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Internal structure of the reproductive system of the deep-sea chaetognath *Eukrohnia hamata* based on histological data and 3D reconstructions

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ABSTRACT: The internal structure of the reproductive system and the reproductive biology of chaetognaths remain poorly understood, especially for deep-sea species. This study investigates the anatomical and histological features of gonad maturation patterns in the deep-sea chaetognath *Eukrohnia hamata* (Möbius, 1875) from the Arctic Ocean, based on histological examination of the late stages of development (from the third to sixth, according to Kosobokova, Isachenko, 2017). A detailed description of the female and male reproductive systems is presented. We found such unusual features as ventral and dorsal lobes of the tail coelom and ciliated funnels around the female genital openings, which emerge during the third stage of development and fully develop by the sixth stage. We assume that the paired lobes of the tail coelom allow *E. hamata* to accumulate a larger number of sperm, and the ciliated funnels increase the chance of capturing male gametes during copulation. For the first time, paired blood vessels associated with the posterior blood sinus have been described in the longitudinal mesentery of the tail part of the body. These blood vessels presumably provide a better supply of nutrients for developing male gametes. A 3D reconstruction is provided to improve visualization of the genital structures in the area of the posterior sept and adjacent trunk and tail parts of the body.
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KEY WORDS: Chaetognatha, Eukrohniidae, lobes of the tail coelom, posterior sept, ciliated funnels, tail blood vessels.

Строение половой системы глубоководной хетогнаты *Eukrohnia hamata* на разных стадиях онтогенеза по данным гистологических исследований и 3D реконструкции

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РЕЗЮМЕ: Внутреннее строение и биология размножения морских стрелок (Chaetognatha), особенно глубоководных представителей этой группы, изучены чрезвычайно плохо. В нашей работе с использованием гистологической техники и современных методов 3D реконструкции исследованы особенности анатомии и гистологии половой системы глубоководной хетогнаты *Eukrohnia hamata* (Möbius, 1875), определяющие конкретные стадии развития. Приведено подробное описание строения женской и мужской систем на световом уровне, а также детали их структуры на разных стадиях онтогенеза. Стадии созревания определялись по классификации Кособоковой и Исаченко (2017): на 3-й и 4-й стадии обе половые системы начинают развиваться, на 5-й стадии мужская половая система достигает зрелости, а на 6-й стадии созревает женская половая система. При изучении морфологии половой системы впервые обнаружены и описаны такие её особенности, как наличие вентральных и дорзальных лопастей хвостового целома, а также ресничных воронок, окружающих женские половые отверстия. Наличие парных лопастей хвостового целома, по-видимому, позволяет созревающим особям *E. hamata* накапливать в них большое количество мужских половых клеток, а ресничные воронки, вероятно, повышают вероятность захвата мужских половых продуктов при копуляции. В продольном мезентерии хвостовой части туловища впервые обнаружены парные кровеносные сосуды, связанные с задним кровеносным синусом. Предположительно, наличие кровеносных сосудов позволяет лучше снабжать развивающиеся мужские гаметы питательными веществами. Представлена 3D-реконструкция части тела *E. hamata* в районе туловищно-хвостовой септы с целью визуализации взаимного расположения генитальных структур в этом отделе тела животных.
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КЛЮЧЕВЫЕ СЛОВА: Chaetognatha, Eukrohniidae, лопасти хвостового целома, задняя септа, ресничные воронки, кровеносные сосуды в хвосте.

Introduction

Chaetognaths, also known as arrow worms, are bilaterial metazoans that are found in every marine environment. They are carnivorous, feeding mostly on small crustaceans like copepods and cladocerans (Sullivan, 1980; Feigenbaum, 1991; Kehayias, Kourouvakalis, 2010, Bonnet *et al*., 2010; Grigor, 2017). In turn, chaetognaths themselves serve as a food source for pelagic fish, playing an important link in the marine food web (David, 1955; Reeve, 1966, Feigenbaum, 1991). The cultivation of planktonic chaetognaths in laboratory is challenging (Murakami, 1959), resulting in limited research on their biology. Only a few species have been studied, and little is known about reproductive biology of deep-sea species in particular.

Among deep-sea chaetognaths, the reproductive biology of the Eukrohniidae is of a particular interest. Species belonging to this family bear eggs and developing embryos in marsupial sacs formed by folded lateral fins. The reproductive biology of one species of this family, the deep-water species *Eukrohnia hamata* (Möbius, 1875), has recently draw significant attention (Timofeev, 1998; Kosobokova, Isachenko, 2017; Kosobokova, Hopcroft, 2021), but several aspects of anatomy of its reproductive system remain unknown. *E. hamata* has a cosmopolitan distribution and occurs in the Arctic (Falkenhaug, 1991;, Grigor, 2017), Southern Ocean (Øresland, 1990, 1995; Kruse *et al*., 2009, Bieleska *et al*., 2016), Pacific (Nagasawa, 1984; Nagasawa, Marumo, 1984; Terazaki, 1998), Atlantic (Feigenbaum, 1979; Reeve, 1980; Bonnet *et al*., 2010) and Indian Oceans (Øresland, 2000), playing a significant role in deep-sea food webs (Feigenbaum, 1991).

The body of chaetognaths consists of a head, trunk, and tail, with all parts containing their own coelomic compartments separated by transverse sept. One or two pairs of lateral fins and a single caudal fin emerge from the flank and the posterior end of the body. The species of the genus *Eukrohnia* have only one pair of lateral fins. Chaetognaths are protandric hermaphrodites (Terazaki, Miller, 1982; Bone, 1991) with only sexual reproduction known. The female reproductive system is located in the trunk, and the male reproductive system is in the tail. The male reproductive system of *E. hamata* consists of testes, male gonoducts, and seminal vesicles. Gametes develop in the testes and tail coelom, while mature spermatozoa accumulate in the seminal vesicles on the lateral sides of the tail. The female reproductive system of *E. hamata* consists of ovaries and gonoducts also known as ovispermaducts. During mating, the vesicles rupture and spermatozoa are released into the water, where they swim towards female genital openings. They then penetrate the female gonoducts to fertilize the mature oocytes. In most species, the zygote is released freely into the water where their development continues. However, in the Eukrohniidae family, eggs and developing embryos are kept in marsupial sacs of adults until hatching (Dawson, 1968; Timofeev, 1998; Kosobokova, Hopcroft, 2021).

The ontogenetic development of chaetognaths, including their gonad maturation, occurs gradually and smoothly, rather than abruptly. However, to simplify the description of their sexual maturation, several researchers have suggested dividing the ontogenesis into several stages based on development of the reproductive system (Russel, 1932; Kramp, 1939; Alvariño, 1963, 1968; Kosobokova, Isachenko, 2017; Kosobokova, Hopcroft, 2021). In this study, we rely on the classification of gonad maturity stages proposed by Kosobokova & Isachenko (2017), who identified eight gonadal stages in *E. hamata*. According to their research, the male reproductive system in *E. hamata* starts to develop at the third stage, continues growing at the fourth stage, and reaches full maturity at the fifth stage (Kosobokova, Isachenko, 2017). The female reproductive system also starts developing at the third stage, progresses through the fourth and fifth stages, and becomes fully mature by the sixth stage. Fertilization takes place during the sixth stage of gonad development, and at the seventh stage, *E. hamata* release eggs into marsupium, where growth of juveniles takes place (Alvariño, 1968; Kosobokova, Hopcroft, 2021). At the eighth stage, the juveniles leave marsupia, and the general degradation of the reproductive system occurs.

Despite the main stages of the development and maturation of the reproductive system in *E. hamata* having been described (Alvariño, 1968; Kosobokova, Isachenko, 2017; Kosobokova, Hopcroft, 2021), the anatomy of the female and male gonads at the microscopic level remains poorly understood. This study aims to investigate the internal structure of the *E. hamata* reproductive system using histological sections and advanced 3D imaging techniques. This method has recently been used for anatomical investigations in various invertebrates (Brenzinger *et al*., 2010; Boone *et al*., 2011; Weber *et al*., 2014; Song *et al*., 2020; Temereva *et al*., 2021), as it has been found to be helpful in visualizing the relative position of the internal structures. Our goal is to understand the interactions between individual structures within the reproductive system, with a particular focus on those involved in mating and egg production.

Materials and methods

Specimens of *Eukrohnia hamata* were collected in the Arctic Ocean during the expedition ARK XXVI/3 of the RV Polarstern in August 2011. The zooplankton samples were collected using a Bongo net with a mouth diameter of 0.5 meters and a mesh size of 300 μm from a depth layer of 0-1,500 m at a location with a bottom depth of 3,593 m and coordinates 83.5° N, 60.3° E. The specimens were then fixed with 4% formaldehyde and later transferred to 70% ethanol.

The external morphology of the reproductive system was studied using specimens with developing male and female reproductive systems (stages from third to sixth, according to Kosobokova, Isachenko, 2017). The specimens were examined using an Olympus XZ7 stereomicroscope, and photographs were taken using a TopCam digital camera.

