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Larval trematodes (Digenea) in molluscs from small water bodies near České Budějovice, Czech Republic

Anna Faltýnková

Faculty of Biological Sciences, University of South Bohemia; Institute of Parasitology, Academy of Sciences of the Czech Republic, Branišovská 31, 370 05 České Budějovice, Czech Republic

Abstract

A total of 2802 molluscs from two fishponds and one swamp in the vicinity of České Budějovice, South Bohemia, were examined between 1998 and 2000. A total of 12 species of molluscs were infected (33.9%, n = 951) with larval trematodes of 28 species (26 species were represented by cercariae, 8 species by metacercariae; 6 species were found both as cercariae and metacercariae). The dominant species found as cercariae were *Tylodelphys excavata* (Diplostomidae), *Plagiorchis elegans* (Plagiorchiidae), *Echinostoma spiniferum* sensu Našincová (1992) and *E. revolutum* (Echinostomatidae). Three cases of double infections with cercariae were found; metacercariae often occurred together in one snail (up to five species). The most infected hosts were *Planorbarius corneus* (78.2%, n = 662), *Lymnaea corvus* (65.5%, n = 55) and *Lymnaea stagnalis* (47.1%, n = 473). Most of the trematode species reported mature in birds and are widely distributed in other parts of Europe. The common species found as cercariae are illustrated.

Key words

Mollusca, Planorbidae, Lymnaeidae, Trematoda, cercariae, metacercariae, Czech Republic

Introduction

Trematodes have complicated life cycles in which molluscs, being used almost obligatorily as first intermediate hosts, play the key role (Esch *et al*. 2001). Their examination makes possible to gain information about the sources of infection of the second and definitive hosts (Vojtek 1962).

Several studies of larval trematodes have been carried out in South Bohemia, which is a region with many systems of fishponds with a long tradition. However, the data are restricted mainly to the 1960's, when Zajíček (1963), Zajíček and Valenta (1964) and Žďárská (1963, 1964) for the first time surveyed the occurrence of cercariae in aquatic molluscs. In the 1980's, Našincová concentrated on surveys of cercariae from omitted regions in Bohemia (Našincová 1986, 1991; Našincová *et al*. 1989, 1993; Našincová and Bušta 1991) and reported 64 species found as cercariae and 27 species found as metacercariae from the Czech Republic (Našincová 1992).

Recently, bird schistosomes (*Trichobilharzia*, *Bilharziella*), some of them causing cercarial dermatitis, were reported also from South Bohemia (Kolářová *et al*. 1992, 1997; Kolářová and Horák 1996; Horák *et al*. 1998, 2002).

The present study provides data on the occurrence of larval trematodes in two small fishponds and one swamp near České Budějovice, South Bohemia, which is the first step in providing new data on the recent distribution of larval trematodes in the Czech Republic. Although it is a survey, drawings of the common species are added for better orientation of the reader and as material for comparison with other literature since the systematic status of some species is often confused. Drawings of metacercariae are omitted since the cysts provide few characteristics that are helpful in determination.

Materials and methods

Study site

Samples of molluscs were taken from two fishponds and one swamp 7 km eastwards from České Budějovice (49°00´N, 14°29´E). The two fishponds are small water bodies of rather eutrophic still water with rich aquatic vegetation.

Kořenský pond (1.6 ha) is surrounded by a small forest from the east. It is swampy and overgrown with vegetation (*Phragmites australis*, *Lemna minor*). The bottom is muddy;

Corresponding address: faltyn@bf.jcu.cz

the maximum depth is 1.3 m; it reaches a maximum of 0.5 m at the shores. In 2000, there were two piles of corn at the shore where the snails fed on and accumulated.

The fishpond Motovidlo is larger (6 ha), deeper (2.5 m), with less aquatic vegetation and less abundant mollusc population than in the Kořenský pond. The shores are steep and rocky; at the northern side is a small forest. The surface is covered with *Utricularia* sp. and *Lemna minor* at two sides near the shore; at the other sides, *Phragmites australis* is dominant.

