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Metamorphosis of the larva of *Halisarca dujardini* (Demospongiae, Halisarcida)

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Abstract

Larval settlement and metamorphosis were investigated in *Halisarca dujardini* from the White Sea, using light and electron microscopy. The larvae settle on the substratum by the anterior pole. Exopinacoderm is the first structure to be formed in the course of metamorphosis. It develops by means of trans-differentiation of the flagellated cells of the larval posterior hemisphere, owing to the fact that a *zonula adhaerens* is retained in this region. This process belongs to the type of epithelial morphogenesis. The development of the basopinacoderm and the aquiferous system of the sponge take place by means of migration of separate cells, surface as well as internal ones, their association and de-differentiation. We suppose that flagellated cells of the larva and their derivatives are the main source of all rhagon structures. Flagellated cells of the larva are polypotent and can transform in two (at the minimum) types of definitive cells: exopinacocytes and choanocytes. The rhagon is characterized by the presence of a single spacious choanocyte chamber. The anterior-posterior axis of the *H. dujardini* larva was shown to become a baso-apical axis of the sponge.

Key-words: Sponges, *Halisarca dujardini*, metamorphosis, axis succession, ultrastructure

Résumé

La fixation des larves au substrat et la métamorphose ont été observés chez *Halisarca dujardini* de la Mer Blanche, en utilisant la microscopie photonique et électronique. Les larves se fixent au substrat par le pôle antérieur. L'exopinacoderme est la première structure à être formée au cours de la métamorphose. Il se développe au moyen de la trans-différentiation des cellules flagellées de l'hémisphère postérieur larvaire, étant donné que la *zonula adhaerens* est retenue dans cette région. Ce processus appartient au type de morphogenèse épithéliale. Le développement du basopinacoderme et du système aquifère de l'éponge se font par la migration de cellules séparées, provenant de la surface aussi bien que de la zone interne, qui s'associent et se dé-différentient. Nous supposons que les cellules flagellées de la larve et leurs dérivés sont la source principale de toutes les structures du rhagon. Les cellules flagellées de la larve sont polypotentes et peuvent se transformer en deux (au minimum) types de cellules définitives: les exopinacocytes et les choanocytes. Le rhagon est caractérisé par la présence d'une chambre choanocytaire unique et spacieuse. Au cours de la métamorphose, l'axe antérieur-postérieur de la larve de *H. dujardini* devient l'axe baso-apical de l'éponge.

Introduction

Metamorphosis — the development of a larva into an adult — is a very important stage of metazoan ontogenesis. In the course of metamorphosis, a larva undergoes a more or less dramatic transformation into an adult, which frequently involves new organogenesis and sometimes reorganization of the basic body plan. Metamorphosis occurs in an overwhelming majority of the representatives of the phylum Porifera. A special interest to the study of this process in sponges is connected with their basic phylogenetic position among Metazoa (LI *et al.*, 1998; KIM *et al.*, 1999). The knowledge of metamorphosis mechanisms in the sponges will enable us to approach the understanding of the first stages of evolution of morphogenesis in multicellular animals, the formation of cell lines in ontogenesis and the mechanisms of cell trans-differentiation.

Morphological descriptions of metamorphosis of several sponge species from different orders and classes have been already published (BERGQUIST & GREEN, 1977; MISEVIC & BURGER, 1982; BERGQUIST & GLASGOW, 1986; KAYE & REISWIG, 1991; MISEVIC *et al.*, 1990; AMANO & HORI, 1993, 1996, 2001; IVANOVA, 1997; KALTENBACH *et al.*, 1999; LEYS & DEGNAN, 2002).

Seven main types of larvae have been described in the sponges: parenchymella, cinctoblastula, hoplitomella, disphaerula, trichimella, amphiblastula and calciblastula (ERESKOVSKY & KOROTKOVA, 1999; MALDONADO & BERGQUIST, 2002). All larvae are characterized by an anterior-posterior polarity, which is expressed in the structure of the layer of external cells, in the organisation of the internal cell mass (if present) and the distribution of spicules.

When studying the metamorphosis in sponges, authors attempt to find out whether there is a link between flagellated cells of the larva and choanocytes of the adult specimens. It

has a direct bearing on the so-called problem of "the inversion of germinal layers" in sponges (DELAGE, 1892; TUZET, 1963; BRIEN, 1967; EFREMOVA, 1997; MALDONADO, 2004). However, there is no univocal answer to this question at present. Some authors think that this succession does exist (DELAGE, 1892; TUZET, 1963; BRIEN, 1967; LÉVI, 1963; AMANO & HORI, 1993, 1996). According to others, the flagellated cells of the larva are differentiated terminally (EFREMOVA & EFREMOV, 1979; MISEVIC & BURGER, 1982; BERGQUIST & GLASGOW, 1986; MISEVIC *et al.*, 1990; WEISSENFELS, 1989; KALTEBACH *et al.*, 1999).

The representatives of the species *Halisarca dujardini* JOHNSTON, 1842 (Halisarcida, Demospongiae) were the objects of the present research. The order Halisarcida unites the most simply organized sponges of the class Demospongiae, lacking mineral skeleton (BERGQUIST, 1996). The development of *Halisarca* is considered to be the most primitive among viviparous Demospongiae (LÉVI, 1956; BOROJEVIC, 1970; KOROTKOVA, 1981; ERESKOVSKY & GONOBOBLEVA, 2000). We have shown that the embryogenesis of *H. dujardini* results in the development of parenchymella - like, coeloblastula - like and disphaerula morphotypes of larvae (GONOBOBLEVA & ERESKOVSKY, 2004), which are different in their internal structure. The *H. dujardini* larvae of all three morphotypes possess a pronounced anterior-posterior polarity (GONOBOBLEVA & ERESKOVSKY, 2004). The metamorphosis of *H. dujardini* and *H metschnikovi* has yet been studied only at light microscopy level (METSCHNIKOFF, 1879; LÉVI, 1953, 1956).

The main attention of our investigation is to describe the morphogenesis and axis succession during metamorphosis of the larvae of *H. dujardini*.

Material and methods

Reproducing specimens of *Halisarca dujardini* were collected in the Chupa Inlet (Kandalaksha Bay, White Sea, Arctic) from the depth of 1.5-5.0 m, in June-July of 1999-2001. For transmission electron microscopy (TEM) samples were prefixed in 1% OsO_4 for 10 min and fixed in 2.5 % glutaraldehyde in phosphate buffer (pH 7.4) at room temperature for 1 h. After fixation, the samples were washed in phosphate buffer (pH 7.4) and postfixed in 1% OsO_4 in phosphate buffer for 1 h. For investigation of the cell contacts in larvae and settlers, either alcian blue or ruthenium red were used. For alcian blue, samples were fixed for 1 h in 1% OsO_4 buffered with phosphate buffer (pH 7.4; 0.1M in final solution) at 4°C. After fixation, samples were washed in phosphate buffer (pH 7.4; 0.2 M; 10 min) and postfixed at 4°C in 2.5% glutaraldehyde to which alcian blue was added at a final concentration of 1%. For ruthenium red, the samples were fixed

