Vestnik Zoologii, **52**(4): 289–294, 2018
DOI 10.2478/vzoo-2018-0030

UDC 595.122:597.38(477./41/.42)

THE FIRST RECORD OF PARTHENITAE AND CERCARIAE OF *PLAGIORCHIS MULTIGLANDULARIS* (TREMATODA, PLAGIORCHIIDAE) IN *LYMNAEA STAGNALIS* IN UKRAINE

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The First Record of Parthenitae and Cercariae of *Plagiorchis multiglandularis* (Trematoda, Plagiorchiidae) in *Lymnaea stagnalis* in Ukraine. Zhytova, E. P. — Parthenitae and cercariae of *Plagiorchis. multiglandularis* Semenov, 1927 are recorded in *Lymnaea stagnalis* (Linnaeus, 1758) for the first time in Ukraine; their morphological characteristics are specified. Diagnostic characters of *P. multiglandularis* parthenitae and cercariae found in Ukrainian Polissia are compared with those from other regions. To confirm the validity of the species, a comparison of the morphometric data of this trematode larvae with the cercariae of *Plagiorchis elegans* (Rudolphi, 1802) Braun, 1902, found in molluscs *L. stagnalis*, *L. ralustris* and *L. corvuses*, was performed. It was determined that *P. multiglandularis* cercariae differ from those of *P. elegans* in size and position of the penetration glands.
Key words: *P. multiglandularis*, *P. elegans*, *L. stagnalis*, *L. palustris, L. corvus*, cercaria, sporocyst, trematode.

Introduction

Trematodes are an inseparable component of ecosystems (Beklemishev, 1970) and as a significant part of its biodiversity (Poulin, 2014) they play an important role in processes occurring in the biosphere (Galaktionov, 2016). Studying species composition of trematodes in aquatic molluscs enables prognostication and timely prevention of the appearance of trematodoses' transmission foci (Zhytova, Korol, 2008). The climate-induced changes in environment may lead to changes in the species composition of trematodes in the region (Zhytova, 2011; Galaktionov, 2016).

The results of the study of *P. multiglandularis* Semenov, 1927 parthenitae and cercariae during the complex research of the trematode species composition in pond snails *L. stagnalis* (Linnaeus, 1758) from the water reservoirs of Ukrainian Polissia are given.

Material and methods

The study was conducted in 2004–2012 and 2016. The molluscs were identified according to Stadnichenko (2004). In total, 6898 specimens of molluscs (*Lymnaea stagnalis*, *L. corvus* Gmelin, 1791, *L. (Stagnicola) palustris* (O. F. Müller, 1774)) were investigated. The morphology of each trematode life cycle stage (sporocysts, rediae, unformed cercariae and mature cercariae leaving the infected mollusc) was studied mainly on living specimens using vital dyes (Ginetsinskaya, 1961; Chernogorenko, 1983). Measurements were made on living specimens of five sporocysts and 22 cercariae of *P. multiglandularis* (table 1), and five sporocyst and 15 cercariae of *P. elegans* (Rudolphi, 1802) Braun, 1902, respectively (table 2). All measurements are given in millimetres. The drawings and description of the trematode larvae were made based on living specimens as well.