The swamp Hluchá Bašta (0.3 ha) is surrounded by a meadow; its depth ranges from 30 cm to 1 m. It is overgrown by *Iuncus* sp., *Phragmites australis* and *Lemna minor*. In late summer (August/September), it becomes dry which makes mollusc sampling impossible, therefore, no further collections were made here in 2000.

Sampling and handling of molluscs

In 1998 and 1999, the samples were taken from April to October from all three localities; in 2000, monthly from February to December from Kořenský pond and Motovidlo. A total of 2802 molluscs belonging to 12 species were collected (Table I). The molluscs were collected with a strainer or located visually and hand picked from the shores (at a maximum distance of 1.2 m). In the laboratory, the molluscs were placed into glass containers with tap water (50–500 ml).

Shedding of cercariae was stimulated overnight by light and heat of a lamp. Cercariae were observed under light microscope; neutral red and Nile blue were used for vital staining. Afterwards, the molluscs were dissected to detect other developmental stages. Metacercariae were excysted mechanically, by coverslip pressure; although they are often damaged by this method, the species mentioned below can be reliably determined. Larval stages found were identified according to Našincová (1992); sporocysts and rediae that could not be identified to species level were also taken into account. Mollusc nomenclature was applied after Jackiewicz (2000).

Results

A total of 951 (33.9%) molluscs were infected; 12 species of them were found to harbour developmental stages of 28 species (cercariae of 26 species, metacercariae of 8 species; 6 species were found both as cercariae and metacercariae) (Tables II and III).

In Kořenský pond, 578 (51.2%) molluscs were infected and harboured cercariae of 19 species and metacercariae of 7 species; in Motovidlo, 282 (48.0%) molluscs were infected with cercariae of 23 species and metacercariae of 6 species; in the swamp near Hluchá Bašta, 91 (8.4%) molluscs were infected and harboured cercariae of 3 species and metacercariae of 3 species.

The dominant cercariae were *Tylodelphys excavata*, *Plagiorchis elegans*, *Echinostoma spiniferum* sensu Našincová (1992) and *Echinostoma revolutum* (Figs 1–3). Three double infections of cercariae were found in *Planorbarius corneus* (*Haematoloechus asper* and *T. excavata*; *Rubenstrema exasperatum*/*Neoglyphe locellus* and *Bilharziella polonica*) (Figs 2 and 3) and in *Lymnaea stagnalis* (*Opisthioglyphe ranae* and *Plagiorchis elegans*) (Fig. 2). The highest number of species found simultaneously as metacercariae was in *P. corneus* (5); usually echinostome metacercariae of 3 species (*Moliniella anceps*, *Echinoparyphium aconiatum* and *Echinostoma* spp.) were found together in the kidney of the snails.

The most heavily infected species of molluscs were *P. corneus* (78.2%), *Lymnaea corvus* (65.5%) and *L. stagnalis* (47.1%). The highest number of cercariae was found in *L. stagnalis* (10 species), *P. corneus* (7 species) and *L. corvus* (5

Table I. Survey of molluscs collected and infected with larval trematodes

Mollusc species	Total		Kořenský pond		Motovidlo		Hluchá Bašta	
	collected	infected	collected	infected	collected	infected	collected	infected
Planorbarius corneus (Linnaeus)	662	518	463	385	199	133	–	–
Planorbis planorbis (Linnaeus)	71	22	28	15	43	7	–	–
Segmentina nitida (Müller)	624	59	239	11	1	1	384	47
Bathyomphalus contortus (Linnaeus)	67	2	36	1	–	–	31	1
Gyraulus albus (Müller)	6	1	1	1	5	–	–	–
Lymnaea stagnalis (Linnaeus)	473	223	304	143	169	80	–	–
Radix peregra (Müller)	472	38	3	1	18	–	451	37
Radix auricularia (Linnaeus)	23	6	–	–	23	6	–	–
Lymnaea corvus (Gmelin)	55	36	11	8	36	27	8	1
Stagnicola turricula (Held)	74	21	34	13	34	7	6	1
Physella acuta (Draparnaud)	57	21	–	–	57	21	–	–
Pisidium sp. Pfeiffer	218	4	10	–	–	–	208	4
Total	2802	951	1129	578	585	282	1088	91

