



RESEARCH NOTE

Adaptations to frequent oocyte release in the ovary of the invasive snail *Pomacea canaliculata* (Caenogastropoda: Ampullariidae)

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The Ampullariidae ('apple snails') are freshwater caenogastropods of probable Gondwanan origin that are now indigenously distributed in tropical and temperate zones of southern North America, South America, Africa, India and South East Asia (Berthold, 1991). The family is a basal offshoot of the phylogenetic tree of the Caenogastropoda and comprises nine extant genera and *c.* 120 valid species (Hayes, Cowie & Thiengo, 2009), several of which are ecologically significant. In particular, *Pomacea canaliculata* (Lamarck, 1822) and *P. maculata* Perry, 1910 have invaded South East Asia (Halwart, 1994) and Spain (Oscoz, Tomás & Durán, 2010), where they have become serious pests of rice and other crops.

In the reproductive season a mature female *P. canaliculata* deposits 2–3 egg clutches per week, each one containing 100–300 bright-reddish eggs (Albrecht, Carreno & Castro-Vazquez, 1996; Estoy *et al.*, 2002; Tamburi & Martín, 2011). The egg colour is mainly due to ovorubin, a carotene glycoprotein present in the perivitelline fluid (Heras *et al.*, 2007), which is the main energy source for developing embryos (Heras, Garin & Pollero, 1998). It protects the embryo against oxidative stress (Dreon *et al.*, 2004) and, with perivitellin protein 2 (PV2), serves as a deterrent to predation (Heras *et al.*, 2008; Dreon *et al.*, 2010).

The testis, spermatogenesis and parasperm generation (including that of an ectoquasperm-like parasperm) have received considerable attention in this species (Martín, 1986; Winik, Schlick & Catalán, 1994; Catalán, Schlick de Santolaya & Winik, 1997; Winik, Catalán & Schlick, 2001; Gamarra-Luques *et al.*, 2006; Winik *et al.*, 2009). However, little is known of its ovary and oogenesis. The ovarian tubular cells appear small in mature females and the accumulation of yolk in developing oocytes seems sparse in light-microscopy preparations (Martín, 1986; Cruz López *et al.*, 2002; Gamarra-Luques, Giraud-Billoud & Castro-Vazquez, 2013).

For the current study we used five adult females of *P. canaliculata* collected during the reproductive season (November, 2010) from ponds communicating with the Salí river (Tucumán, Argentina, 29°50'45"S, 65°10'42"W). Ovarian samples were fixed in Karnovsky's fluid (4% paraformaldehyde and 0.8% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4, at 4°C). One

day later, tissues were washed thrice in phosphate buffer and transferred to 1% osmium tetroxide overnight. Afterwards, they were rinsed in distilled water and treated with an aqueous solution of 2% uranyl acetate for 40 min, gradually dehydrated in a graded ethanol series followed by acetone and finally embedded in Spurr resin. Ultrathin sections mounted on copper grids were stained with uranyl acetate and lead citrate and examined with a transmission electron microscope.

Vitellogenic oocytes had highly dispersed chromatin and a large nucleolus (Fig. 1A), nuclear pores (Fig. 1A), cytoplasmic nucleolus-like bodies ('nuages', Onohara & Yokota, 2012, resulting from the segregation of nucleolar material into the cytoplasm) (Fig. 1B, C), numerous free ribosomes (Fig. 1A–F), some profiles of the rough endoplasmic reticulum (Figs 1E, 2B) and Golgi complexes (not shown), which suggest active protein synthesis. Numerous lipid droplets (Fig. 1A–D) and small oval or elongated mitochondria (Fig. 1B–E) were also seen, as well as numerous membrane-bound, electron-dense granules (Figs 1A, B, 2A), which probably correspond to an early stage of the 'cortical granules' that occur in sea urchins and are extruded after fertilization (Runnström, 2009). Some oval yolk bodies containing a single large protein crystalloid (Fig. 1D and inset) were also seen. Coated endocytic cups, indicative of receptor-mediated endocytosis (Fig. 1E, F) and phagosomes containing materials in different stages of digestion (Figs 1A, B, E, 2A, B) suggest that oocyte development may depend not only on synthesis, but on the incorporation of extra-oocytic materials. Short microvilli extend to the lumen and appear to lie flat on the oolamina (Fig. 1E) or protrude while covered by a conspicuous electron-dense material (Fig. 1F).

Tubular somatic cells are small and appear as cytoplasmic wedges close to the basal and luminal areas and are attached to the adjacent oocytes through desmosome-like junctions close to the lumen (Fig. 2A). They also form thin cytoplasmic laminae between adjacent oocytes (Fig. 2B). An unusual feature of these laminae is the frequent occurrence of myeloid membrane stacks inside them (Fig. 2B, C).