Results and discussion

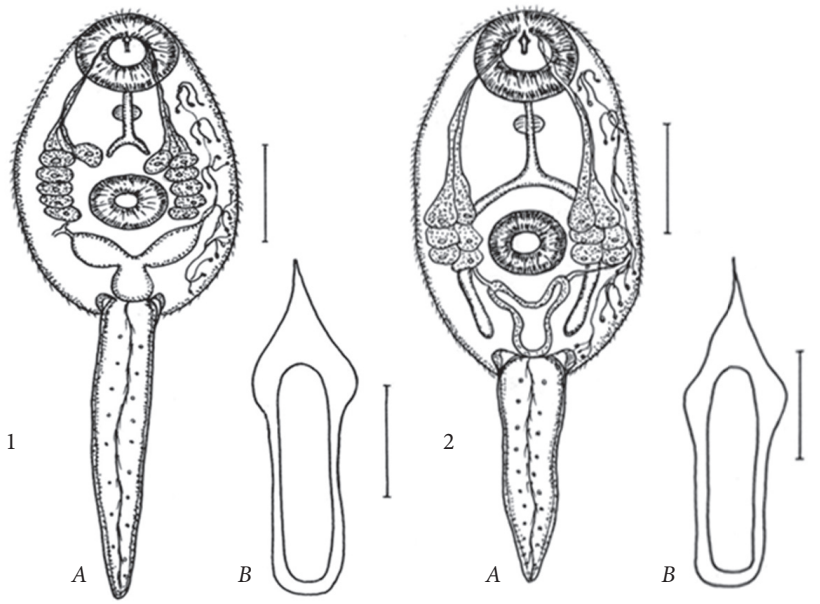
Parthenitae and cercariae morphologically similar to those of *P. multiglandularis* Semenov, 1927 were found only in Petrytskyi pond in Manevitsky District, Volyn Region. This is the first record of *P. multiglandularis* in *L. stagnalis* in Ukraine. Prevalence of *L. stagnalis* infection was 1.29 %.

Cercaria is oval, elongated, covered with small spines; length of the body is 0.3041 ± 0.0100 mm and width is 0.0650 ± 0.0060 mm (fig. 1, *A*). Body parenchyma contains small drops of fat. The oral sucker is 0.0490 ± 0.0010 mm in diameter. The ventral sucker is approximately 1.4 times smaller, 0.0360 ± 0.0010 mm in diameter. The stylet is 0.0290 ± 0.0003 mm in length and 0.0060 ± 0.0010 mm in width (fig. 1, *B*). Digestive system consists of the pharynx, 0.0180 ± 0.0003 mm in diameter, small esophagus and the intestine. There are six pairs of penetration glands, five of them are in lateral rows, the sixth is near the acetabulum. Excretory bladder is Y-shaped. The main excretory channels enter the bladder terminally. The tail lacks swimming membrane. Its length is 0.2240 ± 0.0070 mm, width is 0.0240 ± 0.0010 mm (table 1).

Cercariae develop in yellow elongated sporocysts. The length of the sporocyst is 0.9940 ± 0.0360 mm, and width is 0.2430 ± 0.0230 mm. Each sporocyst contains 15 to 20 cercariae and many embryos. These cercariae do not ripen simultaneously.

Comparison of the morphometric characteristics of parthenitae and cercariae shows (table 1) that the studied *P. multilglandularis* cercariae from Ukrainian Polissia correspond to those described by T. O. Ginetsinskaya (1959), E. N. Frolova (1975), and T. V. Shcherbina (1976). At the same time, the form described by Smirnova and Ibrasheva (1967) almost certainly belongs to different species of the genus *Plagiorchis*.

The *P. multiglandularis* cercariae found by Yu. V. Belyakova (1981) and E. N. Frolova (1975) are identical to those described by T. O. Ginetsynskya (1959) as *Xiphidiocercarie* IV. E. N. Frolova noted that cercariae developed in sporocysts of pink color and not simultaneously (Frolova, 1975). However, other researchers (Smirnova, Ibrasheva, 1967) marked the color of the parthenitae of *Xiphidiocercarie* IV (?) Ginetzinskaya, 1959 as orange. V. O. Smyrnova



Figs 1–2. *Plagiorchis*: *1* — *P. multiglandularis*; *2* — *P. elegans* (*A* — cercaria; stylet; *B* — stylet). Scale bar: *1*— 0.1 mm; *2* — 0.01 mm.

