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Sexual reproduction in demosponges: ecological and evolutive implications

Reproducción sexual en demosponjas: implicaciones ecológicas y evolutivas

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Barcelona 2007

Chapter 6:

Introduction

Sexual reproduction in demosponges may proceed by either brooding the fertilised eggs and the derived embryo (viviparism) or spawning mature eggs for external embryonic development (oviparism) (Fell 1983; Simpson 1984). In viviparous sponge species, sexual reproduction has been studied both by light (Tuzet and Pavans de Ceccatty 1958; Tuzet and Paris 1964; Franzén 1988; Witte 1994) and electron microscopy (e.g., Gaino et al. 1986a, b; Sarà et al. 2002; Riesgo et al. 2007). Comparatively, there is less information in oviparous demosponges about sexual reproduction (Gaino 1980; Gaino and Sarà 1994; Lepore et al. 1995; Sciscioli et al. 1989, 1994; Mercurio et al. 2007) than in viviparous demosponges. Among oviparous species, gonochorism is the most frequent mode of reproduction (Simpson 1984), although some authors have reported oviparous and hermaphroditic sponges: *Aplysina (Verongia) aerophoba* (Scalera-Liaci et al. 1971), *Tetilla* sp. (Scalera-Liaci et al. 1976), *Cliona viridis* (Piscitelli 1997), and *Cinachyra tarentina* (Lepore et al. 2000). However, Reiswig (1973) found that other verongid was dioecious, and also, Watanabe (1978) reported that *Tetilla japonica* and *Tetilla serica* were not hermaphroditic species. Such controversial requires further investigations, since most of the studies were performed only under light microscopy.

Oogenesis is a process relatively well preserved across the phylum Porifera. Oocytes are reported to derive, generally, from archaeocytes (see Fell 1983 and Simpson 1984 for reviews), although in some cases choanocytes has been suggested as the oocyte anlagen, either by direct or indirect (multi-step) transdifferentiation (Fell 1983; Gaino et al. 1986), an also pinacocytes (Borojevic 1969). External morphology and ultrastructure of oocytes are relatively similar in demosponges, being size, presence

of nurse or follicular cells, additional collagenous covers of oocytes, and type and abundance of yolk the main differences between oocytes (Fell 1974, 1983; Simpson 1984). Little is known concerning the synthetic activities of sponge oocytes or the chemical composition of the yolk (Fell 1983; Sciscioli et al. 1991). Vitellogenesis in invertebrates may be: 1) auto-synthetic, partly using pinocytosed basic precursors, 2) hetero-synthetic, with yolk or yolk precursors supplied by somatic cells (nurse cells), or 3) both types simultaneously (Nørrevang 1968; Fell 1974, 1983; Simpson 1984; Sciscioli et al. 1991; Ramírez Llodra 2002). Even though the yolk shows a remarkable uniformity in structure throughout the animal kingdom, possessing a homogeneous appearance (Nørrevang 1968), yolk of both homogeneous and heterogeneous nature and appearance have been reported in sponges (see Fell 1983 and Simpson 1984 for reviews).

The typical celerity of gametogenic cycles (e.g., Fromont 1994; Usher et al. 2004) and the gonochoristic nature of most oviparous demosponges hindered the study of their gametogenesis for a long time. To increase the knowledge of gametogenesis in oviparous demosponges, we selected two different species belonging to different orders for studying their oogenesis: *Axinella damicornis* (order Halichondrida) and *Raspaciona aculeata* (order Poecilosclerida). In previous studies, Siribelli (1962) described the reproductive cycle of a Mediterranean population of *Axinella damicornis*, which was monitored from February to July in the Gulf of Naples. However, the structure of the gametes was studied only under light microscopy. Lévi (1950) reported the spawning of mature eggs of dragged individuals of *Axinella damicornis* in September, but he did not provide any images. The reproduction of *Raspaciona aculeata* has never been examined before. Therefore, we decided to investigate the oogenesis of both sponge species using light and electron microscopy, in order to describe the process of oocyte maturation, with special interest on the morphological inner oocyte organization and the mechanisms of yolk production and storage.

Material and Methods

Sampling

We studied different populations of the oviparous demosponges *Axinella damicornis* and *Raspaciona aculeata*, established at the sublittoral rocky communities

of North-eastern Mediterranean coast of Spain. To conduct a general monitoring of reproductive activity in the population, we tagged 5 large and presumably mature individuals, which were sampled monthly during two consecutive years. Using scuba and surgical scissors, we collected a small tissue piece (approx. 1 x 0.5 x 0.3 cm) from each sponge at each sampling time. In no case tissue collection involved dead or perceptible signs of disease in the injured sponges over the study period. Tissue samples for light microscopy were maintained in ambient seawater for transportation to the laboratory. Samples were divided into two pieces, one assigned to light microscopy, and the other to electron microscopy.

Light microscopy

Tissue samples were fixed within 2 h after collection in 4% formaldehyde in seawater for 24 h. Then, samples were desilicified with 5% hydrofluoric acid for 5 h, rinsed in distilled water, dehydrated through a graded ethanol series, cleared in toluene, and embedded in paraffin to cut them into 5 μm-thick sections with an Autocut Reichert-Jung microtome 2040. After deparaffining with xylene, sections were stained with Hematoxylin-PAS, and studied through a Zeiss Axioplan II compound microscopy connected to a Spot Cooled Color digital camera. When sections of both species revealed oogenic activity we underwent the post-fixation for electron microscopy of selected samples.

Transmission electron microscopy

The protocol followed for TEM fixation is detailed in Chapter 2.

Results

Axinella damicornis

Oogenesis showed an annual cycle with a single peak of oocyte production from October-November to May. Oocytes consistently located scattered within the mesohyl (Fig. 1A, C-D). The youngest oocytes, observed in October and November, measured approximately 30 μm (Fig. 2A) and were lobate because of the numerous pseudopodia (Fig. 1C, 2A-B).

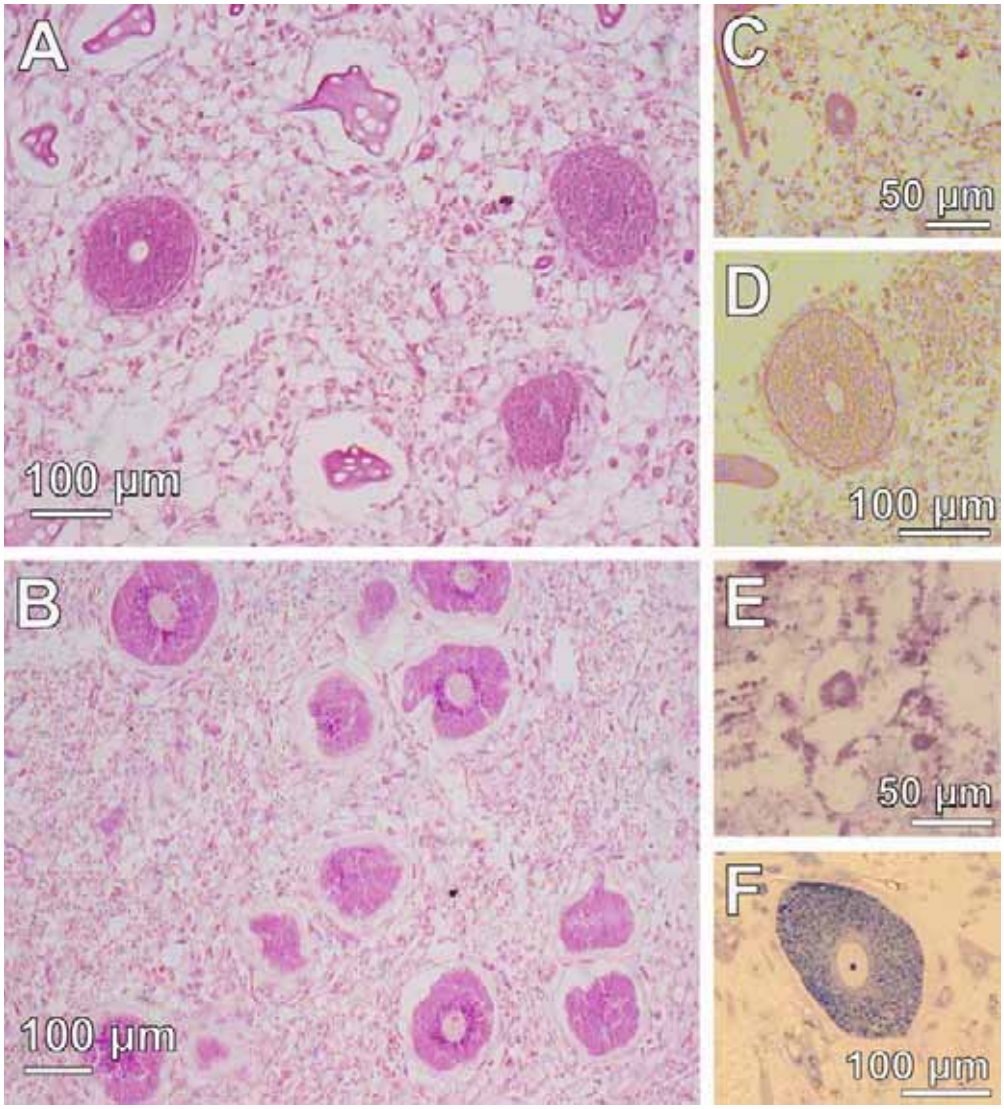

Figure 1. Oocytes of the two studied species. Mature oocytes spread homogeneously within the mesohyl of **(A)** *Axinella damicornis* **(B)** *Raspaciona aculeata*. Young **(C)** and mature **(D)** oocyte of *Axinella damicornis*. Young (E) and mature (F) oocyte of *Raspaciona aculeata*.

