Received: 2 December 2023 | Accepted: 10 September 2024

DOI: 10.1111/ivb.12443

ORIGINAL ARTICLE

Invertebrate Biology WILEY

# Development and reproduction of sporocysts of *Leucochloridium paradoxum* (Trematoda)

Gennady L. Ataev[1] | Regina R. Usmanova[1] | Anna A. Vinogradova[2] | Elena E. Prokhorova[1] | Arina S. Tokmakova[1]

[1]Laboratory of Experimental Zoology, Department of Zoology and Genetics, Faculty of Biology, Herzen State Pedagogical University of Russia, St Petersburg, Russia

[2]Zoological Institute, Russian Academy of Sciences, St Petersburg, Russia

**Correspondence**
Gennady L. Ataev, Department of Zoology and Genetics, Herzen State Pedagogical University of Russia, Moyka River 48, St Petersburg 191186, Russia.
Email: ataev.gennady@gmail.com

**Funding information**
Grant of Ministry of Education of RF, Grant/Award Number: VRFY-2023-0009

## Abstract

We studied the development of sporocysts of the trematode *Leucochloridium paradoxum*. For this purpose, their life cycle was traced experimentally. Eggs obtained from adult trematodes raised in chicks (*Gallus gallus domesticus*) were used to infect snails (*Succinea putris*, laboratory strain). Histological studies of sporocysts were conducted for 95 days after exposure. During this time, the embryos of sporocysts developed into a small stolon filled with numerous metacercarial embryos. We found that miracidia hatched in the stomach and in the proximal part of the snail's midgut. After the larvae migrated into the area of the hepatopancreas, their body was destroyed, and germinal cells were released. They cleaved to form the embryos of sporocysts, in which only metacercarial embryos developed. Our study showed that these trematodes lack the parasitic phase of the mother sporocyst and have only one generation of parthenitae, the daughter sporocysts.

**KEYWORDS**
daughter sporocyst, development, *Leucochloridium paradoxum*, life cycle, trematodes

## 1 | INTRODUCTION

The mechanism and dynamics of development of trematode parthenitae have been broadly debated since the 19th century. The intramolluscan part of the life cycle of trematodes has been described for several dozen species of trematode in experimentally infected snails; nevertheless, new studies focusing on the development of rediae or sporocysts of previously uncharacterized species are important for understanding the evolution of trematode life cycles.

Trematodes of the genus *Leucochloridium* are of particular interest because they belong to the family Leucochloridiidae, one of the basal families of trematodes (Cribb et al., 2011; Olsen et al., 2003), and also because of their life cycle, which includes unique branched sporocysts. Mature sporocysts form broodsacs whose color and shape are species specific (Ataev et al., 2016; Ginetsinskaya, 1988; Usmanova et al., 2023).

The sporocysts of *Leucochloridium paradoxum* Carus, 1835, parasitize the land snail *Succinea putris* (Linnaeus, 1758). The body of mature sporocysts is a strongly branched stolon with several broodsacs, which are green in color and capable of pulsation (Ataev et al., 2016; Usmanova et al., 2023). They are connected to the central part of the stolon by tubular sections. The broodsacs penetrate the tentacles of snails and become clearly visible through their covering tegument. The broodsacs, with their characteristic coloration and pulsation, look like insect larvae and attract various birds. However, they can also detach from the stolon and exit the molluscan host through ruptures in the surface epithelium, remaining mobile in the external environment for about an hour (Ataev et al., 2016). By pecking on the broodsacs, birds become infected with the metacercariae contained in them. The metacercariae transform into adults that reside in the cloaca of the definitive host. The eggs produced by the adults are transported into the environment with the feces of infected birds. Mollusks are infected by ingesting eggs, and a branched sporocyst is formed in them.

Mimicry of sporocysts of *Leucochloridium* species is a textbook example of parasite adaptation to facilitate the infection of the

  

definitive host. For this reason, these trematodes are well known to a broad range of biologists. However, there is no information on the characteristic features of development of different life cycle phases of species of *Leucochloridium* in the modern literature. Particularly, little is known about the mechanism of infection of the mollusk and the development of the sporocysts. It is even unclear whether the branched sporocysts belong to the mother or daughter generation of parthenitae. Their reproduction also requires further study, as there are no reliable data on the place and nature of formation of metacercarial embryos.

The life cycle of trematodes in the genus *Leucochloridium* attracted much attention in late 19th to early 20th centuries (Heckert, 1889; Kagan, 1952; Mönnig, 1922; Woodhead, 1935). These researchers traced the main stages of the sporocyst development using material from naturally and experimentally infected mollusks and made excellent anatomical drawings. They hypothesized about the modes of infection in the snails and even about the mechanism of pigment formation in the broodsacs. However, the analysis of sporocysts of *Leucochloridium* could not be completed at that time because of the limited understanding of the nature of trematode parthenitae and the dynamics of their reproduction and development. Some further studies of the development of sporocysts in *Leucochloridium* (Ataev et al., 2013; Ataev & Tokmakova, 2015; Ginetsinskaya, 1954) were made on naturally infected mollusks, and so the age of parthenitae and the exact sequence of their developmental processes could not be reliably determined. In this study we traced the development of sporocysts of *L. paradoxum* in experimentally infected snails *S. putris*.

## 2 | METHODS

### 2.1 | Trematode species identification

Individuals of the snail *S. putris* ($n = 18$) infected with the trematode *L. paradoxum* were collected in the vicinity of the biological station of the Herzen State University, near Vyritsa Settlement of the Leningrad Region of the Russian Federation (59°24′42.3″N 30°19′08.1″E) in June 2022. The species of trematode (*L. paradoxum*) was identified morphologically and by sequencing.

