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ORIGINAL PAPER

Larval development in *Guancha arnesenae* (Porifera, Calcispongiae, Calcinea)

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Abstract Larval development and follicle structure of a representative of the Calcinea (Calcispongiae) *Guancha arnesenae* from the White Sea have been studied for the first time at the ultrastructural level. The follicle in *G. arnesenae* has an unusual structure: it consists of trapezoid cells rich in phagosomes and a surrounding dense collagen layer. Follicular cells differentiate from choanocytes. Cleavage results in formation of a hollow, equal, non-polarized coeloblastula. Larval morphogenesis occurs by means of direct hollow blastula formation without any individual cell or cell layer movements. The coeloblastula (calciblastula) larva of *G. arnesenae* is completely ciliated. The larva also contains rare non-ciliated cells: vacuolar cells, bottle-shaped cells and free cells in a central cavity. The basal ciliary apparatus of larval cells includes the basal body, an accessory centriole oriented perpendicularly to it, the basal foot, and two cross-striated rootlets. A bundle of microtubules emerges from the side of the basal body, opposite to the basal foot, running parallel to the outer surface. All bundles of cells are parallel to each other and oriented towards the posterior larval pole, forming a transverse cytoskeletal system. Specialized intercellular junctions in the apical regions of all ciliated cells are revealed for the first time in a Calcispongiae larva. The central larval cavity contains symbiotic bacteria, which are included inside the embryo at the blastula stage.

Keywords Calcinea · Embryonic development · Larva · Coeloblastula · Phylogeny

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Introduction

First results of molecular phylogenies provided some evidence for a possible paraphyly of the Porifera (Borchiellini et al. 2001; Medina et al. 2001). The calcareous poriferans were found to branch as the sister group of Eumetazoa, whereas Demospongiae and Hexactinellida were the sister group of all other metazoans including the Calcispongiae. Calcispongiae include two clades: the Calcinea and the Calcaronea (Manuel et al. 2002). Monophyly of Calcispongiae is strongly supported by morphological and molecular phylogenetic data (Manuel et al. 2003, 2004), but no evidence has yet been provided by comparative embryology (Borojevic 1970; Ereskovsky 2004, Leys and Ereskovsky 2006).

Developmental studies of the Calcinea started at the same time with those of the Calcaronea. Pioneer works were done by Schmidt (1877) and Metschnikoff (1879) on development of *Clathrina* (*Ascetta*) *primordialis* (Haeckel, 1872), *Guancha blanca* Miklucho-Maclay, 1868 and *Clathrina clathrus* (Schmidt, 1864). Later, however, these sponges did not get much attention. Almost all information currently available on oogenesis and embryogenesis of the Calcinea was obtained from light microscopic studies (Schmidt 1877; Metschnikoff 1879; Minchin 1896, 1900; Dendy and Frederick 1924; Tuzet 1948; Sarà 1955; Vacelet

1967, 1977; Borojevic 1969; Johnson 1978, 1979). Only their larval structure and the metamorphosis were investigated at the electron microscopic level (Borojevic 1969; Amano and Hori 2001).

During sexual reproduction, some Calcinea species develop peculiar temporary structures, referred to as "nests" (*nids* in French; Borojevic 1969). A nest looks like a disorganized radial canal of the aquiferous system, with its own skeleton. Nests are not present in all calcinean species (Johnson 1979; Minchin 1900).

Monophyly of the Calcispongiae is supported morphologically only by the sharing of a skeleton made of calcareous spicules with comparable symmetry. In contrast, the Calcinea and the Calcaronea diverge deeply from each other with respect to skeleton architecture, the position of the nucleus within choanocytes, and larval type (Bidder 1898; Hartman 1958; Manuel et al. 2003). Unfortunately, such elements of sexual reproduction as spermatogenesis, fertilization, ultrastructural features of oogenesis remain unknown in Calcinea. Comparative embryological analysis of the Calcinea and the Calcaronea revealed profound differences in their development and allowed delimitation of two distinct developmental types: (1) "calciblastula" developmental type (the Calcinea), and (2) "amphiblastula" developmental type (the Calcaronea) (Table 1) (Ereskovsky 2004, 2005; Leys and Ereskovsky 2006).

The Calcinea larva is a calciblastula, a term coined by Maldonado and Bergquist (2002), for the coeloblastula of Calcinea. They are described at light microscopic level (Metschnikoff 1879; Dendy 1891; Minchin 1896, 1900; Tuzet 1948; Borojevic 1969; Johnson 1979). The body wall of larva consists of a single layer of cells, surrounding the larval cavity. Most researchers described two types of cells: the ciliated cells and the granular cells of the posterior pole. The only ultrastructural study of a calcinean, *Soleneiscus* sp. (Amano and Hori 2001, wrongly referred to as "*Leucosolenia laxa* Kirk, 1895"; Manuel et al. 2003) also revealed vacuolar cells, bottle-shaped cells and granular cells of maternal origin.

Table 1 Comparative table of some developmental features in Calcinea and Calcaronea

Embryological features	Calcinea	Calcaronea
Follicle	Follicle of big phagosomes reach cells	Nurse membrane, placenta membrane
Nests	Yes/no	No
Feeding complex with nurse cells	No	Yes
Cleavage	Polyaxial	Incurvational
Blastula type	Equal coeloblastula	Unequal stomoblastula
Larval morphogenesis	Direct hollow blastula formation	Excurvation
Larva	Calciblastula (coeloblastula)	Amphiblastula

Fig. 1 *Guancha arnesenae.* **a** Light microscopy. Semithin section of reproducing sponge. **b** TEM of mesohyl (*m*) of the maternal sponge with a granular amoeboid cell (*gc*), and symbiotic bacteria of type 1 (*b1*). *ag* Golgi complex, *c* collagen layer, *e* embryo, *ex* exopinacoderm, *f* follicle, *mc* mesohylar cells, *n* nucleus ▶

The present study describes in detail larval development of a *Guancha arnesenae* from last stages of cleavage to the larva, using light, transmission and scanning electron microscopy. We will try to provide data to answer the questions: (1) Do the embryological characters support the monophyly of Calcispongiae? (2) What morphological features of larvae are shared, Calcinea with Calcaronea?

