New data on embryonic development of *Halisarca dujardini* Johnston, 1842 (Demospongiae, Halisarcida)

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ABSTRACT

Embryogenesis of *Halisarca dujardini* from the White Sea (Russia) was studied by light and transmission electron microscopy. The cleavage is equal and asynchronous. From the stage of 8-16 blastomeres, the cells divide perpendicularly to the surface of embryos and form a coeloblastula. The few internal cells of the prelarva are derived by multipolar migration of external cells. The larva (disphaerula) consists of two flagellated spheres: external and internal. The internal sphere is formed by invagination of lateral flagellated cells. The disphaerula is completely flagellated, but the flagella on posterior pole are sparse. The peculiarities of *Halisarca* embryonic development indicates the particular position of this genus in subclass Ceractinomorpha. The pattern of development is most primitive in this subclass.

RÉSUMÉ

*Données nouvelles sur l'embryogenèse d'*Halisarca dujardini *Johnston, 1842 (Demospongiae, Halisarcida).*

L'embryogenèse d'*Halisarca dujardini* de la mer Blanche (Russie) a été étudiée en microscopie photonique et électronique. La segmentation est égale et asynchrone. À partir du stade 8-16 blastomères, les cellules se divisent perpendiculairement à la surface de l'embryon et forment une coeloblastula. Les rares cellules internes de la prélarve proviennent d'une migration multipolaire de cellules externes. La larve est une disphaerula formée de deux sphères flagellées, une externe et une interne. La sphère interne se forme par l'invagination de l'épithélium flagellé superficiel. La disphaerula est entièrement flagellée, mais les flagelles du pôle postérieur sont clairsemés. Les particularités du développement embryonnaire des *Halisarca* témoignent de la position particulière de ce genre dans la sous-classe Ceractinomorpha. Le développement des *Halisarca* est le plus primitif des éponges de cette sous-classe.

INTRODUCTION

Sponges are distinguished for high plasticity and variability of their morphological structure. Nevertheless, it is always possible to identity a set of morphological characters typical for a species, genus, family, order, etc. Since each structure is the result of a previous morphogenesis, there arises the requirement for comparative analysis of all the stages of sexual development. Various distinctive taxonomic characters may be found not only in adult specimens but also in the early ontogenesis as well. An excellent example of such analysis was demonstrated by C. Lévi (1956) on higher taxa level within the class Demospongiae.

For the group of Demospongiae lacking a mineral skeleton, and formerly referred to the order Keratosa, the application of comparative embryological methods seems to be required. Presently these sponges are placed into several orders (Bergquist *et al.* 1998).

Due to the simplicity of organization, the genus *Halisarca* Johnston, 1842 stands out among Ceractinomorpha lacking a mineral skeleton. Many authors repeatedly noted significant differences between *Halisarca* and other keratose sponges including the order Dendroceratida, into which this genus was placed for many years (Lévi 1956; Bergquist *et al.* 1979; Bergquist 1980; Vacelet *et al.* 1989). In particular, *Halisarca* differs from Dendroceratida anatomically (Lévi

1956; Bergquist 1996), cytologically (Bergquist 1980; Vacelet & Donadey 1987; Vacelet *et al.* 1989), biochemically (Bergquist & Wells 1983) as well as in specific features of larval morphology (Bergquist *et al.* 1979; Bergquist 1980). This allowed Bergquist (1996) to separate *Halisarca* as a monogeneric order, Halisarcida. However, embryological peculiarities of the order were not included in the diagnosis.

Due to indistinctness of morphological characters required for species identification, ultrastructural characters together with peculiarities of embryological development become particularly important. Thus, on the basis of ultrastructural properties, a new species *Halisarca caerulea* (Vacelet & Donadey 1987) was described. Differences in sexual reproduction combined with ecological features gave opportunity to separate *H. metschnikovi* (Lévi 1953, 1956) and *H. nahantensis* (Chen 1976).

The modern comparative embryology of the Porifera based upon use of contemporary methods has not been worked out yet due to insufficient knowledge of embryonic developmental in species from different orders. Consequently, the features of embryogenesis, in particular Porifera groups, are still neglected when identifying or describing the group. *Halisarca* is no exception, although its embryonic development has been studied for about 100 years (Giard 1873; Barrois 1876; Schulze 1877; Metschnikoff 1879; Lévi

1953, 1956; Chen 1976; Korotkova & Ermolina 1982, 1986; Korotkova & Ereskovsky 1984; Sizova & Ereskovsky 1997).

The objective of the present study is the investigation of embryogenesis and larval development of *H. dujardini* with the use of electron microscopy and optical methods and the elucidation of characteristic features of development for the order Halisarcida on the basis of the obtained data. We have demonstrated that in a set of early ontogenetic features *Halisarca* essentially differs from other Porifera.

MATERIAL AND METHODS

Reproducing specimens of *Halisarca dujardini* were collected in the Chupa Inlet near the Sredniy Island 33º05'E, 66º15'N (Kandalaksha Bay, White Sea) from the depth of 1.5-5 m in June-August 1995-1998.

Material for light microscopy was fixed in Bouin fixative. Then tissue fragments were dehydrated through an ethanol series, placed in a celloidin-castor oil mixture and then in chloroform and embedded in paraffin. 6 µm thick sections were mounted on glass slides and stained with Mayer's hematoxylin, eosin and Heidenheim ferric hematoxylin.

For electron microscopy, the sponges were cut into cubes of about 1 mm. These samples were prefixed in 1% OsO_4 for 10 min and fixed in 2.5% glutaraldehyde in phosphate buffer at room temperature for 1 h. After fixation, sponge samples were washed in the phosphate buffer and postfixed in 1% OsO_4 in phosphate buffer for 1 h. Samples were dehydrated through graded ethanol series and embedded in Epon-Araldite. Semi-thin sections were stained with methylene blue-borax. Ultrathin sections were stained with uranyl acetate and lead citrate.

