers. Detailed parasitological examination was carried out immediately after sacrifice using the protocol of Marcogliese (1996). External organs (fins, skin, gills, and eyes) and internal organs (body cavity, heart, liver, spleen, swim bladder, stomach, gall and urinary bladders, gonads, digestive tract and contents) were examined first by dissecting microscope and subsequently with a compound microscope.

Specimens from each sample were fixed in 70% ethyl alcohol for parasitological examination and in 5% buffered formalin for histological examination. Formalin-fixed tissues were

subjected to standard histological procedures (True, 2001) and 6 μm thick sections were placed on glass slides, stained by haematoxylin-eosin, covered with cover slips and examined by light microscopy. The determination of parasites was performed according to Bauer (1984), Lom and Dykova (1991), Moravec et al. (1991) and Bruno et al. (2006).

Results

Cobitis elongatoides specimens were collected at Sites 1 and 2 on the Sava river as well as on the Drava river (Site 4), while *C. elongata* specimens were sampled on the Kupa river (Site 3). From the Adriatic watershed only the Zrmanja river (Site 5) was included in the study, where a sample of *C. bilineata* was taken (Table 1). Total and standard lengths as well as weights of samples at Sites 1 and 2 collected in the Sava river and Site 4 from the Drava river were normally distributed ($P > 0.05$), while weights of samples from Site 3 in the Kupa river and Site 5 in the Zrmanja river were not ($P < 0.05$).

A total of 51 fish were examined; almost all were parasitized (Table 2). At sampling Site 1, 10 of 13 fish (77%) were parasitized by the ciliates *Trichodina nigra* and *Chilodonella cyprini* and the dinoflagellate *Piscioodinium pillulare*. *T. nigra* was recorded in nine of 13 (69%) fish, while *C. cyprini* was recorded in five of 13 (38%). *P. pillulare* was present in three of 13 (23%) fish. A mixed infection by *T. nigra* and *C. cyprini* was recorded in five of 13 (31%), while *T. nigra* and *P. pillulare* were recorded in two of 13 (15%) examined fish. The Sava river (Site 2) sample consisted of 11 fish; 10 (91%) were parasitized either by the ciliate *T. nigra* or *C. cyprini*. The former was recorded in 10 of 11 (91%) fish, whereby two (18%) were also infected by *C. cyprini*. Histological examination of muscle tissue from a single specimen sampled at Site 2 showed lesions whereby various sections revealed multiple, large, basophilic cysts containing myriad dormant merozoites morphologically compatible with *Sarcocystis* sp. (Fig. 2). Adjacent muscle fibres were compressed, with individual fibres undergoing degeneration; however, no significant inflammatory or fibrosis tissue response was noted. The *Cobitis elongata* sample from the Kupa river (Site 3) consisted of 11 fish, nine (82%) infected by varying developmental stages of *Ichthyophthyrus multifiliis*. On the Drava river (Site 4), seven specimens of *C. elongatoides* were collected; in one (14%) fish a plerocercoid of the tapeworm *Ligula colymbi* was found in the body cavity, and four (57%) fish were infected by the ciliate

Table 1

Cobitis species sampled at five sites in four rivers in Croatia; total (TL) and standard length (SL) and weight (w) with mean values, standard error (SE) and minimum–maximum value for each parameter

River sampling site	Species	n	TL (mm) x \pm SE (range)	SL (mm) x \pm SE (range)	w (g) x \pm SE (range)
1	<i>C. elongatoides</i>	13	88.10 \pm 3.56 (69.4–111.4)	78.2 \pm 3.0 (61.1–97.1)	3.1 \pm 0.44 (1.2–6.3)
Sava					
2	<i>C. elongatoides</i>	11	70.1 \pm 3.88 (45.4–84.5)	61.3 \pm 3.61 (39.7–74.3)	1.9 \pm 0.3 (0.5–3.3)
Sava					
3	<i>C. elongata</i>	11	113.6 \pm 4.55 (95.6–137.7)	98.9 \pm 4.06 (84.7–122.1)	5.0 (3.1–9.6)
Kupa					
4	<i>C. elongatoides</i>	7	66.6 \pm 3.54 (53.5–77.3)	57.3 \pm 3.08 (45.8–66.5)	1.7 \pm 0.29 (1.0–2.9)
Drava					
5	<i>C. bilineata</i>	9	57.7 \pm 3.52 (48.6–76.9)	49.0 \pm 3.12 (40.7–67.4)	0.82 (0.5–2.1)
Zrmanja					

Table 2

Overall prevalence of parasites in different *Cobitis* species sampled from four river systems in Croatia (see Fig. 1)

Parasite	<i>C. elongatooides</i> (n = 31)	<i>C. elongata</i> (n = 11)	<i>C. bilineata</i> (n = 9)
<i>Piscioodinium pillulare</i>	6.45%	–	–
<i>Trichodina nigra</i>	61.29%	–	–
<i>Chilodonella cyprini</i>	22.58%	–	–
<i>Ichthyophthyrus multifiliis</i>	–	81.81%	–
<i>Epistylis</i> sp.	12.90%	–	–
<i>Gyrodactylus cobitis</i>	–	–	22.22%
<i>Ligula colymbi</i>	3.22%	–	–

n = number of specimen studied.