Table 1. Comparison of metrical characters (min–max, mm) of *P. multiglandularis* sporocysts and cercariae described in separate regions

Character	Ukraine	European Russia			Central Asia
	P. multiglandularis (present study)	*Xiphidiocercarie* IV (Ginetsinskaya, 1959)	*Plagiorchis* sp. III (?*multiglandularis* Semenov, 1927) (Frolova, 1975)	*P.multiglandularis* (Shcherbina, 1976)	*Xiphidiocercarie* IV (?) Ginetzinskaya, 1959 (Smirnova, Ibrasheva, 1967)
Sporocysts					
Body length	0.920–1.2	2	1.60	0.50–3.5	3.126
Body width	0.210–0.38	–	–	0.153–0.436	0.625
Cercariae					
Body length	0.231–0.33	0.258	0.230–0.32	0.274	0.238
Body width	0.044–0.077	0.031	0.08–0.10	0.128	0.127
Tail length	0.138–0.269	0.064–0.137	0.1–0.2	0.218	0.16
Tail width	0.022–0.025	–	–	–	0.025
Diameter of oral sucker	0.044–0.055	0.045	0.05	0.059	0.057
Diameter of ventral sucker	0.033–0.04	0.033	0.036	0.039	0.041
Redia length	0.028–0.03	0.021	0.032	0.031	0.017
Redia width	0.005–0.006	–	–	0.005	–
Pharynx diameter	0.017–0.02	–	0.016	0.018	0.02

and S. I. Ibrasheva (1967) noted that the cercariae were identical in structure and size to the *Xiphidiocercarie* IV , but differed from them by weak positive larval geotaxis, whereas in *Xiphidiocercarie* IV it is negative (Ginetsinskaya, 1959). The view was expressed (Smirnova, Ibrasheva, 1967) that they might be different species of the same genus.

Information on the life cycle of this trematode is given in a number of publications (Shtein, 1957; Krasnolobova, 1974; Shcherbina, 1976; Gorman, 1977; Genov, Samnaliev, 1984).

The first intermediate hosts of *P. multiglandularis* are the molluscs of the family Lymnaeidae (*L. stagnalis, L. auricularia* (Linnaeus, 1758); *L. saridalensis* Mozley, 1934; *L. balthica* (Linnaeus, 1758); *L. ovata* Draparnaud, 1805) (Ginecinskaya, 1959; Smirnova, Ibrasheva, 1967; Frolova, 1975; Krasnolobova, 1987, Vodyanitskaya, Yurlova, 2013; Akimova, 2015; Rastyazhenko et al., 2015; Faltynkova et al., 2016). The second intermediate hosts are the larvae of *Heptagenia* sp., *Ephemerella ignita, Cloeon dipterum* and *Limnophilus rhombicus* (Shtein, 1957, Shcherbina, 1976; Krasnolobova, 1982).

The definitive hosts of *P. multiglandularis* are mostly birds, rarely mammals (Krasnolobova, 1987; Borgsteede, Okulewicz, 2000; Ivanov et al., 2013; Akimova, 2015). This trematode maritae were experimentally grown in young chickens and laboratory mice (Krasnolobova, 1982; Genov, Samnaliev, 1984). According to experimental data (Genov, Samnaliev, 1984) *P. multiglandularis* at the age of 10 days are morphologically similar to *P. elegans*, which led the authors to conclude that these species are synonymous. The synonymy of *P. multiglandularis* and *P. elegans* was also supported by Ukrainian parasitologists (Iskova et al., 1985). Other specialists consider *P. multiglandularis* as a valid species (Rastyazhenko, Vodyanitskaya, 2015; Akimova, 2015; Faltynkova et al., 2016), which is consistent with our point of view.

In order to compare the morphometric characteristics of parethenites and cercariae of *P. multilglandularis* with those of *P. elegans* we investigated the latter ones and present their description below. The *P. elegans* larvae were found in the molluscs *L. palustris*, *L. stagnalis* and *L. corvus* from different types of water reservoirs on the territory of the Ukrainian Polissia. In total, the observed prevalence of infection of molluscs by *P. elegans* was 2.44 %.

