

Reproductive organs of trematode parthenitae during the cold season: an ultrastructural analysis using evidence from rediae of *Bunocotyle progenetica* (Markowski, 1936) (Digenea, Hemiuroidea)

K.V. Galaktionov¹, I.M. Podvyaznaya²

¹ Zoological Institute RAS, Universitetskaya emb. 1, St Petersburg 199034, Russia;
St Petersburg State University, Universitetskaya emb. 7/9, St Petersburg 199034, Russia.
E-mail: Kirill.Galaktionov@zin.ru, kirill.galaktionov@gmail.com

² Zoological Institute RAS, Universitetskaya emb. 1, St Petersburg 199034, Russia.
E-mail: Irina.Podvyaznaya@zin.ru

ABSTRACT: The ultrastructure of reproductive organs of trematode parthenitae during the cold season was investigated for the first time. We used as material the rediae of a monoxenous hemiuroid digenean *Bunocotyle progenetica* parasitizing intertidal mud snails *Peringia ulvae* at the White Sea. Infected molluscs were collected in hydrological winter (March) from under the ice at a temperature of near-sediment water of -1.5 – 1 °C. The ultrastructural study of the germinal masses and the brood cavity of “winter” rediae revealed substantial differences from their “summer” state described in Podvyaznaya *et al.* (2019). The mitotic activity in the “winter” germinal masses decreased considerably but did not cease completely. Degenerative processes involving germinal cells and young cercarial embryos were enhanced, while the fully formed cercariae, metacercariae and adult flukes in the redial brood cavity were not affected by degeneration. Degeneration products were absorbed and digested by the cells lining the brood cavity, whose ultrastructural characters indicated an intensification of the digestive function. Feeding on their own degenerating germinal elements during the cold season appears to help the rediae to sustain their own life and that of the advanced stages of the hermaphroditic generation contained in them. In addition, the use of internal food resources presumably makes it possible to decrease the parasitic load on the molluscan host organism during the hardships of the cold season, which promotes the survival of the rediae group. Our data suggest that a traditional view that the functional activity of trematode parthenitae stops completely in winter should be reconsidered.

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KEY WORDS: trematode parthenitae, reproduction, germinal mass, ultrastructure, parasite transmission, low temperature effect, White Sea.

Репродуктивные органы партенит трематод в холодный период – ультраструктурный анализ на примере редий *Bunocotyle progenetica* (Markowski, 1936) (Digenea, Hemiuroidea)

К.В. Галактионов¹, И.М. Подвязная²

¹ Зоологический институт РАН, Университетская наб. 1, Санкт-Петербург 199034, Россия; Санкт-Петербургский государственный университет, Университетская наб. 7/9, Санкт-Петербург 199034, Россия. E-mail: Kirill.Galaktionov@zin.ru, kirill.galaktionov@gmail.com

² Зоологический институт РАН, Университетская наб. 1, Санкт-Петербург 199034, Россия. E-mail: Irina.Podvyaznaya@zin.ru

РЕЗЮМЕ. Впервые изучена ультраструктура репродуктивных органов партенит трематод в холодное время года. Материалом послужили редии моноксенной гемипарзитической трематоды *Bunocotyle progenetica*, паразитирующей в литоральных моллюсках *Peringia ulvae* Белого моря. Зараженных моллюсков собирали в период гидрологической зимы (март) из-под льда при температуре пригрунтовой воды – 1.5–1 °С. Ультраструктурное исследование герминальных масс и выводковой полости «зимних» редий выявило значимые различия от их «летнего» состояния, описанного нами ранее (Podvyaznaya *et al.*, 2019). Существенно ослабевает, но не прекращается, митотическая активность внутри герминальной массы. Заметно усиливаются дегенеративные процессы, которые затрагивают генеративные клетки и ранние эмбрионы церкарий, но не более продвинутые в своем развитии стадии (сформированные церкарии, метацеркарии и мариты), находящиеся в выводковой полости. Продукты дегенерации поглощаются и перевариваются выстилающими полость клетками, которые демонстрируют ультраструктурные признаки интенсификации пищеварительной функции. Использование в качестве пищевого субстрата собственных дегенерирующих генеративных элементов, по-видимому, помогает редиям поддерживать на необходимом уровне свою жизнедеятельность и жизнедеятельность содержащихся в них особей гермафродитного поколения в холодное время года. Кроме того, питание за счет внутренних ресурсов позволяет значительно снизить паразитарную нагрузку на организм моллюска-хозяина в неблагоприятный холодный период, что способствует выживанию группировки редий. Полученные данные заставляют пересмотреть устоявшиеся взгляды на полное прекращение функциональной активности партенит трематод в зимний период.