OBSERVATIONS

Halisarca dujardini Johnston, 1842 (Ceractinomorpha, Halisarcida) is a dioecious sponge. Males begin to produce spermatocysts with spermatocytes in approximately mid-December at water
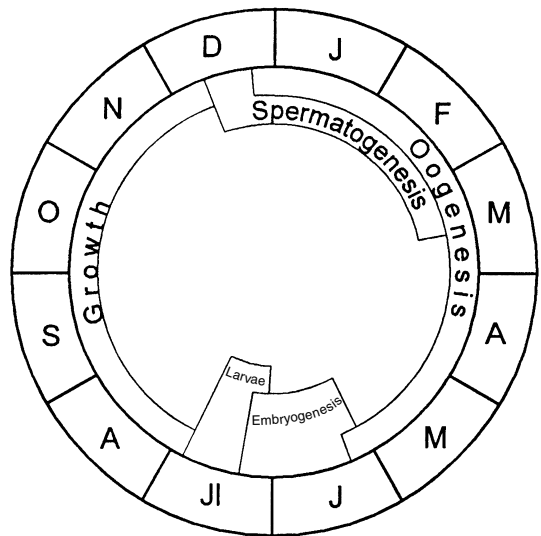

FIG. 1. — Life-history scheme of *Halisarca dujardini* in the White Sea.

temperature of about – 0.1 °C. Early oocytes differentiate in the last decade of December when water temperature fluctuates around – 0.6 °C. Vitellogenesis starts in May at nearly + 2 °C. Eggs mature by the end of May. Cleavage and larval development occur from the second half of June until the end of July when water temperature averages 10-12 °C. The volume of reproductive elements (embryos and prelarvae) reaches its maximum by the end of June – beginning of July and reaches to about 69.5% of sponge volume (Ereskovsky 2000). It is the time when mesohyl destruction and complete disorder of central and basal choanosome, which are largely filled with developing larvae, is taking place. Normal tissue organization remains only in the thin periphery of a sponge. In the studied population, larval emergence is swift, lasting for one and a half to two weeks in the second half of July at about 12 °C. Subsequently slow development of the new generation of sponges and postreproductive revival of maternal specimens is going on until December. The general scheme of the *H. dujardini* life cycle in the White Sea is illustrated on Fig. 1.

Embryogenesis, larval development and metamorphosis of the studied population of *H. dujardini* is continuing generally for four weeks from late June till end of July.

The oocyte development of this viviparous species occurs inside temporary embryonic capsules formed before maturation divisions of oocytes (Korotkova & Apalkova 1975). Every capsule has a double layer structure: the cells of the embryonic capsule stretching along the embryo surface and the layer of collagen filaments extending parallel to the cell surface on the outside. The thickness of fiber layer is 1 ± 0.5 µm. Distal parts of embryonic capsule cells overlay each other forming a continuous cell layer. No special contacts between individual cells have been recorded (Sizova & Ereskovsky 1997).

Before cleavage cell division, a mature egg is a slightly oval body of 129 × 105 µm size. A spherical nucleus about 28 µm in diameter with a distinctly marked nucleolus (8 to 12 µm) is located in the centre of an egg. Vitelline granules are evenly spread over the whole volume of egg (Fig. 5A).

Eggs cleavage in *H. dujardini* is complete, equal and asynchronous. If the egg pole where maturation divisions are taking place is conventionally considered to be the animal one, the first cleavage division in *H. dujardini* occurs in the meridional plane. The size of the first two blastomeres is about 75.3 × 105.6 µm. The nucleus diameter is 16.4 µm. During all cleavage stages, the interphase blastomeres nuclei contain one to three pronucleolar bodies of 3 to 8 µm diameter at their periphery. As a rule these structures are of rounded shape. During the second cleavage division, the relative position of spindles varies from nearly parallel to perpendicular to one to another, while the relative position of blastomeres resembles a tetrahedron.

Similarly during the third cleavage division the spindle position within the embryo is also varying. As a result of asynchronous cleavage, embryos at the stage of five, six and seven cells might be found in one sponge. The octacellular embryo is rounded or slightly oval-shaped in the cross section. All the blastomeres are oval (Fig. 2A).

Subsequent cleavage continues to be asynchronous but cleavage spindles in this case are positioned in parallel to the embryo surface; as a result division planes become radial. A single-layered coeloblastula consisting of 18 to 24 cells sur-

rounding a small cavity is thus formed. Polarity of the embryo is indistinct. Large nuclei with nucleoli are located in the central part of the cells. Rounded vitelline granules of heterogeneous content and ranging between 0.2 to 2.4 µm in diameter occupy most of the volume of blastomere cytoplasm. These structures are pyroninephilous, their dimensions slightly increase with the distance from the nucleus. Beginning after the fourth to fifth cleavage cycle, polarization of the blastomeres occurs with the movement of nuclei in apical direction and vitelline granules in the basal direction (Fig. 5C). Cleavage planes of polarized blastomeres are radially directed resulting in the formation of blastula of 130 to 155 µm diameter with a small cavity (nearly 30 µm in diameter) limited by wedge-form cells. At the stage of about 100 cells, the apical-basal polarity of blastomeres is clearly indicated (Fig. 5D). Such cells are about 70 µm in length and their apical breadth is nearly 25 µm; the rounded nucleus (about 9 µm in diameter) contains a nucleolus (about 2.5 µm). Sparse cisterns of Golgi apparatus and minute mitochondria are arranged around the nucleus.

At this stage some cells of a blastula migrate into the cavity. This process is apolar. Before the beginning of migration, the nucleus is shifted to the basal part of the cell which in this case begins to expand (Fig. 5C). The examination of serial sections has indicated that two to four cells migrate simultaneously. In the central cavity of the blastula those cells become spherical (about 27 µm in diameter), the nucleus with nucleolus occupying the central position (Fig. 2B). The inner cells of embryo do not form a dense mass. Subsequently they start dividing with the ensuing differentiation into amoebocytes. At the same time flagella are formed at the apical end of external cells.

