Sperm morphology of two marine neogastropods from the southwestern Atlantic Ocean (Caenogastropoda: Volutidae and Olividae)

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ABSTRACT

The testes of Caenogastropoda typically produce two types of spermatozoa, euspermatozoa and paraspermatozoa. The structures of both morphological forms of sperm contribute to our understanding of reproductive biology, and also have been useful to elucidate taxonomic and phylogenetic relationships among gastropods. This article describes the ultrastructure and the possible importance for systematics of the euspermatozoa in two species, *Adelomelon beckii*, family Volutidae, and *Olivancillaria deshayesiana*, family Olividae.

The euspermatozoa of these species are characterized by: the presence of an acrosomal vesicle with an apical bleb and accessory membrane; a nucleus that is long and tubular with the axoneme penetrating the nucleus; a midpiece with mitochondrial elements coiled helically around the axoneme; a glycogen piece; and a short end piece. A constriction in the acrosomal vesicle and mitochondrial elements that appear U-shaped and electron dense in cross section are features that are present in the studied taxa, but have not been reported outside of the Neogastropoda.

Additional keywords: Sperm, ultrastructure, Gastropoda, Neaogastropoda

INTRODUCTION

Members of the family Volutidae are active marine predators. The majority of taxa inhabit sandy to silty bottoms in coastal waters of the southern hemisphere, although the family has a global distribution and extends to bathyal and abyssal depths (Clench and Turner, 1970; Poppe and Goto, 1992). More than 200 species are known, with shells that vary substantially in shape and size. Fourteen species of Volutidae are reported from the southwestern Atlantic Ocean, including members of the genus *Adelomelon* (Rios, 1994). *Adelomelon beckii* (Broderip, 1836) is endemic to the southwestern Atlantic Ocean, ranging from Espírito Santo, Brazil, to Tierra del Fuego, Argentina. It is the largest (390 mm maximum length) carnivorous gastropod in the region, and inhabits sandy bottoms at depths of 35 to 70 m (Poppe and Goto, 1992). *Adelomelon beckii* has been caught as a byproduct of trawler fishing, but in the past several years a new market demand appeared for this species. Its large, muscular foot is sold for food, while its shell is sold in local markets as an artisanal product.

The family Olividae encompasses carnivorous, infaunal marine gastropods of medium size (Smith, 1998). Olivids inhabit nearshore waters along the northern coast of Argentina. Twelve species of Olividae are reported off the Argentine coast, spanning the genera *Olivancillaria*, *Olivella*, and *Amalda*. Seven species of *Olivancillaria* are recorded from South America (Castellanos, 1970; Rios, 1994).

Olivancillaria deshayesiana (Ducros de Saint Germain, 1857), with a maximum shell length of 35 mm, is the most common species. It is distributed along the southern coast of Buenos Aires province and lives at depths of 6–12 m, from Rio de Janeiro to Mar del Plata. Together with some volutids (i.e., *Adelomelon*, *Zidona*) and nassariids (i.e., *Buccinanops*), *Olivancillaria* species are among of the most common endemic taxa living in sandy bottoms of the Argentine malacological province.

In contrast to many other neogastropod families, the ultrastructure of the sperm of the Volutidae and Olividae in the southwestern Atlantic Ocean has not been intensively examined, except for Giménez et al., 2008; Zabala et al., 2009. Other studies have relied on traditional anatomical morphology, with most literature on these families still focused on their systematics (Marcus and Marcus, 1959; Klappenbach, 1965, 1966; Weaver and du Pont, 1970; Novelli and Novelli, 1982; Darragh, 1988; Poppe and Goto, 1992; Bondarev, 1995; Bail and Poppe, 2001; Pastorino, 2003; Absalão and Pimenta, 2003).

The caenogastropod testis typically produces two types of spermatozoa: eusperm and parasperm. The structures of both morphological forms of sperm contribute to an understanding of the reproductive biology of these animals, and have also been useful in elucidating the taxonomic and phylogenetic relationships among them (Ponder et al., 2007).

The following account describes the ultrastructure of the euspermatozoa of two neogastropod species, the volutid *Adelomelon beckii* and the olivid *Olivancillaria deshayesiana*, and identifies several features of potential systematic importance.