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КЛЮЧЕВЫЕ СЛОВА: партениты трематод, размножение, герминальная масса, ультраструктура, трансмиссия паразитов, влияние низких температур, Белое море.

Introduction

The acquisition of stages parthenogenetically reproducing in molluscs was a key event of digeneans (Trematoda, Digenea) evolution (Galaktionov, Dobrovolskij, 1998, 2003; Cribb *et al.*, 2002; Galaktionov, 2016; Brooks *et al.*, 2019). The “parasitic signal” is strongly amplified during these stages, ensuring further implementation of the complex life cycle along the chain of hosts. The role of reproductive organs in parthenitae is played by the germinal masses, which seem to be a modified ovary of the hermaphroditic ancestor (Dobrovolskij, Ataev, 2003; Galaktionov, Dobrovolskij, 2003; Podvyaznaya, 2007; Podvyaznaya, Galaktionov, 2014). These structures were first found by Cort and his colleagues (see for review Cort *et al.*, 1954), who investigated their structure in parthenitae of various trematode groups (Cort, Oliver, 1943; Cort, 1944; Cort, Ameel, 1944; Ameel *et al.*, 1949; Cort *et al.*, 1949, 1950). Detailed studies of germinal masses and their functioning were conducted at the Department of Invertebrate Zoology of the Leningrad State University starting from the late 1960ies under the supervision of A.A. Dobrovolskij (Dobrovolskij, Raichel, 1973; Gerasev, Dobrovolskij, 1977; Dobrovolskij, Mukhamedov, 1979; Mukhamedov, 1979; Dobrovolskij *et al.*, 1983; Galaktionov, Dobrovolskij, 1985) and were later continued by his disciples (Ataev, Dobrovolskij, 1990; Kofiadi, 1995; Ataev *et al.*, 1997, 2001, 2006, 2007, 2013, 2016; Isakova, 2011; Ataev, Tokmakova, 2015). These studies, carried out at the light microscopic level, indicated that the organisation of germinal masses is generally similar in parthenitae of different trematode taxa (Dobrovolskij *et al.*, 2000; Dobrovolskij, Ataev, 2003; Galaktionov, Dobrovolskij, 2003; Isakova, 2011). According to the results of this research, germinal mass is a compact structure, which is either associated with the body wall of the parthenita (attached to it or inbuilt into it) or floats freely in the brood cavity. It consists of germinal cells, stem (undifferentiated) cells and supporting cells as well as early embryos of the next generation (partheni-

tae or cercariae) until the formation of the primitive epithelium (that is, until the stage of germinal balls). Supporting cells form numerous branching outgrowths enveloping the germinal elements of the germinal mass and ensuring its structural integrity. In addition, they also seem to be “conducting elements” along which nutrients are transported to other components of the germinal mass. Stem cells divide and differentiate either into germinal or into supporting cells. Germinal cells are only capable of cleavage, and give rise to individuals of the next generation. As embryos mature and grow, they rupture the enveloping outgrowths of supporting cells and get out into the brood cavity of the parthenita, where they mature further. Once their development is completed, fully formed cercariae or young parthenitae leave the mother organism through a birth pore or local ruptures of the body wall.

Recently, we conducted the first ultrastructural studies of germinal masses in parthenitae of different morphotypes (sporocysts with modular organisation, rediae of different types) in species from phylogenetically distant trematode families (Podvyaznaya, 2007; Podvyaznaya, Galaktionov, 2014, 2018; Podvyaznaya *et al.*, 2019). These investigations have confirmed the conclusion about a uniform cellular composition of the germinal masses across different digenean groups. At the same time, electron microscopy has shown that the fine structure of germinal masses is considerably diverse, the differences being mostly associated with the structural variation of their supporting tissue as well as the number and age of embryos inside them. Our studies have also demonstrated similarities in ultrastructure between undifferentiated and germinal cells of the germinal masses in sporocysts and rediae of different trematodes. Germinal cells were found to contain organelles typical of animal oocytes such as germ granules (or nuage) and annulate lamellae, which is an additional evidence that they belong to the germline.