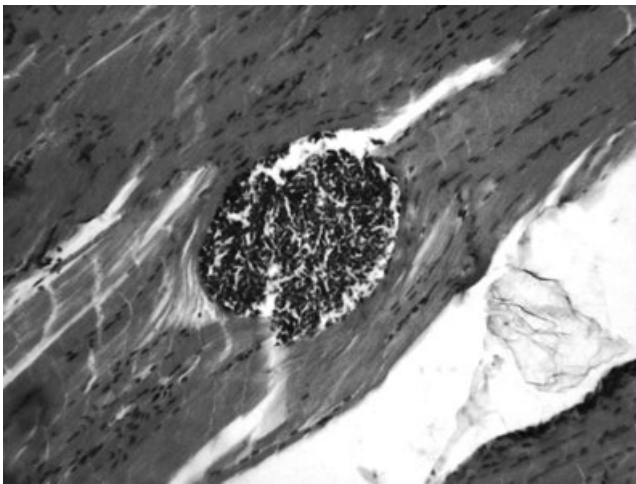


Fig. 2. *Sarcocystis* sp. in muscle of *C. elongatooides* from Sava river. Stage comprised of large tissue cysts containing thousands of dormant merozoites

Epistylis. At Site 5 on the Zrmanja river, nine *Cobitis bilineata* were examined; the monogenean *Gyrodactylus cobitis* was recorded on the skin of two fish (22%). Although the gills as well as internal organs from all sampled fish were examined histologically, most tissues were within normal limits. In some specimens the fusion of lamellae, epithelial cells hyperplasia with the proliferation of adjacent connective tissues was noted.

Discussion

Parasites affect almost every conceivable level of biological organisms; generally, the importance of these abundant, species-rich and wide-spread life forms is not well known (Lindstrom et al., 1994). Recent research has also revealed that the species richness of *Cobitis* characterized by geographic diversity has established scientific interest in all aspects of their biology. Some of the species in this genus are considered as vulnerable to endangered, according to IUCN (1990) methodology. The question then arises as to what extent does the presence of parasites influence the vulnerability or abundance of a particular species?

The ectoparasites recorded in our study did not reveal pathological changes caused by their presence. As to the literature data, most papers deal with the description of endoparasitic helminths of different *Cobitis* species (Bauer,

1984; Moravec, 2001; Popiolek and Kotusz, 2004); monogenean *G. cobitis* was listed in reviews of Bychovskij (1962) and Moravec (2001). The different ciliates recorded in our specimens were either on the skin or the gills, similar to the finding of trichodinid from the gills of *Cobitis taenia* in the Rybinsk Reservoir, USSR (Arthur and Lom, 1984) or the list of parasites recorded in *Cobitidae* that includes *Trichodina nigra* (Bychovskij, 1962). To date, there are no records of digenean in representatives of the *Cobitis* species in Croatia, however, future research should comprise more regular sampling, with higher numbers of particular species.

It is known that cyprinid fish are secondary intermediate hosts for *L. intestinalis*, the occurrence of which in the host body cavity inhibits gonadal development and prevents reproduction (Carter et al., 2005). The finding of the plerocercoid of *L. colymbi* in *C. elongatooides* in the Czech Republic (Halačka et al., 2000) indicates that discovery of this parasite in *C. elongatooides* from the Drava river in Croatia is not an isolated case. A single finding of this parasite in *C. elongatooides* from the Drava river suggests the necessity of further research to determine its prevalence among the *Cobitis* population and the possible influence on gonadal development. *Sarcocystis* in the muscle of *C. elongatooides* from the Sava river was, until now, unknown; its prevalence and possible pathological impact on the host remains to be clarified. The lesions described in the histopathological examination of gills are non-specific and the possibility of parasitic etiology should be further investigated.

It should be evaluated as to whether the parasitic load can additionally endanger the Croatian species of the genus *Cobitis*, as it is known that predators tend to catch prey infected with parasites more often than expected. Lafferty and Morris (1996) proved that herons prey substantially, up to 30 times more, on fish infected with digeneans than on uninfected fish.

The abundance of parasites may influence the number of predators a community can support (Skorping and Hogstedt, 2001). Therefore, parasite-induced trophic transmission deserves greater attention; future research should focus on the abundance of certain parasites and their possible cascade effect with regard to ecologists.

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