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LARVAL STAGES OF *PHYLLODISTOMUM* SP. (DIGENEA, GORGODERIDAE) FROM THE DUCK MUSSELS *ANODONTA ANATINA* IN UKRAINE

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Larval Stages of *Phyllodistomum* sp. (Digenea, Gorgoderidae) from the Duck Mussels *Anodonta anatina* in Ukraine. Kudlai O. S., Yanovich L. N. — Rhopalocercous cercariae were found in the gonads of duck mussels, *Anodonta anatina* (Linnaeus, 1758) collected from the Sluch River (Zhytomyr oblast, Ukraine). The morphological features observed led to conclusion that this species belonged to the genus *Phyllodistomum* Braun, 1899. Obtained results suggested that the second intermediate hosts in the life cycle of this trematode were absent. Free-swimming cercariae were observed encysting in water. This species is similar to *Phyllodistomum pseudofolium* Nybelin, 1926 by overall body proportions and ratio of suckers: lengths of oral to ventral sucker 1 : 1.1; widths of the same organs 1 : 1.2. To confirm the taxonomic position of the species found and establish a possible relationship between it and *P. pseudofolium* an experimental infection of fish *Carassius carassius* (Linnaeus, 1758) as potential definitive host was performed. The infection was not successful. Descriptions and figures of all detected larval stages of *Phyllodistomum* sp. are provided.

Key words: trematodes, Gorgoderidae, *Phyllodistomum*, larval stages, *Anodonta anatina,* Ukraine.

Личинночные стадии *Phyllodistomum* sp. (Digenea, Gorgoderidae) из беззубок *Anodonta anatina* в Украине. Кудлай Е. С., Янович Л. Н. — Булавохвостые церкарии обнаружены в гонадах моллюсков *Anodonta anatina* (Linnaeus, 1758) из р. Случ (Житомирская обл., Украина). Изучение морфологических особенностей позволило сделать вывод, что этот вид принадлежит роду *Phyllodistomum* Braun, 1899. Полученные результаты показали, что второй промежуточный хозяин в жизненном цикле данного вида отсутствует. Инцистирование свободноплавающих церкарий происходило в воде. Данный вид наиболее близок к *Phyllodistomum pseudofolium* Nybelin, 1926 по общим пропорциям тела и соотношению присосок: длины ротовой к брюшной присоске 1 : 1,1; ширины тех же органов, 1 : 1,2. Для подтверждения таксономического положения обнаруженного вида и установления возможной связи между ним и *P. pseudofolium* проведен эксперимент по заражению рыбы *Carassius carassius* (Linnaeus, 1758) как потенциального окончательного хозяина. Экспериментальное заражение не было успешным. Приведено описание и рисунки всех обнаруженных личиночных стадий.

Ключевые слова: трематоды, Gorgoderidae, *Phyllodistomum*, личиночные стадии, *Anodonta anatina,* Украина.

Introduction

The cosmopolitan trematode genus *Phyllodistomum* Braun, 1899 includes approximately 100 freshwater and marine species found primarily in the urinary bladder of teleosts and to a lesser extent in amphibians (Pigulevsky, 1953; Yamagutti, 1971; Cribb, 1987 b). *Phyllodistomum* trematodes show a variety of life cycles with two or three hosts. Much of this variation relates to the morphology and biology of the larval stages. Cercariae produced in the life cycles of *Phyllodistomum* species are assigned to three types: microcercous, cystocercous, rhopalocercous (Cribb, 1987 a).

Rhopalocercous cercariae are a group characterized by a club-shaped, corrugated tail. The tail is capable of expansion to form a balloon-like chamber within which metacercarial encystment occurs. These cercariae typically possess markedly well-developed genital primordia. Life cycles are known for two European species which develop from cercariae of this type: *Phyllodistomum angulatum* Linstow, 1907 from *Anodonta ponderosa* Pfeiffer, 1825 (Ivantsiv, Kurandina, 1985) and *P. elongatum* Nybelin, 1926 from *A. cygnea* (Linnaeus, 1758)

(Orecchia et al., 1975). Nine rhopalocercous species were previously described for North America (Fischthal, 1951, 1954; Coil, 1954; Flook, Ubelaker, 1971).

