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# A comparison of oocyte development pathways in athecate and thecate hydroids

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ABSTRACT: The differentiation of germ cells in most bilaterians occurs once during embryogenesis, while in primitive multicellular organisms gamete differentiation can occur continuously in their adult life. Germ cells in colonial hydroid polyps, for example, are believed to undergo continuous differentiation from interstitial stem cells (i-cells) during reproductive periods throughout their lifespan. However, the exact time and sites of germ cell differentiation remain an intriguing question in evolutionary research. In some colonial hydroids, the reproductive zooids develop only during the reproductive period. In others, medusae or gonophores (reduced medusae) develop directly on the feeding polyps (gastrozooids). For such species, the specific zone of gamete differentiation remains unknown. At the same time, the migration of gametes or their precursors along the coenosarc (the soft body of the colony) to the areas of reproductive zooid formation has been shown for some species of the thecate hydroids. A closely related question is the relationship between the processes of gamete differentiation and the initiation of reproductive structure development. Applying light microscopy, transmission electron microscopy and immunocytochemical methods, we followed the appearance and fate of the female germ cells in two colonial hydroid species. In thecate *Gonothyraea loveni* the growing oocytes migrate along the coenosarc of the shoots towards developing reproductive zooids. It has been experimentally confirmed that the initiation of the development of *G. loveni* reproductive zooids is independent from the presence of oocytes in their tissues. However, the presence of oocytes in reproductive zooid tissues is necessary for the completion of the normal development of these structures. The normally developing oocytes of athecate *Clava multicornis* are first identified in the gastrodermis of the hydranth body wall protrusions, which are the rudiments of gonophores. The abnormal oocytes for the first time were found in *C. multicornis* gastrozooid pedicels. Their investigation allowed to assume that the gamete precursors of *C. multicornis* migrate along the hydranth body to the sites of reproductive structure development, similar to the behavior of oocytes in *G. loveni*. Moreover, there is reason to assume that the differentiation and initial growth of *C. multicornis* oocytes can occur independently of the development of reproductive structures. While the reproductive structures development in *C. multicornis* depends on the presence of oocytes at the sites of their formation.

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KEY WORDS: *Clava multicornis*, *Gonothyraea loveni*, germ cells, ectopic oocytes, oocyte migration, reproductive zooids.

# Сравнение путей развития ооцитов у атекатных и текатных гидроидов

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РЕЗЮМЕ: Дифференцировка половых клеток у большинства билатеральных животных происходит однократно в эмбриогенезе, в то время как у низших многоклеточных организмов дифференцировка гамет может происходить непрерывно в течение всей взрослой жизни. Например, считается, что половые клетки колониальных гидроидных полипов дифференцируются из интерстициальных стволовых клеток (i-клеток) в репродуктивные периоды на протяжении всей их жизни. Однако точное время и места дифференцировки половых клеток остаются интригующим вопросом в эволюционных исследованиях.

У некоторых колониальных гидроидов репродуктивные зооиды развиваются только в репродуктивный период. У других медузы или гонофоры (редуцированные медузы) развиваются непосредственно на питающих полипах (гастрозооидах). Для таких видов специфическая зона дифференцировки гамет остаётся неизвестной. В то же время, для некоторых видов текатных гидроидов показана миграция гамет или их предшественников вдоль ценосарка (мягкого тела колонии) к местам формирования репродуктивных зооидов. Тесно связанным вопросом является взаимосвязь между процессами дифференцировки гамет и инициацией развития репродуктивных структур. Используя световую микроскопию, просвечивающую электронную микроскопию и иммуноцитохимические методы, мы проследили судьбу женских половых клеток у двух видов колониальных гидроидов. У текатного гидроида *Gonothyraea loveni* растущие ооциты мигрируют вдоль ценосарка побегов к развивающимся репродуктивным зооидам. Экспериментально подтверждено, что инициация развития репродуктивных зооидов *G. loveni* не зависит от наличия ооцитов в их тканях. Однако наличие ооцитов в тканях репродуктивных зооидов необходимо для завершения нормального развития этих структур. Нормально развивающиеся ооциты у атекатного гидроида *Clava multicornis* первоначально могут быть идентифицированы в гастродермисе выступов стенки тела гидранта, которые являются зачатками гонофоров. Впервые в ножках гастрозооидов *C. multicornis* были обнаружены аномальные ооциты. Их исследование позволило предположить, что предшественники гамет *C. multicornis* мигрируют вдоль тела гидранта к местам развития репродуктивных структур, аналогично поведению ооцитов *G. loveni*. Более того, есть основания предполагать, что дифференцировка и начальный рост ооцитов *C. multicornis* могут происходить независимо от развития репродуктивных структур. В то время как развитие репродуктивных структур у *C. multicornis* зависит от наличия ооцитов в местах их формирования.

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КЛЮЧЕВЫЕ СЛОВА: *Clava multicornis*, *Gonothyraea loveni*, половые клетки, эктопические ооциты, миграция ооцитов, репродуктивные зооиды.

## Introduction

The germline stem cells (GSCs) of many bilaterians arise once during embryogenesis from precursor cells (primordial germ cells, PGCs). The primordial germ cells undergo the stage of migration to presumptive gonads, where they differentiate into GSCs: oogonia or spermatogonia (Gilboa, Lehmann, 2004; Lacham-Kaplan, 2004; Richardson, Lehmann, 2010; Grimaldi, Raz, 2020). At the same time, the question of a GSC lineage existence remains open for representatives of primitive multicellular animals, for example, for representatives of Cnidaria, which constitute a sister group of Bilateria.

According to recent studies, the existence of an independent GSC lineage, which differentiates during early embryogenesis, has been shown for the solitary polyp *Hydra* (Nishimiya-Fujisawa *et al*., 2023). However, in colonial hydroids, germ cells do not segregate once during development, but differentiate from multipotent interstitial stem cells (i-cells). For example, for the athecate colonial hydroid *Hydractinia symbiolongicarpus* (Anthoathecata), it has been shown that during the lifespan of an adult colony, the germ cells differentiate from the progeny of the pluripotent i-cells, which also give rise to the somatic cells (DuBuc *et al*., 2020). *Hydractinia* colonies are characterized by zooid polymorphism, and the germ cells are first detected in a specific zone, the "neck" of the reproductive zooids (gonozooids), called the "germinal" zone (Frank, 2009; Calder, 2017; Varley *et al*., 2023; Curantz *et al*., 2025). However, for athecate colonial hydroids without zooid polymorphism, whose colonies consist exclusively of feeding polyps (gastrozooids), the site of germ cells differentiation remains unknown.

For the thecate hydroids (Leptothecata), whose reproductive zooids (blastostyles) exist temporarily, developing exclusively during the period of sexual reproduction, the localization of differentiating gamete precursors has not been studied until now. Nevertheless, in *Gonothyraea loveni*, oocytes (de Varenne, 1882; Weismann, 1883; Aisenstadt, Polteva, 1981) and spermatozoid precursors (de Varenne, 1882; Weismann, 1883; Donakov, 1988) were described as migrating along the coenosarc of the shoots, apparently towards the blastostyles.

The relationship between germ cell differentiation and the development of the reproductive structures of the colony remains the intriguing question. Experiments on *Hydractinia* polyps have shown that the presence of a "germinal" zone induces the development of gonophores, as germ cells secrete the Gls (Gonadless) morphogen (Curantz *et al*., 2025). At the same time, there is no obvious answer to the question of induction of the development of reproductive structures by germ cells for the thecate hydroids that most likely do not have a "germinal" zone and whose reproductive zooids are formed temporarily. Moreover, the presence of oocytes in the coenosarc of colonies even before the appearance of reproductive zooid buds has been described (de Varenne, 1882; Weismann, 1883).

In the present study, we analyzed abnormal oocytes found in a colony of a White Sea athecate hydroid without zooid polymorphism: *Clava multicornis* (Forsskål, 1775), taken from its natural habitat. These large oocytes visible to the naked eye were detected for the first time in the basal (cylindrically shaped) parts of the hydranth bodies: hydranth pedicels at a considerable distance from the area of gonophore formation, whereas they are usually observed in this way only within the gonophores (reduced medusae). *C. multicornis* is a colonial athecate hydroid that forms compact colonies on fucoid algae, which consist of densely clustered feeding hydrants (gastrozooids). During the reproductive period, numerous fixed gonophores develop on gastrozooids, just below the tentacles (Edwards, Harvey, 1975; Antsulevich, 2015). Gonophores are highly reduced: they do not have tentacles or radial channels; the manubrium is reduced to a blindly ending gastrodermal outgrowth (spadix) (Weismann, 1883; Goette, 1907). In the research literature, highly reduced fixed gonophores, such as those formed in *C. multicornis*, are sometimes called "gonads" because they only perform the function of gonads (Freeman, 1987; Boero, 1992). We used the term "ectopic oocytes" to refer to abnormal oocytes in the hydranth pedicels because of their "extragonadal" location. This was done by analogy with the term "ectopic germ cells" used to refer to extragonadal germ cells that are also capable of differentiation into oocytes outside the gonads in animals with true gonads (Upadhyay, Zamboni, 1982; Ewen, Koopman, 2010). Ectopic and normally developing oocytes in *C. multicornis* were studied using light and electron microscopy. At the same time, it has been shown using the immunocytochemical approach that normally developing oocytes can be unequivocally detected in the gastrodermis of the hydranth body wall protrusions, which are early stages of the gonophores development. Moreover, gonophores rudiments that do not contain oocytes were not detected.

To compare the mutual influence of germline cells and reproductive structures in two main groups of colonial hydroids, a series of experiments were performed with the thecate hydroid *Gonothyrarea loveni*. This is a colonial thecate hydroid with shoots (hydrocaulus with hydrocladia (Bouillon *et al*., 2006)) bearing numerous hydranths (gastrozooids: feeding zooids). Gonozooids (reproductive zooids bearing gonophores) develop sequentially on the hydranth pedicels during the reproductive period only, and consist of a blastostyle bearing up to 5-6 gonophores along its one side. Each gonozooid with gonophores is assembled into a specific structure covered by a rigid theca and is called gonangium (Miller, 1973; Marfenin, Kosevich, 1984; Antsulevich, 2015). Applying histological and experimental methods, it was confirmed that oocytes in the thecate hydroid *G. loveni* migrate along the shoot coenosarc towards the developing reproductive zooids. During that migration, oocytes grow. Analysis of the obtained data has allowed us to assume that the process of initiating gonangia development in *G. loveni* is to a certain extent independent from oocytes migrating to the areas of gonangia development. Finally, it has been suggested that ectopic oocytes in *C. multicornis* may constitute evidence of oocyte precursor migration along the hydranth bodies in this species too, which is reminiscent of oocyte migration along the coenosarc in the thecate hydroid polyps.

## Material and methods

MATERIAL COLLECTION. The material was collected from the tidal zone of the Eremeevsky rapids between June and July 2018 near the N.A. Pertsov White Sea Biological Station of the Biological Faculty of the M.V. Lomonosov Moscow State University (WSBS MSU) (66° 34′ N, 33° 08′ E).

INVESTIGATED SPECIES. *Clava multicornis* (Forsskål, 1775) (Anthoathecata: Hydractiniidae) is an Atlantic boreal-arctic species of colonial athecate hydroids. This species inhabits the lower part of the littoral zone of the White Sea. Stolonial colonies consist of morphologically identical hydranths called gastrozooids and grow on some species of algae, mainly *Ascophyllum nodosum* and *Fucus serratus*. The body of each gastrozooid consists of an upper, spindle-shaped part that bears tentacles and a lower, cylindrical part called the hydranth pedicel. During the reproductive period, gonophores develop on the hydranths just below the tentacle zone (Fig. 1A, B). The maturation of gametes, as well as the fertilization and development of larvae (planulae), occur inside the gonophores (Naumov, 1960; Schuchert, 2008; Antsulevich, 2015). Most hydroids are dioecious (Bouillon *et al*., 2006). However, for this species, not only the presence of hermaphroditic zooids (that have both female and male gonophores simultaneously), but also intersexual gonophores have been described (Burmistrova, Kosevich, 2022). About 100 female colonies of *C. multicornis* were studied, including one colony containing hydranths with ectopic oocytes. After in vivo observation, these hydranths were fixed for histological and ultrastructural studies using light microscopy and transmission electron microscopy (TEM).

