

## RESEARCH NOTE

### An unusual glandular differentiation of the male gonoducts in bivalves and its possible role in sperm transfer: the case of *Carditamera plata* (Ihering, 1907) (Carditidae)

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As a part of a research project focusing on the relationships between brooding, brood size, space available for brooding and the occurrence of ova resorption in species of Carditoidea, a remarkable and previously undescribed structure was found in the terminal portion of the male ducts of *Carditamera plata* (Ihering, 1907). The discovery is striking, because the gonoducts of bivalves are generally thought to be extremely simple structures, lined with a ciliated epithelium without conspicuous differentiation (Runham, 1993).

*Carditamera plata* is a small bivalve (maximum observed length in the present study = 5.2 mm), occurring from Rio de Janeiro, Brazil to San Matías Gulf, Argentina (Ríos, 1994). Specimens for this study were collected by dredging at 6–12 m depth from San Matías Gulf (40°30'16.2" S, 65°27'29.6" W), Río Negro. Additional specimens were collected by hand during maximum low tides from the rocky intertidal at Las Grutas, San Matías Gulf, where *C. plata* lives in small clusters, sheltered in holes and crevices of consolidated substrates.

Specimens for histology (length range 1.7–5.2 mm) were fixed either in a 10% aqueous formalin solution or in Bouin's fixative. After repeated rinsing in tap water, tissues were dehydrated in an ethanol series, embedded in Histo-resin® (Leica) and sectioned at 3.5 µm with a motorized rotary microtome (Leica RM2255). Besides routine haematoxylin/eosin staining, usual approaches to the histochemical study of mucosubstances (glycans and glycosaminoglycans) were performed: Periodic Acid Schiff (PAS) for glycans, Toluidine blue at pH 2.5 and Alcian blue (AB) at pH 2.5 for acidic mucosubstances (Gabe, 1968).

*Carditamera plata* is a gonochoristic species. Female gonads produce relatively large eggs (about 120 µm diameter) surrounded by a two-layered (12 µm thick) vitelline envelope; male gonads produce only euspermatozoa (present study). The main ducts of the male gonad open into a conspicuous, relatively short, glandular structure, here termed the 'glandular gonoduct' (Fig. 1A–C). The lumen of the glandular gonoduct is lined by a cuboidal or low-columnar, heavily ciliated epithelium (7–9 µm height); beneath, is a very tall columnar subepithelial glandular layer (about 55–63 µm height) supported by a thin connective tissue (Fig. 1D, F). The glandular cells have a basal nucleus and the cytoplasm is filled with densely packed secretion droplets. Usually, at the posterior (proximal) end of the glandular gonoduct, the secretion droplets coalesce into large secretion globules (Fig. 1D, E). A few slender interstitial cells with a rounded or slightly elongate nucleus are scattered among columnar glandular cells (Fig. 1H). The development of the glandular gonoduct was not related to the stage of maturity of the gonads, but