Table II. Survey of trematode species found as cercariae, their total prevalence and prevalence of cercariae in each first intermediate host

Cercariae	Total no. infected (%)	Host	[a]Infected (%)	[b]Infected (%)	[c]Infected (%)
Notocotylus ephemera (Nitzsch, 1807)	3 (0.4)	*P. corneus*	2 (0.3)	1 (0.4)	–
Notocotylus attenuatus (Rudolphi, 1809)	2 (0.2)	*L. stagnalis*	1 (0.2)	1 (0.4)	–
Catatropis sp. of Našincová (1992)	13 (1.4)	*S. nitida*	8 (1.4)	–	5 (5.5)
Diplodiscus subclavatus (Pallas, 1760)	2 (0.2)	*P. planorbis*	–	2 (0.7)	–
Echinostoma revolutum (Frölich, 1802)	26 (2.7)	*L. stagnalis*	6 (1.0)	4 (1.4)	–
		R. peregra	–	–	15 (16.5)
		L. corvus	–	–	1 (1.1)
Echinostoma spiniferum (La Valette, 1855) sensu Našincová (1992)	42 (4.4)	*P. corneus*	28 (4.8)	14 (4.9)	–
Echinoparyphium aconiatum Dietz, 1909	24 (2.5)	*L. stagnalis*	2 (0.3)	21 (7.4)	–
		L. corvus	–	1 (0.4)	–
Echinoparyphium recurvatum (Linstow, 1873)	17 (1.8)	*R. peregra*	–	–	17 (18.7)
Echinoparyphium sp. of Zajíček (1963)	12 (1.3)	*L. stagnalis*	1 (0.2)	11 (3.9)	–
Moliniella anceps (Molin, 1859)	8 (0.8)	*L. stagnalis*	–	2 (0.7)	–
		L. corvus	2 (0.3)	1 (0.4)	–
		S. turricula	2 (0.3)	1 (0.4)	–
Plagiorchis elegans (Rudolphi, 1802)	34 (3.6)	*L. stagnalis*	27 (4.7)	7 (2.5)	–
Opisthioglyphe ranae (Frölich, 1791)	5 (0.5)	*L. stagnalis*	3 (0.5)	1 (0.4)	–
		L. corvus	–	1 (0.4)	–
Plagiorchis maculosus (Rudolphi, 1802)	3 (0.3)	*L. stagnalis*	–	3 (1.1)	–
Haematoloechus asper (Looss, 1899)	7 (0.7)	*P. corneus*	7 (1.2)	–	–
Haematoloechus sp.	1 (0.1)	*P. planorbis*	–	1 (0.4)	–
Rubenstrema opisthovitellinum Soltys, 1954	11 (1.2)	*P. corneus*	3 (0.5)	8 (2.8)	–
Rubenstrema exasperatum (Rudolphi, 1819)/ *Neoglyphe locellus* (Kossack, 1910)	42 (4.4)	*P. corneus*	23 (3.9)	19 (6.7)	–
Diplostomum pseudospathaceum Niewiadomska, 1984	18 (1.9)	*L. stagnalis*	3 (0.5)	14 (4.9)	–
		R. auricularia	–	1 (0.4)	–
Diplostomum paracaudum (Iles, 1959)	16 (1.7)	*L. corvus*	–	16 (5.7)	–
Posthodiplostomum cuticola (Nordmann, 1832)	2 (0.2)	*P. planorbis*	1 (0.2)	1 (0.4)	–
Tylodelphys excavata (Rudolphi, 1803)	64 (6.7)	*P. corneus*	48 (8.3)	16 (5.7)	–
Tylodelphys clavata (Nordmann, 1832)	1 (0.1)	*R. auricularia*	–	1 (0.4)	–
Parastrigea robusta Szidat, 1928	2 (0.2)	*S. nitida*	1 (0.2)	1 (0.4)	–
Trichobilharzia szidati Neuhaus, 1952	2 (0.2)	*L. stagnalis*	1 (0.2)	1 (0.4)	–
Trichobilharzia franki Müller et Kimmig, 1994	2 (0.2)	*R. auricularia*	–	2 (0.7)	–
Bilharziella polonica (Kowalewski, 1895)	4 (0.4)	*P. corneus*	1 (0.2)	3 (1.1)	–
Rediae – echinostomous	8 (0.8)	*L. stagnalis*	3 (0.5)	3 (1.1)	–
		P. corneus	2 (0.3)	–	–
Sporocysts – xiphidiocercariae	20 (2.1)	*L. stagnalis*	11 (1.9)	1 (0.4)	–
		P. corneus	5 (0.9)	1 (0.4)	–
		L. corvus	–	1 (0.4)	–
		P. planorbis	1 (0.2)	–	–
Sporocysts (undetermined)	3 (0.3)	*L. stagnalis*	–	1 (0.4)	–
		S. nitida	2 (0.3)	–	–
Number of species	26		19	22	3