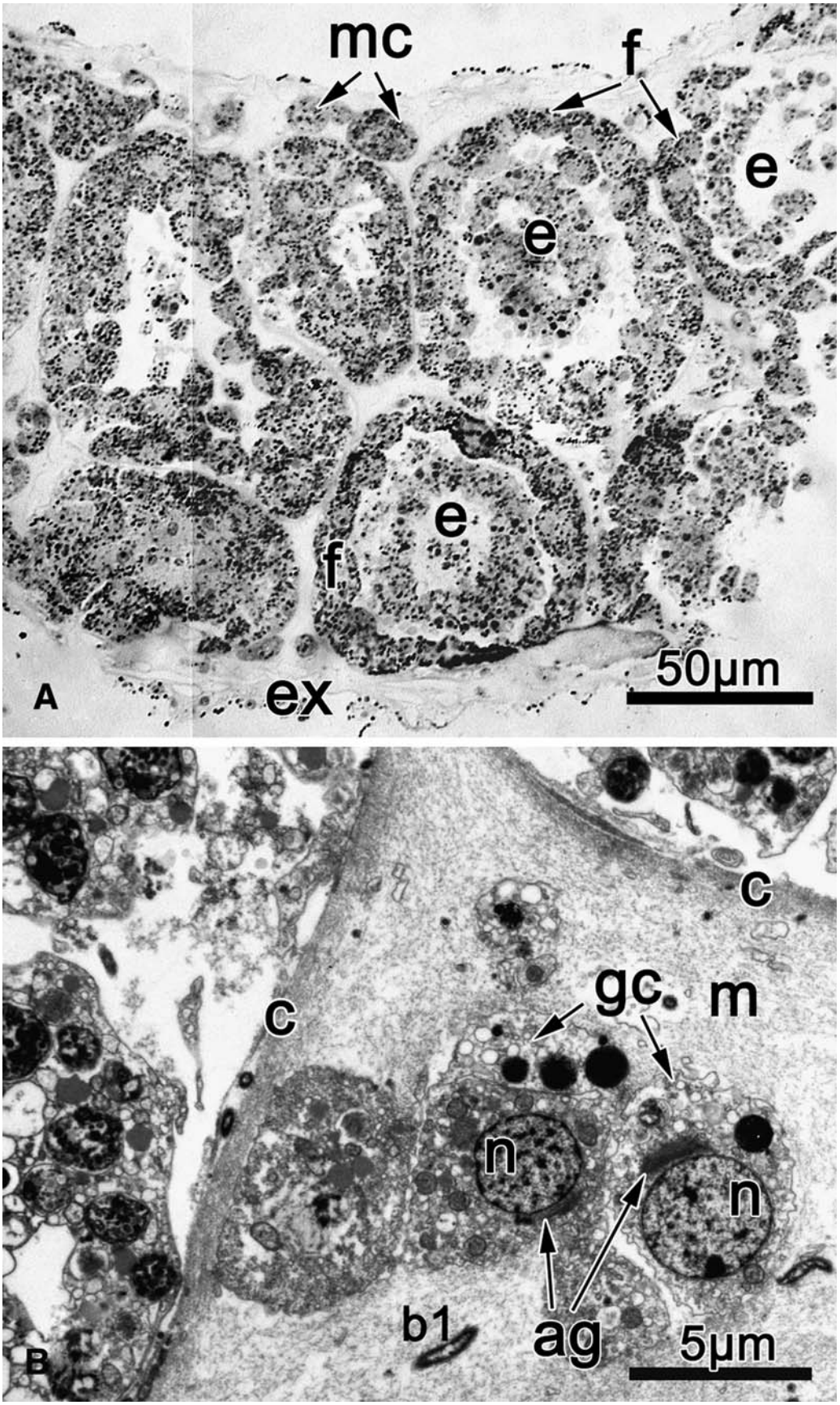
Material and methods

Specimens of *Guancha arnesenae* (Rapp, 2006) (Calcispongiae, Calcinea) were collected by SCUBA diving from June to July 2003, in the Chupa Inlet (Kandalaksha Bay, White Sea, Arctic) at a depth of 9–12 m. Pieces of each specimen were fixed immediately after collection. For transmission (TEM) and scanning (SEM) electron microscopy, samples were prefixed in 1% osmium tetroxide in 0.2 M cacodylate buffer for 10 min and fixed in 2.5% glutaraldehyde in cacodylate buffer (pH 7.4) at room temperature for 1 h. After fixation, samples were washed in cacodylate buffer (pH 7.4) and postfixed in 1% osmium tetroxide in 0.2 M cacodylate buffer for 1 h. Samples were then dehydrated through a graded ethanol series and embedded in Araldite. Semi-thin sections were stained with methylene blue-borax. Ultrathin sections, double contrasted with uranyl acetate and lead citrate according to Reynolds (1963), were observed under a LEO 906 E TEM. For SEM, samples were dried by the critical point method from carbon dioxide, sputter-coated with gold-palladium, and observed under a XL30 ESEM Philips SEM. Poriferan morphology and embryology are described in terms accepted in Boury-Esnault and Rützler (1997).

Results

General characteristic of development

Embryonic development of *G. arnesenae* in the Kandalaksha Bay of the White Sea occurs in late June–first half of July. During embryogenesis the sponge is completely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filled with developing embryos. The aquiferous system (normally of asconoid type) disintegrates, choanocytes are absent. The mesohyl is retained as a narrow layer between the embryos and between the embryos and the exopinacoderm (Fig. 1a); it contains granular and vacuolar amoeboid cells with a well-developed Golgi apparatus and cells with large phagosomes (Fig. 1b).

Follicle

Embryos develop inside a follicle made of two cell layers. The external layer consists of a dense extracellular matrix and, the internal layer is made up of large cells (Fig. 1a, 2a, b), which may be cuboidal, prismatic or flattened (about 10.6 μm high and 13.4 μm width) (Fig. 2a–c). The proximal cell surface, facing the embryo, is smooth, without any projections towards the embryo (Fig. 2a–d). The distal surface, facing the extracellular matrix layer forms rare long projections of the plasma membrane penetrating into the extracellular matrix at an acute angle and may anchor the cell to it (Fig. 2e). No special cell junctions were observed between follicular cells.

The nucleus of the follicular cell may be situated either in the distal zone or in the central zone, or more rarely, in the proximal zone (Fig. 2a, c). The nucleus is rounded, about 4.9 μm in diameter and contains a nucleolus, approximately 1.3 μm in diameter (Fig. 2c). Golgi stacks are situated near the pole of the nucleus (Fig. 2c). In the same region, elements of the ciliary basal apparatus, the basal body and the accessory centriole occur (Fig. 2c). The cytoplasm of the follicular cell is foamy, about one-third of it being filled with electron-transparent vacuoles (from 0.1 to 0.7 μm in diameter). It also contains numerous phagosomes (from 0.8 to 3 μm in diameter) with heterogeneous content and a few lipid droplets.

Cleavage and larval morphogenesis

Cleavage is total and equal, according to the polyaxial pattern. The cleaving embryos retain the hollow blastula structure from the stage of eight cells to the larval stage. As a result, a hollow, one-layered, equal, non-polarized blastula is formed, about 80–95 μm in diameter (Fig. 3a, b). The coeloblastula consists of a columnar epithelium of cells closely apposed to each other (Fig. 3a–d). The thickness of this epithelium is relatively even, ranging from 10.9 to 12.2 μm.

