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Ultrastructure of oogenesis of two oviparous demosponges: *Axinella damicornis* and *Raspaciona aculeata* (Porifera)

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ABSTRACT

We investigated the cytology of the oogenic cycle in two oviparous demosponges, *Axinella damicornis* and *Raspaciona aculeata*, during 2 consecutive years both by light and electron microscopy. Oocytes of both species were similar in their basic morphological features but differences were noticed in time required to complete oocyte maturation and mechanisms of acquisition of nutritional reserves. The oogenic cycle of *A. damicornis* extended for 7–8 months in autumn-spring, while that of *R. aculeata* did it for 3–5 months in summer-autumn. Yolk of *A. damicornis* was predominantly formed by autosynthesis. Oocytes endocytosed bacteria individually and stored them in groups in large vesicles. Bacteria were digested and lipidic material was added to the vesicles to produce a peculiar granular yolk hitherto unknown in sponges. Scarce cells carrying heterogeneous inclusions were observed in the perioocytic space, and were interpreted as putative nurse cells. Such cells were presumably releasing lipid granules to the perioocytic space. In contrast, large numbers of nurse cells were found surrounding the oocytes of *R. aculeata*. They transported both lipid granules and heterogeneous yolk bodies to the oocytes. *R. aculeata* also produced some of their yolk by autosynthesis. The involvement of nurse cells in the vitellogenesis of *R. aculeata* shortened the oocyte maturation, whereas a largely autosynthetic vitellogenesis in *A. damicornis* prolonged the duration of oogenesis.

1. Introduction

Sexual reproduction in demosponges exhibits profuse variation in terms of mode (oviparism/viviparism and gonochorism/hermaphroditism), dynamics (from very short gametogenic cycles to nearly continuous gametogenesis) and morphology of gametes (Fell, 1974; Reiswig, 1983; Simpson, 1984; Boury-Esnault and Jamieson, 1999; Riesgo et al., 2007a,b; Riesgo and Maldonado, 2008a). Origin of gametes in demosponges is also diverse. To date there is no evidence of a predetermined germline, and demosponge gametes are known to derive from at least three types of somatic cells (archaeocytes, choanocytes, or storage cells), depending on the species (Fell, 1974, 1983; Reiswig, 1983; Simpson, 1984; Willenz and Hartman, 2004).

Oocytes usually derive from archaeocytes (see Fell, 1983 and Simpson, 1984 for reviews), although in few cases, choanocytes have been suggested as the oocyte anlagen (Diaz, 1973a,b; Gaino et al., 1986). Oocytes usually develop relatively scattered through the sponge mesohyl (Fell, 1983; Simpson, 1984), though in some species appear clustered or aggregated (e.g., Lévi, 1956; Diaz, 1973a,b; Fell and Jacob, 1979; Fromont, 1994; Riesgo et al., 2007b). Demosponge oocytes usually differ morphologically in aspects such as oocyte size, type and abundance of yolk, presence of enveloping nurse or follicular cells, and collagenous covers (see Fell, 1974, 1983; Simpson, 1984 for reviews).

Yolk formation (i.e., vitellogenesis) in poriferans, has been reported to take place by: 1) autosynthesis, with or without using pino- or endocytosed basic precursors (proteins, lipids, etc); 2) heterosynthesis, with yolk and/or yolk precursors supplied by somatic cells (nurse cells), or 3) both types simultaneously (Fell, 1974, 1983; Simpson, 1984; Sciscioli et al., 1991). All these three types of yok formation patterns have been also reported for most marine invertebrates (Nørrevang, 1968; Anderson, 1974; Eckelbarger, 1994; Ramírez Llodra, 2002). It is postulated that basal invertebrates predominantly form their yolk by the most "primitive" mechanism: autosynthesis (Eckelbarger, 1994). The diverse populations of inclusions traditionally called yolk can be roughly divided into fatty yolk -lipid droplets- and proteid yolk -composed of protein and carbohydrates (Nørrevang, 1968; Anderson, 1974). Proteid yolk shows a remarkable uniformity throughout the animal kingdom, occurring as membrane-bound electron-dense bodies with a homogeneous structure that, in some cases, possess a finely outlined dense core

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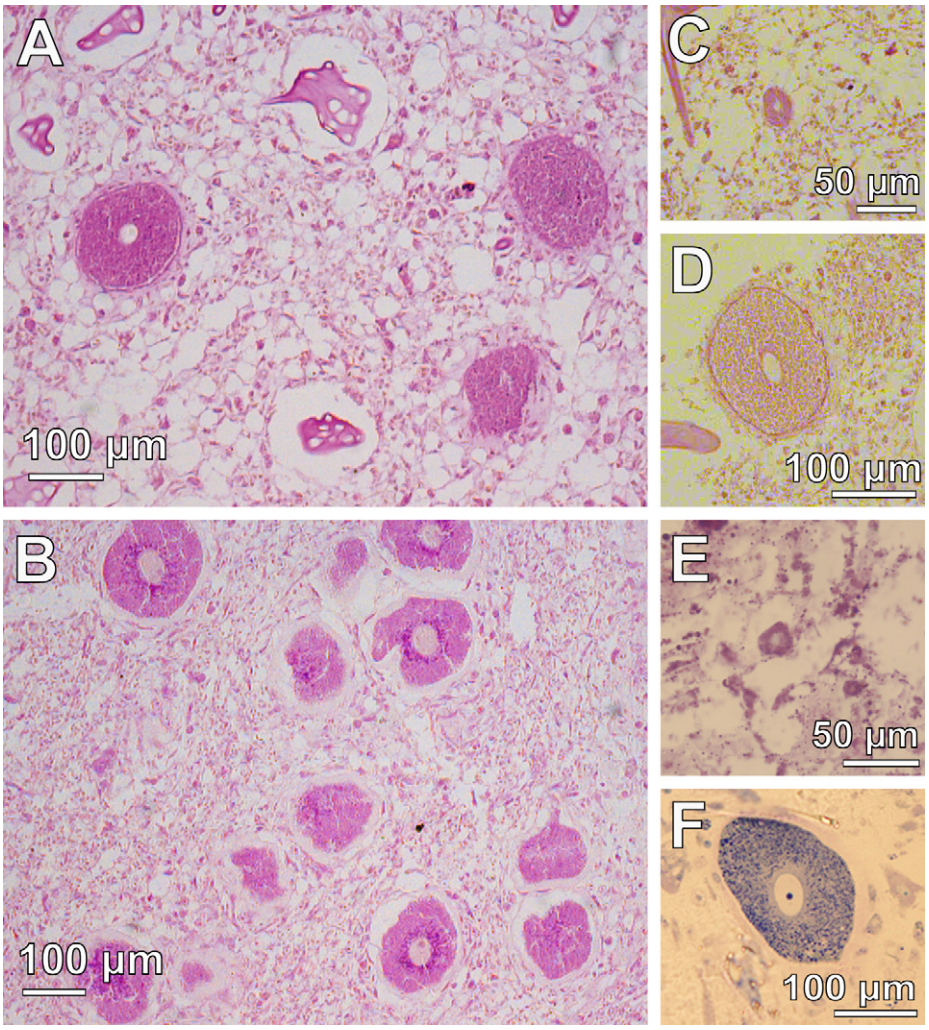

Fig. 1. Morphology and location of oocytes of *Axinella damicornis* and *Raspaciona aculeata* studied by light microscopy. Mature oocytes spread homogeneously within the mesohyl of (A) *Axinella damicornis* and (B) *Raspaciona aculeata*. Young (C) and mature (D) oocytes of *Axinella damicornis*. Young (E) and mature (F) oocytes of *Raspaciona aculeata*.

embedded in a less dense matrix (Nørrevang, 1968; Anderson, 1974; Adiyodi and Adiyodi, 1983). Only in some invertebrates, such as some molluscs, polychaetes, and echinoderms, yolk looks heterogeneous (e.g., ringed or vesiculated) (Anderson, 1974). In demosponges, yolk has been reported to have both homogeneous (e.g., Lévi and Lévi, 1976; Gallissian and Vacelet, 1976; Sciscioli et al., 1989, 1991, 2002; Riesgo et al., 2007a) and heterogeneous appearance (e.g., Diaz et al., 1975; Watanabe, 1978; Gaino et al., 1986; Gaino and Sarà, 1994; Lepore et al., 1995), but the latter is more common and abundant.

Since vitellogenesis remains poorly documented in oviparous demosponges, we selected two species belonging to different orders: *Raspaciona aculeata* (Johnston, 1842) (order Poecilosclerida) and *Axinella damicornis* Esper, 1794 (order Halichondrida). The reproductive cycle of *Raspaciona aculeata* has been studied by Riesgo and Maldonado (2008b), who reported the oogenesis to occur from July to November and described the gametes using light microscopy only. Similarly, little is known about the gametogenesis of *A. damicornis*. The duration of the gametogenic cycle was studied by Siribelli (1962) in the western coast of Italy and by Riesgo and Maldonado (2008b) in the northeastern coast of Spain. Whereas Siribelli (1962) reported the oogenesis to extend from February to July, Riesgo and Maldonado (2008b) documented it from October to May. In contrast, in the western coast of France, Lévi (1950) documented spawning of mature eggs of *A. damicornis* in September. Thus, it appears that timing and duration of oogenesis in *A. damicornis* depend on the particular environmental characteristics of each location. Again, all these studies described only the basic morphology of gametes using light microscopy. Therefore, since ultrastructural features of female gametes of *A. damicornis* and *R. aculeata* are largely unknown, we decided to investigate these oogeneses using light and electron microscopy to describe the process of oocyte maturation, with focus on the mechanisms of yolk production and storage.