To create 3D reconstructions and study the histology of the reproductive system in *E. hamata*, we prepared a series of histological sections. We used parts of chaetognath bodies from the area around the posterior sept that divides the trunk and tail of the body. The material was dehydrated according to standard methods. The process of sample dehydration was as follows: first, the samples were twice submerged into 96% ethanol solution for 30 minutes each time, then into the butanol (2 times for 30 minutes each), then into xylene (2 times for 15 minutes each), then left overnight soaked into paraplast. After that, the samples were again submerged in paraplast for 2-3 hours and finally embedded in it. Next, it was cut into

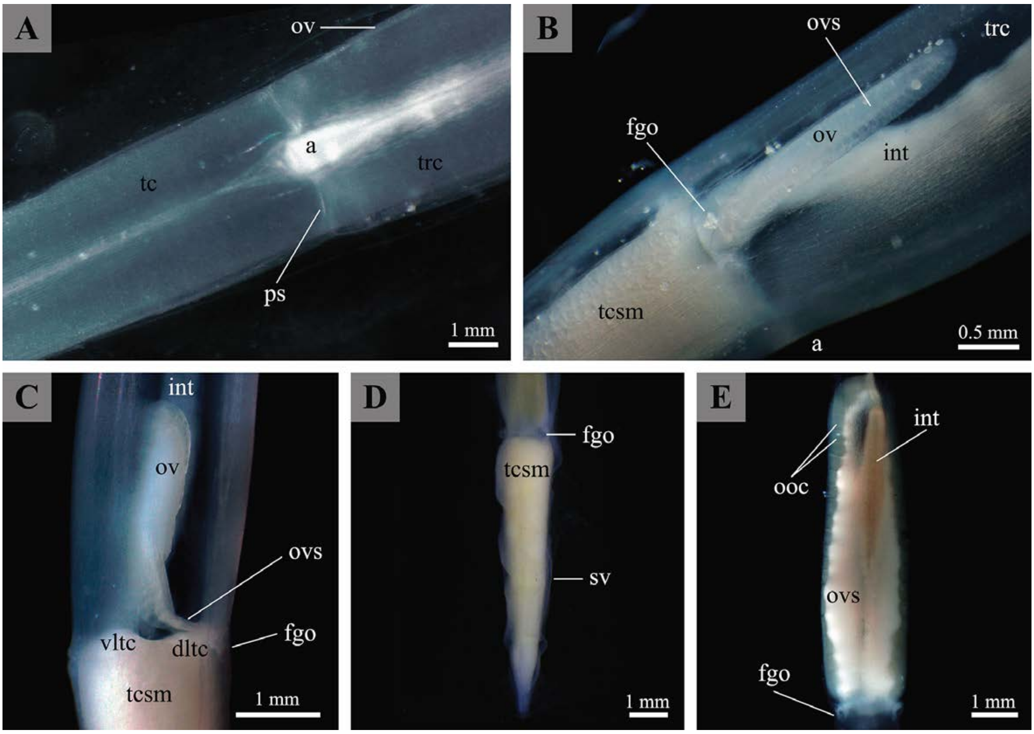

Fig. 1. Parts of the body of *Eukrohnia hamata* at different stages of development in area of posterior sept; photographs of fixed animals. In A–B, the posterior part is on the left; the anterior part is on the right. In C–E, the posterior part is to the down; the anterior part is to the top. A — 3rd stage; B — 4th stage; C — 5h stage; D — 5th stage: tail is filled with spermatogenic masses; E — 6th stage: ovaries containing mature oocytes.
Abbreviations: a — anus; dltc — dorsal lobe of tail coelom; fgo — female genital opening; int — intestine; ooc — oocyte; ov — ovary; ovs — ovispermaduct; pc — posterior sept; sv — seminal vesicle; tc — tail coelom; tcsm — tail coelom filled with spermatogenic masses; trc — trunk coelom; vltc — ventral lobe of tail coelom.

Рис. 1. Внешний вид участков тела в зоне задней септы *Eukrohnia hamata* на разных стадиях развития; фотографии фиксированных животных. На рис. A–B задняя часть находится справа, передняя слева. На рис. C–E задняя часть находится снизу, передняя сверху. A — 3-я стадия; B — 4-я стадия; C — 5-я стадия; D — 5-я стадия: хвост заполнен сперматогенными массами; E — 6-я стадия: в яичниках находятся зрелые ооциты.
Обозначения: a — анус; dltc — дорсальная лопасть хвостового целома; fgo — женское половое отверстие; int — кишечник; ooc — ооцит; ov — яичник; ovs — овиспермадукт; pc — задняя септа; sv — семенной пузырёк; tc — хвостовой целом; tcsm — заполненный сперматогенными массами хвостовой целом; trc — туловищный целом; vltc — вентральная лопасть хвостового целома.

7 µm-thick sections using a Kedee Rotary Microtome KD-2268 model. The sections were then stained according to a standard protocol with hematoxylin and eosin and then filled with Canada Balsam for permanent preparations. In total, four complete series of cross-sections were obtained.

These series of histological sections were photographed using an Olympus BX61VS slide scanner. The images were processed using IrfanView software, and then three-dimensional models were created using Amira software. The final reconstruction was prepared using Blender.

Results

ANATOMY OF THE REPRODUCTIVE SYSTEM. The structure of the reproductive system of *E. hamata* was examined in specimens at the third to sixth maturation stages (Fig. 1A–E). The beginning of the development of the male reproductive system is observed in *E. hamata* at the third stage (Figs 1A; 2A, B). During this period, it consists of compact cord-like testes located laterally in the tail and the rudiments

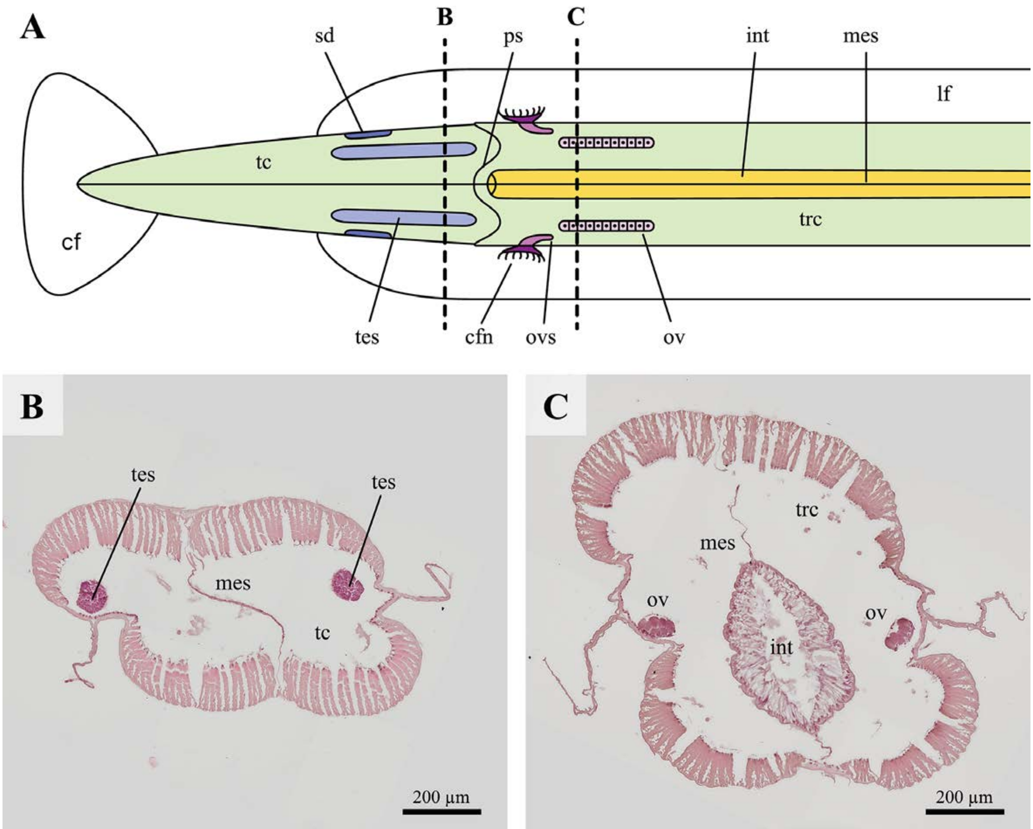

Fig. 2. Anatomy of the reproductive system of *Eukrohnia hamata* at the third stage of development. A — diagram of the frontal section showing relative positions of the male and female reproductive systems, the dashed lines indicate the positions of the cross sections in figures B and C, respectively; B — male reproductive system; C — female reproductive system.

Abbreviations: cf — caudal fin; cfn — ciliated funnel; int — intestine; lf — lateral fin; mes — mesentery; ov — ovary; ovs — ovispermaduct; ps — posterior sept; sd — seminal duct; tc — tail coelom; tes — testis; trc — trunk coelom.

