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# The smallest carnivorous bivalve? Biology, morphology and behaviour of *Grippina coronata* (Anomalodesmata: Cuspidarioidea: Spheniopsidae) preying on epipsammic microcrustaceans in the southwestern Atlantic off Brazil

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## ABSTRACT

The Spheniopsidae are today represented by five living species of *Spheniopsis* and nine of *Grippina*, distributed in the Pacific and Atlantic Oceans. Little is known of their anatomy and the phylogenetic position of the family within the Bivalvia is debated. In order to investigate these questions, the functional morphology of *Grippina coronata* obtained from the continental shelf off Rio de Janeiro and Espírito Santo States, Brazil, has been examined. Less than 2 mm in shell length, the siphonal apparatus of *G. coronata* is complex, with seven sensory papillae, and the ctenidia are reduced to transverse septa pierced by four pairs of ciliated pores. There are no labial palps and the stomach is of Type II with epibenthic harpacticoid and ostracod prey identified inside it. Although there is an intestine producing faeces, the stomach also possesses a unique waste storage pouch for exoskeletal remains of digested prey. Collectively, these features suggest that the Spheniopsidae comprise carnivorous taxa belonging to the Cuspidarioidea within the Anomalodesmata. *Grippina coronata* is a self-fertilizing simultaneous hermaphrodite that, uniquely, broods fertilized oocytes within the ovarian follicles and, thereby, provides the first example of intrafollicular fertilization and brooding in the Bivalvia. Release of the encapsulated oocytes must be by parental death, which coincidentally releases the exoskeletal remains from the storage pouch. Such *post mortem* semelparity creates a life-history trait hitherto unrecognized in the Bivalvia.

## INTRODUCTION

The Anomalodesmata, lying within the basal Heterodonta (Bieler *et al*., 2014), comprises a richly diverse assemblage of exclusively marine bivalves, which vary widely both in overall form and in the highly specialized niches they occupy. As a consequence, they exhibit a high diversity of unique anatomies. The Anomalodesmata comprises some eight superfamilies (Runnegar, 1974; Morton, 1981a, 2012), one of which is extinct (the Ceratomyoidea), although most putative families of the Palaeozoic Pholadomyoidea also occur only as fossils (Runnegar, 1974). The adaptive radiation expressed by the living representatives of the Anomalodesmata is spectacular but still poorly appreciated, because most extant taxa occur typically as solitary individuals in highly specialized niches. Two anomalodesmatan clades stand out, for mention, however. The first includes the adventitious tube-dwelling representatives of the Clavagelloidea (Morton, 2007). The second comprises the remarkable deeper-water predatory bivalves often collectively referred to as the septibranchs. The classification schemes erected for these latter bivalves have undergone many changes over the last 50 years and these were reviewed by Morton (1982: Table II). The septibranchs are today thought to be represented by the fossil Orthonotoidea (Bieler, Carter & Coan, 2010) and three extant superfamilies: the Verticordioidea (Verticordiidae, Euciroidae and Lyonsiellidae), Poromyoidea (Poromyidae) and the Cuspidarioidea (Keen, 1969; Bernard, 1979; Bieler *et al*., 2010). Hitherto, this last superfamily has been considered to comprise but one extant family—the Cuspidariidae—although, as will be described and discussed, the most recent classification by Carter *et al*. (2011) considered the Cuspidarioidea to also include the (unstudied) Halonymphidae, Protocuspidariidae and the Spheniopsidae (similarly unstudied). A fourth family of deep-water predatory bivalves, the Parilimyidae, is believed to be affiliated with the Pholadomyoidea (Morton, 1982).

The predatory anomalodesmatan bivalves have received some attention, focusing largely on anatomy. Prior to a greater appreciation of their familial diversity, such bivalves were judged to belong, collectively, to the Septibranchia, characterized by the presence of a horizontal muscular septum in place of the typical bivalve ctenidium. This generalization, however, is not true—*Halicardia nipponensis* Okutani, 1957 (Verticordiidae), for example, possesses a

ctenidum (but no labial palps), a funnel-shaped mouth and a stomach for the processing and digestion of its copepod prey (Nakazima, 1967). Yonge (1928) was perhaps the first to bring the septibranch bivalves, *Cuspidaria* and *Poromya*, to wider scientific attention although Pelseneer (1888, 1911) and Ridewood (1903) had earlier made comprehensive studies of numerous septibranchs, the latter author describing the structure of the septum in detail.

Later, Reid & Reid (1974) examined aspects of the morphology and behaviour of *Cuspidaria rostrata* (Spengler, 1793) and *C. obesa* (Lovén, 1846) and Reid & Crosby (1980) subsequently undertook a study of how *Cardiomya planetica* (Dall, 1908) captured its prey. Reid (1978) made the only physiological study to date of any carnivorous bivalve, again *C. planetica*, demonstrating the presence of proteolytic enzymes in the stomach to digest its prey. Purchon (1956) examined the stomach architecture of *Cuspidaria cuspidata* (Olivi, 1792), classifying it, mistakenly, alongside that of the Protobranchia in his Type II, the Gastrodeuteia (Purchon, 1958) and 'sub-class' Oligosyringia (Purchon, 1962, 1987). The anatomies of the deep-water representatives of the Verticordiidae (Allen & Turner, 1974) and Cuspidariidae and Poromyidae (Allen & Morgan, 1981) have been described, the latter paper also discussing the evolution of the septibranch condition. In a series of studies, Morton (1981b, 1982, 1984, 2003) undertook investigations of the anatomies of *Poromya granulata* (Nyst & Westendorp, 1839) (Poromyidae), *Parilimya fragilis* (Grieg, 1920) (Parilimyidae), *Lyonsiella formosa* (Jeffreys, 1881) and *Bentholyonsia teramachii* (Habe, 1952) (Lyonsiellidae), also reviewing statocyst and siphon structure in representatives of the various families (Morton, 1985, 1987). Oliveira & Sartori (2013) examined the arenophilic radial glands in the siphons of *Cuspidaria obesa* and a species of *Cardiomya*.

In his comprehensive reviews of the abyssal and hadal bivalves collected from many parts of the earth's oceans, Knudsen (1967, 1970) described the shells of numerous species of the Septibranchia, while Simone & Cunha (2008), Oliveira & Absalão (2010) and Absalão & Oliveira (2011) have reviewed the species of Verticordiidae, Lyonsiellidae and Cuspidariidae, respectively, occurring in the deeper waters off Brazil.

The Spheniopsidae Gardner, 1928, known since the Oligocene, are represented today by a number of small bivalves with shells usually less than 5 mm in length (Boss, 1982). Two genera occur in the Recent fauna, *Spheniopsis* Sandberger, 1861 and *Grippina* Dall, 1912, the first with five and the second with nine species, both recorded from the warm, tropical waters of the Americas, Africa and New Zealand (Verril & Bush 1898; Dall, 1912; Coan, 1990; Cosel, 1995; Coan, Valentich-Scott & Bernard, 2000; Marshall, 2002; Coan & Valentich-Scott, 2012; Machado & Passos, 2015). From the Atlantic Ocean, *Spheniopsis senegalensis* Cosel, 1995 is recorded from off the coast of West Africa (Cosel, 1995), *Spheniopsis triquetra* (Verril & Bush, 1898) from North Carolina, Florida and the Bahamas (Coan & Valentich-Scott, 2012; Machado & Passos, 2015), and *Spheniopsis brasiliensis* Machado & Passos, 2015 and *Grippina coronata* Machado & Passos, 2015 were recently described from the southeastern Brazilian coast. In addition to Recent species, the Spheniopsidae have five fossil species, belonging exclusively to the genus *Spheniopsis* (Coan, 1990).

The anatomy of representatives of the Spheniopsidae is virtually unknown, with little snippets of information available on muscle scars on the shells and the dried tissues of *Grippina californica* Dall, 1912 (Coan *et al*., 2000; Mikkelsen & Bieler, 2008). Associated with this, the phylogenetic position of the Spheniopsidae has been the subject of recent debate (Marshall, 2002; Harper, Dreyer & Steiner, 2006; Bieler *et al*., 2010). The family was considered to be allied to the Heterodonta (Gardner, 1928; Keen, 1969; Coan, 1990; Cosel, 1995), notably between the Corbulidae and the Hiatellidae, i.e. within the Myoidea (Boss, 1982; Amler, 1999; Coan *et al*., 2000), Bieler & Mikkelsen (2006) considering the Spheniinae to be a subfamily of the Myidae. Based on the observations that species of *Grippina* have sunken resilifers with a lithodesma and some have an exterior commarginal microsculpture most similar to that seen in some anomalodesmatans, Marshall (2002) concluded that the Spheniopsidae should be placed in the Cuspidarioidea. Bieler *et al*. (2010) accepted this view, albeit questioningly, and suggested that "the family would profit from molecular analyses" (p. 133). Despite such information not being available, Carter *et al*. (2011) agreed with Marshall's view and placed the Spheniopsidae firmly within the Cuspidarioidea.

In the absence of such a molecular analysis and comprehensive information regarding the anatomy of any spheniopsid, however, uncertainties concerning their relationship with the Myoidea (Amler, 1999; Coan *et al*., 2000) or the Cuspidarioidea still remain. This work is a study of the anatomy of the minute (<2 mm) spheniopsid *G. coronata*—recently collected off the coast of Brazil, described as new and the shell described in detail by Machado & Passos (2015). The aim of the study, therefore, was to reveal those characters that would assist in identifying the systematic affiliations of the Spheniopsidae. In addition, however, it also examines aspects of the species' anatomy that show how such a minute bivalve is adapted to life on the continental shelf and deeper sea bed, and to a carnivorous mode of feeding.

