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RESEARCH NOTE

Shell morphology and sperm ultrastructure of *Solen tehuelchus* Hanley, 1842 (Bivalvia: Solenidae): New taxonomic characters

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Abstract: *Solen tehuelchus* Hanley, 1842 is currently the only razor-clam reported from Brazilian waters. Although its shell is well known and similar to the other Solenoidea, their anatomical aspects are poorly studied. Recently some living specimens of *S. tehuelchus* were obtained from a Brazilian beach, allowing the removal of their testes for an ultrastructural analysis. In order to add a new anatomical feature for this species and provide a brief comparison between some related taxa, the shell and sperm ultrastructure were described in detail. The present study showed that characteristics of the spermatozoa of *S. tehuelchus* are similar to those of other solenids, although certain sperm ultrastructural characteristics such as a short acrosome with an electron-dense base and rounded nucleus are also similar to the species *Tagelus plebeius* (Lightfoot, 1786) (Solecurtidae). Herein, we have added new anatomic and taxonomic data hitherto unknown to science. The sperm morphology of *S. tehuelchus*, might in the near future bring new insights into the systematic of Solenidae.

Key words: razor-clam, sperm morphology, transmission electron microscopy, shell features, Brazil.

The Solenidae Lamarck, 1809 commonly known as razor clams are infaunal and a deep burrowing family (Owen 1959, Simone 2009, Saeedi 2013). They are generally found in soft bottom in shallow and intertidal to sub-tidal region of tropical and temperate seas worldwide (Cosel 1989, 2002, Coan and Valentich-Scott 2012). Genera *Solen* Linnaeus, 1758 (66 species) and *Solena* Morch, 1853 (2 species) composed exclusively by marine species and *Neosolen* Gosh, 1920 a freshwater genus with only one valid species (Huber 2010, Bouchet 2015) are included in Solenidae. For Brazil, specifically, only two species are recorded, *Solen tehuelchus* Hanley, 1842 and *Solen obliquus* Spengler, 1794 (Abbott 1974, Rios 1994, 2009, Passos and Magalhães 2011, Tallarico *et al.* 2014); although according to Huber (2010), *Solena obliqua* (Spengler, 1794) a new combination to *S. obliquus* does not occur in Brazilian waters. Therefore, one of these Brazilian solenids remains without a formal description probably being a putative new species.

Some species of this family have a high commercial value in several Asian and European countries. In Japan and China, particularly, these bivalves are appreciated for their nutritional value as in the case of *Solen gordonis* Yokohama, 1920, which is collected by inhabitants for food and *Solen*

grandis Dunker, 1862 that is also appreciated due to its medicinal properties (Habe 1964, Yuan *et al.* 2012).

Despite their economic importance, solenids are rarely investigated in comparison to other bivalve families, such as the Veneridae, Mytilidae and Ostreidae. While there have been some crucial studies on solenid biology and morphology (Ghosh 1920, Fraenkel 1927, Yonge 1952, Owen 1959, Morton 1989, Pohlo 1989) as well as on their systematics (Dall 1899, Lamy 1932, Cosel 1989, 1990, 1993, 2002) the taxonomy of these organisms, in particular, requires more detailed investigation.

Because of superficial similarities, the shells of solenids, are easily confused with those of species belonging to the families Pharidae and Solecurtidae. The solenid shell is elongated, cylindrical, razor-shaped, asymmetric with terminal or sub-terminal umbones; smoothly sculptured or with commarginal growth lines, periostracum fine to thick with a glossy appearance; external ligament, opisthodontic and parivincular; hinge plate poorly-developed, with only one cardinal tooth in each valve, lateral teeth absent (Owen 1959, Rios 1994, 2009, Coan and Valentich-Scott 2012).

In addition to similarities in their shells, the digging behavior of solenids (rapid and active burrowers) is a common feature shared with other families, such as the Pharidae,

Solecurtidae and some Psammobiidae, which superficially appears to reflect their relatedness, and reinforces the demand for reclassification of their species. These characteristics are directly relevant to phylogenetic position of the Solenidae, which occupies an uncertain placement in the Veneroidea (Bieler *et al.* 2010, Coan and Valentich-Scott 2012, Yuan *et al.* 2012). Molecular and anatomical studies have also suggested that Solenidae (Solenioidea) is closely related to Solecurtidae (Tellinoidea) (Plazzi *et al.* 2011, Yuan *et al.* 2012).