Рис. 2. Анатомия половой системы *Eukrohnia hamata* на третьей стадии развития. A — схема фронтального среза, показано относительное расположение мужской и женской половых систем, пунктирными линиями отмечено расположение поперечных срезов на рис. B и C, соответственно; B — мужская половая система; C — женская половая система.

Обозначения: cf — хвостовой плавник; cfn — ресничная воронка; int — кишечник; lf — боковой плавник; mes — мезентерий; ov — яичник; ovs — овиспермадукт; ps — задняя септа; sd — семенной проток; tc — хвостовой целом; tes — семенник; trc — туловищный целом.

of seminal ducts (Fig. 2B). During the fourth stage, seminal ducts development continues, but seminal vesicles are still absent (Figs 1B; 3B).

The male reproductive system in *E. hamata* consists of paired testes, seminal ducts and seminal vesicles. At the fifth stage of development, the mature male reproductive system becomes visible (Figs. 1C; 4A). During this stage, the sperm is released from the testes and fills the caudal coelom almost completely. The tail coelom then forms ventral and dorsal lobes that protrude into the trunk coelom, giving the posterior sept a curved shape with two dorsal and two ventral invaginations facing the trunk coelom (Fig. 4A). These reproductive ducts connect to the tail coelom and open into the seminal vesicles (Fig. 4A, B). The latter are located laterally in the middle of the tail and are filled with mature spermatophores which are groups of sperm joined by a common sheath. At later stages, male gametes are not found in the tail coelom (Fig. 5B).

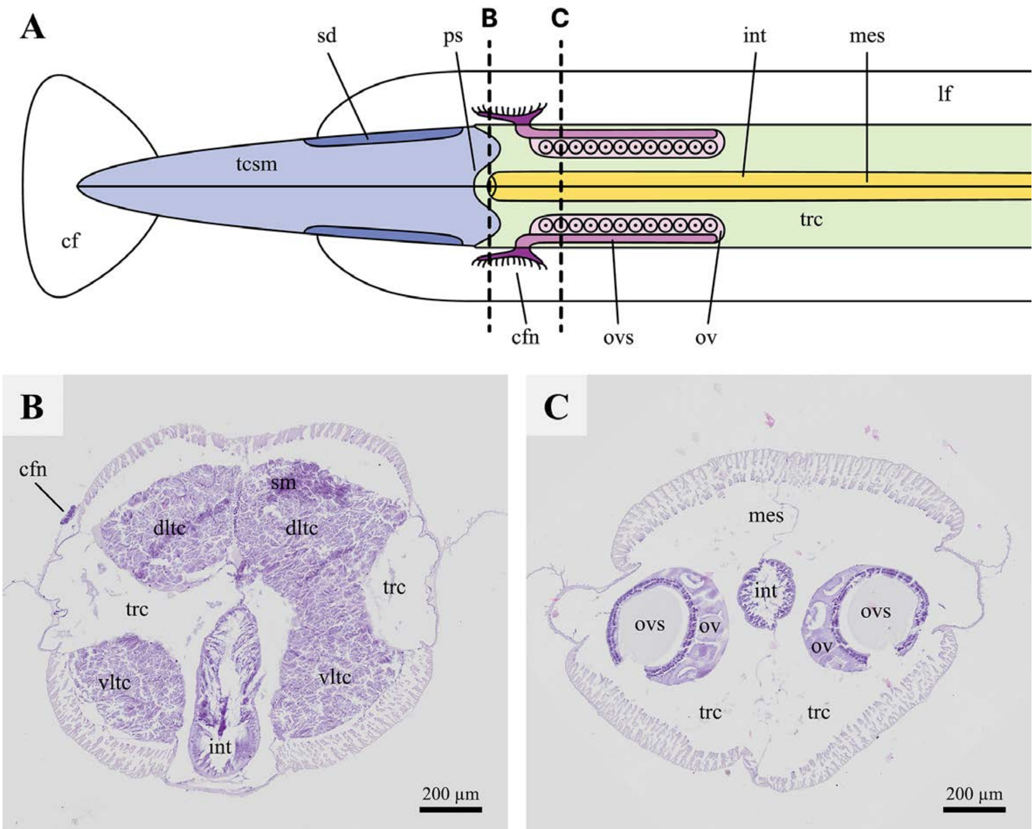

Fig. 3. Anatomy of the reproductive system of *Eukrohnia hamata* at the 4th stage of development. A — diagram of the frontal section showing relative positions of the male and female reproductive systems, the dashed lines indicate the positions of the cross sections in figures B and C respectively; B — male reproductive system; C — female reproductive system.

Abbreviations: cf — caudal fin; cfn — ciliated funnel; dltc — dorsal lobe of tail coelom; int — intestine; lf — lateral fin; mes — mesentery; ov — ovary; ovs — ovispermaduct; ps — posterior sept; sd — seminal duct; sm — spermatogenic masses; tcsm — tail coelom filled with spermatogenic masses; tes — testis; trc — trunk coelom; vltc — ventral lobe of tail coelom.

Рис. 3. Анатомия половой системы *Eukrohnia hamata* на 4-й стадии развития. A — схема фронтального среза, показано относительное расположение мужской и женской половых систем, пунктирными линиями отмечено расположение поперечных срезов на рис. B и C, соответственно; B — мужская половая система; C — женская половая система.

Обозначения: cf — хвостовой плавник; cfn — ресничная воронка; dltc — дорсальная лопасть хвостового целома; int — кишечник; lf — боковой плавник; mes — мезентерий; ov — яичник; ovs — овиспермадукт; ps — задняя септа; sd — семенной проток; sm — сперматогенные массы; tcsm — заполненный сперматогенными массами хвостовой целом; tes — семенник; trc — туловищный целом; vltc — вентральная лопасть хвостового целома.

The female reproductive system consists of three functional parts: the ovaries, the ovispermaducts (female genital ducts) and the external funnels surrounding the genital opening (Fig. 4A, B). The external funnels are located dorsolaterally on the surface of the body, above the fins, and face the dorsal side of the body. Funnels form long ciliated lobes extending along sides of the body up to the posterior sept. The epithelium of the funnels forms a valve that separates the internal space of the ovispermaduct from the outside environment (Fig. 4C). The ovispermaduct and ovary form a single compact tube, surrounded by the modified coelothelium of the trunk coelom. The ovispermaducts pass alongside the ovaries. In the septal region, the ovaries are connected to the posterior blood sinuses, through which developing oocytes are supplied with nutrients (Fig.

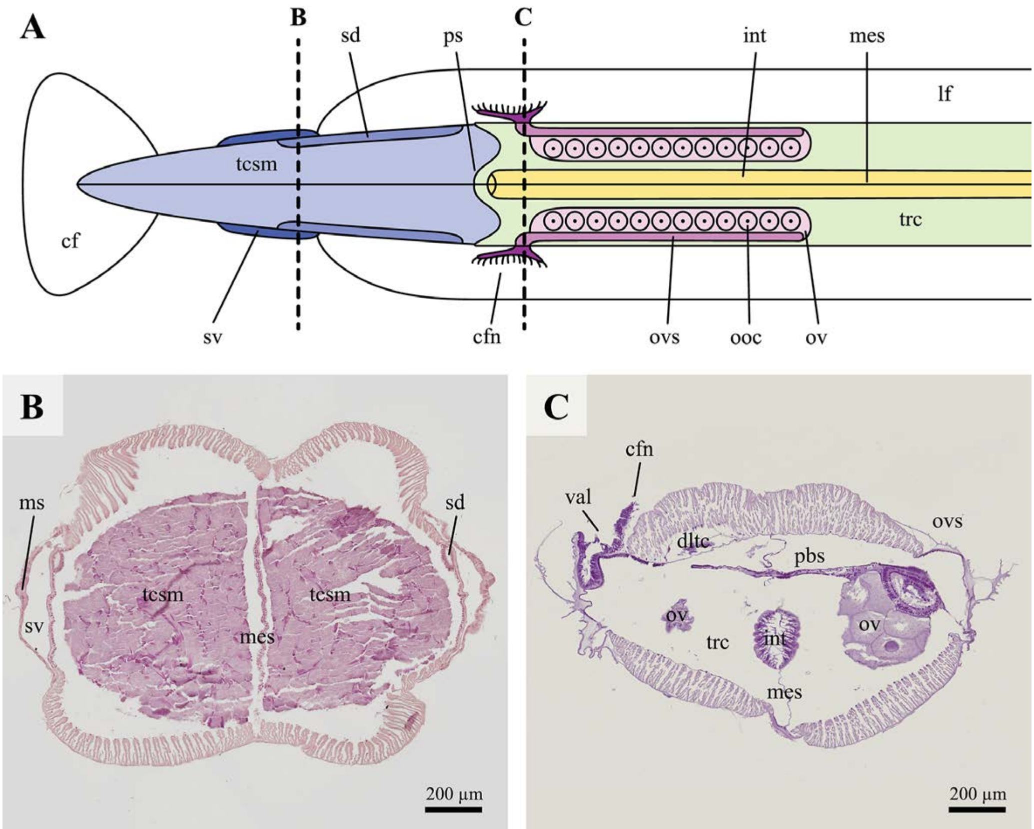

Fig. 4. Anatomy of the reproductive system of *Eukrohnia hamata* at the 5th stage of development. A — diagram of the frontal section showing relative positions of the male and female reproductive systems, the dashed lines indicate the positions of the cross sections in figures B and C respectively; B — male reproductive system; C — female reproductive system.