## MATERIAL AND METHODS

Individuals of *Grippina coronata* were obtained from bottom samples collected by a box corer during research conducted under the 'Habitats Project—Campos Basin Environmental Heterogeneity', in February and July 2009. Sampling was undertaken on the shelf and continental slope of the Campos Basin, an area of oil and natural gas exploitation off the southeastern coast of the Brazilian states of Rio de Janeiro and Espírito Santo. Numerous bottom samples were obtained from depths of 12 to 3200 m and sieved through a 0.5-mm mesh. From 28 of these samples collected at depths of 21 to 53 m, 67 empty shells and 20 living individuals of *G. coronata* were obtained. These were initially fixed in 4% formalin and then preserved in 70% alcohol. Preserved individuals were dehydrated in an ascending series of ethanol, critical-point dried and mounted on aluminum stubs for examination by a scanning electron microscope (SEM). For histological purposes, two individuals of *G. coronata* were decalcified in a solution of 100 ml distilled water containing 0.88 g of NaCl and 1.02 g of ascorbic acid. These were dehydrated in an ascending series of ethanol and embedded in methyl methacrylate (Historesin®) to obtain serial transverse and sagittal sections 5 μm thick. Histological slides and SEM stubs have been deposited in the Museum of Zoology of the State University of Campinas (ZUEC) under accession numbers ZUEC-BIV 6181, 6183, 6203 and 6204.

### *Abbreviations used in figures*

| | |
|---|---|
| aam | Anterior adductor muscle (or scar) |
| an | Anus |
| aprm | Anterior pedal retractor muscle (or scar) |
| asm | Anterior septal muscle (or scar) |
| at | Anterior hinge tooth |
| atl | Anterior hinge lock |
| au | Auricle (of heart) |
| bo | Brooded oocytes |
| cc | Collagen coat |
| ci | Cilia |
| co | Collar around crystalline style sac |
| cs | Crystalline style |
| css | Crystalline style sac |

| | |
|---|---|
| d | Dissoconch |
| dc | Digestive cell |
| dd | Digestive diverticulae |
| ddc | Dehiscing digestive cell |
| do | Developing oocyte |
| dt | Digestive tubule |
| eo | Encapsulated oocyte |
| es | Exhalant siphon |
| f | Foot |
| fimmf | Fused inner and inner surfaces of the middle mantle folds |
| g | Gland in the foot |
| gs | Gastric shield |
| ha | Haemocoel |
| hg | Hindgut |
| iec | Inner epithelium cells |
| ilm | Inner lip of the mouth |
| imf | Inner mantle fold |
| ipi | Ingested prey item |
| i | Intestine |
| is | Inhalant siphon |
| isc | Infraseptal chamber |
| l | Ligament |
| li | Lithodesma |
| m | Mouth |
| ma | Mantle |
| mc | Mantle cavity |
| mg | Midgut |
| mi | Microvilli |
| mm | Mantle margin |
| mmf | Middle mantle fold |
| mo | Mature oocyte |
| o | Oesophagus |
| obe | Ooctye being encapsulated |
| obf | Oocyte being fertilised |
| oc | Oocyte capsule |
| odd | Openings to the digestive diverticulae |
| of | Ovarian follicle |
| omf | Outer mantle fold |
| ommf | Outer surface of the middle mantle fold |
| ov | Ovaries |
| p | Periostracum |
| pam | Posterior adductor muscle (or scar) |
| peg | Pedal groove |
| pega | Pedal ganglia |
| pg | Periostracal groove |
| pga | Pedal gape |
| pl | Pallial line |
| pprm | Posterior pedal retractor muscle (or scar) |
| ps | Pallial sinus |
| psm | Posterior septal muscle (or scar) |
| pr | Prodissoconch |
| pr (I) | Prodissoconch I |
| pr (II) | Prodissoconch II |
| prm | Pallial retractor muscle |
| pt | Posterior hinge tooth |
| ptl | Posterior hinge lock |
| r | Rectum |
| sa | Sorting area in the stomach |
| sc | Secretory cell |
| se | Septum |
| selm | Septal elevator muscle |
| sem | Septal membrane |
| sep | Septal pore |
| sep (1)–(4) | Septal pores (1–4) |
| so | Siphonal open |
| sp | Siphonal papilla |
| spb | Siphonal papilla base |
| spc | Sphincter cells |
| spe | Spermatocyte |
| spg | Septal pedal gape |
| sph | Sphincter |
| spm | Sperm morula |
| spt | Spermatid |
| srp | Skeletal remains of prey |
| ssc | Supraseptal chamber |
| st | Stomach |
| sta | Statocyst |
| stl | Statolith |
| t | Testes |
| tf | Transverse fibres |
| v | Ventricle (of heart) |
| vm | Visceral mass |
| wsp | Waste-storage pouch |

## RESULTS

### *Shell*

In side view, the shell of *Grippina coronata* (Fig. 1A) is subtrigonal, as in *G. californica* (Coan *et al*, 2000: 481, pl. 103), with the posterior face foreshortened and distinctively pointed posterodorsally, but more flattened posteroventrally. The anterior face is more smoothly rounded and oval. The shell is transparent, as illustrated in Figure 2A, which also shows oocytes being brooded (bo) internally, and its surface is smooth with only fine commarginal growth lines. The most characteristic feature is the dorsal prodissoconchs, described below. From the dorsal aspect (Fig. 1B), the shell is slightly sinusoidal with the anteroposterior greatest width (a–b) located through the dorsal umbones to the midventral border. In ventral view (Fig. 1C), the slightly inequivalve shape seen dorsally is not obvious and the left and right valve borders fit together tightly. The posterior shell margin from this aspect is variably flattened, as noted above. Seen from the posterior aspect (Fig. 1D), the slight sinusoidal form of the valve margins is again obvious and the greatest shell width, left to right (*x*–*y*), is somewhat dorsal to the midpoint of the dorsoventral axis of the shell. This is also obvious when seen from the anterior aspect (Fig. 1E). There are also indications of a lunule anteriorly and an escutcheon posteriorly (Machado & Passos, 2015), as identified for *G. californica* (Coan *et al*., 2000), although such structures might have been designated when the Spheniopsidae were considered to have heterodont affinities, since they are only expressed subtly.

The prodissoconchs of *G. coronata* (Fig. 3) are large in relation to the overall size of the shell (<2 mm). Apically, there is a prodissoconch I (pr (I)), about 260 μm in length (254 ± 14 μm, Machado & Passos, 2015), comprising a biconical apex surrounded by a raised rim. Surrounding this is a plain prodissoconch II (pr (II)) about 350 μm in diameter. There does not appear to be a nepioconch and thus the dissoconch (d) forms smoothly around prodissoconch II in a uniformly blended manner. Machado & Passos (2015: fig. 3) provided photographic images of the larval shell stages of *G. coronata*.

The internal hinge plates of the right and left shell valves of *G. coronata* (Fig. 4A, B) were also described by Machado & Passos (2015). That of the right bears anterior and posterior hinge teeth (at, pt), which fit beneath equivalent locking points (atl, ptl) that are merely indentations on the thickened hinge of the left valve. The two hinge teeth flank a central amphidetic ligament (l) located upon a resilifer and ventrally formed into a lithodesma (li), the structure of which has been described for a number of species of the Cuspidariidae by Bernard (1974) and for New Zealand representatives of the Spheniopsidae by Marshall (2002: fig. 21). Yonge & Morton (1980: fig. 11) described the ligament

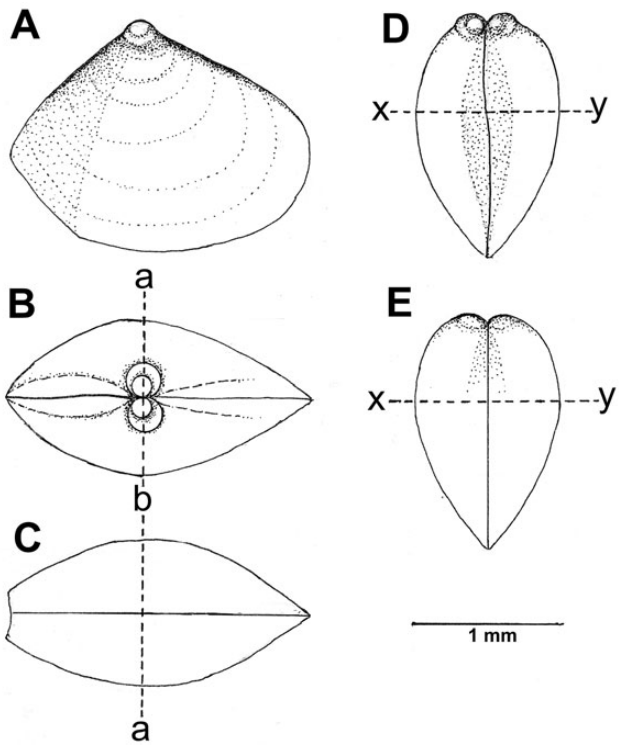


**Figure 1.** Shell of *Grippina coronata*. **A.** Right side. **B.** Dorsal. **C.** Ventral. **D.** Posterior. **E.** Anterior. Abbreviations: a–b, greatest width anteroposteriorly; *x*–*y*, greatest width dorsoventrally.

and lithodesma of *Cuspidaria cuspidata* and showed how it was formed. The lithodesma of *G. coronata* resembles those of the other spheniopsids described above, although it is too tiny to be dissected out and illustrated here.

An interior view of the left shell valve of *G. coronata* (Fig. 5) shows the muscle scars. These are simple and comprise approximately equal-sized anterior and posterior adductor muscles (aam, pam), each internally and dorsally flanked by small septal retractor muscle scars (asm, psm). These are indistinguishable as scars from the anterior and posterior pedal retractor muscle scars (aprm, pprm). The pallial line (pl) is thin and posteriorly forms a shallow pallial sinus (ps).

### *Siphons*

The withdrawn siphons of *G. coronata* (Figs 2D, 6A), as seen from the posterior aspect, are separate and formed by fusion of the inner folds and the inner surfaces of the middle mantle folds and are thus of Type B (Yonge, 1957). They comprise a conical exhalant siphon (es), only 100 μm in diameter, and a larger, 200 μm, inhalant one (is). The former siphon has a single sensory papilla (identified as tentacles by Reid & Crosby, 1980, for *Cardiomya planetica*) dorsally and two others laterally (sp). The latter siphon has four such papillae all located ventral to the siphon and sitting upon a raised base (spb). A single papilla is illustrated in Figure 6B and has an embayed tip from which arise up to approximately 12 long cilia, each with a paddle-shaped tip (Fig. 6C).