*Gonothyraea loveni* (Allman, 1859) (Leptothecata: Campanulariidae) is a boreal-arctic species of colonial thecate hydroids. In the White Sea, it is found in the upper part of the sublittoral zone on brown algae of the genera *Fucus* (*F. inflatus*, *F. serratus*) and *Laminaria* spp., as well as on rocks, mollusk shells, and other hard substrates. *Gonothyraea loveni* is a thecate colonial hydroid with shoots (hydrocaulus with hydrocladia (Bouillon *et al*., 2006)) bearing numerous hydranths (gastrozooids, i.e., feeding zooids). New shoots appear regularly in the course of the elongation of stolons, which are the tubular colony parts growing along the substrate and attaching the colony to the substrate (Marfenin, Kosevich, 1984). Gonozooids (reproductive zooids bearing gonophores) develop only during the reproductive period practically on all shoots of the colony, except the smallest (the youngest) ones (Fig. 1C, D). Gonozooids appear sequentially on the hydranth pedicels, starting from the base of the shoot stem and proceeding towards the tips of its branches, and consist of a blastostyle bearing up to 5–6 gonophores along its one side (Miller, 1973; Marfenin, Kosevich, 1984; Antsulevich, 2015). Blastostyles surrounded by a rigid exoskeleton (gonotheca) are called gonangia (Fig. 2) (Bouillon *et al*., 2006). The maturation of germ cells, as well as the fertilization and planula-larva development, take place in gonophores (Marfenin, Kosevich, 1984; Burmistrova *et al*., 2018). During unfavorable seasons, the colony survives in dormancy in the form of stolons. In most cases, the colony rebuilds after dormancy by forming new shoots on old stolons and by growing new stolons (based on personal observations). About 150 female colonies of *G. loveni* were studied.

LIGHT MICROSCOPY AND ELECTRON MICROSCOPY STUDIES. To prepare the samples

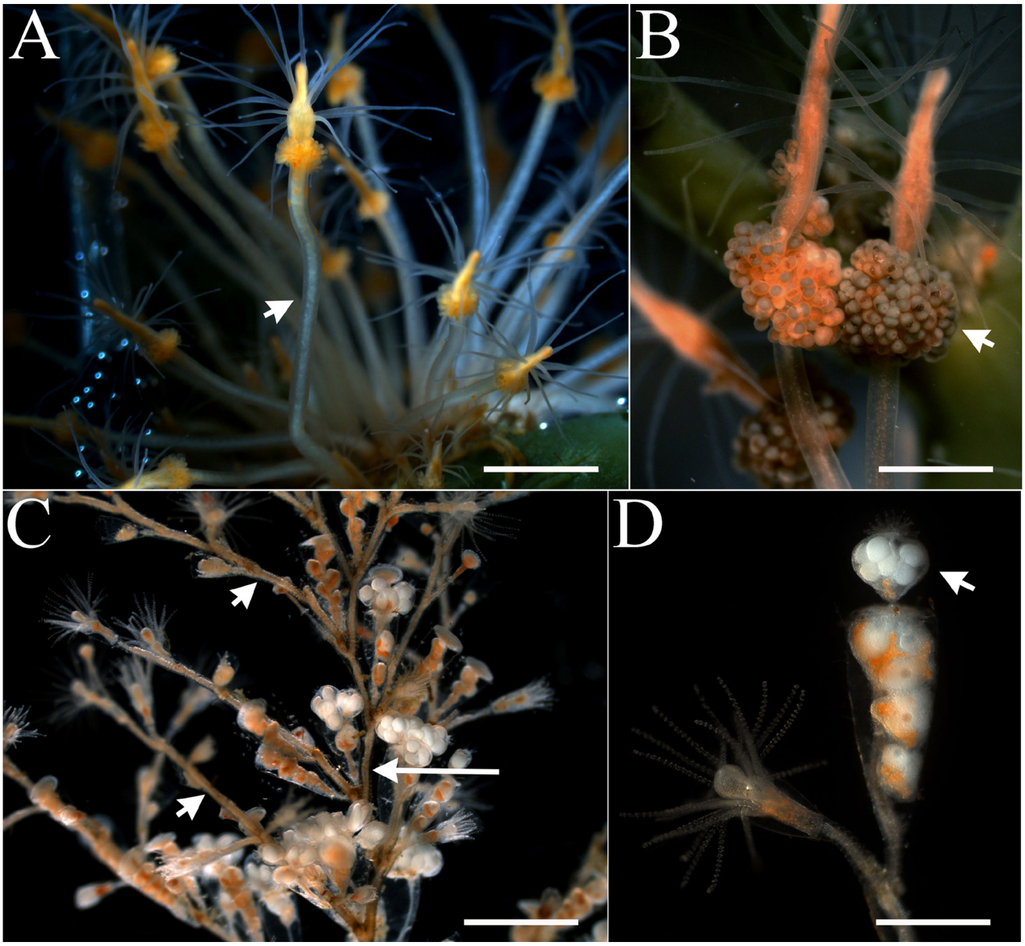

Fig. 1. Female colonies of *Clava multicornis* and *Gonothyraea loveni* during reproductive period. A — colony of *C. multicornis.* Arrow points to the hydranth pedicel; B — hydranths of *C. multicornis* with gonophores. Arrow points to the gonophores; C — shoot of the colony of *G. loveni.* Long arrow points to the shoot stem. Short arrows point to the shoot branches; D — gonangium of *G. loveni* contains gonophores. Arrow points to the gonophore with embryos.
Scale bars: A — 2.5 mm; B, C — 1.5 mm; D — 0.5 mm.

for light microscopy and electron microscopy studies, fragments of colonies were anesthetized in a solution of 2.5% $MgCl_2*6H_2O$ in seawater and fixed with 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) (Millonig, 1964) for 12 hours at 4 °C. The samples were postfixed in 1% osmium tetroxide (OsO4) in the same buffer at room temperature for 1 hour. After washing with the same buffer, the material was dehydrated in an ascending series of ethanol concentrations (10, 20, 30, 40, 50, 60, and 70%). The material was then stored at 4 °C in 70% ethanol until further processing. Before being embedded in Epon-Araldite resin (Epon and Araldite resins (SPI-Pon TM 812 kit, SPi-Chem)) in accordance with the manufacturer's recommendations, the material was dehydrated in ethanol and acetone at 4 °C.

Semi-thin (1 μm thickness) sections were prepared using an LKB III Ultratome. The sections were stained with a mixture of methylene blue and toluidine blue (Mironov *et al*., 1994). The ultrathin sections (70 nm thickness) were prepared using a Leica UC-6 Ultratome, and contrasted with solutions of uranyl acetate (Hall, 1995) and lead citrate (Reynolds, 1963).

CONFOCAL LASER SCANNING MICROSCOPY (CLSM). For immunocytochemical studies, the hydranths of *C. multicornis* with young gonophore buds were cut from the colony, anesthetized, and fixed with 4% paraformaldehyde (PFA (Fluka,

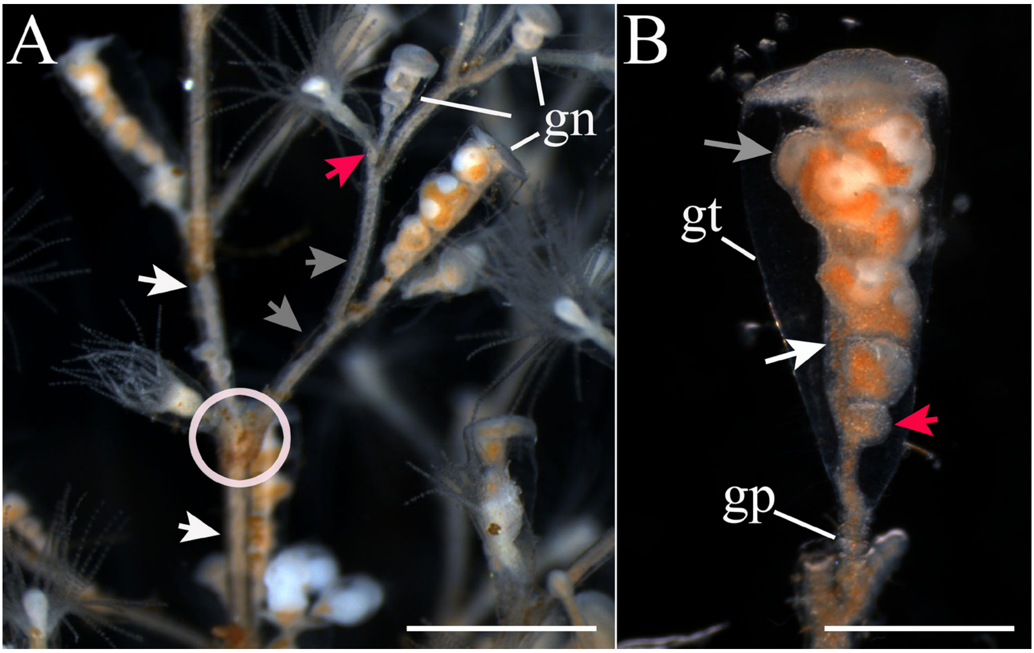

Fig. 2. Female colony of *Gonothyraea loveni* with gonangia. A — part of the shoot. The circular area points to the node of the shoot stem. White arrows point to the internodes of the shoot stem. Grey arrows point to the internodes of the shoot branches. Magenta arrow points to the hydranth pedicel; B — gonangium with gonophores. White arrow points to the blastostyle. Magenta arrow points to the youngest gonophore bud. Grey arrow points to the oocyte.
Abbreviations: gn — gonangia; gp — gonangium pedicel; gt — gonotheca (a rigid exoskeleton that surrounds blastostyle). Scale bars: A — 1.3 mm; B — 0.5 mm.

Germany)) in phosphate buffer (PBS; Sigma, USA) (0.1 M, pH = 7.4) at 4 °C for 12 hours. After fixation, the samples were washed in PBS with 0.1% Triton X-100 (Ferak Berlin, Germany) (PBS (Tr)) three times, for 2 hours each time. Before incubation in a primary antibody mixture, the samples were washed three times in blocking solution (5% bovine serum albumin (BSA; Sigma), 0.3% Triton X-100; 0.05% Tween-20 (Sigma) and 0.05% sodium azide (Sigma) in PBS), for 2 hours each time.

Antibodies (primary and secondary) were diluted in blocking solution. The samples were incubated in primary antibodies (mouse monoclonal antibodies against tyrosinated α-tubulin (1:1000; Sigma, Germany, Cat #T9028) and mouse monoclonal antibodies against acetylated α-tubulin (1:2000; Sigma, USA, Cat #T6793)) for 48 hours at 4 °C. Following washing with blocking solution three times, for 4 hours each time, the samples were incubated in a mixture of secondary antibodies (donkey anti-mouse antibodies labelled with Alexa Fluor 555 (1:500; Molecular Probes, Cat #A-31570) and donkey anti-rabbit antibodies labelled with Alexa Fluor 647 (1:500; Molecular Probes, Cat #A10040)) for 24 hours at 4 °C. After that, the material was washed with blocking solution three times at 4 °C, for 2 hours each time, and was rinsed with PBS solution with 0.3% Triton twice, for 30 minutes each time. The samples were then stained with DAPI nuclear dye (Sigma-Aldrich, Cat #D9542) for 1 hour and were washed with PBS solution with 0.3% Triton twice for 15 minutes.

