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# Ultrastructural and Cytochemical Aspects of the Female Gonad of *Geoplana burmeisteri* (Platyhelminthes, Tricladida, Terricola)

**Alessandra Falleni,[1]* Paolo Lucchesi,[1] Claudio Ghezzani,[1] Marina Silveira,[2] and Vittorio Gremigni[1]**

[1]*Department of Human Morphology and Applied Biology, University of Pisa, 56126 Pisa, Italy*
[2]*Laboratory of Electron Microscopy, Institute of Physics, University of São Paulo, 05315-970 São Paulo, Brazil*

*ABSTRACT* The ultrastructure of the female gonad of the land planarian *Geoplana burmeisteri* was investigated by means of electron microscopy and cytochemical techniques. It consists of two small germaria located ventral to the intestine and of two irregular, lateral rows of vitelline follicles, both enveloped by a tunica composed of an extracellular lamina and an inner sheath of accessory cells. Accessory cell projections completely surround developing oocytes and vitellocytes. The main feature of oocyte maturation is the appearance of chromatoid bodies and the development of the rough endoplasmic reticulum (RER) and Golgi complexes. These organelles appear to be correlated with the production of egg inclusions of medium electron density, about 1.5–1.8 μm in diameter, which remain scattered in the ooplasm of mature oocytes. On the basis of cytochemical tests demonstrating their glycoprotein composition, these inclusions were interpreted as residual yolk globules. Vitellocytes are typical secretory cells with well-developed RER and Golgi complexes that are mainly involved in the production of yolk globules and eggshell globules, respectively. Eggshell globules appear to arise from repeated coalescence of small Golgi-derived vesicles and, at an intermediate stage of maturation, show a multigranular pattern. Later, after vesicle fusion, they reach a diameter of 1.3–1.6 μm when completely mature and show a meandering/concentric pattern, as is typical of the situation seen in most Proseriata and Tricladida. The content of yolk globules is completely digested by pronase, while the content of eggshell globules is unaffected. Mature vitellocytes contain, in addition, a large quantity of glycogen and lipid droplets as further reserve material. On the basis of the ultrastructural characteristics of the female gonad described above and in relation to the current literature, we conclude that *G. burmeisteri* appears to be more closely related to the freshwater triclads, in particular to members of the Dugesiidae, than to the marine triclads. J. Morphol. 267:318–332, 2006.


KEY WORDS: female gonad; oocyte; vitellocyte; accessory cell; Platyhelminthes-Tricladida

Platyhelminthes are free-living, symbiotic, and parasitic flatworms that have long been regarded as the stem species from which more evolved forms with bilateral symmetry arose and, therefore, a key group in the study of metazoan evolution (Baguñà, 2001).

Platyhelminthes "Turbellaria" were subdivided by Karling (1940) into two levels of organization ("Stadiengruppen") according to the structure of the female gonad. The lower level, the Archoophora, have homocellular female gonads consisting of only germaria with entolecithal eggs. The higher level, the Neoophora, possess heterocellular female gonads composed of both germaria, usually with alecithal eggs and vitellaria (vitelline glands) with vitellocytes producing the polyphenolic cocoon shell precursors (shell-forming globules or eggshell globules) and the nutritive substances for the developing embryo. Vitellocytes are released from the vitelline follicles, pass through the vitelloduct, and are enclosed in the cocoon together with one or more fertilized eggs.

Ultrastructural investigations on the female gonad of Platyhelminthes have provided useful information on the reproductive biology and phylogeny of the group (Gremigni and Falleni, 1991). In particular, the fine structure and composition of shell-forming globules in vitellocytes and of peripheral granules in oocytes, as well as the presence or lack of nutritive materials in the ooplasm, have been proven to be a suitable feature for the understanding of phylogenetic relationships in Platyhelminthes (Gremigni, 1988; Sopott-Ehlers, 1997; Gremigni and Falleni, 1998). As far as Tricladida is concerned, even though several studies on the germaria of freshwater and marine triclads are available to date, much less is known about the vitellaria (Rieger et al., 1991). Triclads are neoophoran Platyhelminthes subdivided into three suborders, Maricola, Paludicola, and Terricola, corresponding to the marine, freshwater, and terrestrial planarians, respectively

*Correspondence to: Dr. Alessandra Falleni, Dipartimento di Morfologia Umana e Biologia Applicata, Sezione di Biologia e Genetica, Via A. Volta 4, 56126 Pisa, Italy. E-mail: afalleni@biomed.unipi.it

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(Hallez, 1890; Sluys, 1989). Earlier ultrastructural investigations have shown that in the freshwater triclads belonging to the Dugesiidae family, oocytes produce, by an autosynthetic mechanism, a small quantity of glycoprotein inclusions that have been interpreted as residual yolk (Gremigni, 1969a, 1979). By contrast, the oocytes of the other two families of freshwater triclads (Planariidae and Dendrocoelidae) are devoid of yolk globules and are provided with small granules forming a monolayer in the cortical ooplasm (Gremigni, 1969b, 1979). In the marine species studied to date, the oocytes of *Cercyra hastata* and *Sabussowia dioica* (Cercyridae) both show aggregates of ribonucleoproteins, which have been interpreted as a special kind of yolk, and small cortical granules (Gremigni and Nigro, 1983; Tekaya et al., 1999), while those of *Procerodes dohrni* (Procerodidae) are provided with cortical granules and lack yolk (Gremigni et al., 1986; Gremigni, 1988). The vitellocytes of freshwater triclads are filled with both reserve material (yolk globules, lipids, and glycogen) and cocoon-shell globules with a concentric or convoluted-like pattern of the content that reacts positively to the cytochemical test for polyphenols (Domenici and Gremigni, 1974). A similar content pattern has been observed in the marine triclads *P. dohrni* (Gremigni, 1988) and *S. dioica* (Tekaya et al., 1998), while the other marine triclad studied to date, *C. hastata*, has eggshell globules with a homogeneous content pattern (Gremigni, 1988). To date, no ultrastructural investigation has been performed on the female gonad of land planarians. The present study aims to investigate the germaria and vitellaria of the terrestrial triclad *Geoplana burmeisteri* (Geoplanidae) by means of conventional and cytochemical TEM techniques focusing on the genesis, structure, and composition of oocyte and vitellocyte inclusions.

## MATERIALS AND METHODS

Specimens of *Geoplana burmeisteri* Schultze and Müller, 1857 were collected at the University of São Paulo campus, São Paulo, Brazil, under boards, leaves, and flagstones. The worms were fixed overnight with 2.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.2, at 4°C. Specimens were then cut into small segments, postfixed in 1% osmium tetroxide in the same buffer for 2 h at room temperature, dehydrated in an ethanol series, and embedded in Epon-Araldite. Ultrathin sections, obtained with a Reichert-Jung Ultracut E equipped with a diamond knife, were stained with aqueous uranyl acetate and lead citrate and examined with a Jeol 100 SX electron microscope.

### Cytochemical Tests

**Enzymatic protein extraction.** Ultrathin sections were treated with 2% $H_2O_2$ at room temperature for 10 min, rinsed in distilled water, then incubated for 3–12 h at 37°C in 0.5% protease (pronase E, Sigma, St. Louis, MO) solution adjusted to pH 7.5. Ultrathin sections were stained with uranyl acetate and lead citrate.

**Test for polysaccharides and glycoproteins.** The Thiéry method (1967) was used on ultrathin sections obtained from blocks used for morphological studies. Ultrathin sections were treated with 1% periodic acid for 30 min, incubated with 0.2% thiocarbohydrazide (TCH) in 20% acetic acid for 8–72 h, and treated with 1% silver proteinate in the dark for 30 min at room temperature.

## RESULTS

The female gonad of *Geoplana burmeisteri* is composed of two small germaria located ventral to the intestine, in the anterior, pre-pharyngeal region of the animal, and of paired vitellaria consisting of two irregular rows of oval follicles distributed along the lateral margins of the animal's body from the anterior end, behind the ovaries, to the caudal end.