Cercariae of *P. elegans* are comparatively small. Length of the body is 0.2390 ± 0.0210 mm and width is 0.0890 ± 0.0060 mm (fig. 2, *A*). Most of the body is filled

Table 2. Metrical characters (min–max, mm) of *P. elegans* sporocysts and cercariae

Character	Ukraine		Weatern Europe	European part of Russia	Asian part of Russia
	Own data	*P. elegans* (*Cercaria secunda*) (Zdun, 1961)	Styczynska-Jurevicz, 1962	Krasnolobova, 1982	Vodyanitskaya, 2006
		Sporocysts			
Body length	0.980–1.08	0.1–1.5	1.07–2.14	1.07–2.14	0.794–1.076
Body width	0.280–0.4	–	0.28–0.41	0.28–0.41	0.226–0.295
		Cercariae			
Body length	0.198–0.281	0.180–0.3	0.214–0.252	0.214–0.252	0.239–0.283
Body width	0.090–0.118	0.050–0.12	0.100–0.107	0.100–0.107	0.112–0.126
Tail length	0.090–0.132	0.060–0.25	0.108–0.18	0.108–0.18	0.185–0.21
Tail width	0.018–0.033	0.010–0.030	0.020–0.029	0.02–0.029	0.027–0.029
Diameter of oral sucker	0.04–0.058× 0.058–0.061	0.05 × 0.05	0.058 × 0.061	0.058 × 0.061	0.049 × 0.054
Diameter of ventral sucker	0.038–0.042	0.03 × 0.03	0.04 × 0.04	0.04 × 0.04	0.032 × 0.039
Redia length	0.028–0.032	0.03	0.02–0.03	0.028–0.03	0.026–0.029
Redia width	0.004–0.006	–	0.004–0.006	0.004–0.005	0.005–0.006
Pharynx diameter	0.018–0.029	–	–	–	–

with cystogenic glands. The cuticle is covered with spines. The mouth sucker is 0.0490 ± 0.0021 mm in length and 0.0590 ± 0.0003 mm in width, almost 1.3 times bigger than the ventral one, which diameter is 0.0388 ± 0.0002 mm. The stylet is well developed, without a bulb (fig. 2, *B*). Its length is 0.0300 ± 0.0003 mm and width is 0.0050 ± 0.0002 mm. The tip of the stylet is slightly bent. Six pairs of penetration gland are located on the sides of the ventral sucker. The digestive system consists of the prefarynx, the pharynx (0.0210 ± 0.0003 mm in diameter), the esophagus and two branches of the intestine, which almost reach the end of the body. Excretory formula — 2 [(3 + 3 + 3)] + [(3 + 3 + 3)] = 36. Excretory bladder is Y-shaped, the main excretory channels enter the bladder terminally. The gonopore is located behind the ventral sucker. Tail lacks swimming membrane, its length is 0.1160 ± 0.0080 mm and width is 0.0230 ± 0.0010 mm (table 2).

Cercariae develop in elongated sporocysts. The length of sporocyst is 1.0320 ± 0.0220 mm, and width is 0.3720 ± 0.0230 mm. There are about 19–20 cercariae in a sporocyst.

The second intermediate hosts of this species are the snails *L. stagnalis*, *L. ovata*, *L. auricularia*, the insects *Aedes aegypti*, *Culex pipiens*, *Chironomus plumosus*, *Chaoborus* sp., *Tabanus pecularius*, *Cleon*, *Baetis*, *Limnophilus* sp., *Phryganea* spp., *Aescha grandis*, *Orthetrum cancellatum*, *Enallagma cyathigerum, Dytiscus* sp., 35 species of dragonflies, and the crustaceans *Asellus aquaticus*, *Gammarus pulex* (Gorman, 1977; Krasnolobov, 1982; Iskova et al., 1985; Korobov, 2008; Kirillov, 2010).

Definitive hosts of *P. elegans* are reptiles, birds and mammals (Iskova et al., 1985). The analysis of the literature (Zdun, 1961; Styczynska-Jurevicz, 1962; Genov, Samnaliev, 1984; Krasnolobova, 1987) and our own data reveals similarity of *P. elegans* cercariae to *Cercaria secunda* Sinitzin, 1905. T. Genov and P. Somnaliev (1984) indicated the identity of these cercariae in their work. In Ukraine, *Cercaria secunda* was recorded by V. I. Zdun (1961) in the reservoirs of the western regions and by G. I. Vergun (1957) in *L. stagnalis* molluscs in the Siversky Donets and Molochnaja rivers in eastern Ukraine. In the work of G. I. Vergun (1957) the description of the cercaria is absent. At the same time G. P. Stenko (1986) found cercariae identified as *P. elegans* in *L. stagnalis* only in the Crimea. There are no data on the findings of *P. elegans* larvae in other regions of Ukraine. Carcariae of *P. elegans* found by us, according to the morphometric features, are close to those described by F. Stichinskaya-

Yurevich (1962) and S. N. Vodyanitskaya (2006) (table 2).