[a]Kořenský pond, [b]Motovidlo, [c]Hluchá Bašta.

species). The highest number of metacercariae was found in *L. stagnalis* (7 species), *P. planorbis* (6 species) and *P. corneus* (5 species).

Discussion

In relatively small fishponds, a diverse fauna of cercariae and metacercariae was found in a period of three years (1998–2000); from 64 species found as cercariae by Našincová (1992) 26 were found in the present study; Vojtková (1988) and Vojtek (1989) reported a total of 113 trematode species known as cercariae in the former Czechoslovakia, however, they included species with provisorical names and only 53 were determined to species level. Metacercariae of 27 species were reported by Našincová (1992) from the Czech Republic; in the present study 8 species were found.

Table III. Survey of trematode species found as metacercariae, their total prevalence and prevalence for each second intermediate host

Metacercariae	Total no. infected (%)	Host	Kořenský pond infected (%)	Motovidlo infected (%)	Hluchá Bašta infected (%)
Echinostoma spp.*	199 (20.9)	*L. stagnalis*	57 (9.9)	1 (0.4)	–
		L. corvus	4 (0.7)	3 (1.1)	–
		S. turricula	5 (0.9)	4 (1.4)	–
		R. peregra	1 (0.2)	–	5 (5.5)
		R. auricularia	–	2 (0.7)	–
		P. corneus	48 (8.3)	5 (1.8)	–
		P. planorbis	5 (0.9)	2 (0.7)	–
		B. contortus	1 (0.2)	–	1 (1.1)
		S. nitida	–	–	42 (46.2)
		G. albus	1 (0.2)	–	–
		P. acuta	–	12 (4.3)	–
Echinoparyphium aconiatum	490 (51.5)	*L. stagnalis*	30 (5.2)	32 (11.3)	–
		L. corvus	1 (0.2)	14 (4.9)	–
		S. turricula	3 (0.5)	–	1 (1.1)
		R. peregra	1 (0.2)	–	–
		R. auricularia	–	2 (0.7)	–
		P. corneus	335 (57.9)	54 (19.1)	–
		P. planorbis	8 (1.4)	2 (0.7)	–
		P. acuta	–	7 (2.5)	–
Echinoparyphium recurvatum	21 (2.2)	*L. stagnalis*	1 (0.2)	–	–
		R. peregra	–	–	16 (20.9)
		Pisidium sp.	–	–	4 (4.4)
Moliniella anceps	261 (27.4)	*L. stagnalis*	40 (6.9)	7 (2.5)	–
		L. corvus	5 (0.9)	7 (2.5)	–
		S. turricula	6 (1.0)	4 (1.4)	–
		P. corneus	171 (29.6)	19 (6.7)	–
		P. planorbis	1 (0.2)	1 (0.4)	–
Neoglyphe locellus	368 (38.7)	*L. stagnalis*	46 (7.9)	5 (1.8)	–
		L. corvus	2 (0.3)	3 (1.1)	–
		S. turricula	5 (0.9)	–	–
		P. corneus	230 (39.8)	74 (26.2)	–
		P. planorbis	3 (0.5)	–	–
Opisthioglyphe ranae	4 (0.4)	*P. acuta*	–	4 (1.4)	–
Strigeida gen. sp.	3 (0.3)	*L. stagnalis*	1 (0.2)	–	–
		P. planorbis	1 (0.2)	–	–
		S. nitida	1 (0.2)	–	–
Metacercaria gen. sp.	18 (1.9)	*L. stagnalis*	2 (0.3)	4 (1.4)	–
		L. corvus	1 (0.2)	–	–
		P. corneus	8 (1.4)	1 (0.4)	–
		P. planorbis	1 (0.2)	–	–
		P. acuta	–	1 (0.4)	–
Number of species	8		7	6	3