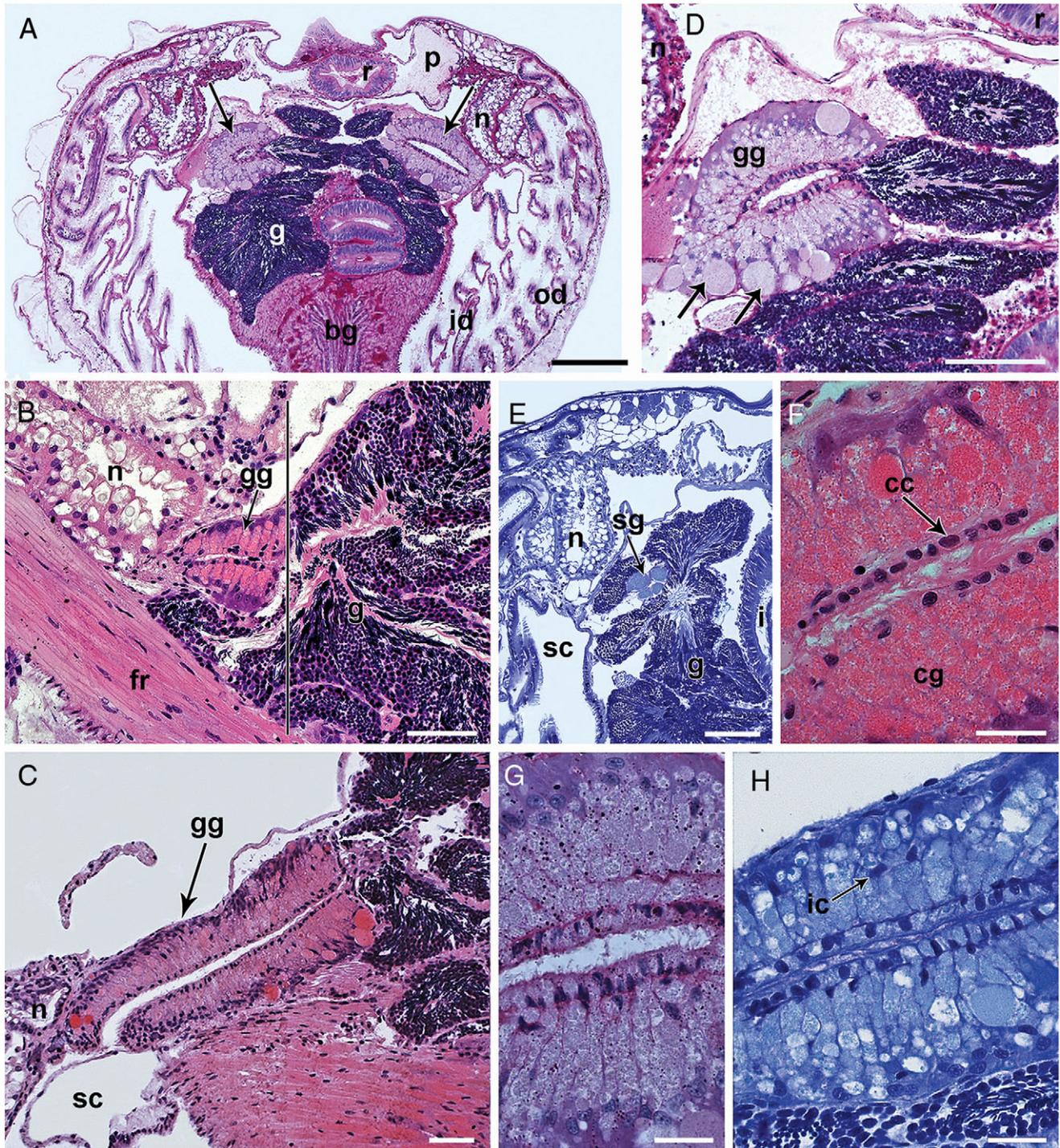
instead to the size of specimens; males above 1.8–2 mm length showed the glandular gonoduct fully developed.

Regarding the chemical nature of the secretions, their strong acidophilic reaction to eosin should be indicative of a prominent proteinaceous content (Fig. 1F). Various dyes such as eosin, orange G and phloxin are currently considered as protein-specific (Exbrayat, 2013). With the PAS technique, the secretion granules were stained a very pale rose colour, a reaction compatible with the presence of neutral mucopolysaccharides and glycoproteins (Fig. 1A, D, G). The negative reaction to AB and the orthochromatic reaction to Toluidine blue indicate the absence of acidic mucopolysaccharides (Fig. 1E, H).

In contrast to that observed in males, the terminal portion of the female gonoduct is nonglandular; the duct wall is formed by a single cell layer of columnar epithelial cells (7.5–12.5 µm high) with rounded basal nucleus, intermingled with interstitial heavily ciliated cells, supported by dense connective tissue (Fig. 2A, B).

