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Sperm ultra-structure of *Odontosyllis ctenostoma* (Polychaeta: Syllidae) with inferences on syllid phylogeny and reproductive biology

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SUMMARY: The analysis of the complex reproductive patterns of Syllidae may drastically change the taxonomic hierarchy of the family. To further contribute to the knowledge of Syllidae we have described the sperm ultra-structure and some steps of spermiogenesis of *Odontosyllis ctenostoma* Claparède, 1868, the first non interstitial eusylline investigated. The mature sperm has a bell-shaped acrosome and contains electron-dense granular material and thin filaments. The barrel-shaped nucleus bears two depressions: one anterior facing the acrosome and the other posterior partially containing the distal centriole and up to six mitochondria. *Odontosyllis ctenostoma* spermatozoa can be ascribed to the ect-aquasperm type typical of species practising external fertilisation. This morphology is not in complete accordance either with the particular brood protection reported for the species or the egg size. The sperm is similar to those of the Syllinae species thus far investigated, but the acrosome resembles that of the exogonine spermatid. Some authors consider *Odontosyllis* to be phylogenetically closer to Syllinae, though it shares epigamy with Exogoninae. Others have hypothesised that the exogonine sperm could have been derived from sylline sperm by simplification. In our hypothesis this could have happened through a gradual passage from an *Odontosyllis*-like eusylline ancestor.

Keywords: sperm morphology, ultrastructure, Polychaeta, Eusyllinae, reproduction, phylogeny.

RESUMEN: Ultra-estructura de los espermatozoides de *Odontosyllis ctenostoma* (Polychaeta: Syllidae) y su aplicación a la filogenia y biología reproductiva de los Sílidos. – La jerarquía taxonómica actualmente aceptada de la familia Syllidae podría cambiar drásticamente después de un profundo análisis de las modalidades reproductivas; especialmente para la subfamilia Eusyllinae que es considerada parafilética. Con el objetivo de dar una mayor contribución al conocimiento de la biología de la familia Syllidae, en este trabajo se ha estudiado la ultraestructura del espermatozoide y algunas fases de la espermatogénesis de *Odontosyllis ctenostoma* Claparède, 1868. El espermatozoide maduro presenta un acrosoma en forma de campana, cuya parte basal contiene material electrón denso donde se originan filamentos. El núcleo es en forma de barril con dos depresiones: una en la base del acrosoma y otra posterior que contiene seis mitocondrias que circundan los centriolos. El espermatozoide de *O. ctenostoma* puede ser adscrito al tipo "ect-aquasperma" que es típico en aquellas especies que tienen una fecundación externa. Esta morfología no concuerda con su biología reproductiva y con el tamaño de los huevos. El espermatozoide es muy similar a los de especies de la subfamilia Syllinae estudiadas hasta el momento, si bien el acrosoma es más similar al acrosoma del espermatidio de Exogoninae. Según algunos autores *Odontosyllis* es filogenéticamente similar a la subfamilia Syllinae si bien comparte la epigamia con la subfamilia Exogoninae. En otros trabajos se ha formulado la hipótesis que el espermatozoide maduro de algunas especies de Exogoninae podría derivar para simplificación del espermatozoide de Syllinae. Se formula la hipótesis que un ancestro similar a *Odontosyllis* para un trayecto gradual podría ser la conexión de uno con otro.

Palabras clave: morfología espermatozoides, ultraestructura, Polychaeta, Eusyllinae, reproducción, filogenia.

INTRODUCTION

The reproductive biology of Syllidae includes a great diversity of phenomena within the different subfamilies (Daly, 1975; Schroeder and Hermans, 1975; Franke and Pfannenstiel, 1984; Garwood, 1991; Bührmann *et al*., 1996; Franke, 1999; Lepore *et al*., 2006), with epigamy typical of Eusyllinae and Exogoninae, and stolonisation (schizogamy) typical of Syllinae and Autolytinae (Nygren, 1999). Variations exist in both reproductive modes. For example, epigamy is accompanied by external gestation in Exogoninae, while, except for some interstitial species, Eusyllinae develop from a free-swimming larva (San Martín, 2003). From a phylogenetic point of view, various authors consider the general reproduction pattern of syllids to be conservative within sub-families (Garwood, 1991; Franke, 1999; Nygren, 1999). In this case the sperm morphology can also probably help in the phylogenetic reconstruction.