Comparative ultrastructure of spermatozoa has been an especially useful tool for the study of different bivalve clades, particularly given the uniform conchological features observed in many families and genera (Franzén 1983, Hodgson *et al.* 1987, Hodgson *et al.* 1990, Healy and Willan 1991, Healy 1995a, 1995b, 1996, Sousa and Oliveira 1994, Gwo *et al.* 2002, Introíni *et al.* 2004, 2009, 2013, Drozdov *et al.* 2009, Cuzzo 2011, Yurchenko 2012, Torroglosa and Giménez 2015). In fact, it seems unlikely that different bivalve species will have morphologically identical spermatozoa (Franzén 1983). Even in very conservative families, such as Ostreidae, the variation in the dimensions of sub-structures of the gametes probably reflects interspecific differences (Yurchenko 2012).

The present study describes the shell and spermatozoan ultrastructure of the bivalve *Solen tehuelchus* Hanley, 1842 with the aim of providing insights into the taxonomy of this

solenid. The sample collection occurred at Engenho D'água beach (lat 23°48' S, long 45°22' W) in Ilhabela, on the northern coast of São Paulo state, Brazil. Small fragments (0.1 to 0.3 mm³) of the male gonads were fixed during 24 hours at 4 °C in 2.5% glutaraldehyde, in 0.1 M of sodium cacodylate buffer at a pH of 7.2, with the addition of 7% saccharose and 5 mM of calcium chlorate. Once fixed, the sample was washed during 12 hours in the same buffer in the conditions described above, and post-fixed in 1% osmium tetroxide (OsO₄). The samples were then dehydrated in a graded (increasing) series of ethanol, before being embedded in EPON 812. Uranyl acetate and lead citrate (Reynolds) were applied for contrast, and the sections were then observed under a Zeiss Leo 906 transmission electron microscope operating at 60 and 80 kV. A total of 20 spermatozoa of the specimen ZUEC 2463 were analyzed. All specimens examined for shell characterization are deposited in the Museum of Zoology of the State University of Campinas (ZUEC) under accession numbers ZUEC-BIV 432, 433, 436, 2463, 1232 and 3779.

Shell morphology

The shell of *Solen tehuelchus* is cylindrical, elongated, equi-convex, inequilateral, gaping at both ends; anterior end broadly rounded and posterior end subtruncated. Dorsal margin (dm) straight and ventral margin (vm) slightly convex.

Outer surface smooth with a translucent to yellowish periostracum; inner surface exhibits small purplish spots in the anterior and posterior ends. Hinge plate weak with one fragile anterior cardinal tooth (at) in each valve; lateral teeth absent. Umbones anterior. External ligament (li), opisthodontic and long. Muscle scars well visible: (i) anterior adductor muscle scar (aam) elongated, (ii) posterior adductor muscle scar (pam) smaller and rounded, (iii) pallial line (pl) scar long, sinuous shape with anterior end produced anteroventrally (apl); scars of pedal retractor muscles not distinguishable probably united with their respective adductors scars. Pallial sinus (ps) small and short with a dorsal limb (dl) united with the posterior adductor scar and a ventral limb (vl) posteroventrally produced (Fig. 1A-E).