Early oocytes were very similar in their size, morphology, and affinity for stains to archaeocytes. Young oocytes had a nucleolate nucleus measuring approximately 10 µm (Fig. 1D). They displayed great number of microvilli (Fig. 2C-D), which were presumably involved in the pinocytosis of many molecules from the mesohyl. Those microvilli were filiform and had a small vesicle in the tip (Fig. 2D). Vitellogenesis started homogeneously within the entire cytoplasm. At this early stage the cytoplasm contained a great number of inclusions: heterogeneous (Fig. 2C) and homogeneous yolk inclusions (Fig. 3A), small vesicles with fibrilar content (Fig. 3B), and vesicles with

bacteria in different stages of digestion (Fig. 3C). It appears that the heterogeneous inclusions (Fig. 3D) were advanced stages of the vesicles with bacteria in digestion (Fig. 3C). Apart from these different types of vesicles, the cytoplasm appeared highly vesiculated because of the great number of small electron clear vesicles (Fig. 3A, C-D), which were particularly very abundant in the periphery of the oocyte (Fig. 2C-D). Glycogen rosettes located scattered within the cytoplasm (Fig. 3D). Lipidic inclusions were scarce (not shown).

As oogenesis progressed, both cytoplasm and nucleus increased in size. Mature oocytes became round, attaining approximately 120-150 μm (Fig. 1A, D). The nucleus, which contained fine-grained chromatin and several chromatin masses (Fig. 4A-B), measured 15 μm and the nucleolus 2 μm (Fig. 4A). A narrow area (approx. 3 μm) with scarce yolk inclusions but very vesiculated surrounded the nucleus (Fig. 4A-B). Multiple dictyosomes located within that perinuclear area, with the lamellae oriented parallel to the nuclear membrane and small vesicles detaching from their ends (Fig. 4B). Mitochondria were elusive because of the high density of inclusions in the cytoplasm. We did not find any endoplasmic reticulum or free ribosomes in the cytoplasm of the oocyte.

In mature oocytes two different types of yolk granules were observed in the cytoplasm, being probably different stages of the formation of yolk inclusions: 1) heterogeneous membrane-bound composites including semi-digested bacteria (Fig. 4C-F) and 2) vesicles of variable size containing highly electron-dense yolk granules (Figs 4F, 6A, D-F). It appeared that the mechanism of vitellogenesis involved the endocytosis of bacteria. Free bacteria of the mesohyl (Fig. 2D, 5A) were endocytosed individually (Fig. 5A-C). Endocytosed bacteria were stored also individually in small vesicles (Fig. 5D), which subsequently fused together, showing evident signs of bacteria digestion (Fig. 6A-C). Then, several vesicles with bacteria in digestion fused together in a large one, also incorporating lipidic material (Fig. 6D-E) in the vesicles together with the product obtained from the digestion of bacteria. The result of the bacterial digestion in those vesicles, were small vesicles containing coarse granular electron-dense yolk (Fig. 6A, D, F). Granular yolk vesicles were comprised of 10-25 granules (Fig. 6F).

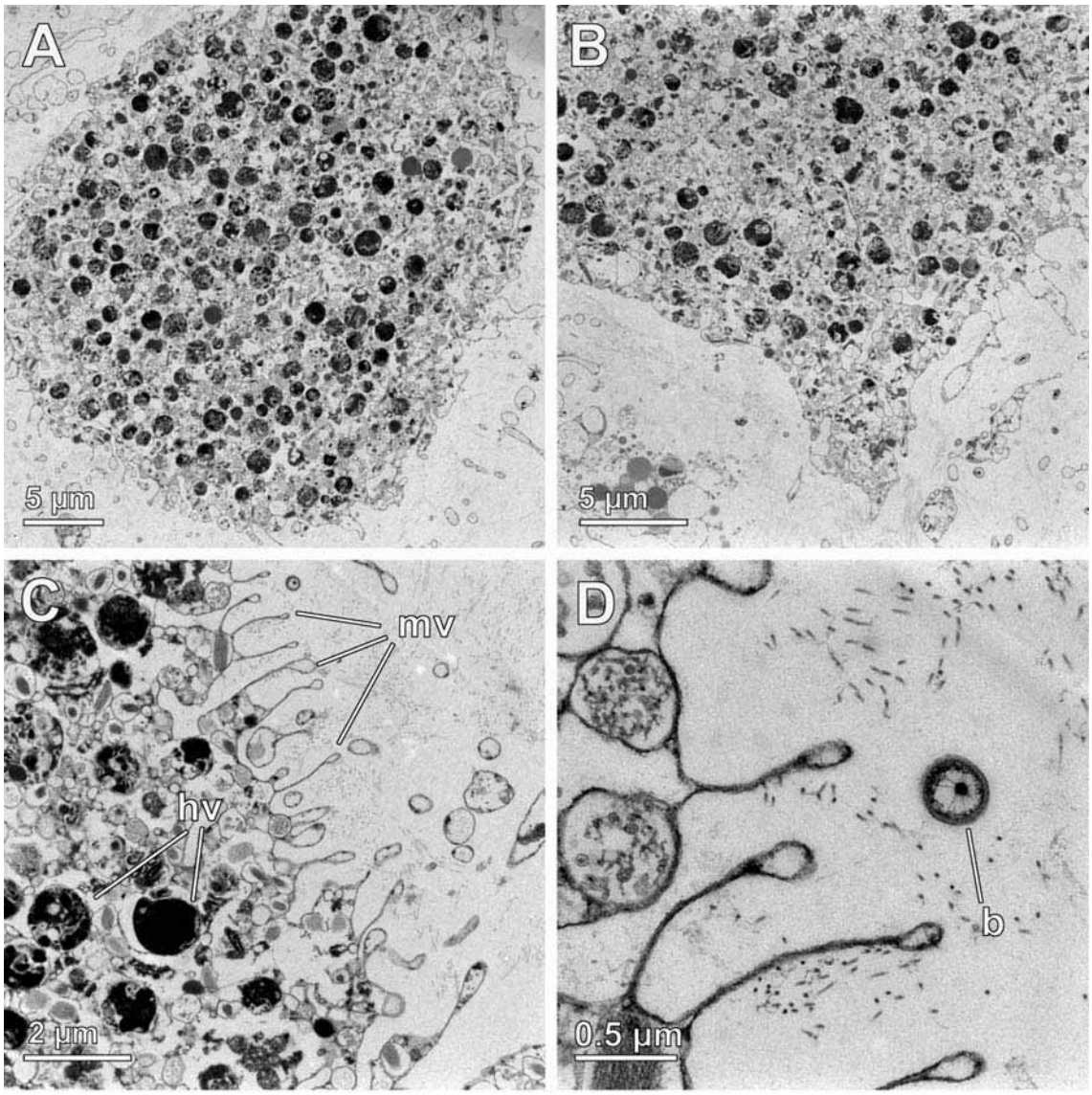

Figure 2. Young oocyte of *Axinella damicornis*. **(A)** General view of a young oocyte. **(B)** Pseudopodium of a young oocyte. **(C)** Microvilli (mv) displayed by the oocyte membrane. The cytoplasm contained heterogeneous yolk (hv). **(D)** Higher magnification of the microvilli approaching a bacteria (b).

Few nurse cells surrounded the oocyte (Fig. 7A). These nurse cells showed a large anucleolate nucleus with chromatin condensations in the inner nuclear membrane (Fig. 7B). Their cytoplasm contained heterogeneous inclusions (Fig. 7B-C), which strongly resembled those of the oocyte. Moreover, nurse cells were presumably exocytosing lipidic inclusions in the vicinity of the oocyte (Fig. 7A-B).