Abbreviations: cf — caudal fin; cfn — ciliated funnel; dltc — dorsal lobe of tail coelom; int — intestine; lf — lateral fin; mes — mesentery; ms — medial seam of seminal vesicle; ooc — oocyte; ov — ovary; ovs — ovispermaduct; pbs — posterior blood sinus; ps — posterior sept; sd — seminal duct; tcsm — tail coelom filled with spermatogenic masses; tes — testis; trc — trunk coelom; val — valve of ciliated funnel.

Рис. 4. Анатомия половой системы *Eukrohnia hamata* на 5-й стадии развития. A — схема фронтального среза, показано относительное расположение мужской и женской половых систем, пунктирными линиями отмечено расположение поперечных срезов на рис. B и C, соответственно; B — мужская половая система; C — женская половая система.

Обозначения: cf — хвостовой плавник; cfn — ресничная воронка; dltc — дорсальная лопасть хвостового целома; int — кишечник; lf — боковой плавник; mes — мезентерий; ms — срединный шов семенного пузырька; ooc — ооцит; ov — яичник; ovs — овиспермадукт; pbs — задний кровеносный синус; ps — задняя септа; sd — семенной проток; tcsm — заполненный сперматогенными массами хвостовой целом; tes — семенник; trc — туловищный целом; val — клапан ресничной воронки.

4C). In their anterior part, the ovispermaducts are blindly closed and surrounded by ovaries.

The immature female reproductive system in the third stage (Fig. 2A, C) consists of compact ovaries located on the lateral sides of the trunk. At this stage, the ovispermaduct and the external genital funnels are beginning to develop in the epidermis near the sept, but they are not yet connected to each other or to the ovary (Fig. 6). The location of these developing structures suggests that they have different origins. The ciliated funnel originates from the epidermis, and the ovispermaduct originates from modified coelothelial cells (Fig. 6B). At the fourth stage, the ovaries appear similar to mature ones, but shorter and contain smaller oocytes (Fig. 3C). At

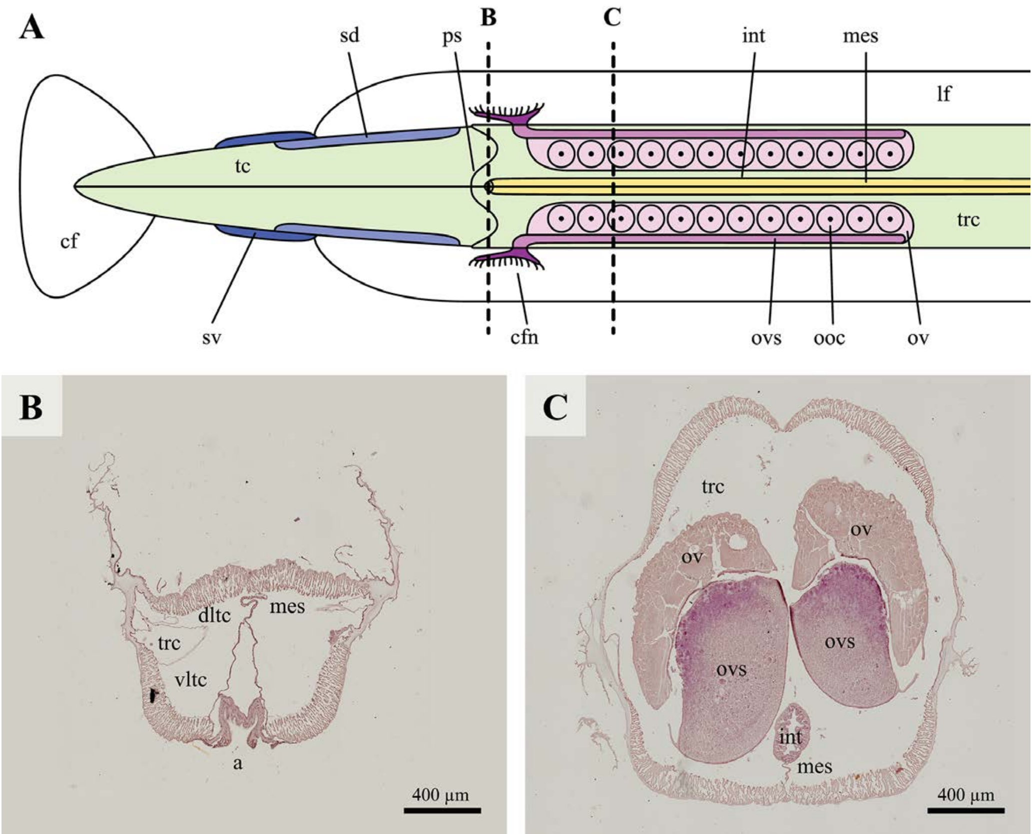

Fig. 5. Anatomy of the reproductive system of *Eukrohnia hamata* at the 6th stage of development. A — diagram of the frontal section showing relative positions of the male and female reproductive systems, the dashed lines indicate the positions of the cross sections in figures B and C respectively; B — male reproductive system; C — female reproductive system.

Abbreviations: a — anus; cf — caudal fin; cfn — ciliated funnel; dltc — dorsal lobe of tail coelom; int — intestine; lf — lateral fin; mes — mesentery; ooc — oocyte; ov — ovary; ovs — ovispermaduct; ps — posterior sept; sd — seminal duct; tc — tail coelom; tes — testis; trc — trunk coelom; vltc — ventral lobe of tail coelom.

Рис. 5. Анатомия половой системы *Eukrohnia hamata* на 6-й стадии развития. A — схема фронтального среза, показано относительное расположение мужской и женской половых систем, пунктирными линиями отмечено расположение поперечных срезов на рис. B и C, соответственно; B — мужская половая система; C — женская половая система.

Обозначения: a — анус; cf — хвостовой плавник; cfn — ресничная воронка; dltc — дорсальная лопасть хвостового целома; int — кишечник; lf — боковой плавник; mes — мезентерий; ooc — ооцит; ov — яичник; ovs — овиспермадукт; ps — задняя септа; sd — семенной проток; tc — хвостовой целом; tes — семенник; trc — туловищный целом; vltc — вентральная доля хвостового целома.

the sixth stage, the ovispermaduct significantly increases in size and is filled with male reproductive products while the eggs become very large and contain clearly visible yolk granules (Fig. 5C).

Based on the data obtained, we have created three-dimensional (3D) reconstructions of the *E. hamata* body region in the area of the posterior sept (Fig. 7). The resulting model allows to visualize the relative positions of the structures of the male and female reproductive systems as described above.

HISTOLOGY OF THE REPRODUCTIVE SYSTEM. In the mature male reproductive system, round spermatocytes with filamentous, complexly shaped nuclei and a diameter of approximately 10 µm can be found in the posterior

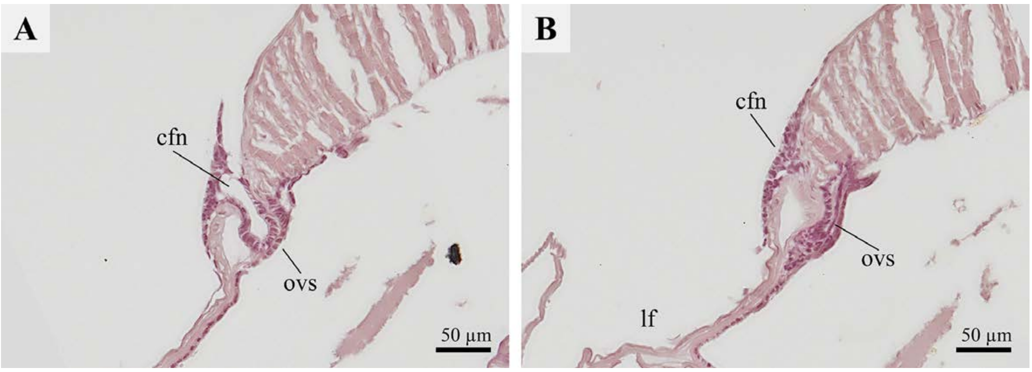

Fig. 6. The details of early development of the female reproductive system of *Eukrohnia hamata*. A — the connection of the developing ciliated funnel and ovispermaduct; B — the rudiments of the ciliated funnel and ovispermaduct.
Abbreviations: cfn — ciliated funnel; lf — lateral fin; ovs — ovispermaduct.
Рис. 6. Детали раннего развития женской половой системы *Eukrohnia hamata*. A — соединение развивающихся ресничной воронки и овиспермадукта; B — зачатки ресничной воронки и овиспермадукта.
Обозначения: cfn — ресничная воронка; lf — боковой плавник; ovs — овиспермадукт.

part of the tail (Fig. 8A). Blood sinuses are clearly visible in the ventral and dorsal parts of the longitudinal mesentery (Fig. 9A, B). At later stages, single spermatogenic masses containing filamentous spermatids can be observed in the germinal zone. The length of these spermatids may reach up to 90 μm. Most of the tail coelom is filled with these developing spermatogenic masses (Fig. 8B), which are not covered by a common sheath and are likely to represent syncytia of male gametes at different stages of differentiation. Spermatids and modified spermatozoa with elongated, filamentous nuclei are clearly visible.