We sequenced the tissues of broodsacs ($n = 6$) of the sporocysts and used these for infection of chicks (*Gallus gallus domesticus*). In all the cases, DNA was isolated using a commercial DNA-sorb-C-M kit (Cat. No. K1-6-50-Mod) (AmpliSens, Russia). A fragment of the *cytochrome c oxidase subunit I* (*cox1*) was sequenced using primers JB3 (5′-TCCTCCCCAGACCAGTCATAG-3′) and CoxL ((5′-TCCTCCCCCCA-GACCAGTCATAG-3′) (Bowles & McManus, 1993; Usmanova et al., 2023). Annealing temperature was 55°C. Polymerase chain reactions were performed using TaqDNA polymerase (Cat. No. EP1712) (ThermoFisher Scientifc, USA) according to the previously described protocols (Prokhorova et al., 2020). PCR products were isolated from the agarose gel and purified using a Wizard® SV Gel and PCR Clean-Up System commercial kit (Cat. No. A9281) (Promega, USA) according to the manufacturer's instructions. Sanger sequencing (3500xL Genetic Analyzer) was performed by OOO Sintol. Analysis, assembly, and alignment of nucleotide sequences were conducted with BioEdit v. 7.2.5 (Hall, 1999) and MEGA v. 10.2.4 software (Kumar et al., 2018).

We obtained and annotated *cox1* sequences of the sporocysts with a length of 772–832 bp (GenBank sequence numbers ON526789.1, ON526790.1, ON526788.1, MZ676725.2, MZ676726.2, and MZ676727.2). The samples of sporocysts were extracted from different snails. The trematodes used in our study were identified as *L. paradoxum* based on morphological (shape and coloration of broodsacs) and genotypic analysis (see Usmanova et al., 2023, for details).

### 2.2 | Laboratory strain of the snail *S. putris*

We collected several dozen snails from the Vyrisa population. Eggs obtained from them were used to establish a laboratory strain of *S. putris*.

Morphological analysis and sequencing of snails with the use of an rDNA fragment (*ITS1–5.8S–ITS2*) and fragments of mitochondrial genes (*cox1* and *cytb*) showed that the individuals collected in Vyritsa belonged to a single one and the same species, *S. putris* (see Prokhorova et al., 2020). The progeny of these mollusks was obtained and later used for cross-breeding. Snails from F3 and F4 generations, obtained as a result of further cultivation, were used in the experiment.

### 2.3 | Experimental infection of snails

Experimental infection of snails with miracidia of *L. paradoxum* was performed in three stages. These stages included the infection of sterile chicks with trematodes to obtain trematode eggs and then infecting intermediate hosts—*S. putris*.

#### 2.3.1 | Infection of chicks

Sterile chicks of four breeds of *G. gallus domesticus* ($n = 38$)—Kuchinskaya Yubileynaya (code 9354849), Lohmann Brown (code 9355905), and Pushkinskaya (code 9358991)—were obtained and infected with metacercariae of *L. paradoxum* extracted from the naturally infected snails. Fertilized chicken eggs were incubated for 23 days under the following conditions: temperature 37.8°C, humidity 70% from Days 1 to 19, and temperature 37.3°C, humidity >85% from Days 19 to 23. The hatched chicks were not fed until the infection. They were infected by ingestion of 40–50 metacercariae (taken from one broodsac) with the help of a pipette. Infected snails were dissected, and the sporocysts with mature pigmented broodsacs were removed (Figure 1A). Mature broodsacs ($n = 24$) were placed into molluscan Ringer solution (98.2 mM NaCl, 11.94 mM KCl, 17.97 mM $CaCl_2$, 27.64 mM $MgCl_2$, and 24.42 mM $NaHCO_3$) and dissected to collect metacercariae (Figure 1B). Because experimental chicks were fed only on sterile food, we can be sure that the adult trematodes raised in them belonged to *L. paradoxum*. Before and after exposure the chicks were kept in brooder houses at 25–30°C, with fresh water and food ad libitum, and a 14:10 h light:dark lighting schedule.

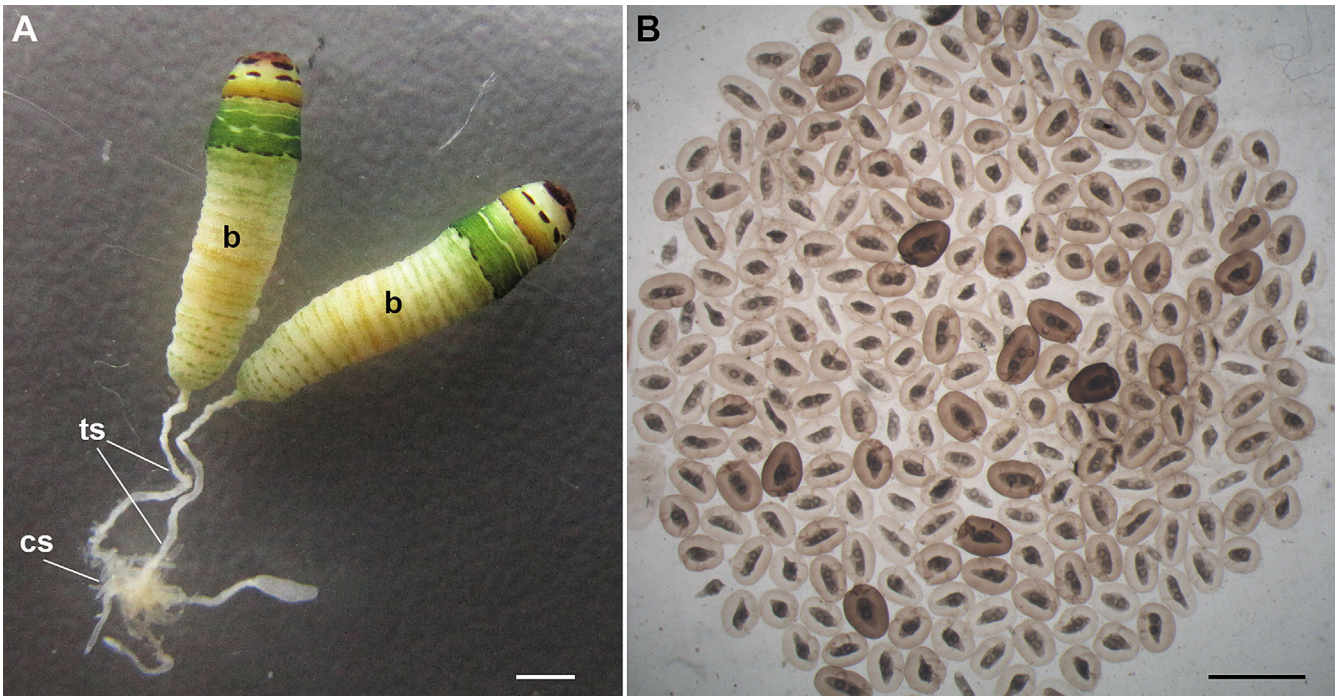


**FIGURE 1** Microphotographs of the trematode *Leucochloridium paradoxum*. (A) Mature broodsac. (B) Metacercariae. Scale bar = 2 mm. b, broodsac; cs, central part of the stolon; ts, tubular part of the stolon