The beginning of larval morphogenesis is indicated by the differentiation of a cilium on the lateral–apical face of future ciliated cells (Fig. 3c, e). During this period, the apical cell surface forms numerous cytoplasmic projections and lobopodia (Fig. 3b, c).

Fig. 2 *Guancha arnesenae*. Follicle ultrastructure. **a** TEM of follicle and coeloblastular embryos, *b1* symbiotic bacteria of type 1, *c* collagen layer, *ec* embryonic cells, *fc* follicular cells, *n* nucleus. **b** SEM of follicle, *c* collagen layer, *ex* exopinacocytes, *e* embryos, *fc* follicular cell. **c** TEM of follicular cell (*fc*) with the pear-shaped nucleus (*n*), Golgi complex (*ag*), basal body (*bb*), electron transparent vacuoles (*tv*) and phagosomes (*ph*), *c* collagen layer. **d** SEM of proximal (facing the embryo) surface of follicle cells (*fc*). **e** TEM of distal (facing the extracellular matrix layer) part of follicular cell (*fc*) with the projections of the plasma membrane (*po*), penetrating into the extracellular matrix (*c*), *ph* phagosomes ▶

The oval or spherical nucleus is located below the ciliary basal apparatus in the apical region of the cell and contains 1 or 2 nucleoli (Fig. 3d, f). A Golgi apparatus is situated near the nucleus (Fig. 3f). Spherical or ovoid mitochondria and numerous ribosomes occur in the cytoplasm. The phagosomes of various sizes (from 1 to 2.6 μm in diameter) with heterogenic inclusions, some lipid droplets (from 0.3 to 1.8 μm in diameter), and vacuoles with inclusions looking like pile of fibres ranging from 1.1 to 1.7 μm in diameter are also abundant (Fig. 3d, g). The sides of the blastula cells are closely adjoined, but no specialized intercellular junctions are present at this stage.

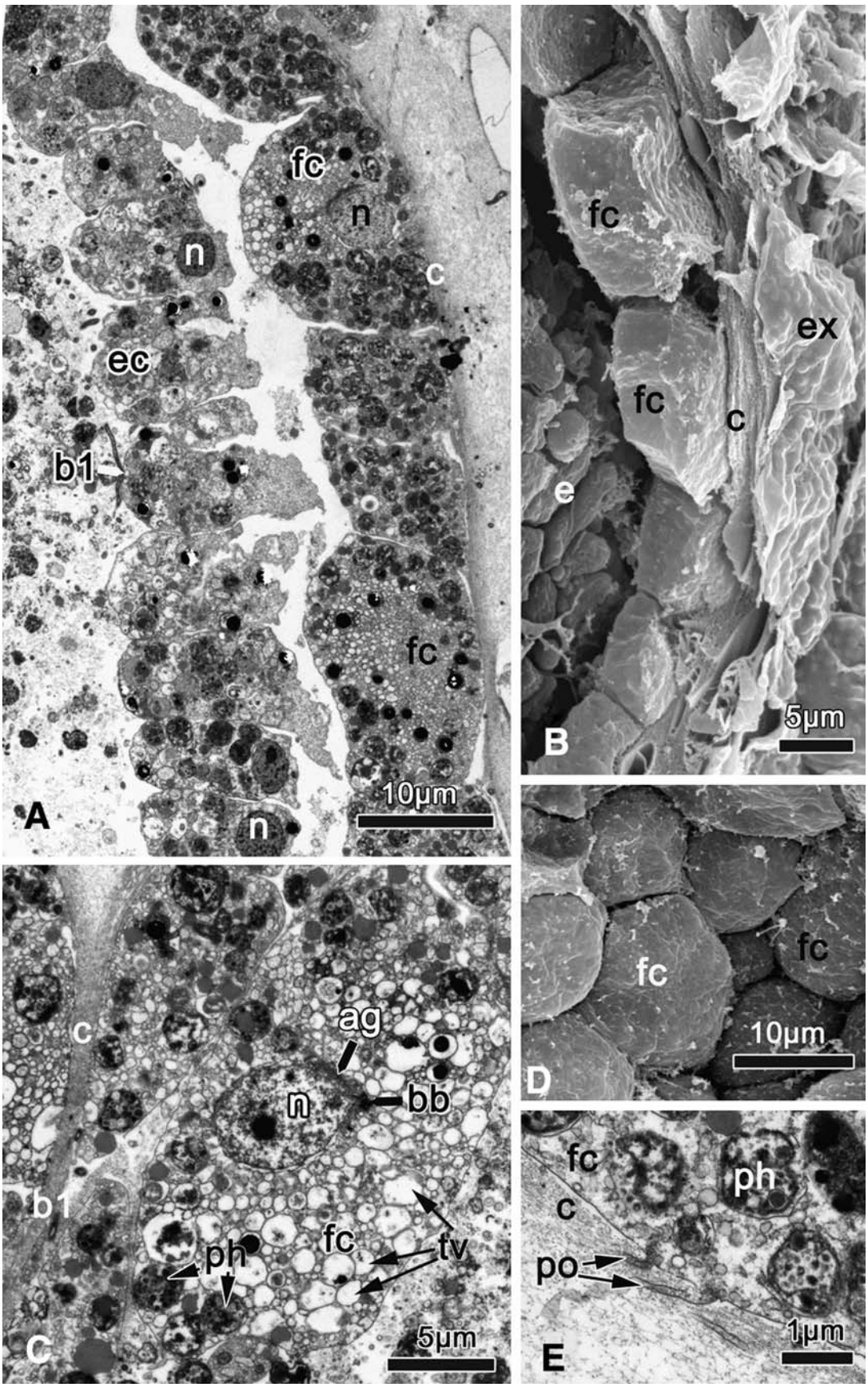
Larva formation proceeds without any cells movements. It is accompanied by an active proliferation of embryonic cells (Fig. 4a, b), resulting in their multiplication and an increased surface area of the embryo. As a result, the blastula becomes wrinkled (Fig. 4b). At a later stage of embryonic development, the larval cells become differentiated.

Larva

The larva of *Guancha arnesenae* is hollow and oval. The central cavity is surrounded by ciliated cells (Fig. 5a) organized in a palisade cell layer that lacks a basal lamina. The larva, about 200 μm in length and 85 μm in width, is entirely ciliated. Besides ciliated cells, the most abundant ones, there are three additional types: bottle-shaped cells, vacuolar cells, and free cells in the central cavity. There are no spicules in the larva.

Ciliated cells are characterized by a clear apical–basal polarity expressed in the localization of cellular organelles and inclusions. The cells are prismatic in shape, with the nuclei in their apical or, rarely, in central parts (Fig. 5b, c). The cell length is about 7.9 μm, the width in the nucleus area is 2.7 μm. The nuclei are pear-shaped, 2.7 × 5.1 μm in size. Nucleolus is single or absent. There are accumulations of heterochromatin in the nuclei (Fig. 5c, d).

