Spermatozoon Ultrastructure of *Prisogaster niger* (Wood, 1828) (Mollusca: Vetigastropoda): Supporting Affinities between the Prisogasterinae and Turbininae

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Gonzalo A. Collado, Claudio L. Correa, Marco A. Méndez, and Donald I. Brown (2011) Spermatozoon ultrastructure of *Prisogaster niger* (Wood, 1828) (Mollusca: Vetigastropoda): supporting affinities between the Prisogasterinae and Turbininae. Zoological Studies 50(6): 773-779. Recent phylogenetic analyses of Vetigastropoda using DNA sequences suggest that the subfamilies Prisogasterinae and Turbininae form a monophyletic group within the recently redefined family Turbinidae. To verify this systematic arrangement, we describe the spermatozoon ultrastructure of *Prisogaster niger*, a reproductive anatomical component that has been successfully used in phylogenetic investigations of vetigastropods, and compare the results with previously published information from other trochoidean taxa based on a phylogeny of the group. *Prisogaster niger* has a primitive spermatozoon, or aquaspermatozoon, typical of invertebrates with external fertilization. The sperm has a conical pointed acrosome which is > 50% of the head length, a barrel-shaped nucleus, a midpiece with 2 centrioles and 5 spherical mitochondria, and a simple flagellum with 9+2 axonemes. Compared to other trochoideans, the spermatozoon of *P. niger* is notably similar in structure to those of the Turbininae, with shared synapomorphies (e.g., an elongated conical pointed acrosome and a broad subacrosomal space) that suggest a close relationship between the Prisogasterinae and this subfamily, and that *Prisogaster* may have originated from an ancestral Turbininae. http://zoolstud.sinica.edu.tw/Journals/50.6/773.pdf

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The genus *Prisogaster* Mörch, 1850 includes the only living species, *Prisogaster niger* (Wood, 1828), and 2 fossil species recently described by DeVries (2006). It is unclear whether *P. elevatus* (Eydoux and Souleyet, 1852) recorded from northern Chile (Ramírez 1981, Valdivieso 1987, Lancelloti and Vásquez 2000, Nicosia and Gaete 2003) also represents a valid taxon (DeVries 2006). *Prisogaster niger* is a common marine snail that is of ecological importance in intertidal and subtidal rocky shore communities along the southwestern coast of South America because of its role as a grazer of algal communities (Vásquez and Vega 2004). Its distributional range extends from Paita, Perú, to the Straits of Magellan, Chile (Marincovich 1973, Osorio et al. 1979, Ramírez 1981, Alamo and Valdivieso 1987). In Chile this snail, popularly termed "lilihuen" (Osorio et al. 1979), is taken in limited quantities for human consumption.

The systematic position of *Prisogaster* is uncertain because different combinations of anatomical characters have been used for its classification. The genus was alternately considered a member of the family Phasianellidae.
(Thiele 1929, Wenz 1938) and Turbinidae; and within the latter, was assigned to the subfamily Turbininae (Robertson 1958, Knight et al. 1960, Ramírez 1981). To resolve this systematic uncertainty, Hickman and McLean (1990) erected the turbinid subfamily Prisogasterinae to include this “unusual” taxon. More recently, 2 phylogenetic analyses of the Vetigastropoda using DNA sequence data showed that the Turbinidae (sensu Hickman and McLean 1990) was a polyphyletic group which was redefined as a subset of those groups diagnosed by Hickman and McLean (1990), including the Prisogasterinae and Turbininae, which were proposed as sister groups (Williams and Ozawa 2006, Williams et al. 2008).


Studies on the reproductive biology of Prisogaster have not been performed to date. However, Hickman and McLean (1990) studied the epipodium, radula, and additional internal anatomical characteristics of *P. niger*. The aims of the present study were: 1) to describe in detail the spermatozoon ultrastructure of *P. niger* and 2) to verify the sister group relationship between the Prisogasterinae and Turbininae by comparing our results to previously published information from other trochoidean taxa.

