

Digenean parasites in prosobranch snail *Lithoglyphus naticoides* population with the morphological description of *Echinochasmus* sp. cercaria

Gražina Stanevičiūtė*,

Romualda Petkevičiūtė,

Valerija Kisilienė

*Institute of Ecology,
Vilnius University, Akademijos 2,
LT-08412 Vilnius, Lithuania*

The prosobranch snail *Lithoglyphus naticoides* (C. Pfeiffer, 1928), originated from the Ponto-Caspian region, was collected in the river Nemunas in Lithuania. The diversity of intramolluscan stages of trematodes associated with *L. naticoides* was examined. A total of nine species of trematodes were detected in the population studied – cercariae of *Apophallus muehlingi* (Jägerskiöld, 1899) (dominant species), *Xiphidiocercaria* sp. 1 of Odening (1971), *Xiphidiocercaria* sp. 2 of Odening (1971), *Sanguinicola* sp., *Palaeorchis* sp., *Notocotylus* sp., *Crowcrocaecum skrjabini* (Iwanitzky, 1928) and *Spelotrema* sp. metacercariae. Intramolluscan stages of echinostomatid trematode as endosymbionts of *L. naticoides* were found for the first time. Red-tailed gigantocercous cercaria, according to the main features of morphology – a discernible collar without collar spines, two subequal suckers, excretory ducts with 8–10 large composites (from 2–3 granules) and 3–4 small irregular concretions, a double excretory vesicle and 16 flame cells were identified as echinochasmid and attributed to the genus *Echinochasmus* Dietz, 1909.

Key words: *Lithoglyphus naticoides*, endosymbionts, cercaria, *Echinochasmus*

INTRODUCTION

The prosobranch snail *Lithoglyphus naticoides* (C. Pfeiffer, 1928) originates from the Ponto-Caspian region. It is considered to be a species of gently flowing waters or water-meadows on silty ground (Šivickis, 1960; Gittenberg et al., 1998). The dispersal of this snail was thought to be a consequence of canal construction in the previous centuries (Bij de Vaate et al., 2002). As part of the food web and as an intermediate host for trematodes, snails exert an impact on freshwater ecosystems. The diversity, distribution or pathology caused by endosymbionts of *L. naticoides* has not been studied sufficiently. Odening (1971) examined intramolluscan stages of trematodes from *L. naticoides* in the vicinity of Berlin (Germany); eight species of trematodes were detected in the population studied. Черногоренко (1983) found six species of larval trematodes in snails inhabiting the river Dniepr, Ukraine. Studies of parasite-invaders of the Volga River basin revealed that the spread of some digenean species (*Apophallus muehlingi*, *Rossicotrema donicus* (Heterophyidae) and *Nicola skrjabini* (Opcoelidae)) is associated with migration of their first intermediate hosts *Lithoglyphus* spp. (Жохов, Пугачева, 2001).

While studying the larval trematodes associated with *L. naticoides* inhabiting the water reservoir of the dammed up Nemunas

river near Kaunas in Central Lithuania, snails were collected that shed a red-tailed gigantocercous cercaria. The cercaria, according to the main features of morphology, was identified as echinochasmid and attributed to the genus *Echinochasmus* Dietz, 1909. The intermediate hosts of *Echinochasmus* spp. cercariae are mainly freshwater prosobranch gastropods (Карманова, 1975; Kostadinova, 2005). The life cycles of less than half from approximately 50 known species of the genus *Echinochasmus* have been studied till now (Карманова, 1975; Dhanumkumari et al., 1991; Fried, 2001). These investigations have indicated that cercariae of the genus *Echinochasmus* form a compact group morphologically distinct from other echinostomatid cercariae (Карманова, 1975; Grabda-Kazubska et al., 1991). The main diagnostic characters of the species *Echinochasmus* cercaria are body proportions and the number of large granules in excretory ducts; collar-spines are absent in the morphology of cercaria. A wide range of fish-eating birds and mammals are known as final hosts of *Echinochasmus* spp. (Карманова, 1975; Kostadinova, Gibson, 2000). The fact that echinochasmid trematodes can infect man orally via the cercarial stage in contaminated water is of considerable interest to the medical community (Taylor et al., 2001).