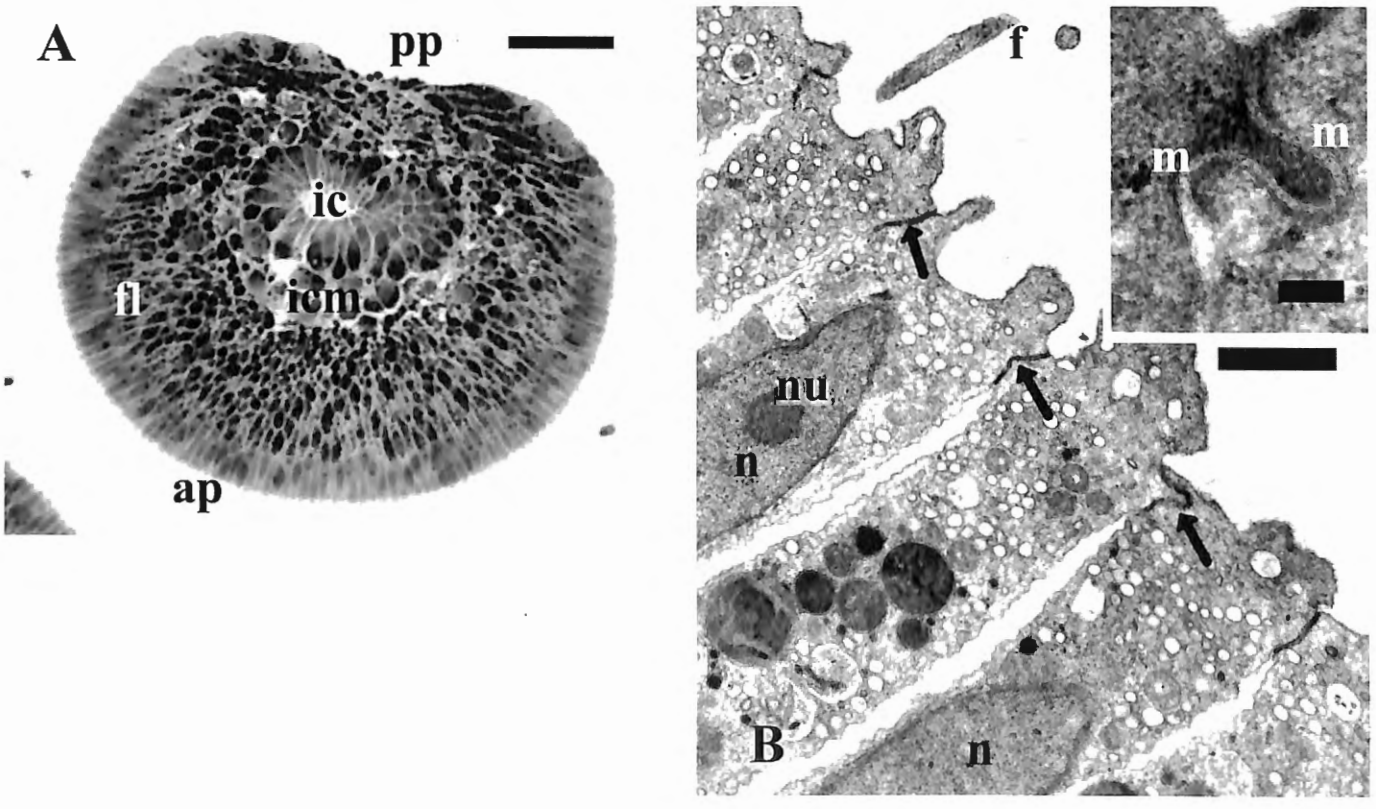

Fig. 1. Disphaerula larva of *Halisarca dujardini*. A) Longitudinal section through a disphaerula larva showing an anterior pole (ap) and posterior pole (pp), external flagellated epithelium (fl), inner cell mass (icm) and internal flagellated chamber (ic). B) Ultrastructure of the apical part of anterior external flagellated cells of the larva. Larva was fixed with ruthenium red which is seen to concentrate in the cell junctions (arrows); n = nucleus. Insert: the adhesion zone. Abbreviations: f = flagellum; m = cell membrane; n = nucleus; nu = nucleolus. Scale bars: A – 30 μm; B – 2 μm; insert – 18 nm.

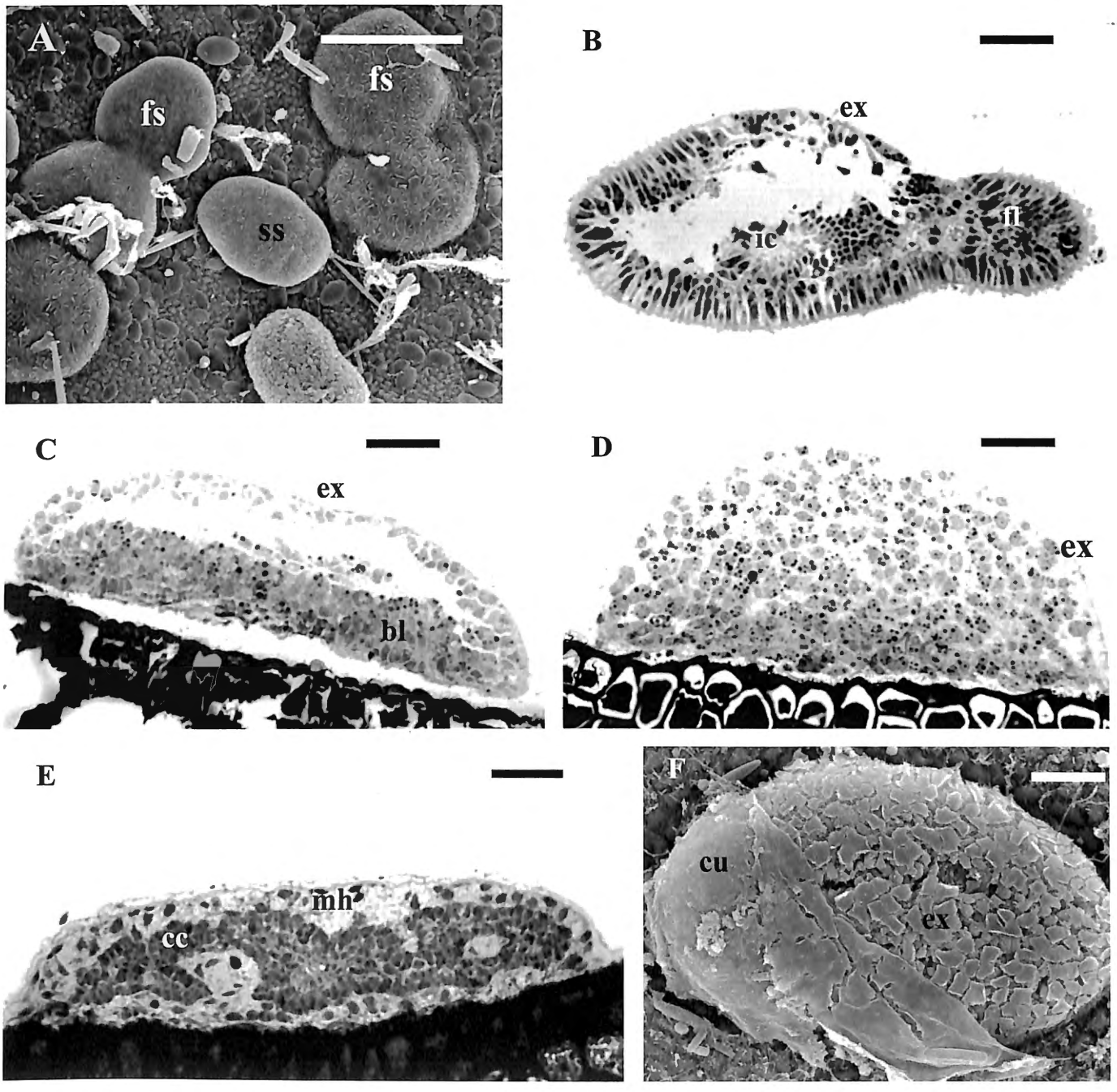

Fig. 2. Consecutive stage of settlement and metamorphosis of a larva of *Halisarca dujardini.* A) scanning electron micrograph of two fused settlers (fs) and singly settler (ss). B) Longitudinal section through 1 h old settler. Flagellated cells on the posterior pole are transformed into exopinacocytes (ex). C) Longitudinal section through 2-3 h old settler showing that exopinacoderm is formed before the basal layer (bl) of settler is completely destroyed. D) Longitudinal section through 8 -10 h old settler-"pupa stage". E) Longitudinal section through a rhagon showing a single choanocyte chamber (cc) and mesohyl (mh). F) Scanning electron micrograph of an 8 h old settler showing an external cuticle (cu) and external plates of exopinacocytes. Scale bars: A – 100 μm; B-F – 30 μm.

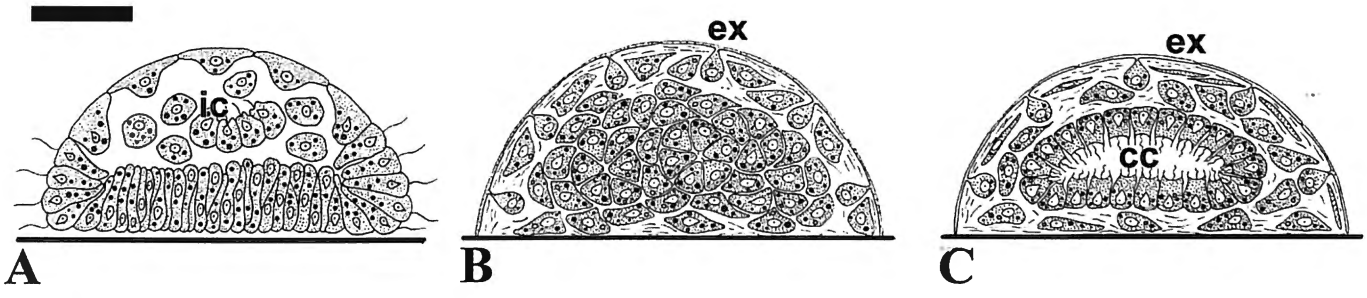

Fig. 3. Diagrams of the main stages of *Halisarca dujardini* larva metamorphosis. A) First stage of the metamorphosis directly after settlement with dismantling inner chamber (ic) of a disphaerula; B) Pupa stage with developing exopinacoderm (ex); C) Early rhagon stage with single choanocyte chamber (cc), well developed exopinacoderm (ex), but without basopinacoderm. Scale bar: 50μm.

for 1 h in 1% OsO_4 buffered with cacodylate buffer (pH 7.4; 0.1M in final solution) at 4°C. After fixation, larvae were washed in the cacodylate buffer (pH 7.4; 0.2 M; 10 min) and postfixed in 2.5% glutaraldehyde to which ruthenium red was added at a final concentration of 1mg/ml at 4°C.
Samples were dehydrated through a graded ethanol series and embedded in Epon-Araldite. Semi-thin sections were stained with methylene blue-borax. Ultrathin sections were contrasted with uranyl acetate and lead citrate. For scanning electron microscopy (SEM) the specimens were fractured in liquid nitrogen, dried by the method of critical point, sputter-coated with gold-palladium, and observed under a Hitachi S 570 SEM.