With a single exception, which will be discussed below, all studies devoted to the analysis of structure and function of germinal masses

were made in the period of their functional activity, that is, during the warm season. This is the time when groups of parthenitae actively produce cercariae and the latter emerge from the molluscan host (see for review Ginetsinskaya, 1968; Smyth, Halton, 1983; Dobrovolskij *et al.*, 1983; Galaktionov, Dobrovolskij, 1998, 2003). Numerous studies of seasonal dynamics of cercarial emergence have shown that it stops during the cold season (e.g., Werdning, 1969; Robson, Williams, 1970; Lauckner, 1980; Hughes, Answer, 1982; Lemly, Esch, 1984; Schmidt, Fried, 1997; Field, Irwin, 1999; Marcogliese, 2001; Peterson, 2007; Thieltges, Rick, 2006; Prokofiev, 2006). The composition of the groups of parthenitae in the molluscan host also changes in winter (e.g., K oe, 1975; Machkevsky, 1982; Rusanov, Galaktionov, 1984; Ataev, 1991; Taskinen *et al.*, 1994; Abdul-Salam *et al.*, 1997; Sendersky *et al.*, 2002; Kube *et al.*, 2002; Galaktionov *et al.*, 2006; Korniyuchuk, 2008; Levakin, 2008; Averbuj, Cremonte, 2010; Fermer *et al.*, 2010; Prinz *et al.*, 2010; Nikolaev, 2012; Ataev, Tokmakova, 2015). If there are only two generations of parthenitae (mother and daughter sporocysts) in the group, the development of embryos in daughter sporocysts is suspended, while mature cercariae, that have not emerged before the drop of temperature, may die and resorb during the winter (our unpublished data). In self-sustaining groups of rediae/daughter sporocysts (the so-called microhemipopulations or self-sustaining infrapopulations — see Galaktionov *et al.*, 2014, 2015), the character of reproduction may change with the onset of the cold spell: instead of producing cercariae, rediae start to produce rediae of the next generation (Chowaniec, 1961; Bednarz, 1962; Kendall, 1965; Dinnic, Dinnic, 1964; Machkevskiy, 1982; Rusanov, Galaktionov, 1984; Korniyuchuk, 2008). For groups of rediae of *Himasthla* spp. parasitising molluscs *Littorina* spp. at the White Sea, Nikolaev (2012) found that a drop of temperature in autumn resulted in the death of the majority of the mature individuals that have actively produced cercariae during the summer. They are substituted by young individuals from the pool of young parthenitae present in the

group, whose development has previously been retarded. In experiments with molluscs *Melanopsis praemorsa* (Linnaeus, 1758) infected with *Philophthalmus rhionica* Tikhomirov, 1980, Ataev (1991) demonstrated that the rate of embryogenesis of both cercariae and rediae decreased at low water temperature (0–2 °C).

All these materials indicate that the reproduction of parthenitae and the development of their progeny is suspended during the cold season. The functional activity of reproductive organs, the germinal masses, should decrease correspondingly, which was indeed found in a comparative study of “summer” and “winter” germinal masses in daughter sporocysts of *Xiphidiocercaria* sp. VII Odening, 1962, carried out under the supervision of A.A. Dobrovolskij (Mukhamedov, 1979, 1981). In winter the germinal masses of sporocysts of this species are considerably smaller than in summer, there are fewer cercarial embryos forming in them, and these embryos are smaller in size. No dividing cells were found in the winter germinal masses, all the cells were in the interphase. It would seem that in winter the development of the parthenitae is arrested, and they enter the state of hypobiosis (developmental arrest), leaving it only when the water warms up in spring.

Since no studies of fine structure of reproductive organs of parthenitae in the winter period have ever been carried out, we decided to perform such a research. The chosen object of our study was *Bunocotyle progenetica* (Markowski, 1936) Chabaud et Buttner, 1959. This parasite of intertidal molluscs *Peringia ulvae* (Pennant, 1777) (syn. *Hydrobia ulvae* (Pennant, 1777)) and *Ecrobia ventrosa* (Montagu, 1803) (syn. *Hydrobia ventrosa* (Montagu, 1803)) is common at the White Sea. This species has a monoxenous life cycle, with all the phases being confined to one and the same molluscan host. The miracidium, after hatching in the gut of the mollusc that has swallowed an egg of the parasite, penetrates the gut wall and, already in the hemocoel, develops into a large, motile mother sporocyst giving birth to rediae. In the rediae, individuals of the hermaphroditic generation are formed, from early embryos to

adult egg-producing flukes. The eggs are released into the environment after the death of the molluscan host (Levakin, 2008). The germinal mass of rediae of *B. progenetica* is attached to the body wall. Its ultrastructural organisation in the period of active functioning (in summer) was described in our earlier study (Podvyaznaya *et al.*, 2019). We have shown that the germinal mass, the brood cavity of the redia and its birth canal opening with a birth pore on the outer surface of the parthenita, are elements of a well-developed integrated reproductive apparatus, in which the germinal mass is a functional equivalent of the gonad and all the other structures mentioned above play the role of efferent ducts. In the Results section below the attention is mainly focussed on the differences in the organisation of reproductive structures in the “winter” and the “summer” rediae.