The samples were mounted onto coverslips coated with poly-L-lysine (Sigma-Aldrich, USA, Cat #LPLL001), dehydrated in an ascending series of isopropanol concentrations (70, 85, 95, and 100%) for 30 seconds in each concentration, and embedded in Murray's clear (a mixture of benzyl benzoate and benzyl alcohol in a ratio of 2:1; Sigma-Aldrich, USA) (Dent *et al.*, 1989). Negative controls included specimens processed without incubation in primary antibodies. The autofluorescence control was prepared without the addition of secondary antibodies.

EXPERIMENTS with *Gonothyraea loveni*. To test the relationship between gonophore formation and the presence of gamete precursors in the developing gonophore, two series of experiments were performed. For experiments, the shoots with developing gonangia were isolated from the colonies collected from the natural environment within 24 hours of collection.

1. Gonangia in the initial stages of formation (when they could be distinguished from the developing hydranth bud) and located most distally on the shoot stem or branch were isolated from the shoots with a minimal part of the shoot node by cutting off the proximally and distally located parts of the shoot. Preliminary studies showed that at that stage of gonangium development (the growing blastostyle has no sign of gonophore rudiments), the oocytes were found only in the coenosarc of the shoot internodes. After isolation, further development of the gonangium was provided by the available volume of tissue, which did not fundamentally affect the course of gonangium development (Kosevich, 1991).

2. In this series of experiments, gonangia at the same stages of their development were cut off from the proximal part of the shoot with the rest of the distally located shoot internodes. This prevented gamete precursor migration from the proximal parts of the shoots, while the possibility of the presence and migration of oocytes already present in the distally located internodes was preserved.

For each series, 10 isolated gonangia, or fragments of shoots containing gonangia, were placed into a 60-mm-diameter plastic Petri dish containing 20 ml of natural seawater. The results of gonangia development were recorded on the second and third days. Each experiment was repeated three times.

OBTAINING AND STUDYING IMAGES OF LIVING AND FIXED MATERIAL. Living colonies were studied and photographed using a Leica M165C stereomicroscope (Leica, Germany) equipped with a Leica DFC 420 digital camera (5.0 MP). Semi-thin sections were analyzed using a Leica DM 2500 microscope with a built-in Leica DMC 2900 digital camera. Ultrathin sections were studied using a JEOL JEM-1011 electron microscope (Japan) equipped with an ORIUS SC1000W digital camera (accelerating voltage 80 kV; camera control program: Digital Micrograph (GATAN)). ICC-stained samples were studied with a Nikon A1 laser confocal microscope (Tokyo, Japan) at the WSBS MSU Microscopy Center.

The primary images were processed using Adobe Photoshop CS2 version 9.0 (Adobe Inc.) and ImageJ 1.43 software. Brightness and contrast corrections were applied to the entire image.

## Results

### *Clava multicornis*

During the reproductive period, the development of gonophores in colonies of *C. multicornis* starts with the emergence of cone-shaped protrusions from the hydranth body wall beneath the tentacle zone. Gonophores appear on all hydranths of the colony, including very young, recently formed hydranths (Fig. 3A, B). As the gonophore bud develops, it divides into a “pedicel” and a “bud”. At the early stages of female gonophore development, when the “bud” just begins expanding, growing oocytes become clearly visible inside it (Fig. 3C, D).

ECTOPIC OOCYTES. Among the collected *C. multicornis* colonies, we found one that differed from any others we had ever observed. In this colony, oocytes could be seen with the naked eye in the body wall of some hydranths due to their bright yellow color (Fig. 4). Oocytes of comparable size are usually distinguishable only in mature gonophores, but are typically gray or gray-blue in color, while yellow coloration is typical of small growing oocytes, as well as planulae. The oocytes had different shapes: some were round, while others were strongly elongated. The elongated oocytes reached the length of 340 μm. Observing the state of the “abnormal” colony for a week revealed no changes in the localization, shape, or size of the oocytes. However, by the end of the observation period, the oocytes began to degrade.

HISTOLOGY AND IMMUNOCYTOCHEMICAL STUDY OF FEMALE HYDRANTHS OF *C. MULTICORNIS*. In histological sections of female hydranths, oocytes are usually found at the base of young gonophore buds. They are located in the gastrodermis of the hydranth body wall evagination tightly adjoining to the mesoglea. Young oocytes are clearly distinguishable from the other gastrodermal cells due to their rounded shape, darker cytoplasm, large nucleus with a distinct nucleolus occupying approximately half the cell volume, as well as overall size (10–25 μm in diameter) (Fig. 5A–C). The epidermis of the protrusion does not differ in thickness from the epidermis of the hydranth body wall in the gonophore budding zone and is about 25 μm thick. However, as the protrusion expands, the epidermis of its distal part (the epidermis of the developing gonophore) becomes thinner (Fig. 5B). The epidermis of the hydranth body wall below the gonophore budding zone is thinner than the epidermis of the hydranth body wall in the gonophore budding zone and is about 20 μm thick. Immunocytochemical staining of hydranths with young gonophore buds clearly visualizes oocytes in the gastrodermis of the developing buds as rounded cells with an unstained nucleus (Fig. 5D, E).

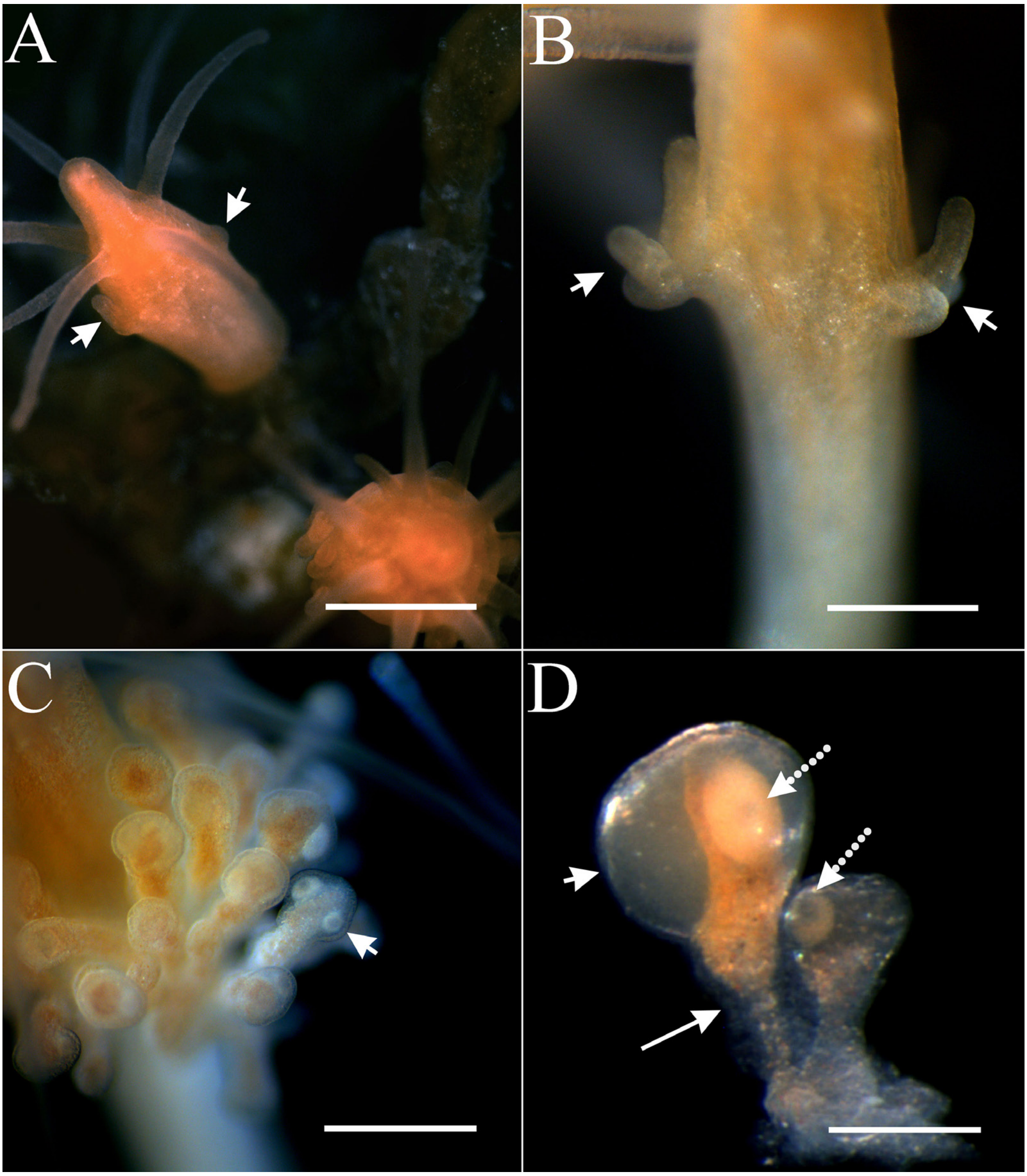

Fig. 3. Hydranths and gonophores of *Clava multicornis*. A — young hydranths with developing gonophores. Arrows point to the gonophore buds; B — hydranth with bunches of gonophore buds (arrows); C — hydranth with young gonophores. Arrow points to the gonophore with growing oocytes; D — intersexual gonophores. Short arrow points to the gonophore "head" containing gametes. Long arrow points to the gonophore pedicel. Dotted arrows point to the oocytes.
Scale bars: A — 0.6 mm; B, C — 0.3 mm; D — 0.1 mm.

The first and largest oocyte is located in the gastrodermis of the apex of the growing gonophore bud. More oocytes may be found in the gastrodermis at the base of such a bud (Fig. 5C). In longitudinal sections throughout the later stages of bud development, the oocytes get out of the gastrodermal layer and are located in the mesoglea separating the gastrodermal and epidermal layers (Fig. 5F). The size (diameter) of mature oocytes varied within the range of

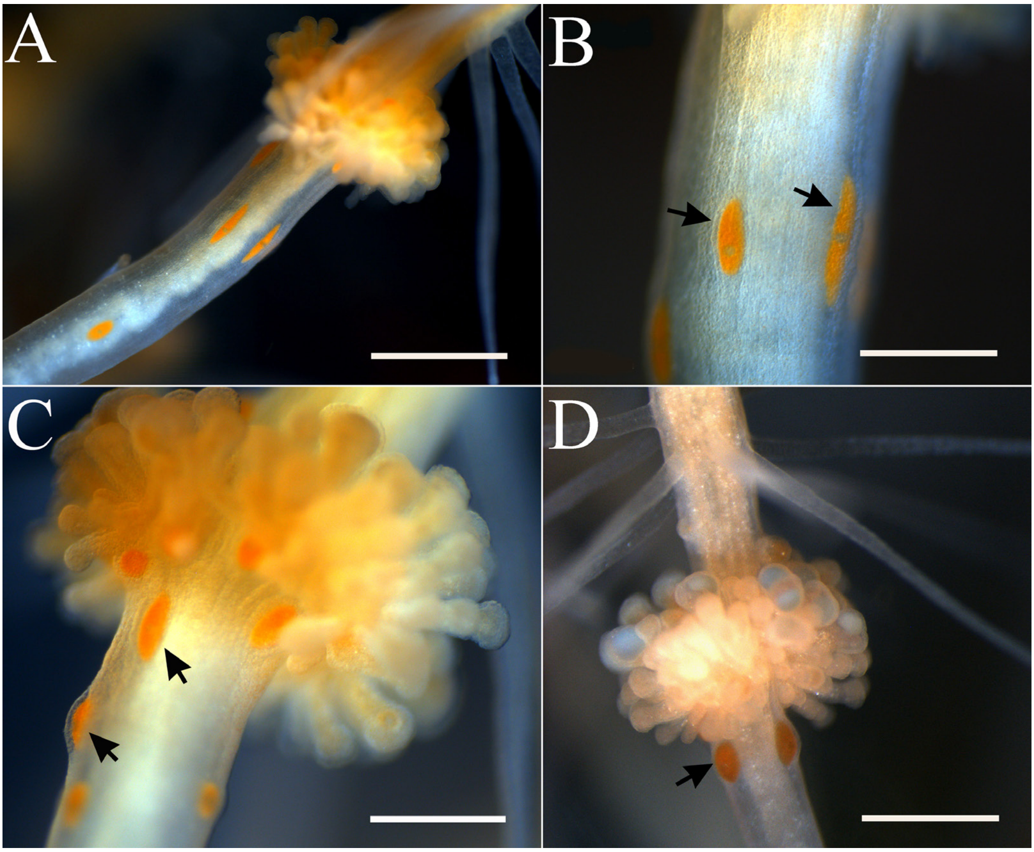

Fig. 4. Hydranths of *Clava multicornis* with ectopic oocytes. A — oocytes in the hydranth pedicel; B — rounded and elongated oocytes in the hydranth pedicel; C, D — oocytes in the hydranth pedicel, below the gonophore budding zone. Arrows point to the oocytes.
Scale bars: A — 0.8 mm; B — 0.4 mm; C — 0.5 mm; D — 0.6 mm.