There are 6 species of *Phyllodistomum* found in the freshwater fishes in Ukraine: *P. folium* (Olfers, 1816), *P. angulatum*, *P. elongatum*, *P. pseudofolium* Nybelin, 1926, *P. simile* Nybelin, 1926, *Phyllodistomum* sp. (Koval, 1969). Detections of larval stages are reliable only for two out of these species. They were found in bivalves of the families Unionidae and Sphaeriidae: *P. elongatum* as *Cercaria duplicata* Baer, 1827 from *A. cygnea, A. anatina* (Linnaeus, 1758) (Zdun, 1961) and as *P. folium* from *A. stagnalis* (Gmelin, 1791) (Stadnichenko, 1983), *P. angulatum* Linstow, 1907 from *A. ponderosa* (Ivantsiv, Kurandina, 1985). Larvae of *Phyllodistomum* sp. from gills of *Pisidium amnicum* (Müller, 1774) were described by Chernogorenko (1983).

During the investigation of parasites of molluscs from the Sluch River, rhopalocercous cercariae were found in the gonads of the duck mussels, *A. anatina*. Detailed study of the morphology of these trematodes revealed that they belonged to *Phyllodistomum* sp. and were found for the first time in Ukraine.

In this paper, larval stages of *Phyllodistomum* sp. are described and some data on the species development are provided and illustrated.

Material and methods

Molluscs were collected for parasitological investigation from the Sluch River (Baranovka, Zhytomyr oblast, Ukraine) from July, 2011 to June, 2012. Totally, 374 specimens of unionid molluscs belonging to the following 6 species were examined: 113 — *Unio pictorum* (Linnaeus, 1758); 127 — *U. tumidus* Philipsson, 1788; 12 — *U. crassus* Philipsson, 1788; 112 — *A. anatina*; 3 — *A. cygnea*; 7 — *Pseudanodonta complanata* (Rossmässler, 1835). In the laboratory molluscs were dissected. Twenty-eight *A. anatina* were placed individually in glass containers with tap water at temperature 18–22 °C. The containers were examined daily under a binocular microscope for detection of spontaneous cercariae emission and their encystation. Three carp fish *Carassius carassius* (Linnaeus, 1758) (grown from the fry) served as potential definitive hosts for experimental infection. In addition, a total of 32 fishes including 7 — *Rutilus rutilus* (Linnaeus, 1758); 1 — *Scardinius erythrophthalmus* (Linnaeus, 1758); 1 — *Blicca bjoerkna* Linnaeus, 1758; 3 — *Gobio gobio* (Linnaeus, 1758); and 20 — *C. carassius* were collected for parasitological investigation by angling from the Sluch River between July to August 2012 in the locality, where molluscs were collected.

Morphology of the larval stages was studied on living and fixed specimens; neutral red and Nile blue stains were used for intravital staining. Afterwards, living worms were rinsed in water, killed with hot water and fixed in 70 % ethanol. For morphological examination, fixed specimens were stained with acetocarmine, dehydrated in a graded ethanol series, cleared in clove oil, and mounted permanently in Canada balsam. Drawings were made with the aid of a drawing tube. Photographs were made using Am Scope microscope equipped with digital camera. All measurements are presented in mm, mean values are given in parentheses. The systematic positions of trematodes are indicated according to the system proposed by Campbell (2008). Nomenclature of mollusc species follows Glöer, Meier-Brook (1998), Vasilyeva (2011) and that of fish species follows Movchan (2011).

Results

Partenitae and cercariae of *Phyllodistomum* sp. were found in the gonads of only six specimens of *A. anatina* (prevalence 5.4 %). Experimental infection of *C. carassius* by metacercariae was not successful. *Phyllodistomum* trematodes were not detected also in the fish from the Sluch River.

Description of *Phyllodistomum* sp. developmental stages

Sporocyst (fig. 2, *1*). Measurements and description are based on 24 live specimens. Motionless, oval, with thick body wall. Body length 0.520–1.620 (0.849), width 0.300–0.870 (0.529). Total number of cercariae per sporocyst 1–7 (3).