According to De Jong-Brink, Boer & Joose (1983), there are cases in molluscs with external or intermediate fertilization (e.g. within the mantle cavity) in which the male or female ducts may have glandular portions that produce substances that could contribute to some kind of gamete agglutination to improve the fertilization success. It is generally accepted that bivalve gonoducts are lined by a simple ciliated epithelium, leading gametes to the suprabranchial chamber through a simple genital pore, sometimes located on a small papilla (Sastry, 1979; Runham, 1993; Gosling, 2015). It has also been claimed that glandular cells are absent from bivalve gonoducts (Runham, 1993), although Grobben (1892) described an accessory genital organ of glandular nature that is functional in the male phase of the protandric bivalve *Cuspidaria cuspidata*. Grobben (1892) described cyclic changes in the morphology and degree of development of the so called 'tubular glands' according to the development of the male phase. The tubular glands described by Grobben (1892) differ from the condition observed herein in *C. plata* in not being part of the gonoduct, but a structure that empties into the male duct. The paired tubular glands in *C. cuspidata* are similar to the male glandular gonoducts of *C. plata* both in the shape of the gland cells (high columnar cells with basal nucleus) and in the overall aspect and nature of the secretions, i.e. large globules of acidophilic material.

The only previously known glandular differentiation in the sperm ducts of a bivalve was reported by Lützen, Jespersen & Russell (2015) in the venerid *Nutricula tantilla*, in which the terminal