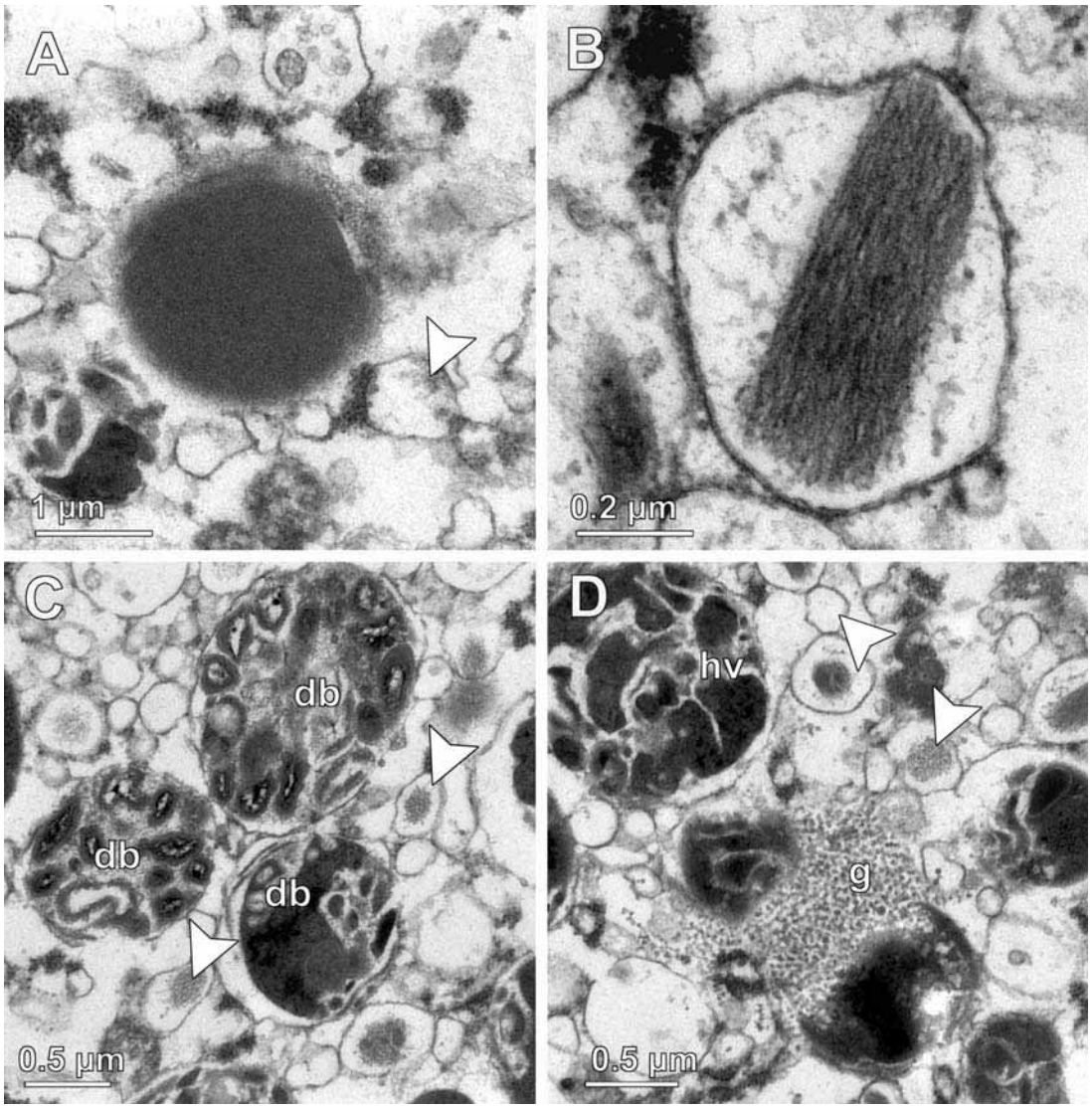

Figure 3. Inclusions of the young oocyte of *Axinella damicornis*. **(A)** Homogeneous yolk inclusion. Note the vesicles (arrow head) within the cytoplasm. **(B)** Vesicle containing fibrilar material. **(C)** Vacuoles showing bacteria in different digestion stages (db) and numerous vesicles (arrow heads) within the cytoplasm. **(D)** Glycogen rosettes (g), heterogeneous yolk inclusions (hv), and multiple vesicles (arrow heads) within the cytoplasm of the oocyte.

Raspaciona aculeata

Oogenesis developed once a year during 3-5 months in late summer and autumn (from July-August to October-November). Young oocytes (25 µm) appeared scattered within the mesohyl (Fig. 1B, E), and were strongly similar in appearance, size, and affinity for stains to archaeocytes. Young oocytes had an oval nucleus measuring 5 µm with a well developed nucleolus (approx. 2 µm) (Fig. 8A).

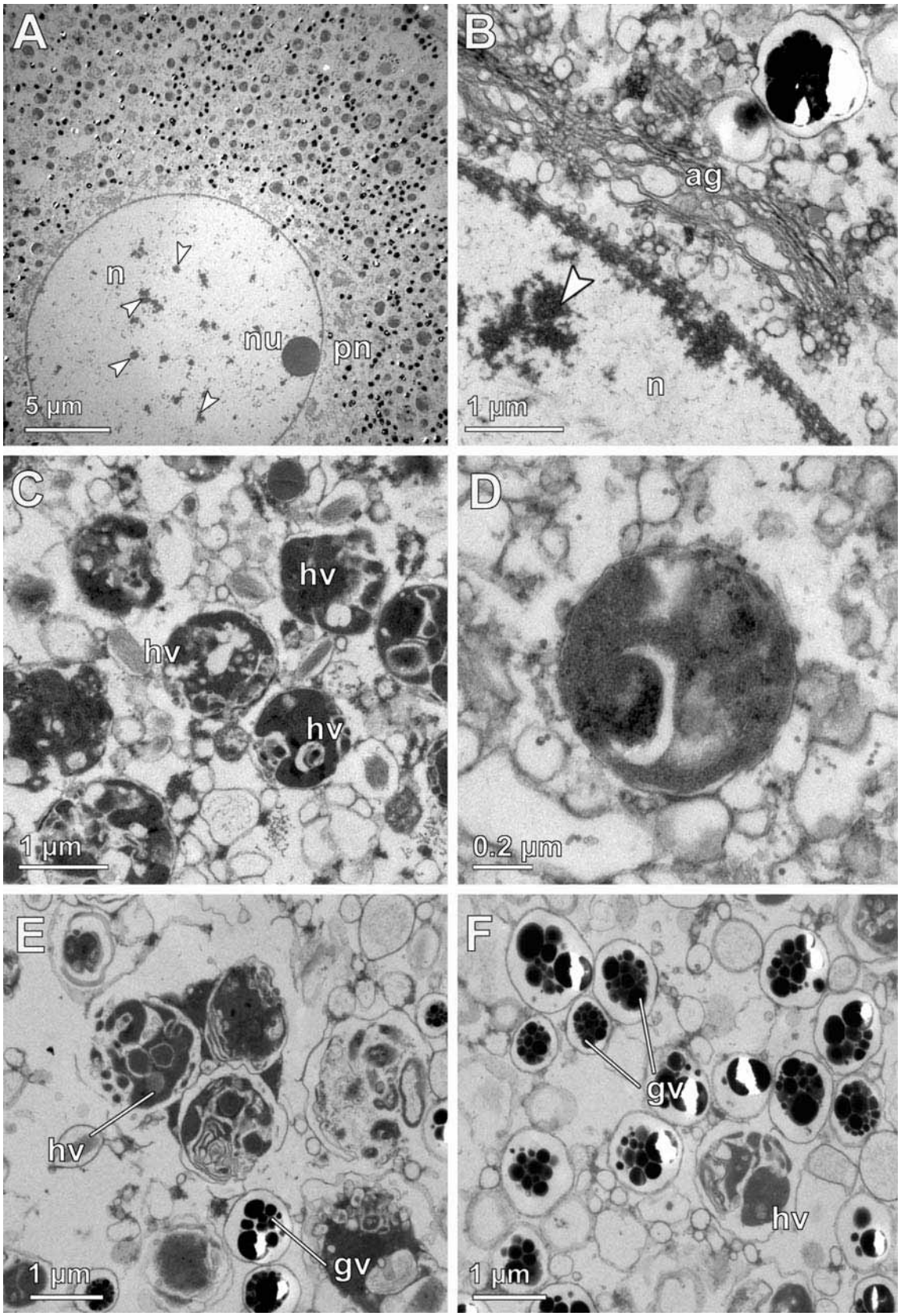
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Figure 4. Mature oocyte of *Axinella damicornis*. **(A)** View of the nucleolate (nu) nucleus (n) and the narrow perinuclear region (pn). Note the chromatin masses (arrow heads) within the nucleus. **(B)** Close up of the nucleus (n) with chromatin masses (arrow heads) and the Golgi apparatus (ag), with the lamellae orientated parallel to the nuclear membrane. **(C)** Heterogeneous inclusions within the cytoplasm (hv). **(D)** Close up of a heterogeneous yolk inclusion. **(E-F)** Vacuoles of granular electron-dense yolk (vg) and heterogeneous inclusions (hv).

Dictyosomes occurred adjacent to the external nuclear membrane, with cisternae oriented parallel to it, and small vesicles detaching from their ends (Fig. 8B). The cytoplasm contained large numbers of small electron-clear vesicles, scarce lipid droplets, and small groups of mitochondria (Fig. 8C-D). At this stage, vitellogenesis started, and small irregular inclusions appeared within the cytoplasm (Fig. 8C).