2. Materials and methods

2.1. Sampling

We studied two populations of the oviparous demosponges *Axinella damicornis* and *Raspaciona aculeata*, in the sublittoral rocky communities of the North-eastern Mediterranean coast of Spain, between the localities of Blanes and Tossa de Mar (41° 11′ 18″ N, 2° 45′ 2″ W). A previous 2-year study based on repetitive monthly

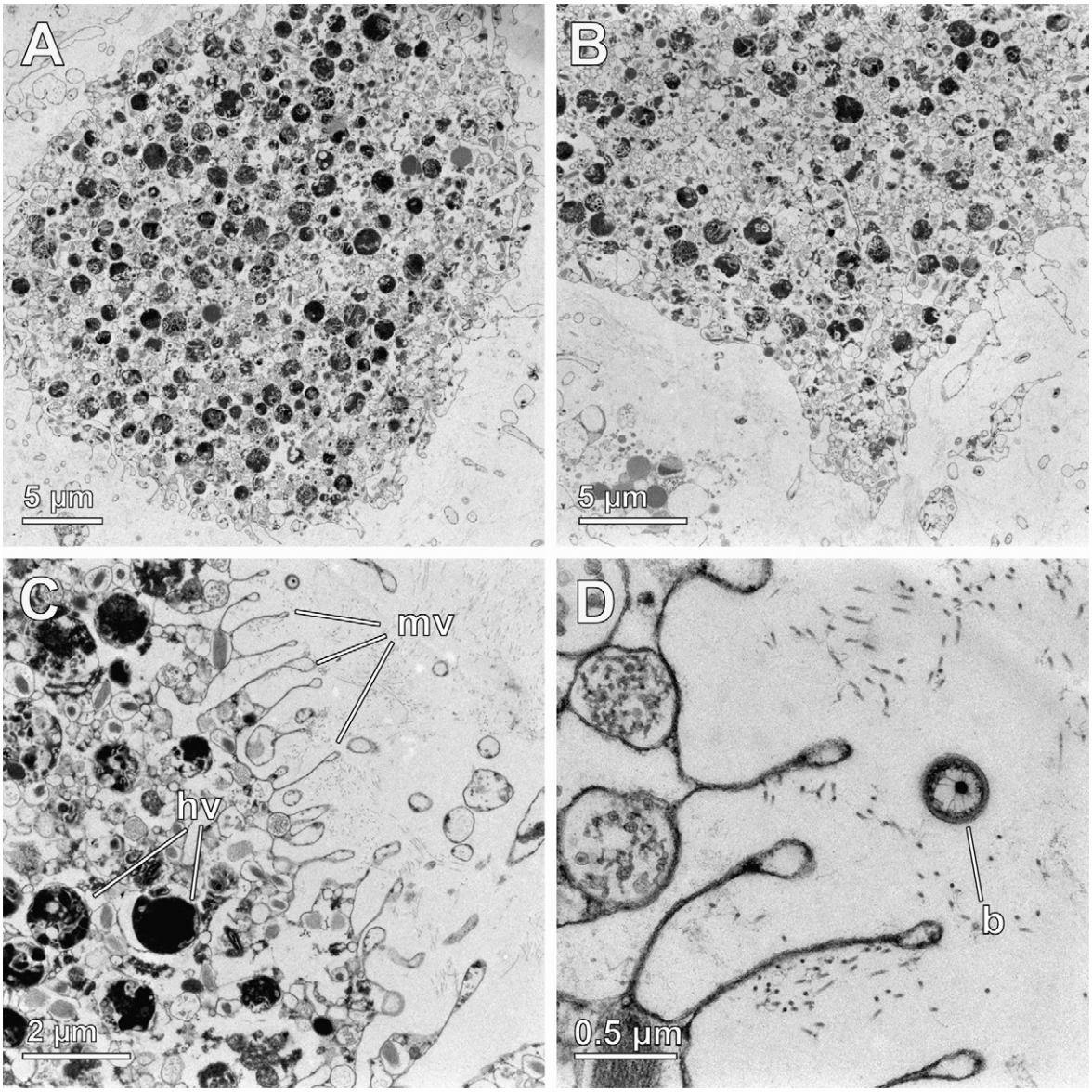

Fig. 2. Young oocyte of *Axinella damicornis*. (A) General view of a young oocyte. (B) Large pseudopodium emitted by a young oocyte. (C) Filiform microvilli with a small vesicle in the tip (mv) displayed by the oolemma. The cytoplasm of the oocyte contained heterogeneous yolk (hv). (D) Higher magnification of the microvilli, in close proximity to a bacteria (b).

sampling of 5 tagged individuals and additional fortnightly sampling of 25 untagged individuals during the maximum reproductive activity revealed that males and females of both species produce synchronically oocytes and spermatic cysts during a brief period of the year (Riesgo and Maldonado, 2008b). From that study, a sex ratio close to 1:1 was inferred for *Raspaciona aculeata* and close to 2:1 for *Axinella damicornis*.

The current cytological study of the oogenesis was based only on the tagged individuals ($n = 5$) of each species mentioned above. All these 5 individuals were large and presumably mature adults. They were sampled monthly during 2 consecutive years (2003–2005). Using scuba and surgical scissors, we collected a small tissue piece (approx. 1 cm × 0.5 cm × 0.3 cm) from each sponge at each sampling time. In no case tissue collection involved death or reproductive interruption in the injured sponges over the study period. Tissue samples were divided into two pieces, one assigned to light microscopy and the other to electron microscopy. Samples for electron microscopy were always pre-fixed in 2.5% glutaraldehyde in 0.2 M Millonig's phosphate buffer (MPB) and 1.4 M sodium chloride, and stored until further examination.

2.2. Light microscopy

Tissue samples for light microscopy were maintained in ambient seawater for transportation to the laboratory and 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 (70%, 96%, 100%), 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 microscope connected to a Spot Cooled Color digital camera. When light-microscopy sections revealed oogenic

activity in any of the species, we resumed processing for samples for electron microscopy.

2.3. Transmission electron microscopy

After primary aldehyde fixation, samples were post-fixed in 2% osmium tetroxide in MPB, dehydrated in a graded acetone series, and embedded in Spurr's resin. Ultrathin sections obtained with an Ultracut Reichert-Jung ultramicrotome were mounted on gold grids and stained with 2% uranyl acetate for 30 min, then with lead citrate for 10 min (Reynolds, 1963). Observations were conducted with a JEOL 1010 transmission electron microscope (TEM) operating at 80 kV and provided with a Gatan module for acquisition of digital images.

3. Results

3.1. Axinella damicornis

First evidence of oogenesis was detected in the sponge tissue in October-November (depending on year). Oogenesis was synchronic in the studied population, and extended through April-May (7–8 months). Most females (2 out of three) that produced oocytes in 2004 year did it also in 2005. Oocytes consistently located scattered throughout the mesohyl (Fig. 1A, C and D), and were very similar in morphology to archaeocytes, even showing a similar affinity for stains. Youngest oocytes, found in October and November, measured approximately 30 μm (Fig. 2A) and were lobate because of the formation of pseudopodia (Figs. 1C and 2A and B). They had a nucleolate nucleus measuring approximately 10 μm (Fig. 1C). They