Materials and methods

Mud snails *P. ulvae* infected with *B. progenetica* were collected in March 2017 in Sukhaya Salma creek of the Chupa Inlet (the Kandalaksha Bay of the White Sea). The sea was covered with ice at that time, with the water temperature in the intertidal being $-1.5 - -1$ °C (Babkov, 1998). The molluscs together with the sediment were sampled from under the ice and transported to the laboratory of the White Sea Biological Station of the Zoological Institute of the Russian Academy of Sciences. There the mud snails were cleared from the sediment with seawater with a temperature of about 0 °C and dissected under a stereomicroscope. Rediae obtained from infected *B. progenetica* molluscs were immediately fixed and treated for electron microscopic studies following a technique described in Podvyaznaya *et al.* (2019). Thin and semithin sections of rediae were made with the help of a Leica EM UC6rt ultramicrotome. Thin sections were stained with an aqueous solution of uranyl acetate and lead citrate after Reynolds and examined with the help of a Morgagni 268 transmission electron microscope at a voltage of 80 kV. Semithin sections were stained with tolui-

dine blue and viewed under Leica DMLS microscope. For TEM studies of the germinal mass and the brood cavity we selected 1) individuals whose development corresponded to young “summer” rediae studied earlier, that is, whose brood cavity mostly contained germinal balls (3 ind.) as well as 2) mature rediae containing fully formed cercariae, metacercariae and adult flukes (2 ind.).

Results

General structural plan of the germinal mass in all the studied “winter” rediae of *B. progenetica* corresponded to that described before in the “summer” rediae (Podvyaznaya *et al.*, 2019). In both cases the germinal mass contained a few undifferentiated cells, germinal cells at various stages of maturation and supporting cells, which formed the interstitial tissue of the organ and its sheath and lined the internal lacunae (Fig. 1A–D). Germinal masses of the “summer” and the “winter” rediae contained numerous early embryos up to the stage of young germinal balls (Fig. 1A–C). Visible seasonal differences in the structure of the germinal masses mainly concerned physiological state of their elements. For instance, we found mitoses — primarily in separate cells (undifferentiated and mature germinal cells) and sometimes in blastomeres of embryos — in all the studied “winter” individuals (Fig. 2A–C). However, they were found on sections much less frequently (from 3 to 5 per germinal mass in total) than in the “summer” rediae (from 2 to 7 per section). At the same time, in the “winter” parthenitae, as compared with the “summer” ones, the number of degenerating cells and pyknotic bodies in the germinal mass increased considerably, with some parts of the organ being almost completely filled with them (Fig. 1B). Besides separate degenerating cells, germinal masses of all the “winter” rediae examined contained many degenerating embryos, which were especially numerous in mature individuals (Fig. 1C). In young “summer” rediae degenerating embryos in the germinal mass were only sporadically found.