The small seminal ducts are located laterally between the body wall and the coelothelium (Fig. 8C). They are lined with a cubic epithelium that is about 9–10 μm in height. Inside the lumen of these ducts, scattered filiform spermatozoa can be observed.

The seminal vesicles form as an extension of the epithelial tissue on the lateral sides of the *E. hamata* tail, between the lateral and caudal fins (Fig. 8D, E). The outer wall of the vesicles is formed by cubic or prismatic cells, measuring approximately 30 μm in height, while the inner wall consists of flattened cells, measuring approximately 5–6 μm. Unlike the epithelial cells that cover the rest of the body, the nuclei in the seminal vesicle cells are easily visible and have a more elongated shape. Along the entire length of the vesicle there is a medial seam formed by prismatic cells with apically positioned nuclei (Fig. 8D).

The outer wall of the ovary is formed by flattened cells of the coelomic epithelium, which lines the outside of both the ovary and ovispermaduct. (Fig. 10A). The peri ovarian coelom contains homogeneous liquid that resembles the fluid of blood sinuses in terms of its histological features (Fig. 11). Both oogonia and oocytes are evenly distributed throughout the ovary. Throughout the length of the ovary, oocytes are mostly at the same stage of development. If the ovary is mature, all or nearly all of the eggs have undergone vitellogenesis. Follicular cells with flattened nuclei are spread over the surface of oocytes.

The ovispermaduct is formed by two layers of epithelium: an inner layer and an outer layer (Fig. 10C). The internal epithelium is represented by a syncytium containing rounded nuclei in its basal part and numerous secretory vesicles in its middle and apical parts. On the inner surface of the ovispermaduct there are accumulations of secretion synthesized by the syncytial layer. The outer layer consists of prismatic cells with elongated nuclei. During the development of the ovispermaduct, the inner layer may peel off from the outer layer or even degenerate (Fig. 10D). During the fourth and fifth stages, the ovispermaduct is empty, and both of its epithelial layers are closely adjoint to each other. However, during the sixth stage, when it is filled with sperm, the

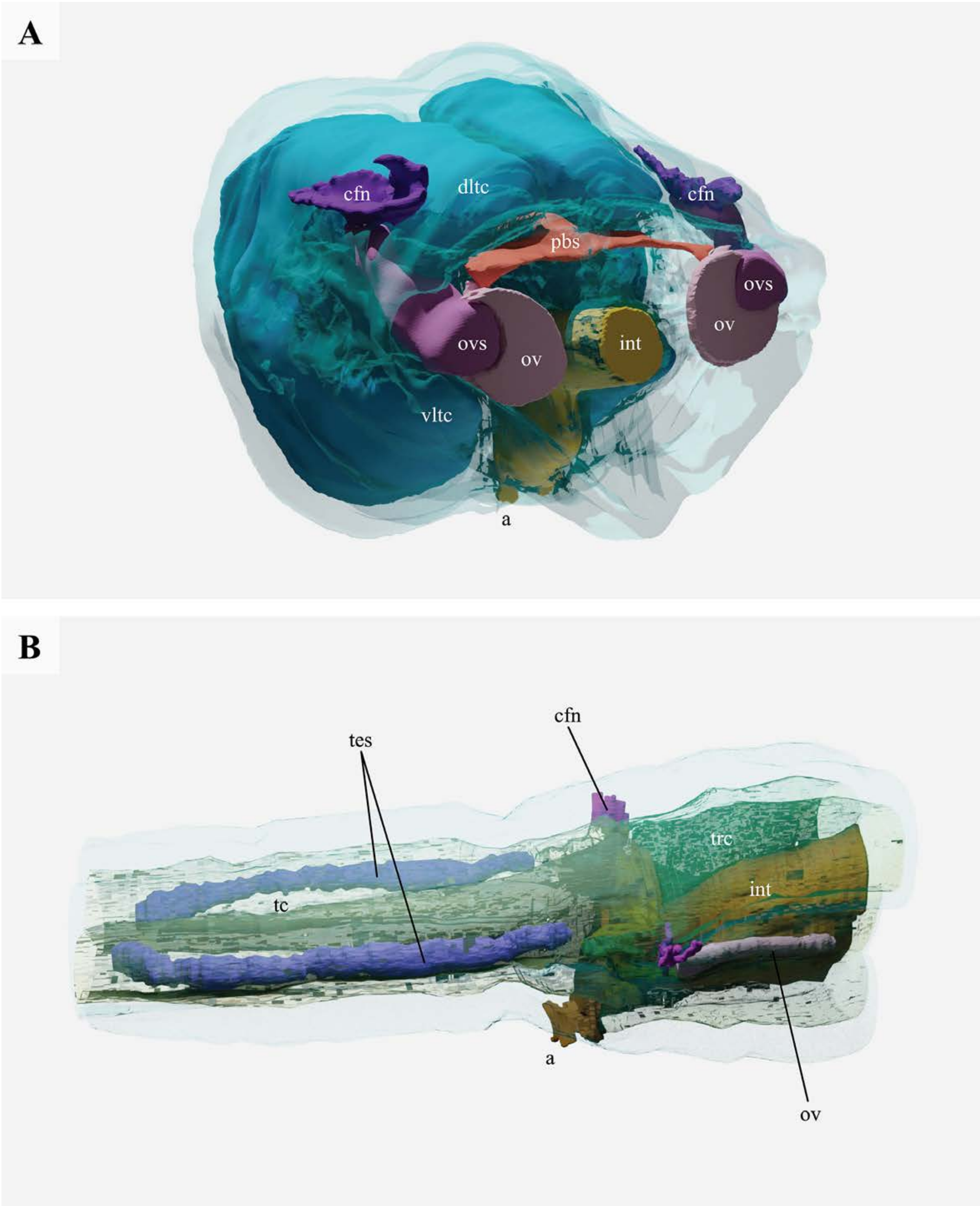

Fig. 7. Three-dimensional reconstructions of the reproductive system of *Eukrohnia hamata*; the anterior side is on the right and posterior is on the left. A — the 4th stage, laterofrontal view; B — the 3rd stage, lateral view. Abbreviations: a — anus; cfn — ciliated funnel; dltc — dorsal lobe of tail coelom; int — intestine; pbs — posterior blood sinus; tc — tail coelom; trc — trunk coelom; ov — ovary; ovs — ovispermaduct; vltc — dorsal lobe of tail coelom.

Рис. 7. Трёхмерные реконструкции половой системы *Eukrohnia hamata*; передняя часть расположена справа, задняя слева. A — 4-я стадия, латерофронтальный вид; B — 3-я стадия, вид сбоку. Обозначения: a — анус; cfn — ресничная воронка; dltc — дорсальная лопасть хвостового целома; int — кишечник; pbs — задний кровеносный синус; tc — хвостовой целом; trc — туловищный целом; ov — яичник; ovs — овиспермадукт; vltc — вентральная лопасть хвостового целома.