The great diversity of reproductive phenomena is connected to an abundant variety of sperm types. Following the terminology of Jamieson and Rouse (1989), the syllid sperms described up to now can morphologically be ascribed in accordance with the reproductive strategies to the ent-aquasperm type such as *Salvatoria* (= *Grubea*) *clavata* (Franzén, 1974), *Autolytus* sp. (Franzén, 1982), *Exogone naidina*, *E. dispar* (Giangrande *et al*., 2002) and (possibly) *Sphaerosyllis hystrix* (Franzén, 1956), or to the intro-sperm type, which seems to be present in some interstitial taxa such as *Neopetitia* (= *Petitia*) *amphophthalma* (Bührmann *et al*., 1996), *Sphaerosyllis hermaphrodita* (Kuper and Westheide, 1997) and in some parasite species whose taxonomic position within Syllidae is under taxonomic debate: *Calamyzas* sp. (Jamieson and Rouse, 1989) and *Asetocalamyzas laonicola* (Vortsepneva *et al*., 2006). Ect-aquasperms were found in the three examined species of the genus *Syllis* (= *Typosyllis*) (Jamieson and Rouse, 1989; Heacox and Schroeder, 1981a; Lepore *et al*., 2006).

Taking into account the high species diversity of the family (about 670 species) (San Martín, 2003), sperm ultrastructure studies are limited to only a small percentage of syllids. This is a gap of knowledge also considering that the sperm structure may be useful for the reconstruction of the phylogenetic patterns of the family (Franke, 1999). Moreover, the analysis of the reproductive patterns, which seem quite fixed at subfamily level, could drastically change the currently accepted taxonomic hierarchy of the group. However, the few analysed species can already give us some information. In fact, apart from interstitial forms whose shared intro-sperm type could be considered a homoplasy (Lepore *et al*., 2006), all the epigamic forms show ent-aquasperm while the schizogamic Syllinae show ect-aquasperm and external fertilisation. Moreover, the acrosome morphology could also be indicative of phylogeny within species sharing the same morphological sperm type (Lepore *et al*., 2006). The acrosome morphology was also helpful in clarifying the phylogeny within Sabellidae (Patti *et al*., 2003).

Therefore, an increase in knowledge on sperm morphology is needed, especially within the very poorly screened Eusyllinae (only the interstitial *Neopetitia amphophthalma*), which is the most problematic subfamily from a taxonomic point of view. On the basis of epigamy and brooding type the Eusyllinae are considered paraphyletic by several authors (Nygren, 1999; San Martín, 2003), and possibly include some taxa more closely related to other subfamilies. Since the beginning of the last century Pierantoni (1903) considered brood protection of some *Pionosyllis* to be an atypical character that may be sufficient to consider them as belonging to a different genus in Eusyllinae. Moreover, as pointed out by Nygren (1999), due to the ambiguous taxonomic position of the genus *Pionosyllis*, the analysis of reproductive patterns in Syllidae could help to drastically change the currently accepted taxonomic hierarchy of the family.

In the present paper the sperm ultra-structure of the eusylline *Odontosyllis ctenostoma* Claparède, 1868, is studied and compared with previous correspondent analyses of the other syllid species.

MATERIAL AND METHODS

Target species and study area

Samplings were performed in the Mar Piccolo of Taranto (northern Ionian Sea; 40°28.4'N-17°16.4'E) during May 2006, in order to collect ripe syllid specimens. The sampling station is located near the Punta Penna bridge, along a cement quay of several km in length, situated on the northwestern coast of the Mar Piccolo first inlet. A rich hard bottom macrozoobenthic community lives on the vertical submerged part of the quay (mean depth 50-70 cm). Samples

were collected by scraping off a surface of 400 cm^2. Soon after collection the samples were transported in cooled bags to the laboratories of the Zoology Department of the University of Bari for examination of live specimens. Polychaetes were carefully sorted using a stereomicroscope; some specimens of *O. ctenostoma* were then examined to evaluate their degree of sexual maturity. The species is common in shallow waters of the Mediterranean and eastern Atlantic areas, particularly inhabiting photophilic infra-littoral hard substrates and *Posidonia oceanica* rhizomes. *Odontosyllis ctenostoma* is a relatively large, dioecious, epigamic syllid (up to 20 mm for 100 chaetigers) that is currently placed within the subfamily Eusyllinae (San Martín, 2003).

Electron microscopy

For transmission electron microscopic investigations, specimens were fixed in a mixture of 2.5% glutaraldehyde, cacodylate buffer (0.4 M) and filtered sea water (pH 7.4). Subsequently, they were postfixed for 1h in 1.0% OsO_4 in sea water at 4°C, rinsed in sea water, dehydrated with increasing acetone, and embedded in Araldite (Taab, Aldermaston, England). Ultrathin sections were cut with an LKB ultratome, stained with 5% uranyl acetate in 50% ethanol and lead citrate in distilled water and examined under a Philips EM 208 microscope. Semi-thin sections (1 μm thick) were heat-stained with toluidine blue borate (Millonig, 1976).