The purpose of the present paper was to present a list of intramolluscan stages of trematodes infecting the Lithuanian population of *L. naticoides* and to provide a comprehensive

* Corresponding author. E-mail: grasta@ekoi.lt

taxonomic description through light microscopy of redia and cercaria of *Echinochasmus* sp., previously not recorded in other populations of *L. naticoides* snail.

MATERIALS AND METHODS

A total of 3665 specimens of *L. naticoides* were collected in the water reservoir of the dammed up river Nemunas near Kaunas in Central Lithuania (coordinates of sampling point: N 54°51.383', E 24°09.077'). Pooled cercariae emitted by naturally infected snails, and almost fully developed cercariae and other intramolluscan stages from crushed snails comprised the study material. Cercariae were observed under a light microscope. The larval stages found were identified according to Odening (1971), Стенько (1976), Черногоренко (1983).

The morphology of *Echinochasmus* sp. cercaria was studied from living specimens emerging from snails. The cercariae, immersed in egg albumin or 2% methylcellulose in a 0.5% NaCl solution, were observed under oil immersion. Measurements (μm) were taken from 10 heat-killed specimens placed in a drop of water, and the means were calculated. Their internal morphology was documented by drawings made with the help of a drawing tube and by microphotograph made with a Carl Zeiss Jena microscope.

RESULTS

In the present study, cercariae of eight species (representatives of six families – Heterophyidae, Sanguinicolidae, Monorchidae, Opecoeloidae, Notocotylidae and Echinostomidae and one of the superfamily Lecithodendrioidea) and metacercariae of one species (family Microphalloidae) were found in 1072 (29.23%) snails *L. naticoides* (Table). Quantitatively, the range of infestation of fluke's larvae in these snails was wide. The dominant cercariae were *Apophallus muehlingi* (Jägerskiöld, 1899) (688 hosts infected), *Xiphidiocercaria* sp. 1 of Odening (1971) (142), *Sanguinicola* sp. (103) and metacercariae of *Spelotrema* sp. (90). A small number of snails were infected with *Palaeorchis* sp. (22), *Notocotylus* sp. (8), *Crowcrocaecum skrjabini* (Iwanitzky, 1928) (2) and *Xiphidiocercaria* sp. 2 of Odening (1971) (2). Six specimens of *L. naticoides* (0.16% of the molluscs) were infected with *Echinochasmus* sp. parthenites. It is the first mention of the intramolluscan stages of *Echinochasmus* sp. from *L. naticoides*. Double infections of cercariae were found in six *L. naticoides* (*A. muehlingi* and *Sanguinicola* sp., *A. muehlingi* and *C. skrjabini*, *Xiphidiocercaria* sp. 1 and *Spelotrema* sp., *Palaeorchis* sp.

and *Xiphidiocercaria* sp. 1, *Sanguinicola* sp. and *Spelotrema* sp., *Xiphidiocercaria* sp. 1 and *Sanguinicola* sp.).

The morphology of *Echinochasmus* sp. redia and cercaria

The cylindrical rediae (Fig. 1a) were embedded in hepatopancreas of snails. Body elongate $840 \times 280 \mu\text{m}$. Apophysis of redia visible. Pharynx 80×80 , caeca 440×84 , filled with orange granular material. Contained cercariae in various stages of development; no mature cercariae with a large tail were observed in rediae.