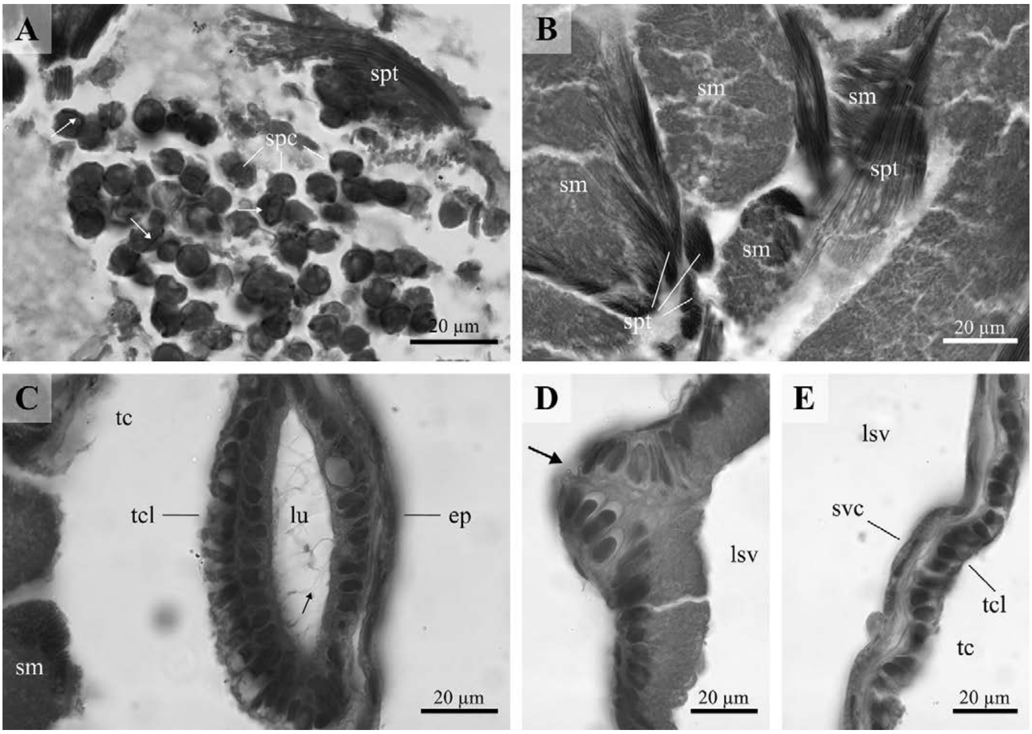

Fig. 8. The histology of the mature male reproductive system in *Eukrohnia hamata* at the 5th stage of development. A — transverse section of the posterior part of the tail, the arrows indicate sperm nuclei; B — spermatogenic masses; C — transverse section through the seminal duct, the arrow indicate spermatozoa; D — the transverse section of the distal wall of the seminal vesicle, arrow indicates the medial seam; E — transverse section of the proximal wall of the seminal vesicle.

Abbreviations: ep — epidermis; lsv — lumen of the seminal vesicle; lu — lumen of the seminal duct; sm — spermatogenic mass; spc — spermatocyte; spt — spermatide; svc — seminal vesicle cells; tc — tail coelom; tcl — tail coelom lining.

Рис. 8. Гистология зрелой мужской половой системы *Eukrohnia hamata* на 5-й стадии развития. A — поперечный срез задней части хвоста, стрелки указывают на ядра сперматозоидов; B — сперматогенные массы; C — поперечный срез семенного канала, стрелки указывают на сперматозоиды; D — поперечный срез дистальной стенки семенного пузырька, стрелка указывает на срединный шов; E — поперечный срез проксимальной стенки семенного пузырька.

Обозначения: ep — эпидермис; lsv –просвет семенного пузырька; lu — просвет семенного канала; sm — сперматогенная масса; spc — сперматоцит; spt — сперматида; svc — клетки семенного пузырька; tc — хвостовой целом; tcl — выстилка хвостового целома.

ovispermaduct significantly increases in size and stretches. Both epithelial layers become flattened, and the nuclei within them become more dispersed.

The external funnel is formed by pseudostratified epithelium composed of prismatic ciliated cells with large nuclei elongated in the apico-basal direction (Fig. 10E). The thickness of this funnel is approximately 25 µm. The ovispermaduct is adjacent to the ciliated funnel and opens into it with female genital pore.

STAGES OF GAMETES DEVELOPMENT. At the third stage of development, male gametes are represented by spermatocytes that fill the compact testes (Fig. 12A). During subsequent stages, the spermatocytes are released into the tail coelom (Fig. 12C), where they differentiate into spermatogenic syncytia, and maturation into sperm occurs within these syncytia. At the sixth stage, the tail becomes empty (Fig. 5B), and sperm fills the ovispermaduct (Fig. 12E). As a result, the ovispermaduct contains both

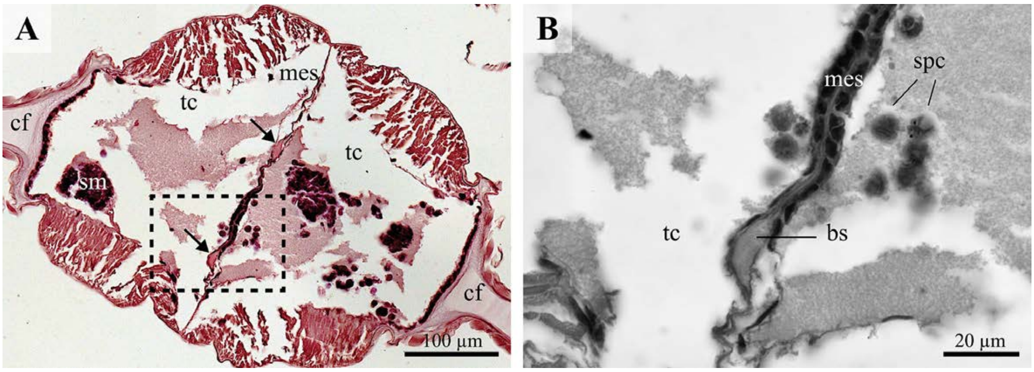

Fig. 9. Tail blood sinuses in the 5th stage of development of *Eukrohnia hamata*. A — transverse section of the posterior part of the tail, arrows indicate blood sinuses; B — a single tail blood sinus within the area highlighted by a frame (dashed line) in panel A.
Abbreviations: bs — blood sinus; cf — caudal fin; mes — mesentery; sm — spermatogenic mass; spc — spermatocyte; tc — tail coelom.
Рис. 9. Хвостовые кровеносные синусы на 5-й стадии развития *Eukrohnia hamata*. A — поперечный срез задней части хвоста, стрелки указывают на кровеносные синусы; B — увеличенный фрагмент поперечного среза с кровеносным синусом, обозначенный пунктирной рамкой на рис. A.
Обозначения: bs — кровеносный синус; cf — хвостовой плавник; mes — мезентерий; sm — сперматогенная масса; spc — сперматоцит; tc — хвостовой целом.

more or less compact masses of spermatozoa and homogeneous sperm (Fig. 12E) at this stage.

Female gametogenesis begins with the bean-shaped oogonia (diameter of 15–25 µm) in the immature ovary at the third stage of development (Fig. 12B). As development progresses, the oocytes gradually increase in size and their cytoplasm becomes evenly filled with yolk granules (diameter of 5 µm) (Fig. 12D, F). The sizes of female gametes at the fourth, fifth, and sixth stages of development are approximately 55–65, 110–120 and 350 µm, respectively.

Discussion

MALE REPRODUCTIVE SYSTEM. It is known that all chaetognaths have two testes located inside the tail coelom. In specimens of *Eukrohnia hamata* with a mature male reproductive system (fifth stage according to Kosobokova, Isachenko, 2017), the male gametes fill the coelomic cavity of the tail, with spermatogonia located posteriorly in relation to the mature sperm. According to previous studies, the male gametes develop in the testes located in the posterior part of the tail and are released into the coelom (Shinn, 1997; Muller *et al.*, 2019). However, in histological sections of *E. hamata*, we did not observe the release of sperm into the cavity of the caudal coelom, which is described for other chaetognath species (Shinn, 1997; Muller *et al.*, 2019).

Three-dimensional reconstruction of the area of the posterior sept has revealed that the paired tail coeloms in *E. hamata* have the shape of cones that expand in the longitudinal direction forming paired dorsal and ventral lobes invaginating into the trunk coelom. This anatomical feature has not been previously described for chaetognaths. However, analysis of the literature suggests that a similar shape of the posterior sept is also present in the bathypelagic species *Heterokrohnia involucrum* (see Dawson, 1968). In Dawson's (1968) work, illustrations of the reproductive system of *H. involucrum* show a structure similar to that observed in *E. hamata*. The anterior lobes of the tail coeloms in *E. hamata* may increase their volume and contribute to the accumulation of a larger quantity of male gametes. Besides, this "intertwining" of the tail and trunk coelomic compartments ensures the most efficient exchange of fluids between them and increases nutrient delivery to developing male gametes.

The appearance, shape, and size of the seminal vesicles in chaetognaths depend on the species and the degree of filling with spermatozoa (Bergey *et al.*, 1994; Ghirardelli, 1968; Tokioka, 1939; Pierrot-Bults, 1976). The seminal vesicles of *E. hamata* do not contain additional secretory or cuticular epithelial structures, but