Cercaria (fig. 1; 2, *2*). Measurements are based on 25 specimens from permanent mounts. Body elongate, slightly narrower at anterior end and broader at posterior end. Body 0.395–0.860 (0.593) by 0.123–0.250 (0.173), maximum width at level of anterior testis. Tegument thin, without spines, with very finely papillate, distributed irregularly from anterior to posterior end of body, more numerous in posterior part. Oral sucker subterminal, 0.063–0.143 (0.096) long, 0.063–0.108 (0.084) wide; mouth subterminal. Ventral sucker round, 0.060–0.145 (0.104) × 0.070–0.138 (0.102). Its center at 0.175–0.500 (0.325) from anterior end. Papillae noticeable on oral and ventral suckers. Sucker ratio: lengths of oral to ventral sucker 1 : 1.1; widths of same organs 1 : 1.2. Pharynx absent. Oesophagus 0.030–0.100 (0.068) long, surrounded by unicellular penetration glands. Intestinal bifurcation at about midway between oral and ventral suckers. Caeca

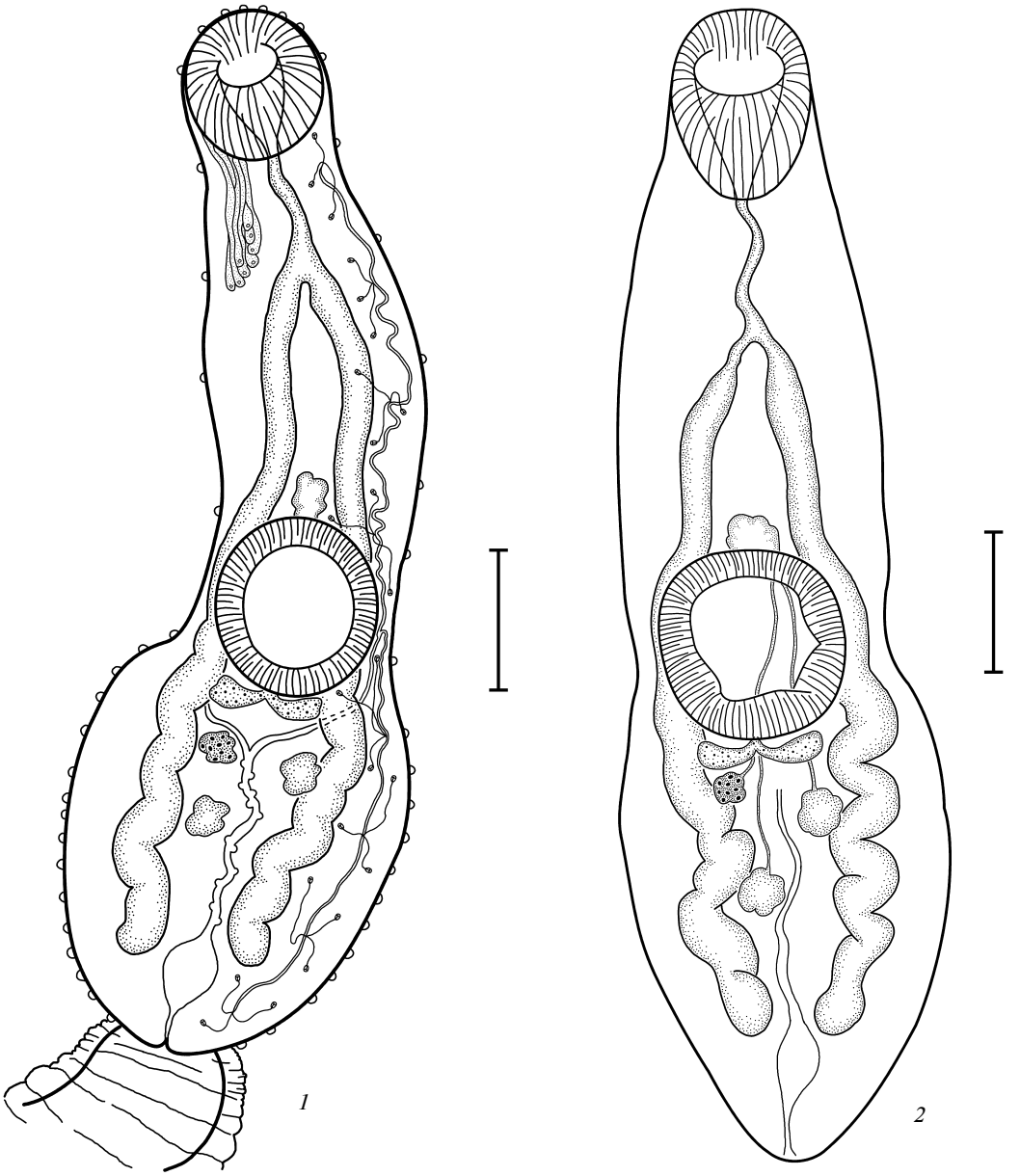

Fig. 1. Cercariae of *Phyllodistomum* sp.: *1* — alive specimen; *2* — fixed specimen. Scale bar 0.1 mm.