### *Mantle*

The ventral mantle margin at the pedal gape of *G. coronata* (Fig. 7A) is simple. Each lobe comprises an elongate inner fold (imf), a tiny middle fold (mmf) and a similarly small outer fold (omf). From the periostracal groove (pg) between the latter two folds arises an exceedingly thin periostracum (p). A pallial retractor muscle is not obvious here but, instead, there is a capacious haemocoel (ha), which is characterized by transverse muscle fibres (tf) that cross-connect the outer and inner mantle surfaces, presumably helping to sustain tonus. The inner surface of the mantle is characterized by an epidermis that has strangely vacuolated cells and one of these is illustrated in greater detail in Fig. 7B. No function can as yet be ascribed to these.

Posterior to the pedal opening, the left and right mantle margins fuse (Fig. 8); Figure 2B shows this posterior fusion forming the siphonal opening (so). In transverse section, fusion is seen to be of the inner folds and the inner surfaces of the middle mantle folds (fimmf). This, therefore, as with the siphons, is of Type B (Yonge, 1948, 1982). In this location, the pallial retractor muscles are more obvious (prm), extending through the haemocoelic spaces (ha) of the outer mantle fold (omf). The middle mantle folds are here still small and comprise the outer surfaces only (ommf). From between these outer surfaces and the inner surfaces of the outer mantle folds, within the periostracal grooves (pg), arises the periostracum (p). Oliveira & Sartori (2013) demonstrated the presence of arenophilic radial mantle glands in some species of *Cuspidaria* (e.g. *C. obesa*) and *Cardiomya* (e.g. *C. cleryana* (d'Orbigny, 1842)), but not others. Such glands have not been identified for *G. coronata*.

### *Organs of the mantle cavity*

The organs of the mantle cavity of *G. coronata*, as seen from the right side after removal of the right shell valve and mantle lobe, are illustrated in Figure 9. The septum (se), with its four pairs of pores (sep), is suspended in the mantle cavity by anterior (asm) and posterior septal retractor muscles (psm), the latter larger than the former. As shown earlier, these muscles attach to the shell internal to the anterior (aam) and posterior (pam) adductor muscles and share an attachment with the minute and equivalent anterior and posterior pedal retractor muscles. The visceral mass (vm) is situated above the septum and has a posterior foot (f) that extends through a gape in the septum anteriorly and can, presumably, also be extended out from the similarly anterior pedal gape (pga) in the mantle margin (mm) to effect burrowing. Posteriorly, there are the siphons, the inhalant (is) opening into the infraseptal chamber (isc), the exhalant (es) into the supraseptal (ssc). Within the visceral mass, the digestive diverticulae (dd) are located anterodorsally with brooded oocytes (bo) present virtually everywhere else, left and right. The heart with a central ventricle (v), penetrated by the rectum (r), with left and right auricles (au), is situated posterodorsally between the visceral mass and the posterior adductor muscle. The rectum terminates in an anus (an) on the posterior face of this muscle, so that faeces exit from the individual via the exhalant siphon.

Also shown in Figure 9 (arrows) are the postulated cleansing currents of the infraseptal chamber. The positions and orientations of these cleansing currents are based on the locations of ciliary activity in this chamber, shown in subsequent figures. Having such short siphons, *G. coronata* must be feeding on prey items close to the sediment-water interface (discussed below) and it is inevitable, therefore, that some unwanted inorganic particles will be taken into the infraseptal chamber of the mantle cavity and need to be removed from it. This must be via the inhalant siphon, as in all bivalves.

Superficially, the presence of pedal retractor muscles within the visceral mass of *G. coronata* is undetectable. However, a transverse section (Fig. 10) through the left half of the visceral mass just posterior to the septum shows the posterior adductor muscle (pam) and the left posterior pedal retractor muscle (pprm). In this region of the body too, the inner fold (imf) of the mantle

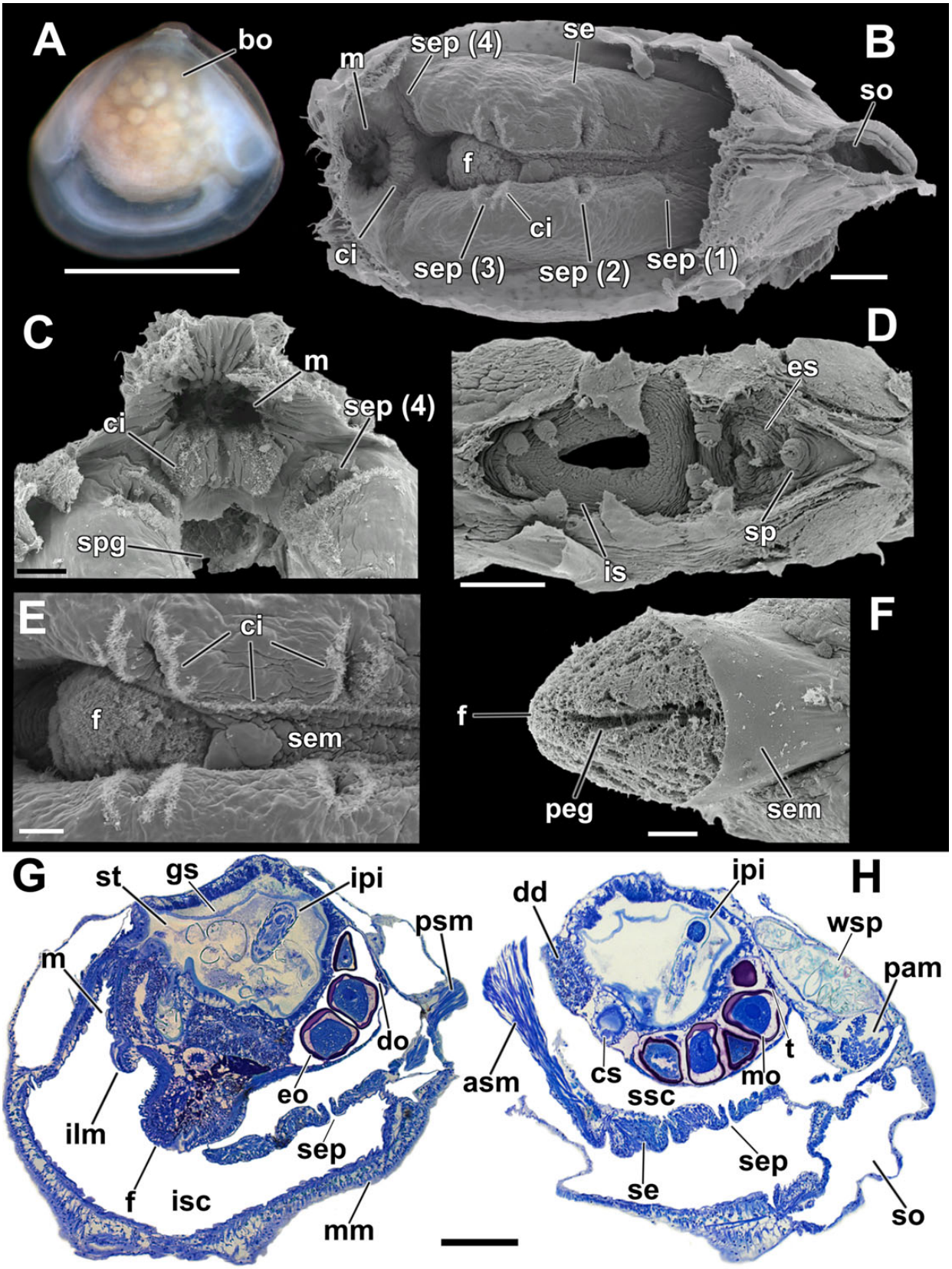


**Figure 2.** *Grippina coronata*. **A.** Photomicrograph of whole individual (from left side) showing transparency of shell and brooded oocytes in visceral mass. **B–F.** SEM images. **G, H.** Photomicrographs of histological sections. **B.** Ventral view. Mantle margin was removed showing mouth with cilia, septum with four pairs of pores, foot and siphonal opening. **C.** Higher magnification of mouth. **D.** External view of inhalant and exhalant siphons showing sensory papillae. **E.** Higher magnification of septal pores surrounded by cilia. **F.** Detailed ventral view of foot. **G, H.** Sagittal sections of same individual showing organs of mantle cavity and visceral mass, respectively. Abbreviations: see Material and Methods. Scale bars: **A =** 1 mm; **B, C =** 100 μm; **D, E, F =** 30 μm; **G, H =** 200 μm.

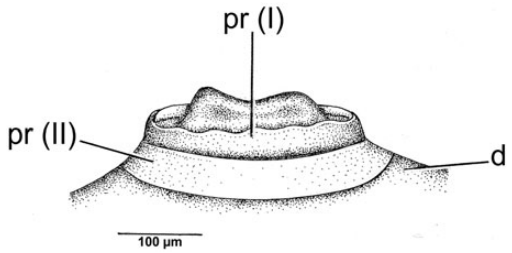


**Figure 3.** *Grippina coronata*: prodissoconchs seen from right side. Abbreviations: see Material and Methods.

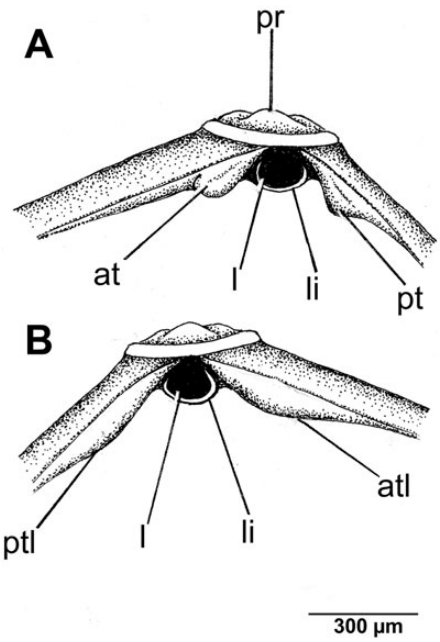


**Figure 4.** *Grippina coronata*: hinge plates of right (**A**) and left (**B**) shell valves. Abbreviations: see Material and Methods.