### Germarium Morphology

The ovaries of *Geoplana burmeisteri* consist of two pyriform or oval-shaped organs measuring about 80 × 180 μm in diameter (Fig. 1A). Each ovary is enveloped by a tunica consisting of a thin extracellular lamina, about 60 nm thick, composed of fibrogranular material of medium electron density and a sheath of accessory cells (Fig. 1B). It contains germ cells at different stages of maturation (Fig. 1C). Oogonia and early oocytes lie at the periphery of the gonad, adjacent to the extracellular lamina, while developing and larger, presumably mature, oocytes occupy the growth area of the gonad close to the oviduct opening.

**Accessory cells.** Accessory cells are found both under the extracellular lamina, at the periphery of the gonad (Fig. 1B), where they often form long cytoplasmic processes (Fig. 2A,B), and between oocytes (Fig. 2B). The irregularly shaped nucleus is located in the space between two or three oocytes and displays scattered clumps of chromatin mainly adjacent to the inner nuclear envelope and a prominent nucleolus (Fig. 2B). The cytoplasm contains free ribosomes, cisternae of rough endoplasmic reticulum (RER), a few Golgi complexes, some irregular electron-dense lysosome-like bodies, lipid droplets, and glycogen particles (Fig. 2C). Neither intercellular bridges nor specialized junctional complexes have been observed between oocytes and accessory cells or between adjacent accessory cells.

**Oocyte differentiation.** Oogonia and early oocytes are ovoid, irregularly shaped cells measuring about 6 × 11 μm in diameter. Their large nuclei contain small patches of dense heterochromatin and one or two eccentrically located nucleoli with intermingled fibrillar and granular components (Fig. 3A,B). Some synaptonemal complexes indicating the zygotene/pachytene stage of the first meiotic division are occasionally observed in the nucleoplasm (Fig. 3C). The narrow and scarcely differentiated cytoplasm is packed with free ribosomes and contains some mitochondria with poorly developed cristae and short, principally individual cisternae of ER (Fig. 3A,B). Small aggregates of finely granular

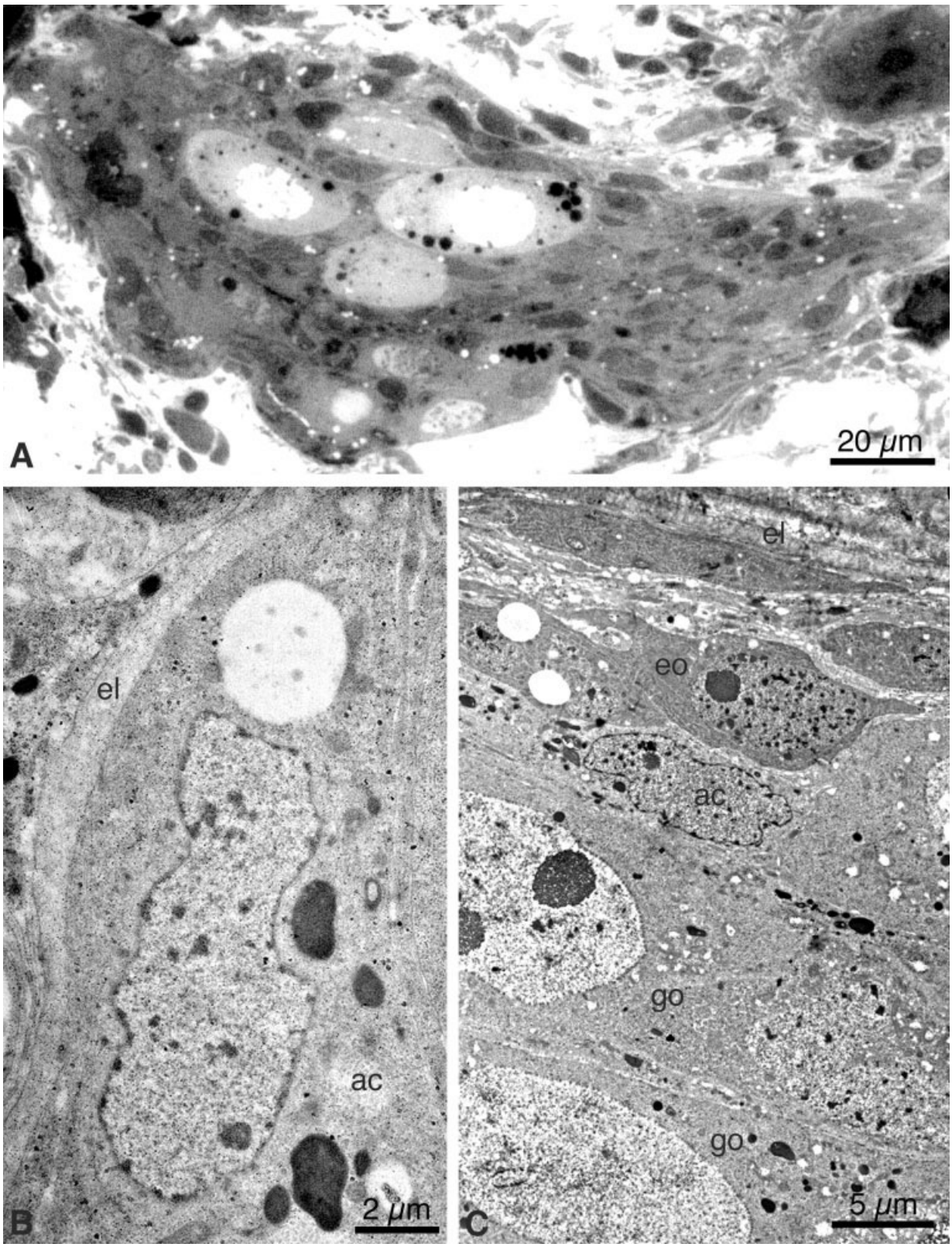


Fig. 1. *Geoplana burmeisteri*. **A:** An ovary showing oocytes at different stages of maturation. Larger oocytes are localized in the central part of the gonad. LM. **B:** Outer portion of the germarium showing the extracellular lamina (el) and an underlying accessory cell (ac). TEM. **C:** Portion of the germarium showing an early oocytes (eo) in the outer part and growing oocytes (go) in the inner part. TEM. ac, accessory cell; el, extracellular lamina.