Thus, the study of the morphometric characteristics of partenitae and cercariae of *P. multiglandularis* confirms the validity of this species and its distinction from *P. elegans*.

An identification key of *Plagiorchis* spp. cercariae, recorded in freshwater molluscs in Ukraine is given below.

Key for the determination of cercariae *P. multiglandularis* and *P. elegans* of the genus *Plagiorchis*

1 (6). Twelve penetration glands, six on each side of the ventral sucker. The oral sucker is about 1/4 larger than the ventral one. Stylet has no clearly defined shoulders, the lateral ridges are smoothed.

2 (3). Four anterior penetration glands form pairs lying slightly in front of the ventral sucker; the remaining eight glands lie one after another, forming "chains" of four links, enveloping the sucker on both sides. The body length of the cercaria and tail are roughly equal. Stylet length is up to 0.03 mm. .... *Plagiorchis multiglandularis* Semenov, 1927.

3 (2). Penetration glands are located differently. The tail is shorter than the body. Stylet is of different length.

4 (5). Three pairs of penetration glands lay in pairs one after another on each side of the ventral sucker. The branches of the intestine almost reach the end of the body. The length of the tail is about 0.6 times the length of the body. Styletto length is about 0.03 mm. ..... *Plagiorchis elegans* (Rudolphi, 1802) Braun, 1902

5 (4). Penetration glands are located preacetabularly, forming bunches arranged in two or three transverse rows (3 + 3 or 2 + 3 + 1). The length of the tail is about 0.4 times the length of the body. Stylet is up to 0.04 mm. .....*Plagiorchis* sp.

6 (1). Number of penetration gland is bigger. The ratio of the sizes of the oral and abdominal suckers is different. Stylet has well-defined shoulders.

7 (8). Eighteen penetration glands form clusters resembling grapes, on each side of the ventral sucker. The oral sucker is slightly larger than the ventral sucker, the difference is 0.1. The branches of the intestine are short, slightly extending beyond the posterior edge of the ventral sucker. The tail is slightly shorter than the body of cercariae (about 1/4). Styletto length is up to 0.03 mm. ..... *Plagiorchis mutationis* Panova, 1927

8 (7). Penetration glands form grouchlike groups on the sides of the ventral sucker mostly preacetabularly, they consist of 7 and 8 glands, arranged respectively 1 + 3 (2) pairs one after another +1 (2). The oral sucker is 1.5 times larger than the ventral sucker. The tail is almost twice as short as the body. Intestinal ceca reach the posterior end of the body. Stylet length is 0.033 mm. ..... *Plagiorchis laricola* Skrjabin, 1924*

* after T. Krasnolobova (1987).

According to N. Iskova et al. (1985), 14 species of the genus *Plagiorchis* occur in the country: *P. arvicolae* Schulz et Skworzow, 1931, *P. elegans*, *P. koreanus* Ogata, 1938, *P. laricola* Skrjabin, 1924, *P. maculosus* (Rudolphi, 1802), *P. marii* Skrjabin, 1920, *P. molini* Lent et Freitas, 1940, *P. motacillae* (Yamaguti, 1939), *P. muelleri* Tkach et Sharpilo, 1990, *P. mutationis* Panova, 1927, *P. nanus* (Rudolphi, 1802), *P. notabilis* Nicoll, 1909, *P. triangularis* (Diesing, 1850), and *P. vespertilionis* (Muller, 1780). The larval stages were previously described only for two species: *P. elegans* and *P. mutationis* (Zhytova, 2010). Now this list is replenished with *P. multiglandularis* larvae.

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Received 7 February 2018
Accepted 7 May 2018