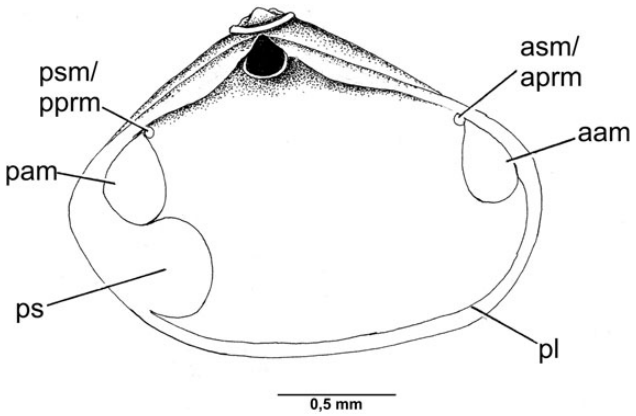


**Figure 5.** *Grippina coronata*: interior view of left shell valve showing muscle scars. Abbreviations: see Material and Methods.

(ma) margin cavity extends across the mantle cavity (mc). The stomach (st), lined by a gastric shield (gs) secreted from the stomach epithelium (sc), is here approaching its posterior extremity and associated with it is a waste-storage pouch (wsp) containing the skeletal remains of prey (srp), which will be described in greater detail below. The base of the foot possesses

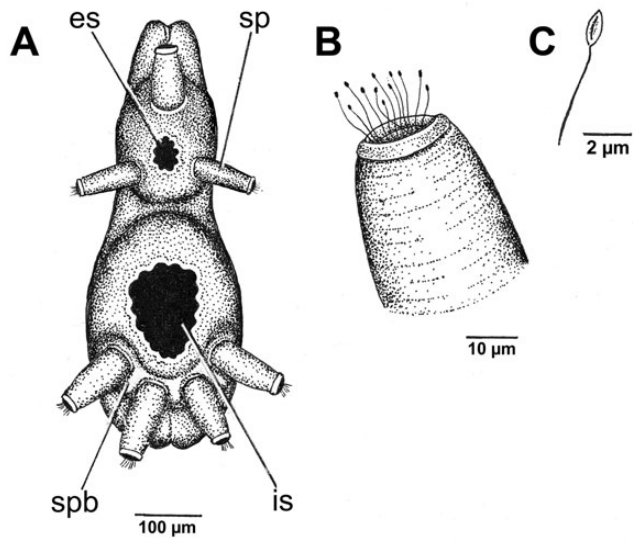


**Figure 6.** *Grippina coronata*. **A.** Withdrawn siphons seen from posterior aspect. **B.** One of siphonal papillae. **C.** One of papilla's terminal array of paddle-shaped cilia. Abbreviations: see Material and Methods.

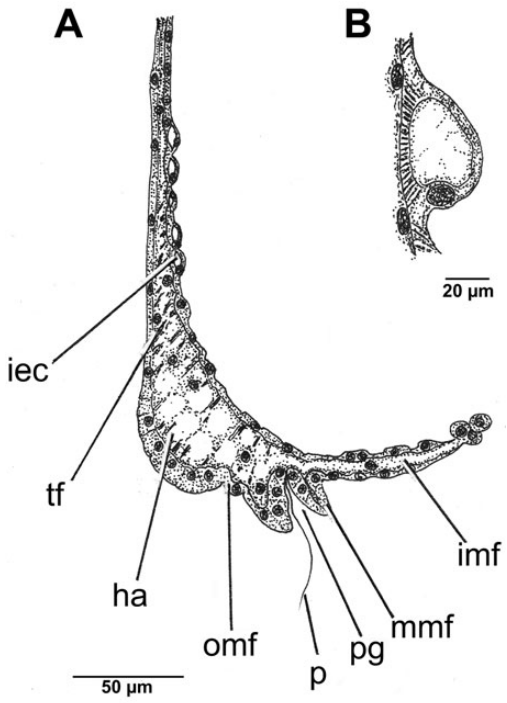


**Figure 7.** *Grippina coronata*. **A.** Transverse section through left mantle margin. **B.** Detail of inner surface of mantle showing vacuolated cells. Abbreviations: see Material and Methods.

glands (g) and this region of the visceral mass also sees the posterior termination of the gonads with, most obviously, mature oocytes (mo) and oocytes being encapsulated (obe), also to be described subsequently.

### *Septum*

The septum of *G. coronata* is illustrated from the ventral aspect in Figures 2B and 11A. The muscular septum (se) is narrow posteriorly and wide anteriorly and has an overall length of 500 to

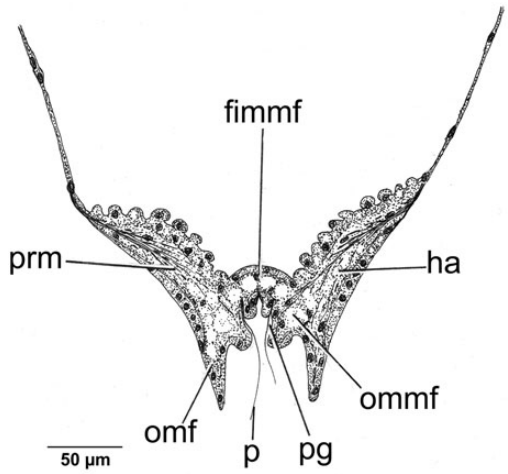


**Figure 8.** *Grippina coronata*. **A.** Transverse section through fused posteroventral mantle margin. Abbreviations: see Material and Methods.

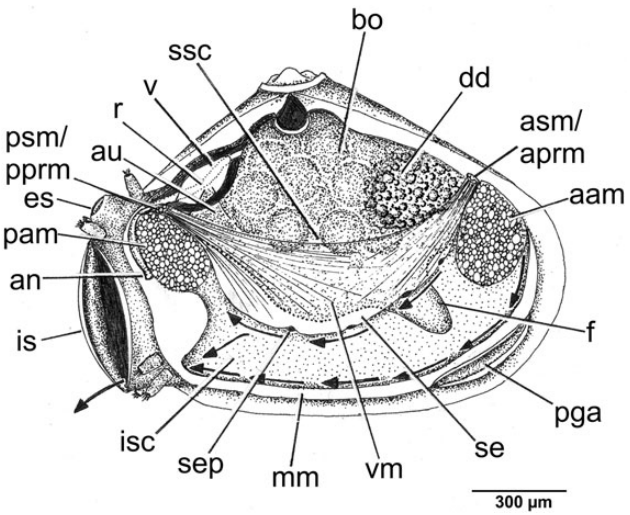


**Figure 9.** *Grippina coronata*: organs of mantle cavity seen from right side after removal of right shell valve and mantle lobe. Also shown (arrows) are postulated cleansing currents of mantle cavity based on occurrence of ciliary bundles. Abbreviations: see Material and Methods.

800 µm and a maximum width of 150 to 200 µm. Left and right halves are united by a septal membrane (sem), which separates anteriorly to create an opening (spg) through which the foot (f) can protrude. Along the length of the septum are four pairs of pores (sep(1) to sep(4)), which each have diameters of about 20 µm. Anteriorly, the septum ends at the mouth (m). There are no labial palps. Figure 11B shows a single septal pore in greater detail. Each one (sep) opens into the supraseptal chamber and is surrounded by a fringe of cilia (ci). The margin of the septal membrane (sem) is also fringed by cilia. It is probable that these cilia around the septal pores and along the margins of the septal membrane serve a cleansing function to keep the prey-capturing septum and its valves, the septal pores, free of accidentally inhaled sediment, as described and discussed above.

The foot (Figs 2B, E, F, 11C; f) of *G. coronata* extends through the septal pedal gape (spg) in the septal membrane (sem). Ventrally, the densely ciliated foot, with an overall length (when contracted) of 240 to 300 µm, has a pedal groove (peg) that may

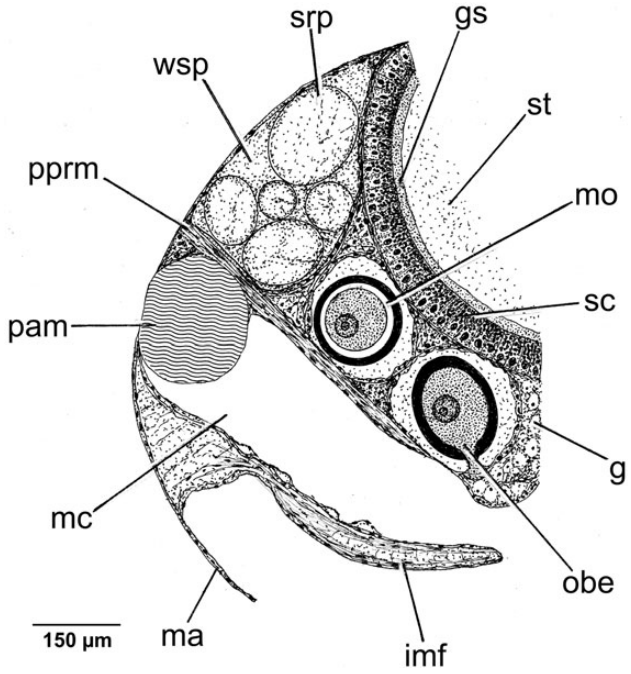


**Figure 10.** *Grippina coronata*: transverse section through left half of visceral mass posterior to septum, showing posterior adductor and posterior pedal retractor muscles. Abbreviations: see Material and Methods.