Two zones are apparent in the ciliated cells, the apical–central and the basal one. The apical–central region of the cell is characterized by the presence of a cilium with a rootlet apparatus and a nucleus (Fig. 5d–f). The cilium emerges from the apico-lateral cell side (Fig. 5b); neither a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ciliary pit nor a small collar of short microvilli is present. The rootlet apparatus includes the basal body (kinetosome), an accessory centriole, oriented perpendicular to it, and a pair of rootlets, originating at the basal body (Fig. 5d–f). The rootlets are associated with the nuclear membrane. They consist of cross-striated fine fibrillar material, with a distance of about 43 nm between the midpoints of two neighbouring striae. The Golgi stack is always located parallel along one of the rootlets. A basal foot is formed approximately in the middle of the basal body. It is ovoid and lacks a pedicle (Fig. 5d, e). A well-pronounced bundle of microtubules starts from the side of the basal body, opposite to the basal foot, running parallel to the outer surface (Fig. 5d–f). The distal end of this bundle is curved towards the basal cell part and goes to the lateral plasmalemma at the level of the intercellular junctions (Fig. 5d–f). Horizontal bundles of all cells are situated at the same level. They are parallel to each other and directed towards the posterior larval pole.

Specialized adhesion contacts have been found in the apical regions of all ciliated larval cells. They consist of electron-dense thickenings of filamentous material along the internal membrane (Fig. 5e–g). The fine electron-dense strands found are in the contact area between the terminal region of the bundle of microtubules and the lateral plasmalemma (Fig. 5g). The apical cell part contains numerous electron-transparent vesicles and oval fibrous granules. The latter are yolk granules filled with a fibrous substance (Fig. 5f, g). In the apical part there are oval mitochondria and spherical so-called "glutinous granules" of about 0.8 μm in diameter (Fig. 5c). The surface layer of some of these granules is very electron-dense, while the inner part is made of the loose network of a fine granular substance. Analogous structures were named "glutinous granules" by Amano and Hori (2001) in *Soleneiscus* sp. (possibly pigment, granules).

The basal part of ciliated cells contains rare phagosomes (ranging from 0.4 to 1.5 μm in diameter) with heterogenous contents and electron-transparent vacuoles (Fig. 5c). The surface membrane forms short filopodia, arranged in a loose network. Besides, a loose extracellular matrix is secreted at the basal side of the cell into the larval cavity.

Vacuolar cells (Fig. 6a) are not numerous. Each larva contains only 3–5 vacuolar cells. In most cases, these cells are situated in the larval cavity below the basal part of ciliated cells (Fig. 6a). They are oval to irregular; their sizes are within the range of 9.3 × 7.3 μm. These cells are filled with electron-transparent vesicles (1.33 ± 0.9 μm in diameter), osmiophilic granules, phagosomes, and, rarely, include one big lipid droplet 2.5 μm in diameter (Fig. 6a). Their nucleus is large (5 ± 0.9 μm in diameter), oval, with heterochromatin masses.

Fig. 3 *Guancha arnesenae*. Embryos on the cell differentiation stage inside of the follicle. **a** Light microscopy, semithin section of coeloblastula embryo (*e*) inside of the follicle (*f*), *ex* exopinacoderm, *b1* blastocoel, *mc* cells of the mesohyl. **b** SEM of coeloblastula embryo (*e*) inside of the follicle (*f*), *ex* exopinacoderm. **c** SEM of coeloblastula ciliated cells (*cc*) with cilium (*ci*) and cytoplasm outgrowths (*co*) on the apical surface. **d** TEM of coeloblastula ciliated cells with nucleolated nucleus (*n*), phagosomes (*ph*), lipid droplets (*l*), *b1* symbiotic bacteria of type 1 inside of embryo cavity. **e** TEM of apical part of ciliated cell of coeloblastula with the developing cilium (*ci*), *ag* Golgi complex. **f** TEM of the nucleus (*n*) of coeloblastula ciliated cell with nucleolus (*nu*), *ag* Golgy complex. **g** TEM of vacuoles with inclusions having the shape of piles of fibres in the ciliated cell of coeloblastula ▶

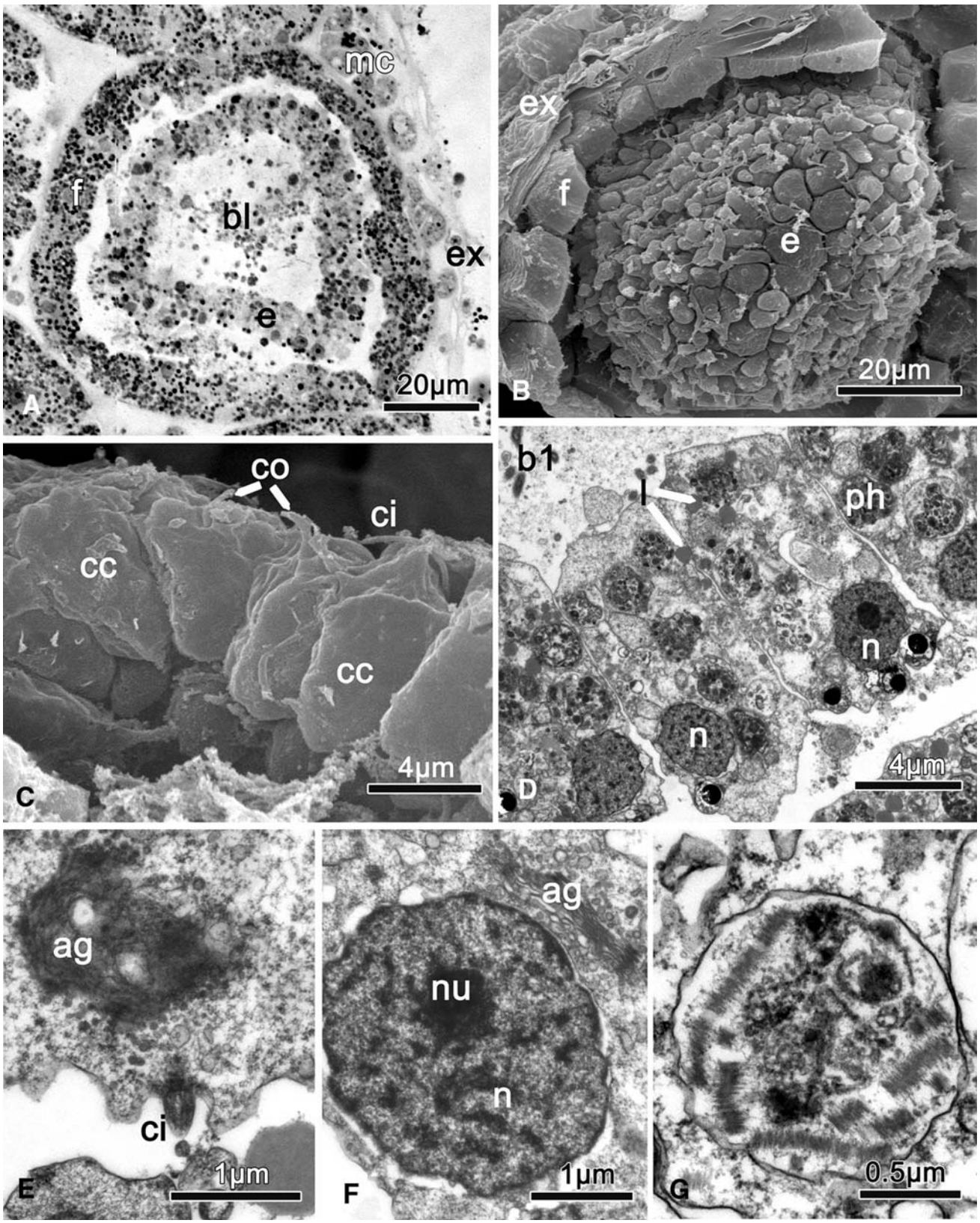
Large and rare bottle-shaped cells are located between ciliated cells (Fig. 6b). Their sizes are within the range of 9.4 × 6.9 μm. They are longer than ciliated cells and protrude outside and into the central cavity and bear no cilium. Their nucleolated nucleus (4.7 μm in diameter) is located in the central or basal region. The cytoplasm has vast clear areas without any inclusions. There is a polarization in the distribution of organelles: the apical and middle part is filled with membranous structures, while the basal part contains phagosome-like electron dense granules in diameter (Fig. 6b).