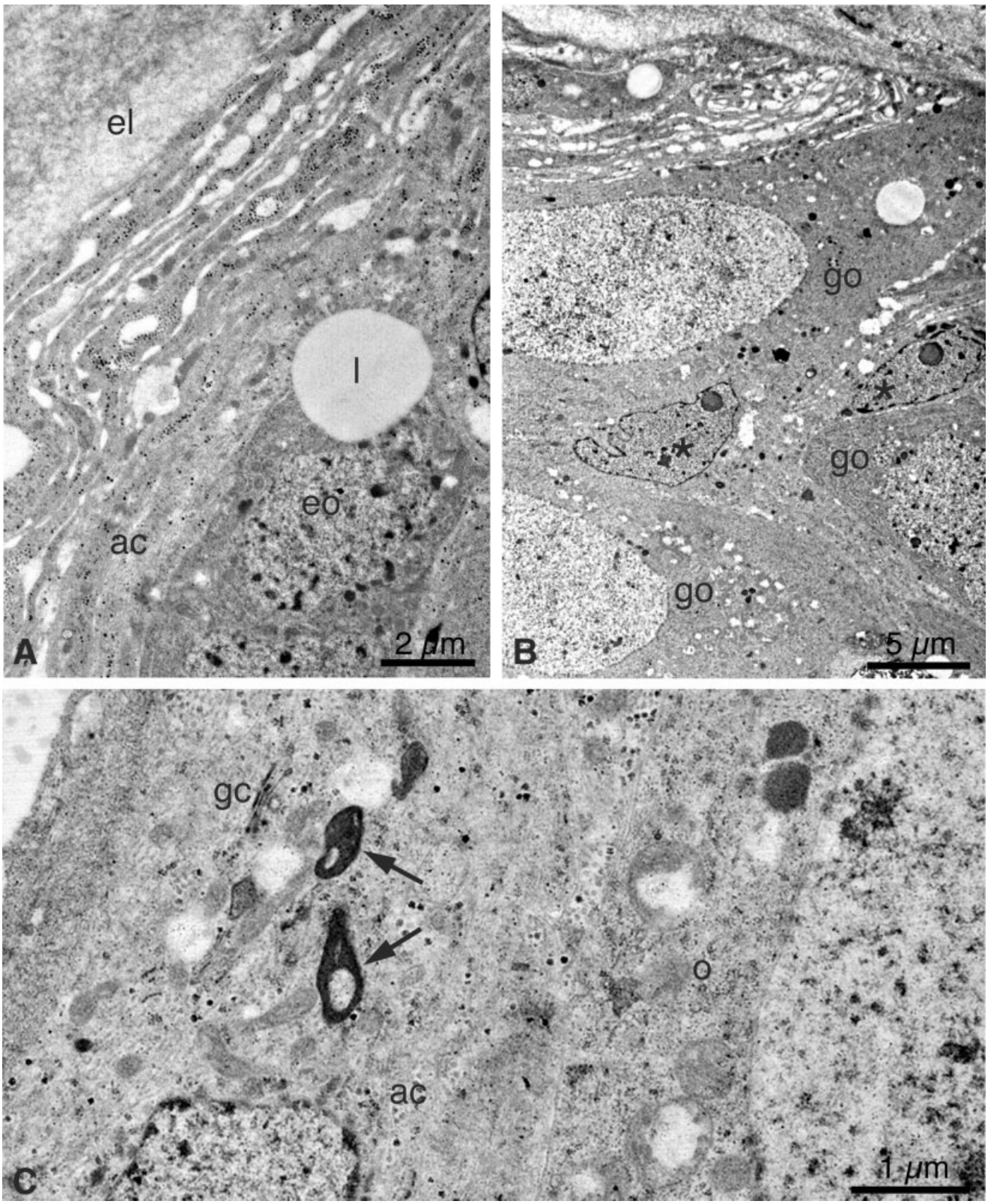


Fig. 2. *Geoplana burmeisteri*. **A:** Long cytoplasmic processes of the accessory cells (ac) containing mitochondria and rich in glycogen are visible under the extracellular lamina (el) at the periphery of the germarium. TEM. eo, early oocyte; l, lipid droplet. **B:** Accessory cell nuclei (*) are present between growing oocytes (go). TEM. **C:** Accessory cell cytoplasm with Golgi complexes (gc), electron-dense inclusions (arrow), and glycogen particles. TEM. ac, accessory cell; o, oocyte.

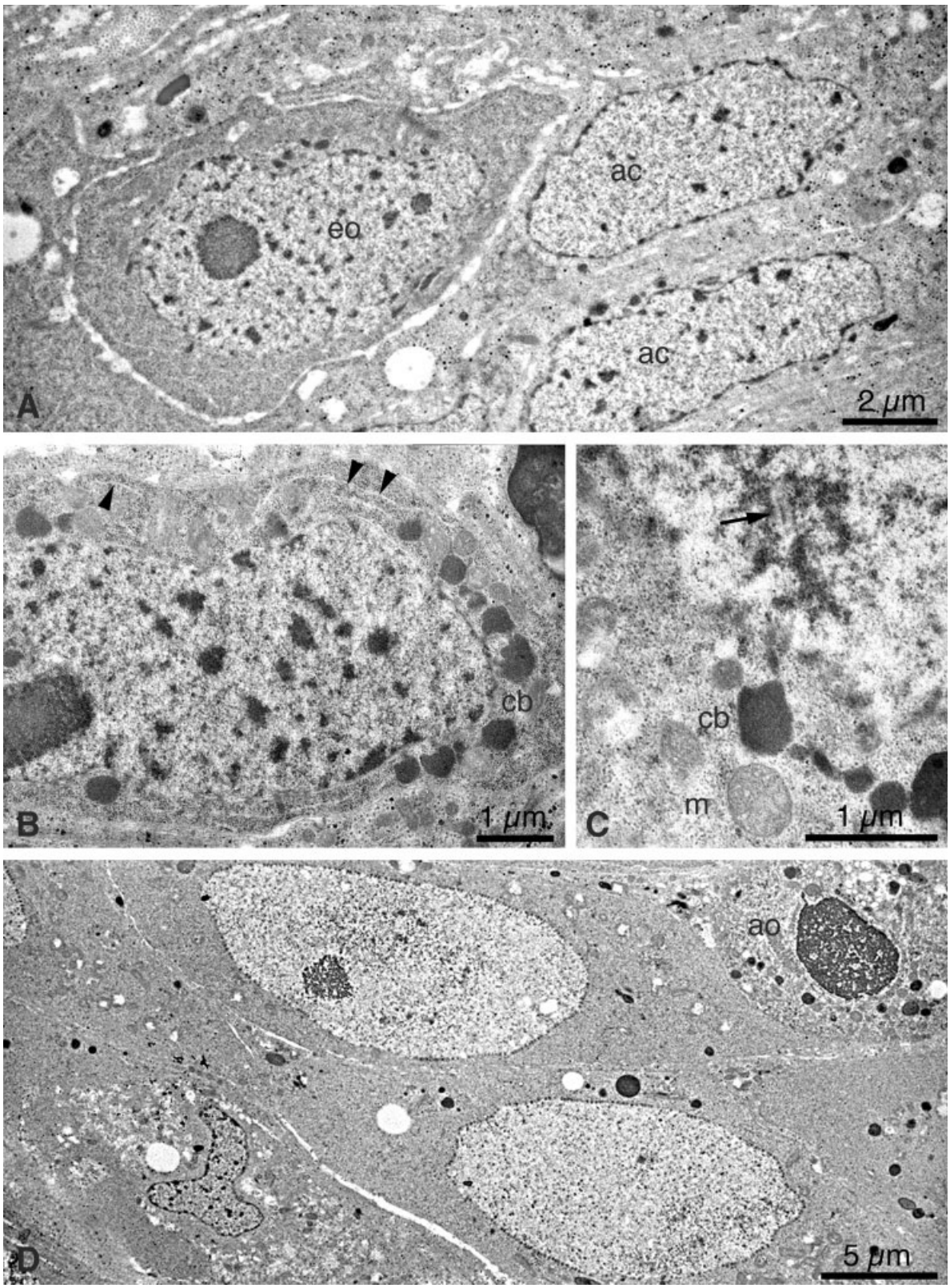


Fig. 3. *Geoplana burmeisteri*. **A:** Early oocyte (eo) showing a large nucleus with small patches of condensed chromatin and a prominent nucleolus. The narrow cytoplasm is rich in ribosomes. TEM. ac, accessory cell. **B:** Early oocyte with numerous chromatoid bodies (cb) close to the nuclear envelope and some elongated cisternae of RER (arrowhead). TEM. **C:** A synaptonemal complex (arrow) with the characteristic tripartite structure. TEM. cb; chromatoid body; m, mitochondrion. **D:** Detail of the growth area of the ovary showing oocytes with a nucleus containing diffuse chromatin and a cytoplasm with different types of inclusions. An apoptotic oocyte (ao) with very condensed chromatin and shrunken cytoplasm is visible in the upper right corner. TEM.