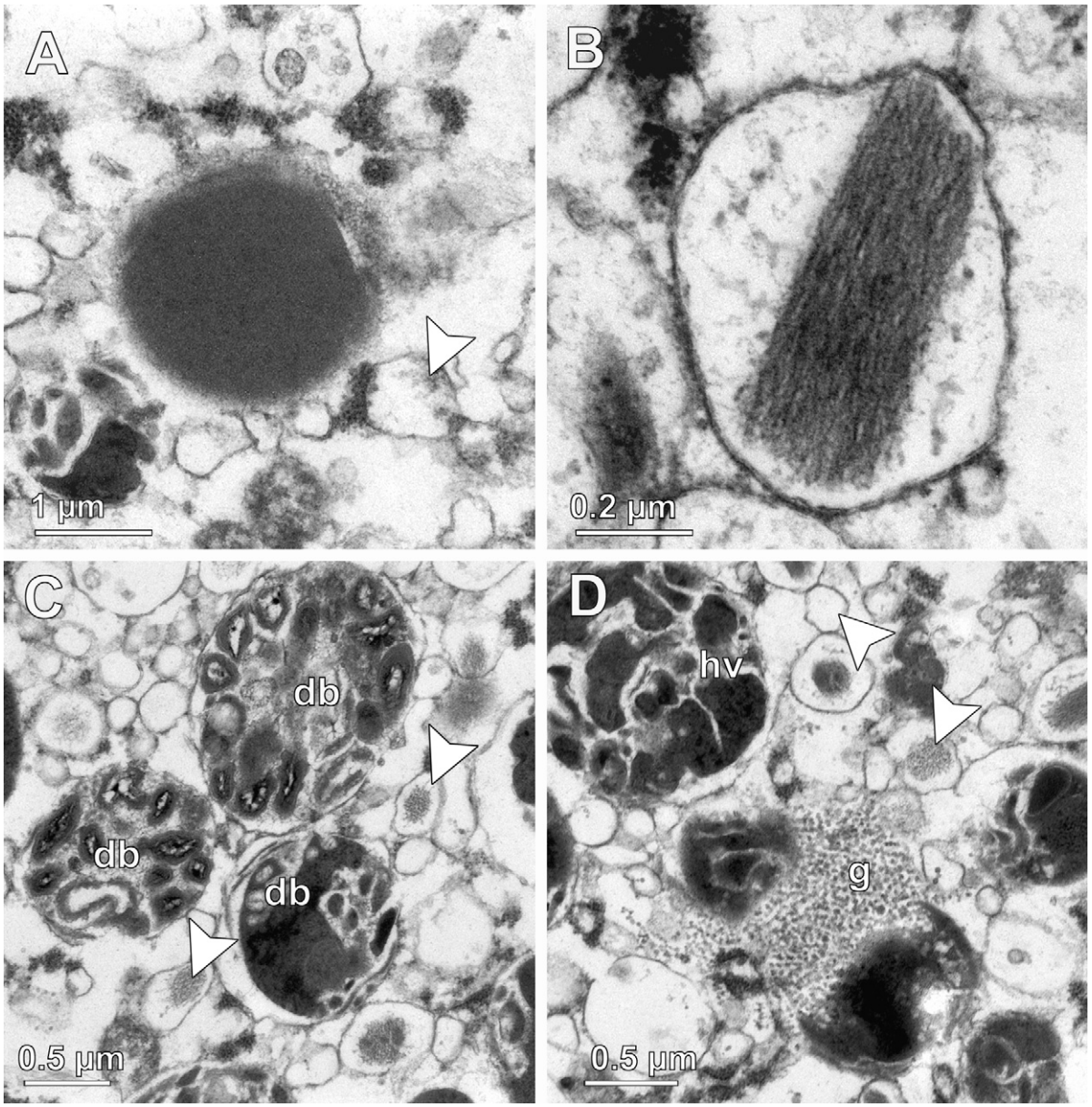

Fig. 3. Inclusions of the young oocyte of *Axinella damicornis*. (A) Homogeneous yolk inclusion. Note the numerous vesicles (arrow head) surrounding the yolk body. (B) Vesicle containing fibrous material in the cytoplasm of the oocyte. (C) Vacuoles showing bacteria in different digestion stages (db) and numerous vesicles (arrow heads) with a fine-grained content in the cytoplasm. (D) Glycogen rosettes (g), heterogeneous yolk inclusions (hv), and multiple vesicles (arrow heads) within the cytoplasm of the oocyte.

displayed great number of filiform microvilli with a small vesicle in the tip (Fig. 2C and D).

In early oocytes, vitellogenesis involved obvious changes in most of the ooplasm, except for the perinuclear space. The oocyte cytoplasm progressively filled with different types of inclusions: heterogeneous and homogeneous yolk bodies (Figs. 2C and 3A, respectively), small vesicles with fibrous content (Fig. 3B), and vesicles with bacteria in different stages of digestion (Fig. 3C and D). Bacteria contained in these vesicles have previously been endocytosed from the mesohyl of the sponge (Figs. 2D and 4A) and stored individually in small vesicles (Fig. 4B–D). Later, such small vesicles fused together and showed evident signs of bacteria digestion (Figs. 3C and D and 4A). The ooplasm also showed great number of small electron-clear vesicles (Fig. 3A, C and D), which were particularly abundant in the periphery of the oocyte (Fig. 2C and D). Glycogen rosettes located scattered within the ooplasm (Fig. 3D). Lipid droplets were scarce (not shown).

Oocyte maturation progressed synchronously within and between individuals during both years of study. During oocyte growth, both cytoplasm and nucleus increased in size. Mature oocytes became round, attaining approximately 120–150 μm (Fig. 1A and D). The nucleolated nucleus (15 μm in diameter) contained fine-grained chromatin and several chromatin masses (Fig. 5A and B). A narrow area (approx. 3 μm) with scarce yolk inclusions and highly vesiculated surrounded the nucleus (Fig. 5A and B). Multiple dictyosomes located within this perinuclear area, with the lamellae oriented parallel to the nuclear membrane and small vesicles detaching from their ends (Fig. 5B). Mitochondria were hard to see because of the high density of inclusions in the ooplasm, as well as endoplasmic reticulum and free ribosomes.

In mature oocytes, different types of yolk inclusions were observed in the ooplasm, presumably being correlative stages of yolk formation: (1) heterogeneous membrane-bound composites containing semi-digested bacteria (Fig. 5C–F), (2) large vesicles con-

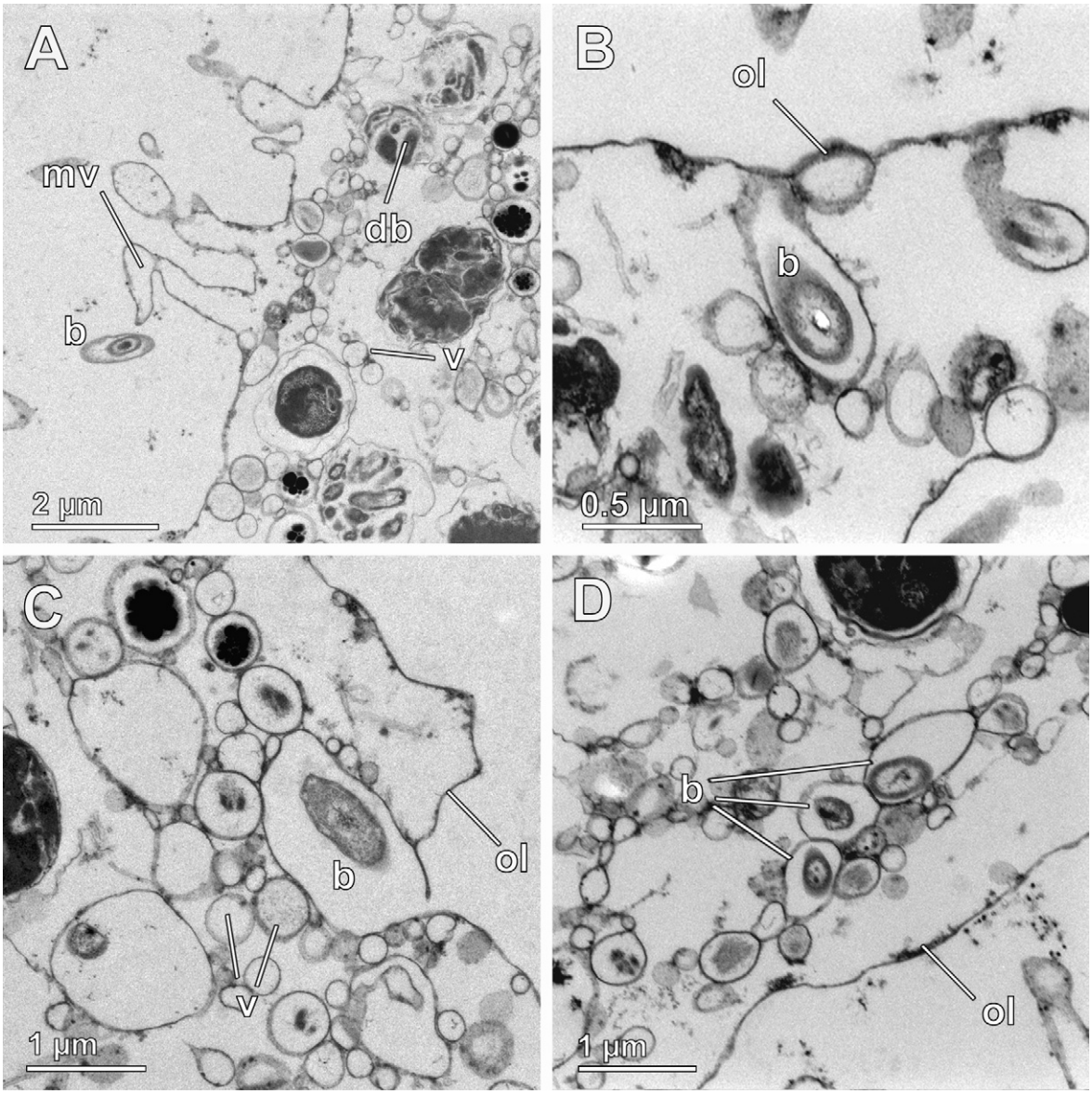

Fig. 4. Endocytosis of bacteria in *Axinella damicornis*. (A) Microvilli (mv) of the oolemma close to a free bacteria (b). The cytoplasm of the oocyte showed numerous microvesicles (v) and large vesicles with bacteria in different stages of digestion (db). (B and C) Bacteria (b) endocytosed by a mid-stage oocyte. Note the oolemma (ol) and the great number of microvesicles (v) in the periphery of the cytoplasm of the oocyte. (D) Small vesicles containing single bacteria (b) in the cytoplasm of an oocyte. Note the proximity to the oolemma (ol).