Few free cells occur in the central cavity (Fig. 6c). They are small, oval, about 5.8 μm in diameter with a large nucleolated nucleus (4.1 μm in diameter), their cytoplasm is filled with phagosomes and lipid droplets (Fig. 6c).

Discussion

Follicle

In oviparous and (ovo)viviparous species of the Porifera, embryos develop in temporary brood chambers called follicles. These special capsules form at the end of vitellogenesis, from flattened choanocytes, amoebocytes or endopinacocytes (Simpson 1984; Ereskovsky 2005). During sexual reproduction, *Ascandra minchini* Borojevic, 1966, *A. falcata* Haeckel, 1872, *Clathrina contorta* Minchin, 1905 develop temporary structures, referred to as "nests" (Borojevic, 1969). They are formed in the body wall, evenly throughout the sponge. Each nest has its own developmental cycle. During its formation, normal organization of choanoderm is locally disrupted, the neighbouring mesohyl areas and radial tubes are destroyed, and their cells dedifferentiate. However, in *Clathrina coriacea* (Montagu, 1818) and *Guancha blanca* nests are not formed (Minchin 1900; Johnson 1979). During the development of *Guancha arnesenae* no nests were found either. Nevertheless, the choanoderm of the parent sponge is completely destroyed, as happens in nest formation (Borojevic, 1969).



Follicle structure in *G. arnesenae* is rather unusual for Calcinea: a single layer of trapezoid cells with numerous phagosomes is surrounded with a dense layer of extracellular matrix. The retention of the basal ciliary apparatus and the polarized position of the Golgi stacks can indicate that follicular cells may be derived from choanocytes. These

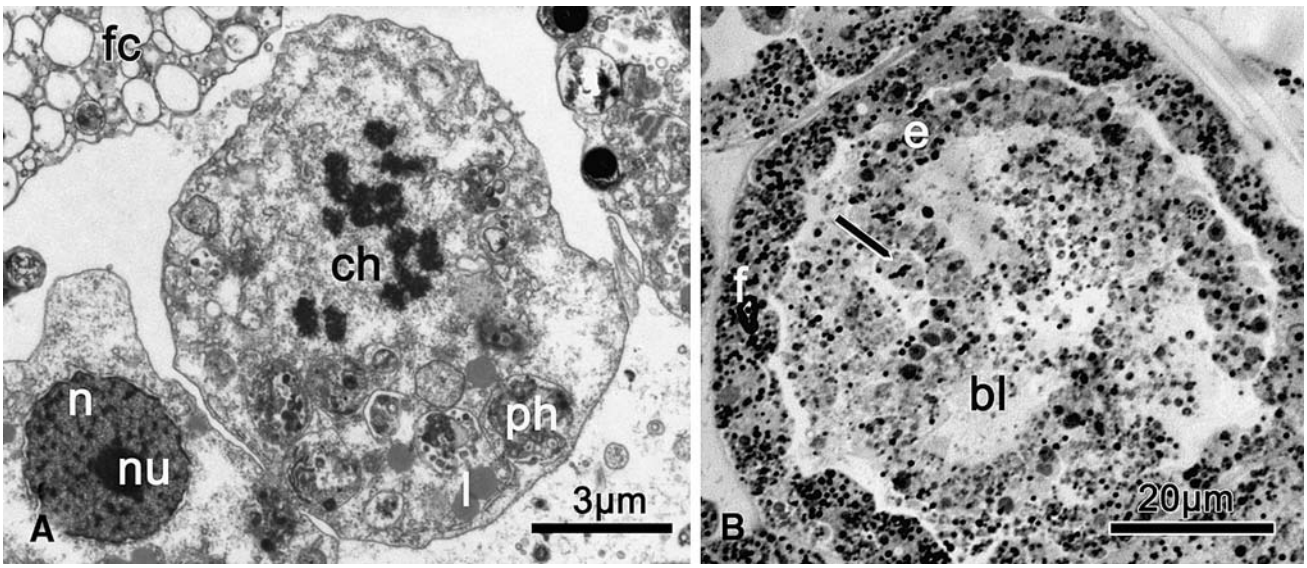


Fig. 4 *Guancha arnesenae.* Coeloblastula embryos with dividing cell inside of the follicle. **a** TEM of mitotic coeloblastula ciliated cell with the chromosomes (*ch*), phagosomes (*ph*), lipid droplets (*l*), *fc* follicular cell, *n* nucleus of the cell in interphase stage, *nu* nucleolus. **b** Light microscopy, semithin section of coeloblastula embryo (*e*), inside of the follicle (*f*) with mitotic cells (*arrow*), *bl* blastocoel

morphological characters are peculiar features of calcinean choanocytes (Amano and Hori 2001).

Development

Cleavage pattern in *Guancha arnesenae* is polyaxial, as in all Calcinea. This cleavage pattern was described also for *Halisarca dujardini* Johnston, 1842 (Demospongiae) (Ereskovsky 2002). After cleavage, in *G. arnesenae* a hollow blastula is formed directly, without any movements of individual cells or cell layers. The same morphogenetic mode was described in coeloblastula larva formation of *Polymastia robusta* (Bowerbank, 1861) (Borojevic 1967) and *Halisarca dujardini* (Gonobobleva and Ereskovsky 2004a).