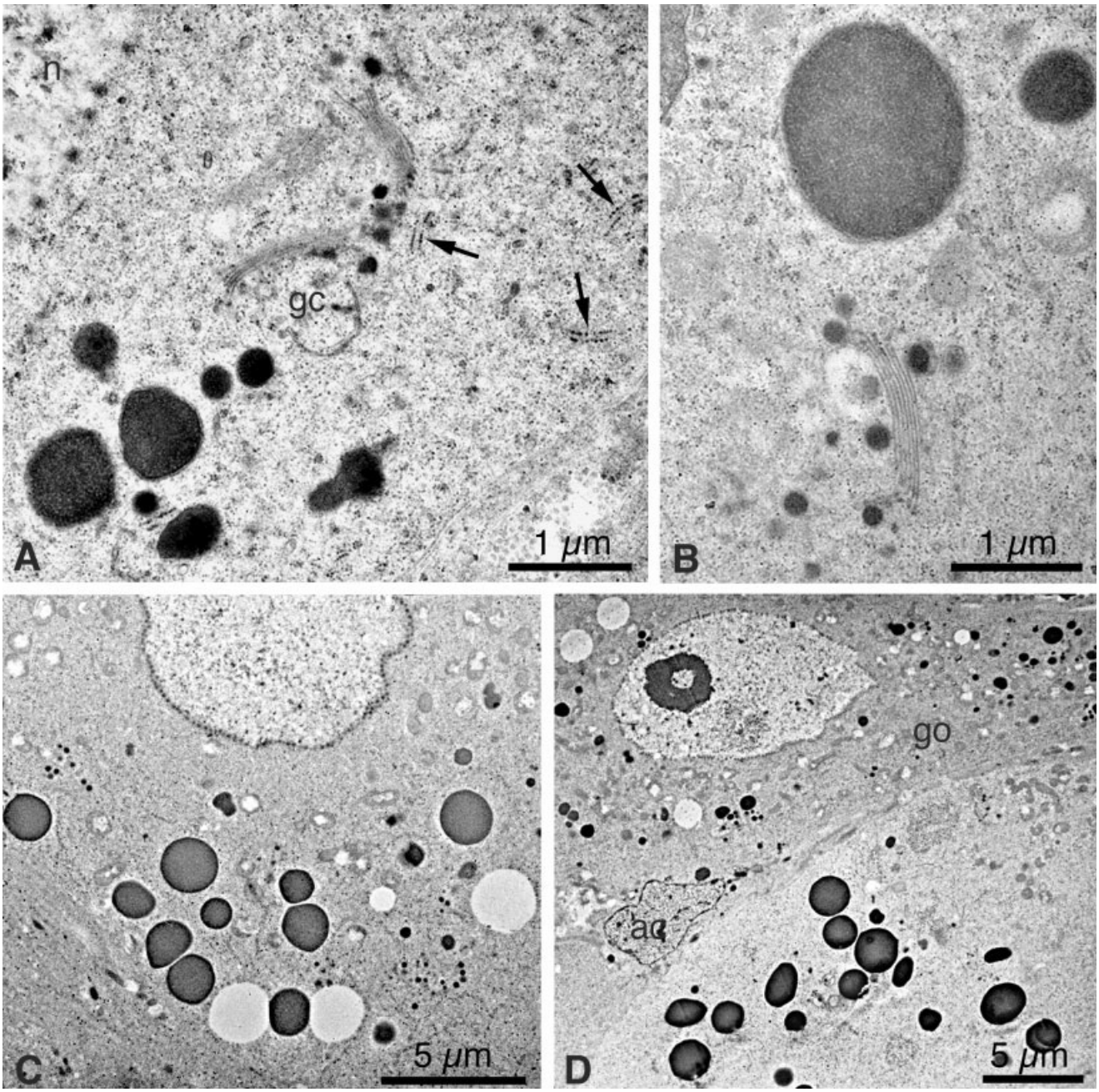


Fig. 4. *Geoplana burmeisteri*. **A:** Perinuclear cytoplasm of a growing oocyte with Golgi complexes (gc) and short cisternae of RER (arrow). TEM. n, nucleus. **B:** A Golgi complex and a mature egg globule with a finely granular content of medium electron density delimited by a smooth membrane. TEM. **C:** Growing oocyte at an intermediate stage of maturation showing scattered egg globules and lipid droplets. TEM. **D:** Growth area of the ovary. Portion of a mature oocyte containing numerous dense globules is visible in the lower part of the micrograph. TEM. ac, accessory cell; go, growing oocyte.

electron-dense material devoid of a limiting membrane (chromatoid bodies) are observed in the perinuclear region (Fig. 3B,C).

Growing oocytes at an intermediate stage of maturation are elongated in shape with a large nucleus (up to 15–18 μm in diameter) having mainly diffuse chromatin and a nuclear envelope very rich in pores (Fig. 3D). Moreover, some apoptotic oocytes are visible among growing oocytes (Fig. 3D). Cytoplasmic differentiation is characterized by the development of RER profiles, Golgi complexes (Fig. 4A), and the appearance of lipid droplets. The Golgi complexes are involved in the production of small vesicles containing an electron-dense material (Fig. 4A,B). Repeated coalescence of these vesicles give rise to larger membrane-bound inclusions with a homoge-

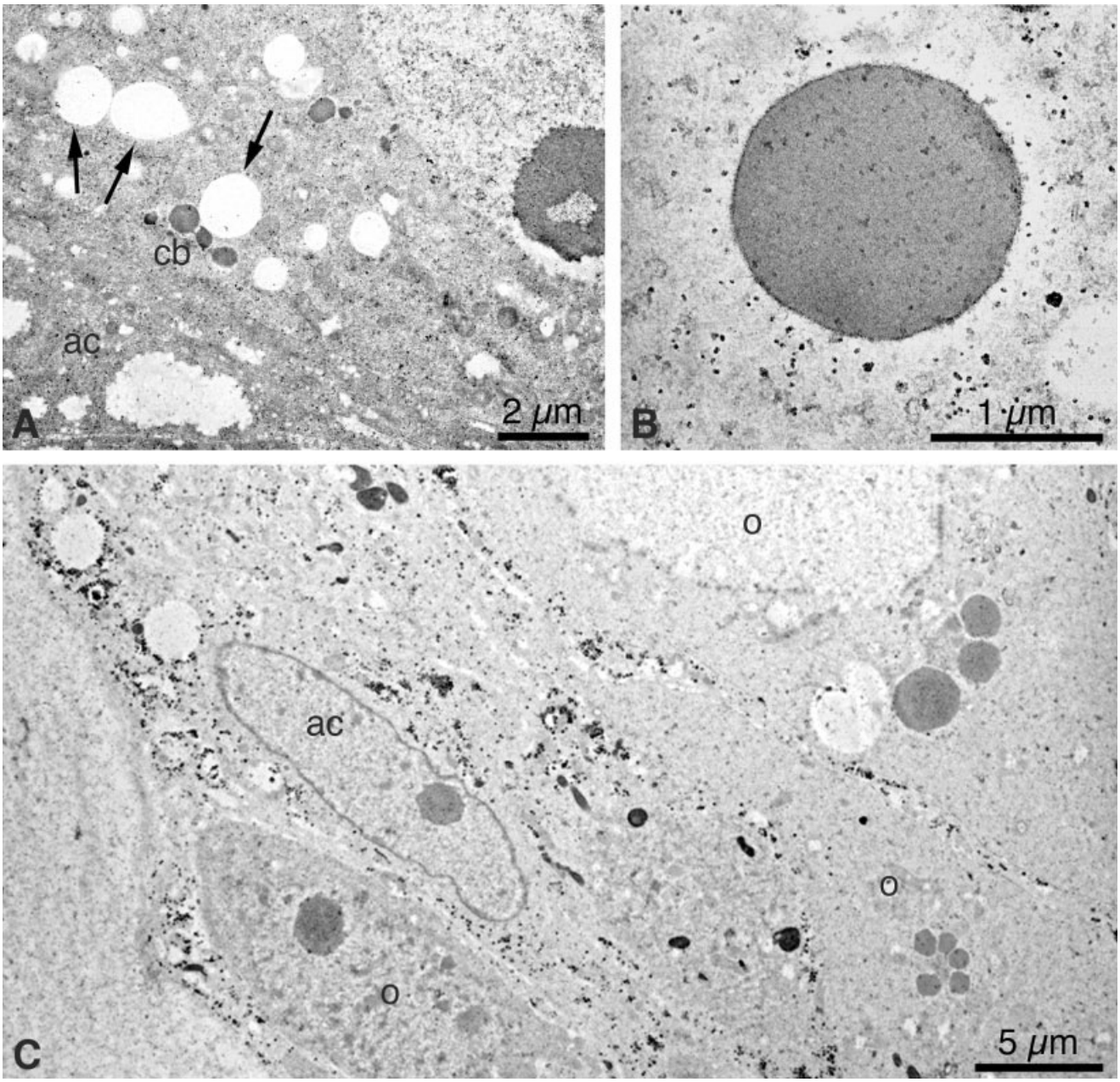


Fig. 5. *Geoplana burmeisteri*. **A:** Protease extraction. The content of the egg globules (arrow) is completely digested. TEM. ac, accessory cell; cb, chromatoid body. **B:** Thiéry test, 72-h incubation in thiocarbohydrazide (TCH), unstained section. A fine silver precipitate is visible on the content of the egg globule as well as on randomly distributed glycogen particles in the ooplasm. TEM. **C:** Thiéry test, 8-h incubation in TCH, unstained section. A strong silver precipitate is visible on glycogen particles in the accessory cell cytoplasm (ac). TEM. o, oocyte.

neous and/or finely granular material of medium electron density, delimited by a smooth membrane (Fig. 4B). When completely mature, they are round in shape, measure about 1.5–1.8 μm in diameter, and are dispersed throughout the ooplasm (Fig. 4C).