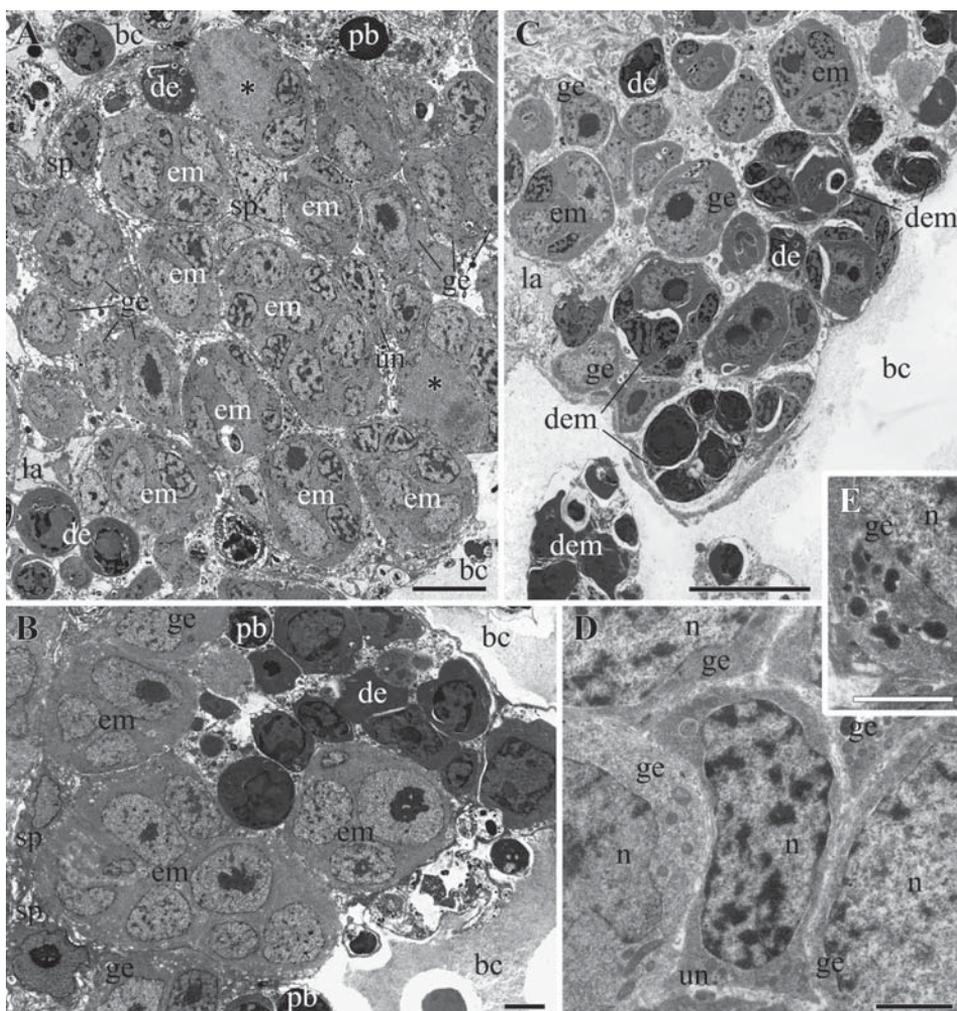


Fig. 1. General structure of the germinal masses in "summer" and "winter" rediae of *Bunocotyle progenetica*. A — TEM section of the germinal mass of the "summer" redia; B — TEM section of the germinal mass of the "winter" redia, containing germinal balls; note numerous degenerating cellular elements; C — TEM section of the germinal mass of the "winter" mature redia; note degenerating cells and embryos inside the organ; D — undifferentiated and germinal cells in the germinal mass of the "winter" mature redia; E — autolysosome-like vesicles in the germinal cell of the "winter" mature redia.

Abbreviations: asterisk (*) — mitotic cell; bc — brood cavity; de — degenerating cells; dem — degenerating embryos; em — embryos; ge — germinal cells; la — internal lacunae of the germinal mass; n — nucleus; pb — pyknotic body; sp — supporting cells; un — undifferentiated cell. Scale bars: A — 5 μ m; B — 2 μ m; C — 8 μ m; D, E — 1 μ m.

Рис. 1. Общее строение герминальных масс у «летних» и «зимних редий *Bunocotyle progenetica*. А — ТЕМ-срез герминальной массы «летней» редии; В — ТЕМ-срез герминальной массы «зимней» редии, содержащей зародышевые шары; отметьте многочисленные дегенерирующие клеточные элементы; С — ТЕМ-срез герминальной массы «зимней» зрелой редии; отметьте дегенерирующие клетки и эмбрионы внутри органа; D — недифференцированные и генеративные клетки герминальной массы «зимней» зрелой редии; E — везикулы похожие на аутолизосомы в генеративной клетке «зимней» зрелой редии.

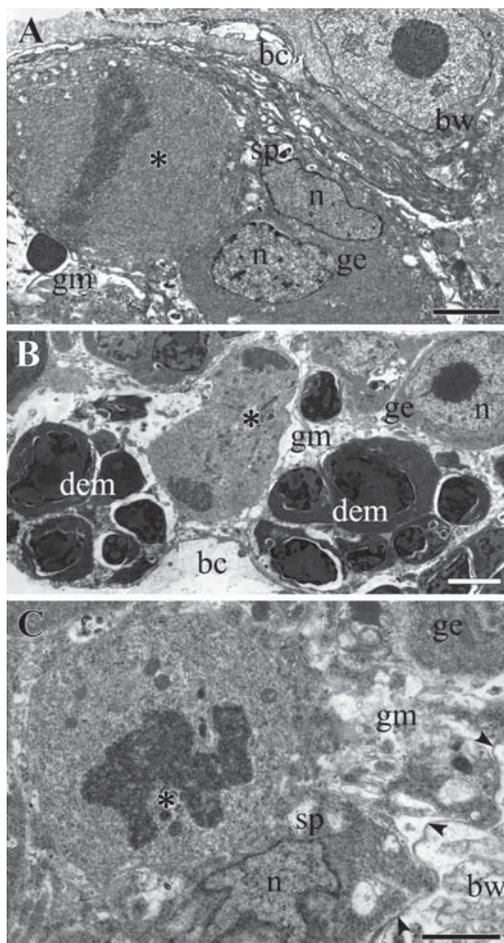
Обозначения: астериск (*) — митотические клетки; bc — выводковая полость; de — дегенерирующие клетки; dem — дегенерирующие эмбрионы; em — эмбрионы; ge — генеративные клетки; la — внутренние лакуны герминальной массы; n — ядро клетки; pb — пикнотическое тело; sp — поддерживающие клетки; un — недифференцированные клетки. Масштабные линейки: А — 5 мкм; В — 2 мкм; С — 8 мкм; D, E — 1 мкм.