MATERIALS AND METHODS

Reproductively mature males of *Adelomelon beckii* and *Olivancillaria deshayesiana* were trawled off Mar del Plata, Argentina (38°20′ S, 57°37′ W) (Figure 1) at depths of 35–40 m and 8–12 m, respectively. Small
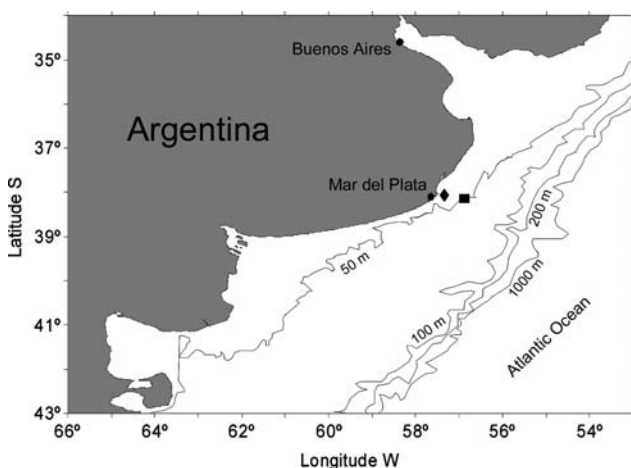

Figure 1. Map showing presence of the studied species in sampled sites ♦ = *Olivancillaria deasayesiana* location and ■ = *Adelomelon beckii* location.

pieces of the testis were fixed in 2% glutaraldehyde in phosphate buffer [0.1 M, pH 7.0] for 4 hours at 4°C. Subsequently, the tissue pieces were placed in a 1% solution of osmium tetroxide (in 0.1M phosphate buffer) for 1.5 h and washed in buffer. Tissues were dehydrated using an ascending series of ethanol concentrations (20% to absolute ethanol), placed in a 1:1 ethanol: propylene oxide solution for 15 min and embedded in Spurr's epoxy resin. Ultrathin sections were cut using either a Reichert or an LKB IV ultramicrotome and stained with uranyl acetate and lead citrate (Reynolds, 1963). All sections were examined and photographed using Zeiss (Oberkochen, Germany) EM 109T, Hitachi 300 and Jeol 1010 transmission electron microscopes operated at 75–80 kV.
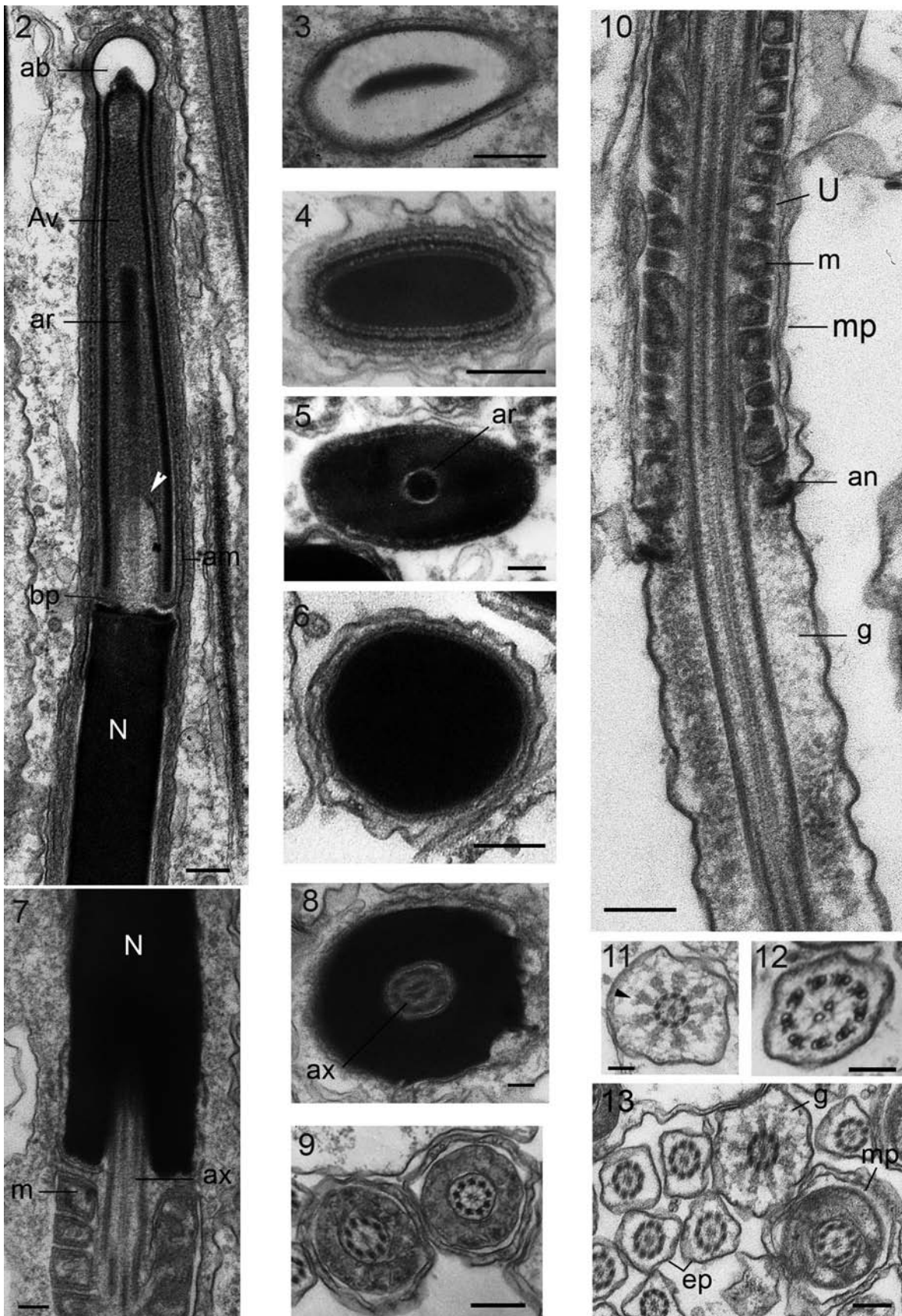
RESULTS