Mature oocytes are elongated and measure about 50–60 μm in maximum diameter; they show a decreased nucleo/cytoplasmic ratio due to a remarkable increase in volume of the ooplasm. The nucleus contains only diffuse chromatin and the nucleolus is ring-shaped or absent. The membrane-bound inclusions of medium electron density are increased in number and are still scattered throughout the ooplasm (Fig. 4D).

**Cytochemical tests.** The content of the round membrane-bound inclusions is completely digested by pronase, as are the granules of accessory cells (Fig. 5A). After the Thiéry test, a loosely dispersed fine silver precipitate is observed on the content of oocyte inclusions after 72 h of treatment in TCH

(Fig. 5B). In addition, a positive reaction is observed on glycogen particles in the accessory cell cytoplasm (Fig. 5C) and, to a lesser extent, in the ooplasm (Fig. 5B,C) after 8 h incubation in TCH.

### Vitellarium Morphology

Each vitelline follicle (about 50–80 μm in maximum diameter) contains vitelline cells at different stages of maturation and is enveloped by a tunica composed of a thin extracellular lamina and several accessory cells (Fig. 6A,B). Vitellocytes are distributed along a maturation axis from the distal germinative area to the proximal area of the follicle near the origin of the vitelloduct. The extracellular lamina is about 200–400 nm thick and appears moderately electron-dense.

**Accessory cells.** Accessory cells are distributed peripherally under the extracellular lamina (Fig. 6C) and their cytoplasmic projections surround early and growing vitellocytes (Fig. 6A,D). They have an irregularly shaped nucleus with scattered patches of chromatin (Fig. 6C). The cytoplasm contains free ribosomes, lipid droplets, glycogen, and two main types of inclusions. One type is larger and has a lysosome-like structure with a heterogeneous dense content; the other type of inclusion is smaller and has the same electron density as the extracellular lamina (Fig. 6E). These inclusions are observed close to or fusing with the plasma membrane (Fig. 6E,F).

**Vitellocyte differentiation.** Early vitellocytes are located adjacent to the extracellular lamina in the germinative area of the follicle (Fig. 6A,B). They are roundish in shape, measure about 10–12 μm in diameter, and have a high nucleo/cytoplasmic ratio and a smooth surface. The nucleus (about 8 μm in diameter) contains small clumps of heterochromatin and a well-developed excentrally located nucleolus. The narrow cytoplasm displays numerous free ribosomes and scattered mitochondria. Growing vitellocytes are located in the middle region of the follicle, are elongated in shape, and show small cytoplasmic protrusions facing the accessory cells (Fig. 7A). These protrusions increase in number and size to become well-developed and branched in larger growing vitellocytes (Fig. 7B). Vitellocyte differentiation is characterized by the development of ER profiles and the appearance of Golgi areas and lipid droplets (Fig. 7A). The Golgi complexes usually consist of a few short cisternae with enlarged ends filled with a dense material (Fig. 7C). The repeated coalescence of the Golgi-derived vesicles gives rise to large membrane-bound inclusions (forming eggshell globules) which, in early stages of maturation, show a multigranular electron-dense content with granules of different sizes. When completely mature, they have a round shape, measure 1.3–1.6 μm in diameter, and their contents show an irregularly ring-shaped/meandering pattern delimited by a smooth membrane (Fig. 7D).

Long cisternae of RER with enlarged ends filled with a material of medium electron density are often found close to nascent yolk globules (Fig. 8A,B). Mature yolk globules have a roundish shape, measure about 2.5–3 μm in diameter, and show a homogeneous content of medium electron density delimited by a smooth membrane (Fig. 8C).

Mature vitellocytes are elongated in shape and always show cytoplasmic protrusions limited to one side of the cells. They have an increased volume due to the accumulation of different types of inclusions and to the large amount of glycogen (Fig. 8D).

**Cytochemical tests.** The electron-dense content of eggshell globules is unaffected by pronase, while the content of yolk globules, as well as that of the accessory cell inclusions, is digested (Fig. 9A,B). A strong positive reaction to the Thiéry test is observed on the glycogen lacunae in the vitellocyte cytoplasm after 8 h of incubation in TCH (Fig. 9C).

## DISCUSSION

The heterocellular female gonad of *Geoplana burmeisteri* consists of well-separated germarian and vitellarian areas, both enveloped by a tunica composed of an outer extracellular lamina and an inner sheath formed by accessory cells. This finding (extracellular lamina + accessory cells) has been described for the first time in Tricladida and is similar to that observed in some Proseriata (Sopott-Ehlers, 1986, 1990, 1994, 1995), Rhabdocoela (Lucchesi et al., 1995; Sopott-Ehlers, 1997, Falleni et al., 1998, 2002), and in the parasitic Neodermata (Xylander, 1987; Cifrian et al., 1993; Martinez-Alos et al., 1993). A different situation has been described in the marine triclad *Sabussowia dioica*, where the extracellular lamina is absent and accessory cells are surrounded by flattened cells constituting a dense multilayered gonadal wall (Tekaya et al., 1999), and in some Lecithoepitheliata where the germovitellarium is surrounded solely by an extracellular lamina or by accessory cells (Falleni et al., 1995; Falleni, 1997).

In *Geoplana burmeisteri*, accessory cells have also been observed among growing germ cells, where they tightly encompass oocytes with their long cytoplasmic processes throughout oogenesis. This feature is similar to that observed in some Proseriata (Sopott-Ehlers, 1994, 1995) and in other Tricladida (Gremigni and Nigro, 1983; Tekaya et al., 1999), and differs from that reported in some rhabdocoelan Typhloplanida and Temnocephalida (Falleni and Lucchesi, 1992; Falleni et al., 1998), where accessory cells are only peripherally located in the germarium. As previously suggested, accessory cells could play a trophic role in transferring low molecular weight precursors from the surrounding tissue to the growing oocytes (Nigro and Gremigni, 1987; Falleni and Gremigni, 1992; Falleni et al., 2002). In addition, the presence of a large quantity of glycogen in the ac-