As they became larger, mid-stage oocytes displayed large numbers of pseudopodia (Fig. 9A, 10C-D). Their cytoplasm started filling, from the nucleus to the periphery, with heterogeneous inclusions of complex nature (presumably lipidic and proteinaceous) (Fig. 9A-B, 10A), leaving a perinuclear region devoid of yolk inclusions where large dictyosomes located (Fig. 9B-D). At this stage the nucleus measured approximately 25 μm, and the nucleolus increased in size up to 5 μm (Fig. 9B). Inside the nucleus some chromatin masses located close to the inner membrane (Fig. 9C). The nuclear membrane of the nucleolate nucleus showed numerous pores, probably facilitating the exchange of large molecules (Fig. 9C-D). Clusters of 15-20 mitochondria appeared scattered within the entire cytoplasm (Fig. 10B). The periphery of the oocyte membrane showed numerous small electron-clear vesicles (Fig. 10C-D), presumably indicating multiple events of pinocytosis. Surrounding the oocyte some collagen microfibrils were perceivable (Fig. 10C), as well as some free-living bacteria (Fig. 10D).

Mature oocytes measured approximately 190 μm (Fig. 1F) and located in large groups within the mesohyl (Fig. 1B). The nucleus was oval, measuring 30 μm in its largest diameter, with a 4-5 μm nucleolus (Fig. 11A). A 5 μm-wide area rich in small vesicles and devoid of yolk inclusions appeared surrounding the nucleus (Fig. 11A-B). Larger dictyosomes than in the mid-stage oocytes appeared in the vicinity of the

nucleus, most of them with the lamellae oriented parallel to the nuclear membrane (Fig. 11B). Few microvilli were produced by the oolemma (Fig. 11C). Large clusters of mitochondria were no longer found. Instead, groups of only 6 to 8 mitochondria occurred in the periphery of the oocyte cytoplasm (Fig. 11D). Glycogen rosettes distributed within the cytoplasm intermingled with diverse inclusions (Fig. 11E). Two types of yolk inclusions occurred in large numbers: one with a heterogeneous electron-dense content, and other with a more homogeneous electron-dense material (Fig. 11F). Both types appeared to correspond to different stages of formation of yolk. Many lipid droplets were intermingled with yolk inclusions, as well as granular inclusions (Fig. 11F).

Numerous nurse cells, containing heterogeneous yolk and other inclusions, approached the oocyte during the entire growth period (Fig. 12A). We identified two potential types of nurse cells in the vicinity of growing oocytes. Type I were amoeboid cells (Fig. 12B) that flattened against the oocyte membrane (Fig. 12A, C); they measured approximately 10 μm in their largest diameter and contained heterogeneous inclusions (Fig. 12A-D) similar to the oocyte's inclusions. When these cells became in contact with the oocyte, numerous small electron-dense vesicles were released to the perioocytic space (Fig. 12D) to be presumably incorporated by the oocyte. Type II were round, approximately 4 μm in diameter, and less numerous than type I (Fig. 12E). Type II nurse cells were charged with large vacuoles of granular content and lipid droplets. These cells embedded so deeply in the oocyte that the possibility that they are phagocytosed entirely can not be discounted (Fig. 12E).

Occasionally, collagen fibrils were endocytosed (Fig. 12F), as well as other nutritive material presumably exocytosed by nurse cells in the perioocytic space.

Discussion

Oocytes of the 2 studied species appeared to derive from archaeocytes, because of their similarities in size, morphology, and affinity for stains. A similar origin has been postulated for the majority of sponges (see Fell 1983 and Simpson 1984 for reviews). Oocytes of *Axinella damicornis* and *Raspaciona aculeata* were similar in appearance during early stages of growth, but differentiated from each other as growth proceeded, particularly regarding reserve material.

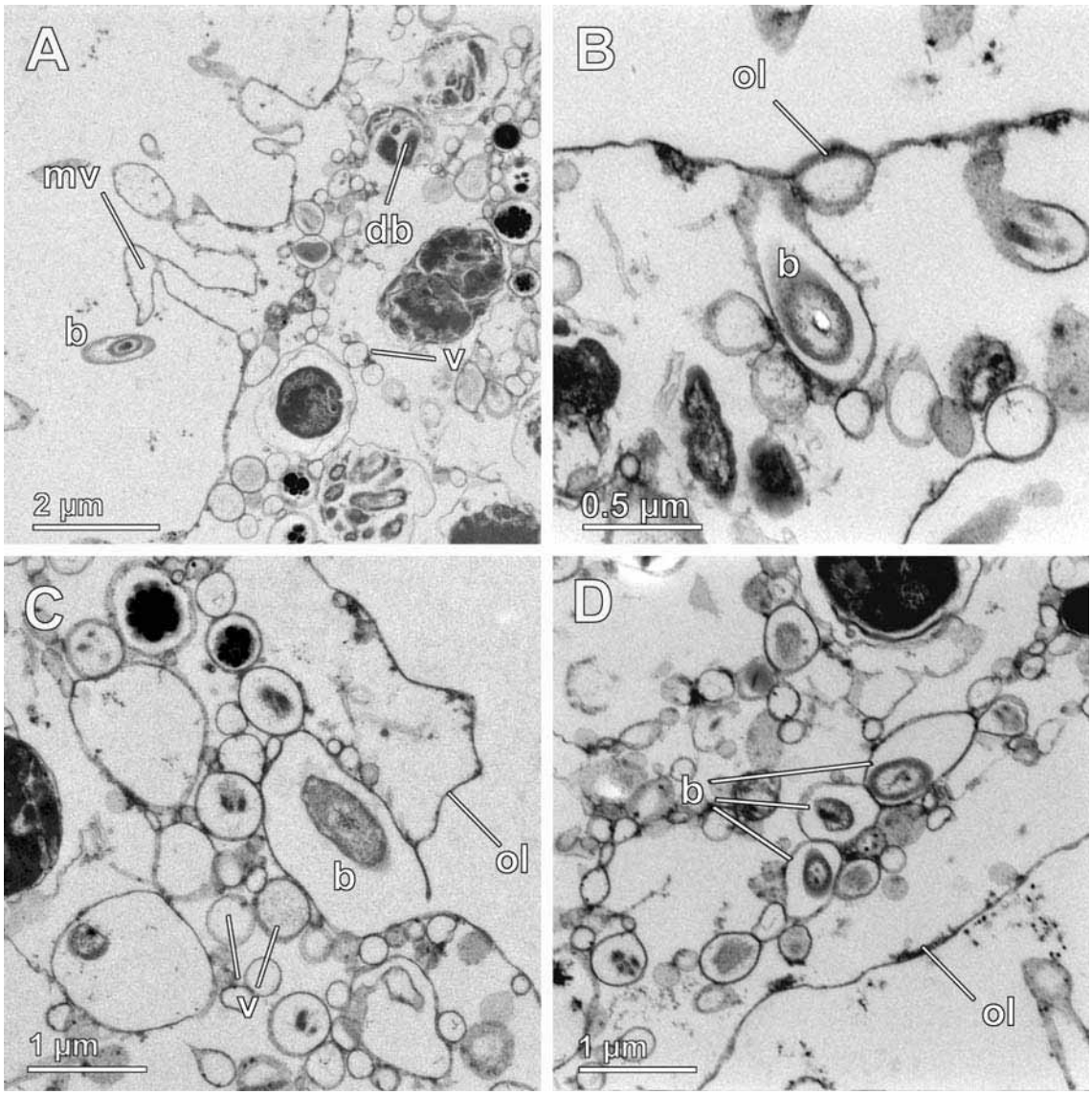

Figure 5. Endocytosis of bacteria in *Axinella damicornis*. **(A)** Microvilli (mv) of the oolemma close to a free bacteria (b). The cytoplasm of the oocyte showed numerous microvesicles (v) and vesicles with bacteria in different stages of digestion (db). **(B-C)** Bacteria (b) endocytosed in the periphery of the oocyte. Note the oolemma (ol), and the great number of microvesicles (v) in the periphery of the cytoplasm of the oocyte. **(D)** Small vacuoles containing single bacteria (b) close to the oolemma (ol).