Larva

The structure of the coeloblastula (calciblastula), characteristic of Calcinea, was mostly described at the light microscopic level (Metschnikoff 1879; Dendy 1891; Minchin 1896, 1900; Tuzet 1948; Johnson 1979, Borojevic 1969). Only the larva of *Soleneiscus* sp. was investigated under TEM (Amano and Hori 2001). Ultrastructure and cell composition of *Guancha arnesenae* larvae are very similar to that of *Soleneiscus* sp. (Amano and Hori 2001). In both cases, ciliated cells are the main cell type, with vacuolar cells, bottle cells and free cells in a central cavity also present. Vacuolar and bottle cells of *G. arnesenae* are identical ultrastructurally to those of *Soleneiscus* sp. (Amano and Hori 2001). Free cells in the central cavity in *G. arnesenae* differ from those in *Soleneiscus* sp. in the presence of numerous phagosomes and lipid drops.

Ciliated cells of *G. arnesenae* larva have fibrous vacuoles, which are absent in the developing blastula, similarly to all other Calcispongiae studied. Ciliated cells of *G. arnesenae* larvae have a unique feature: cilia arise from the apico-lateral part and not apically, as in all other Demospongiae and Calcispongiae larvae so far described. There are no structures near the axoneme basis, such as the ciliary pit described, for example, in *H. dujardini* (Gonobobleva and Ereskovsky 2004b), small collar of short microvilli as in *Ircinia oros* (Schmidt, 1864) (Ereskovsky and Tokina 2004), or deep depression in the cell's apical surface as in *Leucandra abratsbo* Hozava, 1929 and *Scypha* sp. (Amano and Hori 1992; Elliot et al. 2004).

The ciliary basal apparatus of the ciliated cells of *G. arnesenae* calciblastula is not essentially different from similar structures in other calcarean species (Borojevic 1969; Amano and Hori 2001). A horizontal bundle of microtubules located parallel with the overlying plasmalemma and directed towards the lateral cell wall, starts from the basal body part opposite to the basal foot in larvae of *G. arnesenae*, as in many parenchymellae of Demospongiae (Woollacott and Pinto 1995; Ereskovsky and Tokina 2004) and calciblastulae of Calcinea (Borojevic 1969; Amano and Hori 2001). Woollacott and Pinto (1995) referred to these structures as a transverse cytoskeletal system. Horizontal bundles of all ciliated cells in *G. arnesenae* are directed to the posterior larval pole. A similar orientation of an analogous structure was described for the larvae of some calcinean species (Borojevic 1969; Amano

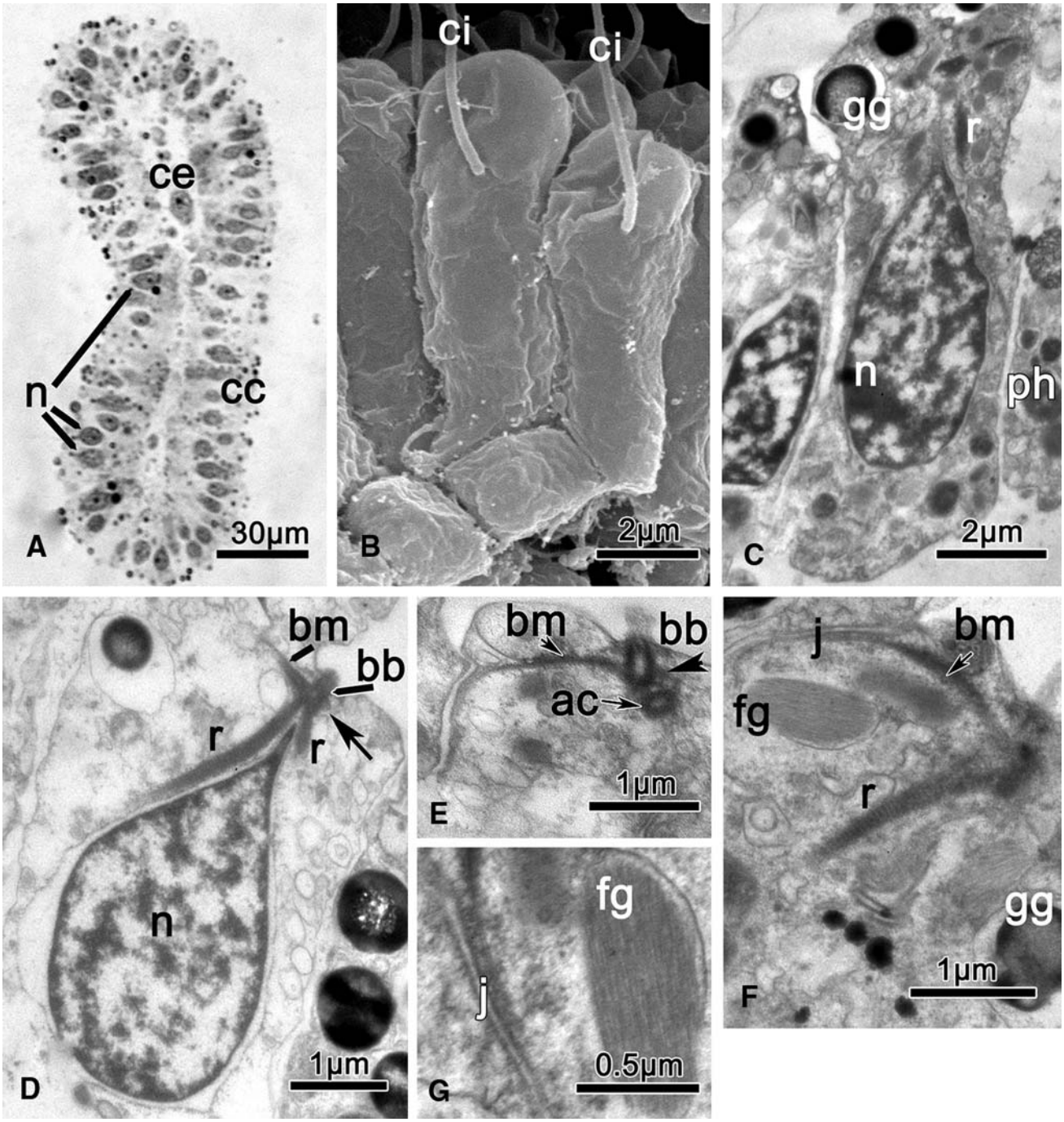


Fig. 5 *Guancha arnesenae.* Calciblastula larva. **a** Light microscopy, semithin section of calciblastula *ce* free cells in a central cavity, *cc* ciliated cells, *n* nucleus. **b** SEM of larval ciliated cells with cilia (*ci*). **c** TEM of larval ciliated cells, *gg* glutinous granule, *n* nucleus, *ph* phagosomes, *r* ciliary rootlet. **d** TEM of nucleus (*n*) and ciliary basal apparatus of larval ciliated cells, *bb* basal body, *bm* bundle of microtubules, *r* ciliary rootlets, *arrow* basal foot. **e** TEM of ciliary basal apparatus of larval ciliated cells with accessory centriole (*ac*), basal body (*bb*), basal foot (*arrow head*), bundle of microtubules (*bm*). **f** TEM of apical part of larval ciliated cells with bundle of microtubules (*bm*), fibrous granules (*fg*), glutinous granule (*gg*), specialized adhesion contacts between cells (*j*), cross-striated ciliary rootlet (*r*). **g** TEM of specialized adhesion contacts between two ciliated cells (*j*), *fg* fibrous granule

and Hori 2001) and parenchymella of the dictyoceratid *Ircinia oros* (Ereskovsky and Tokina 2004). The transverse cytoskeletal system in Eumetazoa is generally directed posteriorly and such an orientation is probably essential for aligning the direction of the effective stroke of the cilia (Sanderson 1984).