Results

Larval anatomy, settlement and general features of metamorphosis

Larvae of *Halisarca dujardini* are ovoid, 125-130 μm in diameter, and completely flagellated (Fig. 1A). The external layer is composed of a single row of highly polarized, monoflagellated cells. We distinguished three main cell types in the larvae differing by their origin and fine structure: flagellated cells, nucleolated amoeboid cells and maternal granular cells (Ereskovsky & Gonobobleva, 2000).
External flagellated cells join together by belt desmosome-like junction (zonula adhaerens) located at the apical part of cells (Fig. 1B). In cross-section, the membranes of adjacent cells run exactly parallel to each other, separated usually by a 16 – 18 nm intercellular space. Intercellular space of junctional areas contained ruthenium red positive material (Fig. 1B). The glycocalyx of apical membrane of flagellated cells is also detected by ruthenium red staining.
Larvae of *H. dujardini* swim rotating clockwise around the anterior-posterior axis. A larva settles on and attaches to a suitable substratum by the anterior pole. For a time, a settled larva rotates around its axis as if trying to screw itself into the substratum (about 30-40 minutes). During this time, the larva flatten along the anterior-posterior axis (Figs 2B, C; 3A). Sometimes, two or more larvae, which have settled close to each other, may fuse (Fig. 2A). The flagellated cells of the posterior larval hemisphere form the external epithelium of the postlarva, the exopinacoderm (Figs 2B, C, D; 3A, B). The differentiation of exopinacocytes starts immediately after the larvae has settled on the substratum and is completed after its complete attachment (40 min – 1 hour). The flagellated cells of the anterior hemisphere flatten parallel to the substratum (Figs 2C; 3A). At this time, the amorphous material, debris and flagellated acsonemes are found in the space between the apical part of flagellated cells and the substratum.
During the first 8-10 hours after settlement, an inner conglomerate is formed below the exopinacoderm of the larva (Figs 2D; 3B). It comprises the flagellated cells of the anterior larval hemisphere, its internal cells, as well as the cells of the internal flagellated chamber of the disphaerula, which is anarchized (Figs 2B; 3A). It results in the "pupa" formation (Figs 2D; 3B). During metamorphosis, the conglomerate cells dedifferentiate into choanocytes, endopinacocytes, basopinacocytes and the cells of the mesohyl.
The metamorphosis of *H. dujardini* is completed by the third - forth day and results in the development of the rhagon. It is characterized by the presence of one osculum and a functioning aquiferous system. The main volume of the rhagon is oc-

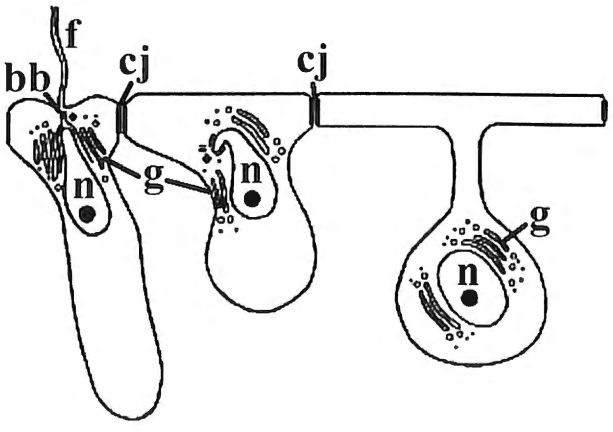

Fig. 4. Scheme of the transformation of larval flagellated cells of posterior hemisphere into the exopinacoderm of juveniles during the metamorphosis of *Halisarca dujardini*. Abbreviations: g = Golgi complex; bb = basal body; cj = intercellular junction; f = flagellum; n = nucleus.

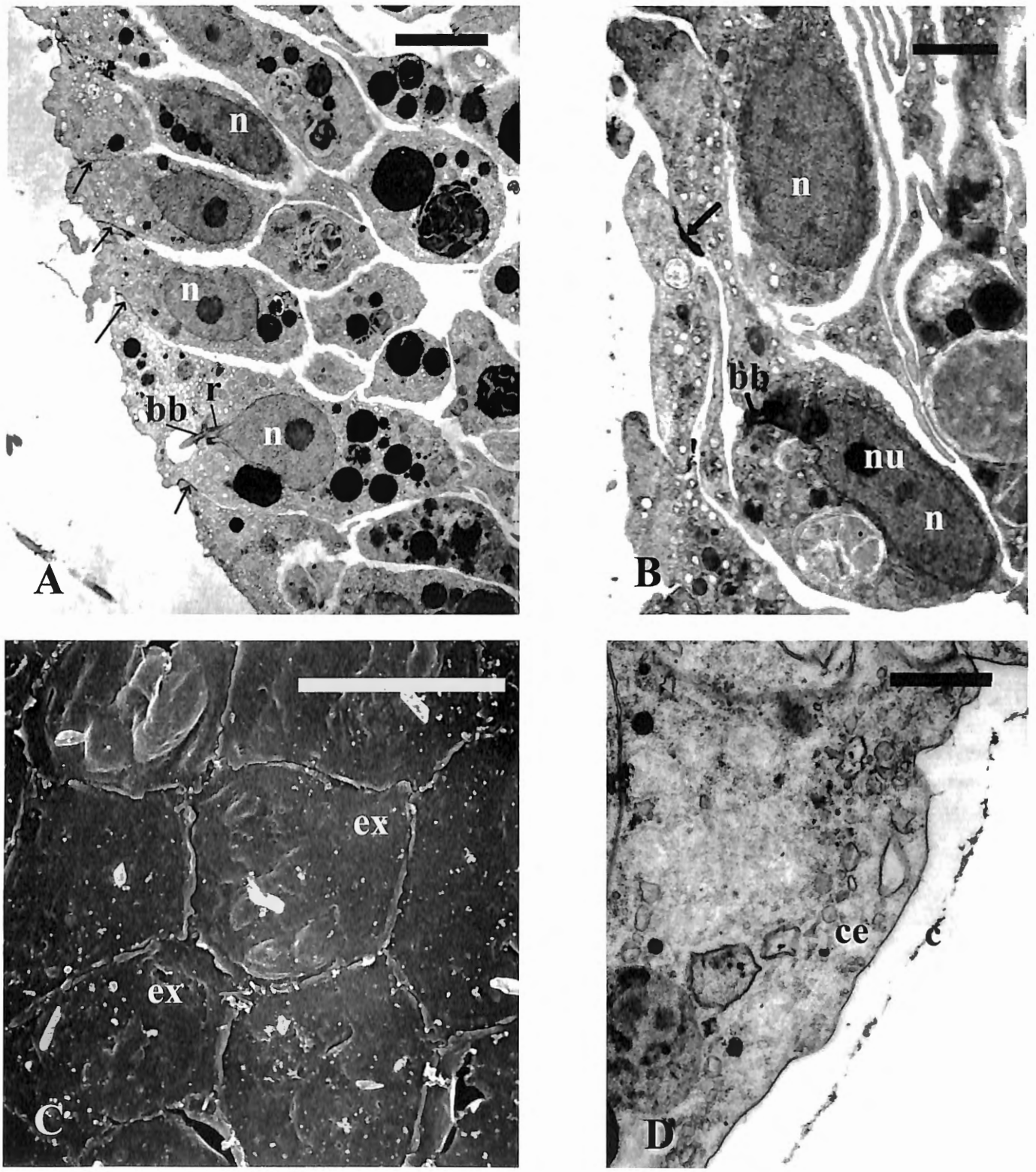

Fig. 5. Exopinacoderm formation. Settlers were fixed with alcian blue. A) Progression of exopinacoderm formation in the apical part of 15 min old settler. Cell junctions (arrows) connected these flagellated cells. B) 2-3 h old settler. External flagellated cell transformation into the exopinacocyte. Cell junctions (arrows) connected these flagellated cells. C) Scanning electron micrograph showing external hexagonal plates of exopinacocytes (ex). D) Cell in the apical part of the 30 min old settler with the cuticle (posterior pole of the larva). Abbreviations: bb = basal body; n = nucleus; nu = nucleolus; r = rootlet. Scale bars: A – 2.8 μm; B – 3.8 μm; C – 10 μm; D – 1 μm.