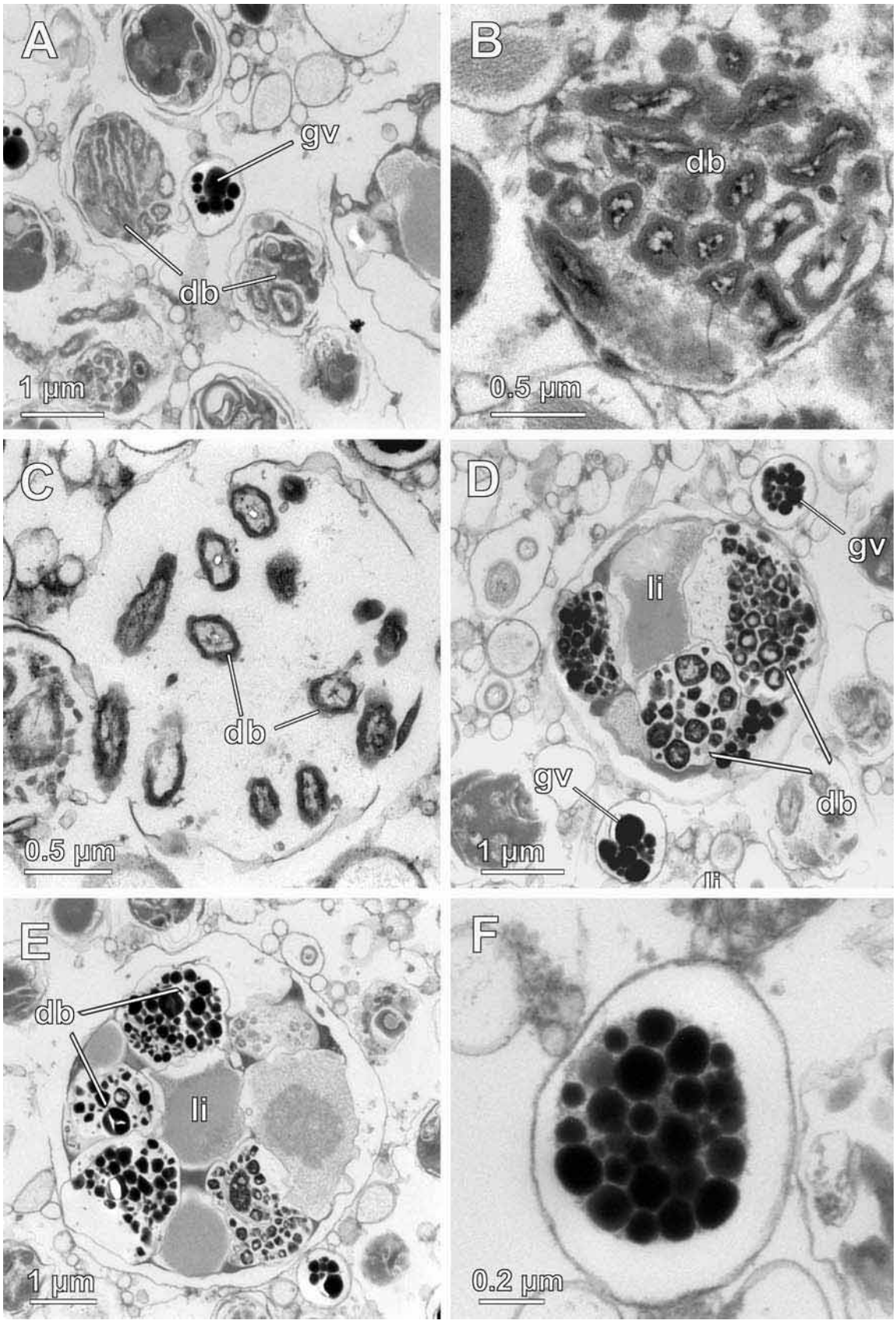
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Figure 6. Yolk elaboration in *Axinella damicornis.* **(A)** Periphery of the oocyte showing numerous vacuoles with bacteria in different digestion stages (db) and granular yolk inclusions (gv). **(B)** Close up of a vacuole containing bacteria in digestion (db). **(C)** Vacuole containing several bacteria in digestion (db). **(D-E)** Intermediate stages of formation of granular yolk (gv). Note the lipidic material (li) and the digested bacteria (db). Close to the bacteria, a completely formed vacuole of granular yolk is observed (gv). **(F)** Granular yolk inclusion within a vacuole.

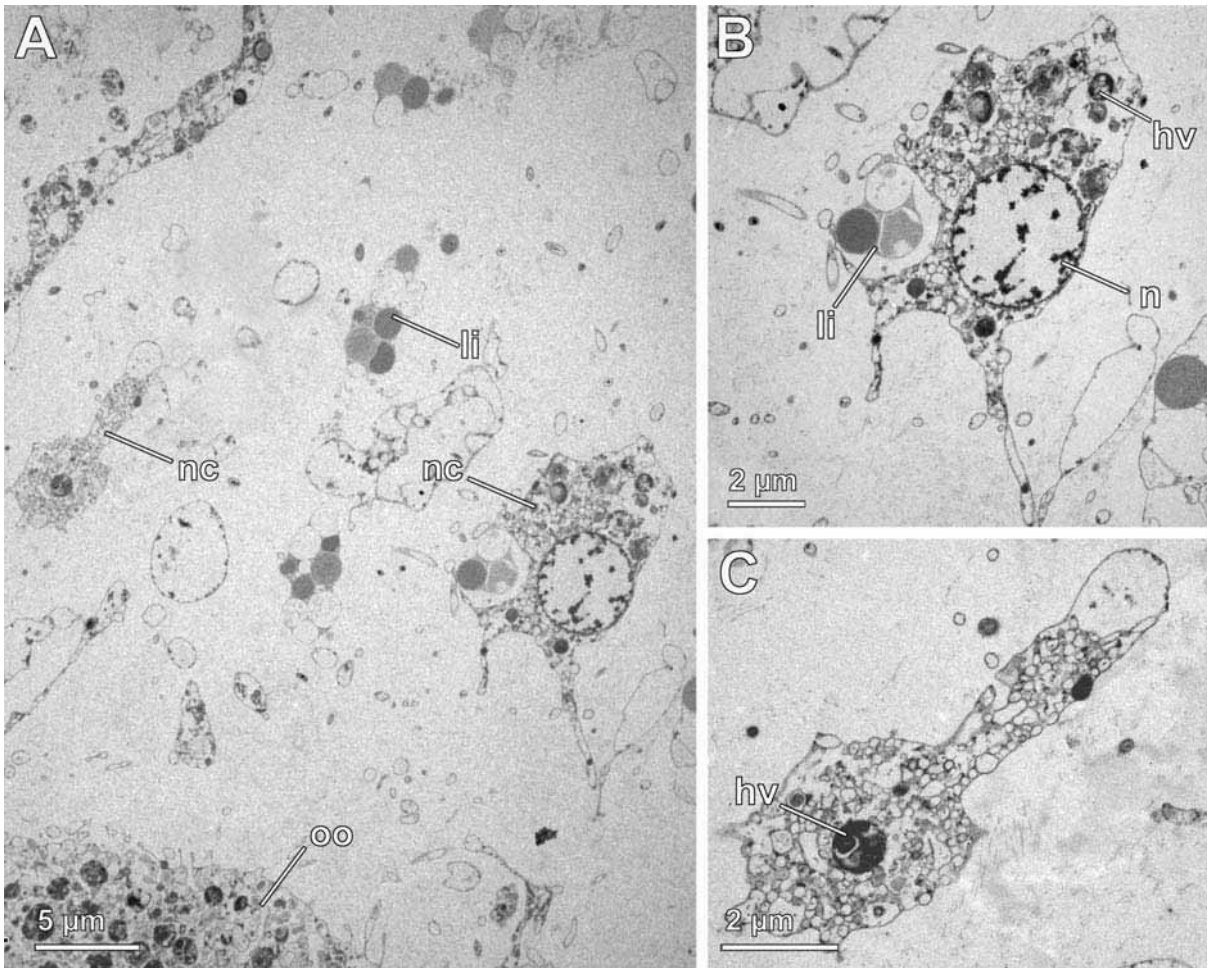

Figure 7. Nurse cells in *Axinella damicornis*. **(A)** Nurse cells (nc) approaching an oocyte (oo). Note the numerous lipidic granules (li) scattered within the mesohyl. **(B-C)** High magnification of the nurse cells in Fig. 7A. Note the large nucleus (n), the heterogeneous inclusions (hv), and the lipid granules (li) that appeared to be exocytosed by the nurse cell.