145–167 µm (100 measurements within 50 colonies).

HISTOLOGY AND ULTRASTRUCTURE OF *C. MULTICORNIS* OOCYTES. The wall of the distal part of the developing gonophore "bud" consists of two epithelial layers: the epidermis and the layer derived from the medusary nodule — the key structure in the development of the reproductive stages in Hydrozoa. The epidermal epithelial layers surround the spadix constituting a gastrodermal outgrowth (a reduced manubrium of medusa) with adjacent oocytes (Fig. 6A). The surface of the premature oocyte is covered by microvilli, and the cytoplasm contains numerous large vesicles with electron-dense material (Fig. 6B). The microvilli of the oocytes protrude into the gap separating the oocyte and the epidermal and gastrodermal layers. The space between the microvilli looks more electron-translucent compared with the part of the mesoglea adjacent to the surface of the epithelial layers. The nucleus of the premature oocyte is shifted towards the epidermal layers.

The ectopic oocyte, found in the wall of the hydranth pedicel, is located in the mesoglea, forming an outward protrusion from the epidermal layer. The nucleus of this oocyte is displaced toward the outer surface of the protrusion (Fig. 6C). The oocyte bears numerous microvilli, and its cytoplasm contains numerous translucent as well as large electron-dense vacuoles (Fig. 6D). At the ultrastructural level, the gastrodermal cells adjacent to the oocytes possess outgrowths getting in contact with the oocyte surface. These outgrowths contain rounded electron-translucent vesicles (Fig. 6E). The entire ectopic oocyte is

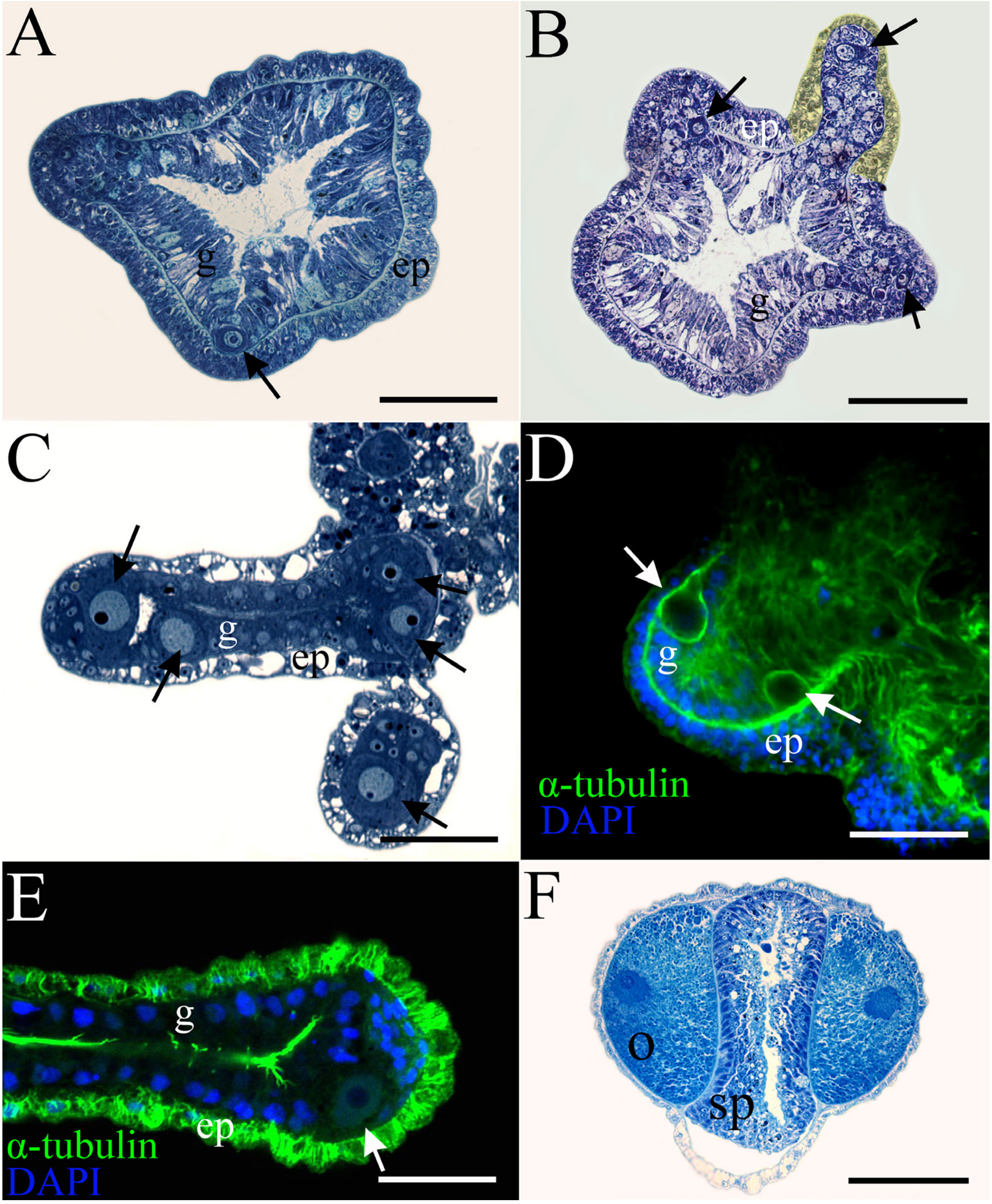

Fig. 5. Hystology of *Clava multicornis* gonophore buds. A, B — transverse sections of the hydranths at the gonophore budding zone. In B epidermis of the hydranth body protrusion is colored; C — longitudinal section of the gonophore buds; D, E — developing gonophore buds containing growing oocytes (staining with antibodies to the tyrosinated and acetylated α-tubulin and DAPI nuclear dye, CLSM); F — longitudinal section of the gonophore which contains oocytes adjoining the gastrodermal outgrowth (spadix).
Abbreviations: ep — epidermis; g — gastrodermis; o — oocyte; sp — spadix. Black and white arrows point to the growing oocytes. Scale bars: A, B — 100 µm; C — 50 µm; D, E — 20 µm; F — 120 µm.

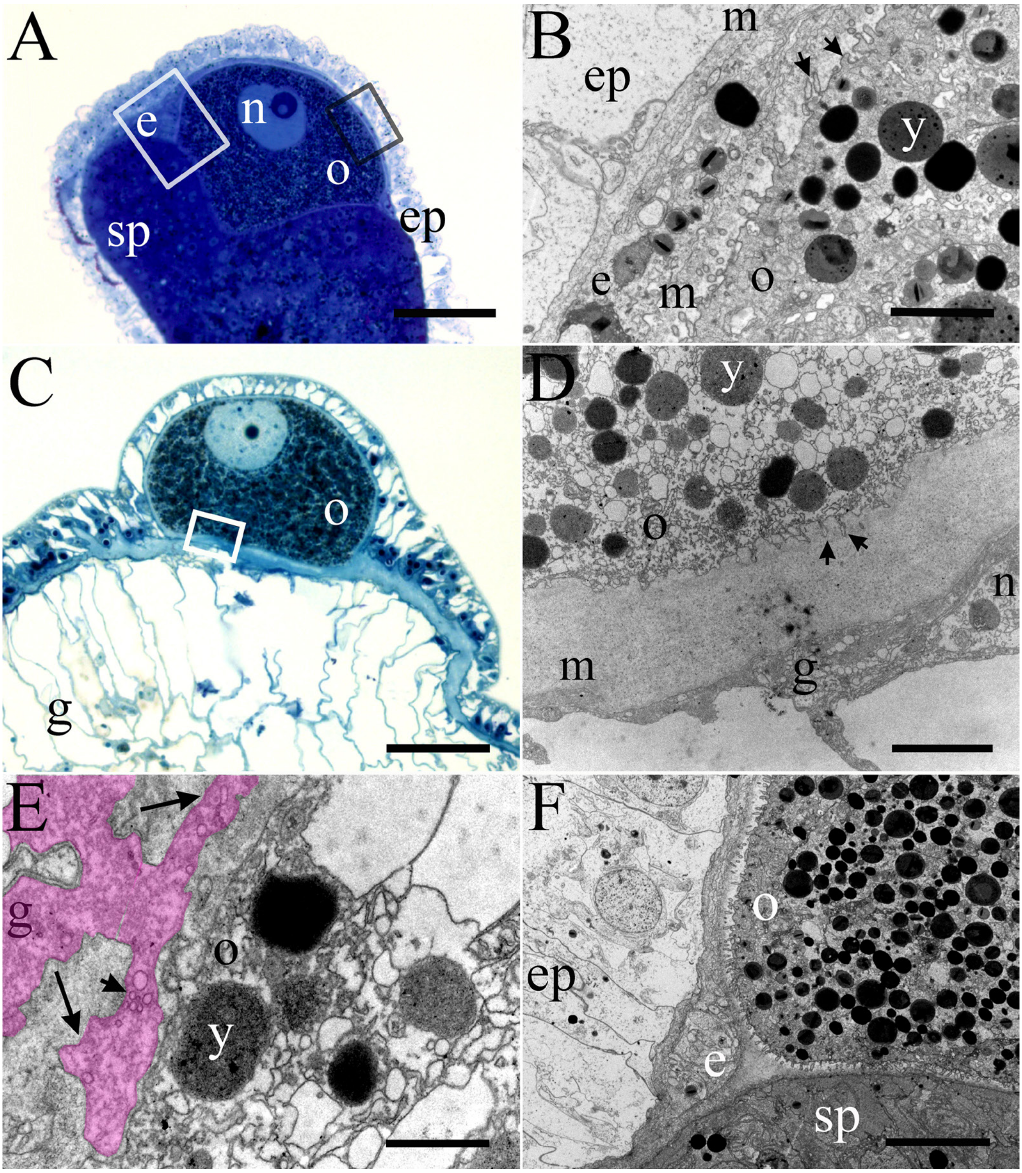

Fig. 6. Histology and ultrastructure of oocyte from the gonophore bud and of ectopic oocyte of *Clava multicornis*. A, C — histological sections (light microscopy); B, D–F — ultrastructure (TEM). A — longitudinal section of the female gonophore bud with oocyte; B — area of the gonophore bud showed in A by black frame presents the oocyte lying in the mesoglea and covered by the epithelial layer formed by the entocodon (medusary nodule) and the epidermis of the gonophore; C — transverse section of the hydranth pedicel containing the ectopic oocyte; D — ultrastructure of the hydranth pedicel framed in C presents the area containing the oocyte and the hydranth gastrodermis separated by thick dense mesoglea. Arrows point to microvilli of the oocyte; E — area of the hydranth pedicel containing gastrodermal cell (colored) and the oocyte. Long arrows point to the outgrowths of the gastrodermal cell (colored). Short arrow points to the accumulation of vesicles within the cytoplasm of the gastrodermal cell outgrowths; F — area of the gonophore bud framed in A by white frame presents oocyte that adjoins to spadix and surrounded by thin loose mesoglea.