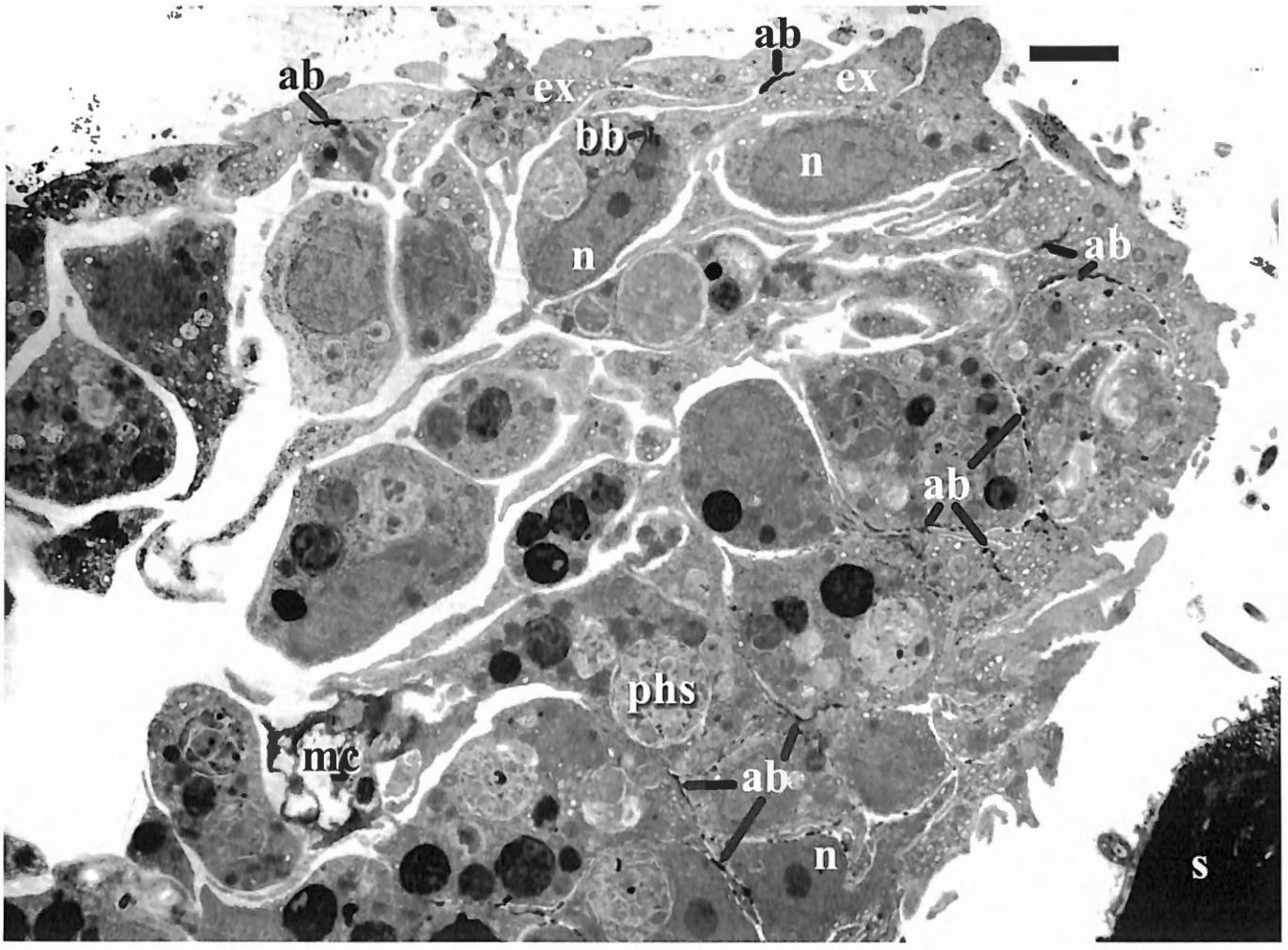

Fig. 6. Ultrastructure of the marginal part of the 2-3-h-old settler. Settler was fixed with alcian blue. Micrograph shows the gradual formation of exopinacoderm, different morphogenetic processes in the apical part of settler (exopinacoderm formation) and basal ones (conglomerate formation). Micrograph shows that tracer (ab) penetrates into deep region of intercellular spaces in the basal part of settler. Abbreviations: bb = basal body; ex = preexopinacocyte; mc = maternal granular cell; n = nucleus; phs = phagosome; s = substrate (*Fucus ramosus*). Scale bar: 2.9 μm.

cupied by a single spacious choanocyte chamber. (Figs 2E; 3C). There are sporadic amoeboid cells, symbiotic bacteria, and the extracellular matrix in the mesohyl.

EXOPINACODERM FORMATION - POSTLARVA STAGE

During larva settlement, the flagellated cells of the posterior hemisphere begin to transform into T-shaped exopinacocytes, characteristic of the adult sponge (Figs 3B, C; 4). This process starts in the region of the posterior pole of the settled larva and spreads towards its base. The flagellar basal apparatus of larval flagellated cells can be observed in the preexopinacocytes during period of exopinacoderm formation (Figs 5A, B).

The cell contacts between the flagellated cells of the posterior hemisphere are not disrupted in the course of the dedifferentiation into exopinacocytes (Figs 5A, B; 6). The marginal parts of apical plates join together by areas of adhesion. Intercellular space near junction areas contains the electron dense tracer (alcian blue) (Figs 5A, B, 6). The apical parts of preexopinacoblasts extend into large hexagonal plates, their flagellae are resorbed and completely disappear (Fig. 5C). The main cytoplasmic volume with the cell nuclei and centrioles immerse inside the pupa (Fig. 5B). A fine cytoplasmic bridge is retained between the apical plate and the immersed part of the cell. Autophagous vacuoles, residual yolk granules and phagosomes with fragments of maternal granular cells are present in the cytoplasm of the exopinacocytes (Fig. 5B). Slimy substance enveloped the forming rhagon (Figs 2F; 5D).

Then, the process of exopinacoderm formation is completed and the marginal exopinacocytes are attached to the substratum – *Fucus ramosus* – which is covered by a bacterial film. The adhesion takes place between cell membranes and this bacterial film (Fig. 7 B). In the early pupa stage, the amorphous material is present in the contact area (Fig. 7A).

INNER CONGLOMERATE FORMATION - PUPA STAGE

After the larva settlement, the basal layer of flagellated cells of the postlarva (anterior larval hemisphere) is gradually disorganized (Figs 2D; 3B). Intercellular contacts are disrupted: the electron-opaque tracer (alcian blue) penetrates the intercellular space (Figs 6; 8A, B). The nuclei migrate into the basal part of the cells. The cells acquire amoeboid shape and migrate into the inner part of the larva, forming a dense conglomerate (Fig. 8B).

Some cells retain the nucleus shape, the localization of centrioles and Golgi complexes, characteristic of the flagellated cells of the larva (Fig. 9A). One of the centrioles can have some ultrastructural characteristics of the basal body: connection with nuclear membrane and surface cells membrane, residual basal rootlets and basal foot. This type of cells occurs at the same time the primary choanocyte differentiation begins (Figs 10C; 9B-D).

All cells in the inner conglomerate have nucleolated nucleus. The chromatin is fine-grained with heterochromatin regions. Numerous ribosomes and rough endoplasmic reticulum occur in the cytoplasm. Mitochondria are found in different regions of the cytoplasm (Fig. 9A). Large autophagous vacuoles with heterogeneous content, residual yolk granules and phagosomes with fragments of maternal granular cells and symbiotic bacteria are present in the cytoplasm of these cells (Fig. 10A). In the autophagosomes we found cytoplasmic fragments with yolk granules, cells debris but fragments of nucleoli, acsonemes or basal bodies are never found in the autophagosomes. We suppose that inner cells of the larva are included into the conglomerate.

DIFFERENTIATION OF THE INNER CONGLOMERATE CELLS – RHAGON FORMATION

Differentiation starts with a regionalization of the inner conglomerate of the pupa cells. The main part of the cells (about 80%) is concentrated in their central part. Here, the differentiation of choanocytes and the formation of a unique choanocyte chamber take place (Figs 10A, B).

Amoeboid cells underneath the exopinacoderm divide in two types. Collencytes, secreting collagen fibers, which are oriented parallel to the pupa surface and form the cortex (Figs 10A; 12A), and nondifferentiated nucleolar amoeboid cells.