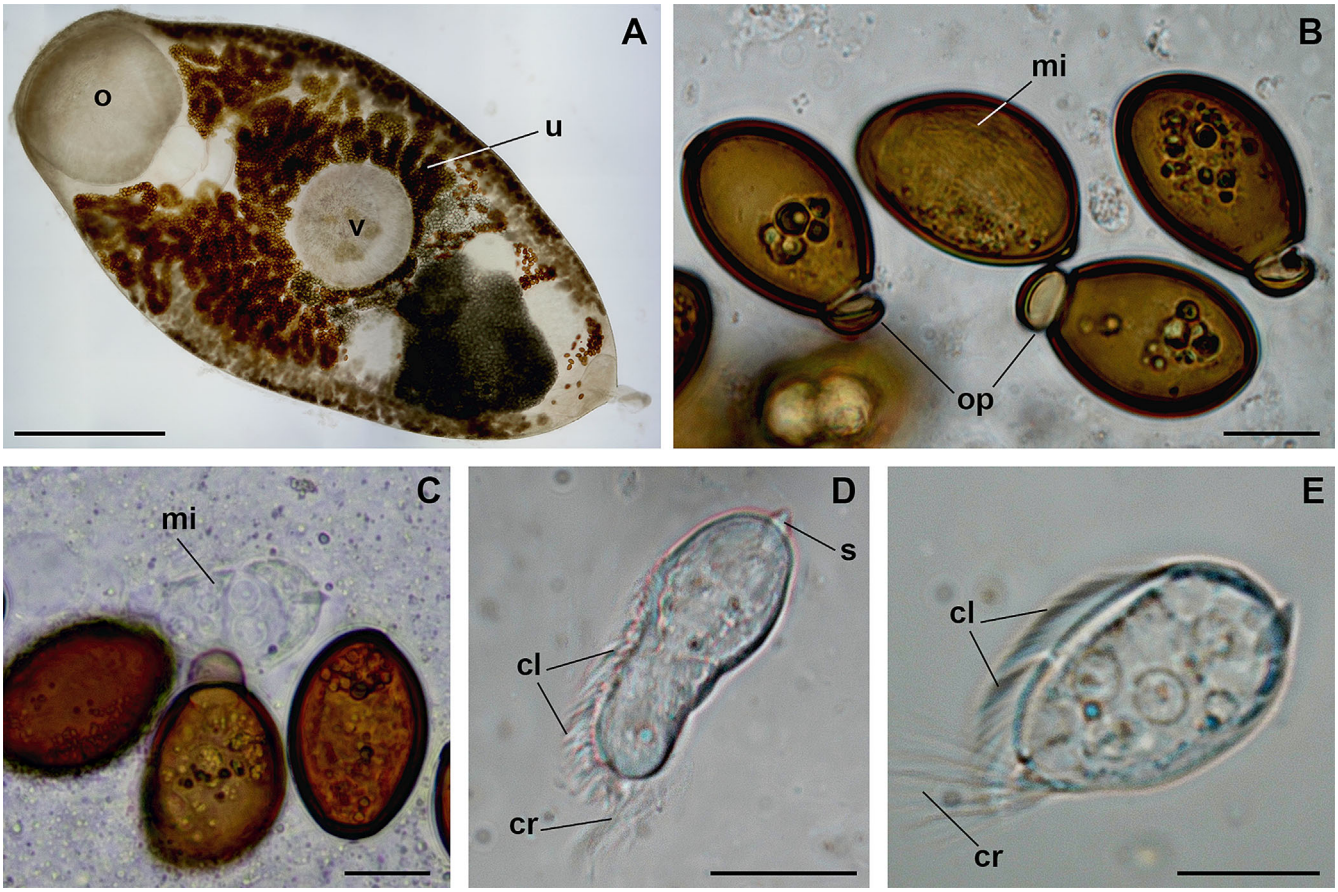


**FIGURE 2** Microphotographs of the trematode *Leucochloridium paradoxum*. (A) Adult. (B) Egg. (C–E) Miracidium. Scale bars: A = 235 μm; B–E = 10 μm. cl, cilia; cr, cirri; mi, miracidium; o, oral sucker; op, operculum; s, stylet; v, ventral sucker; u, uterus

#### 2.3.2 | Obtaining the eggs of *L. paradoxum*

Coproscopic examination of the chicks' feces was performed with the use of Leica DM 500 microscope. The eggs were first found in the feces at 13 days post-exposure. The chicks were fed only on plant fodder (millet), which eliminated the possibility of accidental infection by ingesting parasite eggs. The chicks were dissected at 21–23 days post-exposure. Adult trematodes ($n = 96$) were found in the cloaca and the bursa of Fabricius in 16 chicks (92% of chicks were infected). These trematodes were placed into molluscan Ringer solution (Figure 2A). Some of them ($n = 27$) were then dissected to obtain eggs (Figure 2B).

#### 2.3.3 | Infection of snails with eggs of *L. paradoxum*

The laboratory strain of *S. putris* was used ($n = 74$); we used a range of snail sizes, with a shell height of 11.0–16.2 mm in 2022 and 3.2–13.6 mm in 2023. The snails were kept in aerated 2-L cages filled with mineral soil and fed on cucumbers and lettuce leaves. The conditions in the cages were constant: humidity 80%, temperature 18 ± 1°C, and lighting schedule 12:12 h light:dark. The snails were starved for 2 days prior to the exposure. Then, individual snails were offered thin cucumber slices smeared with the trematode eggs. The infective dose made up ~600 eggs per snail (~60% of snails became infected). After infection, the snails were kept under the same conditions as before.