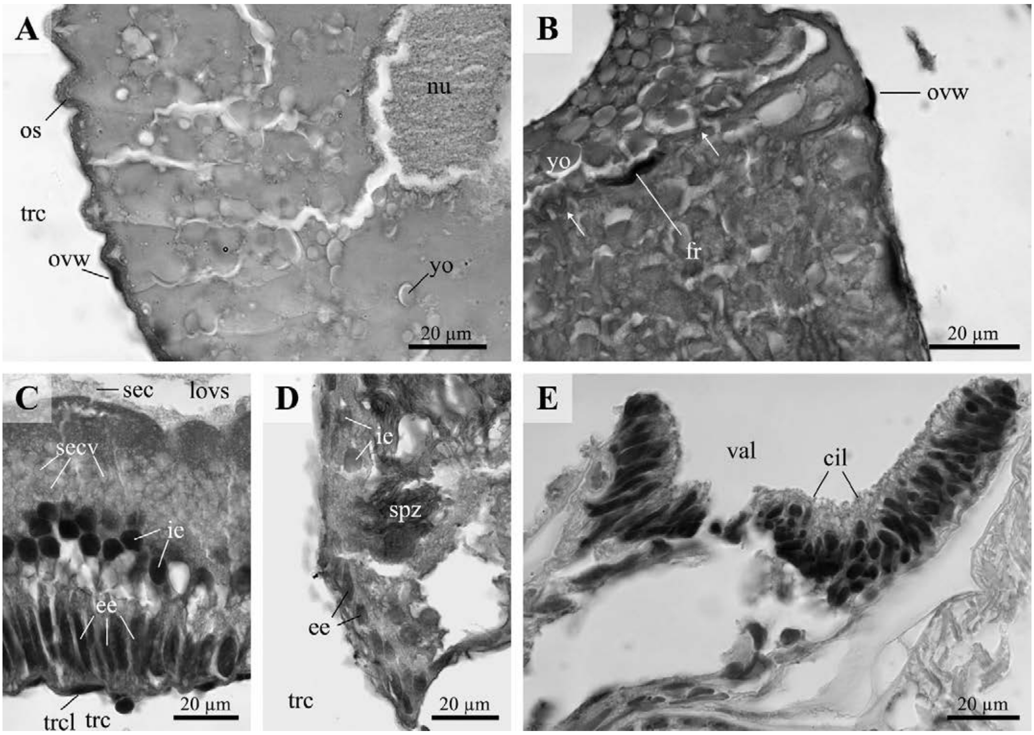

Fig. 10. The histology of the female reproductive system in *Eukrohnia hamata* at the 6th (A, B, D), 5th (C) and 4th (E) stages of development. A — a mature oocyte; B — the ovary with follicular reticulum visible; C — transverse section through an empty ovispermaduct; D — transverse section of the ovispermaduct filled with sperm; E — transverse section of the ciliated funnel.

Abbreviations: cil — cilia; ee — external epithelium of ovispermaduct; ie — internal epithelium of ovispermaduct; fr — follicular reticulum cell; lovs — lumen of the ovispermaduct; nu — nucleus; os — ovarian space (peri-ovarian coelom); ovw — ovarian wall; sec — secretion of the internal epithelium of ovispermaduct; secv — secretory vesicles; spz — spermatozoa; trc — trunk coelom; trcl — truck coelom lining; val — valve; yo — yolk granule.

Рис. 10. Гистология женской половой системы *Eukrohnia hamata* на 6-й (A, B, D), 5-й (C) и 4-й (E) стадиях развития. A — зрелый ооцит; B — яичник, виден фолликулярный ретикулюм; C — поперечный срез пустого овиспермадукта; D — поперечный срез овиспермадукта, заполенного спермой; E — поперечный срез ресничной воронки.

Обозначения: cil — реснички; ee — внешний эпителий овиспермадукта; ie — внутренний эпителий овиспермадукта; fr — клетка фолликулярного ретикулюма; lovs — просвет овиспермадукта; nu — ядро; os — внутреннее пространство яичника (пери-овариальный целом); ovw — стенка яичника; sec — секрет внутреннего эпителия овиспермадукта; secv — секреторные везикулы; spz — сперматозоиды; trc — туловищный целом; trcl — выстилка туловищного целома; val — клапан; yo — гранула желтка.

they do have a medial seam. According to our histological data, the cells in this seam adjoin each other with their basal ends. This allows us to envision the seminal vesicle as a sac formed by the dorsolateral and ventrolateral folds of the epithelium which interlock with each other. Subsequent release of spermatophores occurs when these folds diverge (Shinn, 1997).

The presence of a modified type of spermatozoa with filamentous nuclei in *E. hamata* allows us to suggest internal fertilization, where spermatozoa are forced to overcome obstacles on their journey to the oocyte. In *E. hamata*, these obstacles may be represented by inner and outer layers of the ovispermaduct.

Spermatozoa at all stages of the reproductive cycle are gathered in spermatogenic masses, which can be interpreted as potential spermatophores. Small spermatophores were previously observed in living *E. hamata* specimens (Kosobokova, Hopcroft, 2021), but they were mistakenly identified as amoeboid spermatozoa.

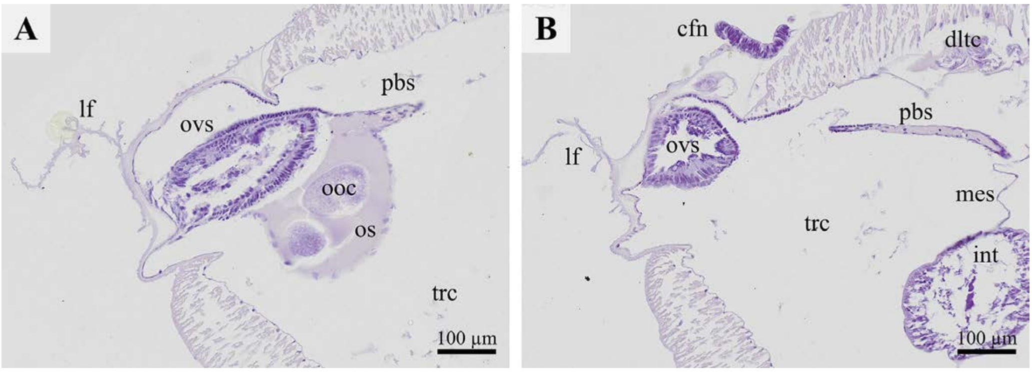

Fig. 11. Ovary of *Eukrohnia hamata* connecting with the posterior blood sinus. A –transverse section of the trunk showing the posterior blood sinus connecting to the ovarian space; B — transverse section of the trunk close to the sept showing that the posterior blood sinus is separated from the ovary.
Abbreviations: cfn — lobe of the ciliated funnel; dltc — dorsal lobe of the tail coelom; int — intestine; lf — lateral fin; mes — mesentery; ooc — oocyte; os — ovarian space; ovs — ovispermaduct; pbs — posterior blood sinus; trc — trunk coelom.
Рис. 11. Соединение яичника *Eukrohnia hamata* с задним кровеносным синусом. A — поперечный срез туловища, видно соединение заднего кровеносного синуса с внутренним пространством яичника; B — поперечный срез туловища рядом с септой, задний кровеносный синус и внутреннее пространство яичника разъединены.
Обозначения: cfn — лопасть ренсичной воронки; dltc — дорсальная лопасть хвостового целома; int — кишечник; lf — боковой плавник; mes — мезентерий; ooc — ооцит; os — внутреннее пространство яичника; ovs — овиспермадукт; pbs — задний кровеносный синус; trc — туловищный целом.

In the posterior region of the tail, paired blood sinuses were observed in the mesentery during our study for the first time (Fig. 9). The details of the anatomical organization of the circulatory system vary among different Chaetognatha families (Malakhov, Berezinskaya, 2001). The structure of the circulatory system in the trunk and head of chaetognaths is described in detail in the literature (Sninn, 1997; Malakhov, Berezinskaya, 2001; Muller *et al.*, 2019). However, information about the circulatory system of the caudal region is scares, and there is no specific data available for *E. hamata*. It can be assumed that paired blood sinuses provide better nutrition to developing male gametes.

FEMALE REPRODUCTIVE SYSTEM. The general plan of the female reproductive system in *E. hamata* is similar to that described for other species of chaetognaths. It is worth noting, however, that *E. hamata* lacks “wings” in the ovispermaduct, which were described in other species such as *Ferosagitta hispida* and *Sagitta bipuncata* (Shinn, 1997; Carr, 1991). The “wings” are flat, dorsal and ventral outgrowths of the outer epithelium of the ovispermaduct that partially encircle the ovary.

The external funnels that we described as part of the female reproductive system of *E. hamata* have not been previously observed in other species. Their ciliated epithelium may help capture spermatophores more efficiently during mating. This feature may be specific to deep-sea species, although more accurate interpretation of this requires data on the presence or absence of the external funnels in other species. The purpose of the valve in the genital funnel remains unknown.