**MATERIALS AND METHODS**

**Sample collection**

Mature adults of *P. niger* were collected at low tide from rocky shores at La Herradura, Coquimbo region, Chile (29°57'56.4"S, 71°21'09"W). After collection, snails were transported to the laboratory and kept in aerated seawater. Voucher adult specimens were deposited in the Museo Nacional de Historia Natural de Chile, registration number MNHNCL 7615-7617.

**Spermatozoon**

For transmission electron microscopy (TEM), the shell of 1 mature individual was removed and 1 mm³ pieces of the testes were fixed for 2 h in 2% glutaraldehyde in 0.25 M pH 7.4 cacodylate buffer (with 17.5% sucrose) in a solution isomolar to seawater. The samples were subsequently rinsed with the same buffer and postfixed for 2 h in 2% osmium tetroxide and buffer containing 25% sucrose. Fixation and rinsing were carried out at 4°C. Samples were dehydrated in a series of ascending concentrations of ethanol, ethanol-acetone, and acetone and embedded in Medcast hard-mixture resin. Ultrathin sections, obtained in a Reichert OM-U2 ultramicrotome (Vienna, Austria), were stained with uranyl acetate and lead citrate, and observed in a Zeiss 900 TEM (Oberkochen, Germany) at 80 kV. For ultrastructural dimensions of organelles, a suspension of spermatozoa was obtained by agitating a small piece of testis fixed in an 85% alcohol, 10% formalin, and 5% acetic acid (AFAA) solution. Samples of the spermatozoa suspension were deposited on glass microscope slides and stained with hematoxylin and erythrosine-orange G. Images of 20 individual spermatozoa were taken at 1000x using a Leitz-Leica model DMRBE light microscope (Wetzlar, Germany) equipped with a Leica DFC 290 digital camera (Wetzlar, Germany). Ultrastructural measurements, made using the software NIH 1.6 (US National Institutes of Health), are given as the mean and standard deviation. Measurements included the length of the head, the length of the head plus the midpiece, the length and width of the nucleus, and the length and width of the acrosome.

**Comparison between morphological and molecular data**

Characters of the spermatozoon of *P. niger* and representatives of the Turbinidae (sensu stricto) were compared and mapped onto the phylogeny of the group recovered by Williams et al. (2008) using molecular data. For this analysis, we restricted the tree to taxa at the subfamily level within the Turbinidae redefined considering the genus *Tectus* as a sister group of the Tegulinae (Williams et al. 2008). Although the monophyly of the subfamily Skeneinae needs to be further evaluated, we tentatively assumed that *Zalipais laseronti* Kershaw is a representative of this group. Longitudinal sections through...
spermatozoa were also compared with 3 families classically recognized within the Trochoidea (sensu lato): Trochidae, Phasianellidae and Turbinidae (Hodgson and Foster 1992, Hodgson 1995).

RESULTS

Spermatozoon ultrastructure

The spermatozoon of *P. niger* is of the primitive (Franzén 1955) or "aquaspermatozoon" type (Jamieson 1987), typical of species that release their gametes into the surrounding aquatic medium. The spermatozoon is bullet-shaped, and on the antero posterior axis is composed of a head with a conical acrosome and a barrel-shaped nucleus (Fig. 1A), a midpiece (Fig. 1B) of 5 mitochondria (Fig. 1C) organized around the proximal and distal centrioles, and a flagellum with a 9+2 microtubule arrangement arising from the distal centriole. The head of the spermatozoon of *P. niger* is 5.30 ± 0.10 µm long and 1.2 ± 0.08 µm wide. The head with the midpiece (0.5 ± 0.07 µm long) is 5.79 ± 0.07 µm long. The nucleus, located in the base of the head, is 2.09 ± 0.07 µm long by 1.2 ± 0.08 µm wide, and has highly condensed chromatin, although with some internal lacunae where the chromatin is less compact. The extreme apex of the nucleus has a slight invagination, while its base has a marked invagination near the proximal centriole. The elongated and conical acrosome at the anterior of the head is 3.21 ± 0.10 µm long by 1.05 ± 0.06 µm wide, and makes up about 60.4% of the length of the head. The acrosome is invaginated at its base to form the subacrosomal space. The internal acrosomal content is differentiated into a more-electron-dense region in the basal zone, which occupies more than 1/4 of its total length.