### 2.4 | Study of miracidia

The snails ($n = 6$) were dissected 15 min after exposure. The contents of the crop, the stomach, and the midgut were removed and diluted with Ringer solution. The preparations were examined under a phase-contrast microscope (Leica DM 5000).

### 2.5 | Histological study

The infected snails were dissected and fixed in Bouin's fixative at 7 ($n = 1$), 14 ($n = 2$), 22 ($n = 2$), 55 ($n = 2$), 72 ($n = 1$), and 95 ($n = 1$) days post-exposure. Histological studies were made on paraffin sections 4–5 μm thick, stained first with Ehrlich's hematoxylin and then with an aqueous solution of eosin. Histological treatment was performed in a standard way. The sections were analyzed with the use of Leica DM 5000 microscope. Morphometric analysis was performed with the help of ImageScope software.

## 3 | RESULTS

### 3.1 | Miracidia

The entire uterus of adults of *L. paradoxum* were filled with eggs, but developed eggs were located only in its distal part (Figure 2A,B). The size of mature eggs was 26.0 ± 0.7 × 16.7 ± 0.6 μm (mean ± SEM, $n = 15$). They were oval, slightly asymmetrical, and had a brown shell with an operculum at the upper pole.

The eggs remained intact while in the environment. Hatching was triggered in the alimentary tract of the snail. Miracidia hatched in the stomach or in the proximal part of the midgut. The operculum opened, and a drop-shaped miracidium left the egg. Miracidia size was 23.2 ± 2.0 × 10.7 ± 0.5 μm ($n = 10$) (Figure 2C,E). The cilia did not cover the miracidium uniformly but were arranged in two longitudinal rows on one side of the body. An average length of the cilia was 4–5 μm, but the cilia in the caudal part of the larva formed cirri reaching 8–10 μm in length. In the apical part of the miracidium (where the terebratorium is located in miracidia of other trematodes), there was a refractive stylet about 3.3 ± 0.2 μm in length. It is probably used by larva to penetrate the intestinal wall. Altogether, a miracidium was found to consist of about 10 cells. In its anterior part, there were four large cells, which had light nuclei 3.3 ± 0.2 μm in diameter (Figure 2E). They resembled secretory cells of miracidia of other trematode species (Ataev & Tokmakova, 2018). There were several smaller cells that also looked like somatic cells. However, in the caudal part of the miracidium, there were 1–2 small cells resembling germinal cells in shape and coloration.

### 3.2 | Development of daughter sporocyst

#### 3.2.1 | Seven days post-exposure

At 7 days post-exposure, rounded accumulations of cells about 15 μm in diameter could be seen in the lumen between the acini of the snail hepatopancreas (Figure 3A). These accumulations were embryos at the stage of 10–12 blastomeres, which were mostly represented by macromeres (cell dimensions 6.9 ± 0.4 × 5.7 ± 0.5 μm; nucleus dimensions 5.1 ± 0.5 × 4.2 ± 0.2 μm; $n = 6$). The character of the cleavage (complete, unequal) and the size and arrangement of the blastomeres were typical of the embryonic development of trematodes (see Galaktionov & Dobrovolskij, 2003). The presence of these embryos indicated that the body of the miracidium disintegrated after penetration, releasing germinal cells. The cleavage of germinal cells resulted in the formation of the embryos of the daughter sporocysts. This means that the trematode *L. paradoxum* lacks the parasitic phase of the mother sporocyst.

#### 3.2.2 | Fourteen days post-exposure

At 14 days post-exposure, the embryos of daughter sporocysts became oval and grew to reach 34.1 ± 2.1 × 23.5 ± 2.5 μm ($n = 8$) (Figure 3B). They consisted of 26–38 blastomeres with an average dimensions of 5.6 ± 0.8 × 4.5 ± 0.7 μm; the dimensions of their nuclei were 4.6 ± 0.4 × 3.6 ± 0.3 μm ($n = 17$).