The paucity of yolk accumulation in oocytes, which otherwise show indications of active protein synthesis, may be explained by the continuous formation and release of eggs that occur in reproductively-active individuals of *P. canaliculata* (Albrecht *et al.*, 1996). The same may be said of the membrane stacks within tubular somatic cells, which may play the role of membrane

[†]deceased.

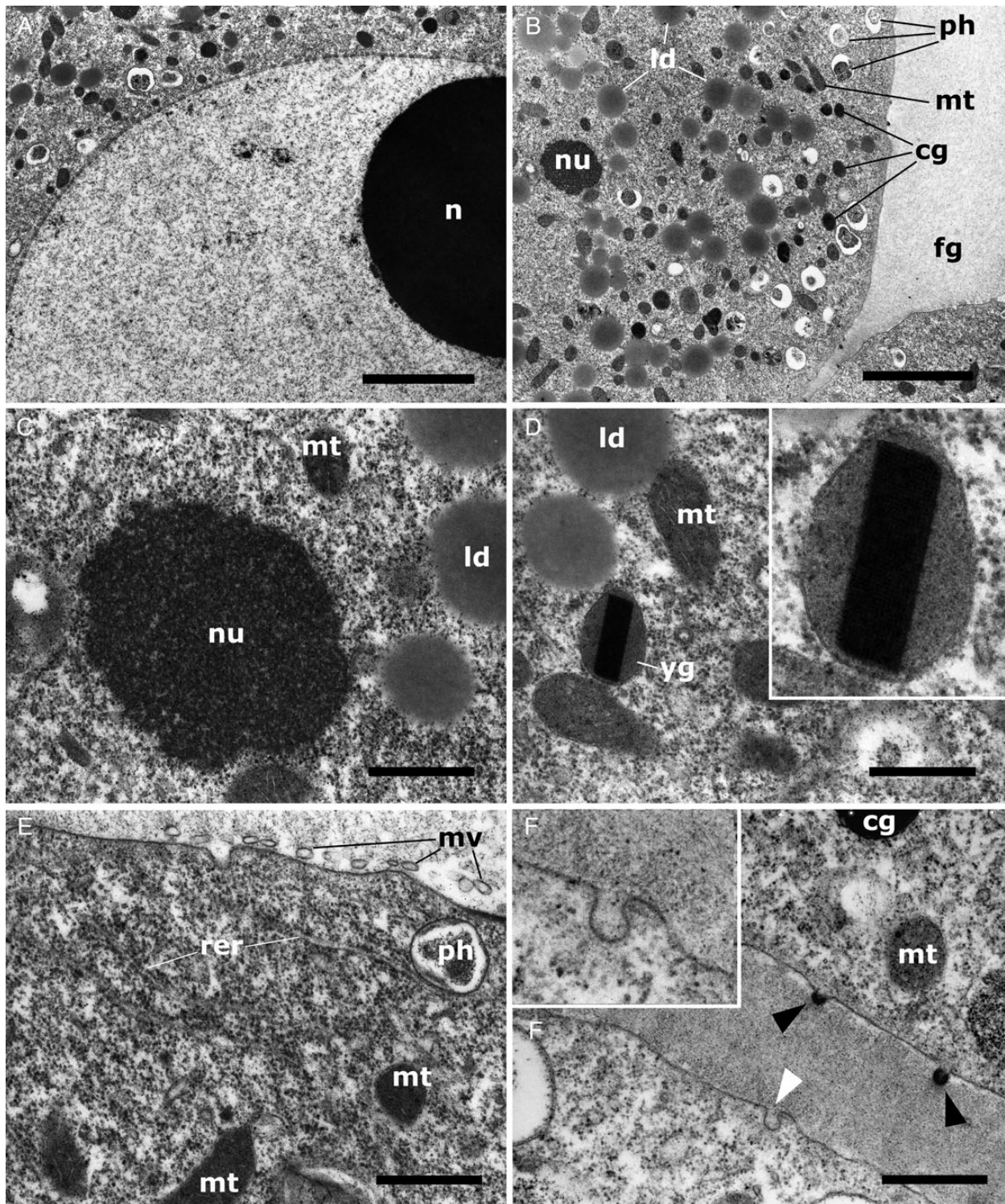


Figure 1. **A.** Vitellogenic oocyte, showing nucleus with highly dispersed chromatin, a large and electron dense nucleolus (n) and numerous pores of the nuclear envelope (for cytoplasmic structures, see **B**). **B.** Cytoplasm of two vitellogenic oocytes protruding into the tubular lumen (same animal as in **A**). The lumen contains fibrogranular material. In all micrographs, the cytoplasm is loaded with free ribosomes. Numerous lipid droplets and ‘cortical granules’ are also seen, together with small mitochondria and rather large phagosomal vesicles containing partly-lysed material. A nucleolus-like body (‘nuage’) is seen in the cytoplasm. **C.** Detail of **B**, showing a ‘nuage’ formed by numerous and densely-packed small fibrous granules that are not membrane-bound. **D.** Vitellogenic oocyte in a different female showing a yolk granule containing a crystalloid. The mitochondria exhibit an electron-dense matrix and few and scattered cristae. Inset: reticular structure of crystalloid within yolk granule. **E.** Luminal region of a vitellogenic oocyte in a third female, showing transversely-sectioned microvilli (mv) lying on plasma membrane, close to an endocytic cup; the underlying cytoplasm shows two profiles of rough endoplasmic reticulum (rer). **F.** Luminal regions of two vitellogenic oocytes (same females as in **E**); upper one shows microvilli coated with an electron-dense material (black arrowheads); lower one shows a coated endocytic cup (white arrowhead) in plasma membrane. A detail of the same cup is shown in inset. Abbreviations: cg, cortical granule; fg, fibrogranular material; ld, lipid droplet; mt, mitochondria; mv, n, nucleus; nu, ‘nuage’; ph, phagosomal vesicle; yg, yolk granule. Scale bars **A, B** = 5 μ m; **C–F** = 1 μ m.