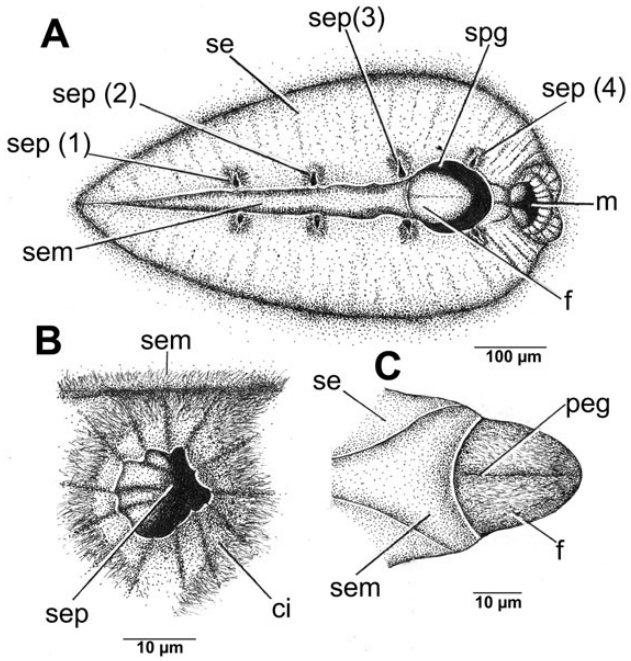


**Figure 11.** *Grippina coronata*. **A.** Septum, seen from ventral aspect. **B.** A single septal pore. **C.** Detail of tip of foot. Abbreviations: see Material and Methods.

be the remnant of a juvenile byssal groove if, as in many bivalves, such a structure is produced to assist in the establishment of the juvenile in its chosen habitat. Morton (1981b) suggested for *Poromya granulata* that the foot, in addition to being responsible for burrowing, probably also served to push captured prey items into the mouth and seal the opening; it is likely that the same functions are served by the foot of *G. coronata*.

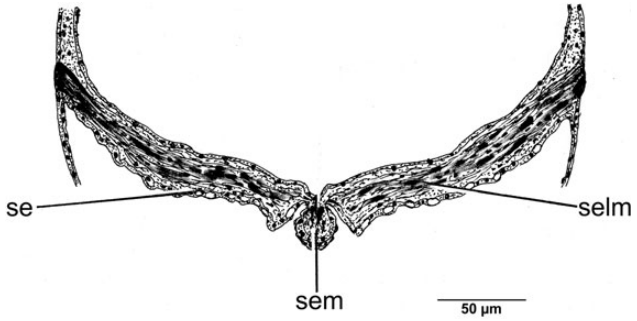


**Figure 12.** *Grippina coronata*: transverse section through septum. Abbreviations: see Material and Methods.

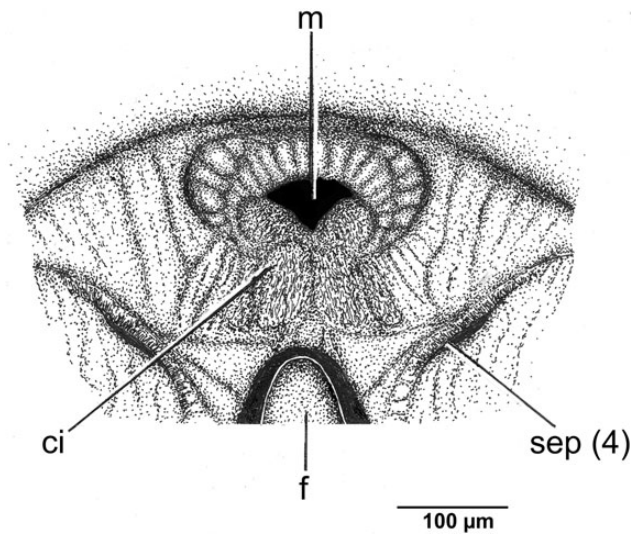


**Figure 13.** *Grippina coronata*: mouth. Abbreviations: see Material and Methods.

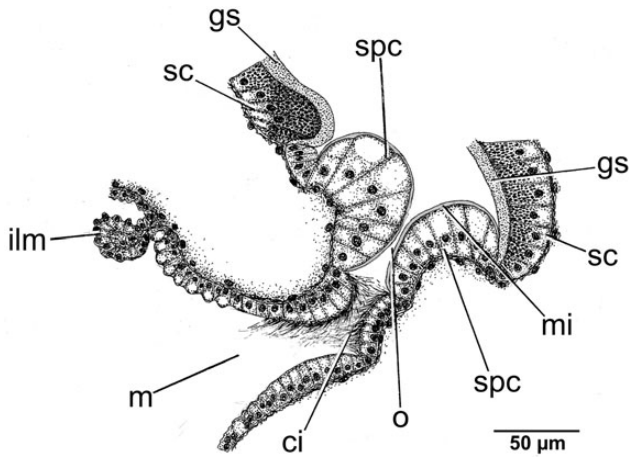


**Figure 14.** *Grippina coronata*: A sagittal section through mouth and oesophagus showing oral sphincter. Abbreviations: see Material and Methods.

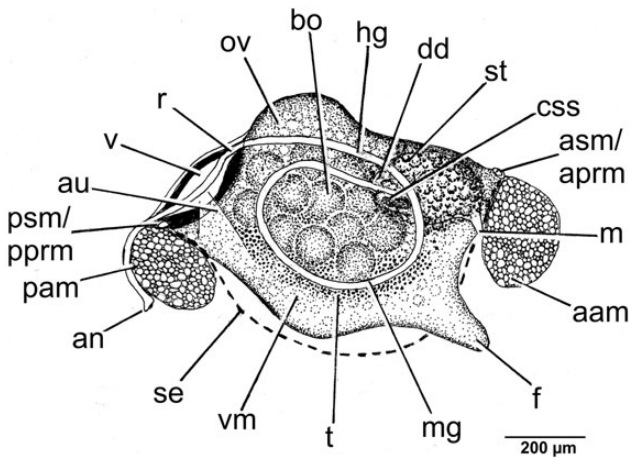


**Figure 15.** *Grippina coronata*: organs of visceral mass, seen from right side showing course of intestine and location of gonads. Abbreviations: see Material and Methods.

Figure 12 is a transverse section through the septum (se) of *G. coronata*. The central septal membrane (sem) divides the septum into left and right halves, which are connected to the shell by a rich array of septal elevator muscles (selm), responsible for the lifting of the septum to create the negative pressure inside the infraseptal chamber and thereby facilitate prey capture, as originally described for cuspidariids by Yonge (1928) and elaborated upon by Reid & Reid (1974).

### *Mouth*

The mouth (m) of *G. coronata* is shown in more detail in Figures 2C and 13. It has dimensions of 60 by 160 µm and is pleated dorsally and densely ciliated (ci) internally at its entrance, both ventrally and laterally. It has a structure similar to that described for *Halicardia nipponensis* by Nakazima (1967). There are no labial palps, which in the suspension-feeding bivalves process particles of collected seston or detritus prior to ingestion (Stasek, 1963). The anteriormost septal pores (sep(4)) are situated close to the mouth as is the medial foot (f). Figure 14 is a sagittal section through the mouth and oesophagus of *G. coronata*. Externally, the mouth has an inner lip (ilm), but not an outer. As noted above, the mouth (m) is lined internally by ciliated cells (ci) and opens into an oesophagus (o). This is surrounded by elongate (*c.* 50 µm) sphincter cells (spc) that seem to be lined distally by microvilli (mi). The gastric shield (gs) lining the stomach and secreted by underlying cells (sc) abuts the mouth closely, but is separated from it by these swollen sphincter cells. The function of the cilia within the mouth thus seems to keep it clean, whereas the oesophagus can be closed by the sphincter cells from contact with the mouth, possibly when prey items have been pushed into the stomach, to stop them getting out before being digested. The sphincter may also create a closed sac—the stomach—in which digestion can proceed more efficiently.

### *Alimentary system*

The organs of the mantle cavity and visceral mass of *G. coronata* are illustrated in sagittal section in Figure 2G, H and from the right side in Figure 15 (vm). The dotted line (Fig. 15) shows the approximate position of the septum (se) with the foot (f) penetrating it anteriorly at the septal gape. The visceral mass itself is situated between and defined by the anterior and posterior adductor muscles (aam, pam). The mouth (m) opens into the stomach (st), which is located anterodorsally in the visceral mass and is surround by its digestive diverticulae (dd). From the stomach's ventral border arises the crystalline style sac (css), which for a short distance is conjoined with the midgut (mg).

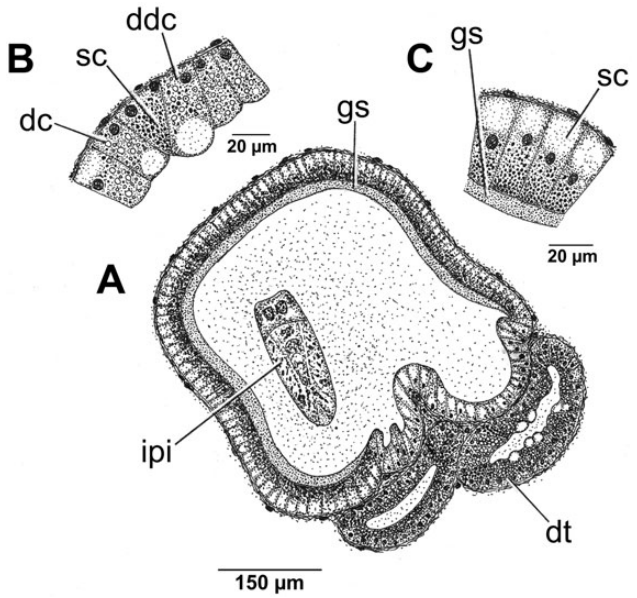


**Figure 16.** *Grippina coronata*. **A.** Transverse section through stomach. **B, C.** Cells of digestive tubules and gastric shield, respectively. Abbreviations: see Material and Methods.