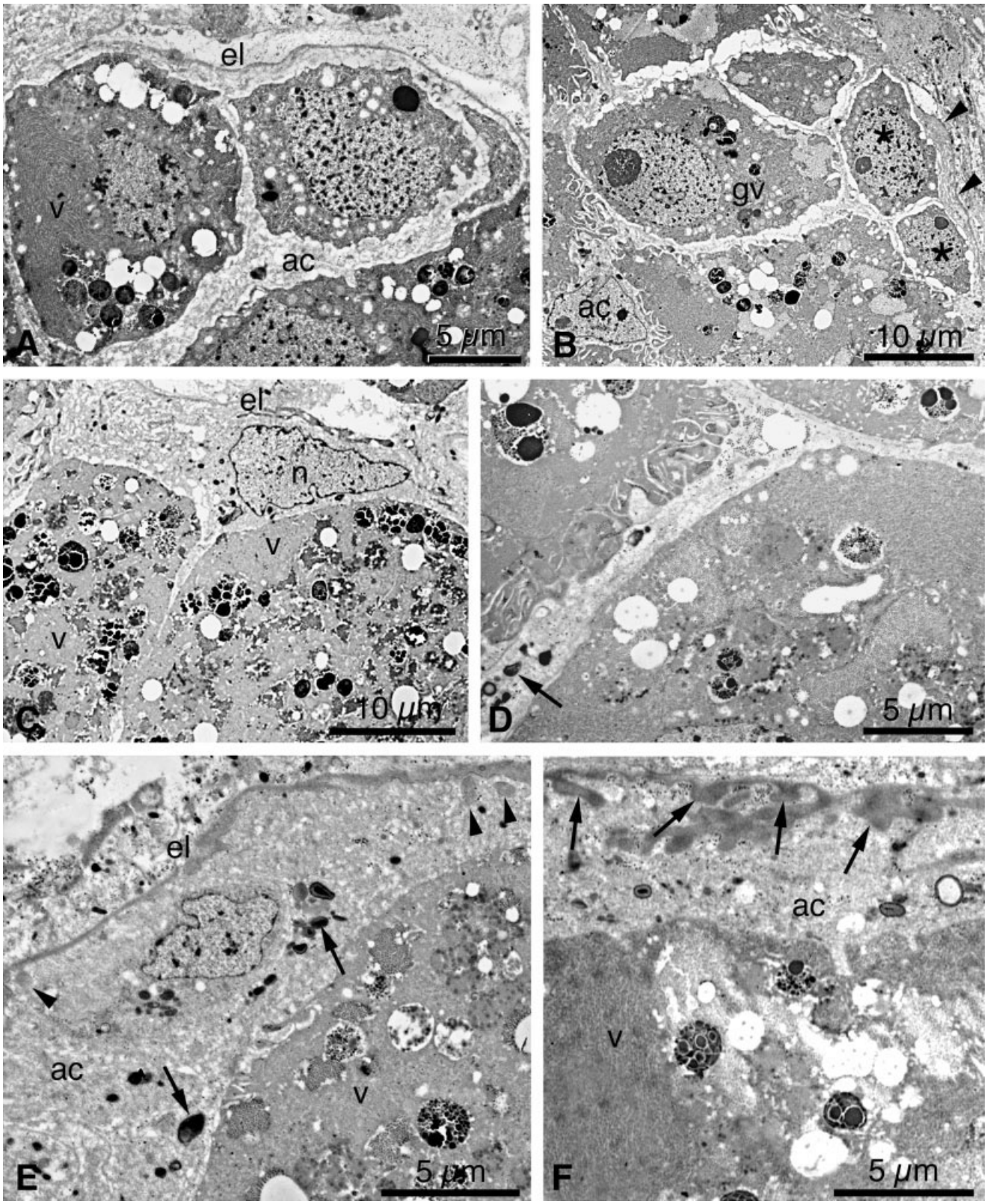


Fig. 6. *Geoplana burmeisteri*. **A:** Outer portion of a vitelline follicle delimited by a thin extracellular lamina (el). TEM. ac, accessory cell cytoplasm; v, developing vitellocytes. **B:** Portion of the germinative area of a vitelline follicle. Early vitellocytes (*) are localized at the periphery of the follicle close to the extracellular lamina (arrowhead) and show a large nucleus with clumps of condensed chromatin. TEM. ac, accessory cell; gv, growing vitellocyte. **C:** Outer portion of a vitelline follicle. The irregularly shaped nucleus (n) of an accessory cell is visible under the extracellular lamina (el). TEM. v, mature vitellocyte. **D:** Cytoplasmic projections of an accessory cell containing lysosome-like bodies (arrow), lipids, and glycogen are visible among three developing vitellocytes. TEM. **E:** Outer portion of a vitelline follicle. Two main types of inclusion are visible in the accessory cell cytoplasm (ac): dense lysosome-like bodies (arrow) and less dense inclusions (arrowhead) with the same electron density as the extracellular lamina (el). TEM. v, vitellocyte. **F:** Accessory cell inclusions of medium electron density in the process of fusing with the plasma membrane (arrow) and releasing their content in the extracellular space. TEM. ac, accessory cell; v, vitellocyte.

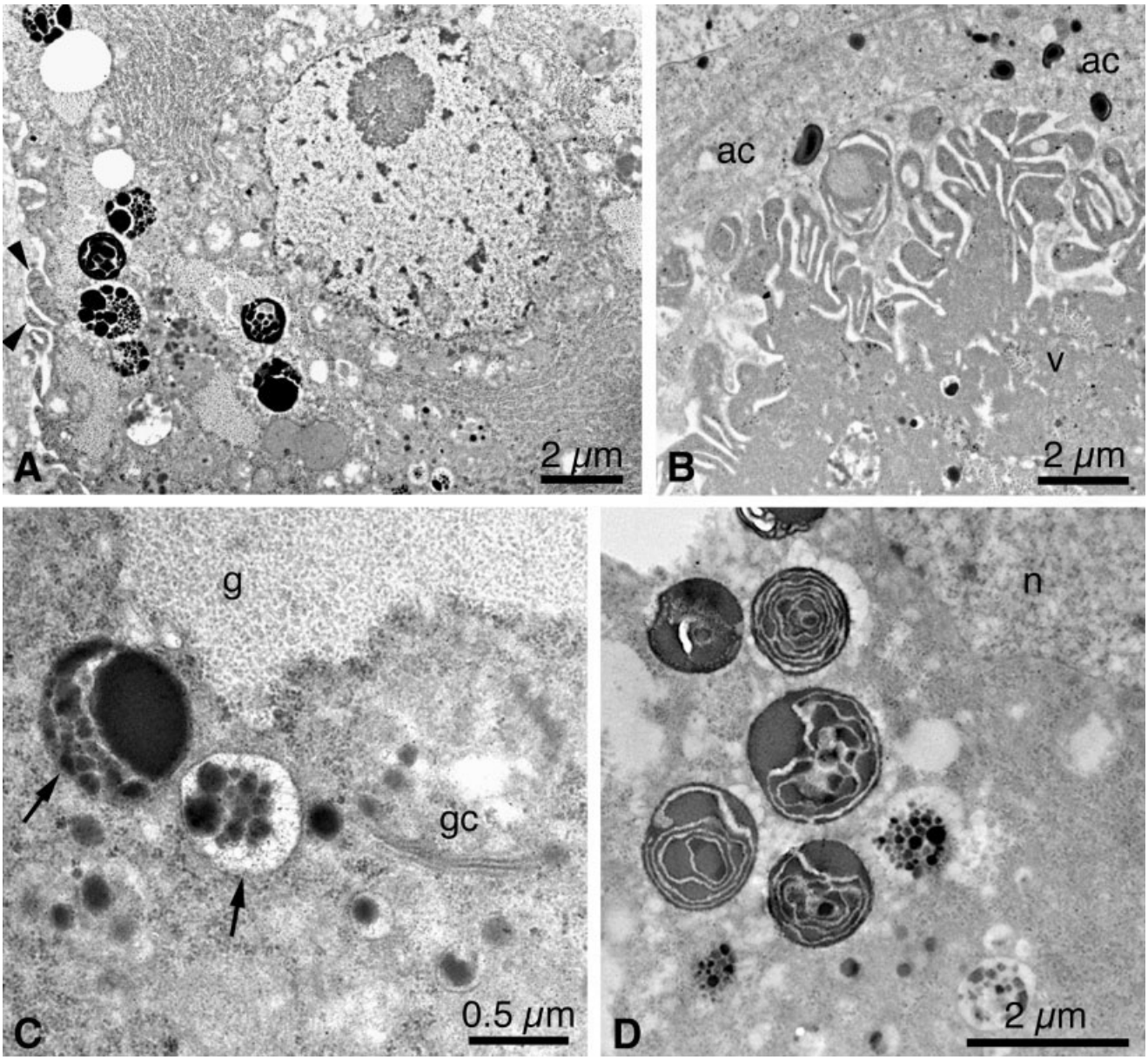


Fig. 7. *Geoplana burmeisteri*. **A:** Developing vitellocyte showing a nucleus with a well-developed nucleolus and a cytoplasm with extensive RER cisternae, Golgi areas and different types of inclusion. Note short cytoplasmic protrusions (arrowhead) at one side. TEM. **B:** Branched cytoplasmic protrusions facing an accessory cell (ac) in a growing vitellocyte (v). TEM. **C:** A Golgi complex (gc) and nascent eggshell globules (arrow) with a poligranular electron-dense content in a growing vitellocyte. TEM. g, glycogen lacunae. **D:** Mature eggshell globules with an irregularly ring-shaped pattern of the content. TEM. n, nucleus.

cessory cell cytoplasm of *G. burmeisteri* could represent further reserve material immediately available for the growing oocytes and presumably for the embryo during the early stages of development that precede the incorporation of vitelline cells. Moreover, in *G. burmeisteri* cytoplasmic projections of accessory cells also fill the space between developing vitellocytes in the vitellarium, as is typical in other neoophoran Platyhelminthes (Falleni et al., 1998), even though some differences have been observed. Indeed, in *G. burmeisteri* the accessory cell cytoplasm contains lysosome-like bodies, glycogen, and lipids, while in most platyhelminths it is generally scarcely differentiated. In addition, the increased surface area of vitellocytes through formation of cytoplasmic protrusions facing the accessory cells, observed for the first time in this species, suggests a trophic role also for the accessory cells of the vitellarium. A further function of these cells seems to be a contribution to the formation of the extracellular lamina through exocytosis, as is the case in some Rhabdocoela (Falleni et al., 1998, 2002).