The formation of a choanocyte chamber starts with the formation of local spherical clusters of amoeboid cells (Figs 10A, B). The cells in these clusters polarize, with the formation of a glycocalyx, the development of microvilli, and later of a flagellum on the apical side (Figs 10A, B, C). Certain cells reveal features characteristic of the flagellated cells of the larva: nucleus shape, localization of the Golgi complex and centrioles, residual basal rootlets and sometimes – flagellum (Figs 10C, D). Apical structures of these cells are oriented into the cavity of the clusters and this side of the cells become the apical part of choanocytes (Figs 10A, B). The area of lateral contacts between neighboring cells increases. A pronounced adhesion zone of the basal areas is formed (Fig. 10A). At the first time of differentiation we

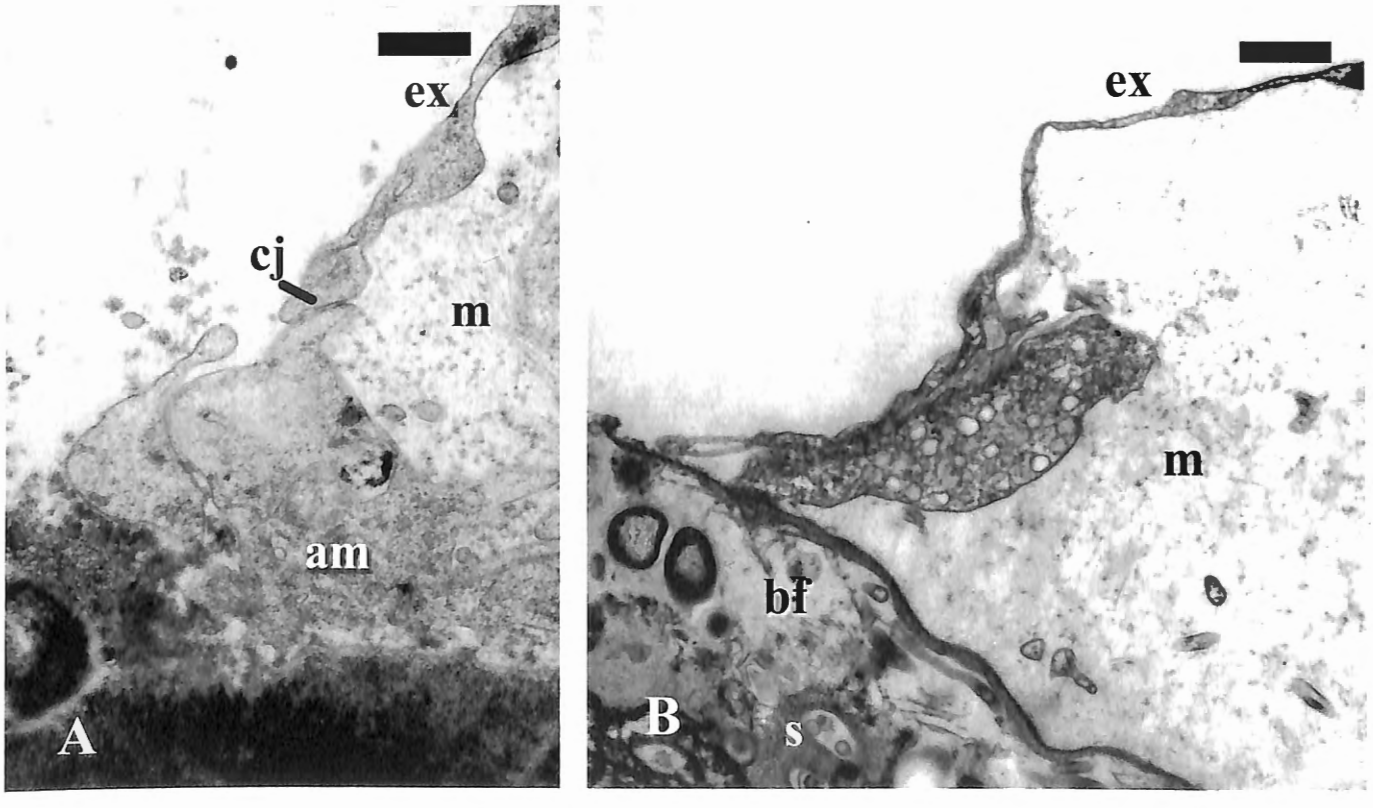

Fig. 7. Adhesion of exopinacocyte to a substratum. A) Pupa stage. Micrograph showing the basal amorphous material. B) Rhagon stage: adhesion to a bacterial film. Abbreviations: am = amorphous material; bf = bacterial film; cj = cell junction; ex = exopinacocyte; m = mesohyl; s = substrate. Scale bars: A, B – 1.5 μm.

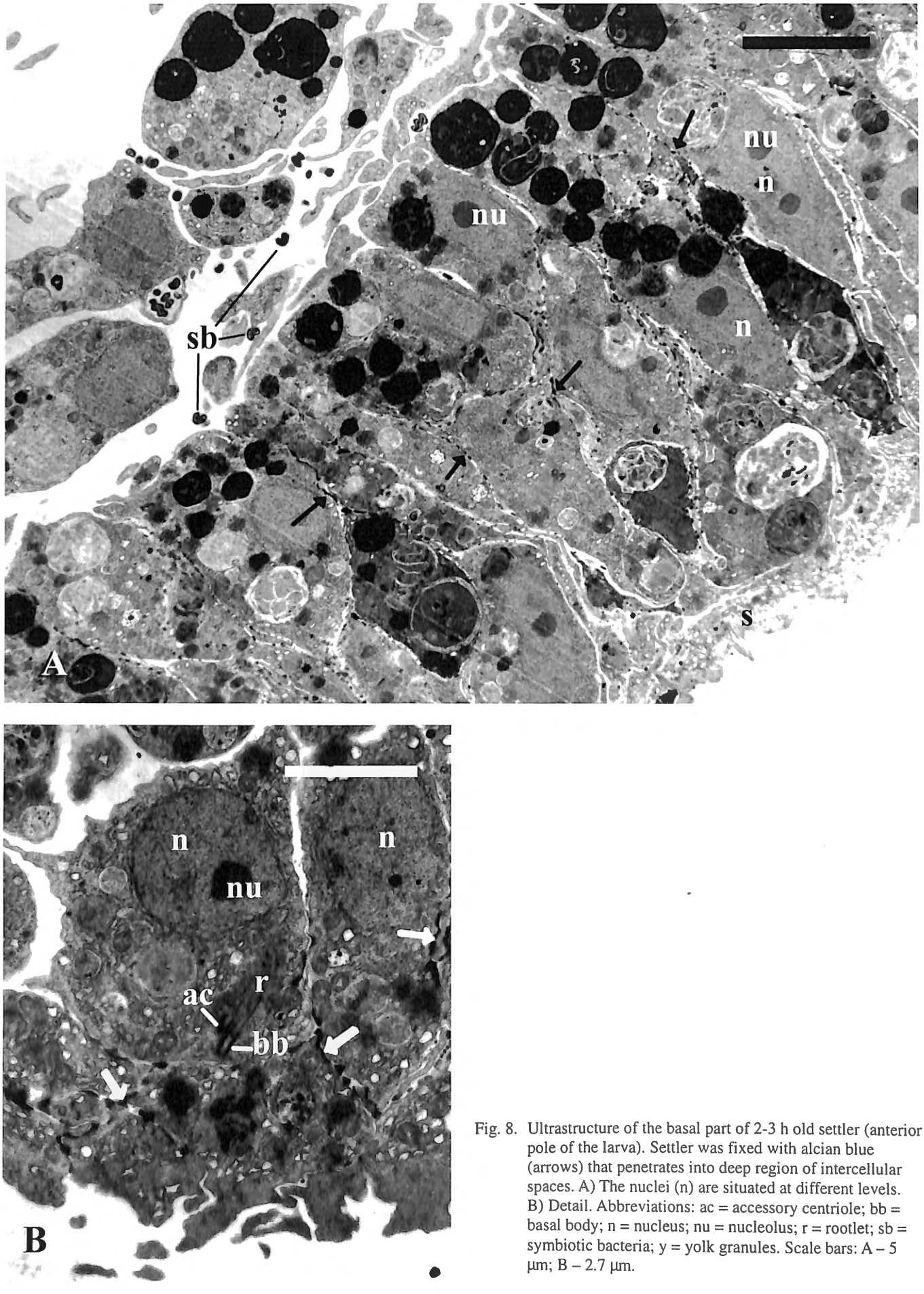

Fig. 8. Ultrastructure of the basal part of 2-3 h old settler (anterior pole of the larva). Settler was fixed with alcian blue (arrows) that penetrates into deep region of intercellular spaces. A) The nuclei (n) are situated at different levels. B) Detail. Abbreviations: ac = accessory centriole; bb = basal body; n = nucleus; nu = nucleolus; r = rootlet; sb = symbiotic bacteria; y = yolk granules. Scale bars: A – 5 μm; B – 2.7 μm.