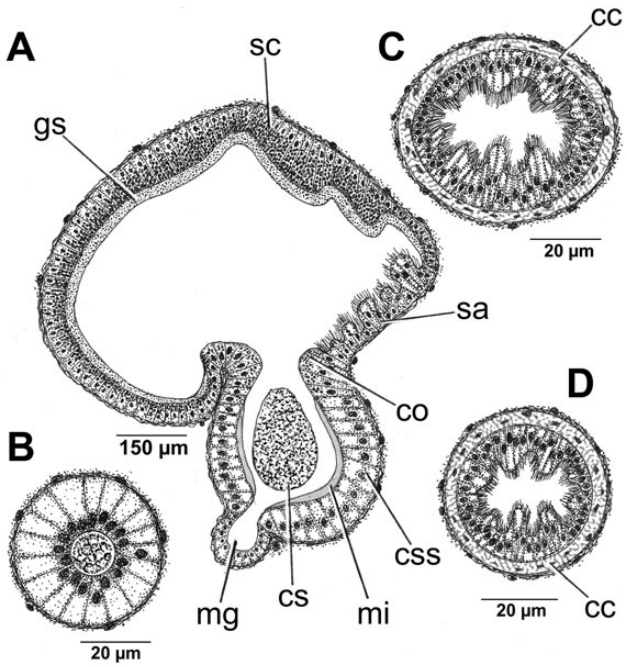


**Figure 17.** *Grippina coronata*. **A.** Transverse section through stomach in region of conjoined crystalline sac and midgut. **B, C, D.** Transverse sections though separated midgut, hindgut and rectum, respectively. Abbreviations: see Material and Methods.

The narrow midgut (*c*. 40 µm) makes a complete loop in the visceral mass and dorsally becomes the somewhat wider (*c*. 50 µm) hindgut (hg). This penetrates the ventricle (v) of the heart, passes between left and right posterior septal muscles (psm), over the posterior adductor muscle and terminates in an anus (an) on this structure's posterior face. This figure also shows brooded oocytes (bo), occupying most of the visceral mass and parts of the ovaries (ov) and testes (t) although, as will be shown later, these germinal tissues are, in fact, scattered widely in the visceral mass.

Figure 16A is a transverse section through the stomach of *G. coronata*. It shows how the stomach is almost entirely covered internally by the gastric shield (gs) and partly surrounded here by digestive tubules (dt). Inside the stomach of this individual was an ingested prey item (ipi), possibly a copepod. The cells of the digestive tubules are illustrated at higher resolution in Figure 16B. They show more darkly staining, pyramidal, secretory cells (sc) interspersed with digestive cells (dc) full of absorbed globules of different sizes and structure. Some of these cells appear to be dehiscing (ddc), i.e. releasing back into the tubule lumen vacuoles of, presumably, unwanted material that will eventually be returned to the stomach and midgut for eventual defaecation. The gastric shield (gs) (Fig. 16C) is produced by specialized secretory cells (sc) that are vacuolated basally and have darkly staining granules apically. A few studies, but not of cuspidarioids, have shown that the gastric shield is itself a source of digestive enzymes that are incorporated into its structure by such secretory cells (Halton & Owen, 1968; McQuiston, 1970).

A transverse section (Fig. 17A) through the stomach (also Fig. 2G, H, in sagittal section), here less than 1 mm in diameter, in the region of the conjoined crystalline sac and midgut shows once again the gastric shield (gs) overlying its secretory cells (sc). The opening to the crystalline style sac (css) is surrounded by a collar (co) of raised cells and flanked on the right side by a sorting area (sa) of convoluted ridges comprising ciliated cells (described for *C. cuspidata* by Purchon, 1956, 1958). The style sac (css) itself contains a stubby crystalline style (cs) and is composed of vacuolated cells that seem to be lined by microvilli (mi). The midgut (mg) separates quickly and basally from the crystalline style sac to pursue an independent course within the visceral mass (Fig. 15). Figure 17B illustrates the separated midgut in transverse section and shows it to comprise tall columnar cells, which appear vacuolated and have apical nuclei such that the lumen of this region of the intestine is exceedingly narrow. The hindgut (Fig. 17C) is wider and surrounded by a coat of smooth muscle and collagen (cc). Its epithelium is convoluted into about eight ridges and is ciliated. The rectum is narrower, but also surrounded by a thick coat of muscle and collagen (cc) and, like the hindgut, has a ciliated and convoluted epithelium (Fig. 17D).

Figure 18A is a transverse section through the stomach in the region of the waste-storage pouch (wsp) arising from its left posterior end. This is situated directly under the mantle (ma) and contains the exoskeletal remains of ingested prey (ipi). In this location too, posterior to the sorting area (sa) and the collar (co) around the crystalline style sac, the stomach continues to be lined by the gastric shield (gs), secreted from specialized cells beneath it (sc) and contains an identifiable ingested prey item (ipi)—in this case a harpacticoid copepod. Also seen ventrally in the visceral mass (vm) is an ovarian follicle (of) containing mature oocytes (mo). Figure 18B is a detail of the junction between the stomach (st) and the waste-storage pouch (wsp). The two organs are connected by a sphincter (sph), which must regulate passage of the skeletal remains of ingested and digested prey from the stomach into the pouch. Such a structure has never before been identified for any other carnivorous (or protobranch) bivalve, although a left pouch is characteristic of all other bivalves, (Purchon, 1962). Whether or not these structures are analogous or homologous is unknown.

Histological sections through prey items in the stomachs of *G. coronata* (Fig. 2G, H; ipi) show benthic harpacticoid copepods (Fig. 19A) and benthic ostracods (Fig. 19B). These taxa (hc, os) can also be identified from their skeletal remains in the waste-storage pouch (Figs 2H, 19C; wsp), along with other unidentifiable skeletal remains (srp).

Figure 20 is a reconstruction of the internal architecture of the stomach as seen from the right side. The gastric shield (gs)

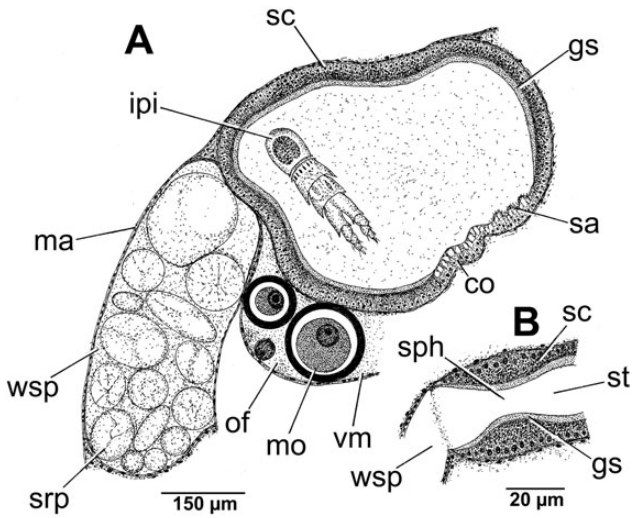


**Figure 18.** *Grippina coronata*. **A.** Transverse section through stomach in region of waste-storage pouch arising from its left posterior end. **B.** Sphincter between stomach and waste-storage pouch. Abbreviations: see Material and Methods.

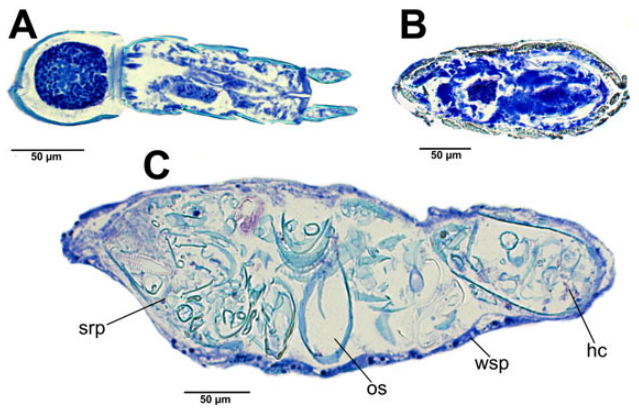


**Figure 19.** *Grippina coronata*: histological sections through prey items in stomach. **A.** Harpacticoid copepod. **B.** Ostracod. **C.** Exoskeletal remains in waste-storage pouch. Abbreviations: see Material and Methods.

covers most of the dorsal and posterior surfaces of the stomach. The crystalline style sac (css) with its stubby style (cs) and the conjoined midgut (mg) depart the stomach from its ventral border. Anterior to the collared entrance (co) to the crystalline style sac (css) is an anteroventral series of ciliated ridges that constitutes a sorting area (sa) and within this are two, left and right, openings into the digestive diverticulae (odd). The mouth (m) is located anteroventrally and opens into the stomach as the oesophagus (o), which has internal sphincters (sph). Recently, Tëmkin & Strong (2013) described the internal architecture of the stomach of *Bathyneaera demistriata* (Allen & Morgan, 1981) (Cuspidariidae) and showed an essentially similar structure to that of *G. coronata*, although without identifying a sorting area as such. Regardless, their illustration (Fig. 3) of *B. demistriata* shows extensive internal folds in the same region as seen in *G. coronata*.

### *Statocysts*

It is not possible to detail the structure of the nervous system of *G. coronata*. Transverse sections through the tiny (50 µm) paired pedal ganglia (pega) in the visceral mass are illustrated in Figure 21. Separate from these is a pair of statocysts (50 µm)

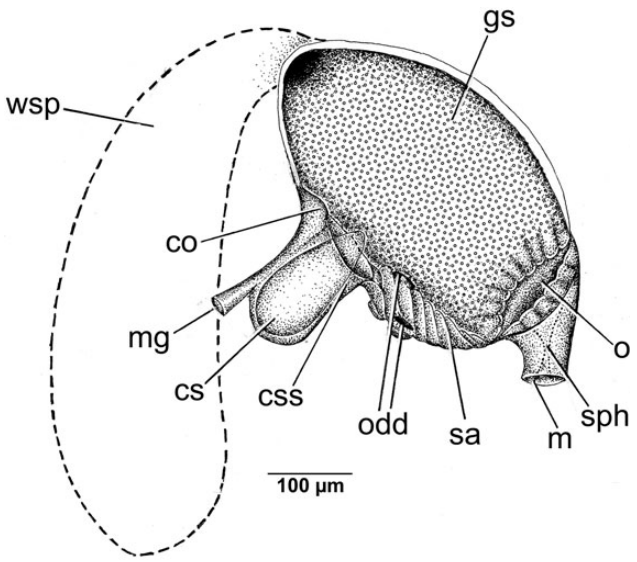


**Figure 20.** *Grippina coronata*: reconstruction of internal architecture of stomach seen from right side. Abbreviations: see Material and Methods.