The euspermatozoa of *Adelomelon beckii* and *Olivancillaria deshayesiana* share the same general morphology, being composed of an acrosomal complex, nucleus, midpiece, glycogen piece, and end piece.
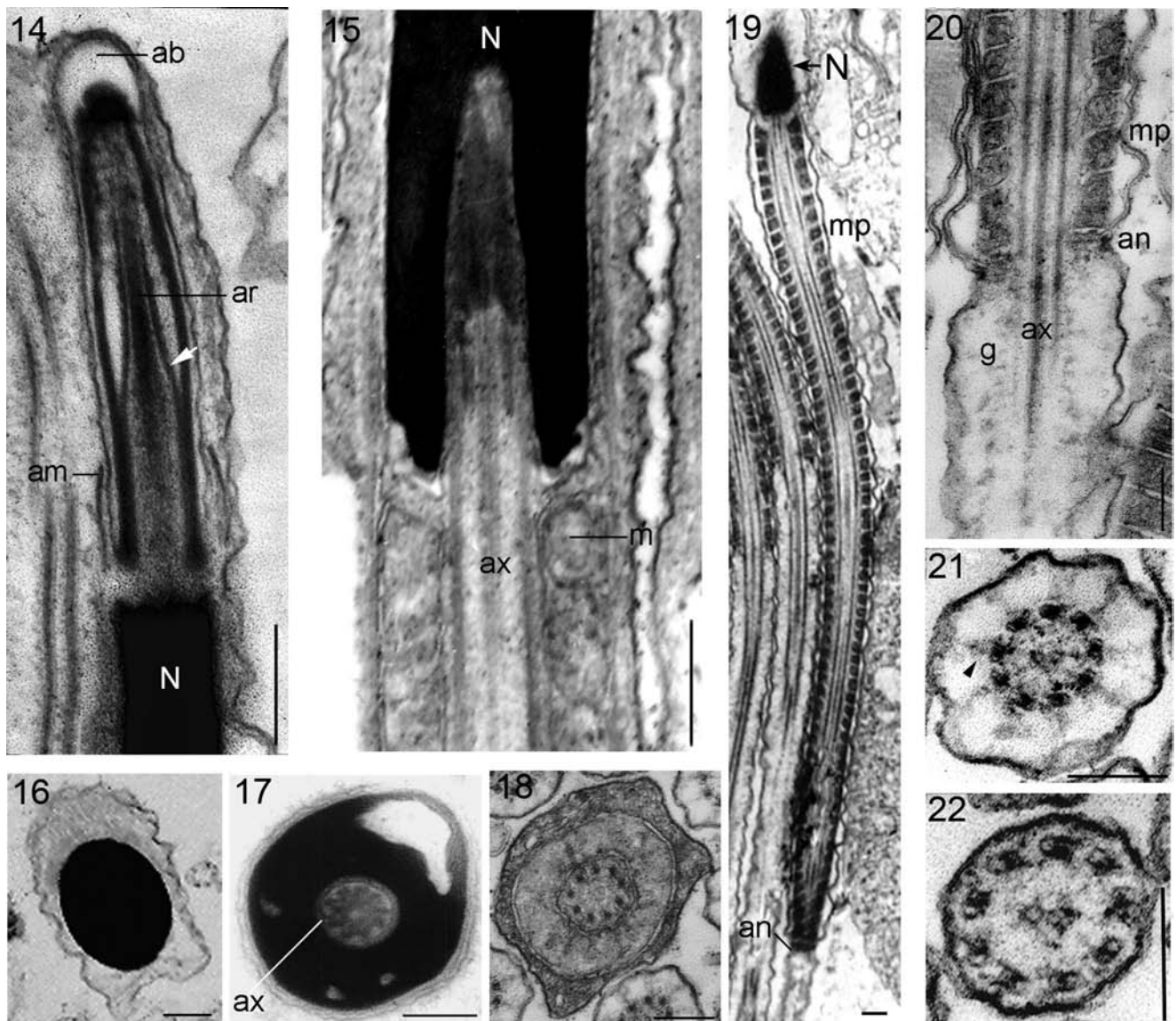
ACROSOMAL COMPLEX: The acrosomal complex consists of a tall-conical, membrane-bound acrosomal vesicle, an axial rod and a basal plate (Figures 2, 14). The acrosomal vesicle is approximately 7.39 ± 0.95 μm long in *A. beckii* and 0.47 ± 0.018 μm long in *Olivancillaria deshayesiana*. Apically, the vesicle membrane is separated from the vesicle contents by the apical bleb. The acrosomal vesicle bears a very deep invagination that contains the axial rod (subacrosomal material). In *Adelomelon beckii* and *O. deshayesiana,* longitudinal sections show a constriction of this invagination. These constrictions measure 1.26 ± 0.26 μm in *A. beckii* and 0.20 μm in *O. deshayesiana.* An accessory membrane is closely associated with the base of the acrosomal vesicle in *A. beckii* but not in *O. deshayesiana*. The acrosomal vesicle is oval in transverse section near its base, but is laterally compressed within the apical bleb (Figures 3, 4, 5).