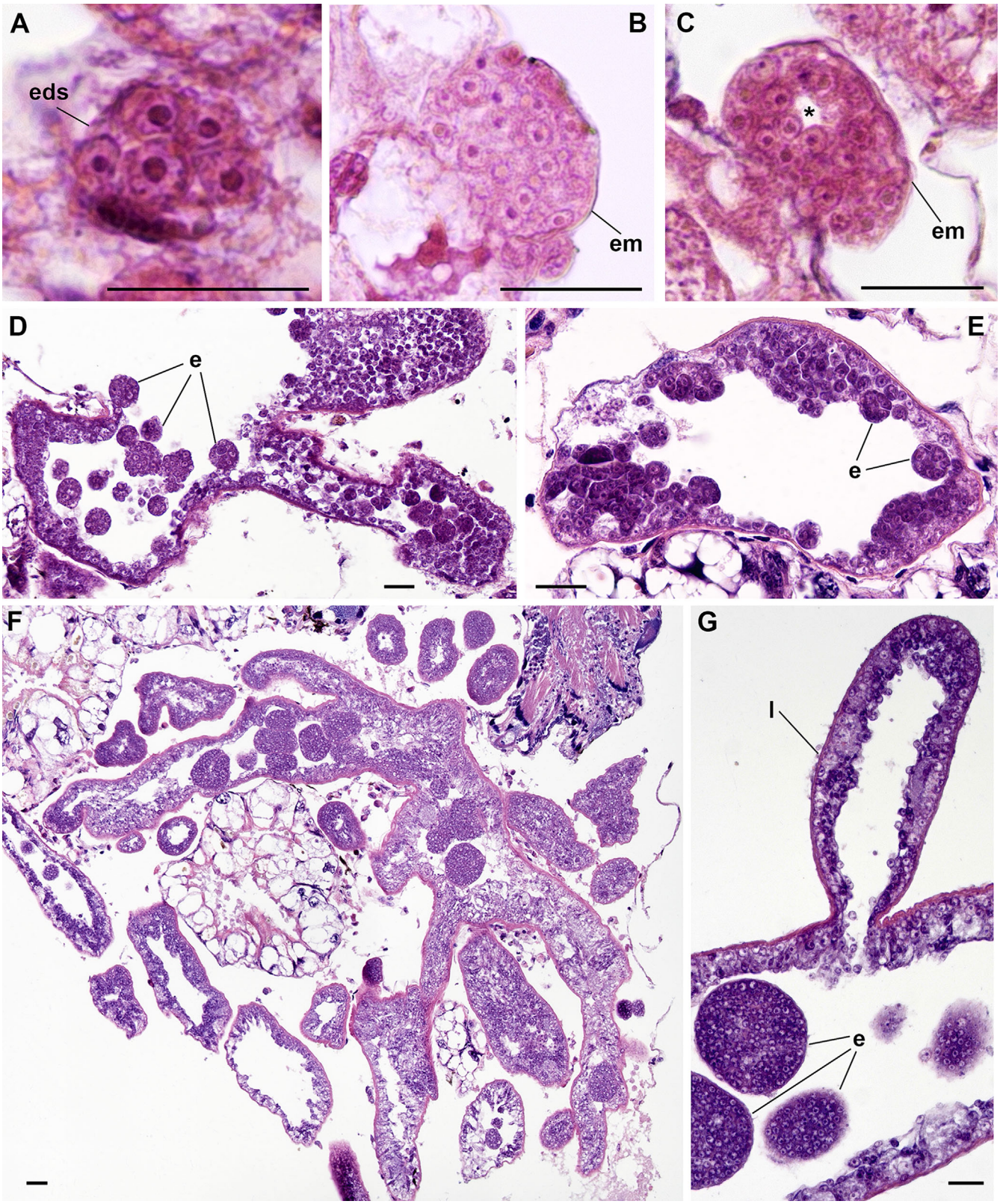


**FIGURE 3** Development of sporocysts of *Leucochloridium paradoxum* in the snail *Succinea putris*. Histological sections of the sporocyst at (A) 7 days post-exposure; (B) 14 days post-exposure; (C) 22 days post-exposure; (D) 55 days post-exposure; (E) 72 days post-exposure; and (F, G) 95 days post-exposure. Scale bar = 20 μm. *, schizocoel; e, metacercarial embryos; eds, embryos of daughter sporocysts; em, embryonic membrane; l, lateral outgrowths

### 3.2.3 | Twenty-two days post-exposure

At 22 days post-exposure, daughter sporocysts increased in size to 37.7 ± 4.0 × 29.7 ± 3.4 μm ($n = 10$). They consisted of more than 90 blastomeres. Their dimensions were 5.7 ± 0.3 × 4.8 ± 0.5 μm, and the dimensions of their nuclei were 4.3 ± 0.4 × 3.6 ± 0.3 μm ($n = 17$). A small cavity (an incipient schizocoel) appeared in the center of the embryo (Figure 3C).

### 3.2.4 | Fifty-five days post-exposure

At 55 days post-exposure, the body of daughter sporocysts was already represented by a stolon, whose central part was about 380 × 130 μm in size. A common schizocoel cavity was formed in the stolon, but the broodsacs had no lumen yet. Daughter sporocysts had not more than 5 broodsacs, the largest reaching 260 μm in length (Figure 3D). The germinal material of sporocysts of this age was represented by germinal cells and by metacercarial embryos. They developed both in the subtegumental parenchyma and within the germinal masses, which are specialized reproductive organs of parthenitae (Figure 4A–C).

The number of cells in small embryos (Figure 4D) did not exceed 10–15. They lacked protective structures. Such embryos are referred to as "naked cell embryos" (Cheng, 1961). However, macromeres could be seen on the surface of the embryos already at the stage of 10 blastomeres. Later, these macromeres formed an embryonic membrane (Figure 4E). When the membrane was formed, the embryos consisted of 25–30 blastomeres reaching the stage of the "germinal ball" (Cheng, 1961).

Germinal embryos whose formation and development proceeds outside the germinal masses are referred to in our study as free germinal cells and free embryos. They were located in different parts of the parietal parenchyma of the central part of the stolon and in the parenchymatose matrix of the broodsacs.

In addition to free germinal elements, the subtegumental layer contained germinal masses. At first, a germinal mass was represented by an accumulation of 15–20 cells, which did not exceed 14–18 μm (Figure 4A). Large germinal masses of this age were oval and reached 30 × 40 μm in size. As they grew, embryos were formed in them, and germinal masses acquired a radial structure, with germinal cells and small embryos in the center and larger embryos in the periphery (Figure 4B). Embryos continued to develop in the germinal mass until the stage of the germinal ball, after which they entered the schizocoel cavity. The largest embryos consisting of 70–80 blastomeres reached 25 μm in diameter.

The development of the schizocoel was accompanied by the destruction of the parietal layer of the parenchyma. Numerous pyknotic bodies and stellate cells could be seen at this stage. As a result, microcavities, where germinal elements developed, were formed in the parenchymatose matrix.

As a result of the destruction of the parenchyma, one or several germinal masses separated from the sporocyst wall and started floating in the schizocoel near the embryos (Figure 4C). The embryos and the germinal masses did not separate instantaneously but remained connected with the body wall for some time by cytoplasmic outgrowths of the stellate cells. For this reason, numerous germinal masses and embryos were arranged at the border of the schizocoel. In small broodsacs, free germinal cells, embryos, and germinal masses were still embedded in the parenchymatose matrix.

### 3.2.5 | Seventy-two days post-exposure

At 72 days post-exposure, the central part of the stolon of daughter sporocysts reached 270 × 145 μm. All sporocysts had more than five broodsacs, the largest being 350 μm long. These broodsacs had a lumen connected with the schizocoel of the stolon's central part. The schizocoel cavity was separated from the parietal parenchyma with a lining formed by the cytoplasmic outgrowths of the stellate cells.