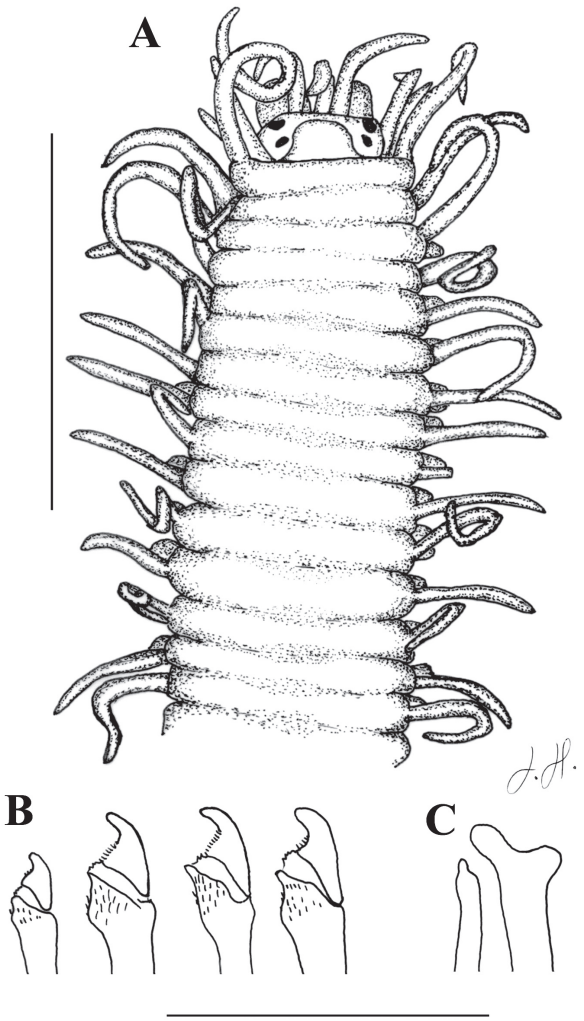

FIG. 1. – *Odontosyllis ctenostoma*. A, prostomial region, dorsal view, scale bar: 2 mm; B compound chaetae from mid-body, scale bar: 0.05 mm; C, aciculae from mid-body, scale bar: 0.05 mm.

RESULTS

Apart from a larger number of chaetae per parapodium (c.a 20 *vs* 12) and a slight dorsal-ventral gradation of their length, the specimens of *O. ctenostoma* examined herein (Fig. 1) fit the available descriptions of the species (see San Martín, 2003), particularly in the chaetal and typical acicular shape (Fig. 1B, C), nuchal lobe and prostomial organisation (Fig. 1A).

The length of the 10 mature specimens (6 males and 4 females) examined ranged from 12 mm to 14 mm for 60-70 chaetigers. The sexually ripe region of the body extended from about chaetiger 30 to the posterior end. The chaetigers of ripe males and, particularly females were wider and no elongated swimming chaetae were observed. The mature males were distinguishable from the mature females by the different coloration of the chaetigers containing gametes. The ripe region was orange in males, but olive-green in females. In ripe specimens the germinal cells entirely filled the coelomic cavity, compressing and reducing the gut lumen.

The coelomic cavity of the ripe females was filled with large oocytes measuring about 100 μm in diameter.

As regards the males, some specimens showed many spermatocytes, others few spermatocytes and numerous spermatids together with a few sperms, and others only sperms (Fig. 2A, B).

The spermatocytes had a large and elliptic nucleus with scattered condensations of chromatin and scarce cytoplasm (Fig. 2C). They appeared as closely packed and irregularly shaped cells, joined by desmosomes (Fig. 2D).