**Echinostoma revolutum*, *E. spiniferum.*

The species represented by cercariae and metacercariae in the present report are common in the Czech Republic and were reported by most of the authors mentioned above. The species found, occur frequently in Europe. It may be due to a diverse spectrum of migrating birds, visiting annually the systems of fishponds in South Bohemia (Šťastný *et al*. 1996). The bird hosts serve as the main source of infection for the snails. In this study, from the 26 species found as cercariae, 20 species are known to use birds as definitive hosts.

However, cercariae of the family Strigeidae, which is a common group of trematodes, were not found except for *Parastrigea robusta*, although suitable species of intermediate and definitive (birds) hosts were present. In South Bohemia Žďárská (1963) found four species with *Cotylurus cornutus* being the most prevalent (2–30%), Zajíček and Valenta (1964) found five species with *Cotylurus erraticus* and *Apatemon gracilis* occurring most frequently, and Našincová (1992) found 7 species. One explanation for the absence of these species in the present study could be that the number of observed species increases with the time of observation as stated by Niewiadomska *et al*. (1997), and the time of study probably could be too short to find rare species. However, the authors mentioned above reported prevalences indicating that some of the species were common. The absence of this group

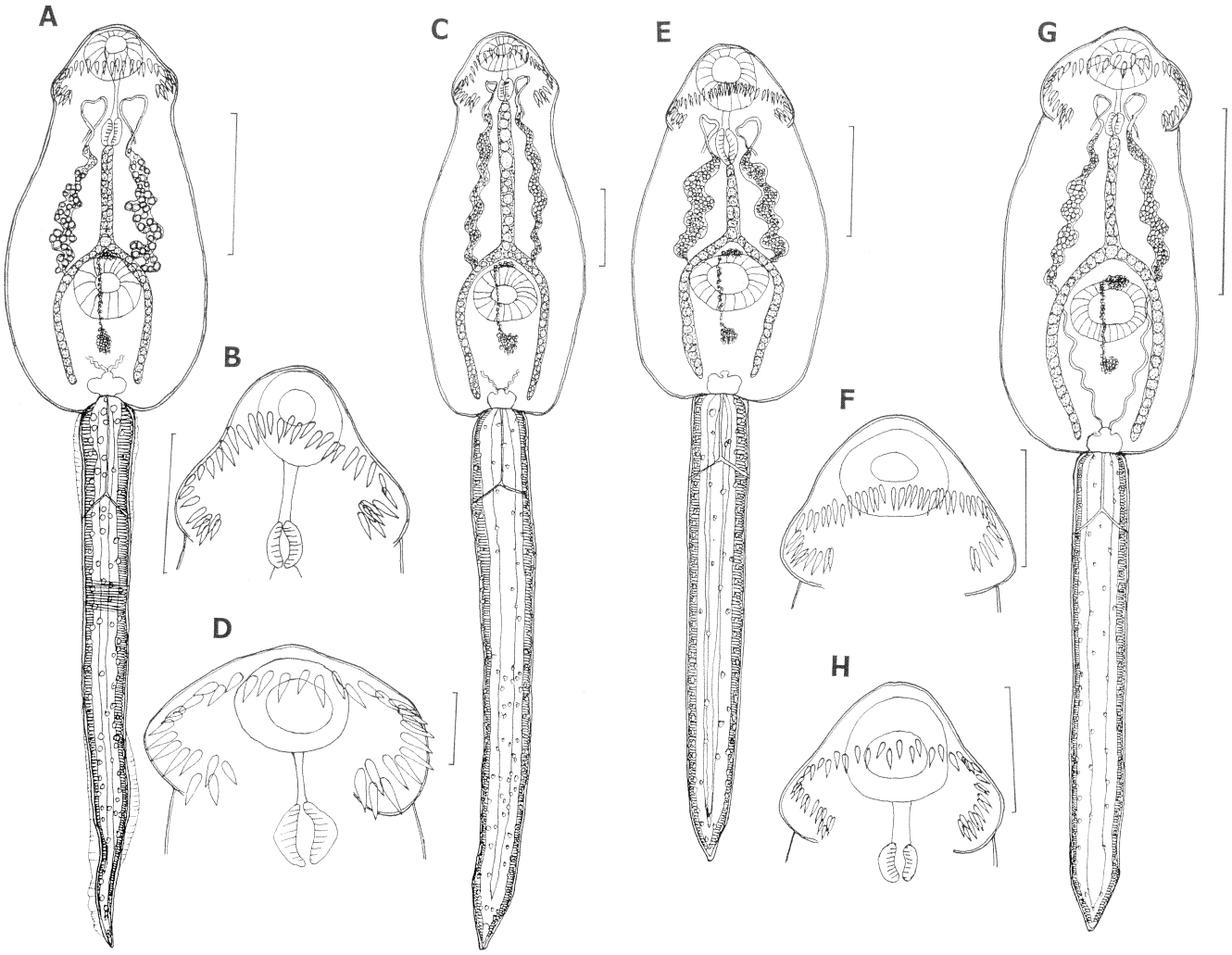