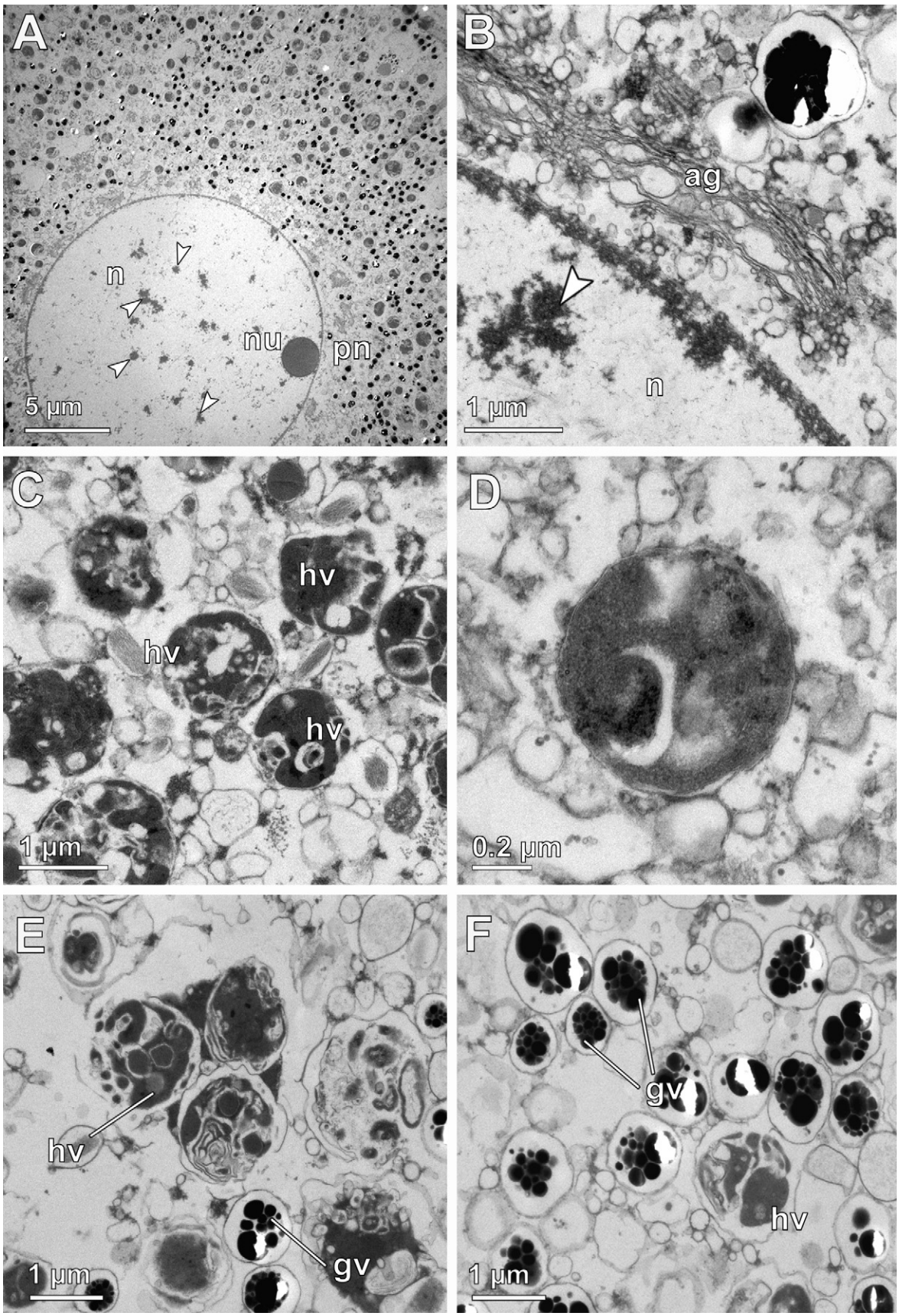

Fig. 5. Mature oocyte of *Axinella damicornis*. (A) View of the nucleolate (nu) nucleus (n) and the narrow perinuclear region (pn) devoid of inclusions. Note the chromatin masses (arrow heads) within the nucleus. (B) Close up of the nucleus (n) with chromatin masses (arrow head) and the Golgi apparatus (ag), with the lamellae orientated parallel to the nuclear membrane and numerous microvesicles detaching from its end. (C) Heterogeneous inclusions (hv) within the cytoplasm of the oocyte. (D) Close up of a heterogeneous yolk inclusion. (E and F) Vesicles of granular electron-dense yolk (gv) and heterogeneous inclusions (hv) in the cytoplasm of the oocyte.

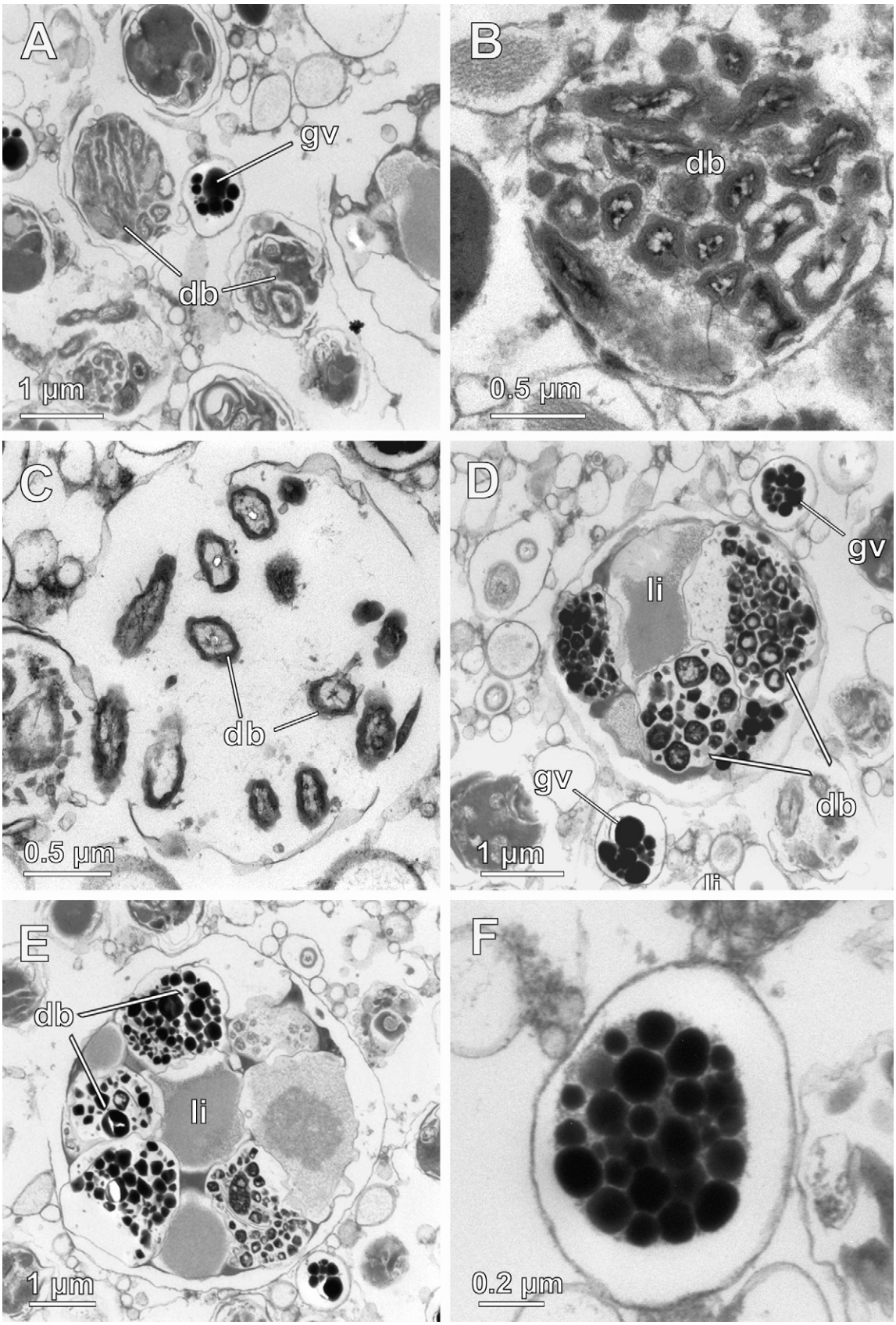

Fig. 6. Yolk elaboration in *Axinella damicornis*. (A) Periphery of the oocyte showing numerous vesicles with bacteria in different digestion stages (db) and granular yolk inclusions (gv). (B and C) Close up of large vesicles containing bacteria in digestion (db). (D and E) Intermediate stages of formation of granular yolk bodies (gv). Note the lipidic material (li) and the digested bacteria (db) within the same large vesicle. (F) Granular yolk inclusion within a small vesicle.