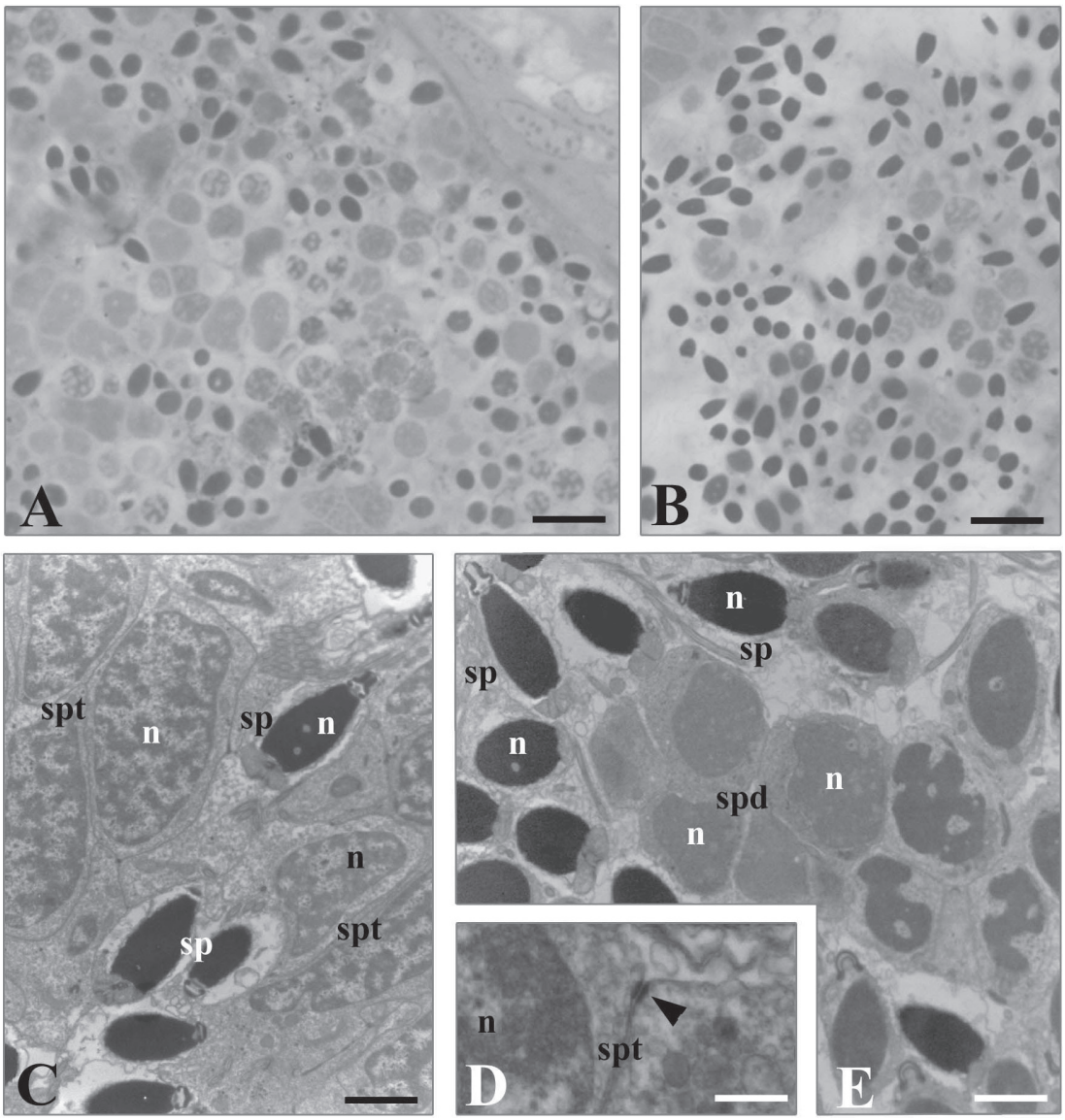

FIG. 2. – A and B, semi-thin sections; C, D and E, ultra-thin sections. A, coelomic cavity with male germinal elements at different phases of maturation, scale bar: 9 μm; B, coelomic cavity of a ripe specimen mainly containing spermatozoa, scale bar: 9 μm; C, spermatocytes (spt) with partially condensed chromatin and spermatozoa (sp), n, nucleus, scale bar: 2 μm; D, portions of two spermatocytes (spt) joined by desmosomes (arrowhead), n, nucleus, scale bar: 0.4 μm; E, early spermatids (spd) joined by cytoplasmic bridges and spermatozoa (sp), n, nucleus, scale bar: 3 μm.

The passage from spermatocytes to early spermatids was marked by a progressive condensation of the chromatin from the periphery of the nucleus toward the centre. The early spermatids formed tetrads of cells connected by cytoplasmic bridges (Fig. 2E).