Fig. 2. TEM images of mitotic cells in the germinal masses of “winter” rediae of *Bunocotyle progenetica*. A — mitotic division (asterisk) at the periphery of the germinal mass in the “winter” redia, containing germinal balls; B — mitotic division (asterisk) at the periphery of the germinal mass in the “winter” mature redia; C — mitotic division (asterisk) at the base of the germinal mass in the “winter” mature redia; note the basal laminae (arrowheads) separating the germinal mass from the redial body wall.

Abbreviations: bc — brood cavity; bw — redial body wall; dem — degenerating embryos; ge — germinal cells; gm — germinal mass; n — nucleus; pb — pyknotic body; sp — supporting cell. Scale bars: A, B — 2 μ m; C — 1 μ m.

Рис. 2. ТЕМ-изображения митотически делящихся клеток в герминальной массе «зимних» редиий *Bunocotyle progenetica*. А — митотическое деление (астериск) в периферической части герминальной массы редиий, содержащей зародышевые шары; В — митотическое деление (астериск) в периферической части герминальной массы зрелой редиий; С — митотическое деление (астериск) в базальной части герминальной массы зрелой редиий; отметьте базальную пластинку (стрелки), отделяющую герминальную массу от стенки тела редиий.

Обозначения: bc — выводковая полость; bw — стенка тела редиий; dem — дегенерирующие эмбрионы; ge — генеративные клетки; gm — герминальная масса; n — клеточное ядро; pb — пикнотическое тело; sp — поддерживающая клетка. Масштабные линейки: А, В — 2 мкм; С — 1 мкм.



In mature “winter” rediae ultrastructural changes were also noted in the germinal cells unaffected by pyknosis (Fig. 1E). They often contained accumulations of vesicles which looked like autophagosomes and autolysosomes.

Evident seasonal changes were also observed in the contents of the brood cavity and in the structure of its cellular lining (Fig. 3A–E). As compared with young “summer” rediae, the brood cavity of young “winter” ones contained much more cellular debris and many more degenerating early embryos (Fig. 3A, B), including those disintegrating into separate cells. Curiously, fully formed cercariae, metacercariae and adults in the brood cavity of mature “winter” rediae did not show any salient features of degeneration. On the contrary, the adults were

even active and discharged spermatozoa, which were regularly seen in the brood cavity (Fig. 3E), often near the germinal mass.

Perikarya of the cells lining the brood cavity in “winter” rediae (Fig. 3D, E) had a much greater volume than in the “summer” individuals (Fig. 3C) and were filled with numerous residual bodies of different outlook and size, vesicular structures resembling lysosomes and endosomes as well as degenerating cells and pyknotic bodies absorbed from the brood cavity (Fig. 3D, E).

Discussion

Our data indicate that the organisation of reproductive structures in the rediae of *B. pro-*