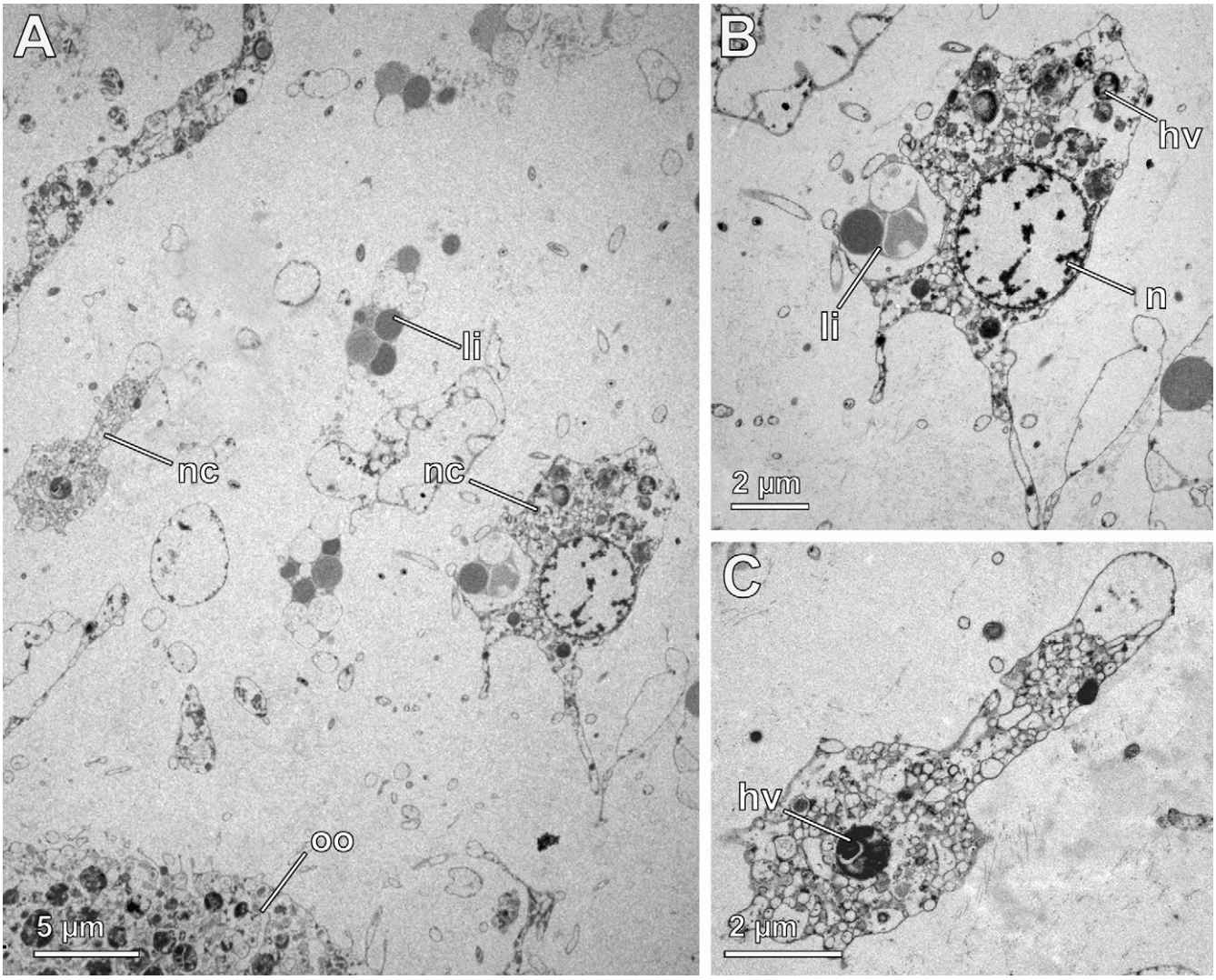

Fig. 7. Nurse cells in *Axinella damicornis*. (A) Nurse cells (nc) approaching an oocyte (oo). Note the numerous lipidic granules (li) scattered within the mesohyl in the vicinity of the oocyte. (B and C) High magnification of the nurse cells in (A). Note the large nucleus (n) and the heterogeneous inclusions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

taining semi-digested bacteria and lipidic material, which resulted from the fusion of several small ones (Fig. 6A–E), and 3) small vesicles containing coarse granular electron-dense yolk (Fig. 6A, D and F). Such granular yolk vesicles were comprised of 10–25 granules (Fig. 6F).

Scarce cells surrounded the oocyte during oogenesis (Fig. 7A). Their cytoplasm contained heterogeneous inclusions (Fig. 7B and C), which strongly resembled those of the oocyte. These cells showed a large anucleolate nucleus with chromatin condensations in the inner nuclear membrane (Fig. 7B). In addition, such cells appear to exocytose lipidic inclusions in the vicinity of the oocyte (Fig. 7A and B).

3.2. *Raspaciona aculeata*

In 2004, oocytes were found in the sponge tissue for 3 months (from August to October). However, in 2005 oogenesis occurred during 5 months, from July to November. Onset of oogenesis was synchronic within the studied population in both years. All females ($n = 3$) that produced oocytes in 2004 did it also in 2005. The smallest cells identified as oocytes were 25 μm in diameter. These young oocytes appeared scattered within the mesohyl (Fig. 1B and E), and strongly resembled to archaeocytes because of their similarities in morphology and affinity for stains. Young oocytes had an oval 5 μm-nucleus provided with a 2 μm-well developed nucleolus (Fig. 8A). Dictyosomes occurred adjacent to the external nuclear membrane, with cisternae oriented parallel to it (Fig. 8B). The remaining ooplasm contained large numbers of small electron-clear vesicles, scarce lipid droplets, and small groups of mitochondria (Fig. 8B–D). Vitellogenesis started at this early-stage (25 μm in diameter), and few small heterogeneous inclusions appeared within the ooplasm (Fig. 8C).

Oocyte development was highly synchronous within and between individuals in both years. Mid-stage oocytes (approximately 65 μm in diameter) emitted numerous pseudopodia (Figs 9A and 10C and D). Their ooplasm started filling with heterogeneous inclusions of complex nature (presumably lipidic and proteinaceous) from the nucleus to the periphery (Figs. 9A and 10A), but leaving a perinuclear region devoid of yolk bodies where large dictyosomes located (Fig. 9B–D). The nucleus measured approximately 25 μm, and the nucleolus increased up to 5 μm in diameter (Fig. 9B). Some chromatin masses were seen apparently attached to the inner nuclear membrane (Fig. 9C). Numerous pores were evident in the nuclear membrane (Fig. 9C and D). Mitochondria were abundant

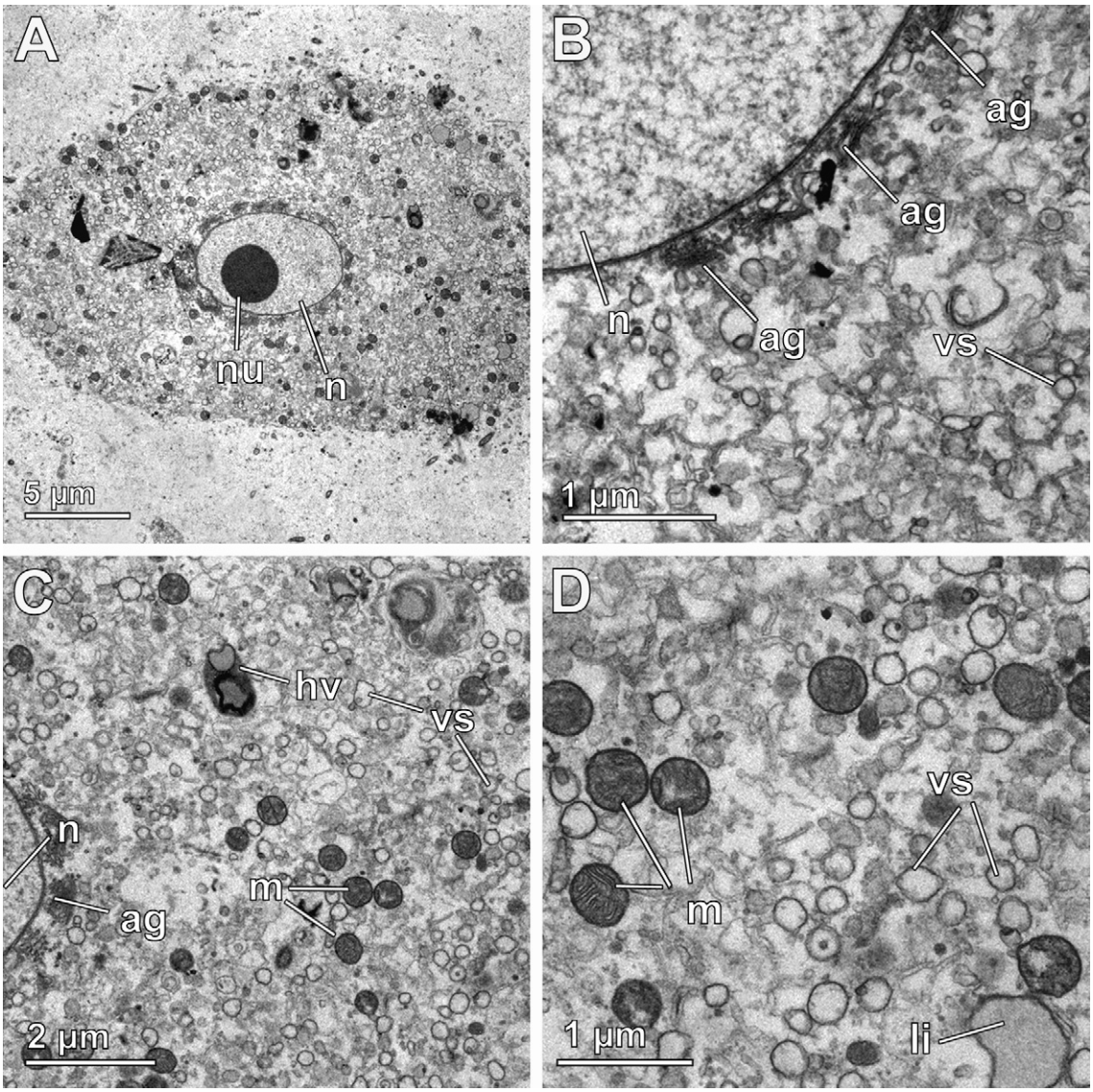

Fig. 8. Young oocyte of *Raspaciona aculeata*. (A) General view of a nucleolate (nu) oocyte. Note the oval shape of the nucleus (n). (B) Close up of the nucleus (n) and the Golgi apparatus (ag) close to the external nuclear membrane. Note the numerous electron-clear microvesicles (vs) found in the ooplasm. (C) General view of the ooplasm showing the nucleus (n), small dictyosomes located close to the nucleus (ag), mitochondrial clusters (m), electron-clear microvesicles (vs), and heterogeneous yolk inclusions in formation (hv). (D) Detail of the ooplasm containing small clusters of mitochondria (m), electron-clear microvesicles (vs), and lipid droplets (li).

through the entire ooplasm, usually in clusters of 15–20 organelles (Fig. 10B). The periphery of the oolemma showed numerous small electron-clear vesicles (Fig. 10C and D). At this stage, moderate amounts of collagen microfibrils (Fig. 10C) and free-living bacteria, were surrounding the oocytes.