There were numerous embryos in the embryonic cavity and in the lumina of the broodsacs. The largest of them were 25 μm in diameter. The size of the germinal masses increased to 50 × 30 μm, about 10 embryos developed in them.

The parenchyma continued to degenerate, thinning and becoming amorphous. Large empty cavities arose in the parenchymatose matrix, and accumulations of pyknotic bodies could be seen. As a result, the germinal material was unevenly distributed in the subtegumental layer (Figure 3E).

### 3.2.6 | Ninety-five days post-exposure

At 95 days post-exposure, the central part of the stolon grew in various directions, reaching 554 × 1,120 μm in size (Figure 3F). The number of broodsacs did not increase but the largest became as long as 675 μm. Lateral outgrowths could be seen in the proximal part of the large broodsacs (Figure 3G).

The sporocyst wall became thinner as a result of degeneration of the parenchyma, and there were no large accumulations of germinal cells there. The reproductive processes of daughter sporocysts proceeded mainly in the embryonic cavity, where germinal masses and metacercarial embryos of various age could be seen. Most of them were early embryos but in some morphogenetic transformation had already started (Figure 4F). These embryos reached 70–80 μm in diameter and consisted of more than 600 blastomeres. It is important to note that the number of free germinal cells in the sporocysts decreased considerably by this time.

Germinal masses were oval, about 90 × 50 μm in size. One germinal mass contained 20–40 developing embryos. The largest of them, reaching 35 μm in diameter, were located closer to the periphery.

## 4 | DISCUSSION

We conducted a study of naturally and experimentally infected individuals of the snail *S. putris* and obtained new data on their infection and on the development and reproduction of parthenitae of the

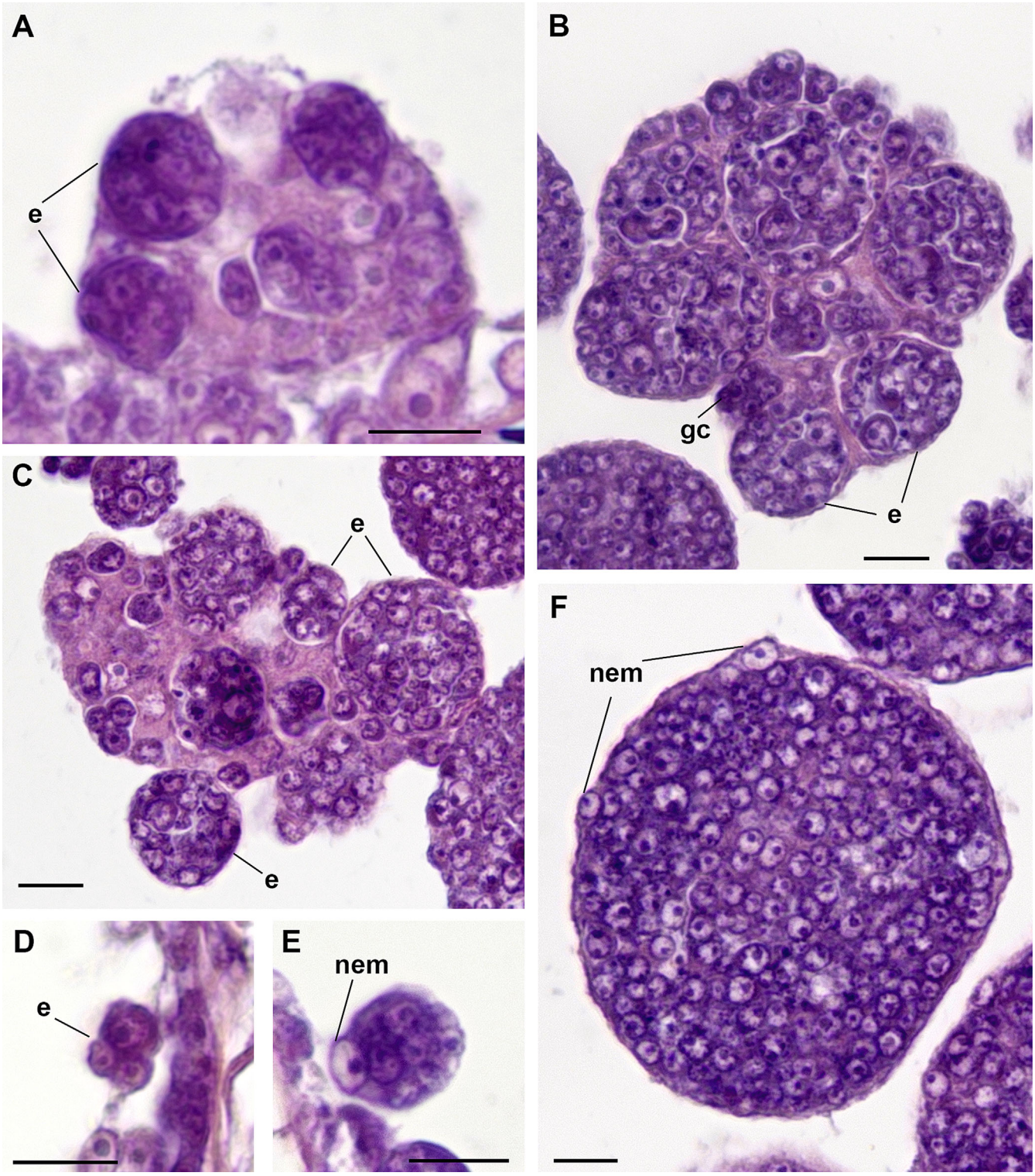


**FIGURE 4** Reproduction of sporocysts of *Leucochloridium paradoxum*. (A–C) Histological sections of germinal masses. (D–F) Histological sections of embryos at various stages of development. Scale bar = 10 μm. e, metacercarial embryos; gc, germinal cell; nem, nucleus of embryonic membrane

trematode *L. paradoxum*. The most important result of our study is the evidence that the branched sporocyst belongs to the daughter generation. Before the analysis of our results, we first discuss the main types of production of mother sporocysts.