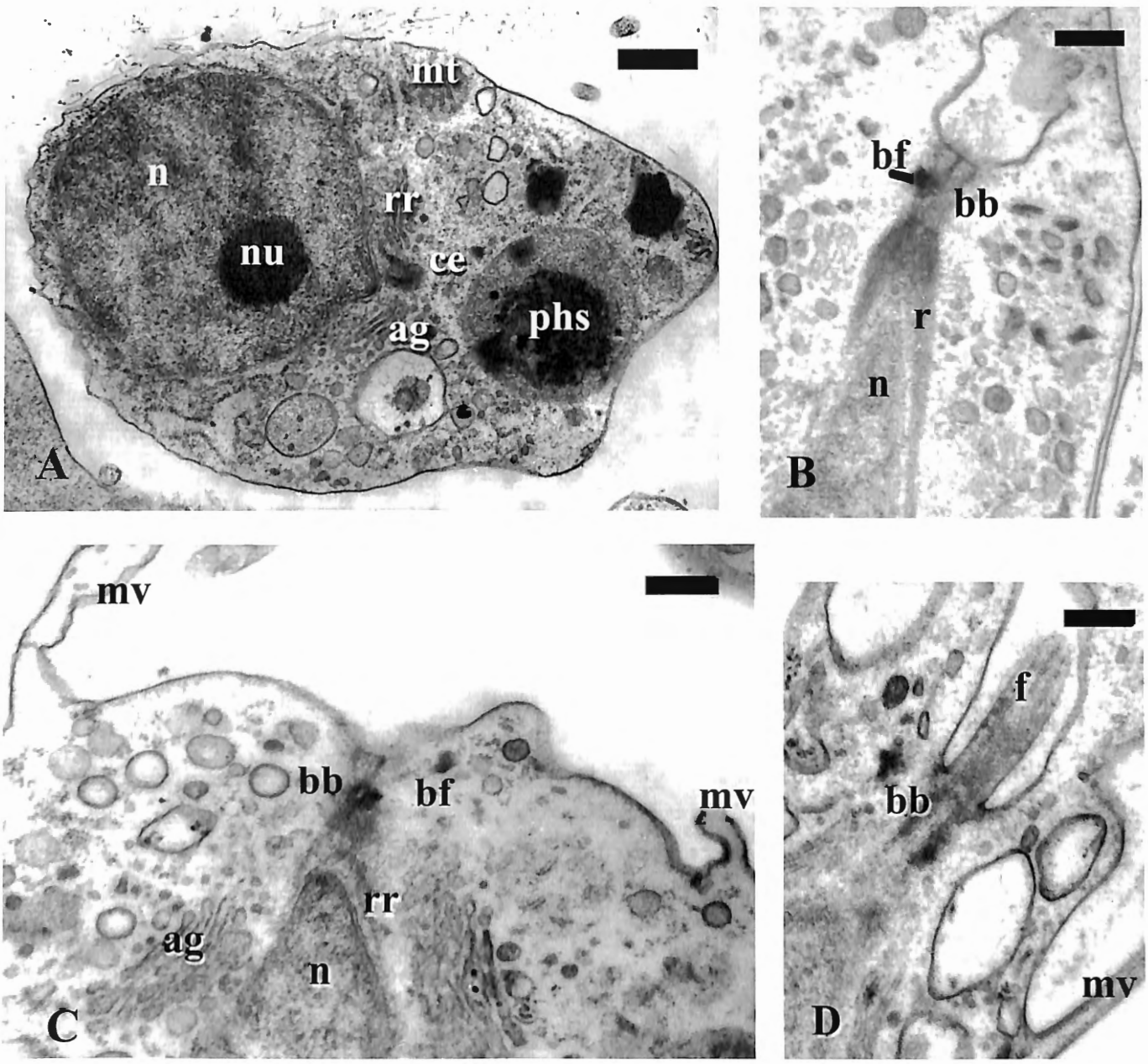

Fig. 9. Inner conglomerate formation - pupa stage (8 – 10 hours). A) Amoeboid cell in the apical part of a conglomerate. B) Apical part of an external flagellated cell of a larva. C) Apical part of a prechoanocyte. D) Apical part of a choanocyte. Abbreviations: ag = Golgi apparatus; bb = basal body; bf = basal foot; ce = centriole; f = flagellum; mt = mitochondria; mv = microvilli; n = nucleus; nu = nucleolus; phs = phagosome; r = rootlet; rr = residual rootlet. Scale bars: A – 1.2 μm; B – 0.7 μm; C, D – 0.6 μm.

found 5 to 6 clusters in the central conglomerate. Part of the cells is not included in these clusters. During metamorphosis, new cells build into the clusters. At the final stages of the metamorphosis, separate chambers fuse to form a single round choanocyte chamber of the rhagon (Figs 2E; 3C; 11B). During organogenesis, the choanocyte chamber forms from 5 to 6 lobes.

The canals of the aquiferous system are formed at the periphery of the pupa at late stages of metamorphosis (48-56 hours), after the isolation of the choanocyte chamber. Amoeboid cells in this region form numerous filopodia, contacting with neighboring cells and fibrillar matrix. Later, they are united into a layer of flattened endopinacocytes (Fig. 11A). In the basal part of the pupa, a collagen layer develops, secreted by the flattened cells. Collagen is secreted both in the direction of the substratum and of the inner cell conglomerate (Figs 12B-D). No regular layer of collagen occurs in the basal part of the pupa. An amorphous groundmat is present in the basal part of the pupa (Fig. 12E).

At that stage, the maternal granular cells are degenerating. The chromatin found in the nucleus is condensed, the cytoplasm becomes highly vacuolated and the volume of these cells decreases. These ultrastructural characteristics are similar for apoptotic cells death.

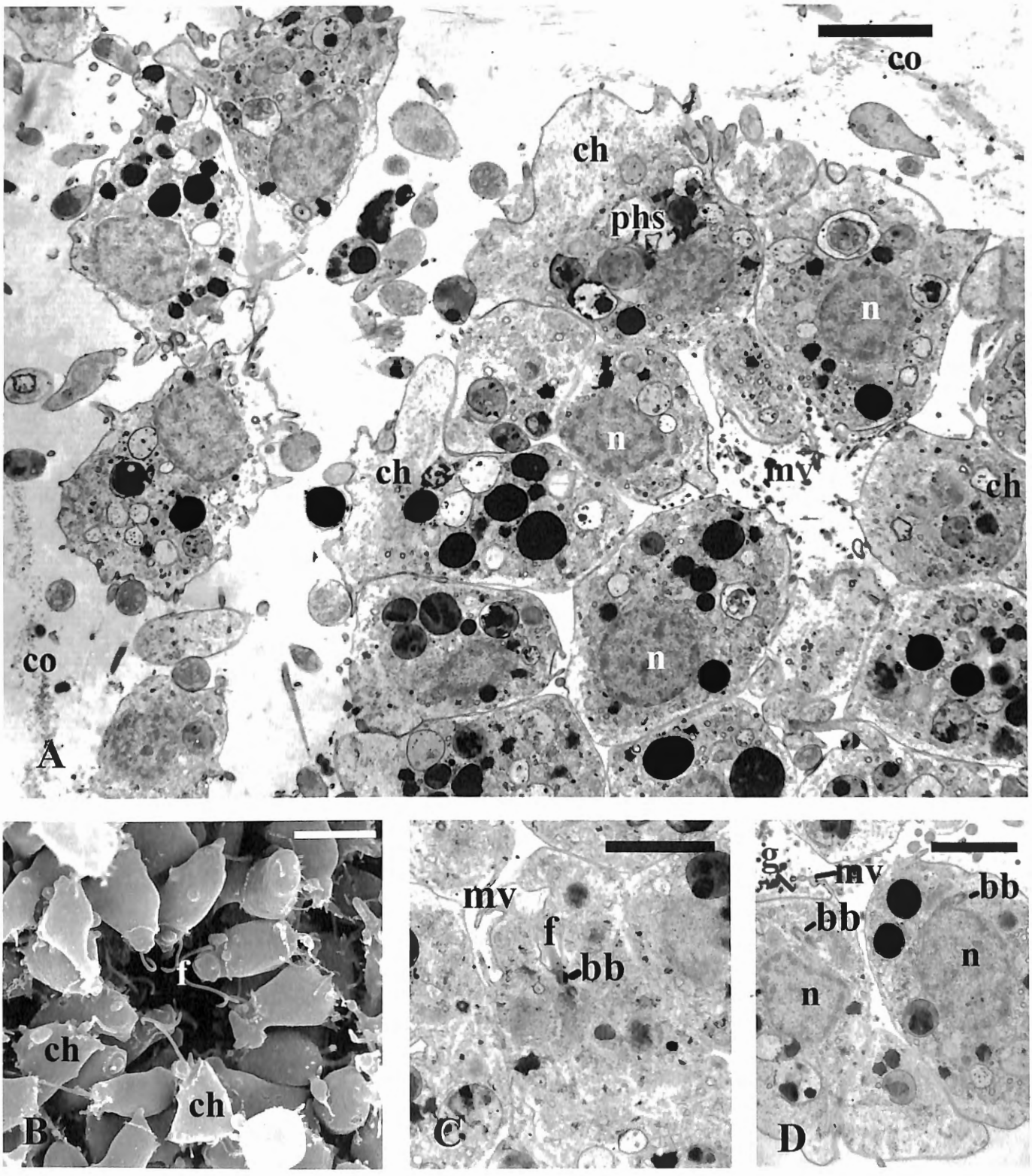

Fig. 10. Pupa 24 – 36 hours old after settlement. Choanocyte differentiation and formation of the choanocyte chamber. A) Ultrastructure of prechoanocytes cluster. Notice the phagosomes (phs) with maternal granular cells fragments. B) Scanning electron micrograph of choanocyte (ch) cluster. C, D) Ultrastructure of young choanocytes. Abbreviations: bb = basal body; ch = choanocyte ; co = collagen; f = flagellum; g = glycocalyx; mv = microvilli; n = nucleus; phs = phagosome. Scale bars: A – 4 μm; B – 11 μm; C – 2.5 μm; D – 2.7 μm.

We did not reveal any mitotically dividing during metamorphosis.