Abbreviations: e — entocodon (medusary nodule); ep — epidermis; g — gastrodermis; o — oocyte; m — mesoglea; n — nucleus; sp — spadix; y — yolk granule. Scale bars: A — 30 μm; B — 1.7 μm; C — 42 μm; D — 2.3 μm; E — 0.6 μm; F — 7 μm.

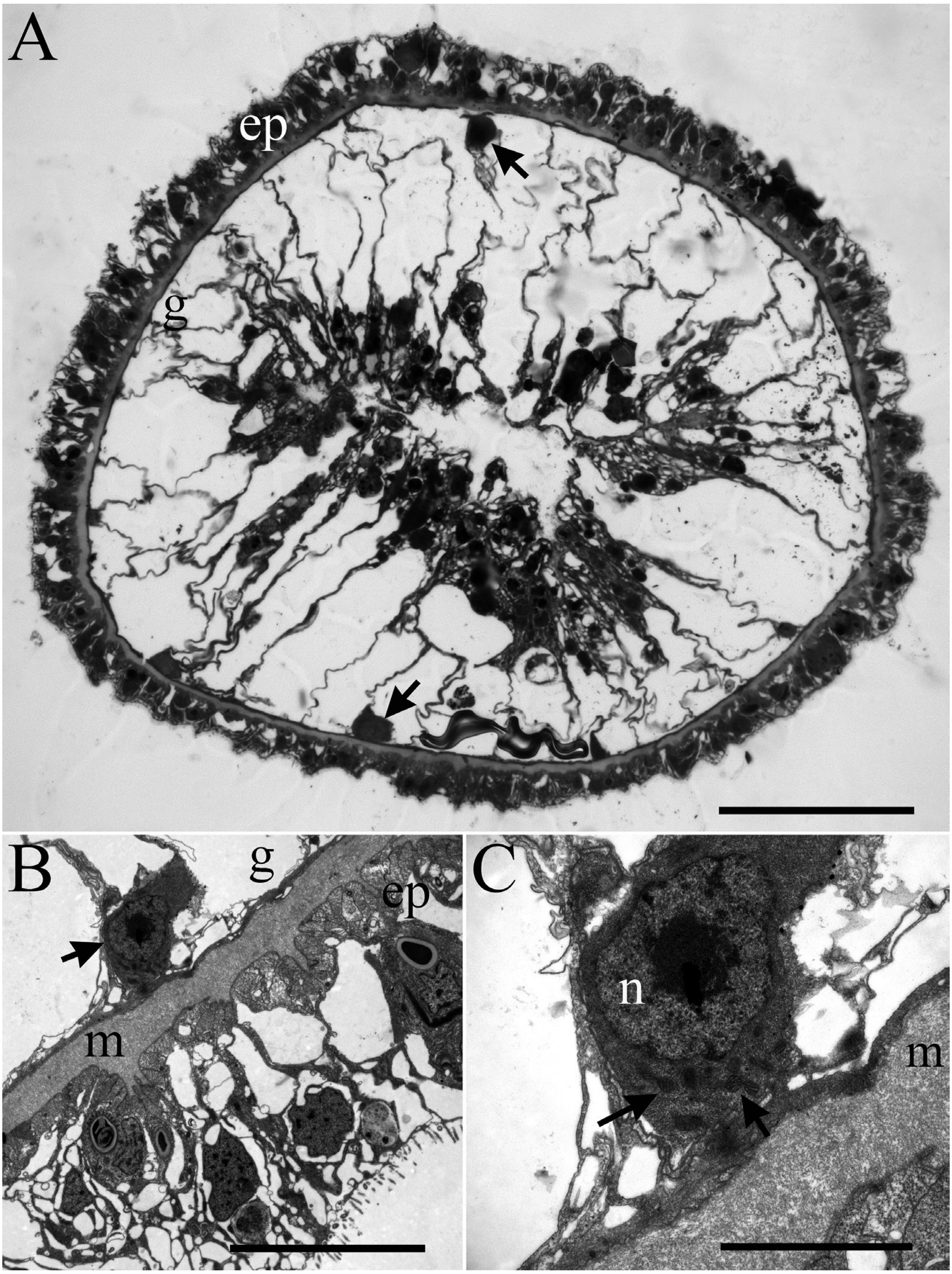

Fig. 7. Histology and ultrastructure of the transverse sections of the hydranth pedicel of *Clava multicornis.* A — histological section of the hydranth pedicel. Arrows point to i-cells in the gastrodermis; B — ultrastructure of the area of hydranth pedicel containing the i-cell (arrow); C — ultrastructure of the i-cell presented in B. Black arrows point to the mitochondria in the i-cell cytoplasm.

Abbreviations: ep — epidermis; g — gastrodermis; m — mesoglea; n — nucleus. Scale bars: A — 50 µm; B — 10 µm; C — 3 µm.

surrounded by mesoglea that is particularly thick from the side of the gastrodermis. The mesoglea looks uniformly dense all across its thickness, even between the oocyte microvilli, while the mesoglea surrounding the oocyte in the gonophore is loose and thin (Fig. 6F).

HISTOLOGY AND ULTRASTRUCTURE OF *C. MULTICORNIS* HYDRANTH PEDICEL. At the transverse sections, the hydranth body below the zone of gonophore development has a rounded shape (Fig. 7A). From the outside, it is covered by a uniform epidermal layer about 20 µm thick. Epidermal epitheliomuscular cells (EMCs) are vacuolated and possess pronounced contractile processes that are oriented longitudinally (Fig. 7B). Numerous nematoblasts are scattered among EMCs at the base of the epidermis. The gastrodermis of the pedicel is composed predominantly of strongly vacuolated EMCs whose cytoplasm is shifted toward the apical surfaces adjacent to the gastrovascular cavity (Fig. 7A). The thickness of the gastrodermis is about 80 µm. The epidermis and gastrodermis are separated by uniformly dense mesoglea that is about 3 µm thick. From the side of the gastrodermis, the mesoglea surface looks more or less even, while at the epidermal side, it forms longitudinal ridges between the bundles of muscular processes.

Within the gastrodermal layer, individual i-cells are visible, which are adjacent to the mesoglea (Fig. 7A). These i-cells have a large nucleus with a clearly distinguishable nucleolus (Fig. 7B). An accumulation of mitochondria is present in the cytoplasm of i-cells (Fig. 7C).

### *Gonothyraea loveni*

During the reproductive period, colonies of *Gonothyraea loveni* form gonangia at the nodes of shoots (Fig. 2A). The gonophores develop sequentially over the blastostyle one by one. The most developed gonophore is always located at the distal end of the blastostyle, while the youngest gonophore bud is located at the proximal end of the blastostyle (Fig. 2B).

HISTOLOGY AND IMMUNOCYTOCHEMICAL STUDY OF OOCYTES IN COLONIES OF *G. LOVENI*. Growing oocytes are easily distinguished in the gastrodermis of the coenosarc of the shoots (stem and branches), gonangia pedicels, and blastostyles of developing gonangia (Fig. 8A–E). Oocytes are found in the gastrodermis of the blastostyle of young gonangia that do not contain gonophore buds yet (Fig. 8A). The oocyte nuclei are centrally located and contain large nucleoli. Areas of darkly colored perinuclear cytoplasm are visible near the oocyte nuclei (Fig. 8B). Some oocytes have pseudopodia (Fig. 8C). Oocytes of different sizes are found along the coenosarc. The largest of them (30 µm) are present in the gonangia pedicels (Fig. 8E). Among the gonangia studied, we found several gonangia containing two gonophores. The younger proximal gonophore did not contain oocytes, while a large oocyte was located opposite this gonophore in the gastrodermis of the blastostyle (Fig. 8F). Young gonophores, not yet containing oocytes, were also visualized by immunocytochemical staining. At the same time, oocytes were clearly visible at the base of these gonophores (Fig. 9A). Small oocytes were found in the pedicels of developing gonangia (Fig. 9B). Interestingly, oocytes were also sometimes observed in the upper young internodes of colony shoots (distal to the youngest gonangia) (Fig. 9A).

HISTOLOGICAL STUDIES OF STOLONS AND SHOOT INTERNODES OF *G. LOVENI*. Histological cross-sections of stolons and shoot internodes from colonies in the sexual reproduction phase were analyzed. Cells with rounded vacuoles resembling secretory and phagocytosis vacuoles are present in the gastrodermis of stolons (Fig. 10A). Numerous small and large i-cells are present in the epidermis of stolons. The large i-cells have an elongated shape. Large nuclei and areas of darkly colored perinuclear cytoplasm are clearly distinguishable in such cells (Fig. 10B, C). There are numerous cells that resemble large i-cells in morphology and contain forming stinging capsules (also known as nematocysts) (Fig. 10D). Small and large i-cells are also present in the epidermis of the internodes (Fig. 10E), and small oocytes (8µm) are found in the gastrodermis of the most proximal internodes (adjacent to the stolon). Nematocysts are also present in the internode epidermal cells, which resemble large i-cells in morphology (Fig. 10F).

STOLON ULTRASTRUCTURE IN *G. LOVENI*. Large secretory vesicles with electron-dense material and vacuoles with digested nematocysts are observed in the gastrodermal cells of the stolons (Fig. 11A). Cells with rounded vesicles with electron-dense material also are

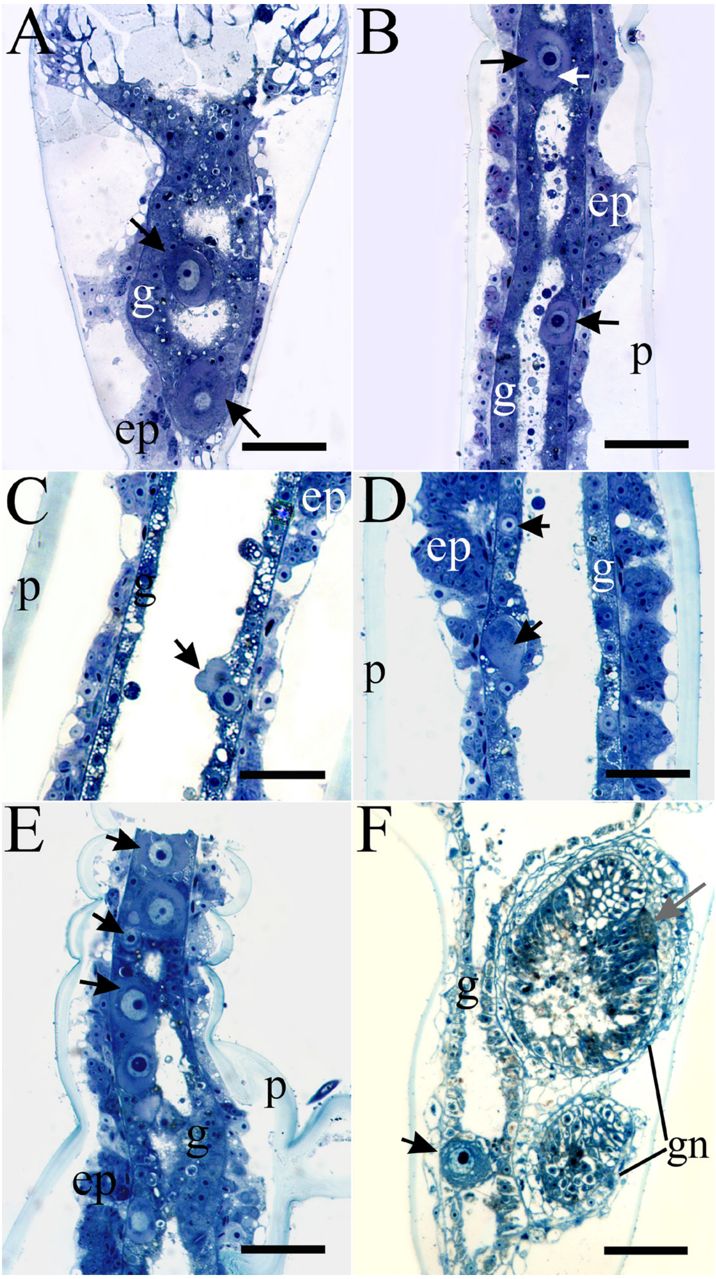

Fig. 8. Hystological longitudinal sections of *Gonothyraea loveni* shoot. A — developing gonangium contains oocytes in the blastostyle (arrows); B — area of the shoot brunch internode containing oocytes (black arrows). White arrow points to the darkly colored oocyte perinuclear cytoplasm; C — area of the shoot stem internode containing oocyte with pseudopodia (arrow); D — area of the shoot stem internode containing oocytes of different size (arrows); E — pedicel of gonangium containing predominantly large oocytes (arrows); F — gonangium with two gonophores. Proximal gonophore does not contain oocytes yet, migrating oocyte is nearby (black arrow). Distal gonophore contains oocyte (grey arrow).
Abbreviations: ep — epidermis; g — gastrodermis; gn —gonophore; p — perisarc. Black arrows point to the oocytes. Scale bars: A — 50 µm; B, D, E — 40 µm; C — 30 µm; F — 70 µm.