Рис. 1. Церкарии *Phyllodistomum* sp.: *1* — живой экземпляр; *2* — фиксированный экземпляр. Масштабная линейка 0,1 мм.

passing posteriorly beyond testes primordium. Genital primordia (n = 15) well developed, within posterior third of body. Testes primordium lobed, placed diagonally. Anterior testis 0.025–0.035 (0.029) long, 0.018–0.038 (0.026) wide, posterior testis 0.018–0.038 (0.030) long, 0.018–0.038 (0.027) wide. Seminal vesicle primordium located at anterior margin of ventral sucker. Ovary primordium slightly lobed, 0.018–0.028 (0.023) × 0.015–0.028 (0.021), anterior to testes on the same side as posterior testis. Paired primordium of vitelline masses transversely elongated, located between ventral sucker and ovary and anterior testis. Excretory system mesostomate. Excretory vesicle I-shaped, thin-walled. Total number of flame cells 48–24 on each side (fig. 1, *1*). Excretory pore terminal. Tail club-shaped, 0.423–0.613 × 0.154–0.166, in cross-section consisting of two distinct layers: outer corrugated cuticle, and beneath and only loosely associated

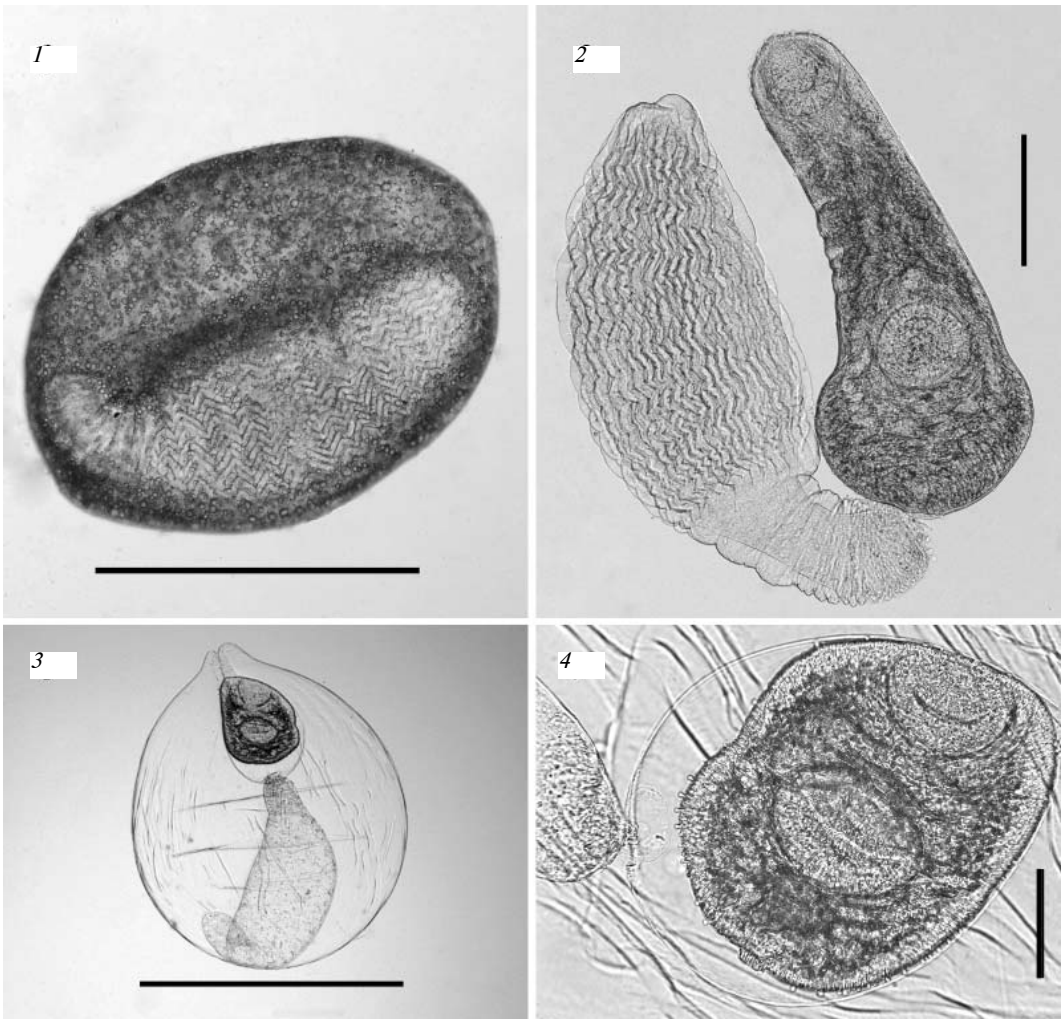

Fig. 2. Larval stages of *Phyllodistomum* sp.: *1* — sporocyst; *2* — cercaria; *3* — metacercaria in the chamber; *4* — metacercaria. Scale bars: *1, 2* — 0.5 mm; *3* — 1 mm; *4* — 0.1 mm.