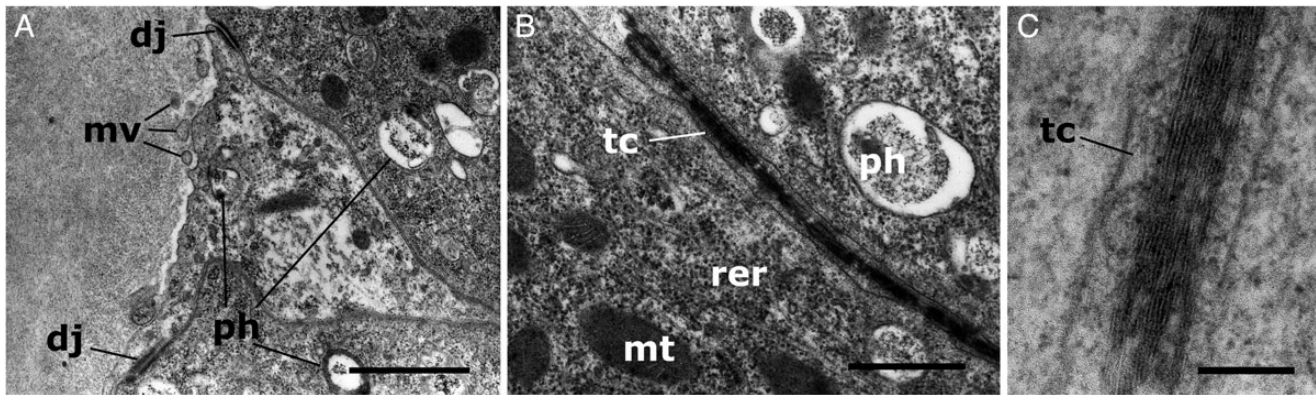


Figure 2. **A.** Cytoplasmic wedge of a tubular cell between the luminal regions of two vitellogenic oocytes (same animal as in Fig. 1A–C). Desmosome-like junctions occur between the tubular cell and the oocytes. The tubular cell also shows short microvilli and a phagosome; the cytoplasm is otherwise inconspicuous. **B.** Cytoplasmic extension of a tubular cell containing myeloid membrane stacks between two oocytes. **C.** A similar membrane stack at higher magnification (same female as in Fig. 1E, F). Abbreviations: dj, desmosome-like junction; mt, mitochondria; mv, microvilli; ph, phagosomal vesicle; tc, tubular cell; rer, rough endoplasmic reticulum. Scale bars: **A** = 2 μm ; **B** = 1 μm ; **C** = 0.2 μm .

storage, serving as a dynamic adaptation to the continuously changing population of growing oocytes during the breeding season, when large egg clutches are laid about twice a week (Albrecht *et al.*, 1996). Similarly, myelin-like (but circular) membrane structures occur in Sertoli cells in this species (Winik *et al.*, 1994) and may also be an adaptation to the frequent sperm release (about three times a week) that occurs in this species (Albrecht *et al.*, 1996).

The paucity of yolk, even in pedunculate (detaching) vitellogenic oocytes, is overwhelmingly compensated by the massive endowment of perivitellines (Heras *et al.*, 2007) that eggs receive in their passage through the albumen and capsule glands (Catalán, Fernandez & Winik, 2002; Catalán *et al.*, 2006). In fact, most nutritional requirements for embryo development are met by perivitellines and not by yolk in *P. canaliculata* (Heras *et al.*, 1998).

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