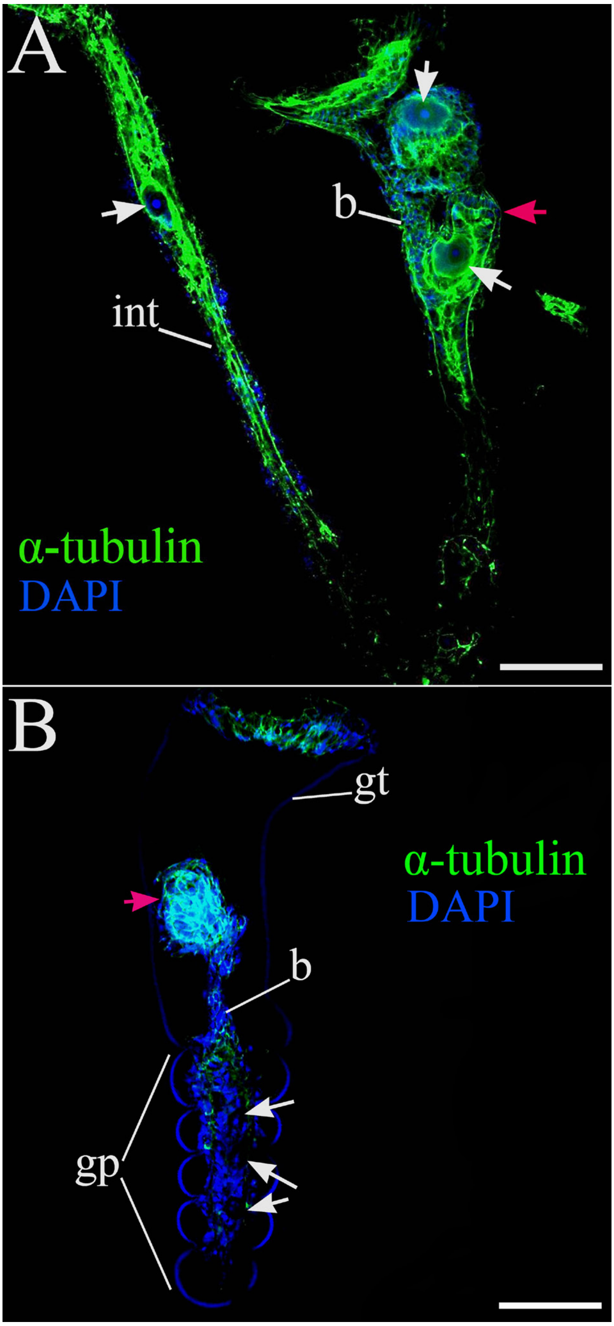

Fig. 9. Visualization of oocytes in the upper (youngest) parts of *Gonothyraea loveni* shoot (staining with antibodies to the tyrosinated and acetylated α-tubulin and DAPI nuclear dye, CLSM). A — developing gonangium and the young internode located distally. Magenta arrow points to the gonophore bud, which does not contain oocytes yet, while oocyte is visible at the base of this bud. White arrows point to the oocytes; B — young gonangium. Magenta arrow points to the gonophore bud, which does not contain oocytes yet. White arrows point to the oocytes in the gonangium pedicel.

Abbreviations: b — blastostyle; gp — gonangium pedicel; gt — gonotheca; int — internode. Scale bars: A — 150 µm; B — 100 µm.

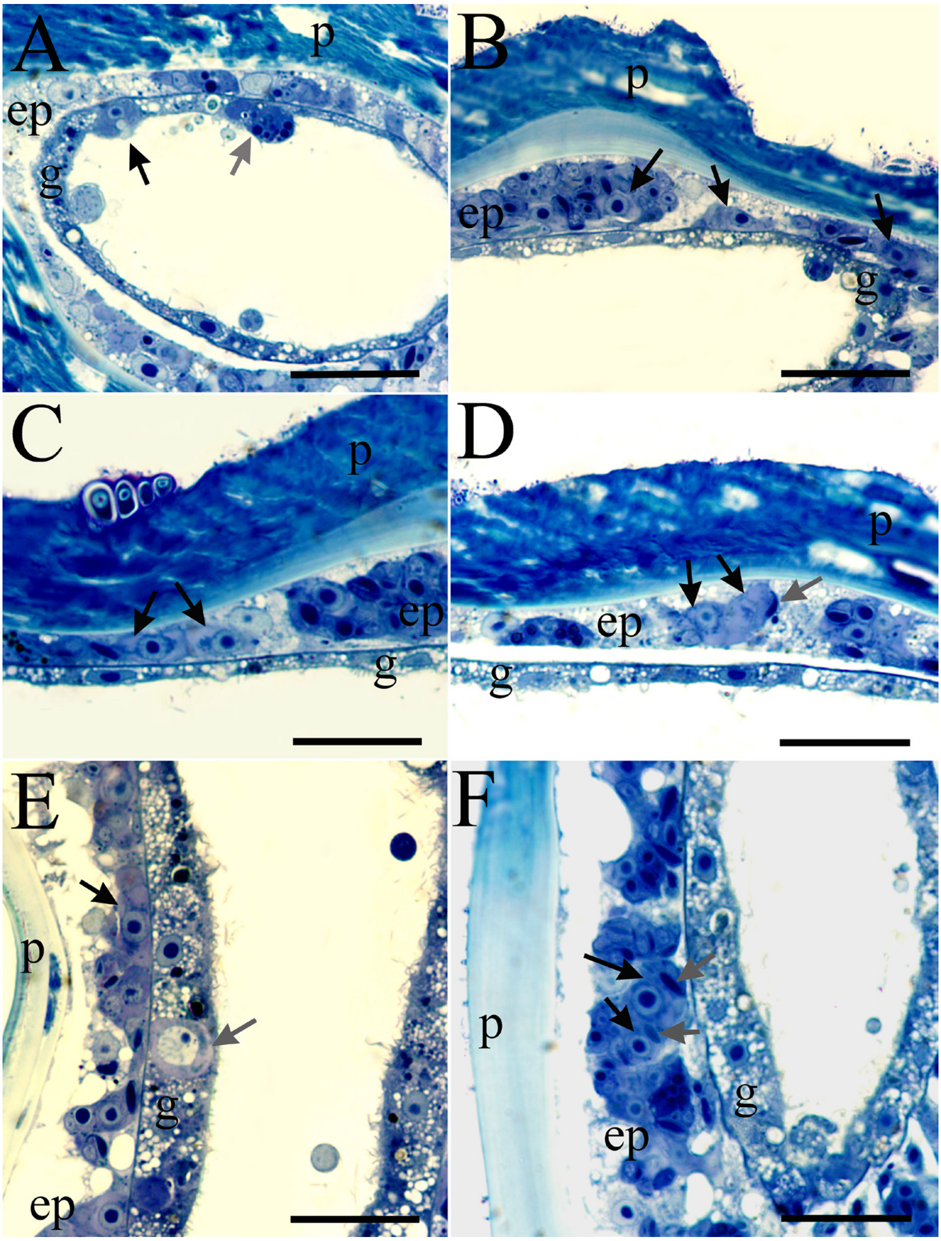

Fig. 10. Histological sections of the stolon and the shoot stem internode of *Gonothyraea loveni*. A — cells, containing vacuoles, in the gastrodermis of the stolon, transverse section. Grey arrow points to the cell containing large darkly colored vacuoles. Black arrow points to the cell presumably with the phagocytic vacuole; B, C — large elongated i-cells (arrows) in the stolon epidermis containing areas of darkly colored perinuclear cytoplasm, transverse section; D — one of the large i-cells (black arrows) in the stolon epidermis that contains the forming nematocyst (grey arrow), transverse section; E — large elongated i-cell (black arrows) in the epidermis and young oocyte (grey arrow) in the gastrodermis of the shoot stem internode containing areas of darkly colored perinuclear cytoplasm, longitudinal section; F — large i-cells (black arrows) containing areas of darkly colored perinuclear cytoplasm and nematocysts (grey arrows) in the epidermis of the shoot stem internode, longitudinal section.

Abbreviations: ep — epidermis; g — gastrodermis; p — perisarc. Scale bars: A, D, E, F — 24 µm; B — 28 µm; C — 20 µm.