The spermatozoon of *Solen tehuelchus* shows a short and conical acrosomal vesicle, with a mean length of 0.35 μm and width of 0.49 μm and zones of different electron density (Table 1). The acrosomal base is filled with a granular

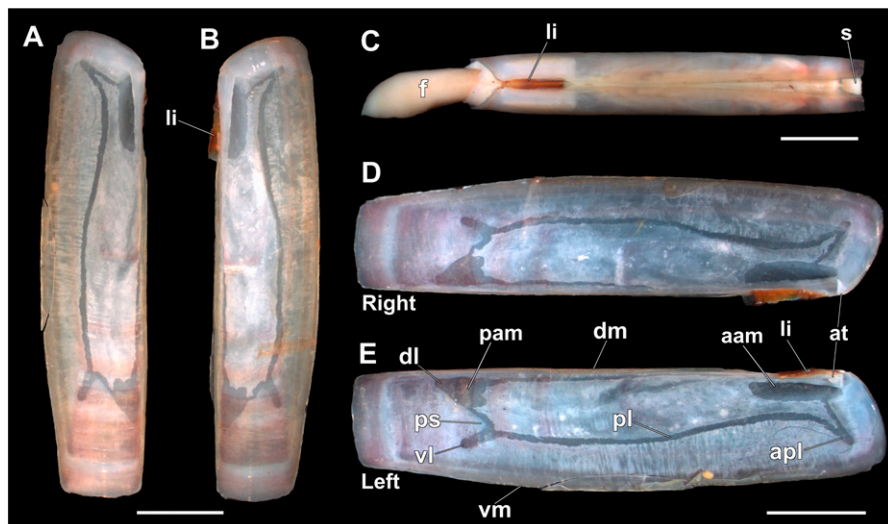


Figure 1. *Solen tehuelchus*, details of the shell. **A.** Outer view of the left valve. **B.** Outer view of the right valve, showing the yellowish periostracum and ligament. **C.** Dorsal view, showing the foot, anterior ligament and posterior siphons. **D.** Inner view of right valve. Note the small purplish spots in the anterior and posterior ends. **E.** Inner view of left valve, showing the ligament, anterior cardinal tooth and muscle scars. Abbreviations: **aam**, anterior adductor muscle scar; **apl**, anterior end of pallial line scar; **at**, anterior tooth; **dl**, dorsal limb; **dm**, dorsal margin; **f**, foot; **li**, ligament; **pam**, posterior adductor muscle scar; **pl**, pallial line scar; **ps**, pallial sinus scar; **s**, siphons; **vl**, ventral limb; **vm**, ventral margin. Scale bars: A-E = 5 mm.

Table 1. Measures of some structures of spermatozoan of *Solen tehuelchus*. SD = Standard deviation; N = Number of spermatozoa analyzed.

SPERM STRUCTURES				
N	Nucleus		Acrosomal vesicle	
	Length	Width	Length	Width
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
20	1.42 ± 0.13 μm	1.37 ± 0.13 μm	0.36 ± 0.04 μm	0.49 ± 0.05 μm

material (Fig. 2A-D). The apex is slightly convex, and composed of homogeneous material, less electron-dense than the base, while the central region contains a subacrosomal area occupied by ultra-fine particles (Fig. 2B). The nucleus is barrel-shaped, 1.42 μm long and 1.36 μm wide, with subtle anterior and posterior nuclear grooves (Table 1). Among the strongly condensed chromatin strands, conspicuous electron lucent lacunae are visible (Fig. 2B, D). The midpiece is made up of four spherical mitochondria, organized in a ring around a pair of short centrioles, perpendicularly arranged (Fig. 2E, G). The flagellum is simple, with a 9+2 arrangement of microtubules originating from the distal centriole (Fig. 2F-H). This analysis indicates that the gamete is primitive (Fig. 3) or into the “ect-aquasperm” category (Rouse and Jamieson 1987), a spermatozoon that fertilizes the oocyte directly into the surrounding water where it is spawned. The morphometric and structural characteristics of the male gametes are generally associated with the mode of fertilization, oocyte size and coverings (Souza and Oliveira 1994); sperm competition, and the environment where they are released (Birkhead *et al.* 2007).