Morphological comparison with other trochoidean taxa and molecular evidence

Compared to representatives of the classical Trochoidea, the spermatozoon morphology of *P. niger* is most similar to members of the Turbinidae; it notably differs from spermatozoa of the Phasianellidae and Trochidae (Fig. 2A). Furthermore, the spermatozoon of *P. niger* is very similar in structure to those of the Turbininae compared to other representatives of the Turbinidae sensu stricto; the comparison agrees with the tree based on molecular data which show that the Prisogasterinae is sister to the Turbininae (Fig. 2B).

DISCUSSION

Characters of diagnostic value for the Prisogasterinae

*Prisogaster niger* was initially described as *Turbo niger* Wood, 1828 and subsequently included by Mörch (1850) in the new genus *Prisogaster* without providing any basis for this decision (DeVries 2006). Later, the subfamily Prisogasterinae was erected by Hickman and McLean (1990) based on the following characters present in *P. niger*: “Peristome interrupted. Operculum with a thick, convex exterior calcareous pad. Ctenidium bipectinate with long dorsal afferent membrane. Left neck lobe digitate, small cephalic lappets present, lip split midventrally,
Fig. 2. (A) Longitudinal sections through spermatozoa characteristic of 3 families of the Trochoidea, based on classical taxonomy following Hodgson and Foster (1992) and Hodgson (1995). Representative species from genera and families selected by these authors were Oxystele sinensis (Gmelin), Tricolia capensis (Dunker), and Turbo sarmaticus L. (B) Congruence between morphological characters of the spermatozoa of trochoidean taxa and the topology of the combined tree constructed by Williams et al. (2008) using molecular data. Taxa in the topology were restricted to subfamilies within the Turbinidae sensu stricto, and the genus Tectus was considered a sister group to the Tegulinae (Williams et al. 2008). Sources of the genera selected: Tectus: Tec. pyramis (Shimoda) from Koike (1985), Tegula: Teg. quadricostata (Wood) from Collado et al. (2008) (left) and Teg. argyrostroma from Koike (1985) (right); Zalipais: Z. laseroni Kershaw from Healy (1990) (Modeled after Hodgson 1995); Prisogaster: P. niger (Wood), present study; Lunella: Lun. cinerea (Ishigaki I.) (left), Lun. granulata (Irimote I.) (right), and Batillus: B. cornutus (Shimoda) from Koike (1985); and Turbo: Tur. coronatus (Gmelin) (left) and Tur. cidaris natalensis Krauss (right) from Hodgson and Foster (1992). a, acrosome; af, anterior nuclear fossa; d, differentiated acrosomal content; f, flagellum; m, mitochondrion; n, nucleus; pm, plasma membrane; s, subacrosomal space. Scale bar = 1 µm.
but lacking pseudoproboscis”. The differentiated internal content at the base of the acrosome of *P. niger*, slightly exceeding the subacrosomal space, is a derived character state of additional diagnostic value for the Prisogasterinae, considering that it has not been observed in other vetigastropods. According to DeVries (2006), the morphology of the operculum and the quadripartite structure of the columella are also distinctive characters for the genus and subfamily.