Flagellate cells continue to proliferate actively. Before its division, the cell becomes rounded and migrates to the periphery of the body where it starts to divide. The division plane is perpendicular to the larval surface (Figs 3A; 5E, F). After the division, the daughter cells elongate and embed themselves into the columnar epithelium. At that time the epithelial cells are about 35 µm long and 5.6 µm wide near the nucleus, which itself is
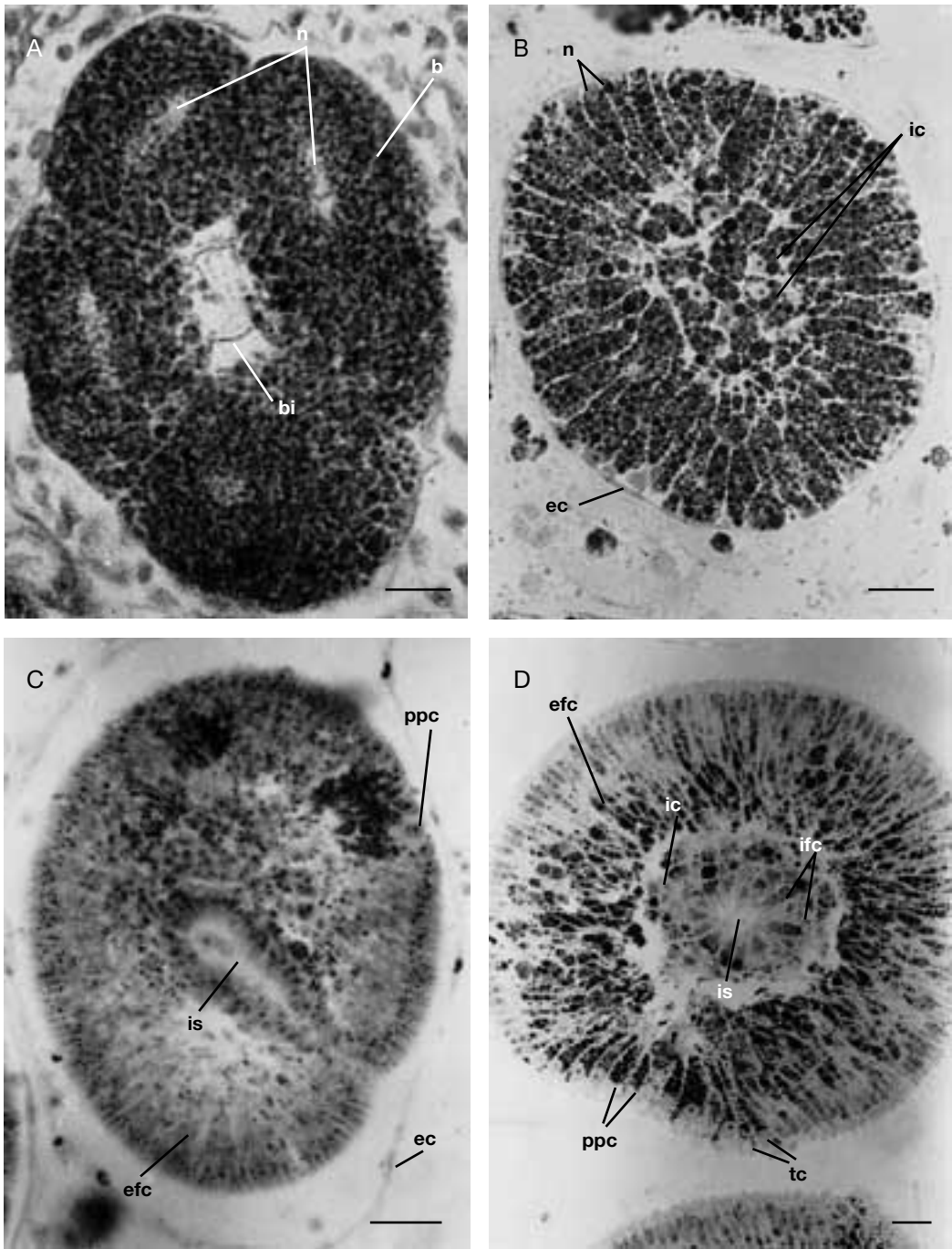
FIG. 2. — **A**, cleaving embryos in 8-cell stage; **B**, blastula after immigration of external cells; **C**, stage of invagination of flagellate cells forming internal sphere; **D**, disphaerula of *H. dujardini*. Abbreviations: **ec**, embryonic capsule; **ic**, internal cells; **n**, nucleus; **efc**, external flagellate cells; **b**, blastomere; **bi**, blastocoel; **ifc**, internal flagellate cells; **is**, internal sphere; **ppc**, posterior pole cells; **tc**, transition cells. Scale bars: A, 20 µm; B, 15 µm; C, 20 µm; D, 10 µm.
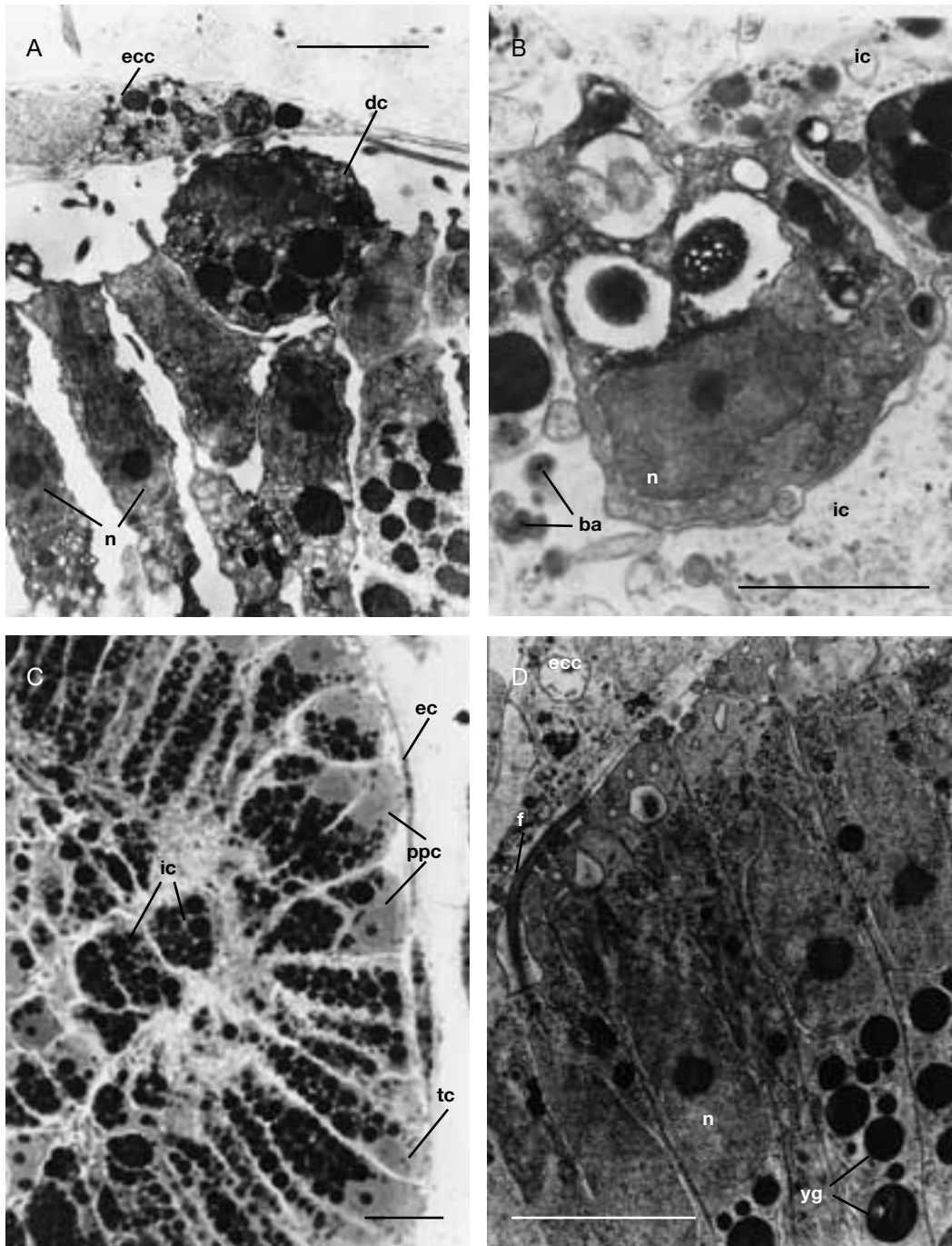
FIG. 3. — **A**, division of external flagellate cells in prelarva; **B**, maternal eosinophilic granular amoebocyte between internal cells of disphaerula; **C**, posterior pole of larva; **D**, external flagellate epithelium of larva. Abbreviations: **ba**, bacteria; **ic**, internal cells; **ec**, embryonic capsule; **ppc**, posterior pole cells; **tc**, transition cells; **dc**, divided cell; **ecc**, embryonic capsule cell; **f**, flagellum; **n**, nucleus; **yg**, yolk granules. Scale bars: A, 5 µm; B, 5 µm; C, 10 µm; D, 5 µm.