Mature oocytes measured approximately 190 µm (Fig. 1F) and located homogenously within the entire mesohyl (Fig. 1B). Their nucleus was ovoid, measuring 30 µm in its largest diameter, with a 4–5 µm nucleolus (Fig. 11A). A 5 µm-wide area rich in small electron-clear vesicles and devoid of yolk bodies surrounded the nucleus (Fig. 11A and B) like in mid-stage oocytes. Larger dictyosomes than in previous stages occurred in the vicinity of the nucleus, most of them with their lamellae in parallel to the nuclear membrane (Fig. 11B). Large clusters of mitochondria were no longer found. Instead, groups of only 6–8 mitochondria occurred in the periphery of the ooplasm (Fig. 11D). Glycogen rosettes were common throughout the cytoplasm (Fig. 11E), as well as yolk inclusions with heterogeneous appearance (Fig. 11C, D, and F). Yolk inclusions with an electron-dense, homogeneous appearance also occurred, but less frequently (Fig. 11F). Both types of yolk inclusions appeared to correspond to different stages in the formation of proteinaceous yolk. Abundant membrane-bound lipid droplets were intermingled with yolk bodies, as well as additional membrane-bound granular inclusions (Fig. 11F). At this stage, few microvilli were produced by the oolemma (Fig. 11C).

We identified two potential types of nurse cells in the vicinity of growing oocytes during the entire oogenesis. Type I were amoeboid cells (Fig. 12A–B) that occurred in high numbers around the oocytes. They measured approximately 10 µm in their largest diameter and contained heterogeneous inclusions (Fig. 12A–D) similar to those within oocytes. As they approached the oolemma, they started to flatten against it (Fig. 12A and C). At those areas, numerous small electron-dense vesicles occurred in the perioocytic space (Fig. 12D). Type II cells were round, approximately 4 µm in diameter, and less numerous than type I cells (Fig. 12E). Type II nurse cells were charged with large vacuoles of granular content and lipid droplets, vaguely resembling spherulose cells. These cells embedded so deeply in the oocyte that the possibility that they

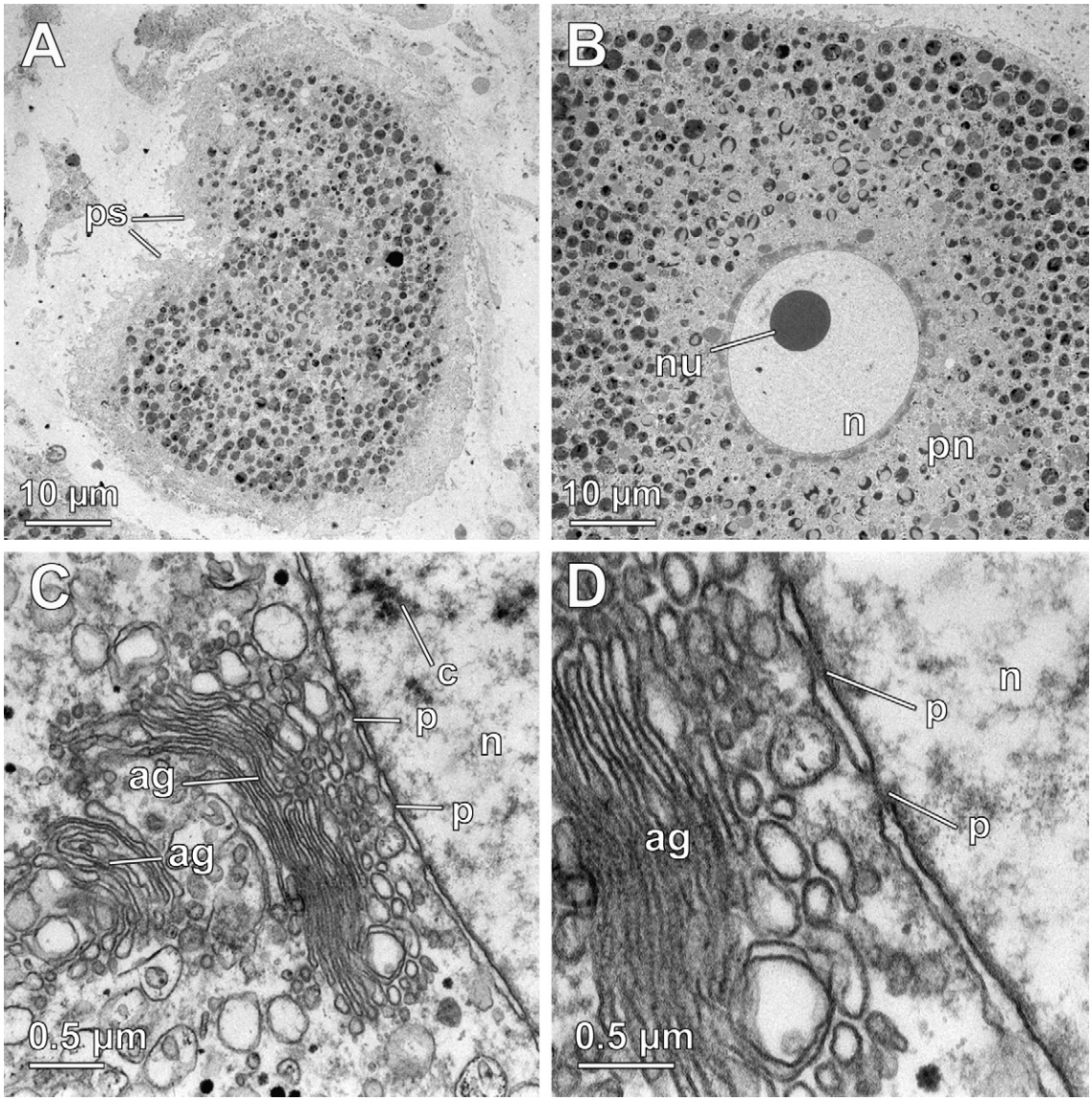

Fig. 9. Mid-stage oocyte of *Raspaciona aculeata*. (A) General view of a complete mid-stage oocyte showing the numerous pseudopodia (ps). (B) Mid-stage oocyte showing the nucleolate (nu) nucleus (n), and the perinuclear region (pn) devoid of inclusions. (C and D) Details of the nucleus (n) and the perinuclear region of a mid-stage oocyte, which contained numerous dictyosomes (ag). Note the nuclear pores (p) displayed by the nuclear membrane and the chromatin masses (c) inside the nucleus.

are phagocytosed entirely cannot be discounted (Fig. 12E). Nevertheless, we never found clear evidence that such phagocytosis takes place.

Occasionally, vesicles containing collagen microfibrils and other material of unknown nature were found in the periphery of the ooplasm of mature oocytes (Fig. 12F). Similar collagen microfibrils occurred in the mesohyl close to the oolemma (Fig. 12F). We have not enough evidence to conclude whether such collagen microfibrils were being secreted or endocytosed by the oocyte.

4. Discussion

Although our data are not conclusive, oocytes of both *Axinella damicornis* and *Raspaciona aculeata* appeared to derive from archaeocytes, because of their similarities in size, morphology, and affinity for stains. A similar origin of oocytes has been postulated for the majority of sponges (see Fell, 1983 and Simpson, 1984 for reviews). Oocytes of the 2 studied species were very similar in morphology during the course of the maturation process. Both oocytes were lobate at early stages, emitting numerous pseudopodia, and microvilli presumably involved in pinocytosis of dissolved compounds from the mesohyl, as previously described for many demosponges (e.g., Fincher, 1940; Diaz, 1979; Gaino et al., 1986; Gallissian and Vacelet, 1992; Sciscioli et al., 1991) and other invertebrates (Nørrevang, 1968). Nevertheless, microvilli were more numerous in *Axinella damicornis* than in *Raspaciona aculeata*. The cytoplasm of both oocytes contained glycogen rosettes, as well as homogeneous and heterogeneous yolk, which have also been described in many other demosponges (Borojevic, 1967; Fell, 1974; Diaz et al., 1975; Aisenstadt and Korotkova, 1976; Lévi and Lévi, 1976; Simpson, 1984; Gaino et al., 1986; Gaino and Sarà, 1994; Sciscioli et al., 1989; Lepore et al., 1995). In both studied species, the nucleus possessed a perinuclear region devoid of yolk inclusions, as shown by the oocytes of the demosponges *Suberites massa* (Diaz et al., 1975), *Tetilla serica* (Watanabe, 1978), *Stelletta grubii* (Sciscioli et al., 1991), and *Halichondria panicea* (Witte and Barthel, 1994).