Рис. 2. Личиночные стадии *Phyllodistomum* sp.: *1* — спороциста; *2* — церкария; *3* — метацеркария в камере; *4* — метацеркария. Масштабные линейки: *1*, *2* — 0,5 мм; *3* — 1 мм; *4* — 0,1 мм.

with it, layer of cells with large, dense nuclei. Anterior part of tail with slight narrowing from the remaining part. Attachment of tail to body fragile, quite often tail separated after emergence from sporocyst.

Available experimental results suggest that the second intermediate hosts in the life cycle of this species are absent. After emerging from mollusc, cercariae encysted inside the chamber formed from their tail. As a result of contractile movement of tail, its upper membrane transformed into round balloon-like chamber, containing cercarial body and tail (fig. 2, *3*). Simultaneously, the cyst was forming around the body of the cercaria (fig. 2, *4*). The formation of the chamber lasted from 1 to 3 minutes. Encysted cercariae sunk to the bottom of the glass container. Encystment and tail transformation in *Phyllodistomum* sp. conform to Fischthal's (1951), Flook and Ubelaker's (1971) descriptions.

Discussion

In the present study rhopalocercous cercariae were found in *A. anatina* in one location in Ukraine. In accordance with the system of gorgoderid trematodes proposed by Pigulevsky (1953), Yamaguti (1971) and Campbell (2008), the cercaria described

here showed the common characteristics of the genus *Phyllodistomum*, specifically the forebody tapered anteriorly, hindbody broad, pharynx absent, testes two diagonal in widest part of hindbody, ovary posterior to ventral sucker. Development and morphology of *Phyllodistomum* sp., including the encystment with in transformed tail in water without second intermediate host, are most similar to two European *Phyllodistomum* species, *P. angulatum* and *P. elongatum* (Orrechia et al., 1975; Ivantsiv, Kurandina, 1985) and nine other rhopalocercous cercariae, which have been studied in North America by Fischthal (1951, 1954), Coil (1954), Flook and Ubelaker (1971).

Two European species appear to be closest to *Phyllodistomum* sp. Cercariae of *P. angulatum* described from *A. ponderosa* (Ivantsiv, Kurandina, 1985) from Ukraine, differ from *Phyllodistomum* sp. mainly in the presence of the lateral protuberance on each side in the posterior part of body; ratio of suckers: 1 : 1.4 in *P. angulatum* and 1 : 1.2 in the species described herein. Another differential feature is a total number of flame cells: 46 in *P. angulatum* and 48 in *Phyllodistomum* sp. Cercaria of *Phyllodistomum* sp. differs from *P. elongatum* described from *Pisidium amnicum*, *A. cygnea* by Sudarikov et al. (2002) by location of ventral sucker, and ratio of length of fore to hind parts of body.

Despite of negative results of experimental infection of fish, in our opinion, the morphological characters suggest that species described herein is most similar to *P. pseudofolium* by overall body proportions and ratio of suckers' size. Adults of *P. pseudofolium* are parasites of *Sander lucioperca* (Linnaeus, 1758), *Perca fluviatilis* Linnaeus, 1758, *Gymnocephalus cernuus* (Linnaeus, 1758) in the Palaearctic (Pigulevsky, 1953; Bauer, 1984). The life cycle of *P. pseudofolium* is unknown. Its metacercariae were registered from mantle tissue of *A. stagnalis* in Russia (Sudarikov et al., 2002).

Our results demonstrate that the life cycle of *Phyllodistomum* sp. involves the freshwater molluscs *A. anatina* as the first intermediate host. Metacercarial encystment occurs within transformed cercarial tail in water and matacercariae may be eaten by fish. The natural definitive host of *Phyllodistomum* sp. has not been identified during the present study.

The possibility of using a combination of morphological, molecular and experimental investigations in further study can help, in our opinion, to identify the species affiliation of *Phyllodistomum* sp. and fully elucidate its life-cycle.

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