about 4.3 μm in diameter. Differentiation of cells of the posterior pole is occurring simultaneously with differentiation of anterior-lateral cells (Fig. 5E). No division of internal cells has been recorded at this stage.

During the period of active proliferation of the external cells, the larval surface becomes corrugated. The internal part of larva is a cavity surrounded by basal parts of flagellate cells. At the same developmental stage, granular eosinophilic (fuchsinophilic) amoebocytes migrate from the maternal sponge mesohyl into the larva via the embryonic capsule (Figs 3B; 5F).

Gradually the larval surface becomes flattened, proliferation is discontinued and the cells acquire the shape and size characteristic of a mature larva. Before the surface becomes finally smoothed, a deep and narrow invagination of the flagellate layer occurs, closing near its mouth (Fig. 2C). Invagination of this layer proceeds perpendicular or at an angle to the anterior-posterior axis of the larva. A single-layered closed structure surrounding a small cavity is formed by the internalized flagellate cells as a result of this process. The blastocoel is reduced, persisting in the form of narrow gaps between the internal and external flagellate cell layers.

The form and shape of the closed structures newly formed by flagellate cells inside the larva may be widely variable. These formations have mostly the form of C- or Σ- shaped, curved cylinders; sometimes they are oval or spherical; their cross-sections are tubular-shaped. The inner cavity diameter ranges between 8.5 μm and 12.5 μm while inner cavity length varies from 28 μm to 61 μm. One or two cells may be often found inside the cavity. This stage terminates the larval development. The walls of embryonic capsules which contain newly formed larvae fuse with the walls of exhalant channels and larvae escape through the osculum. Milk white-colored larvae of *H. dujardini* emerged from the maternal organism are oval or have the shape of a spheroid slightly depressed in the anterior-posterior direction with flat or concave posterior pole (Figs 2D; 5G). They are completely covered with flagella, however in the posterior pole region, the flagella are sparse. The diameter of flagella-sparse cell layer is about 45 μm. Anterio-lateral larval flagellate cells are nearly cylindrical, 38 to 45 μm long

and 2.6 to 3.0 μm wide in the nucleus region. The prolate nucleus (2.6×6.6 μm) with small nucleolus and the flagellum emerging from a pocket-shaped cytoplasm depression are located in their apical part (Fig. 3D). The basal two thirds of these cells is filled with abundant vitelline granules. This cell layer lacks a basal membrane.