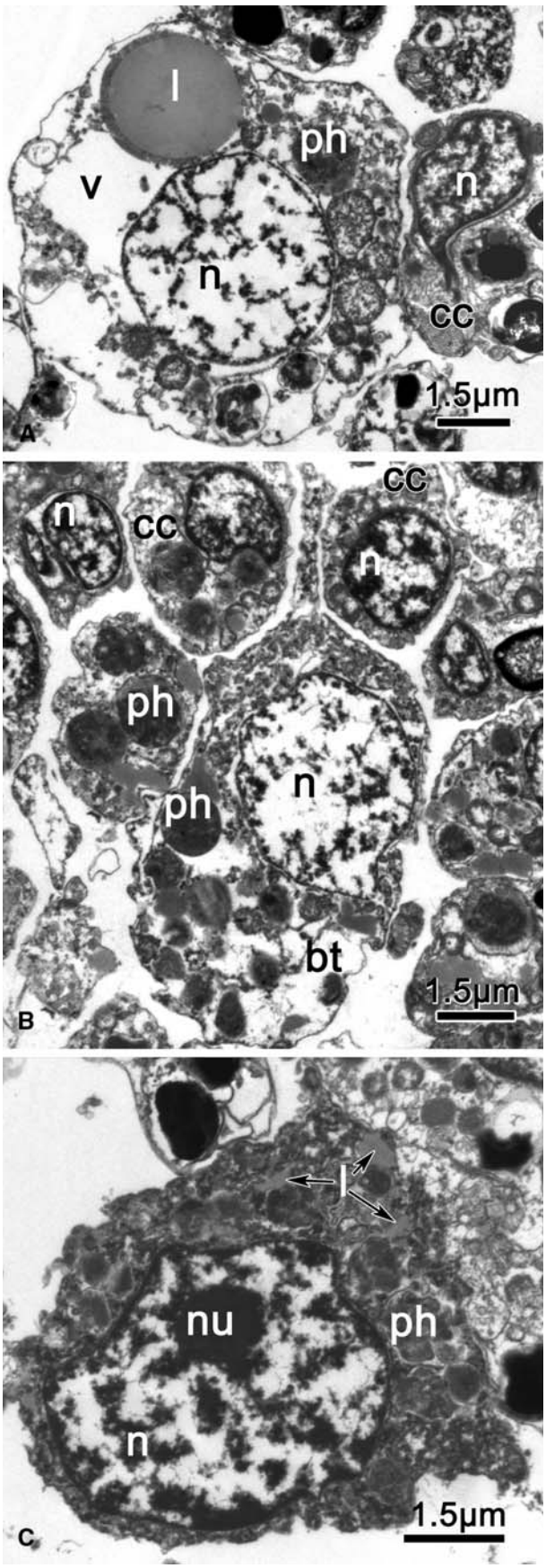


Fig. 6 *Guancha arnesenae.* Transmission electron microscopy (TEM) of additional types of larval cells. **a** Vacuolar cells under larval ciliated cells (*cc*), *l* big lipid droplet, *n* nucleus, *ph* phagosome, *v* vacuole. **b** Bottle cells (*bt*), *cc* larval ciliated cells, *n* nucleus, *ph* phagosome. **c** Free cells in a central cavity, *l* lipid droplet, *n* nucleus, *nu* nucleolus, *ph* phagosome

Cell junctions

We have shown that the larvae of *Guancha arnesenae* have specialized intercellular junctions (of the *zonula adhaerens*-like or the belt desmosome-like type) in the apical regions of all ciliated cells. This is the first time that specialized intercellular contacts have been revealed in calcareous sponge larvae. They are electron-dense thickenings of filamentous material along the internal membrane. A bundle of microtubules goes to the apicolateral plasmalemma at the level of the intercellular junctions. The fine electron-dense strands are between the terminal part of the bundle of microtubules and the lateral plasmalemma in the contact zone. Belt desmosomes have also been shown in the apical parts of the ciliated cells in some demosponge larvae: in the parenchymellae of *Dysidea etheria* De Laubenfels, 1936 (Dendroceratida) (Rieger 1994), *Ircinia oros* and *Pleraplysilla spinifera* (Schulze, 1878) (Dictyoceratida) (Ereskovsky and Tokina 2004; Ereskovsky 2005), in the disphaerula of *Halisarca dujardini* (Halisarcida) (Gonobobleva and Ereskovsky 2004b), in the coeloblastula of *Chondrilla australiensis* Carter, 1873 (Chondrosida) (Usher and Ereskovsky 2005), and in the cinctoblastula larva of Homoscleromorpha (Boury-Esnault et al. 2003). *Zonula adhaerens* newer has been shoved in adult calcareous sponges. Nevertheless, other type of specialized cell junctions, the septate junctions appear in some special cases: between the sclerocytes accumulating calcite in *Sycon ciliatum* (Fabricius, 1780) (Calcaronea) (Ledger 1975) and between choanocytes during deposition of collagen in *Clathrina* sp. (Calcinea) (Green and Bergquist 1979).