Cercarial body elliptical, $240\text{--}280 \mu\text{m}$ in length and $136\text{--}144 \mu\text{m}$ in width. Tail very large, 5–6 times longer than body – $1120\text{--}1360 \times 132 \mu\text{m}$, reddish and opaque (Fig. 1b); contracted tail much smaller (Fig. 1c), highly wrinkled. Body covered with thick tegument armed with occasional spines. Collar discernible, but collar spines not developed. Oral sucker subterminal, $40\text{--}44 \times 44\text{--}42$, with two pairs of openings of penetration glands. Ventral sucker $40\text{--}45 \times 42\text{--}44$ in diameter, situated in posterior third of body (Figs. 1d, 2). Borders of both suckers deeply wrinkled. Large cystogenous glands containing rod-like or laminar secretion lie on both sides of pharynx in two parallel groups extending from pharynx to posterior border of ventral sucker. Ducts of three pairs of penetration glands open on anterior border of oral sucker. Alimentary tract not completely developed, consists of bulbus prepharynx, pyriform pharynx of $17 \times 14\text{--}17 \mu\text{m}$ and relatively long oesophagus bifurcating just anterior to ventral sucker. Oesophagus filled with 4–5 large cells. Caeca very short, hardly visible, except for anterior region. Excretory system composed of transversally elongate vesicle, which gives rise at opposite corners to two dilate ducts containing 8–10 large ($45 \times 55 \mu\text{m}$) composite of 2–3 granules and 3–4 small irregular concretions; at the level of bulbus prepharynx these ducts become narrow and turn back; they bifurcate at the level of ventral sucker into anterior and posterior ducts, each leading to four flame cells. The flame cells formula $2 [(1 + 1 + 1 + 1) + (1 + 1 + 1 + 1)] = 16$. Excretory vesicles dilate bipartite with one part in body and one in tail. The caudal excretory duct, with dilated anterior part forming vesicle-like opens dorsally and ventrally in the first fifth of tail. Genital primordium composed of little differentiated aggregation of germinal cells, situated at anterior and posterior margins of ventral sucker.

DISCUSSION

Invasive prosobranch snail *Litoglyphus naticoides* as the first intermediate host of some digenean species is assumed to be im-

Table. Prevalence of digenean species in population of *Litoglyphus naticoides* from Lithuania. Data are presented from 3665 snails sampled

Digenean species	Number of infected mollusks	Prevalence (%)
<i>Apophallus muehlingi</i> (Jägerskiöld, 1899) (Heterophyidae)	688	18.77
<i>Sanguinicola</i> sp. (Sanguinicolidae)	103	2.81
<i>Xiphidiocercaria</i> sp. 1 (Odening, 1971) (Lecithodendrioidea)	142	3.87
<i>Xiphidiocercaria</i> sp. 2 (Odening, 1971) (Lecithodendrioidea)	2	0.05
<i>Palaeorchis</i> sp. (Monorchidae)	22	0.60
<i>Notocotylus</i> sp. (Notocotylidae)	8	0.22
<i>Echinochasmus</i> sp. (Echinostomidae)	6	0.16
<i>Crowcrocaecum skrjabini</i> (Iwanitzky, 1928) (Opecoeloidae)	2	0.05
<i>Spelotrema</i> sp. (metacercaria) (Microphalloidae)	90	2.70
Total:	1072	29.23



Fig. 1. Morphology of redia and cercaria *Echinochasmus* sp.: a – redia of *Echinochasmus* sp. from naturally infected *Lithoglyphus naticoides*, b–d – fully developed cercaria of *Echinochasmus* sp. with contracted body (b) and contracted tail (c), cercarial body (d). Scale bars: a – 0.1 mm, b – 0.2 mm; c – 0.1 mm; d – 0.05 mm