Several groups of trematodes differ in the character of reproduction of mother sporocysts (Ataev, 2017; Dobrovolskij & Ataev, 2003). The differences mainly concern the germinal elements present in miracidia (the larvae of mother sporocysts). These elements may be

represented by undifferentiated cells, germinal cells, and embryos, and the combinations are fairly diverse. However, this diversity can be roughly classified into two main types.

In trematodes of the first type, miracidia contain undifferentiated cells of germinal nature, which reproduce at the parasitic phase of the mother sporocyst in the mollusk. During this phase, the proliferation of these cells results in the multiplication of germinal cells. The latter cleave to form the embryos of parthenitae of the daughter generations: mother rediae or daughter sporocysts. The majority of trematodes belongs to this type.

Miracidia of trematodes of the second type lack undifferentiated cells. Their germinal material is represented only by germinal cells and (or) embryos, which complete their development in the mother sporocysts. However, they do not multiply, which means there is no reproduction at the parasitic phase of the mother sporocyst. Functionally, the mother sporocyst acts a brood chamber for the germinal material established during the formation of the larvae in the egg (see Dobrovolskij & Ataev, 2003).

Examples of such pedogenetic miracidia can be found in the families Philophthalmidae (Nollen, 1990; Tikhomirov, 2000) and Cyclocoelidae (Szidat, 1932). In these trematodes, a single mother redia develops in a viviparous miracidium. The redia is injected into the mollusk in the process of infection, while the miracidium dies outside. Another example can be found in trematodes from the families Microphallidae (see Galaktionov & Dobrovolskij, 2003), Plagiorchiidae (Schell, 1965), and Brachylaimidae (Bargues & Mas-Coma, 1991), which are characterized by egg parasitism. After penetrating the mollusk, the miracidia disintegrate, and the released germinal cells develop independently in the hemocoel of the molluscan host.

A common feature of trematodes of the second type is the disintegration of the body of the miracidium in the process of infection of the molluscan host. This means that there is no parasitic phase of the development of the mother sporocyst and that the first generation of the parthenitae is represented by mother redia or daughter sporocysts.

In redioid species, the absence of the parasitic phase of the mother sporocyst can be easily ascertained based on the morphology of the parthenitae of the first generation, which are represented by mother rediae. In sporocystoid species, the generation of the parthenitae cannot be inferred from their morphology. In this case, an experimental study of the early stages of the development of parthenitae after the penetration of the miracidium in the molluscan host is required.

In previous studies of species of *Leucochloridium*, the question about the generation of sporocysts in this genus was usually ignored. In some studies, it was assumed that sporocysts were mother sporocysts (Ginetsinskaya, 1988; Woodhead, 1935), but there was no evidence provided to support this.

In this study, we obtained histological data on the early stages of the development of sporocysts of *L. paradoxum* after experimentally infecting snails with this parasite. We found that miracidia hatch in the alimentary tract of the molluscan host, in the stomach or the proximal part of the midgut. After penetration, these larvae migrate into the area of the hepatopancreas, where their body disintegrates. Germinal cells (presumably one cell from each larva) find themselves in the lumen between the acini and start to develop into an embryonic daughter sporocyst. We traced the development of the sporocysts starting from 7 days post-exposure (when the embryo is at the first stages of cleavage) to 95 days post-exposure (when the stolon is already forming). A common embryonic cavity filled with numerous metacercarial embryos and germinal masses was formed in the central part of the sporocyst and in the broodsacs by this time.

Our results indicate that branching sporocysts with colored broodsacs belong to the daughter generation of the parthenitae. This is the only generation of parthenitae in the life cycle of the trematode *L. paradoxum*.

The following general scheme of daughter sporocyst development is supported by our results. One or two germinal cells are formed in the miracidium, but only one of them develops further. After the dissolution of the larval body, this germinal cell is released and starts to cleave, and a daughter sporocyst is formed as a result. A schizocoel cavity lined with stellate cells is formed inside the embryos in the process of embryogenesis, so that only parietal parenchyma is left. Undifferentiated cells capable of differentiating into germinal cells are located in the parietal parenchyma. Free germinal cells, which are formed inside the parenchymatose matrix, can be seen in it in the beginning of the daughter sporocyst development. The embryos formed by these free germinal cells also develop independently.

Later, the germinal masses become responsible for reproduction. Undifferentiated cells divide there, and germinal cells are formed and start to cleave. Larger metacercarial embryos develop in the embryonic cavity. This process is accelerated owing to the degeneration of the parietal parenchyma. Even the germinal masses are released from the parenchymatose matrix and start to float in the schizocoel of daughter sporocysts alongside embryos of various age.

## 5 | CONCLUSION

Snails *S. putris* become infected with trematodes *L. paradoxum* by ingesting parasite eggs. Miracidia hatch from eggs in the stomach and the midgut of the snail. Through the epithelium of the digestive system, the larvae penetrate into the hepatopancreas, where the soma of the miracidium dissolves. As a result, germinal cells are released and develop into branched daughter sporocysts. The sporocysts reproduce by multiplication of germinal cells. The latter either lie freely in the subtegumentary parenchymatose matrix (free germinal cells) or form part of specialized reproductive organs, germinal masses. Accordingly, germinal cells and embryos of metacercariae can also develop either independently or in germinal masses. As the metacercariae mature, they fill the broodsacs that function as brood chambers in the sporocyst. Mature broodsacs, which become green, contain infective metacercariae.

### ACKNOWLEDGMENTS

The authors are grateful to Natalia Lentsman for her help with the preparation of the English version of the manuscript. The study was

funded by the Grant of Ministry of Education of RF (VRFY-2023-0009).

## CONFLICT OF INTEREST STATEMENT

The authors declare no competing interest.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

## ORCID

*Gennady L. Ataev* https://orcid.org/0000-0002-4740-2117

*Arina S. Tokmakova* https://orcid.org/0000-0003-3202-4827

## REFERENCES

Ataev, G. L. (2017). *Reproduction of trematoda parthenites*. Nauka. https://doi.org/10.21072/978-5-02-039707-1

Ataev, G. L., Dobrovolskij, A. A., & Tokmakova, A. S. (2013). Reproduction of trematode *Leucochloridium paradoxum* sporocysts (Trematoda: Leucochloridiidae). *Parazitologiya*, *47*, 178–182.