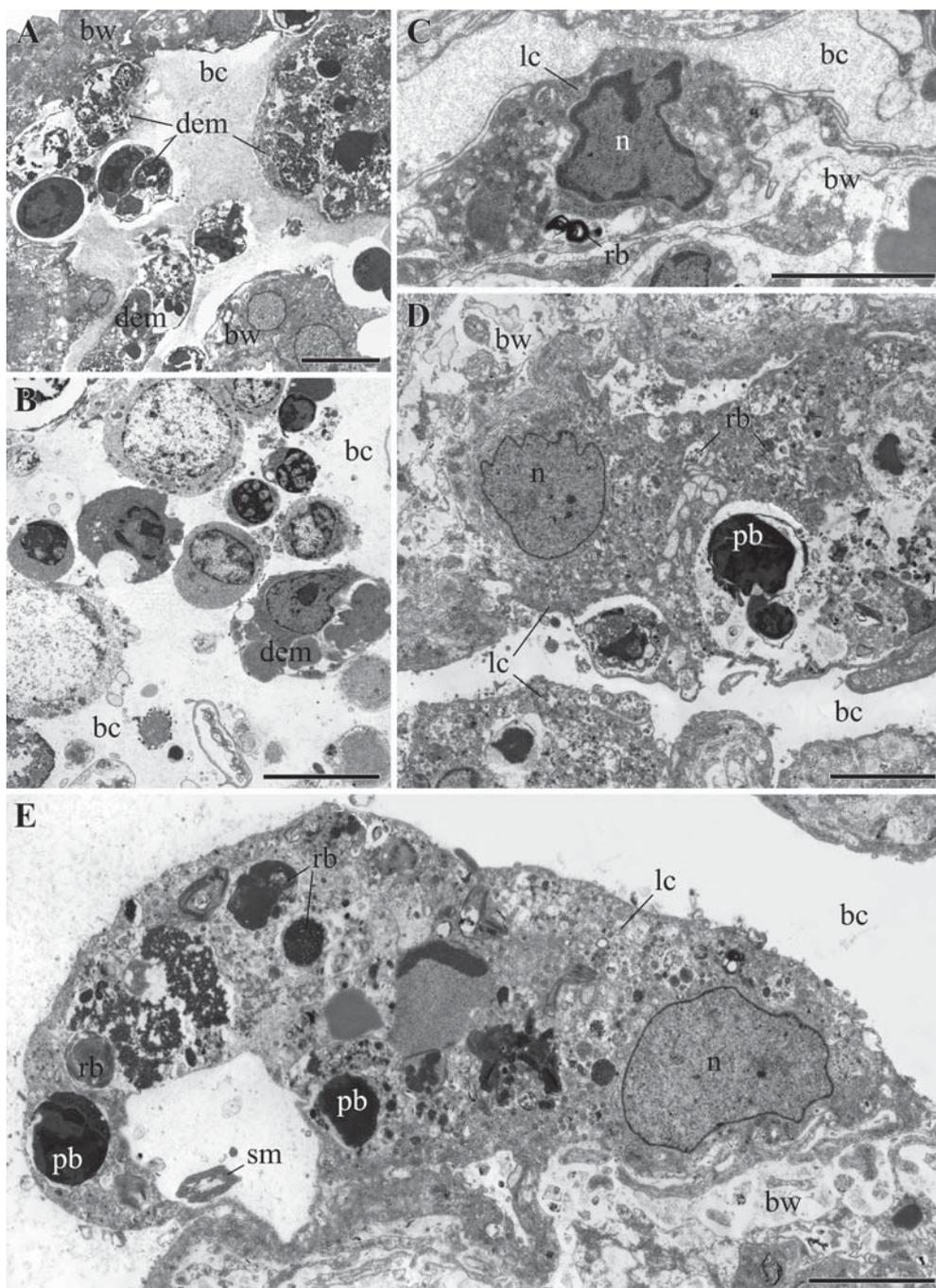


Fig. 3. Brood cavity and its lining in “winter” and “summer” rediae of *Bunocotyle progenetica*. A — degenerating early embryos in the brood cavity of “winter” redia; B — cellular debris in the brood cavity of “winter” redia; C — the cell lining the brood cavity in “summer” young redia; D, E — the cells lining the brood cavity in “winter” mature rediae.

Abbreviations: bc — brood cavity; bw — redial body wall; dem — degenerating embryos; lc — lining cells; n — nucleus; pb — pyknotic body; rb — residual body; sm — sperm cell. Scale bars: A, B — 5 μ m; C — 2 μ m; D, E — 3 μ m.

genetica in the cold season undergoes significant changes as compared with their summer state. Mitotic activity in the germinal masses declines considerably, though it does not cease completely. This means that the reproductive organ continues to function but with a lower intensity. At the same time, there is a noticeable surge of degenerative processes, which now involve not only germinal cells as in “summer” rediae (see Podvyznaya *et al.*, 2019) but also the early embryos, both inside the germinal mass and in the brood cavity. As a result, the number of viable young embryos in “winter” individuals does not increase; it may even decrease as compared with “summer” rediae at the same developmental stage. Products of degeneration that pass from the germinal mass into the brood cavity as well as those that are formed in the cavity itself are absorbed and digested by its lining cells. In “winter” rediae, ultrastructural characters of these cells indicate an intensified digestive function: the volume of their cytoplasm increases dramatically and so does the number of various endosome- and lysosome-like vesicles, residual bodies and absorbed degenerating cells, including pyknotic bodies.