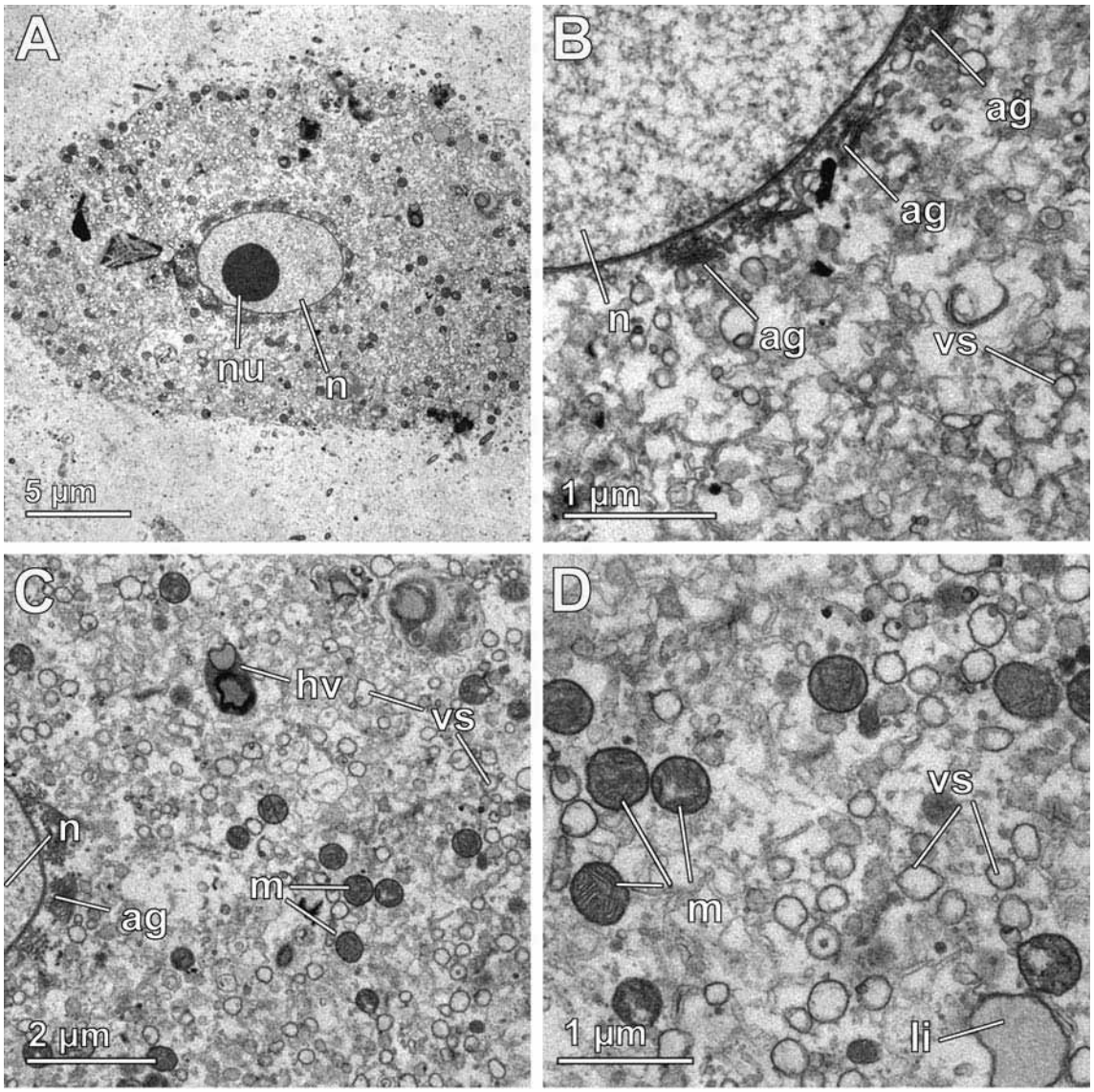

Figure 8. Young oocyte of *Raspaciona aculeata*. **(A)** General view of a nucleolate (nu) oocyte. Note the oval shape of the nucleus (n). **(B)** Close up of the nucleus (n) and the Golgi apparatus (ag), with the lamellae orientated parallel to the nuclear membrane. Note the numerous microvesicles (vs) present in the cytoplasm. **(C)** General view of the cytoplasm showing the nucleus (n) and the dictyosomes close to it (ag), several mitochondrial clusters (m), microvesicles (vs), and heterogeneous yolk inclusions in formation (hv). **(D)** Detail of the oocyte cytoplasm containing small clusters of mitochondria (m), microvesicles (vs) and lipid droplets (li).

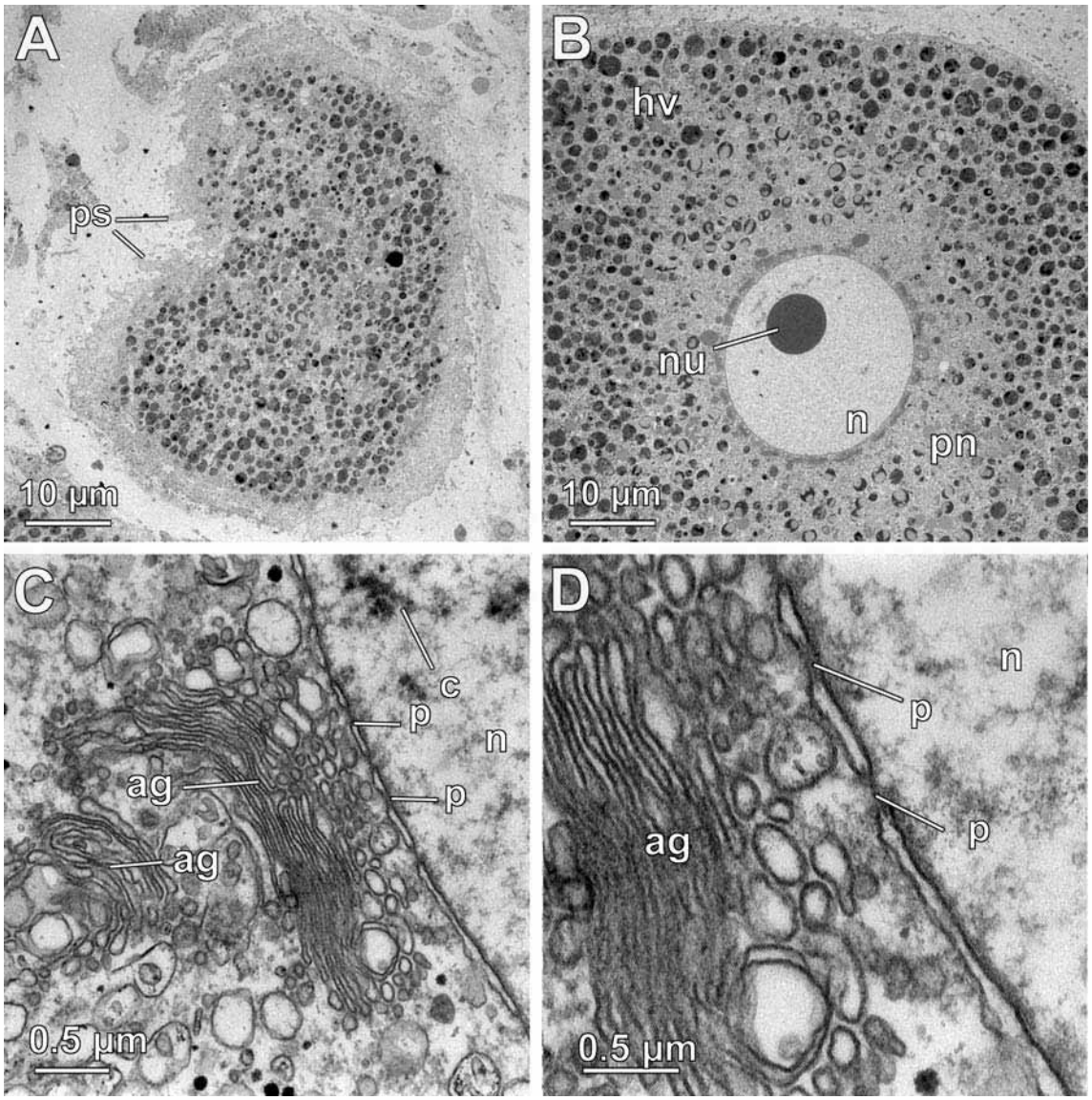

Figure 9. Mid-stage oocyte of *Raspaciona aculeata*. **(A)** General view of a complete mid-stage oocyte showing the numerous pseudopodia (ps). **(B)** Mid-stage oocyte showing the nucleolate (nu) nucleus (n), the perinuclear region (pn) devoid of inclusions, and numerous heterogeneous yolk inclusions in the periphery (hv). **(C-D)** Details of the nucleus (n) and the perinuclear region of a mid-stage oocyte, containing numerous dictyosomes (ag) and microvesicles (vs). Note the nuclear pores (p) displayed by the nuclear membrane and the chromatin masses (c).