NUCLEUS: The nucleus in both species is filiform and highly electron-dense (Figures 6, 7, 15, and 16). The basal invagination contains a centriolar derivative that is continuous with the initial portion of the $9+2$ microtubule pattern axoneme (Figures 8, 17). The length of this basal invagination is 2.46 ± 0.03 and 0.38 ± 0.05 μm in *Adelomelon beckii* and *Olivancillaria deshayesiana,* respectively.

MIDPIECE: Posterior to the nucleus, the axoneme is enclosed in a mitochondrial sheath to form the midpiece region. Oblique longitudinal sections show that the mitochondrial elements are disposed helically (Figures 7, 19). In *Adelomelon beckii*, an electron-dense, U-shaped outer layer is observed in the mitochondrial elements (Figures 9, 10) that is not evident in *Olivancillaria deshayesiana* (Figure 18).

ANNULAR COMPLEX AND GLYCOGEN PIECE: An annular complex is located at the immediate junction of the midpiece

Figures 14–22. Euspermatozoa of *Olivancillaria deshayesiana.* **14.** Longitudinal section (LS) through the acrosomal complex showed the apical bleb (**ab**), the axial rod (**ar**), the accessory membrane (**am**) and the anterior portion of nucleus (**N**). **15.** LS through the nucleus (**N**) and anterior portion of midpiece with mitochondria (**m**) spiraling around the axoneme (**ax**). **16.** TS through the nucleus. **17.** TS through the nucleus with axoneme (**ax**). **18.** TS through the midpiece. **19.** LS through the midpiece (**mp**) showing the nucleus (**N**) and the annular complex (**an**). **20.** LS through the junction of the midpiece (**mp**) and glycogen piece (**g**). Note the annular complex (**an**) and the axoneme (**ax**). **21.** TS through the glycogen piece showing radiating and longitudinal rows (**arrowhead**). **22.** TS through the endpiece. Scale bars = 0.10 μm.