Discussion

The studied larvae of *Halisarca dujardini* attach to the substratum by their anterior pole. At that time, they keep rotating around their axis. Such larval behavior was previously described by LÉVI (1956) in *Halisarca metschnikovi* and *H. dujardini*. The beating of the flagella and the rotation of the larva during attachment may be one of the mechanisms ensuring its flattening and attachment. Axial rotation during attachment is also characteristic of the larvae of Haplosclerida (LEYS & DEGNAN, 2002), some larvae of Halichondrida (BERGQUIST & SINCLAIR, 1968) and Dictyoceratida (KAYE & REISWIG, 1991). The beating of the flagella was noted at the first stages of attachment of amphiblastulae of Calcaronea (AMANO & HORI, 1993).

It was difficult to investigate the larval attachment process. Occasionally, the larvae lifted from the substratum during the fixation and dehydration steps. We suppose that primary adhesion of *H. dujardini* larva to the substratum takes place by means of secretion of a slimy substance by the external flagellated cells of its anterior hemisphere. The secondary adhesion takes place by means of the formation of focal junction areas between marginal exopinacocytes and the substratum. Definitive attachment takes place after the formation of the conglomerate and the differentiation of collagen-secreting cells. Secretion of a slimy substance by the larval cells in the course of attachment was also observed in parenchymellae of different Demospongiae (BOROJEVIC & LÉVI, 1965; BOURY-ESNAULT, 1976; BERGQUIST *et al.*, 1979; KAYE & REISWIG, 1991). It was shown in a number of articles that the substance secreted by the larval cells during primary adhesion contains carbohydrates (acid mucopolysaccharides) and collagen fibrils (BOROJEVIC & LÉVI, 1965; BOURY-ESNAULT, 1976; EVANS, 1977; BERGQUIST & GREEN, 1977). Thus, the sponge larvae possess a universal mechanism of primary adhesion, characteristic of many larvae of marine benthic invertebrates, e.g., Cnidaria and Bryozoa (WOOLLACOTT & ZIMMER, 1978; STRICKER, 1985). The secondary cell-substrate adhesion is similar to the process of *Tetilla* "outgrowth" (WATANABE, 1978).

Larvae of different morphotypes of *H. dujardini* settle on the substratum and undergo the first steps of metamorphosis. After the conglomerate formation, it is difficult to make morphological investigations of metamorphosis of different larvae. However, in one case we found direct transformation of internal flagellated cells of disphaerula into choanocytes. The layer of internal flagellated cells of larva is not disorganized and integrated with the new formed choanocyte chamber. More data would be necessary to discuss this problem.

Morphogenetic events occurring after attachment of the larva can be divided into three stages. The first stage is marked by the early formation processes at the posterior larval pole, where the exopinacoderm develops. At the second stage, the layer of flagellated cells, in contact with the substratum, at the anterior larval pole, is disorganized and the cells migrate inside the postlarva. This process takes place only after the formation of the exopinacoderm. In the course of the third stage, regionalization of the inner cell conglomerate takes place and the elements of the aquiferous system are developed.

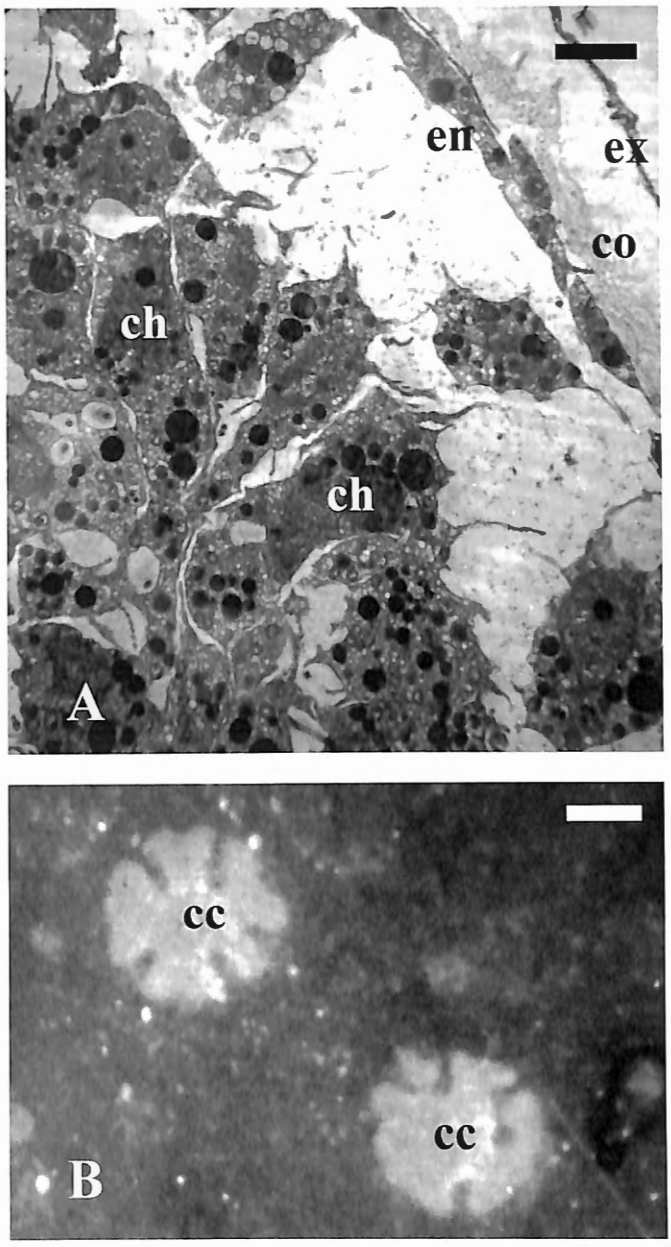

Fig. 11. Rhagon. A) Ultrastructure of the upper part of rhagon and endopinacoderm formation. B) *In vivo* light microscopy view of a rhagon with single spacious, lobated choanocyte chamber (cc). Abbreviations: cc = choanocyte chamber; ch = choanocytes; co = collagen; en = endopinacocytes; ex = exopinacocytes. Scale bars: A – 2.5 μm; B – 50 μm.

Primary exopinacoderm formation was also reported for amphiblastulae and coeloblastulae of calcareous sponges (AMANO & HORI, 1993, 2001), as well as in the parenchymellae of some demosponges (BERGQUIST & GREEN, 1977). At the same time, in parenchymellae larvae from the order Dictyoceratida, families Spongillidae and Lubomirskiidae (Haplosclerida), the substitution of flagel-