Comparison between morphological characters and molecular data

The spermatozoon morphology of *P. niger* follows the structural pattern found in representatives of the Turbinidae considering its classical taxonomy (Fig. 2A). In this group, spermatozoa have a nucleus with a wide anterior invagination and large conical acrosome. The size of the acrosome is > 50% of the total head length and has a differentiated internal content (Hodgson 1995). These characteristics represent an evolutionary novelty of the subfamily compared to spermatozoa of the Phasianellidae and Trochidae (Hodgson and Foster 1992, Hodgson 1995), which are more plesiomorphic (Hodgson 1995). At the subfamily level within the Turbinidae sensu stricto, spermatozoa of the Prisogasterinae and Turbininae are markedly similar; and this agrees with the phylogeny of Williams et al. (2008) which showed that these are sister taxa (Fig. 2B). The shared characteristics which support this assemblage based on observations of spermatozoa include an elongated, conical, pointed acrosome and broad subacrosomal space with a tendency to become triangular at the base. Also, in more-inclusive terms, *Prisogaster* shares characters with members of the genus *Turbo*, including a wide anterior depression in the nucleus, a subacrosomal space which does not surpass the differentiated acrosomal internal content, and a more-conical acrosome compared to other members of the Turbininae. Additionally, the Prisogasterinae and Turbininae are the only 2 subfamilies of the Turbinidae sensu stricto which produce calcareous opercula (Williams et al. 2008).

Hickman and McLean (1990) placed the Tegulinae in a basal position within the Trochidae, but also suggested that an alternative interpretation using morphological characters could support it being basal to the turbinids. Spermatozoa of the Tegulinae studied by Collado et al. (2008) are similar to those of the Prisogasterinae and the Turbininae, but also apparently simpler, which is congruent with the phylogeny of the group (Williams et al. 2008). Hickman and McLean (1990) placed the Turbininae as sister to the Prisogasterinae, but they also recognized a close relationship between this subfamily and the Tegulinacea based on elaborations of the left neck-lobe of the epipodium and radular characters. Williams et al. (2008) suggested a close relationship between the Margaritinae and Skeneinae, and these taxa being sister to the Prisogasterinae-Turbininae clade. The Margaritinae also have affinities with the Tegulinacea on the basis of shared anatomical characters (a ctenidium with a short dorsal afferent membrane) (Hickman and McLean 1990). The spermatozoon ultrastructure; however, does not show similarities between these clades. Although the spermatozoon ultrastructure of those Margaritinae (sensu stricto) species has not been studied, the spermatozoon of *Zalipais laseroni*, as representative of the Skeneinae, shows independent evolution from other Turbinidae sensu stricto; however these incongruities could be a consequence of specialization of *Z. laseroni* for internal fertilization, the reproductive strategy adopted by the Skeneinae (Hodgson 1995). Bouchet et al. (2005) and Kano (2008) also suggested the close affinity of the Turbinidae, Tegulinacea, and Skeneidae sensu stricto. All representatives of the Skeneinae studied to date produce euspermatozoa, while *Z. laseroni* also produces paraspermatozoa (Healy 1990). The euspermatozoa of this species is of the modified (Franzén 1956) or introsperrm (Rouse and Jamieson 1987) type. It consists of a long helically coiled nucleus, a small anterior conical acrosome, and a midpiece which has a mitochondrial sleeve that surrounds a long electron-dense rod (Healy 1990). The fact that skeneins have introsperrm makes it difficult to use spermatozoon morphology to assess their phylogenetic relationships with other vetigastropods; they are unlike other trochoideans (Hodgson 1995) and were suggested to be an ancestral source of the caenogastropods (Healy 1990).

In this study, we showed that the spermatozoon of *P. niger* is similar in structure to those described for species of the subfamily Turbininae, with which it shares synapomorphic characters, supporting the systematic placement of this taxon within the family Turbinidae as recovered by Williams and Ozawa (2006) and Williams et al. (2008). Hickman and McLean (1990) and Hickman (1996) suggested the possibility that the Prisogasterinae and Turbininae were closely
related groups, and that *Prisogaster* may have originated from an ancestral turbinine. The shared derived characters shown by the Prisogasterinæ-Turbininæ and *Prisogaster-Turbo* in characteristics of the spermatozoa provide independent morphological evidence to support this hypothesis.

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