(sta) that are capsules formed by 4–5 cells (in transverse section), a few possessing cilia (ci) and each having a single darkly staining statolith (stl), about 20 µm in diameter, in the centre. Morton (1985) examined statocyst structure in numerous representatives of the Anomalodesmata and those of *G. coronata* are closely similar to those of other representatives of the Cuspidarioidea, i.e. of Type C in which they are distinctively separate from the pedal ganglia.

### *Reproductive system*

A transverse section through the visceral mass (Fig. 22) shows the intestine (i), the tubules of the digestive diverticulae (dt), the foot (f) with a gland (g) at its base and elements of the reproductive system. *Grippina coronata* is a simultaneous hermaphrodite. The paired female ovaries are located virtually everywhere in the visceral mass, including the epithelium within which it is enclosed and, within its central region, separating two exceptionally large, what are here termed ovarian follicles (of). As will be seen, however, the tissues of the testes (t) are also present as mesodermal elements scattered similarly throughout the visceral mass and occur in association with the developing oocytes in the walls of the ovarian (and thus also testicular) follicles and elsewhere (Fig. 2G, H).

The paired ovarian follicles contain oocytes at various stages of maturity, including fertilized ones (Fig. 22). These developmental stages include developing oocytes (do), some of which are being encapsulated (eo) and measure up to *c.* 50 µm in diameter. The number of encapsulated oocytes (oc) within each ovarian follicle varies but, generally, there are about ten each, or a maximum of 20 (not including the many developing oocytes in the follicle walls). No oviducts or seminal ducts connecting the paired gonads to the supraseptal chamber have been identified. Similarly, no fertilized oocytes have been identified within the supraseptal chamber.

In a histological section through a single ovarian follicle (Fig. 23; of) the production of oocytes and spermatozoa and fertilization process can seen. In this section is a mature, fertilized and encapsulated, oocyte (mo). The fertilized oocytes of any anomalodesmatan have only been identified and described hitherto for *Entodesma cuneata* (Gray, 1828) by Campos & Ramorino (1981) and *Laternula elliptica* (King & Broderip, 1832) by Ansell

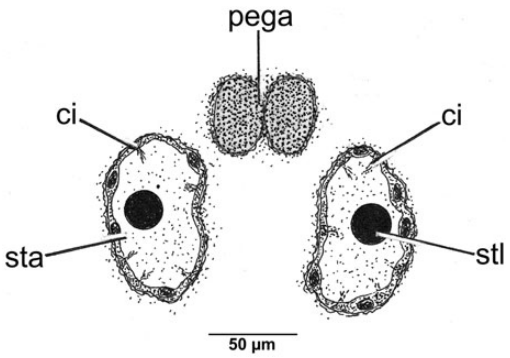


**Figure 21.** *Grippina coronata*: transverse sections through paired statocysts and pedal ganglia. Abbreviations: see Material and Methods.

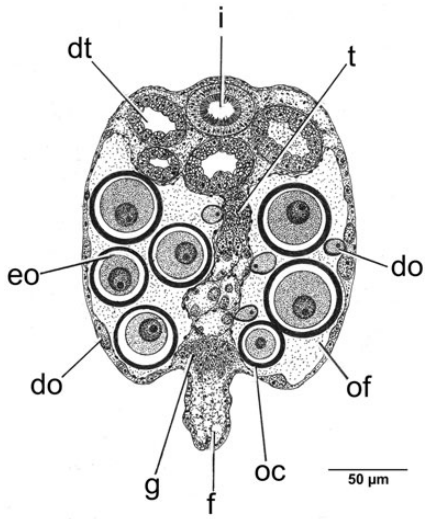


**Figure 22.** *Grippina coronata*: transverse section through visceral mass showing regions of intestine and reproductive system. Abbreviations: see Material and Methods.

& Harvey (1997) and for both of which a capsule (as in *G. coronata*) is surrounded by a fine sheet of mucus that, upon release from the parent, swells to become a thick, still mucoid, envelope. Figure 23 also shows how the capsule is formed around the oocyte (obe) as it develops from the follicular wall. In addition, Figure 23 shows that the follicle wall possesses germinal areas for spermatozoa. These groupings of testis tissues (t) comprise spermatocytes that are seen to develop into strings of spermatids (spt), which develop, in turn, into spermatozoa (spe) that are occasionally seen as clusters or newly divided sperm morulae (spm). The spermatozoa have elongate acrosomal heads. The spermatozoa of *Cuspidaria latesulcata* (Tenison-Woods, 1878), also with long acrosomal heads, have been described by Healey, Bieler & Mikkelsen (2008). Finally, Figure 23 shows an oocyte that is being fertilized (obf) as it buds off from the germinal follicle wall—which it must be, before the capsule can be laid down around the enlarging oocyte.

## DISCUSSION

Until recently, representatives of the Spheniopsidae had only been recorded from Pacific and North Atlantic waters. The occurrence of *Grippina coronata* in Brazilian waters (Machado & Passos, 2015), however, expanded the distribution of this family

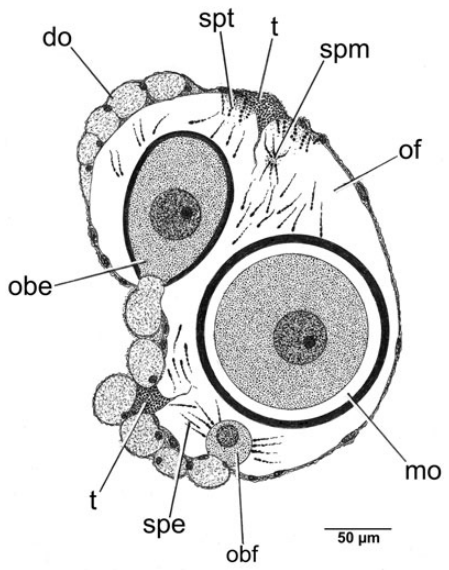


**Figure 23.** *Grippina coronata*: Single follicle of gonad showing production of oocytes and spermatozoa. Abbreviations: see Material and Methods.

to the shallow and tropical waters of the South Atlantic. Major interest in the hitherto poorly studied Spheniopsidae, however, centres around four other aspects of the family: (1) taxonomic affinities, (2) lifestyle, (3) reproductive strategy and (4) life history.

### *Taxonomic affinities*

Based on dried specimens of *G. californica*, Coan *et al.* (2000) described the Spheniopsidae as being characterized by a fused mantle margin with a small pedal gape, two short conjoined siphons and with incubation of fertilized eggs in the mantle cavity. Only the description of the mantle margin is correct, however, as this study shows. The same authors considered that the Spheniopsidae were allied with the Heterodonta and placed them between the Corbulidae and Hiatellidae, i.e. within the Myoidea. The reason for this, it is suggested, is because spheniopsids possess hinge teeth, which are characteristically absent in all, save a few, anomalodesmatans (Morton, 1981a), including representatives of the Cuspidariidae. Similarly, the myoid ligament is typically amphidetic and located on either resilifers or chondrophores as in many anomalodesmatans such as species of *Laternula* (e.g. *L. truncata*, Morton, 1973), although no myoid possesses a ligamental lithodesma. Though this classification was later amended to place the Spheniopsidae in the Anomalodesmata—principally and firstly in the Cuspidarioidea by Marshall (2002) and subsequently by Bieler *et al.* (2010), Carter *et al.* (2011) and Coan & Valentich-Scott (2012)—there has not been any anatomical (or molecular) evidence provided in support of this until the present research.

Table 1 provides a comparison between the anatomy of *G. coronata* and of representatives of the Cuspidariidae. There are a few significant differences, notably with regard to a nonrostrate shell and the presence of hinge teeth in *G. coronata*, an inhalant siphon that is relatively short and the absence of any vestiges of labial palps. Nevertheless, the overriding picture confirms a closer affinity with the Cuspidariidae than with any of the other septibranch bivalves (Morton, 1981a). Importantly, Allen & Morgan (1981) noted that all species of *Cuspidaria* have seven siphonal tentacles, four along the ventral edge of inhalant siphon and three around the exhalant, an arrangement identical to that seen in *G. coronata* (Fig. 6).

**Table 1.** Morphological comparison between *Grippina coronata* (Spheniopsidae) and representatives of the Cuspidariidae (*Cuspidaria cuspidata*, *C. obesa* and *Cardiomya cleryana*)

| Character | Spheniopsidae | Cuspidariidae | References |
|---|---|---|---|
| Shell | Inequilateral, sub-trigonal | Typically rostrate | Coan & Valentich-Scott (2012), Absalão & Oliveira (2011) |
| Hinge teeth | Present, right valve only | Present, right valve only | Allen & Morgan (1981) |
| Ligament | Internal, amphidetic | External, opisthodetic or amphidetic | Allen & Morgan (1981), Coan & Valentich-Scott (2012) |
| Lithodesma | Present | Present | Marshall (2002) |
| Foot | Short stubby, pedal/byssal groove present | Short, stubby, pedal/byssal groove present | Allen & Morgan (1981), Coan & Valentich-Scott (2012) |
| Byssal groove | Present | Present | Allen & Morgan (1981), Coan & Valentich-Scott (2012) |
| Byssus | Possibly present in the post-larva | Post-larva only | |
| Siphons (category) | Separate (Type B) | Separate (Type B) | Reid & Reid (1974), Yonge (1982) |
| Siphonal papillae/tentacles | 7: 3 around the exhalant and 4 around the inhalant | 7: 3 around the exhalant and 4 around the inhalant | Allen & Morgan (1981), Coan & Valentich-Scott (2012) |
| Ciliated sensory papillae | Present | Present | Reid & Reid (1974) |
| Arenophilic glands | Absent | Present (in some) | Oliveira & Sartori (2013) |
| Mantle fusion | Type B | Type B | Yonge (1982) |
| Musculature | Isomyarian | Isomyarian | Allen & Morgan (1981) |
| Septal retractor muscles | Present | Present | Yonge (1928), Reid & Reid (1974) |
| Septal elevator muscles | Present | Present | Yonge (1928), Reid & Reid (1974) |
| Septal pores | Four pairs | Typically, four pairs | Yonge (1928), Morton (1987) |
| Pedal musculature | Reduced | Reduced | |
| Labial palps | Absent | Absent | Yonge (1928), Coan & Valentich-Scott (2012) |
| Stomach type | Type II | Type II | Purchon (1958) |
| Intestine | Long | Short | |
| Style sac and mid gut | Conjoined | Conjoined | Morton (1987) |
| Rectum | Penetrates ventricle of heart | Penetrates ventricle of heart | Yonge (1928) |
| Waste-storage pouch | Present | Absent | Bernard (1974) |
| Statocysts | Present, Type C | Present, Type C | Morton (1985) |
| Sexuality | Simultaneous hermaphrodite | Dioecious | Bernard (1974), Allen & Morgan (1981), Coan & Valentich-Scott (2012) |
| Fertilization | Internal | External | |
| Development | Direct | Direct | Bernard (1974) |
| Brooding | Internal, in visceral mass | Not known | |