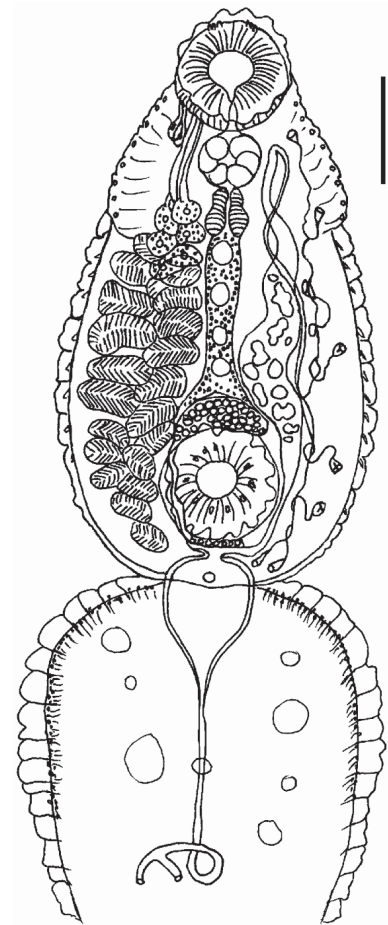


Fig. 2. Cercaria of *Echinochasmus* sp. drawn from living slightly flattened specimens. Scale bar: 0.05 mm

portant in commercial fishery because of the disease it causes among fish (Жохов, Пугачева, 2001). Digenea *A. muehlingi* is known as an agent of “blackspot disease” (Курочкин, Бисерова, 1996). Emission of cercaria *A. muehlingi* and the following transformation into a metacercaria – invasion of free living larvae into the tissues of the second intermediate host – cyprinid fish – is especially lethal to young fish. *Apophallus muehlingi* is associated only with the distribution of the molluscs *L. naticoides* (Šimkova et al., 2003). Adult helminths *A. muehlingi* parasitize in the intestine of several fish-eating animals – birds and mammals.

In the previous studies, exactly *A. muehlingi* was the most abundant species in the various populations of *L. naticoides*, as well as in the Lithuanian population. A survey of larval trematodes from snails in the vicinity of Berlin (Germany) was conducted by Odening (1971); the estimated prevalence of *L. naticoides* invasion by *A. muehlingi* parthenites was 16.64%. *Apophallus muehlingi* was also most abundant species in various water bodies of the Dniepr river basin (Ukraine) (Черногоренко, 1983). The other registered dominant species were *C. skrjabini* (6.95%), *Sanguinicola* sp. (6.48%), metacercariae of *Spelotrema* sp. (6.40%). Intramolluscan stages of echinostomatid trematodes were never found during studies of *L. naticoides* endosymbionts. (Odening, 1971; Черногоренко, 1983).

The current survey of larval trematodes from snails in Lithuania and detection of new *Echinochasmus* sp. cercaria demonstrate that the diversity and distribution of *L. naticoides* endosymbionts has not been studied sufficiently.

Numerous species have been described in the genus *Echinochasmus*. This genus is one of the species-rich genera in Echinostomatidae (Kostadinova, Gibson, 2000). Cercariae so far recorded for most species in the genera whose biology has been studied are usually of gymnocephalous type and have a fairly short tail equal to or slightly longer than the body (Yamaguti, 1975). The most conspicuous feature of the described cercaria is the presence of a very large tail 5–6 times longer than the body, reddish and opaque. Large-tailed or gigantocercous echinostome cercariae have previously been described (see Yamaguti, 1975). According to Карманова (1975), cercariae of the genus *Echinochasmus* form a compact group morphologically distinct from other echinostomatid cercaria. The main diagnostic traits of *Echinochasmus* cercaria are body proportions and the number of large granules in excretory ducts. In various species, 12–20 flame cells were counted, most frequently 16. The ontogenesis of these species (lacking collar with collar spines in the morphology of cercaria) indicates echinochasmids as a more ancient group than echinostomatids (Судариков, Карманова, 1977).