Oocyte maturation occurs completely during the prophase of the first meiotic division, as shown by

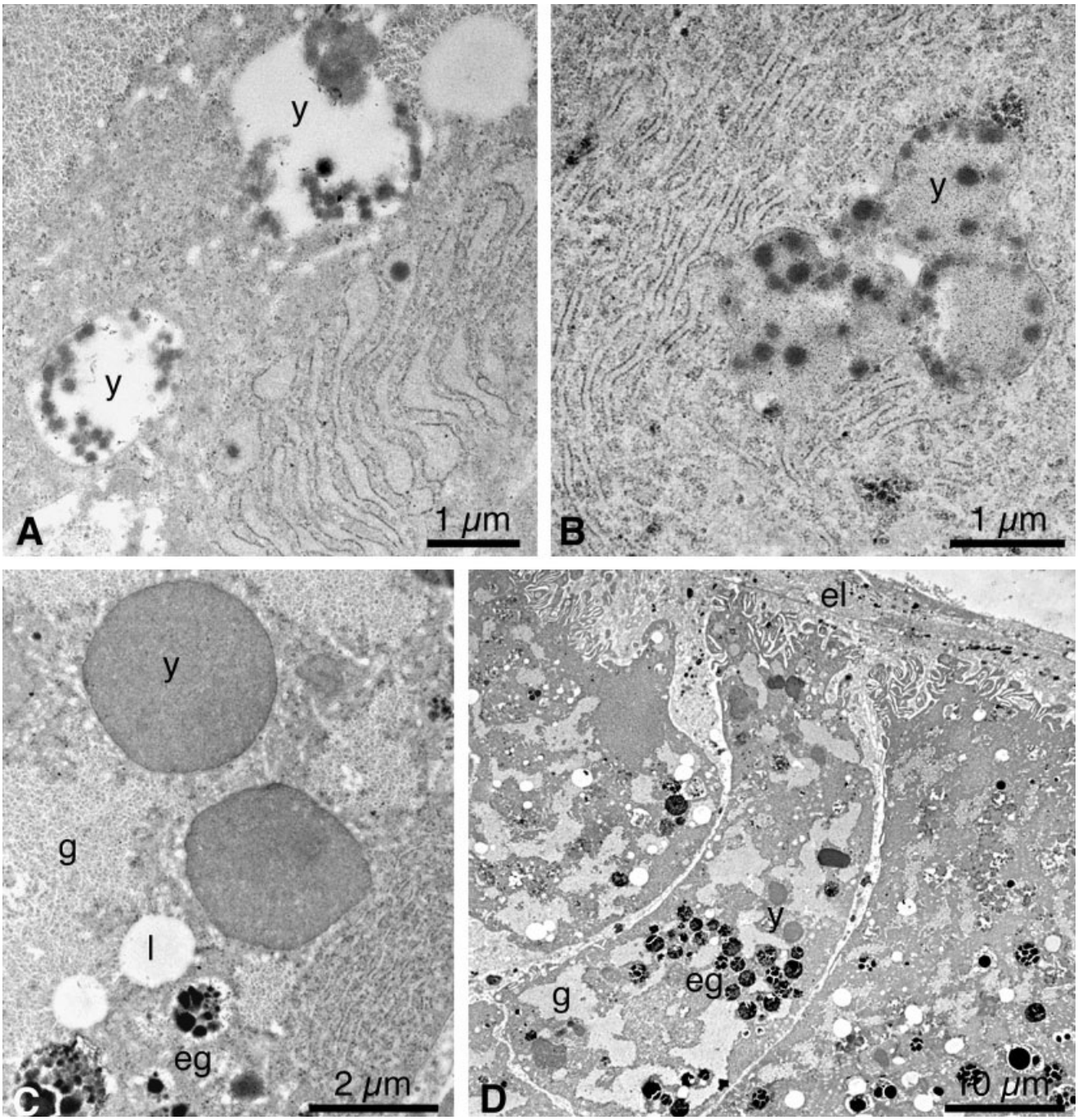


Fig. 8. *Geoplana burmeisteri*. **A:** Growing vitellocyte. Long profiles of ER with enlarged ends filled with a material of medium electron density are visible close to nascent yolk globules (y). TEM. **B:** Growing vitellocyte. Maturing yolk globules (y) showing a granular content of medium electron density with some spots of denser material. TEM. **C:** Growing vitellocyte. Mature yolk globules (y) with a homogeneous content of medium electron density delimited by a smooth membrane. TEM. eg, eggshell-forming globules; g, glycogen; l, lipid droplet. **D:** Nearly mature vitellocytes showing long cytoplasmic protrusions at the side of the cell facing the extracellular lamina (el). The cytoplasm contains different types of inclusion. TEM. eg, eggshell globules; g, glycogen; y, yolk globules.

the presence of synaptonemal complexes even in the nuclei of large oocytes. The presence of chromatoid bodies is a common feature in differentiating cells, in particular germ cells, as is the case in the oocytes of many platyhelminths (Gremigni, 1976; Justine and Mattei, 1986; Falleni and Gremigni, 1992; Falleni and Lucchesi, 1992; Falleni et al., 2002) as well as in other organisms (Wallace and Selman, 1990). According to current general opinion, chromatoid bodies may represent different substances in different cells and organisms and their role still seems to be controversial. It has been shown that many sub-