Fig. 1. *Echinostoma spiniferum* sensu Našincová (1992): **A** – cercaria, **B** – collar spines; *Echinoparyphium aconiatum*: **C** – cercaria, **D** – collar spines; *Echinoparyphium* sp.: **E** – cercaria, **F** – collar spines; *Echinoparyphium recurvatum*: **G** – cercaria, **H** – collar spines. Scale bars = 100 µm (A, C, E, G), 50 µm (B, D, F, H)

could correspond to changes in the abundance of intermediate and definitive hosts; fishes and leeches serve as second intermediate hosts and especially the latter could be affected by environmental changes which could affect the life cycle completion.

From the species found in the present study as most prevalent *Tylodelphys excavata* was not found by Našincová (1992) that often and with a low prevalence (0.8%) more often *T. clavata* was found by Zajíček (1963), Žďárská (1963) and Našincová (1992); *Diplostomum pseudospathaceum* still remains abundant, Zajíček (1963) found it frequently in South Bohemia and also Žďárská (1963) and Našincová (1992) report it with a high prevalence (35%). From the plagiorchiids *Plagiorchis elegans* was found frequently also by Zajíček (1963), Žďárská (1963) and Našincová (1992), also *Opisthioglyphe ranae* was found frequently by the authors mentioned (with prevalences up to 32%). *Echinostoma revolutum* from lymnaeid snails was reported by Zajíček (1963) only from one locality in South Bohemia, Žďárská (1963) found it more frequently with higher prevalences (up to 32%), Našincová (1992) reported lower prevalences (4%) but she found more often *E. spiniferum* (up to 16%) from *Planorbarius corneus*.