In longitudinal section the flagellate cells of the posterior pole are wedge-shaped; they are shorter and wider than the anterio-lateral cells (26 μm long and 6-7 μm wide in the nucleus region). Numerous vitelline granules are spread not only in basal and central parts of the cell, but near the nucleus as well. Rounded or drop-shaped nuclei are about 5.3×4.2 μm in diameter and contain large nucleoli (near 1.6 μm) (Fig. 3C). A ring of tapering flagellate cells is arranged between cells of the posterior pole and covering flagellate cells. The length of transitional cells fluctuates around 30 μm, the width in the nucleus region is about 4 μm; nuclei with nucleoli are oval-shaped (3.5×4.9 μm) (Fig. 3C). The number of cells in the transitional zone does not exceed four to five. There are no specialized contacts between the larval cells.

A most striking feature of the larval structure of *H. dujardini* is the presence of a large closed formation composed of flagellate cells (derivative of the prelarval flagellate layer) inside the larva. This structure is spherical and has a cavity delimited by the apical tips of the internalized flagellate cells. The external diameter (over basal cell apexes) of spherical layer ranges from 49 μm to 54 and the diameter of its cavity from 13.8 μm to 17.2 μm (Fig. 2D). The cells forming this structure are wedge-shaped with the extended basal part concentrating numerous vitelline granules. the flagellum, submerged into a pocket-shaped depression, and the nucleus are located near the narrow apical pole. Cell length varies from 13.8 μm to 17.2 μm, cells width from 3.4 μm to 5.7 μm. Drop-shaped or egg-form nuclei (2.3 μm $\times 5,8$ μm) in these cell contain clearly distinguished nucleoli of about 0.9 μm diameter (Fig. 4A).

Before larval emergence out of maternal tissues, the interior chambers become spherical and their dimensions decrease. The process of cell ejection seems to be the cause of these changes. Numerous intermediate cells are located among internal cells in the space between the internal and external
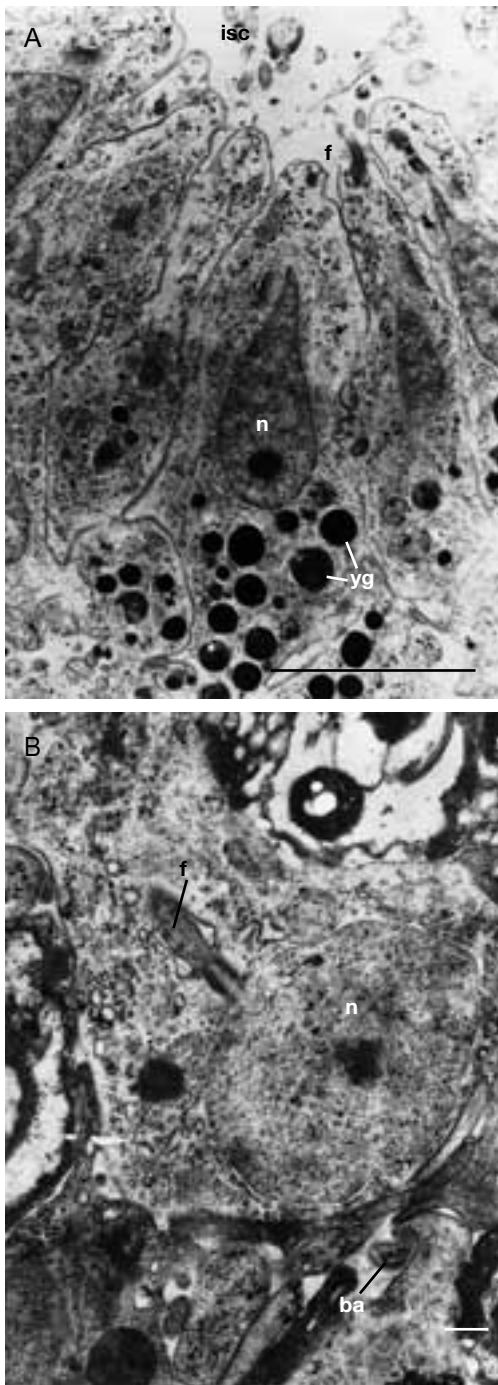
FIG. 4. — **A**, internal flagellate cells of disphaerula; **B**, flagellated cell that migrated from internal sphere. Abbreviations: **f**, flagellum; **isc**, internal sphere cavity; **n**, nucleus; **yg**, yolk granules; **ba**, bacteria. Scale bars: A, 4 µm; B, 1 µm.

epithelia. They are oval-shaped, one pole has a flagellum, near which a rounded nucleus is located. The cytoplasm contains numerous vitelline granules (Fig. 4B).

Interspaces between outer and inner flagellate cells are occupied by the internal cells of the larva: nucleolate amoebocytes. In addition, granular eosinophilic cells of maternal origin are included into the larva. They may be located in different zones: apical, central, basal regions of flagellate layer, in the central part of larva, between the cells of the posterior pole.

Larvae have a spiral swimming movement counter-clockwise at the same time rotating themselves clockwise around the anterior-posterior axis. Sometimes they perform twirling movements. The free-living period of the larva lasts for 4 to 36 hours.

Larvae settle on a substrate with their anterior pole. However, even on a substrate, larvae continue to rotate around their anterior-posterior axis for 30 to 45 minutes before attachment. This rotation is finished after complete attachment.

Preparation for metamorphosis begins already during the swimming period as evidenced in larval behaviour and structure. Premetamorphic larvae are slower in their swimming, may attach themselves to surface film or sink and slowly move over the substrate. Whether attached to surface film or moving over the substrate, the larvae proceed slowly rotating.

Shape variation of the external flagellate cells and violation of their mutual contacts may primarily be observed on ultrastructural level. The lateral surfaces of cells become corrugated and the cells are contracted in the apical-basal direction. However, flagellate cells remain interconnected in their apical parts. In this state larvae settle down on substrate and attach to it.

During the first stages of settling and metamorphosis, the larva slightly flattens in the anterior-posterior direction; its anterior pole flattens on the substrate, while the posterior one remains rounded. The total disintegration of the internal spherical chamber and external flagellate layer takes place during the first 12 hours after the attachment.