Cercaria of *Echinochasmus* sp. from *L. naticoides*, described herein, resembles cercariae of some other echinostomatid species: *Stephanoprora denticulata* (Rudolphi, 1802) Odner, 1910, *Schiginella colymbi* (Schigin, 1956) Karmanova, 1974 (= *E. schigini* (Schigin, 1956)), *Mesorchis denticulatus* (Rudolphi, 1802) Dietz, 1909, *E. milvi* Yamaguti, 1939 and *E. macrocaudatus* Ditrich, Scholz & Vargas-Vázquez, 1996 in possessing an extremely large tail (see Nasir, Scorza, 1968; Карманова, 1974a; 1974b; Kõie 1986; Беспрозванных 1989; Ditrich et al. 1996). But it appears that only *M. denticulatus* described by Kõie (1986) from snail *Hydrobia ulvae* from the Danish fjords has a heavily pigmented tail like that of *Echinochasmus* sp. However, cercaria tails as phylogenetic indicators are very misleading (Cable, 1965). According to body dimensions and proportions, our cercaria resembles slightly the cercaria of *E. macrocaudatus* studied by Ditrich et al. (1996) and *M. denticulatus* described by Kõie (1986). Nevertheless, the structure and morphometry provided for previously described echinostomatid cercariae only partially agree with the observations recorded by us. The smaller suckers and pharynx, bulbous prepharynx, the number of granules in excretory ducts distinguish the described cercaria of *Echinochasmus* sp. from *E. macrocaudatus* studied by Ditrich et al. (1996). When compared with *M. denticulatus* cercaria, the following features distinguish the cercaria of *Echinochasmus* sp. described herein: the number of penetration glands (not observed in *M. denticulatus*), the caeca terminated at the level of the posterior rim of the acetabulum, the number of granules in excretory ducts and the excretory duct bifurcated at about two-thirds of the length from the tail (Kõie, 1986).

Echinochasmus sp. cercariae can be considered as rare species of larval trematodes occurring in *L. naticoides*. The trematoda fauna found in the present study cannot be considered complete because the life cycles of most of the detected cercariae and metacercariae are not yet studied and some species are rare. However, it can be stated that the same spectrum of the most common species of cercariae parasitising in European populations of *L. naticoides* still occurs in the Nemunas river population studied therein.

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Gražina Stanevičiūtė, Romualda Petkevičiūtė, Valerija Kisielienė

PRIEKIAŽIAUNIO MOLIUSKO *LITHOGLYPHUS NATICOIDES* POPULIACIJOJE PARAZITUOJANČIOS DIGENETINĖS SIURBIKĖS IR *ECHINOCASMUS* SP. CERKARIJOS MORFOLOGINIS APRAŠYMAS

S a n t r a u k a

Tirta Nemuno upėje surinkto priekiažiaunio moliusko *Lithoglyphus naticoides* (C. Pfeiffer, 1928), kilusio iš Ponto Kaspijos regiono, parazitų faunos įvairovė. Ištirtoje populiacijoje buvo aptiktos devynios trematodų rūšys – cercarijos *Apophallus muehlingi* (Jägerskiöld, 1899) (vyraujanti rūšis), *Xiphidiocercaria* sp. 1 Odening (1971), *Xiphidiocercaria* sp. 2 Odening (1971), *Sanguinicola* sp., *Palaeorchis* sp., *Notocotylus* sp., *Crowcrocaecum skrjabini* (Iwanitzky, 1928) ir *Spelotrema* sp. metacercarijos. Pirmą kartą aptiktas naujas *L. naticoides* endosimbiontas – echinostomatidinių trematodų partenitos. Cercarija su labai didele raudona uodega pagal pagrindinius morfologinius požymius – aiškiai matomą nedrygliuotą adoralinį diską, apylygius siurbtukus, šalinamuosius latakus, pripildytus 8–10 stambių (po 2–3) ir 3–4 smulkių granulių, bei 16 virpamųjų ląstelių, išsidėsčiusių poromis – buvo apibūdinta kaip echinostominė cercarija ir priskirta *Echinochasmus* Dietz, 1909 genčiai.

Raktažodžiai: *Lithoglyphus naticoides*, endosimbiontai, cercarija, *Echinochasmus*