It seems probable that an active utilization of considerable amounts of products of degeneration of embryos and germinal cells in the cold season has, primarily, a trophic function. The use of their own degenerating germinal elements as a food substrate seems to help rediae to sustain at the necessary level their own life activity and that of fully formed cercariae, metacercariae and adults contained in them. The above mentioned stages of the hermaphroditic generation, found in “winter” rediae, have formed in them before the onset of the cold season and are unaffected by the degeneration. It is reasonable to suppose that they should be provided with nutrients in the first place: further

implementation of the life cycle depends on these individuals, and all the systems of rediae are designed to ensure their normal development and reproduction in the adult phase. The number of adults in mature rediae in infected *P. ulvae* does not exceed 10–15 (our data), and when they mature, embryos that lagged behind in their development are resorbed anyway, and so are even fully formed cercariae (Levakin, 2008).

An enhancement of degenerative processes in winter and, thus, an increased role of internal resources in the feeding of rediae and advanced stages of hermaphroditic generation, also makes it possible to considerably decrease the parasitic load on the host organism during the adverse cold season. This idea is supported by experimental data of Levakin (2005, 2008) on the survival of “winter” molluscs *P. ulvae* (collected in March from under ice at the White Sea). The author showed that the infection with *B. progenetica* did not influence the mortality rate of molluscs at low temperatures (below zero °C) and suggested that this was associated with a seasonal decrease of the parasitic load on the host organism (Levakin, 2005, 2008).

There is nothing unusual in the degeneration of germinal elements of parthenitae. In some degree it is observed in almost all sporocysts and rediae whose development has been studied (Dobrovolskij *et al.*, 1983; Galaktionov, Dobrovolskij, 2003). Furthermore, degeneration of some cells resulting in the formation of pyknotic bodies is observed during the development of any trematode embryo. The products of the degeneration are probably used as food both by parthenitae themselves and by the embryos. Kofiadi (1995) observed a more active degeneration of early cercarial embryos in winter and at certain stages of ontogenesis in daughter sporocysts of Hemiuridae gen. sp. parasitizing the

Рис. 3. Выводковая полость и ее выстилка у «зимних» и «летних» редий *Bunocotyle progenetica*. А — дегенерирующие ранние эмбрионы в выводковой полости «зимней» редии; В — клеточный дебрис в выводковой полости «зимней» редии; С — клетка выстилки выводковой полости молодой «летней» редии; D, E — клетки, выстилающие выводковую полость «зимней» зрелой редии. Обозначения: bc — выводковая полость; bw — стенка тела редии; dem — дегенерирующие эмбрионы; lc — выстилающие клетки; n — клеточное ядро; pb — пикнотическое тело; rb — остаточное тело; sm — спермий. Масштабные линейки: А, В — 5 мкм; С — 2 мкм; D, E — 3 мкм.

caenogastropode *Nucella lima* (Gmelin, 1791). He considered it as an adaptation to surviving adverse environmental conditions as well as an additional adaptation to prolongation of the individual life of the daughter parthenitae.

Our data do not agree quite well with those of Mukhamedov (1979), who did not find any mitoses in the winter germinal masses of *Xiphidiocercaria* sp. VII. At the same time, this author noted that there were dividing cells in cercarial embryos, though their number was infinitesimal as compared to the summer state (dividing cells made up 0.15% of the total number of cells in the embryos) (Mukhamedov, 1979). To note, daughter sporocysts of *Xiphidiocercaria* sp. VII can produce only cercariae, which do not leave the parthenitae in winter, and their transmission to the second intermediate host is impossible. There are no such limitations in the transmission of *B. progenetica*, with its monoxenous life cycle. Therefore, a certain continuation of the development in winter makes sense, as far as physiological mechanisms allow. That such a continuation indeed takes place is indicated by our data presented here as well as by the results of detailed studies of seasonal dynamics of the composition of groups of parthenitae of *B. progenetica* (Levakin, 2008). Comparison of their composition in late autumn (November), winter (March) and early spring (May) showed that some growth and development of mother sporocysts and adults in mature groups of rediae occurs in winter, too (Levakin, 2008).

In conclusion, our data on the ultrastructure of reproductive organs in the rediae of *B. progenetica* make one reconsider the traditional view that the functional activity of parthenitae stops completely in the winter period. At least in some species it may be sustained, albeit in a strongly curtailed version, during the cold season, too.

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