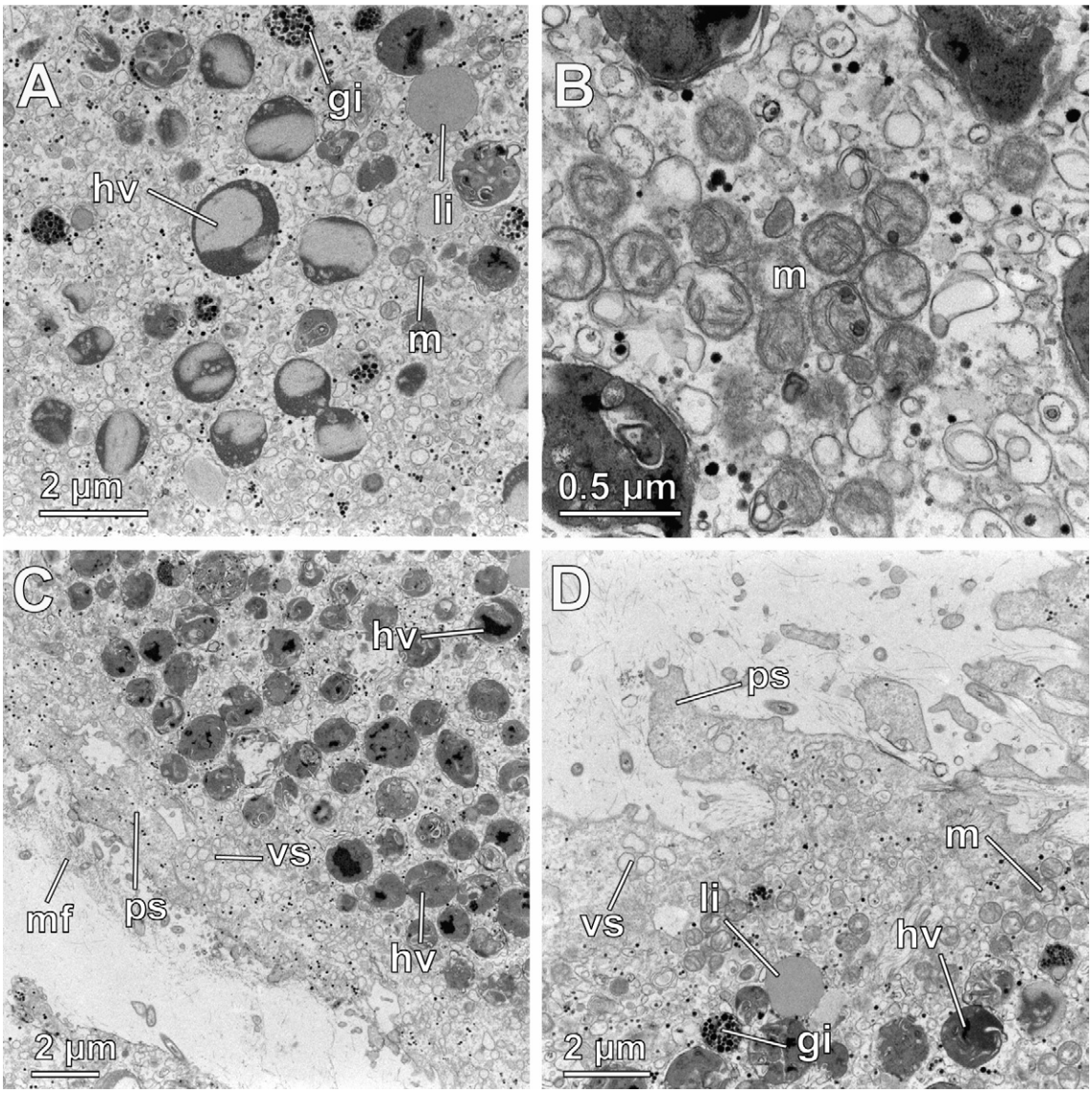

Fig. 10. Organelles and inclusions of the mid-stage oocyte of *Raspaciona aculeata*. (A) Detail of the region immediately close to the nucleus, showing early stages of yolk inclusions (hv), granular inclusions (gi), mitochondria (m), and lipid droplets (li). (B) Detail of a mitochondrial cluster (m). (C) Periphery of the ooplasm showing the pseudopodia (ps), electron-clear microvesicles (vs), and heterogeneous yolk (hv). Note the occurrence of collagen microfibrils (mf) in the vicinity of the oocyte. (D) Small pseudopodium (ps) emitted by a mid-stage oocyte towards the mesohyl. Note the clusters of mitochondria (m), lipid droplets (li), numerous microvesicles (vs), heterogeneous yolk (hv), and granular inclusions (gi) within the ooplasm.

Golgi apparatuses, usually involved in the synthesis and/or accumulation of yolk (Nørrevang, 1968), were very abundant in both studied species. Dictyosomes arranged their lamellae in parallel to the external nuclear membrane, as reported from many other sponge oocytes (Diaz, 1979; Sciscioli et al., 1991; Gallissian and Vacelet, 1992; Lepore et al., 1995). Dictyosomes of mature oocytes of *R. aculeata* were slightly larger than those of *A. damicornis*.

Major differences between the oogenesis of both studied species were basically restricted to the process of formation and accumulation of nutritional reserves. Oocytes of demosponges have been reported to acquire their reserves using autosynthesis with or without endocytosis and heterosynthesis with transfer of nutritive material by nurse cells and/or phagocytosis of complete nurse cells (Simpson, 1984; Sciscioli et al., 1991). Many authors have suggested that endocytosed microbes (bacteria and other symbiotic microbes) may be used to elaborate yolk by the oocytes (Gaino and Sarà, 1994; Sciscioli et al., 1989, 1991, 1994). Ultrastructural examinations of growing oocytes of *Axinella damicornis* allowed the corroboration that the granular electron-dense yolk was elaborated from endocytosed bacteria subsequently combined with other fine-grained granular material -presumably lipidic. Bacteria appeared to be endocytosed individually by the oocyte and stored in small vesicles, a mechanism widespread within demosponges (Diaz et al., 1975; Aisenstadt and Korotkova, 1976; Gallissian and Vacelet, 1976; Gaino et al., 1986, 1987; Sciscioli et al., 1989, 1991; Ereskovsky et al., 2005; Maldonado et al., 2005; Maldonado, 2007). Then, some of the vesicles fused to form a large, single one, containing approximately 15-20 bacteria that were progressively digested. Lipidic material was then incorporated and mixed with the subproduct of bacteria digestion to complete the formation of complex electron-dense granular yolk. Such complex yolk bodies, containing 10-25 yolk granules, have never been reported in other demosponges to date. These observations suggest that most yolk of *A. damicornis* is elaborated by the oocytes from the phagocytosed materials, being a process close to an autosynthetic vitellogenesis (Nørrevang, 1968; Anderson, 1974; Eckelbarger, 1994). Nevertheless, not all the yolk

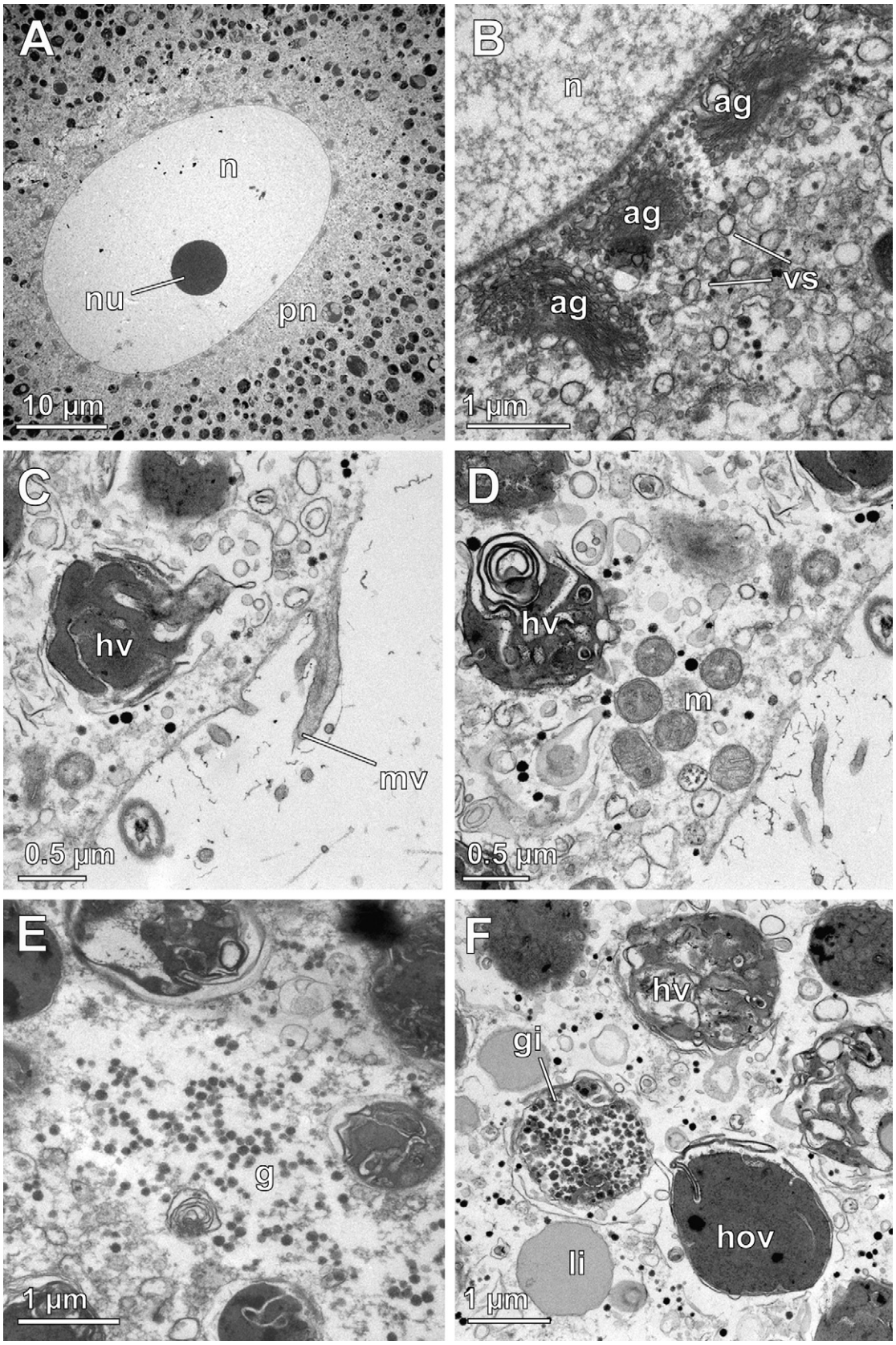

Fig. 11. Mature oocyte of *Raspaciona aculeata*. (A) View of the nucleolate (nu) nucleus (n) and the wide perinuclear region (pn). (B) Detail of the nucleus (n) and the large dictyosomes (ag) located in the perinuclear region, with the lamellae orientated parallel to the nuclear membrane. Note the abundant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