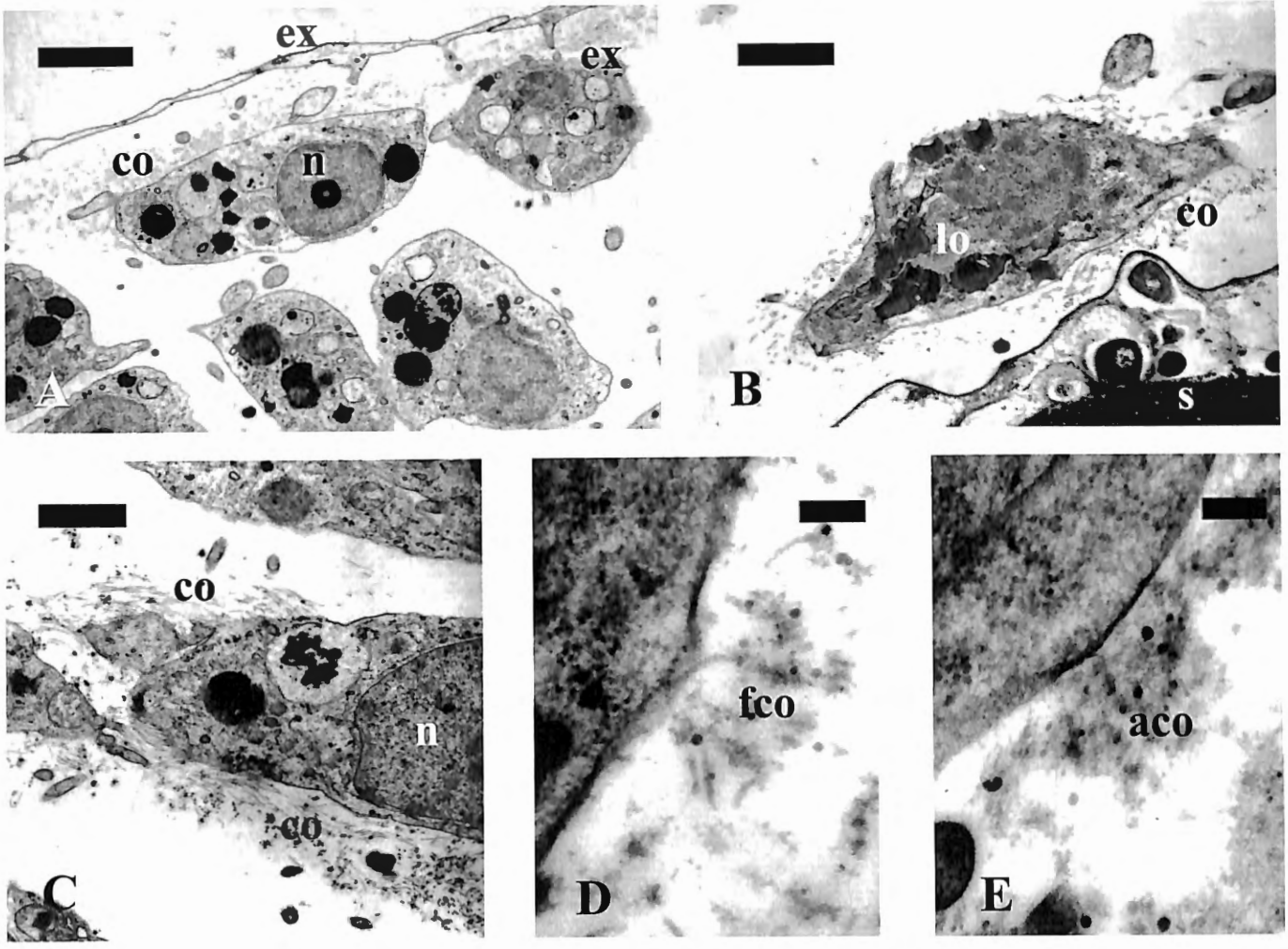

Fig. 12. Pupa. Apical (A) and basal (B, C) parts of mesohyl: collagen secretion. C) Detail of a cell (lophocyte) secreting collagen on all cell surface. D, E) basal extracellular matrix in the mesohyl: fibrillar collagen (fco), and amorphous groundmat (aco). Abbreviations: lo = lophocyte; co = collagen; ex = exopinacocyte; n = nucleus; s = substratum. Scale bars: A – 2.6 μm; B – 2.3 μm; C – 1.5 μm; D, E – 0.9 μm.

lated cells by pinacocytes also occurs at the anterior larval pole, where the basopinacoderm of the juveniles is formed (KAYE & REISWIG, 1991; IVANOVA, 1997; EFREMOVA, pers. comm.).

Morphogenesis of surface structures of adult *H. dujardini* is unusual for sponges. It belongs to the type of «oblique» contact cell polarization. This type is very common for Eumetazoan morphogenesis (BELOUSSOV, 1987). Here, it is possible, owing to the fact that apical regions of the flagellated cells in the posterior larval hemisphere retain specialized intercellular contacts of the *zonula adhaerens* type. The apical adhesion area have been described in different sponge larvae: *Halichondria moorei* (EVANS, 1977), *Ulosa* sp., *Microciona* sp., (BERGQUIST & GREEN, 1977), *Leucosolenia laxa* (AMANO & HORY, 2001), Homoscleromorpha (BOURY-ESNAULT *et al.*, 2003), *Ircinia oros* (ERESKOVSKY & TOKINA, 2004). We previously demonstrated, that ruthenium red (a specific dye for acid mucopolysaccharides) staining makes it possible to reveal zones of adhesion in different species of sponge larvae (GONOBOBLEVA & ERESKOVSKY, 2002). Preservation of intracellular junctions during exopinacocyte formation in larval metamorphosis of *H. dujardini* demonstrated their integrative function. It is known that it is through the zones of specialized cell contacts that integration of cytoskeletons of the metazoan epithelia takes place (KOLEGA, 1986).

In contrast to exopinacoderm, basopinacoderm formation in *H. dujardini* follows mesenchymal type and is accompanied by the disruption of intercellular contacts. The development of elements of the aquiferous system in *H. dujardini* (inhalant and exhalant canals and choanocyte chambers) in the inner conglomerate of the pupa takes place by means of migration of separate cells and their association, followed by differentiation, i.e. it belongs to mesenchymal type.

Surface structures of sponges may form in different ways during metamorphosis. Epithelial morphogenesis following involution type, resulting in the formation of endopinacoderm, choanoderm and an aquiferous system, was described only for the metamorphosis of cinctoblastulae – the larvae of Homoscleromorpha (MEEWIS, 1938; ERESKOVSKY

et al., personal observation.). These larvae possess a true basal membrane and a system of specialized intercellular contacts (BOURY-ESNAULT *et al.*, 2003). In all investigated species from other Porifera groups, the development of surface structures takes place by means of association of amoeboid cells into an epithelial layer, i.e. morphogenesis follows mesenchymal type.

Flagellated cells of larva can transform into two (at the minimum) types of definitive cells: choanocytes and exopinacocytes. We suggest that flagellated cells of larva of *H. dujardini* are the main source of structure of adults for the following reasons: 1) the flagellated cells take up 60-70% of larva volume; 2) the number of larval cells correspond to the number of "pupa" cells; 3) we did not reveal necrosis, apoptosis or phagocytosis of flagellated cells in the course of metamorphosis; 4) we did not reveal mitotically dividing cells in the course of metamorphosis. 5) the ultrastructural characteristics of larval flagellated cells transformation into different adult cells was followed up during metamorphosis.

Transformation of larval flagellated cells into choanocytes of adult specimens has been shown for different parenchymellae of demosponges (BOROJEVIC & LÉVI, 1965; AMANO & HORI, 1996; IVANOVA, 1997). However, ultrastructural details of this process were only described by BOROJEVIC & LÉVI (1965) in *Mycale contarenii*. The authors showed that rhagon formation from the conglomerate of larval cells is accompanied by the transformation of the flagellated cells into choanocytes. During this process, the flagellated cells retain their flagellae and basal apparatus, as well as their polarity.

The metamorphosis of *H. dujardini* is characterized by a clear succession between the layer of flagellated cells at the posterior larval pole and the exopinacoderm of the sponge. At the same time, the basopinacoderm and the inner conglomerate of the *H. dujardini* pupa have a mixed origin. Both internal and surface larval cells take part in their formation. However, this can be shown only with the help of special labeling methods.

The succession of cells of the posterior pole and the exopinacoderm of the rhagon is also characteristic of coeloblastulae of Calcinea (BOROJEVIC, 1969; AMANO & HORI, 2001), amphiblastulae of Calcaronea (AMANO & HORI, 1993) and cinctoblastulae of Homoscleromorpha (MEEWIS, 1938; ERESKOVSKY *et al.*, personal observation.). At the same time, during metamorphosis, the flagellated cells of the anterior hemisphere of these larvae differentiate into all basic cell type of the definitive sponge: endo- and basopinacocytes, choanocytes and mesohyl cells (MEEWIS, 1938; BOROJEVIC, 1969; AMANO & HORI, 1993, 2001). During metamorphosis of demosponges parenchymellae, the external epithelium (exopinacoderm and basopinacoderm) of the rhagon is formed from archaeocytes, underlying the external layer of flagellated cells. Archaeocytes of the anterior hemisphere differentiate into basopinacocytes and take part in the larva adhesion to the substratum, whereas similar cells localized in the posterior larval hemisphere differentiate into exopinacocytes. The regionalization of the inner conglomerate of the pupa and the development of elements of the aquiferous system of the rhagon take place mostly by means of differentiation of the larval archaeocytes; although, the participation of other larval cells is also possible (BERGQUIST & GREEN, 1977; MISEVIC & BURGER, 1982; BERGQUIST & GLASGOW, 1986; MISEVIC *et al.*, 1990; WEISSENFELS, 1989; KAYE & REISWIG, 1991; IVANOVA, 1997; KALTEBACH *et al.*, 1999).

Recently, a special attention has been paid to the problem of formation of the main axis of the animal body and its fate in ontogenesis and phylogenesis (GILBERT, 1997; DAVIDSON, 2001). Porifera, which are at the basis of the phylogenetic tree of Metazoa, are of paramount interest in this respect.

As mentioned above, all sponge larvae possess a clear anterior-posterior polarity. The results of our studies of *H. dujardini* metamorphosis as well as the data of other authors testify to the fact that larval anterior-posterior axis becomes the baso-apical axis of the adult regardless of the larval type. Thus, the sponges hardly differ in this respect from other benthic water invertebrates, e.g., Cnidaria (FREEMAN, 1981).

The analysis of our results, as well as of those obtained from the literature, allow us to conclude that the sponges have two different types of morphogenesis during metamorphosis.

The first type of metamorphosis is observed in parenchymella larvae of Demospongiae, which are characterized by the presence of different cell in the internal cavity. A common feature of the metamorphosis of parenchymellae is the degeneration of the covering epithelium of the larva, and the formation of surface structures of the juvenile by internal cells. During metamorphosis, the main structure-forming role belongs to the inner larval cells. The surface layers are formed by archaeocytes, underlining external flagellated cells, their position relatively to the larval anterior-posterior axis corresponds to the baso-apical position in the juvenile sponge.

The second type of metamorphosis is characteristic of amphiblastulae of Calcaronea, coeloblastulae of Calcinea, cinctoblastulae of Homoscleromorpha and *Halisarca* larvae. A common feature of these larvae is the only differentiation of external cells during their embryogenesis. These cells play the main structure-forming role in metamorphosis. External larval cells form surface structures and their localization relatively to the larval anterior-posterior axis corresponds to the baso-apical position in the juvenile sponge.

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