Subsequent processes associated with metamorphosis will be described in a special study.
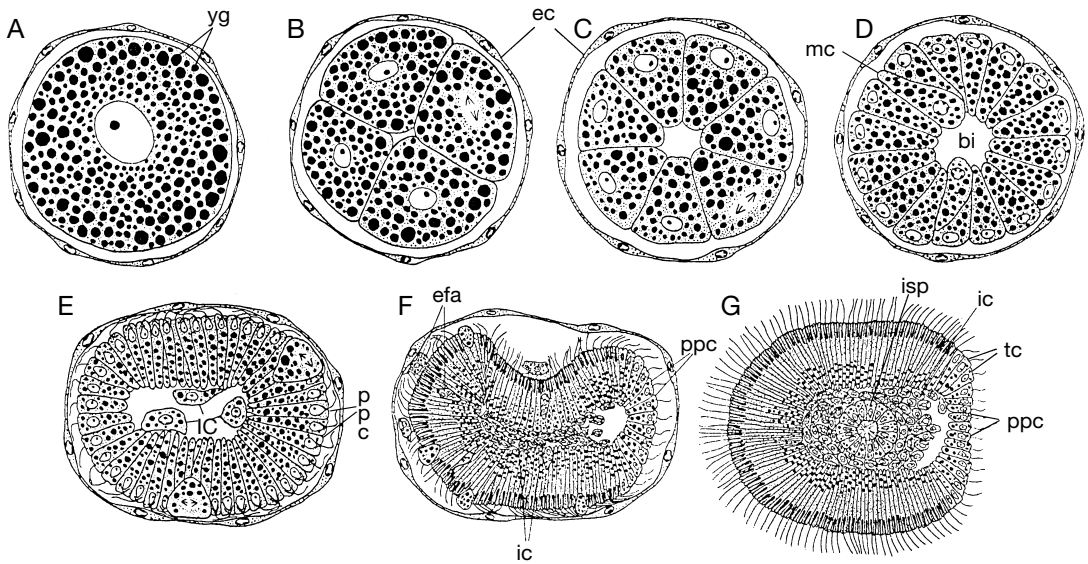
FIG. 5. — Scheme of *H. dujardini* embryonic development; **A**, mature egg; **B**, early cleavage; **C**, beginning of blastomere polarization; **D**, multipolar immigration; **E**, stage larval polarization; **F**, formation of internal sphere and migration of maternal eosinophilic granular amoebocytes into larva; **G**, disphaerula, larva of *H. dujardini*. Abbreviations: **bi**, blastocoel; **ec**, embryonic capsule; **efa**, eosinophilic amoebocytes; **ic**, internal cells; **isp**, internal sphere; **mc**, migrated cells; **ppc**, posterior pole cells; **tc**, transition cells; **yg**, yolk granules.

DISCUSSION

Considering the basic criteria for complexity of animal organization to be the level of differentiation and specialization of body anatomy, organ systems, tissues, and cells, including those involved in integration, one may assume necessarily that similar criteria are valid for the estimation of complexity of the individual development process. The rate of morphogenesis specialization during particular ontogenetic stages, the level of specialization of cells participating in those processes, stability of type of reproduction process and its determinacy may serve as examples of such criteria (Ereskovsky & Korotkova 1997).

Data obtained from the present investigation show that both primitive and specialized characters are present in the early embryogenesis and larval development of *H. dujardini*. Equal cleavage (Fig. 5B-D), variability of similar cleavage stages, and absence of a predetermined arrangement of the blastomeres are considered primitive features of cleavage. This was stated by Barrois (1876), Korotkova & Ereskovsky (1984) and

Sizova & Ereskovsky (1997). Those peculiarities may be explained by the absence of pronounced characters of promorphology in the mature egg, which retains its homogeneous structure in different parts (Lévi 1956; Korotkova & Apalkova 1975; Aisenstadt & Korotkova 1976). The nucleus of mature egg occupies the central position; this position is retained during the early cleavage period (Fig. 5A).

The early origin of uniform radial cell division planes (beginning from the fourth cycle) shall be considered an original cleavage feature. The direction of these planes remains radial until the larva is formed (Fig. 5C-F). Such pattern of egg cleavage is unique among Ceractinomorpha but is typical of Calcinea: *Clathrina blanca* (Miklucho-Maclay, 1868), *C. cerebrum* (Haeckel, 1872), *Ascandra falcata* (Haeckel, 1872), *A. minchini* Borojevic, 1966 (Borojevic 1969). A single-layered flagellate blastula is formed as the result of cleavage. The radial type of larval cell division in *H. dujardini* results in the formation of a blastula. It is characteristic that the coeloblastic organisation is retained until the formation of the larva as

indicated by Korotkova & Ermolina (1982) and Korotkova & Ereskovsky (1984). This evidence disagrees with some authors insisting on the existence of a morula stage in *H. dujardini* (Giard 1873; Metschnikoff 1879; Lévi 1956; Chen 1976). The coeloblastic organisation of *H. dujardini* in the prelarval stage also seems to be unique, not recorded in any species of subclass Ceractinomorpha.

Inner larval cells originate at the 100 to 120 cell stage by multipolar immigration of single cells as is clearly demonstrated by the analysis of serial, semi-thin sections. Internal cells in *H. dujardini* do not form dense aggregations which we nevertheless are not considering as morula stage. According to Lévi (1956), in representatives of *H. dujardini* from the different population studied by the authors, the internal cells are formed as the result of unipolar migration, determining the future posterior pole of the larva.

The formation of aggregations of internal larval cells due to immigration of some of the surface cells is mostly characteristic for embryogenesis of the subclass Calcinea (class Calcarea) (Borojevic 1969). However, in this case the process occurs in the swimming larva and is associated with its preparation for metamorphosis. The formation of an internal cell mass by the process of ingression leading to the development of a stereoblastula has been reported only for the relict Sphinctozoa *Vaceletia crypta* (Vacelet, 1977) (Vacelet 1979) among Demospongiae. Some authors treat this process as gastrulation (Lévi 1956; Efremova 1997).