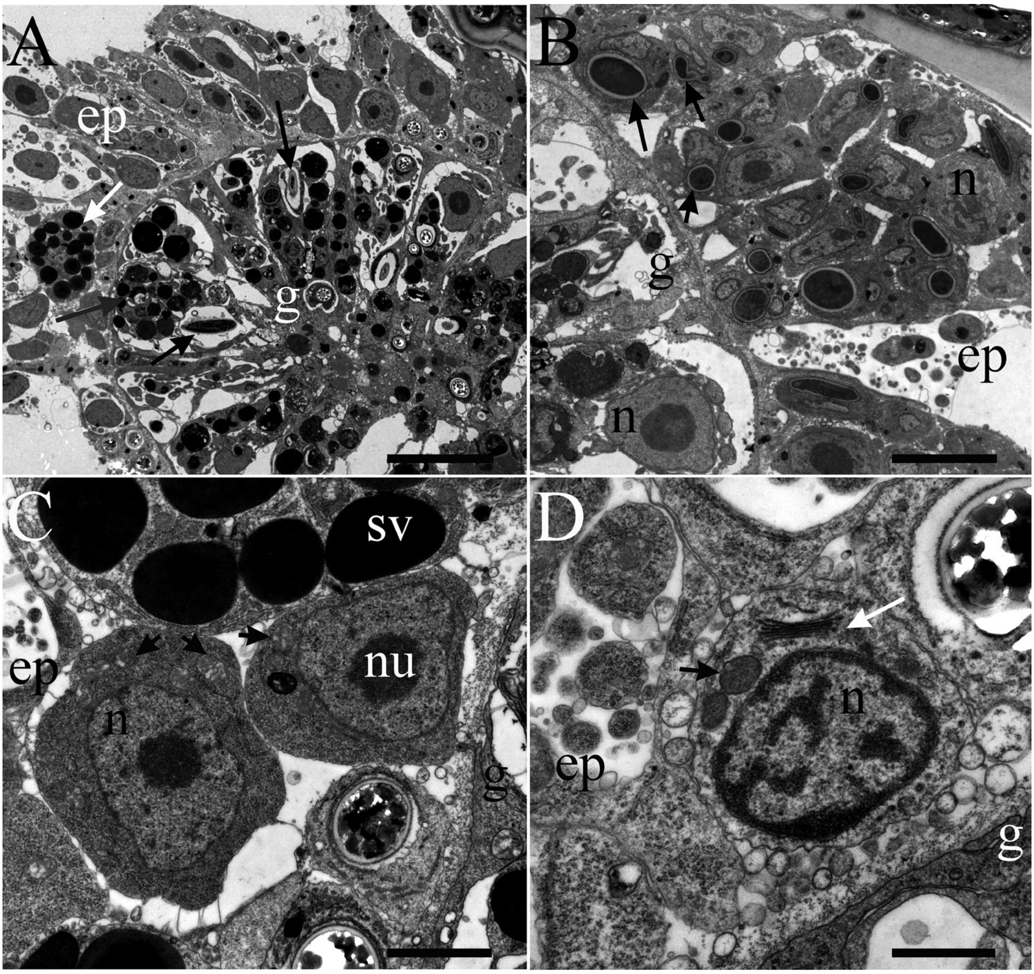

Fig. 11. Ultrastructure of the transverse sections of stolon and of the small i-cells from the stolon epidermis of *Gonothyraea loveni*. A — gastrodermal cells (grey arrow) contain rounded vacuoles with electron dense material. Digesting nematocysts (black arrows) observed in some of the gastrodermal cells. White arrow points to the epidermal cell with secretory vesicles; B — numerous nematoblasts containing forming nematocysts (black arrows) observed in the epidermis; C, D — small i-cells in the epidermis. Black arrows point to the mitochondria in cytoplasm of the small i-cells. White arrow points to the Golgi apparatus.
Abbreviations: ep — epidermis; g — gastrodermis; n — nucleus; nu — nucleolus; sv — secretory vacuole. Scale bars: A — 12 µm; B — 5.6 µm; C — 2 µm; D — 0.9 µm.

present in the epidermis of the stolon. Numerous developing nematoblasts with developing nematocysts are present in the epidermis (Fig. 11B). Rounded small cells with large nuclei occupying half of the cell volume are distinguishable among epidermal cells (Fig. 11C). Mitochondria as well as the Golgi apparatus are clearly distinguishable in the cytoplasm of such cells (Fig. 11D).

EXPERIMENTS WITH CUTTING OFF FEMALE GONANGIA IN *G. LOVENI*. The experiments were designed to test the hypothesis that gonangium development is induced by oocytes migrating to the areas of gonangium formation.

**The first series of experiments**. When gonangia were cut off from the shoots at the very beginning of their development with a small fragment of the shoot, there were two possibilities: either a few oocytes were already present in the coenosarc remnants of the internodes and in the blastostyle, or there were no oocytes at all in the severed structure. Following isolation,

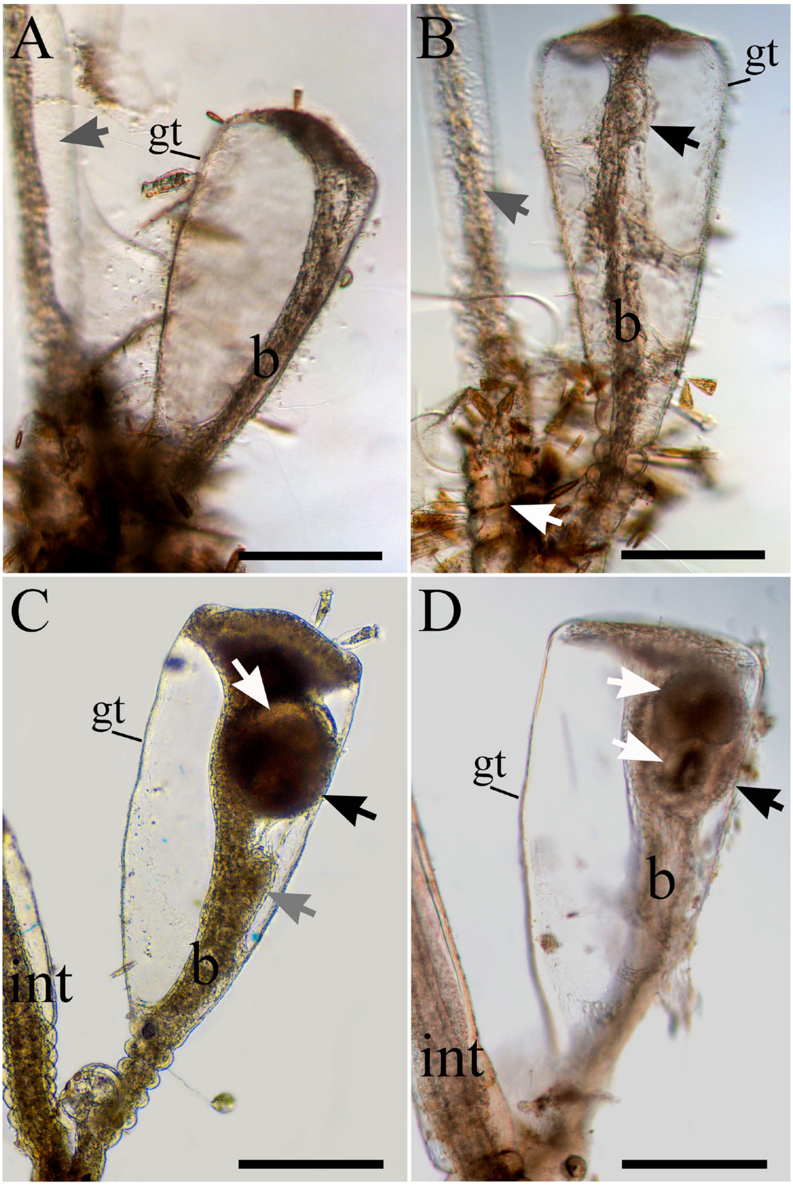

Fig. 12. Experiments with cutting off female gonangia in *Gonothyraea loveni*. A — gonangium developed after dissection from the shoot with small fragments of internodes (proximal and distal internodes were cut off) contains blastostyle, but has no gonophore buds. Arrow points to the newly formed distal internode; B — blastostyle with one gonophore bud (black arrow) developed in the gonangium after its dissection from the shoot with small fragments of internodes. Grey arrow points to the internode formed after dissection. White arrow points to the old part of the shoot node; C — gonangium developed after dissection from the shoot with distal internode (proximal internode was cut off). The blastostyle with one gonophore (black arrow) containing oocyte (white arrow) and gonophore bud arrested in the development (grey arrow) were formed in this gonangium; D — the blastostyle with one gonophore (black arrow) containing two oocytes (white arrows) developed in the gonangium after latter was dissected from the shoot with the distal internode (proximal internode was cut off). There no more gonophore buds developed in this gonangium.

Abbreviations: b — blastostyle; gt — gonotheca; int — distal internode that was saved after the gonangium dissection. Scale bars: A, B — 200 µm; C, D — 230 µm.

two variants of abnormal gonangia development were observed. In the first case, blastostyles in the gonangia formed without gonophore buds (Fig. 12A). In the second case, blastostyles with gonophore buds formed in the gonangia, but their development was arrested (Fig. 12B). In both cases, new distal internodes formed instead of the ones that had been cut off (Fig. 12A, B).

**The second series of experiments**. As mentioned earlier, migrating oocytes were also found in the coenosarc of internodes distal to the youngest gonangium. To test the possibility of "retrograde" migration of oocytes to the developing gonangia, a series of experiments with cutting off the developing gonangium by making a cut just below its base while preserving the distal part of the shoot was conducted. After the cutoff, there were two possibilities: either a few oocytes were already present in the coenosarc of the gonangium pedicel, in the blastostyle, and in the coenosarc of distally located shoot internodes (Fig. 9B), or there were no oocytes at all in the severed structure. In all cases, only one gonophore bearing one or two oocytes developed in each gonangium (Fig. 12C, D). Some of these gonangia additionally contained one or two gonophore buds that stopped developing and did not contain oocytes (Fig. 12C). In both series of experiments, the formed gonangia did not reach the "maximum" size and stopped at 50–80% of the normal gonangium size. During the experiment no new gonangia appeared on the distally located shoot internodes. It is noteworthy that when the gonangia were cut off at the later stages of their development (when they already contained the rudiments of gonophores with oocytes), they appeared to develop "normally," reaching their typical sizes.

## Discussion

### *Gonothyraea loveni*

The data of a recent study and previous investigations (de Varenne, 1882; Weismann, 1883; Aisenstadt, Polteva, 1981, Donakov, 1988) unambiguously testify to the absence of a localized germinal zone in thecate *G. loveni*. First, if it existed, there would be a localized zone of cell proliferation, which was never found in *G. loveni* colonies. Second, our histological studies of female colonies of *G. loveni* during the reproductive period confirm the presence of oocytes in the coenosarc of the colonies, as described in previous studies on this species (de Varenne, 1882; Weismann, 1883; Goette, 1907; Aisenstadt, Polteva, 1981). The smallest oocytes were found at the base of the shoots, while within the higher internodes and closer to the gonangia, the oocytes are larger. At the same time, no evident oocytes were found within the stolon coenosarc.

The i-cells in the epidermis of the stolon represent populations of large and small i-cells: the pluripotent large i-cells give rise to the germ cells and small i-cells — somatic stem cells (Campbell, 1974; Aisenstadt, Polteva, 1981; Polteva, Aisenstadt, 1980; Polteva, 1983). The large i-cells were found in the coenosarc of stolons throughout the colony life (Polteva *et al.*, 1987). Hence, we can assume that they undergo slow multiplication and occupy the coenosarc of the newly formed stolons and shoots during colony growth.

Histological studies revealed large i-cells within the stolon as well as the shoot epidermis. They had an amoeboid shape and their perinuclear cytoplasm contained darkly colored areas similar to those of oocytes. We did not study the ultrastructure of large i-cells; however, according to the research literature data, in some species of hydroids, darkly stained cytoplasm areas of oocytes and of interstitial cells correspond to accumulations of cell organelles and germinal granules, which are markers of stem germ cells (Isaeva *et al.*, 2011; Bilinski *et al.*, 2017).

During the reproductive period, some kind of activation signal induces part of the progeny of the large i-cells to become germ cells (Curantz *et al.*, 2025). From that time onward, the newly committed germ cells (primary germ cells) multiply and travel through the mesoglea to the gastrodermis, as is known to occur with i-cell progeny in *Hydra* (Siebert *et al.*, 2019) and in *Hydractinia* (Curantz *et al.*, 2025). The cells then migrate along the coenosarc towards the developing gonangia. During their migration, the oocytes undergo vitellogenesis and growth, becoming easily distinguished from the somatic cells of the coenosarc. The oocytes are distinguishable at the histological level only in the coenosarc of the shoots, starting from the most proximal shoot internodes, and are not distinguishable in the coenosarc of the stolons, where their precursors are found. Therefore, it is quite possible that the majority of germ cell precursors start their migration towards gonangia from the stolon coenosarc, where they undergo commitment and the primary stages of growth.

Oocyte observation in colonies before the appearance of gonangia, especially their presence in the zones of regular gonangium bud development, led to the idea that the presence of oocytes initiates the development of reproductive structures (de Varenne, 1882). However, observation of "empty" gonophores in hydranths that do not contain oocytes, such as in *Eudendrium racemosum* (Weismann, 1883) or *Aglaophenia myriophyllym* (Goette, 1907), contributed to the consideration of another point of view: an independent development of the somatic part of the gonangium (blastostyle and gonophores), initiated by a distant signal of a different nature.