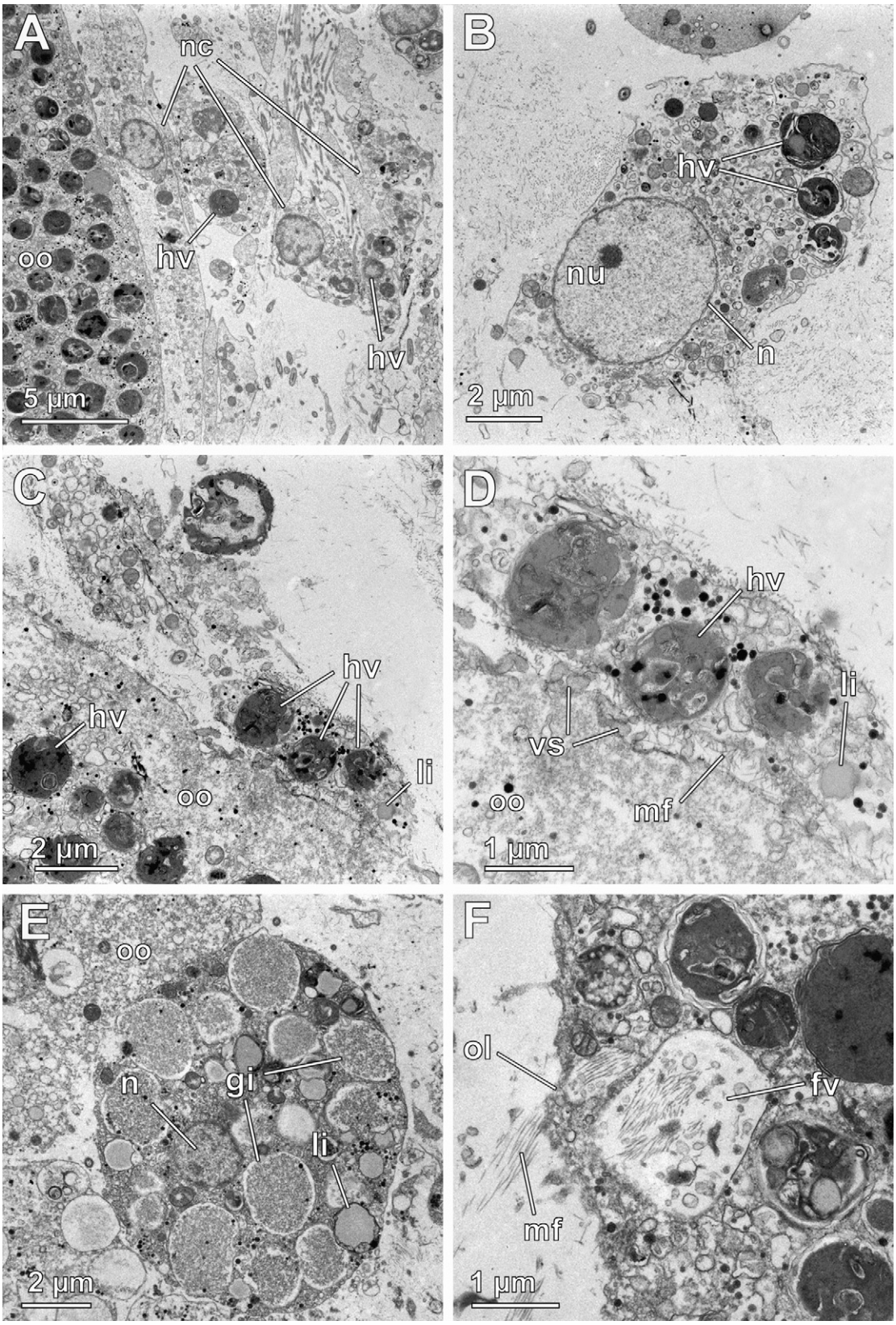

Fig. 12. Nurse cells and fibrilar vacuoles of *Raspaciona aculeata*. (A) Nurse cells (nc) containing heterogeneous yolk (hv) approaching a mid-stage oocyte (oo). (B) Type I of nurse cell in the surroundings of the oocyte. Note the nucleolate (nu) nucleus (n) and the heterogeneous yolk inclusions (hv). (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) and the collagen microfibrils (mf) occurring in the space between the oocyte (oo) 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) from that of type I nurse cells. (F) Vacuoles of fibrilar content (fv) close to the oolemma (ol). Note the similarity of the fibrilar content with mesohyl collagen (mf).

in the oocyte cytoplasm was granular yolk elaborated as described above. An additional autosynthetic mechanism of vitellogenesis cannot be discarded in the oocytes of *A. damicornis*, since we have found small vesicles with incipient yolk formation (Fig. 3 A, C, and D) that may be early-stages of the homogeneous yolk bodies (Fig. 3A). Furthermore, we found numerous small vesicles, presumably Golgi-derived, surrounding homogeneous yolk platelets, which in many other invertebrates is evidence indicating autosynthetic yolk formation (Nørrevang, 1968; Eckelbarger 1979; Ramírez Llodra, 2002). Although the autosynthesis of yolk appeared to be the predominant mechanism in *Axinella damicornis*, some yolk may have been elaborated by heterosynthesis. Few cells approached the developing oocytes. They were interpreted as "putative" nurse cells since they contained heterogeneous inclusions that appeared to be phagosomes involved in synthesis of proteinaceous and lipidic inclusions from unknown material. Furthermore, they appeared to release lipid droplets to the perioocytic space. Nevertheless, such cells cannot be unequivocally considered nurse cells, since many mesohyl cells contain heterogeneous inclusions and are not involved in oocyte nutrition. However, since they were very close to the oocyte and their inclusions were similar to those of the oocyte, our impression is that they are playing a similar role to that of conventional nurse cells. The mechanism of yolk elaboration in which nurse cells are involved, known as heteronomous yolk formation (Nørrevang, 1968) or heterosynthetic vitellogenesis (Anderson, 1974; Eckelbarger, 1994), has been previously reported in many demosponges (Tuzet and Pavans de Ceccaty, 1958; Fell, 1974, 1983; Simpson, 1984).

In the process of yolk elaboration of *Raspaciona aculeata* bacteria played no obvious role. Yolk was elaborated by both autosynthesis, which started in mid-stage oocytes as described in many other demosponges (e.g., Diaz et al., 1975; Gallissian and Vacelet, 1976; Watanabe, 1978) and heterosynthesis, i.e., transference from nurse cells. Incipient yolk granules located close to the perinuclear region, whereas completely formed yolk granules were found in the periphery of ooplasm. Such a gradual distribution may be related to the elaboration of yolk in the Golgi cisternae (e.g., Nørrevang, 1968) which were predominantly located in the perinuclear ooplasm. Additionally, numerous nurse cells of two types were involved in the intense transference of yolk precursors to the oocyte. Transference intensity was inferred from the great number of nurse cells that approached the oocytes and the numerous microvesicles that occurred in the space between the oocyte and the nurse cell. Whether these microvesicles resulted from exo- or endocytotic processes carried out by the oocyte cannot be unequivocally stated, although the latter option appears to be more likely. Such transference of material to the oocyte by nurse cells has previously been reported from other demosponges (e.g., Fell, 1969; Witte and Barthel, 1994; Lepore et al., 1995) and many other invertebrates (e.g., Eckelbarger, 1979; Blades-Eckelbarger and Youngbluth, 1984; Ramírez Llodra, 2002).

Differences between *Axinella damicornis* and *Raspaciona aculeata* in the involvement of nurse cells in yolk elaboration may account for the 5-month lapse in the extension of both oogeneses. Supply of abundant reserve material by nurse cells (heterosynthesis) usually allows a comparatively rapid completion of vitellogenesis and a fast egg production (Eckelbarger, 1994; Ramírez Llodra, 2002). Heterosynthetic vitellogenesis was the predominant mechanism used by *Raspaciona aculeata,* while a largely autosynthetic yolk elaboration with uptake of exogenous material (bacteria) resulted in a slower egg production in *Axinella damicornis*. Although vitellogenic mechanisms have often been considered useful traits to decipher the phylogeny in invertebrates (Eckelbarger, 1994), the reasons after the evidence of sponges having both types of mechanisms of yolk formation (i.e., autosynthetic and heterosynthetic) are not likely to be correlated with phylogenetic position, as suggested for other invertebrates (Eckelbarger and Larson, 1993).

Intracellular symbionts were not observed in the oocytes of the two selected species, although free intercellular microorganisms were abundant in the mesohyl of adults. All engulfed microorganisms were digested and catabolised. Therefore, we discard vertical transmission of microbes through the oocytes in both species. Vesicles of 2 μm in diameter, filled with fibrous material (that strongly resembled collagen microfibrils), were observed in the periphery of the oocyte of *Raspaciona aculeata*. We lack evidence to conclude whether such collagen is incorporated or secreted by the oocyte. Similar vesicles containing collagen have been reported in the oocytes of *Scypha ciliata* (Franzén, 1988), *Sycon ciliatum* (Gaino et al., 1987), *Stelletta grubii* (Sciscioli et al., 1991), and *Geodia cydonium* (Sciscioli et al., 1994). However, while in *Sycon* 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 (*formerly *Verongia) cavernicola* (Gallissian and Vacelet, 1976). The ooplasm of *Axinella damicornis* also showed vesicles containing fibril bundles of collagen similar to those reported for *Tethya seychellensis* (Gaino and Sarà, 1994). Nevertheless, since they did not appear in the periphery of the ooplasm in *A. damicornis*, they cannot be directly linked to exo- or endocytosis processes.

In summary, the oogenesis of both *Axinella damicornis* and *Raspaciona aculeata* follows the general pattern described in other demosponges (see Fell, 1974, 1983; Simpson, 1984 for reviews) and many other invertebrates (Nørrevang, 1968; Anderson, 1974; Adiyodi and Adiyodi, 1983). Nevertheless, both species also show distinct features regarding the formation of oocyte reserves, suggesting that some aspects of sponge oogenesis are specifically adapted.

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