Hodgson *et al.* (1987) described the sperm ultrastructure of *Solen cylindraceus* Hanley, 1843 and *Solen capensis* P. Fischer, 1881. The basic structure of these gametes is closely similar to that of *S. tehuelchus*, that is, an aquasperm type, with a short, conical acrosome and electron-dense material at the base, barrel-shaped nucleus, midpiece formed by mitochondria, and a simple flagellum. The only differences are found in the relatively long, thin acrosome in *S. cylindraceus* (0.4 μm in length, 0.5 μm in width) in comparison with *S. capensis* (0.3 μm in length, 0.7 μm in width). However, the acrosome of *S. tehuelchus* presents a distinct ultrastructure, in which the electron-dense material of the base is not homogeneous exhibiting a granular aspect. The acrosome of the present species is different from that of the other *Solen* species for which data are available, being conical and elongated, reaching a length of 18 μm, with electron-dense material extending throughout its structure. The nucleus may be spherical to slightly elongate, with a length of 3.2 μm.

In *Solen*, the morphology of the spermatozoa appears to be conserved, although there are important distinctions in the acrosome, in particular in its length, especially when compared

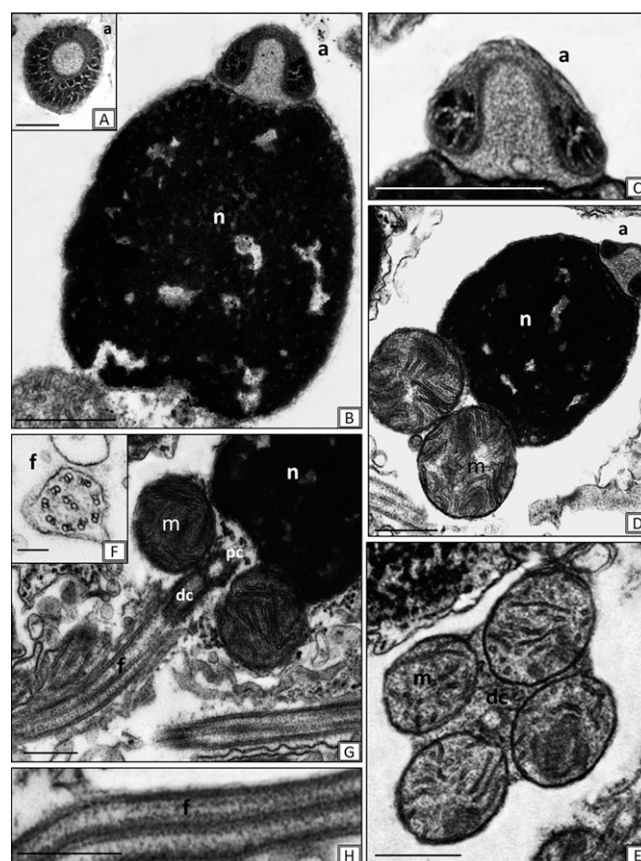


Figure 2. *Solen tehuelchus*, sperm structures. **A.** Cross section of the acrosome, showing its base filled with a granular material. **B.** Longitudinal section of the spermatozoon head. **C.** Higher magnification of the acrosome, showing the structures mentioned in Fig. A. **D.** Longitudinal section of the spermatozoon. **E.** Transverse section of the middle piece, showing mitochondria and distal centriole. **F.** Transverse section of the axoneme, showing 9+2 microtubular structure. **G.** Longitudinal section of the middle piece, showing mitochondria, proximal centriole, distal centriole and flagellum. **H.** Longitudinal section of flagellum. Abbreviations: **a**, acrosome; **dc**, distal centriole; **f**, flagellum; **m**, mitochondrion; **n**, nucleus; **pc**, proximal centriole. Scale bars: A, F = 0.25 μm; B, C, D, E, G, H = 0.5 μm.

with *Ensis ensis* Linnaeus, 1758 (Pharidae), and the distinct pattern of electron density in the base of the acrosome in *S. tehuelchus* (Casas and Subirana 1994). The male gametes of the *Solen* species are generally similar to those of the solecurtid *Tagelus plebeius* (Lightfoot, 1786), which also has a short acrosome with an electron-dense base and rounded nucleus (Matos *et al.* 1997). Despite this general similarity, there are diagnostic differences, primarily in the architecture of the acrosome. According to Matos *et al.* (1997), acrosomal vesicles of *T. plebeius* spermatozoa were dome-shaped in sagittal sections. These domes had larger bases in comparison with their tops. They also described the presence of a perforatorium.