Our experiments have unambiguously demonstrated that the initial stages of gonangium development are independent from the presence of germ cells in the tissues involved in gonangium formation. At the beginning of the reproductive period, there must be some kind of inductive signal emerging in the colony that induces germ cell differentiation and gonangium development. The question about the nature of the signal that induces germ cell differentiation and gonangium development remains unanswered. There could be two different yet correlated signals or the same signal could induce both processes. The only thing we know for sure is that the signal that induces gonangium development is not produced by the growing, migrating oocytes (germ cells): excessive oocyte production in *A. myriophyllym*, a significant portion of which did not reach the gonangia, was observed in the shoot coenosarc (Goette, 1907); and germ cells remained in the shoot coenosarc after the termination of sexual reproduction in the *Obelia* species (*G. loveni*) (Polteva *et al*., 1987; Donakov, 1988). Moreover, in the present study, oocytes were found in the distal parts of the shoots without developing gonangia.

As shown in the present study, the initiation of the gonangium development is independent from the presence of germ cells in the involved tissues. A small blastostyle, sometimes even with one or two gonophore rudiments, can develop for some time without the presence of germ cells. However, it will achieve full-fledged development — reaching its final size with a regular number of gonophores — only if germ cells enter its tissues and finalize their maturation within gonophores. In addition, preliminary experiments gave the evidence that gonangia develop to normal size if they already contain gonophores with oocytes before cutting off proves that the development of small blastostyles in gonangia that were cut off during the initial stages of development (without gonangia buds) has not been caused by possible damage to these gonangia during cutting off or by insufficient tissue for their normal growth completion. If few oocytes arrive to the developing gonangia, only the gonophores that contain oocytes will complete their formation, while the others will either cease their development (Fig. 12), or will not appear at all. The situation with oocytes can be the same: the migrating and growing oocytes reach their final size and maturation only within the gonophore (DuBuc *et al*., 2020), which is also confirmed by the presence of oocytes in the coenosarc of shoots without gonangia and after the end of the sexual reproduction period and the disappearance of gonangia from the colonies (Polteva *et al*., 1987; Donakov, 1988).

The data obtained in present study show that oocytes migrate not only directly to the forming gonangia, but also more distally, to the upper parts of the shoot stem and branches that do not yet contain the gonangium buds. The complete isolation of the gonangium at the very beginning of its development leads to the formation of an underdeveloped gonangium with a blastostyle without gonophores: this happens because oocytes cannot arrive there and are probably absent from the gonangium pedicels. Cutting off a forming gonangium from the shoot while preserving the distal part of the shoot sometimes results in the formation of a normal gonangium with a smaller number of gonophores and oocytes. The formation of normal gonophores with oocytes in gonangia that have been cut off from a shoot with a distal internode during the initial stages of their formation indicates the possibility of oocytes "returning" to the developing gonangia from the distal parts of the shoots. Thus, we assume that oocytes are capable of directed migration in response to a specific signal from developing reproductive structures.

Such interactions between the initiation of gonophores development and the oocytes migrating to them are consistent with the data for another species of the thecate hydroids: *Laomedea flexuosa*. For this species, the development of abnormal gonangia was experimentally demonstrated when the stolons were cut off and the internodes under the gonangia were ligated (Tucker, Wyttenbach, 1975). It is noteworthy that in experiments with the *Campanularia flexuosa* (*Laomedea flexuosa,* Alder, 1857), the authors observed the resorption of most gonangia that were cut off during the early stages of their formation. In contrast, our experiments have shown that all of *G. loveni* gonangia develop after being cut off in the early stages of formation. This may indicate species-specific interactions between oocytes and reproductive structures. However, the gonangia of *G. loveni*, which developed after being cut off, demonstrated smaller sizes compared with the sizes of normal gonangia, which indicates that oocytes are necessary for the normal development of gonangia.

### ***Clava multicornis***

THE LOCALIZATION OF OOCYTE PRECURSORS IN *C. MULTICORNIS*. In *C. multicornis*, the hydranth pedicel performs a supporting function. Specifically, the pedicel constitutes the lower part of the hydranth. It is the largest part and can be clearly distinguished from the others by its strongly vacuolated gastrodermis and thin gastrovascular cavity channel. The remaining parts include the gastric region, which carries the hypostome and tentacles, and gonophores at the base of the gastric region, which is the transitional zone leading to the pedicel. Thus, the hydranth pedicel in *C. multicornis* can be considered to be the analogue of the internode coenosarc in thecate hydroids.

Relying upon our data and by analogy with *G. loveni*, we assume that in *C. multicornis*, germ cell precursors migrate from the base of the hydranth pedicel or even stolon to the gonophore budding zone. Nevertheless, in contrast to thecate hydroids, it is not the differentiated oocytes, but rather their precursors committed to germ cell fate, that migrate to the zone of reproductive structure budding in *C. multicornis*. Only upon reaching this zone, the precursors finally differentiate and start growth.

Since small oocytes are found only in protrusions from the hydranth body wall in the gonophore budding zone and there are no hydranth body wall protrusions without oocytes, a possible explanation is that in *C. multicornis*, the initiation of gonophore formation and oocyte growth are interconnected processes, which is a phenomenon known for some bilaterian species (Rios-Rojas *et al*., 2015; Cao *et al*., 2019). At the beginning of the reproductive period, the first gonophores in *C. multicornis* start forming when oocyte precursors reach the appropriate place and start growing. The number of gonophores is limited simply by the size of the budding zone. Therefore, any subsequent oocytes that reach the budding zone either initiate new gonophore development or are incorporated into an already developing gonophore. Observation of simultaneous gonophore development in all hydranths of the colony including newly formed ones can attest to the possibility of an initiating signal that induces germ cell differentiation and activates the gonophore budding zone. Moreover, such a signal spreads throughout the entire colony. Colonies of *C. multicornis* studied outside the reproductive season had no signs of gonophores at any hydranth. At the same time, the discovered ectopic oocytes did not initiate the formation of gonophores in hydranth pedicels: for gonophore development, germ cells at a certain stage of their development must be located in the appropriate part of the hydranth body. This area must be susceptible to signals from the germ cells.

ECTOPIC OOCYTES. For the first time, ectopic oocytes from the hydranth pedicels of the colonial hydroid *C. multicornis* were studied. The discovery of bright, visible oocytes outside the reproductive structures allowed us to draw attention to the phenomenon of ectopic oocytes in hydroids. In recently published papers, we did not find descriptions of ectopic oocytes in hydroids, with the exception of a study where ectopic oocytes in *Hydractinia symbiolongicarpus* were obtained experimentally (DuBuc *et al*., 2020).

However, 19th- and 20th-century publications on hydroids do contain information about ectopic oocytes. For example, de Varenne (1882) mentioned large, almost developed oocytes in the body wall of one of the hydranths of *Podocoryne carnea* (*Podocoryna carnea* M.Sars, 1846), in the hydranth region where the medusa was supposed to develop. However, such a hydranth lacked some organs (the hypostome and tentacles). We assume that de Varenne was observing the phenomenon of “reproductive exhaustion” (as opposed to a true polymorphism of the hydranths), known for athecate hydroids when the colony’s resources for the formation of reproductive structures are exhausted (Allman, 1871; Schuchert, 2004). During “reproductive exhaustion” in the gonozooids of *Podocoryne carnea*, a reduction of some organs occurs (Edwards, 1972). In the case of our observation, ectopic oocytes in *C. multicornis* were found in several morphologically intact gastrozooids of the colony, and not only in the zone where reproductive structures typically bud off, but also significantly below that zone.

It is noteworthy that ectopic oocytes are not rare in hydroids overall. However, oocytes in ectopic locations usually do not grow large enough to be visible to the naked eye. For example, Goette (1907) observed young ectopic oocytes in the gastrodermis or in the epidermis of different hydroid species in histological sections. He found them in the gastrodermis of *C. multicornis* hydranths above the gonophore budding zone and above the tentacle zone, as well as in the epidermis of *Podocoryne carnea* medusae (*Podocoryna carnea* M. Sars, 1846) and in the epidermis of *Pennaria cavolinii* Ehrenberg, 1834 (at present time — *Pennaria disticha* Goldfuss, 1820) hydranths, etc.

In addition, an interesting fact is the location of the ectopic oocytes found in the colony of *C. multicornis* in this study. The ectopic oocytes were located at a very remote distance from the gonophore budding zone. Moreover, morphologically and ultrastructurally, the ectopic oocytes were similar to the oocytes developing in gonophores, with the exception of the strongly elongated shape of some ectopic oocytes, their more vacuolated cytoplasm, and their predominantly epidermal location.

In the case under consideration, we assume that oocyte precursors differentiated prematurely and achieved an advanced stage of oocyte development during their normal migration along the hydranth pedicel. A disruption of the genetic program and an untimely expression of germ cell commitment regulators, as studied recently in *H. symbiolongicarpus*, could be the reason (DuBuc *et al*., 2020). This premature maturation led to the cessation of their migration and to their displacement from the gastrodermis into the mesoglea, under the hydranth epidermis. Normally, such a displacement of the oocyte takes place only in the gonophore. The mesoglea surrounding oocytes in gonophores is thin and loose, allowing for oocyte nutrition. In comparison with the mesoglea surrounding oocytes in gonophores, the mesoglea around the ectopic oocytes in hydranth pedicels was thick and dense. Nevertheless, contact between the ectopic oocytes and the gastrodermis was retained in the form of outgrowths of the gastrodermal cells, towards the surface of the oocyte. Perhaps due to such contacts with the gastrodermis, the ectopic oocytes were able to receive the necessary nutrition and reached the size corresponding to or even exceeding the size of mature oocytes from the gonophores.

Thus, the detection of ectopic oocytes in *C. multicornis* indicates that the initiation of oocyte development and growth in hydroids is not associated with the localization of precursors in reproductive structures. However, a “gonad” (the gonophore), an appropriate niche, is necessary for the completion of the oocyte development program, since ectopic oocytes do not reach maturity (DuBuc *et al*., 2020). Moreover, experiments with *Hydractinia* oocytes isolated from gonophores have shown that a substance secreted by the gonophores is necessary for oocyte maturation (Freeman, 1987).

Ectopic oocytes are characteristic of cnidarians as well as of bilaterians. However, ectopic PGCs and young ectopic oocytes usually undergo apoptosis (Upadhyay, Zamboni, 1982; Stallock *et al*., 2003; Ewen, Koopman, 2010). In the described case, the ectopic oocytes disintegrated too, possibly due to apoptosis. The death of germ cells in ectopic sites is an extremely important process, since it is believed that germ cell tumors in humans are caused by surviving PGCs that have not reached the embryonic gonads (Schneider *et al*., 2001; Schmoll, 2002).

Thus, the data obtained in this study indicate that the interaction between oocyte development and reproductive structure development varies among different hydroid species. In some species, such as *G. loveni*, these two processes are initiated independently of one another. Nevertheless, an interrelation between reproductive structures and oocytes is necessary for the normal development of both. The study of this interrelation is very important for understanding the evolution of sexual reproduction in cnidarians.

## Conclusion

The data obtained in this study indicates the similarity of the oocyte development pathways in the investigated representatives of colonial hydroids. In thecate hydroid *Gonothyraea loveni* the commitment and beginning of oocyte differentiation occur in the stolons of the colony. Subsequently the growing oocytes migrate into the colony shoot along the coenosarc gastrodermis towards the growing gonangia – the reproductive structures that develop on the colony only during the reproductive period. In athecate hydroid *Clava multicornis* the gonophores – the reproductive structures — emerge on the feeding zooids in restricted zone only during the reproductive period. In this species, the oocyte precursors also migrate from the colony stolons.

On the other hand, the interaction between oocyte development and reproductive structure development varies among investigated hydroid species. The initiation of the gonangia formation in *G. loveni* is independent of the oocyte presence in the gonangia tissue. Whereas in *C. multicornis* the obligatory oocytes presence in the gonophores rudiments indicate the necessity of the oocytes presence in gonophores tissues for the initiation of gonophores development. At the same time, final oocyte maturation and reproductive structures development are interdependent events in both hydroid species.

**Compliance with ethical standards**

CONFLICT OF INTEREST: The authors declare that they have no conflict of interest.

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