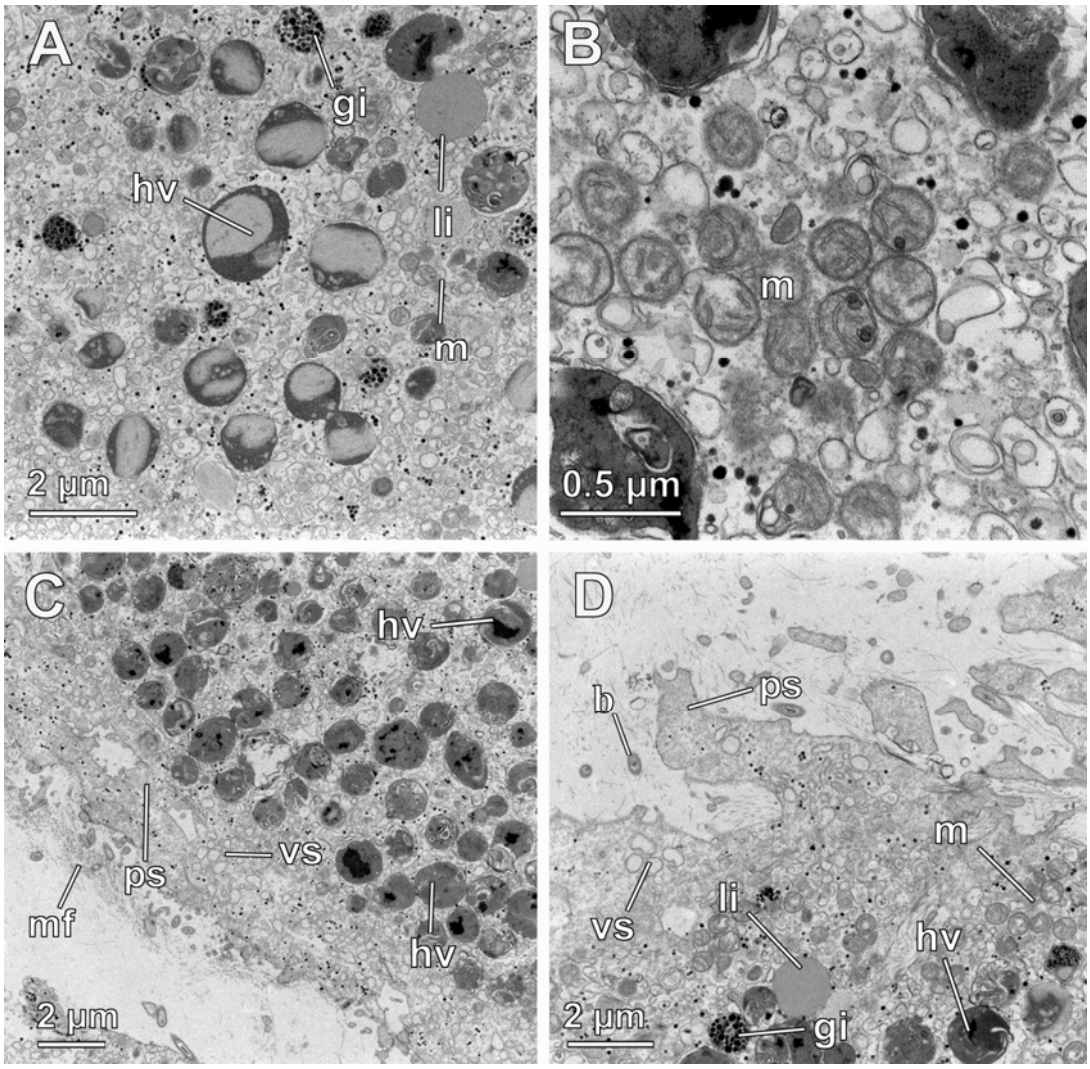

Figure 10. Organelles and inclusions of the mid-stage oocyte of *Raspaciona aculeata*. **(A)** Detail of the region immediately close to the nucleus, showing early stages of yolk inclusions (hv), granular inclusions (gi), mitochondria (m), and lipid droplets (li). **(B)** Detail of a mitochondrial cluster (m). **(C)** Periphery of the oocyte's cytoplasm showing the pseudopodia (ps), small microvesicles (mv), and heterogeneous yolk (hv). Note the occurrence of collagen microfibrils (mf) in the vicinity of the oocyte. **(D)** Pseudopod (ps) emitted by a mid-stage oocyte towards the mesohyl. Note the clusters of mitochondria (m), the lipid droplets (li), the numerous microvesicles (mv), the heterogeneous yolk (hv), and the granular inclusions (gi). In the mesohyl, free-living bacteria are observed (b).

Both oocytes were lobate at early stages, emitting numerous pseudopodia, a feature previously described for other demosponges (Fincher 1940; Gaino et al. 1986a;

Gallissian and Vacelet 1992; Sciscioli et al. 1991) and many invertebrates (Nørrevang 1968). In both species, at all times during development, the oolemma emitted microvilli involved diversely in endocytic processes, as described in some other species (Diaz 1979; Gaino et al. 1986a). However, the microvilli were more numerous in *Axinella damicornis* than in *Raspaciona aculeata*. The cytoplasm contained glycogen rosettes, which have been frequently reported in sponge oocytes (Fell 1974; Simpson 1984; Gaino et al. 1986a), and heterogeneous yolk, as known for many demosponges (Borojévic 1967; Fell 1974; Diaz et al. 1975; Aisenstadt and Korotkova 1976; Lévi and Lévi 1976; Lepore et al. 1995) and some calcareous species (Gaino and Sarà 1994).

In both studied species, the nucleus possessed a perinuclear region devoid of yolk inclusions, as shown by oocytes of the demosponges *Suberites massa* (Diaz et al. 1975), *Tetilla serica* (Watanabe 1978), *Stelletta grubii* (Sciscioli et al. 1991), or *Halichondria panicea* (Witte and Barthel 1994). Golgi apparatuses, which are involved in the synthesis and/or accumulation of yolk (Nørrevang 1968), were very abundant in both *Axinella damicornis* and *Raspaciona aculeata*. Dictyosomes arranged their lamellae in parallel to the external nuclear membrane, which has been observed in many sponge oocytes (Diaz 1979; Sciscioli et al. 1991; Gallissian and Vacelet 1992; Lepore et al. 1995). Dictyosomes of *R. aculeata* were larger than those of *A. damicornis*.

Differences between both oogenesis were basically restricted to the process of reserve supplying and yolk formation (i.e., vitellogenesis). Sponge oocytes are known to acquire their reserves using a variety of methods: 1) phago-, pino-, and endocytosis, 2) assimilation of material from adjacent cells, 3) transfer of nutritive material by nurse cells, and 4) capture of symbiotic bacteria (Simpson 1984; Sciscioli et al. 1991). The possibility that endocytosed bacteria and other symbiotic microbes are used for the formation of oocyte reserves (yolk) have been suggested in a number of cases (Gaino and Sarà 1994; Sciscioli et al. 1991, 1994), but details of the process have never been described.

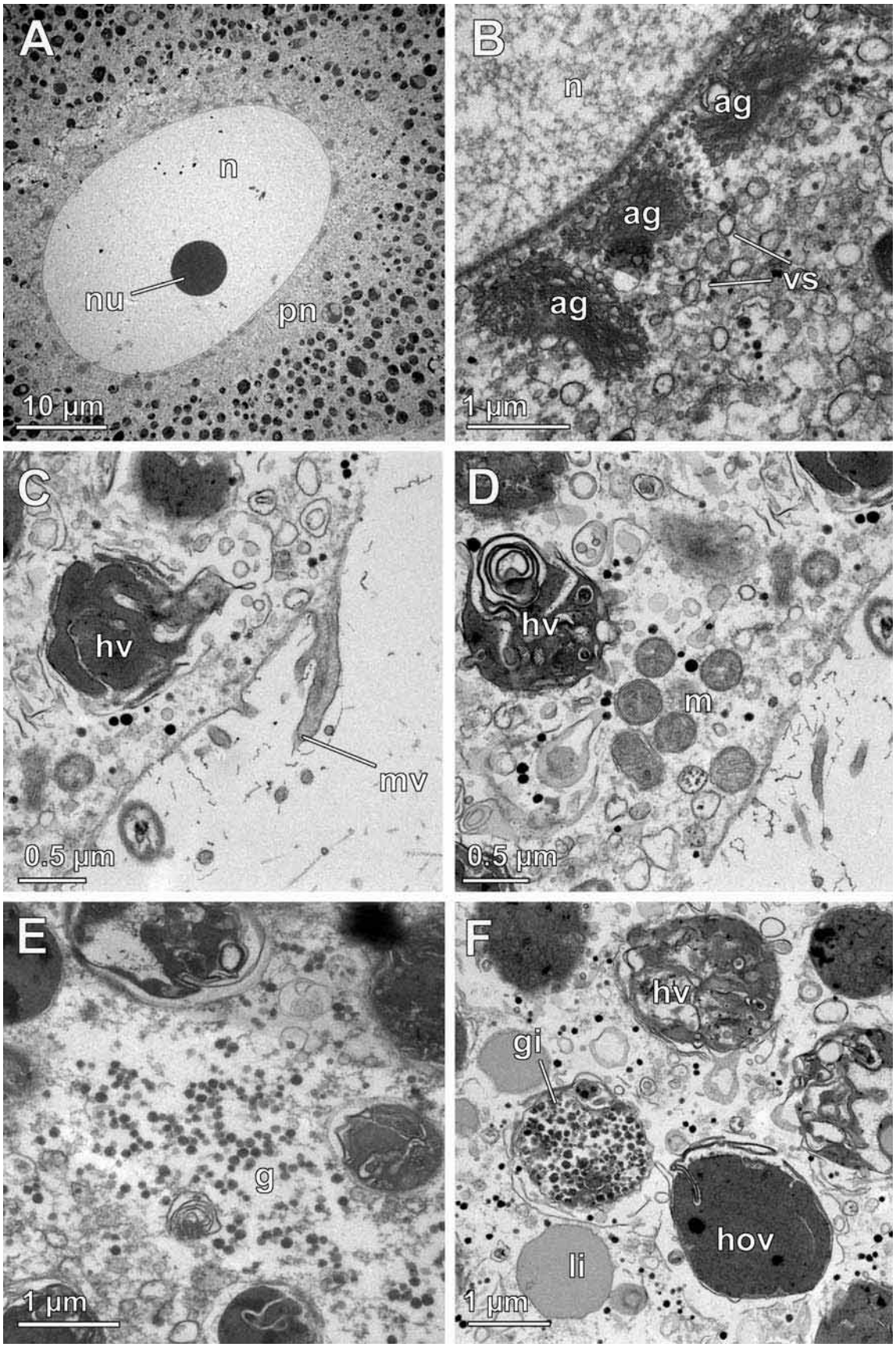
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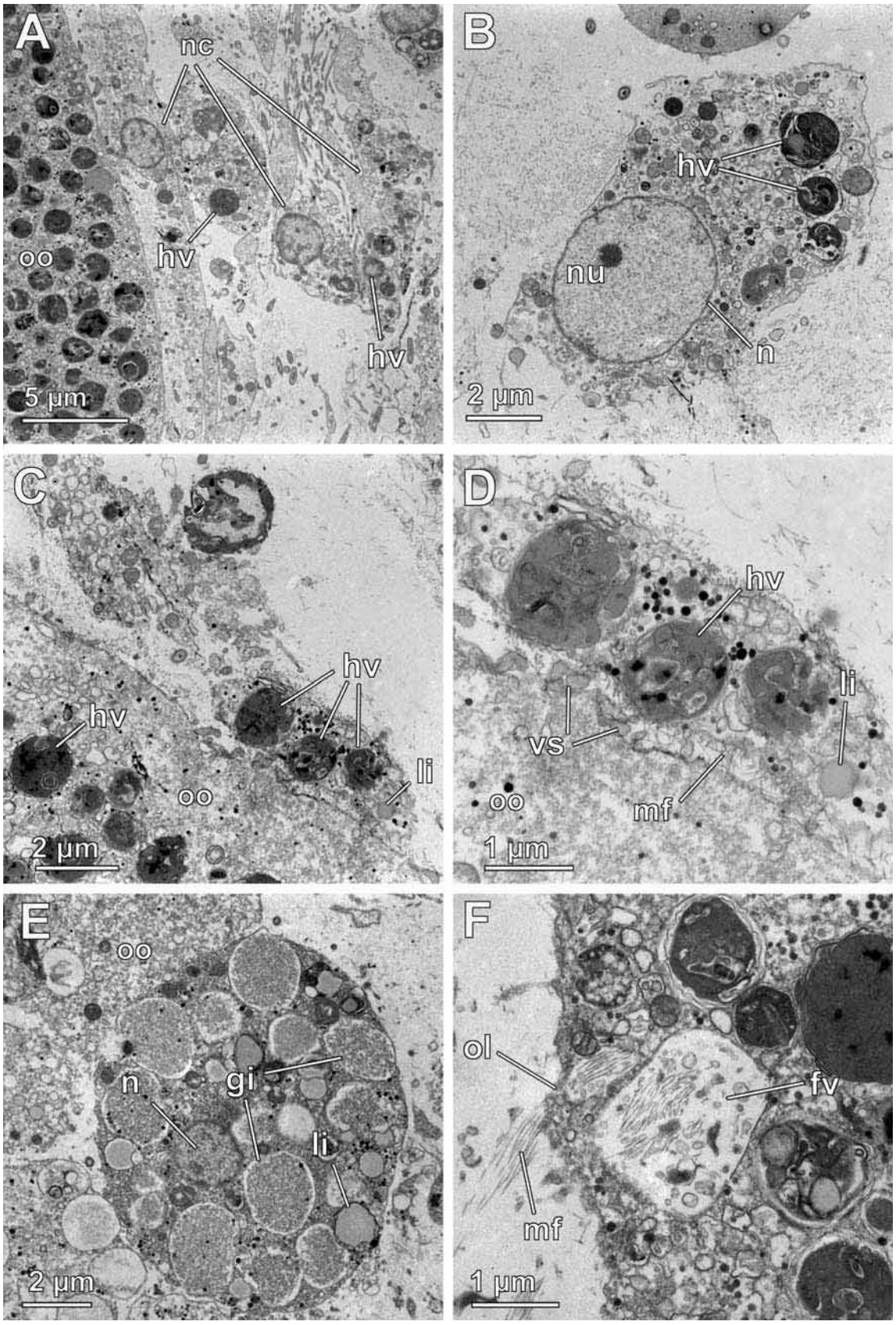
Figure 11. Mature oocyte of *Raspaciona aculeata*. **(A)** View of the nucleolate (nu) nucleus (n) and the wide perinuclear region (pn). **(B)** Detail of the nucleus (n) and the large dictyosomes (ag), with the lamellae orientated parallel to the nuclear membrane. **(C)** Microvilli (mv) displayed by the oocyte membrane. Note the heterogeneous yolk inclusions of the cytoplasm (hv). **(D)** Detail of the peripheral cytoplasm showing small clusters of mitochondria (m) and heterogeneous yolk inclusions (v). **(E)** Glycogen rosettes (g) within the cytoplasm. **(F)** Different types of inclusions observed in the oocyte cytoplasm: granular inclusions (gi), homogeneous yolk (hov), heterogeneous yolk (hv), and lipid droplets (li).