Ataev, G. L., & Tokmakova, A. S. (2015). Seasonal changes in the biology of *Leucochloridium paradoxum* (Trematoda, Leucochloridiomorphidae). *Parazitologiya*, *49*, 200–207.

Ataev, G. L., & Tokmakova, A. S. (2018). Reproduction of *Echinostoma caproni* mother sporocysts (Trematoda). *Parasitology Research*, *117*, 2419–2426. https://doi.org/10.1007/s00436-018-5930-7

Ataev, G. L., Zhukova, A. A., Tokmakova, A. S., & Prokhorova, E. E. (2016). Multiple infection of amber snails *Succinea putris* with sporocysts of *Leucochloridium spp*. (Trematoda). *Parasitology Research*, *115*, 3203–3208. https://doi.org/10.1007/s00436-016-5082-6

Bargues, M. D., & Mas-Coma, S. (1991). Intracellular development in digenean sporocyst early stages. *Research and Reviews in Parasitology*, *51*, 111–124.

Bowles, J., & McManus, D. P. (1993). Rapid discrimination of Echinococcus species and strains using a polymerase chain reaction-based RFLPmethod. *Molecular and Biochemical Parasitology*, *57*, 231–240. https://doi.org/10.1016/0166-6851(93)90199-8

Cheng, T. C. (1961). Studies on the morphogenesis, development and germ cell cycle in the sporocysts and cercariae of *Clypthelmins pennsylvaniensis* Cheng, 1961 (Trematoda: Brachyocoeliidae). *Journal of the Pennsylvania Academy of Science*, *35*, 10–22.

Cribb, T. H., Bray, R. A., Littlewood, D. T. J., Pichelin, S. P., & Herniou, E. A. (2011). The Digenea. In D. T. J. Littlewood & R. A. Bray (Eds.), *Interrelationships of the Platyhelminthes* (pp. 168–185). CRC Press.

Dobrovolskij, A. A., & Ataev, G. L. (2003). The nature of reproduction of digenea rediae and sporocysts. In C. Combes & J. Jourdane (Eds.), *Taxonomy, ecology and evolution of metazoan parasites* (pp. 249–272). Université de Perpignan.

Galaktionov, K. V., & Dobrovolskij, A. A. (2003). *The biology and evolution of trematodes*. Kluwer Academic Publisher. https://doi.org/10.1007/978-94-017-3247-5

Ginetsinskaya, T. A. (1954). Questions of ecology and systematics of parthenogenetic generations of flukes of the genus *Leucochloridium*. *Trudy Leningradskogo Obshchestava Estestvoispytatelei*, *72*, 38–56.

Ginetsinskaya, T. A. (1988). *Trematodes, their life cycles, biology and evolution*. Amerind Publishing Private Limited.

Hall, T. A. (1999). BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series*, *41*, 95–98.

Heckert, G. A. (1889). Untersuchungen über die Entwicklungs- und Lebensgeschichte des *Distomum macrostomum*. *Bibliotheca Zoologica*, *1*, 5–66.

Kagan, I. (1952). Revision of the subfamily Leucochloridiinae Poche, 1907 (Trematoda, Brachylaemidae). *American Midland Naturalist*, *48*, 257–301. https://doi.org/10.2307/2422256

Kumar, S., Stecher, G., Li, M., Knyaz, C., & Tamura, K. (2018). MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution*, *35*, 1547–1549. https://doi.org/10.1093/molbev/msy096

Mönnig, H. O. (1922). *Über Leucochloridium macrostomum (Leucochloridium paradoxum Carus), ein Beitrag Zur Histologie der Trematoden*. Gustav Fischer.

Nollen, P. (1990). Escape of rediae from miracidia of *Philophthalmus megalurus* and *Philophthalmus gralli* during invitro culture. *Journal of Parasitology*, *76*, 275–279. https://doi.org/10.2307/3282989

Olsen, P. D., Cribb, T. H., Tkach, V. V., Bray, R. A., & Littlewood, D. T. J. (2003). Phylogeny and classification of the Digenea (Platyhelminthes: Trematoda). *International Journal for Parasitology*, *33*, 733–755. https://doi.org/10.1016/s0020-7519(03)00049-3

Prokhorova, E. E., Usmanova, R. R., & Ataev, G. L. (2020). An analysis of morphological and molecular genetic characters for species identification of amber snails *Succinea putris* (Succineidae). *Invertebrate Zoology*, *17*, 1–17. https://doi.org/10.15298/invertzool.17.1.01

Schell, S. C. (1965). The life history of *Haematoloechus breviplexus* Stafford, 1902 (Trematoda: Haplometridae McMullen, 1937) with emphasis on the development of the sporocysts. *Journal of Parasitology*, *51*, 587–593. https://doi.org/10.2307/3276238

Szidat, L. (1932). Zur Entwicklungsgeschichte der Cyclocoeliden. Der Lebenszyklus von Tracheophilus sisotvi Skrj. *Zoologischer Anzeiger*, *100*, 205–213.

Tikhomirov, I. A. (2000). Microanatomy of the *Philophthalmus rhionica* miracidium (Trematoda: Philophthalmidae). *Parazitologiya*, *34*, 210–219.

Usmanova, R. R., Ataev, G. L., Tokmakova, A. S., Tsymbalenko, N. V., & Prokhorova, E. E. (2023). Genotypic and morphological diversity of *trematodes Leucochloridium paradoxum*. *Parasitology Research*, *122*, 997–1007. https://doi.org/10.1007/s00436-023-07805-7

Woodhead, E. (1935). The mother sporocysts of *Leucochloridium*. *Journal of Parasitology*, *21*, 337–346. https://doi.org/10.2307/3271943

**How to cite this article:** Ataev, G. L., Usmanova, R. R., Vinogradova, A. A., Prokhorova, E. E., & Tokmakova, A. S. (2024). Development and reproduction of sporocysts of *Leucochloridium paradoxum* (Trematoda). *Invertebrate Biology*, e12443. https://doi.org/10.1111/ivb.12443