The overall prevalences of infection in the molluscs from the two fishponds were relatively high (48.1% for Motovidlo and 51.2% for Kořenský pond). This is in accordance with the findings of other authors (Žďárská 1962, Fashui 1981, Crews and Esch 1986) who observed the highest prevalences (up to 80%) in smaller ponds and pools with shallow, still water overgrown by rich aquatic vegetation. The low infection rate (8.4%) in the swamp Hluchá Bašta may be given by the depauperate fauna of molluscs that contained few small species of molluscs usually harbouring a low number of cercariae of different species while the most suitable species such as *L. stagnalis* and *P. corneus* were missing. The small size of the

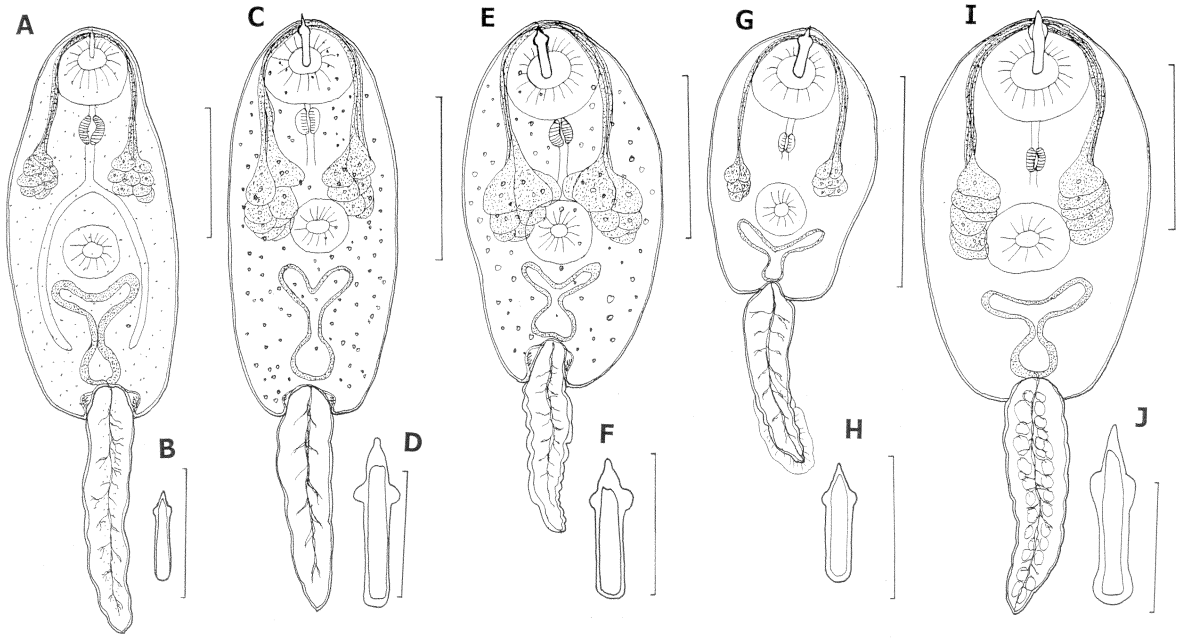

Fig. 2. *Opisthioglyphe ranae*: **A** – cercaria, **B** – stylet; *Plagiorchis elegans*: **C** – cercaria, **D** – stylet; *Plagiorchis maculosus*: **E** – cercaria, **F** – stylet; *Haematoloechus asper*: **G** – cercaria, **H** – stylet; *Rubenstrema exasperatum*/*Neoglyphe locellus*: **I** – cercaria, **J** – stylet. Scale bars = 100 µm (A, C, E, G, I), 50 µm (B), 30 µm (F), 25 µm (D, H, J)