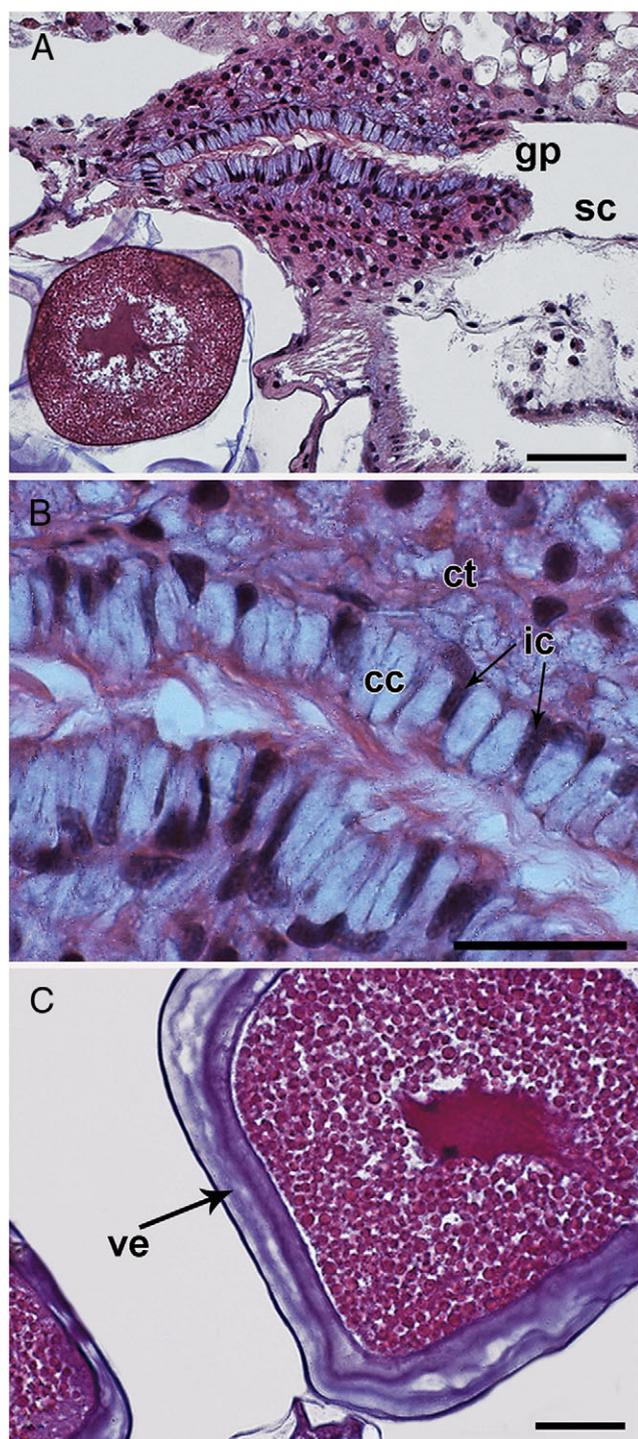


**Figure 1.** *Carditamera plata*. **A.** Transverse section at level of the terminal male gonoducts (arrows). **B.** Sagittal section of posterior half of visceral mass showing relative position of glandular gonoduct (line shows level of section in **E**). **C.** Sagittal section of glandular gonoduct at level of gonopore. **D.** Detail of glandular gonoduct in transverse section with globules of secretion at base (arrows). **E.** Transverse section of a main sperm duct in upper right half of male gonad, at point of its connection to the glandular gonoduct. **F–H.** Details of histology of duct epithelium and columnar glandular cells showing acidophilic (**F**), light Periodic Acid Schiff (PAS) positive (**G**) and orthochromatic (**H**) reactions of the glandular secretions. Abbreviations: bg, byssus gland; cc, cuboidal ciliated cells; cg, columnar glandular cells; fr, foot-retractor muscle; g, gonad; gg, glandular gonoduct; i, intestine; ic, interstitial cell nucleus; id, inner demibranch; n, nephridium; od, outer demibranch; p, pericardium; r, rectum; sc, suprabranchial chamber; sg, secretion globule. Scale bars: **A** = 200  $\mu$ m; **B, C** = 50  $\mu$ m; **D, E** = 100  $\mu$ m; **F–H** = 20  $\mu$ m.

portion of the male ducts are lined by tall and slender glandular, nonciliated cells with basal, rounded nucleus. Contrary to that observed in *C. plata*, the secretion product in *N. tantilla* consists of strongly basophilic spherical droplets. As in *C. plata*, the female

ducts of *N. tantilla* are nonglandular; however, in the latter the epithelium is squamous to cuboidal and nonciliated.

It was not possible to determine the role of the glandular differentiation in the male gonoducts of *C. plata*. Nothing is known



**Figure 2.** *Carditamera plata*. **A.** Transverse section of dorsal visceral mass showing the nonglandular female gonoduct. **B.** Detail of epithelium of female gonoduct. **C.** Late vitellogenic oocyte surrounded by two-layered vitelline envelope. Abbreviations: cc, columnar epithelial cells; ct, connective tissue; gp, genital pore; ic, interstitial ciliated cells; sc, suprabranchial chamber; ve, vitelline envelope. Scale bars: **A** = 50 µm; **B**, **C** = 20 µm.

about how sperm are transferred to females in this species. It could be speculated that the secretions contribute to holding the spermatozooids together after spawning, thus allowing sperm transfer in bulk, improving the efficiency of fertilization. Ó Foighil (1985) proposed that bulk sperm transfer by means of spermatophores or other massive transfer media could be beneficial in

ctenidial-brooding species such as *Mysella tumida* and other Galeommatoidea that usually live aggregated in small clusters on or around a host animal. In this regard, *C. plata* lives in small aggregations, sheltered in small holes and crevices of partly consolidated sediments on intertidal and subtidal rocky shores; if the secretions of the glandular male gonoducts do indeed contribute to the agglutination of spermatozooids, this could be beneficial in such an environment, for example in avoiding sperm dilution after spawning. However, at present there is no information on the occurrence of any kind of brood protection in *C. plata*, either ctenidial as in other carditoidaeans (Yonge, 1969), or clustered in gelatinous envelopes as reported in *Carditamera floridana* by Harvey (1995), in the only previous study on the reproduction of a *Carditamera* species. *Carditamera floridana*, a species occurring from south Florida to Quintana Roo, Mexico (Simone, Mikkelsen & Bieler, 2015), retains its eggs in an external gelatinous egg mass attached to the posterior end of the shell of the maternal individual, in which embryos are protected until released as crawling juveniles; no information is available on the origin of the agglutinating material. Furthermore, Harvey (1995) made no mention in *C. floridana* of the presence of any glandular differentiation in the male ducts as reported here for *C. plata*. Other reproductive traits of *C. floridana* are similar to those of *C. plata*, as for example in the morphology of the relatively large yolky eggs (120 µm, present study), which are covered by a thick (12 µm) two-layered vitelline envelope (Fig. 2C). The size of the ripe oocytes and of the prodissocoonch (180 µm, present study) is indicative of lecithotrophic development. Perhaps future studies might reveal parental care similar to that in *C. floridana*, supporting the speculation of a relationship between mass sperm transfer (suggested by the secretions of the glandular gonoduct) and brooding, as was proposed by Ó Foighil (1985) for *M. tumida*.

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