Some phylogenetical implications

In recent years, several molecular phylogenetic studies suggested that Calcispongiae is the sister group of non-poriferan metazoans (Ctenophora, Cnidaria, Placozoa, and Bilateria) (Cavalier-Smith et al. 1996; Collins 1998; Kruse et al. 1998; Zrzavý et al. 1998; Adams et al. 1999; Borchiellini et al. 2001; Medina et al. 2001). This has stimulated a revival of interest in taxonomy and phylogeny of Calcispongiae (Wörheide and Hooper 1999, 2003; Manuel et al. 2003, 2004; Klautau and Valentine 2003; Dohrmann et al. 2006; Manuel 2006; Rapp 2006). The monophyly of Calcispongiae is strongly supported in the

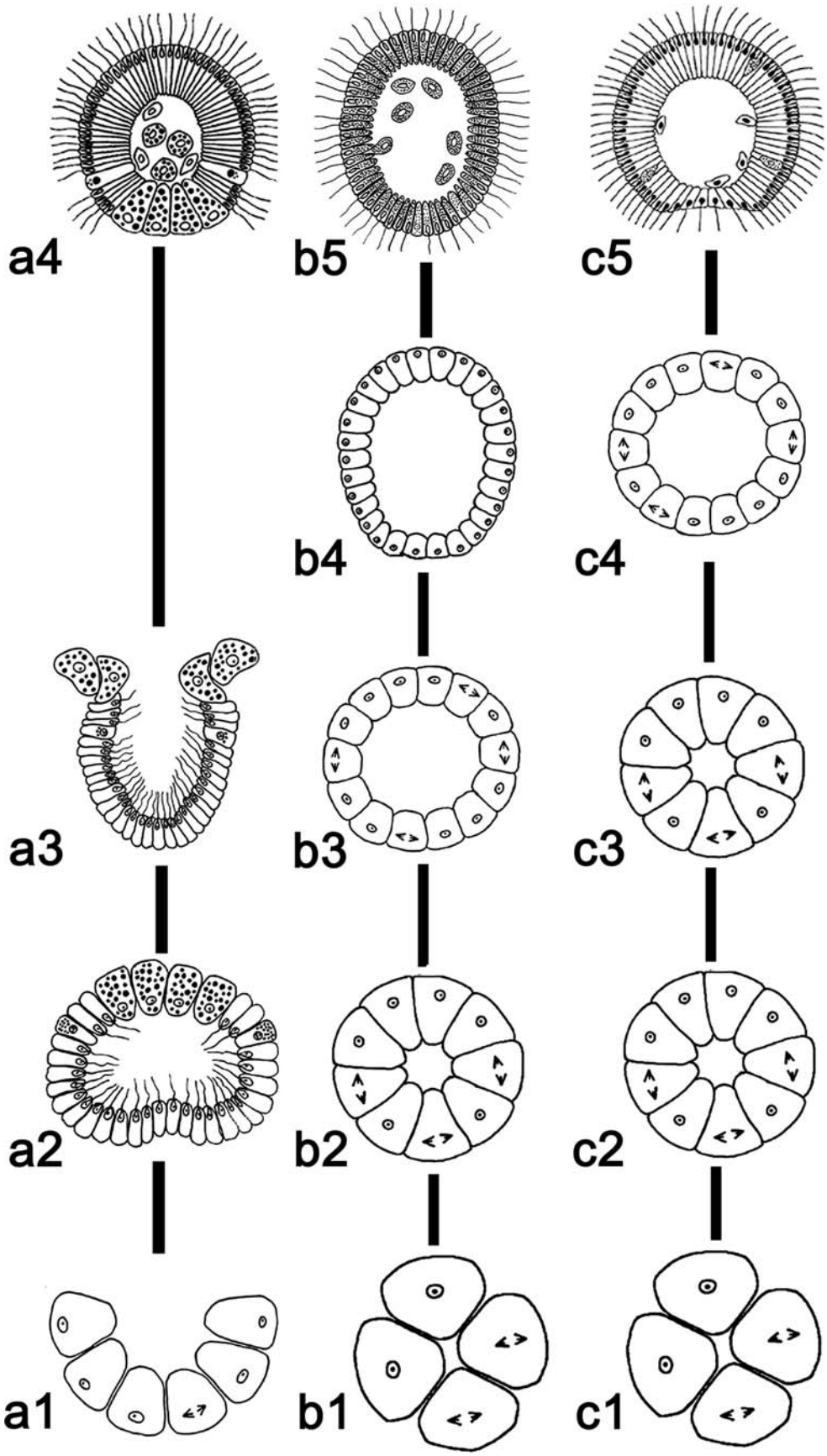


Fig. 7 Comparative diagram of cleavage and morphogenesis, leading to the larvae in Calcaronea (Calcispongiae), Calcinea (Calcispongiae), and Halisarcida (Demospongiae). *a1–a4* Calcaronea. *a1* incurvational cleavage, *a2* stomoblastula, *a3* incurvation of the stomoblastula, *a4* amphiblastula larva. *b1–b5* Calcinea. *b1* polyaxial cleavage, *b2* coeloblastula, *b3–b4* blastula cells proliferation and differentiation, *b5* calciblastula larva. *c1–c5* Halisarcida. *c1* polyaxial cleavage, *c2* coeloblastula, *c3–c4* blastula cells proliferation and differentiation, *c5* coeloblastula larva

works relying on molecular phylogeny and biochemical data (Manuel et al. 2003, 2004; Manuel 2006; Dohrmann et al. 2006; Schreiber et al. 2006). The same works demonstrate their subdivision into two clades, the Calcinea and the Calcaronea, and their early divergence. However, phylogenetic relationships within calcareous sponges for the most part remain enigmatic, and classification schemes currently in use do not rest upon well-supported hypotheses about the underlying phylogeny.

Ultrastructural evidence confirms that the development of Calcinea differs widely from that of Calcaronea. It is interesting that Calcinea embryonic development in general (Fig. 7b1–b5) is much closer to the development of some Halisarcida with coeloblastula larvae (Demospongiae) (Fig. 7c1–c5) than that to Calcaronea (Fig. 7a1–a4). Nevertheless, the big differences between these developmental types do not call into question the monophyly of Calcispongiae.

Larval morphological features are important characteristics in the classification and phylogeny of Porifera (Lévi 1956; Bergquist et al. 1979; Wapstra and van Soest 1987; Maldonado and Bergquist 2002; Ereskovsky 2004; Boury-Esnault et al. 2003; Ereskovsky and Tokina 2004). Moreover, recently the key role in early evolution of Metazoa is emphasizing to poriferan larvae (Maldonado 2004; Nielsen 2008).

It is noteworthy that larvae from both the Calcinea and the Calcaronea, coeloblastulae (calciblastulae) and amphiblastulae, are characterized by a distinct contact between the ciliary rootlet and the nucleus. The basal body also often contacts the apical part of the nucleus in Calcinea and Calcaronea (Amano and Hori 1992, 2001; Gallissian and Vacelet 1992; this paper). In this connection, it is important that a typical character of the Calcinea is basinucleate choanocytes: the basal body of the flagellum is not adjacent to the nucleus (Manuel et al. 2002). On the contrary, the Calcaronea have apinucleate choanocytes, and the basal system of the flagellum is adjacent to the apical region of the nucleus (Manuel et al. 2002). Metamorphosis in both calcarean groups is accompanied by the transformation of the ciliated larval cells into choanocytes and archaeocytes (Amano and Hori 1993, 2001). As a consequence, an apical nucleus linked to the cilium in larvae is probably plesiomorphic for the calcareous sponge's larvae and has been lost during the evolution of adults.

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