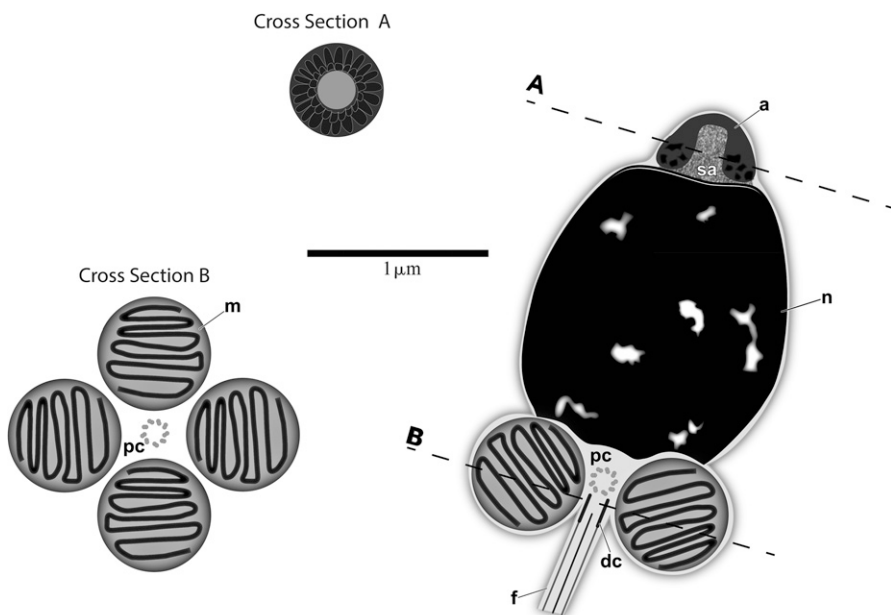


Figure 3. *Solen tehuelchus*, a schematic representation of sperm cell. **A.** Transversal section of the acrosome. Note the electron dense structures compounding the marginal area of the posterior half of the acrosomal vesicle. **B.** Transversal section of the middle piece. Abbreviations: **a**, acrosome; **dc**, distal centriole; **f**, flagellum; **m**, mitochondrion; **n**, nucleus; **pc**, proximal centriole; **sa**, subacrosomal area. Scale bar = 1 μm . Drawing by Alterson Luiz Cação and Ariane Campos.

We were not able to recognize the perforatorium based on the figure provided in Matos *et al.* (1997). Even so, the general uniformity between the spermatozoa of the *Solen* species and those of the solecurtids may reflect the close phylogenetic relationships between these two groups. Guerra *et al.* (1994) described the morphology of the spermatozoa of *Gari solida* Gray, 1828 (Psammobiidae) using light microscopy, and found a completely different morphological pattern in comparison with gametes of *S. tehuelchus* (present work), in particular the conical and elongated nucleus, which reaches a length of 8 μm , a characteristic that is found in modified spermatozoa (Rouse and Jamieson 1987). The acrosome is very small, with a length of no more than 0.4 μm . A preliminary comparison of these features could indicate that the species are not closely related. However, the diagnosis of Psammobiidae family is not based on synapomorphy, being grounded exclusively on conchological characters. Thus, it would be arbitrary to describe the sperm morphology of Psammobiidae considering male gametes of a single species, *Gari solida*.

The present study has shown that characteristics of the shell and spermatozoa of *Solen tehuelchus* are similar to those of other solenids, although certain sperm ultrastructural features are also similar to those of *Tagelus plebeius* (Solecurtidae). On its own, then, the analysis of the ultrastructure of the gametes of these bivalves is insufficient to clarify the phylogenetic

relationships among the families Pharidae, Solecurtidae, and Psammobiidae. However, together with molecular and anatomical data, these findings may provide important and complementary insights into the relationships among these taxa. In addition, the analysis of the male gametes of these bivalves contributes to the understanding of their reproductive biology, which will be fundamentally important for future research into the ecology of these organisms and, ultimately, into the effects of environmental changes and the conservation of these species.

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