The general structure of oocytes in *E. hamata* corresponds to one described in previous studies. Oocyte growth is most intensive between the fifth and sixth stages of development, suggesting that vitellogenesis occurs during this period. Indeed, in oocytes at the sixth stage of development, numerous lipid droplets and yolk granules can be seen (Fig. 12D, F). The diameter of eggs in *E. hamata* (50–70 µm) is slightly larger than in other chaetognath species such as *Bathyspadella oxydentata* (Muller *et al.*, 2019) and *Spadella cephaloptera* (Goto, 1999). This may be due to the need for extended nutrient provision for the developing embryos retained in the marsupial cavity until hatching (Kosobokova, Hopcroft, 2021). Based on our observations, we believe that copulation most likely occurs between the

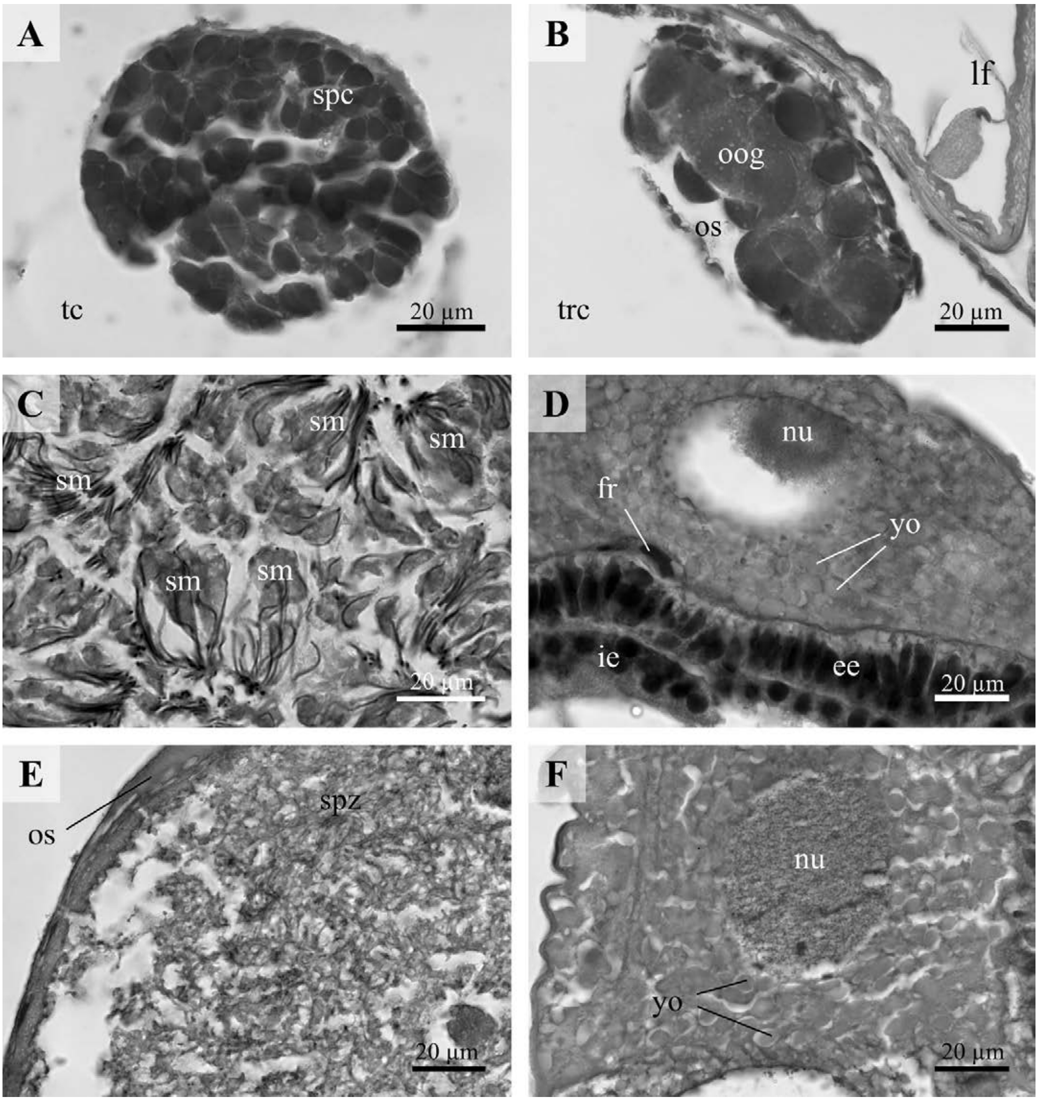

Fig. 12. Some stages of gamete development in *Eukrohnia hamata*, transverse sections. A — the testis at 3rd stage; B — the ovary at 3rd stage; C — the testis at 4th stage; D — the ovary at 4th stage; E — the ovispermaduct filled with sperm at 6th stage; F — the oocyte at 5th stage.

Abbreviations: ee — external epithelium of the ovispermaduct; ie — internal epithelium of the ovispermaduct; fr — follicular reticulum; lf — lateral fin; nu — nucleus; oog — oogonia; os — ovarian space; sm — spermatogenic mass; spc — spermatocyte; spz — spermatozoa; tc — tail coelom; trc — trunk coelom; yo — yolk granule.

Рис. 12. Некоторые стадии развития гамет *Eukrohnia hamata*, поперечные срезы. A — семенник на 3-й стадии; B — яичник на 3-й стадии; C — семенник на 4-й стадии; D — яичник на 4-й стадии; E — заполненный спермой овиспермадукт на 6-й стадии; F — ооцит на 5-й стадии.

Обозначения: ee — внешний эпителий овиспермадукта; ie — внутренний эпителий овиспермадукта; fr — фолликулярный ретикулюм; lf — латеральный плавник; nu — ядро; oog — оогоний; os — внутреннее пространство яичника; sm — сперматогенная масса; spc — сперматоцит; spz — сперматозоиды; tc — хвостовой целом; trc — туловищный целом; yo — гранула желтка.

fifth and sixth stages. This is supported by the fact that the female genital ducts in animals at the fifth stage of development are still empty, and at the sixth stage they are already filled with sperm.

The peeling of the syncytial epithelium from the cellular epithelium of ovispermaduct at the sixth stage, which is described here for the first time, suggests a loose connection between the layers during this stage. This observation is consistent with current knowledge about the fertilization process in chaetognaths. According to literature, fertilization takes place at stages 6–7, and after that, the oocyte is released into the space between the outer and inner epithelium layers of the ovispermaduct (Shinn, 1997). It is possible that degeneration of the junctions between these two layers begins at the sixth stage. However, the composition of the heterogeneous content of the ovispermaduct remains an open question.

This structure has also been described for other chaetognaths (*Adhesisagitta hispida*: Shinn, 1997). However, in *E. hamata* the peri-ovarian coelom is filled with a substance that is similar to the liquid in the lumen of the blood vessels, particularly the posterior blood sinus, in terms of its histological characteristics. Single small cells with rounded nuclei are also observed in the lumen of the vessels. Undoubtedly, the ovarian cavity corresponds to the coelomic cavity and, according to generally accepted ideas (Ruppert *et al*., 2008), it should not directly connect with the circulatory system (hemocoel). Unfortunately, the available data from electron microscopy studies (Muller *et al*., 2019) have not yet revealed the nature of the phenomenon that we have discovered. Nevertheless, the histological similarities between the fluids in the ovary and the posterior blood sinus need to be further explored and interpreted using more advanced methods.

POSSIBLE ECOLOGICAL SIGNIFICANCE. All said above aligns with current data on environmental adaptations in deep-sea organisms and r/K selection theory. It is a common knowledge that r-strategy, characterized by high fecundity, small body size and high mortality rates at the early stages of development allows species to adapt to rapidly changing conditions. K-strategy, on the other hand, is common for species inhabiting stable and predictable environments. K-selected species generally exhibit greater body size than r-selected species and are characterized by low fecundity and greater offspring survivability.

Deep-sea environment is a rather stable one, characterized by low temperature fluctuations throughout the year and almost constant levels of water salinity and lighting. Thus, deep-sea species are generally K-selected. K-strategy has been noted in various invertebrates, including copepods (Auel, 2004), several species of gastropods (Rex, 1979) and bivalves (Gauldron, 2021). We believe that *E. hamata* is also inclined to K-stategy. This hypothesis is supported by the traits we have discovered: the presence of spermatophores and ciliated funnels which serve to increase the number of male gametes captured into the female gonoducts and oocytes which are greater in size than known for planktonic species.

It is also important to note that currently chaetognaths are known to reproduce only sexually. However, all studies on chaetognaths mating behavior have been conducted either on benthic (*Spadella cephaloptera*: Ghirardelli, 1968; Duvert *et al*., 2000; Goto, Suzuki, 2001); *Paraspadella gotoi*: Goto, Yoshida, 1895) or planktonic species inhabiting upper layers of the ocean (*Ferrosagitta hispida*: Reeve, Walter 1972; *Aidanosagitta crassa*: Murakami, 1959). Such species have high population density and high potential to meet a mate. In contrast, scanty populations of deep-sea species like *E. hamata* might take advantage of self-fertilization (autogamy). The results of our study allow to speculate that fertilization in *E. hamata* could also occur through autogamy. This is supported by the pronounced protandry in the reproductive system maturation cycle, as well as the fact that at sixth stage the ovispermaduct is filled with sperm while tail coelom and seminal vesicles are completely empty of male gametes.

Conclusion

The study of reproductive system of *E. hamata* has revealed adaptations to the deep-sea environment, including the gathering of spermatozoa in spermatophores and the presence of ciliated funnels surrounding the female gonopores. These features likely increase the efficiency of sperm capture during copulation, which occurs between the fifth and sixth stages of maturation in *E. hamata*. The anterior lobes of the tail coelom may also increase its volume to contribute to the accumulation of a larger quantity of male gametes. Moreover, the intertwining of the tail and trunk compartments ensures efficient fluid exchange and nutrient delivery to the developing gametes. In addition, this is facilitated through paired blood vessels in the tail mesentery.

Compliance with ethical standards

CONFLICTS OF INTEREST: The authors declare that they have no conflicts of interest.

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