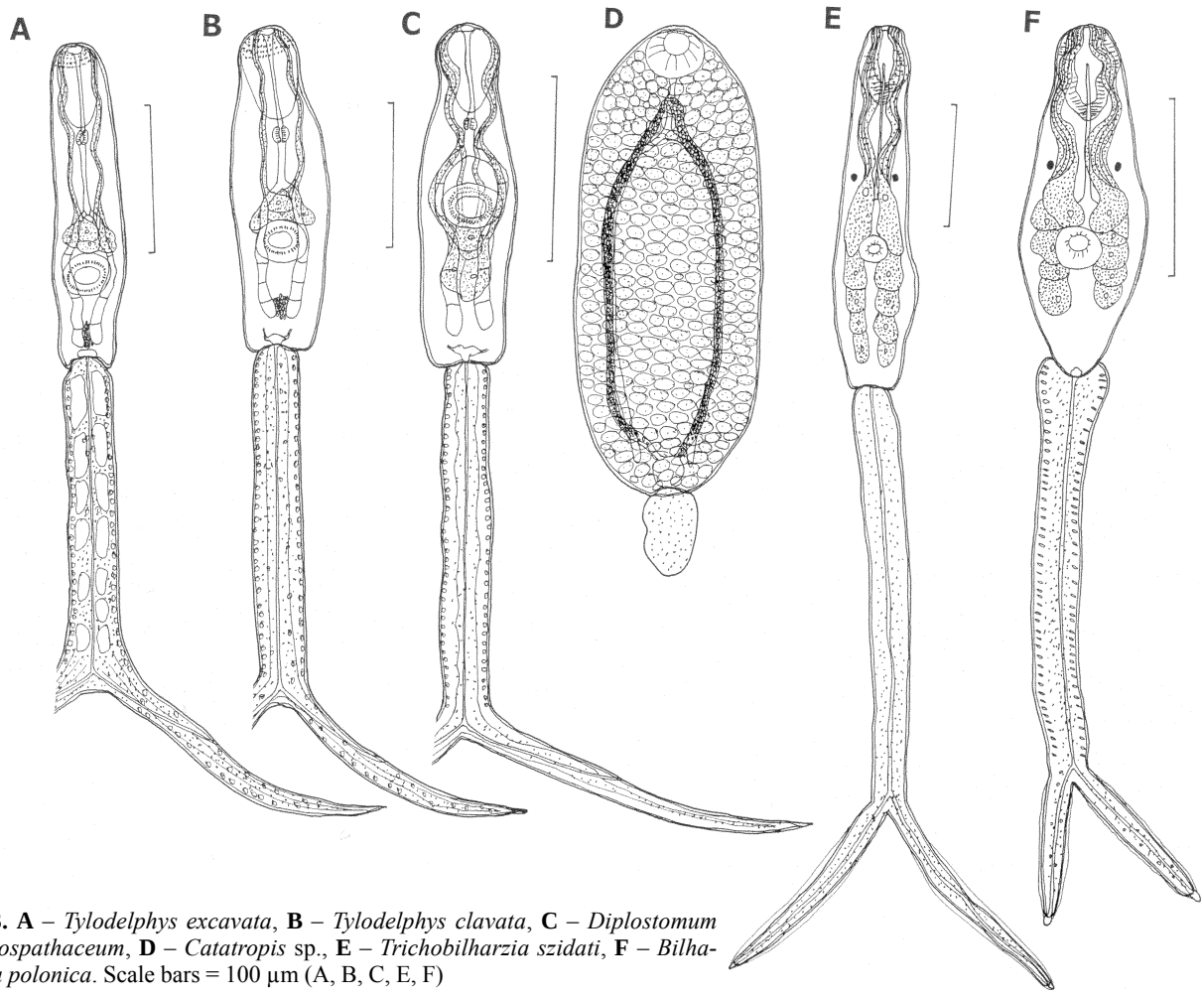

Fig. 3. A – *Tylodelphys excavata*, **B** – *Tylodelphys clavata*, **C** – *Diplostomum pseudospathaceum*, **D** – *Catatropis* sp., **E** – *Trichobilharzia szidati*, **F** – *Bilharziella polonica*. Scale bars = 100 µm (A, B, C, E, F)

swamp, that provides less space for definitive hosts releasing eggs may be also of importance. Another fact is that there was a long dry period when molluscs could not be found, which probably, together with the cold period in winter, reduced the mollusc populations dramatically. Therefore, the mollusc populations had to establish again every year together with their parasitofauna.

In South Bohemia no prosobranchiate snails occur (Beran 2002), therefore some trematode families (Lecithodendriidae, Psilostomidae, Microphallidae, Opecoelidae and Cyathocotylidae), using snails of this group are missing in the area under study. In the regions of North Bohemia and South Moravia, which have a more diverse mollusc fauna, a larger spectrum of larval trematodes can be found.

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