A delayed formation of the anterior-posterior polarity in the larva of *H. dujardini* (after cleavage and during immigration of inner cells) may be also considered a primitive feature of its development. In this case the posterior pole is not determined by the ejection place of inner cells as it occurs in *H. dujardini* studied by Lévi (1956). The rate of cell division in the future posterior pole of the larvae is lower than that of the anterior-lateral parts. As a result the posterior cells retain the features of blastomeres for a longer time: nuclei are rounded and cytoplasm near the nucleus contains conspicuous vitelline granules. The earlier anterior-posterior polarization of the embryo is characteristic for all Ceractinomorpha

(Lévi 1956, 1963; Borojevic 1970; Korotkova 1981).

During the process of cell division the flagellate cells of *H. dujardini* migrate to the surface, round up and only then begin to divide. A similar pattern of cell division is characteristic for some columnar epithelium of Metazoa. Thus during the development of the neural tube in Vertebrata, division is started after migration of the nucleus and concentration of cytoplasm near the surface of epithelium (Sauer 1935). Such a mode of cell division among the Porifera is described, for instance, in *Ephydatia fluviatilis* (Linnaeus, 1758) (Brien & Meewis 1938), *Haliclona limbata* (Montagu, 1818) (Meewis 1939), *Octavella galangaui* (Tuzet & Paris, 1963) (Tuzet & Paris 1964), *Spongilla lacustris* (Linnaeus, 1758) (Saller & Weissenfels 1985).

The studies by Korotkova & Ermolina (1982, 1986), as well as our observations, have demonstrated that the migration of granular eosinophilic amoebocytes (= fuchsinophilic amoebocytes) from the maternal mesohyl through the embryonic membrane into the larva occurs during the formation of the larvae in *H. dujardini* of the White Sea (during the period of fold formation). Chen (1976) described a similar process in *H. nahantensis* Chen, 1976. Granular eosinophilic cells in *H. dujardini* (Fig. 3B) are similar in ultrastructural organization to spherulous cells of other Ceractinomorpha, for instance, *Crambe crambe* (Schmidt, 1862) (Uriz *et al.* 1996). Concerning the question of the function of those cells, it has been demonstrated that in various Demospongiae species the metabolites are contained in their spherulous cells (Thompson *et al.* 1983; Bretting *et al.* 1983; Uriz *et al.* 1996) that might be also involved in sexual reproduction (e.g. Simpson 1984). These cells in *H. dujardini* may possibly transport biologically active substances (Korotkova & Ermolina 1986), since the beginning of larval mobility inside the embryonic capsule is correlated with the invasion of these cells into the larva. In any case the granular eosinophilic cells remaining in the *H. dujardini* larva may not be nutrient cells as suggested by Chen (1976), since those cells are retained in the larval composition of *H. dujardini* until the first stages of metamorphosis.

Proliferation of the flagellate cells of the early larva gives rise to numerous folds and invaginations, evidently associated with the increase of larval surface area. The formation of a plicate larva is described by C. Lévi (1956) and Korotkova & Ermolina (1982, 1986) in *H. dujardini*, and by Chen (1976) in *H. nahantensis*. The stage of rugose prelarva is common in various Ceractinomorpha from different orders: Dictyoceratida: *Hippospongia communis* (Lamarck, 1814), *Spongia reticulata* (Lendenfeld, 1886) (Tuzet & Pavans de Ceccatty 1958; Bergquist *et al.* 1970); Halichondrida: *Halichondria panicea* (Pallas, 1766) (Ivanova 1981); Haplosclerida: *Ochridaspongia rotunda* (Arndt, 1937), *Baikalospongia bacillifera* (Dybowski, 1880) (Gilbert & Hadziscie 1977; Ropstorf & Reitner 1994; Efremova, pers. comm.); Poecilosclerida: *Iophon piceus* (Vosmaer, 1881), *Mycale lobata* (Bowerbank, 1866) (Ereskovsky 1986; Ereskovsky unpubl.), as well as to Homoscleromorpha forming blastular larva, for instance, in *Oscarella lobularis* (Schmidt, 1862) (Meewis 1938) and *O. galangaui* (Tuzet & Paris 1964).

Externally the larvae of the analysed *H. dujardini* population do not differ from their description made by previous authors. They have an oval shape or a shape of hemisphere slightly flattened in anterior-posterior direction with a flattened posterior pole. Larger flagellate cells are located near the posterior pole.

The internal larval structure of *H. dujardini* may be considered one of the most striking features of its early ontogenesis. Almost all the authors that have studied the development of *Halisarca* regarded its larva to be of the parenchymella type (Schulze 1877; Metschnikoff 1879; Lévi 1953, 1956; Chen 1976; Bergquist *et al.* 1979; Bergquist 1996). The only researchers who considered the larva of *H. dujardini* to be blastula-form were Korotkova & Ermolina (1982).

The spherical chamber formed by the flagellate cell layer is located inside the larva. There is a small gap inside the chamber into which flagella are directed. The flagellate cells of the internal sphere cannot be referred to as choanoblasts (Ereskovsky & Gonobobleva unpubl.). The outer diameter of the chamber is so great (about 52 µm) that only a narrow space (12 µm to 18 µm) occupied by the internal cells remains between the basal ends of flagellate cells of the inner sphere and those of the external larval layer (Figs 2D; 5G). Such a larval type has not been described in Porifera. It cannot be referred to as a parenchymella or blastular type. Therefore we propose to name this larva a disphaerula. The internal spherical layer of flagellate cells is formed in *H. dujardini* during the termination of active proliferation by the external cells. This layer is formed as a deep lateral invagination of one of the prelarval surface folds and its subsequent shrinkage. Thus, the internal larval spherical chamber is the derivative of the surface flagellate layer. The invagination of the external cells of the *H. dujardini* larva is represented by a type of plate curve: a mechanism that is typical for epithelial morphogenesis (Fristom 1988). The morphogenesis in *H. dujardini* is associated with the larval surface flattening. In our opinion, this invagination as well as the immigration of blastula cells cannot be regarded as gastrulation. The problem of gastrulation in sponges is difficult and there are different opinions on this point (Efremova 1997; Ereskovsky & Korotkova 1997).