### *Lifestyle*

In the deep sea a carnivorous lifestyle has been adopted by representatives of three bivalve clades, the Propeamussiidae (e.g. *Propeamussium lucidum*, Morton & Thurston, 1989), the Bathymodiolinae (e.g. *Idas argenteus*, Ockelmann & Dinesen, 2011) and four families of the Anomalodesmata—the Parilimyidae (Morton, 1982) and the three extant superfamilies of the clade Septibranchia: Verticordioidea, Poromyoidea and Cuspidarioidea (Bieler *et al.*, 2010). This study adds another family, the Spheniopsidae, to this list of predatory bivalves, albeit at the most miniature (<2 mm) of scales.

Having determined the shell structure and form of the siphonal apparatus of *G. coronata*, it is possible to reconstruct not just its life position, shallowly buried in the sediment (Fig. 24A), but also to understand how it captures its prey (Fig. 24B, C). As described, there is incontrovertible evidence that *G. coronata* is a predator, because within the mantle cavity there is a septum typical of, particularly, cuspidariids (Reid & Reid, 1974) and a stomach that has, again, a structure typical of cuspidariids (Type II, Purchon, 1956) and that contains prey items, identifiable among which are a harpactocoid copepod and an ostracod. These two taxa are typically benthic (Bell, Walters & Hall, 1987; Gage & Tyler, 1991; Huys & Boxshall, 1991; Mackiewicz, 2006), the harpacticoids either excavating into (endopsammic) or living on the surface of the sediment (epipsammic), in both cases being accessible to *G. coronata* as prey.

Accordingly, given the short siphons of *G. coronata* and their associated ciliated sensory apparatus, we can hypothesize that such prey items are detected by these (Fig. 24B) and captured by the rapid eversion of the inhalant siphon (Fig. 24C), as described for *Cuspidaria rostrata* by Reid & Reid (1974: fig. 1), although that species has a much longer siphonal structure, encased within the shell's posterior rostrum. Moreover, Reid & Crosby (1980: fig. 5a) showed that the siphonal papillae (or tentacles) of *Cardiomya planetica* possess bunches of sensory cilia at their apex, as in *G. coronata*, and which were presumed to be mechanoreceptors.

An important distinction between cuspidariids and *G. coronata* is that the shells of the former are invariably rostrate to accommodate the longer siphons and create a tube, like a gun barrel, to focus rapid siphonal eversion and the capture of swimming prey, e.g. copepods, as illustrated for *C. rostrata* by Reid & Reid (1974: fig. 1). Lacking this shell rostrum and possessing only short siphons, it seems probable that *G. coronata*, and possibly

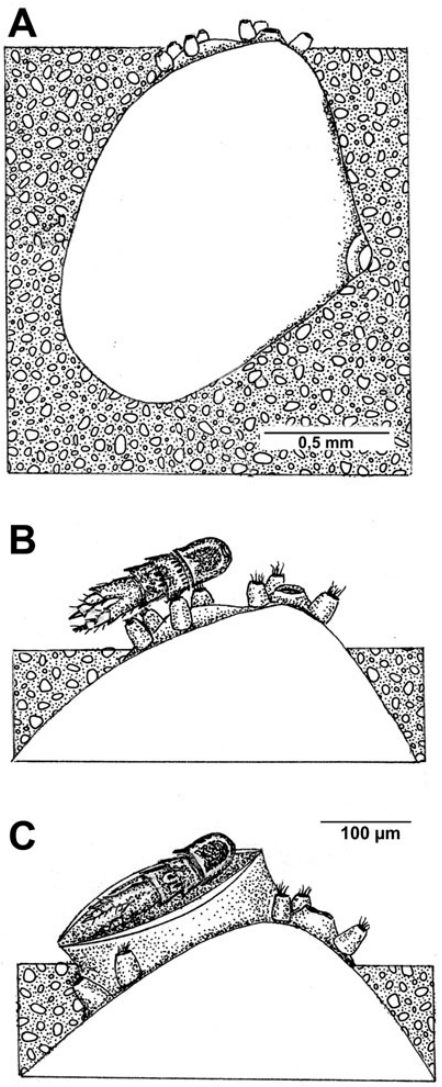


**Figure 24.** *Grippina coronata*: hypothesized mode of life. **A.** An individual in life position. **B.** The siphonal papillae being stimulated by a passing epibenthic harpacticoid copepod. **C.** Prey capture by the everted and extended inhalant siphon.

other spheniopsids with a similarly foreshortened posterior shell face, feeds on surface-dwelling taxa such as the benthic harpacticoid and ostracod identified from the stomach contents. They therefore exploit a different food resource from that of the Cuspidariidae. Spheniopsids and cuspidariids are both, however, immobile predators that 'lie-in-wait' to ambush prey.

A unique feature of the intestine and stomach of *G. coronata* is the presence in the latter of a waste-storage pouch. As described, the intestinal tract itself is narrow (midgut lumen <40 µm) with no evidence of the skeletal remains of these relatively large prey items within it. Instead, such remains appear to be stored in a pouch accessed via a sphincter from the left wall of the stomach. As described, harpacticoid copepods and ostracods are found in the stomach and their exoskeletal remains in this storage pouch, thereby confirming their digestion. Fine, digested remains must therefore be transported from the stomach to the intestine and expelled from the anus as in all, more typical, suspension-feeding bivalves. The skeletal remains of prey in such a tiny spheniopsid as *G. coronata* cannot, however, be removed in this manner and, accordingly, are stored in the waste-storage pouch from which they will never be evacuated in life. As will be described below, *G. coronata* can only release large oocytes after death and the same strategy seems to have been adopted for defaecation also, creating an altogether remarkable life-history trait.

### *Reproductive strategy*

Representatives of the Cuspidariidae are generally considered to be dioecious (Bernard, 1974). This is clearly not the case with *G. coronata*, which is here demonstrated to be a simultaneous hermaphrodite. Oocytes are budded off from mesodermal epithelia into large left and right follicular lobes within the visceral mass. The same germinal epithelia that produce the oocytes also produce spermatocytes, so that fertilization is not just internal, it is intrafollicular. As far as is known, this is unique in the Bivalvia. Fertilized oocytes are enclosed in a thick capsule. It is unknown if *G. coronata* possesses gonopores, the sectioned individuals being simply too small to determine this. Regardless, however, such large (>50 µm) fully-mature oocytes would be far too big to exit the parent in the usual manner. Indeed, they would even have trouble being discharged from the exhalant siphon. The oocytes are, in fact, so well developed when they are released from the parent that the prodissoconch I, at least, is fully formed and probably prodissoconch II also (Machado & Passos, 2015: fig. 3).

*Grippina coronata* thus exhibits direct development to produce a lecithotrophic larva (as defined by Ockelmann, 1965) that almost certainly has an exceedingly short benthopelagic life, if at all. Since it would seem impossible for *G. coronata* to discharge fertilized oocytes in any conventional manner, it is suggested that fully mature individuals of this this species die, thereby releasing their fertilized and encapsulated oocytes within the protection of their mucoid outer capsule, *post-mortem*. Fertilized but unencapsulated oocytes would die at this time. The only other anomalodesmatans that are known to produce such encapsulated oocytes are *Entodesma cuneata* (see Campos & Ramorino, 1981) and the stenothermal *Laternula elliptica* from Antarctic waters (Ansell & Harvey, 1997). The oocytes of both these species, further surrounded by a thick, gelatinous envelope, are released (by a means unknown) and remain in the plankton for only a few days. In the water column, the gelatinous layer surrounding the eggs forms into a strong, sticky and elastic capsule within which development proceeds. It seems possible that, since its intrafollicular oocytes are essentially the same as those described above for the other anomalodesmatans, a similar developmental strategy is adopted by *G. coronata*, albeit following parental death.

### *Life history*

The pioneering research of Ockelmann (1965) showed that at greater depths in the Arctic North Sea an increasing number of bivalve species produce lecithotrophic fertilized eggs with either a short pelagic larval life or with maternal, typically ctenidial, brooding. The greater incidence of species showing direct development with brood protection was, however, more common in shallower-shelf species. This generalization seems applicable to *G. coronata*, recorded by Machado & Passos (2015) from depths of 21 to 53 m off the coast of Brazil. An example of miniaturization having a dramatic effect upon reproduction was provided by Sanders & Allen (1973) in a deep-water species of *Microgloma* (Protobranchia: Pristoglomidae) which, at any one time, only has two mature oocytes in left and right ovarian follicles. These authors attributed this reduction in egg numbers to the small size of these protobranchs, which consequently are unable to produce a sufficient number of planktotrophic larvae and therefore produce a small number of eggs that undergo nonplanktotrophic development. The life history of this minute septibranch, *G. coronata*, however, releasing fertilized and encapsulated

oocytes following parental death, is even more extreme. This *post-mortem* semelparity and similarly *post-mortem* release of undigested food remains, represent life-history traits hitherto unseen in the Bivalvia.

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