The ultrastructural examination of growing oocytes of *Axinella damicornis* allowed inferring part of such a process. The granular electron-dense yolk was elaborated from aggregates of endocytosed bacteria with other fine-grained granular material - presumably lipidic. Unlike in *Corticium candelabrum* (Riesgo et al. 2007), transfer of bacteria from nurse cells to the oocyte was not observed in *Axinella damicornis*. Instead, bacteria appeared to be endocytosed individually by the oocyte, and stored in small vesicles. Subsequently, groups of vesicles fused to form a large, single one containing 15-20 bacteria. Later, granular inclusions were incorporated to form the granular electron-dense yolk. Such vesicles finally contained 10-25 yolk granules. However, not only digestion of bacteria served as the basis for elaborating reserve material, some nurse cells containing yolk inclusions and lipid droplets, approached the oocytes and exocytosed their contents in the perioocytic space. These contents were later incorporated by the oocytes. A similar mechanism has been reported in many demosponges (Tuzet and Pavans de Ceccatty 1958; Fell 1983; Simpson 1984). We can not discard also the auto-synthesis of yolk by the oocyte, since numerous vesicles were observed surrounding the yolk platelets, as described in many other invertebrates (Nørrevang 1968).

In *Raspaciona aculeata* the process of yolk elaboration was fairly different. Auto-synthesis of yolk started in mid-stage oocytes, which soon showed a gradual distribution of yolk granules, depending on their maturation stage, from the perinuclear region to the periphery of the oocyte. Auto-synthesis of yolk has been reported in many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1 µm

Figure 12. Nutrition of the oocyte of *Raspaciona aculeata*, and fibrilar vacuoles. **(A)** Nurse cells (nc) containing heterogeneous yolk (hv) approaching a mid-stage oocyte (oo). **(B)** Type I of nurse cell in the surroundings of the oocyte. Note the nucleolate (nu) nucleus (n) and the heterogeneous yolk inclusions (hv). **(C)** Nurse cell approaching an oocyte (oo) and containing lipid droplets (li) and heterogeneous yolk (hv) strongly similar to those of the oocyte (hv). **(D)** Detail of the heterogeneous inclusions (hv) and lipid droplets (li) carried by nurse cells. Note the microvesicles (vs) detaching from the oocyte membrane, and the collagen microfibrils (mf) between the oocyte and the nurse cell. **(E)** Type II of nurse cell attached to the oocyte (oo) containing granular inclusions (gi) and lipid droplets (li). Note the different appearance of the nucleus (n). **(F)** Vacuoles of fibrilar content (fv) close to the oolemma (ol). Note the similarities with mesohyl collagen (mf).

demosponges (e.g., Gallissian and Vacelet 1976; Watanabe 1978). Additionally, numerous nurse cells (types I and II) were involved in the transference of heterogeneous yolk inclusions, lipid droplets and other inclusions to the oocytes. No phagocytosis of bacteria was observed. Such differences in the vitellogenesis mechanisms between *Axinella damicornis* and *Raspaciona aculeata* may account for differences in oogenesis duration, which were up to 5 months of time lag. Supply of abundant reserve material by nurse cells usually allow a rapid completion of vitellogenesis and a fast egg production (Ramírez Llodra 2002), as occurred in *Raspaciona aculeata*, while auto-synthetic yolk elaboration, which implies an uptake of exogenous material (bacteria in the case of *Axinella damicornis*), result in a slow egg production.

Intracytoplasmic symbionts were not observed in the oocytes in any species, although free intercellular microorganisms were detected in the mesohyl, some of them being reported as high concentrations of Archaea in the particular case of *Axinella damicornis* (Margot et al. 2002). Therefore, we discard maternal transmission of symbiotic bacteria in both species. Instead, we suggest that acquisition of microbial symbionts in both cases takes place probably after settlement and once pumping activity starts (Reiswig 1971).

Vesicles of 2 μm in diameter, filled with fibrilar material (that strongly resembled collagen microfibrils), were observed in the oocyte of *Raspaciona aculeata*.

Similar vesicles have been reported in the oocytes of *Scypha ciliata* (Franzen 1988), *Sycon ciliatum* (Gaino et al. 1987), *Stelletta grubii* (Sciscioli et al. 1991), and *Geodia cydonium* (Sciscioli et al. 1994). However, while in *Sycon* and *Raspaciona* the fibrilar content seemed to be endocytosed to help formation of yolk, in the rest of species collagen fibrils seemed to be secreted by the oocyte itself to form a collagenous envelope, as reported also in *Tetilla serica* (Endo et al. 1967), *Tetilla japonica* (Watanabe 1978), and *Aplysina (Verongia) cavernicola* (Gallissian and Vacelet 1976).

In summary, ultrastructural features found in the oocytes of *Axinella damicornis* and *Raspaciona aculeata* account for the general pattern of oogenesis described not only in demosponges (see Fell 1974, 1983 and Simpson 1984 for reviews) but in many other invertebrates (Nørrevang 1968), with the unique exception of yolk elaboration and composition.