Before the larval escape from the maternal organism, the shape of the inner chamber becomes spherical and its dimensions decrease. This phenomenon is evidently associated with the ejection of part of cells of the internal sphere into the remnants of the blastocoel which is confirmed by the intermediate cells found outside the epithelia. These cells are rounded and flagellate.

The natural question might arise about why the specific features of larval structure have escaped the attention of the researchers who studied the larval development of *Halisarca* (Schulze 1877; Metschnikoff 1879; Lévi 1953, 1956; Chen 1976; Bergquist *et al.* 1979; Korotkova & Ermolina 1982; Bergquist 1996). It can be by a consequence of two reasons. First, for the larvae of *H. dujardini* the polymorphism is typical (Ereskovsky & Gonobobleva, in prep.). Besides disphaerula, blastula type and parenchymella type larvae exists. They differ from disphaerula by absence of the internal flagellated chamber and different quantity of internal cells. The internal cells of the blastula type larvae are not numerous and settle down along the basal parts of

epithelial cells. For larvae of parenchymella type a lot of internal cells and more dense packing of these cells is characteristic. Second, this might be due to the fact that all the mentioned authors dealt with histological preparations made of paraffin blocks no less than 5 µm thick and stained with hematoxylin. The borders between the tightly interpressed cells of both flagellate layers are practically indistinguishable, as well as the borders between the basal ends of cells of both epithelia. These frontiers may be identified only on serial semi-thin sections or by using electron microscopy. This is probably the reason why the internal part of larva looked like a homogenous mass of poorly discernible cells.

Lévi (1956, 1963), Chen (1976) and Korotkova & Ermolina (1982) noted the presence of flagellate chambers inside the larva. However, those authors did not attach special significance to those structures. Korotkova & Ermolina (1982) considered them cross-sections of deep surface layer invaginations. Lévi (1956, 1963) considered small inner hollows inside the larva limited by flagellate cells to be temporary formations originated as the result of inward migration of individual cells. Finally, Chen (1976) only mentioned that the inner flagellate chambers in *H. nahantensis* were formed as the result of invagination of the outer layer.

The larval flagellate cell chambers are described only in demosponges of the order Haplosclerida, both marine and freshwater (Ereskovsky 1999). However, small chambers in the larvae of these sponges have nothing in common with the flagellate sphere of the *Halisarca* larva because they are formed as the result of choanoblast differentiation and represent larval choanocyte chambers. The invagination mechanism of formation of the inner cell layer in the *Halisarca* larva is unique not only among Ceractinomorpha but among all Porifera as well.

Spiral gram-positive bacteria were found in *H. dujardini* embryos at various developmental stages. They could be located both between and inside the blastomeres; in embryo cells bacteria were always inside vacuoles (Sizova & Ereskovsky 1997). Beginning with the 8-cell stage, the bacteria are concentrated in the forming cavity of blastula. During the subsequent development, they remain in the central part of the larval and in intercellular spaces (Figs 3B; 4B). Bacteria found in the developing embryo of *H. dujardini* are probably endosymbionts which are abundant in the mesohyl of adult specimens. This transembryonic method is used to transfer symbiotic bacteria to sponges of a new generation. This process is described in some Demospongiae: *Hamigera hamigera* (Schmidt, 1862) (Boury-Esnault 1976), *O. lobularis* (Lévi & Porte 1962), in four species of Dictyoceratida (Kaye & Reiswig 1991).

The present investigation has indicated the following peculiarities in the embryonic development of *H. dujardini* that distinguish this species from other Ceractinomorpha: 1) equal asynchronous cleavage; 2) radial character of cell division between the 8-16 cell stage and the end of cytodifferentiation; 3) formation of blastula and preservation of blastocoel until the end of larval development; 4) internal larval cells arising as the result of multipolar ingression of individual cells starting the 100-130 cell stage; 5) delayed formation of the anterior-posterior polarity during cytodifferentiation of the surface layer of cells; 6) invagination mechanism responsible for the formation of the inner spherical cellular layer in the disphaerula larva, represented by two spheres, one enclosed within the other and formed by flagellate cells; 7) Presence of the polymorphism of larvae.

Until recently the genus *Halisarca* was included in the order Dendroceratida. By the set of its characters this genus always stood apart from the other dendroceratids. For instance, unlike other Dendroceratida, *Halisarca* lacks a fibrous skeleton (Bergquist 1996), possesses unique ramifying, branching tubular choanocyte chambers (Lévi 1956; Vacelet *et al.* 1989) and a range of secretory-cells types of which fuchsinophilic cells of unique structure are the most abundant (Vacelet & Donadey 1987; Bergquist 1996), contains no terpenoid metabolites (Bergquist & Wells 1983), and its larvae have a uniform flagellation with posterior polar areas sparse in flagella (Lévi 1956; Bergquist *et al.* 1979; Bergquist 1980).

Comparison between embryonic development of *Halisarca* and embryogenesis of other Ceractinomorpha leads to the conclusion that the representatives of this genus have the most primitive development within subclass Ceractinomorpha. This conclusion agrees with some authors (Lévi

1956; Borojevic 1970; Korotkova & Ermolina 1982). However, we do not consider the type of development of *Halisarca* to be original for subclass Ceractinomorpha. Recent sponges have evolved a long way and therefore it is not reasonable to seek any single initial prototype. In any vast taxonomical group of sponges, more advanced, as well as more primitive species might be distinguished. Furthermore, highly specialized characters and primitive characters might be found to coexist in development processes of the same group of sponges.

Acknowledgements

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