Figures 2–13. Euspermatozoa of *Adelomelon beckii.* **2.** Longitudinal section (LS) through the apical bleb (**ab**), the constriction (arrowhead) of the acrosomal vesicle (**Av**) and the axial rod (**ar**) in the acrosomal complex. Note the presence of the accessory membrane (**am**), the basal plate (**bp**) and the nucleus (**N**). Scale bar = 0.58 μm. **3–5.** Series of transverse sections (TS) at different levels of the acrosomal complex: **3.** The apical bleb; **4.** The middle of the acrosomal vesicle; and **5.** The axial rod (**ar**) in the region of the invagination of the acrosomal vesicle. Scale bar = 0.10 μm. **6.** TS section through the nucleus. Scale bar = 1.0 μm. **7.** LS through the junction of nucleus (**N**) and anterior portion of midpiece, showing mitochondria (**m**) spiraling around the axoneme (**ax**). Scale bar = 0.35 μm. **8.** TS of nucleus with axoneme (**ax**). Scale bar = 0.23 μm. **9.** TS of midpiece. Scale bar = 0.58 μm. **10.** LS at the junction between the midpiece (**mp**) and glycogen piece (**g**). Note the presence of the annular complex (**an**). The helical mitochondria (**m**) elements are defined by dense U-shaped profiles (**U**). Scale bar = 0.58 μm. **11.** TS through the glycogen piece showing radiating rows of putative glycogen granules (arrowhead). Scale bar = 0.58 μm. **12.** TS through the end piece. Scale bar = 0.10 μm. **13.** TS showing midpiece (**mp**), glycogen piece (**g**) and end pieces (**ep**). Scale bar = 0.58 μm.

and glycogen piece in both species studied (Figures 10, 20). Beyond the midpiece, the axoneme is associated with nine longitudinal, radiating tracts of dense granules within the glycogen piece (Figures 11, 21).

END PIECE: This region of the eusperm is situated posterior to the glycogen piece, and consists of the axoneme, with a 9+2 pattern of microtubules surrounded by a plasma membrane. The diameter of the end piece is 0.70 ± 0.06 µm in *Adelomelon beckii,* and 0.16 ± 0.02 µm in *Olivancillaria deshayesiana* (Figures 12, 13, 22).

DISCUSSION

In this paper we report new and preliminary information about the ultrastructure of the sperm of one species of the family Volutidae and one species of Olividae. Our study indicates that the euspermatozoa of *Adelomelon beckii* and *Olivancillaria deshayesiana* are similar to the euspermatozoa type 2 described by Healy (1996). This eusperm presents an acrosomal vesicle with an apical bleb and accessory membrane, a solid, electron-dense nucleus a midpiece with mitochondrial elements helically coiled around the axoneme, a glycogen piece with nine tracts of granules, and a dense ring structure at the midpiece-glycogen piece junction. In *A. beckii,* the outer layer of each mitochondrial element is considerably more electron-dense than in *O. deshayesiana.* The outer layer, with its bilaminar appearance, possibly represents a partial "crystallization" of the mitochondrial elements, analogous to that occurring in certain rissoidean caenogastropods (Healy, 1983). This particular U-shaped profile of each mitochondrion is very distinctive and has not been observed in any study of caenogastropod euspermatozoa, except in three members of the family Volutidae: *Zidona dufresnei* (Donovan, 1823), *Provocator mirabilis* (Finlay, 1926) (both Giménez et al., 2008), and *Adelomelon ancilla* (Lightfoot, 1786) (Zabala et al., 2009). The glycogen pieces and end pieces of these three species and *A. beckii* and *O. deshayesiana* are the same as those observed in other caenogastropods, and show the characteristic axoneme of the group (Giménez et al., 2008; Zabala et al., 2009).

The size structure pattern of the acrosomal complex in the mature eusperm from *Olivancillaria deshayesiana* is very small when compared to the acrosomal complex of *Adelomelon beckii.* We suggest the existence of a correlation between the size of individual animals and the length of the acrosomal complex, but additional studies are needed to confirm this observation.

In *Adelomelon beckii*, the constriction in the acrosomal vesicle invagination is situated at 0.2 of the acrosomal length, measured from the posterior margin of the acrosomal complex, while in *Olivancillaria deshayesiana* the constriction is at 0.4 of the acrosomal length. We postulate that the relative position of the constriction of the acrosomal vesicle relative to the length of the acrosomal complex may be of systematic significance. Additional sampling is required to determine if these values are diagnostic of the families Volutidae and Olividae, and if this character has broader utility in clarifying phylogenetic relationships within the Neogastropoda.

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LITERATURE CITED

Absalão, R.S. and A.D. Pimenta. 2003. A new subgenus and three new species of Brazilian deep water *Olivella* Swainson, 1831 (Mollusca, Gastropoda, Olivellidae) collected by the Marion Dufresne in 1987. Zoosystema 25: 177–185.