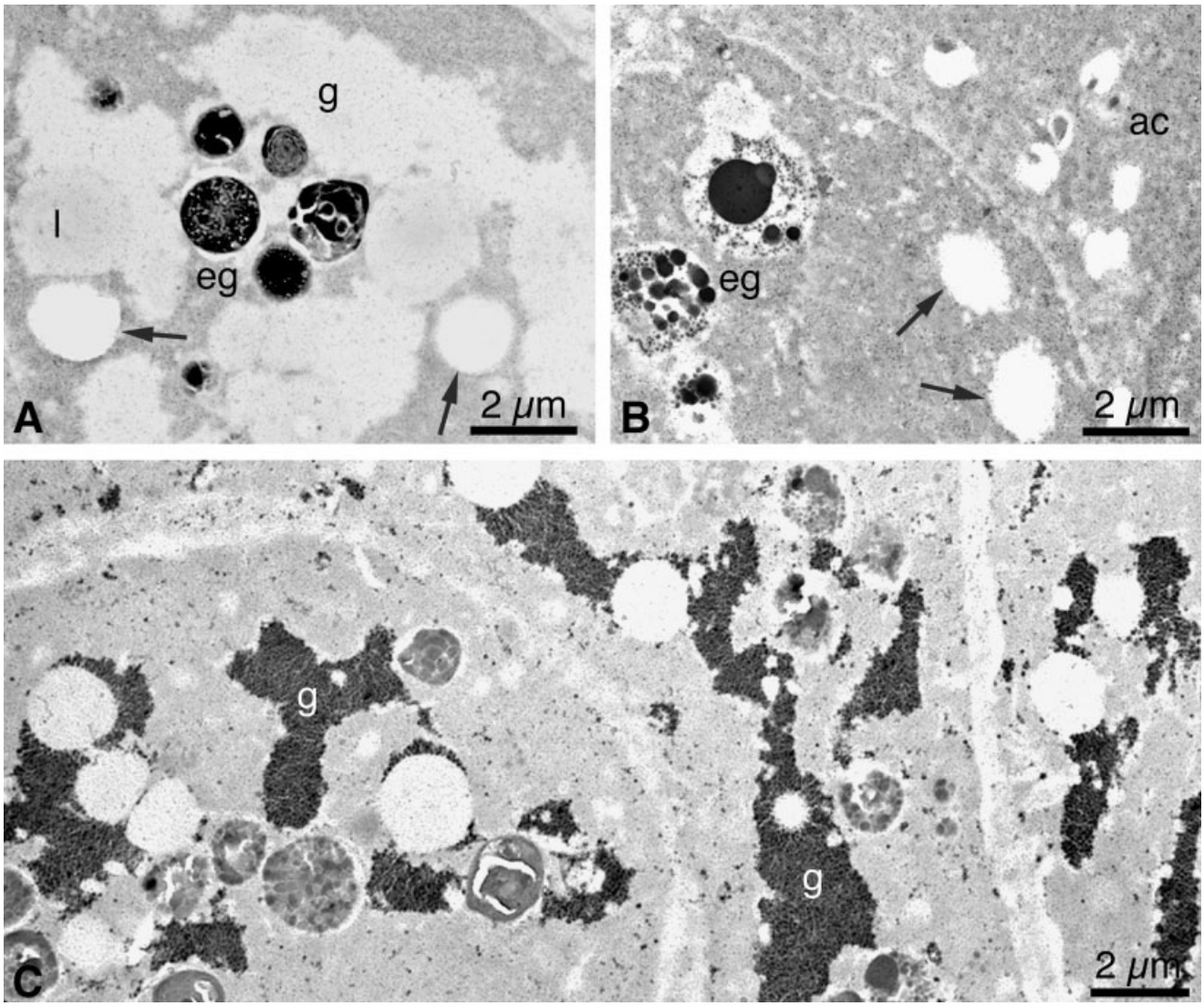


Fig. 9. *Geoplana burmeisteri*. **A,B:** Protease extraction. The yolk globule content (arrow) is digested by pronase while eggshell globules (eg) remain unaffected. TEM. ac, accessory cell; g, glycogen lacunae; l, lipid droplet. **C:** Thiéry test, 8-h incubation in thiocarbohydrazide (TCH), unstained section. A strong silver precipitate is visible on the glycogen lacunae (g). TEM.

stances such as RNA (Walt and Armbruster, 1984; Saunders et al., 1992; Auladell et al., 1993; Figueroa and Burzio, 1998), subunits of ribonucleoprotein (Biggiogera et al., 1990; Moussa et al., 1994), are present in these structures. For this reason it has been proposed that chromatoid bodies are both information-storage structures where mRNA molecules should be stored until they are required and sites of protein synthesis. A recent article (Haraguchi et al., 2005) has shown that chromatoid bodies have aggresomal features with markers such as Hsp70, ubiquitin, and ubiquitin-conjugating enzyme (E2) besides proteasome subunits and proteins of all the subcellular compartments. These findings suggest that chromatoid bodies are not a synthetic site, as proposed previously, but a degradation site where unnecessary DNA, RNA, and proteins are digested. It has also been speculated that chromatoid bodies may change their molecular components, depending on the cell state (Shibata et al., 1999; Sato et al., 2001).

The presence of RER and Golgi complexes is correlated with the production of inclusions with a content of medium electron density that remain scattered in the ooplasm throughout oogenesis. The present cytochemical investigations have shown that these inclusions have a glycoprotein content in common with the situation in some Proseriata (Falleni and Gremigni, 1992) and in the Tricladida belonging to the Dugesiidae, where they have been interpreted as residual yolk (Gremigni, 1969a, 1988). The absence of a detectable endocytotic activity in the cortical ooplasm suggests that yolk in *Geoplana burmeisteri* oocytes is produced by an au-

tosynthetic mechanism. A similar yolk production mechanism involving both the RER and Golgi complex has been previously described in other platyhelminths belonging either to the archoophoran or to the neoophoran level of organization (Gremigni, 1969a; Gremigni and Falleni, 1992). Yolk production in the oocytes of *G. burmeisteri* is scarce if compared with that of archoophoran platyhelminths with no vitellaria. The appearance of vitellaria in neoophoran Platyhelminthes has dispensed germaria from producing yolk and shell-forming substances, in contrast to the case in archoophoran platyhelminths. However, some neoophoran oocytes contain a small amount of yolk, as in *G. burmeisteri.* The presence of yolk in neoophoran species has been interpreted as a remnant inherited from an ancestor with an archoophoran organization of the female gonad (Gremigni, 1988; Gremigni and Falleni, 1992).

No types of peripheral granules have been observed in *G. burmeisteri* mature oocytes, while small peripheral inclusions with a glycoprotein content, interpreted as cortical granules, have been found in the peripheral ooplasm of marine (Gremigni and Nigro, 1983; Tekaya et al., 1999) and freshwater triclads belonging to the Planariidae and Dendrocoelidae (Gremigni, 1969b, 1979; Gremigni and Domenici, 1975) and in some proseriates (Gremigni and Nigro, 1984; Gremigni et al., 1986; Sopott-Ehlers, 1995). Peripheral ooplasmic inclusions with a polyphenolic content, interpreted as residual eggshell granules, have been observed in most prolecithophorans and rhabdocoels (Gremigni, 1988; Falleni and Lucchesi, 1992; Lucchesi et al., 1995).

The occurrence of apoptotic oocytes in *Geoplana burmeisteri* is like that of other invertebrate and vertebrate organisms and part of a normal germline development necessary for fertility and maintenance of germline homeostasis (Matova and Cooley, 2001).

The maturation process of vitellocytes in *Geoplana burmeisteri* follows a pattern similar to that described in other neoophoran Platyhelminthes (Rieger et al., 1991) and is characteristic of biosynthetically active cells with an extensive development of RER and Golgi complexes involved in the production of membrane-bound inclusions. The Golgi complexes are mainly involved in the production of eggshell globules that, when completely mature, show a meandering/concentric pattern. As far as shell globules are concerned, three content patterns have been evidenced in neoophoran platyhelminths to date: the homogeneous pattern typical of Lecithoepitheliata and some Proseriata (Gremigni, 1988; Falleni et al., 1995; Falleni, 1997); the convoluted/meandering or concentric pattern typical of Proseriata and Tricladida (Gremigni and Domenici, 1974; Sopott-Ehlers, 1990, 1991, 1995; Gremigni and Falleni, 1991); and the multigranular/mosaic-like pattern typical of the Prolecithophora and Rhabdocoela (Gremigni, 1988; Gremigni and Falleni, 1991, 1998; Sopott-Ehlers, 1997). The latter have been considered a synapomorphy of these two taxa (Gremigni and Falleni, 1998). The substructure of the eggshell globules in *G. burmeisteri* resembles that of some proseriates and triclads (Gremigni, 1988), where the electron-dense content represents the polyphenolic component, while the less dense areas represent non-phenolic proteins (Tekaya et al., 1998).

The ER is strongly involved in the production of the second type of membrane-bound inclusion in the vitellocytes. These inclusions have a protein content of medium electron density that is digested by pronase and represents yolk. They do not show any peculiarities and are similar to those observed in the other triclads studied so far and in most neoophoran species (Gremigni and Falleni, 1992).

From the data described and discussed above, it can be concluded that the female gonad of the land planarian *Geoplana burmeisteri* shows ultrastructural features that are typical of the basic pattern of Tricladida. The presence of a small quantity of yolk globules, as well as their substructure and composition, the lack of cortical granules in the peripheral ooplasm, along with the presence of eggshell globules with a meandering/concentric pattern in the vitellocytes, make this species more closely related to the freshwater triclads rather than to the marine triclads. In particular, *G. burmeisteri* shows ultrastructural characteristics similar to those found in the freshwater triclads belonging to the Dugesiidae rather than to the Planariidae and Dendrocoelidae. These ultrastructural findings lend further strength to the hypothesis proposed by Carranza et al. (1998) supporting the monophyly of the clade Dugesiidae and Terricola. The authors, on the basis of molecular data from complete sequences of 18S rDNA, suggested that the Paludicola are a paraphyletic group, since the Terricola and one paludicolan family, the Dugesiidae, share a more recent common ancestor than the Dugesiidae with the other paludicolan families (Dendrocoelidae and Planariidae).

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