Bail, P. and G.T. Poppe. 2001. A Taxonomic Introduction to the Recent Volutidae. A Conchological Iconography. ConchBooks, Hackenheim, 158 pp.

Bondarev, I. 1995. A phylogenetic classification of Australian Volutidae. La Conchiglia 276: 25–38.

Castellanos, Z.J.A. de. 1970. Catálogo de los moluscos marinos bonaerenses. Anales de la Comisión de Investigaciones Científicas de la provincia de Buenos Aires 8: 9–365.

Clench, W.J. and R.D. Turner. 1970. The family Volutidae in the western Atlantic. Johnsonia 4 (48): 369–372.

Darragh, T.A. 1988. A revision of the Tertiary Volutidae (Mollusca: Gastropoda) of south-eastern Australia. Memoirs of the Museum of Victoria 49: 195–307.

Giménez, J., J.M. Healy, G. Hermida, F. Lo Nostro, and P.E. Penchaszadeh. 2008. Ultrastructure and potential taxonomic importance of euspermatozoa and paraspermatozoa in the volutid gastropods *Zidona dufresnei* and *Provocator mirabilis* (Caenogastropoda, Mollusca). Zoomorphology 127: 161–173.

Healy, J.M. 1983. Ultrastructure of euspermiogenesis in the mesogastropod *Stenothyra* sp. (Prosobranchia, Rissoacea, Stenothyridae). Zoologica Scripta 12: 203–214.

Healy, J.M. 1996. Molluscan sperm ultrastructure: correlation with taxonomic units within the Gastropoda, Cephalopoda and Bivalvia. In: Taylor J. (ed.) Origin and Evolutionary Radiation of the Mollusca. Oxford University Press, Oxford, pp. 99–113.

Klappenbach, M.A. 1965. Consideraciones sobre el género *Olivancillaria* d'Orbigny, 1840 y descripción de dos nuevas especies de aguas argentinas y uruguayas. Comunicaciones Zoológicas del Museo de Historia Natural de Montevideo 8 (104): 1–10.

Klappenbach, M.A. 1966. *Olivancillaria vesica* (Gmelin, 1791) has priority over *Olivancillaria auricularia* (Lamarck, 1810) Moll. Gastr. Archiv für Molluskenkunde 95: 75–77.

Marcus, E. and E. Marcus. 1959. Studies on Olividae. Boletim da Faculdade de Filosofia, Ciências e Letras da Universidade de São Paulo 232 Zoologia 22: 99–188.

Novelli, R. and A. Novelli. 1982. Algumas consideracoes sobre a subfamilia Zidoninae e notas sobre a anatomía de *Adelomelon brasiliana* (Lamark, 1811), Mollusca, Gastropoda, Volutidae. Atlântica 5: 23–34.

Pastorino, G. 2003. A new species of Ancillariinae (Gastropoda: Olividae) from the southwestern Atlantic Ocean. The Nautilus 117: 15–22.

Ponder, W.F., D.J. Colgan, J.M. Healy, A. Nützel, R.L.L. Simone, and E.E. Strong. 2007. Caenogastropoda. In: Ponder, W.F. and D.L. Lindberg (eds.) Molluscan Phylogeny. University of California Press, Los Angeles, pp. 331–383.

Poppe, G. and Y. Goto. 1992. Volutes. Mostra Mondiole Malacologia, Cupra Maritima, Italy, 348 pp.

Rios, E.C. 1994. Seashells of Brazil. Editora da Fundação Universidade do Rio Grande, Rio Grande, 368 pp.

Reynolds, E.S. 1963. The use of lead citrate at high pH as an electron–opaque stain in electron microscopy. Journal of Cell Biology 17: 208–212.

Smith, B.J. 1998. Family Olividae. In: Beesley, P.L., G.J.B. Ross, and A. Wells (eds.) Mollusca: The Southern Synthesis. Fauna of Australia. Vol. 5. CSIRO Publishing, Melbourne, Part B. viii, pp. 565–1234.

Weaver, C.S. and J.E. du Pont. 1970. The Living Volutes: A monograph of the recent Volutidae of the world. Monograph Series N° 1, Delaware Museum of Natural History, Greenville, 375 pp.

Zabala, S., G.N. Hermida, and J. Giménez. 2009. Ultrastructure of euspermatozoa and paraspermatozoa in the volutid snail *Adelomelon ancilla* (Mollusca: Caenogastropoda). Helgoland Marine Research 63: 181–188.