In the late spermatids the condensed chromatin had a granular structure and in some of them some central electron-lucent areas were visible (Fig. 3A, B). Small electron-dense granules and mitochondria already placed at the base of the nucleus were observable (Fig. 3A). In the later stages the amount of heterochromatin increased gradually and finally the nucleus was completely electron-dense and homogeneous. The shape of the nucleus changed from an irregular structure to an elongated cylindrical body. Then the spermatozoon had a strongly condensed, lengthened nucleus measuring about 3.5 μm in length and 2 μm in diameter. The nucleus had two depressions, one anterior at the base of the acrosome and the other posterior containing the proximal centriole and in part the mitochondria (Fig. 3B). The acrosome was bell-shaped and contained a large sub-acrosomal space with fibrillar material (Fig. 3B, C). A superior narrower part measuring 0.5 μm and a wide base measuring 0.8 μm were distinguished in the acrosome. The height of the acrosome was 1 μm.

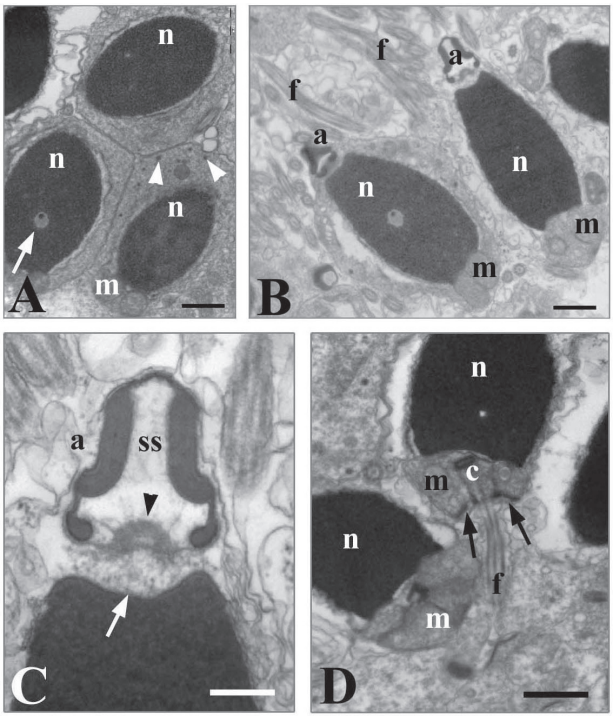

FIG. 3. – Ultra-thin sections. A, late spermatids. In one of them a central electron-lucent area is visible (arrow). Small electron-dense granules (arrowheads) are present in the cytoplasm. Mitochondria (m) are located at the base of the nucleus (n). Scale bar: 1 μm; B, spermatozoa. Note the slight depressions of the nucleus of the spermatozoon at the two extremities. a, acrosome, m, mitochondria, f, flagellum. Scale bar: 1 μm; C, Acrosome (a). It is possible to observe the subacrosomal space (ss) and the large opening with accumulation of electron-dense material in the basal region (arrowhead). Note the anterior depression of the nucleus (arrow). Scale bar: 0.3 μm; D, longitudinal section of the basal portion of the spermatozoa head. Two thin electron-dense structures (arrows) are visible at the base of the mitochondria. n, nucleus; c, centrioles; m, mitochondria; f, flagellum. Scale bar: 0.8 μm.

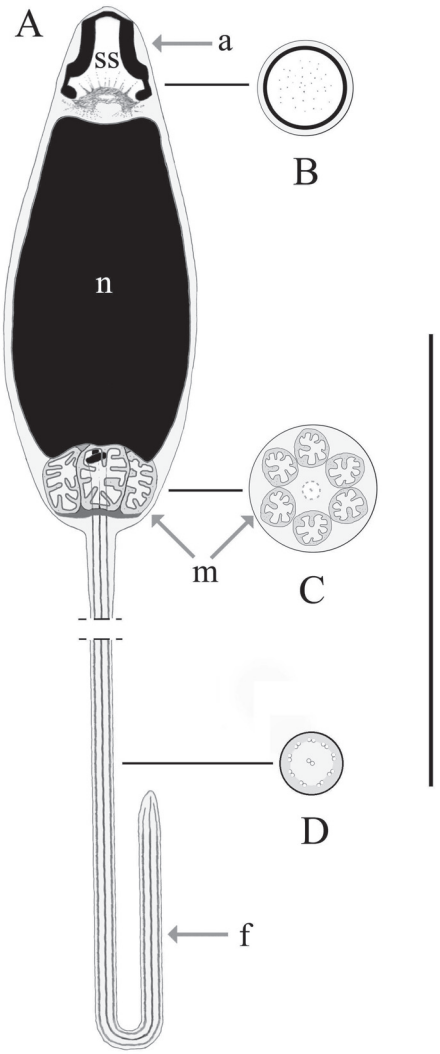

FIG. 4. – Reconstruction of the spermatozoon. A, longitudinal section; B, transverse section through the acrosome; C, transverse section through the mid-piece; D, transverse section through the flagellum; a, acrosome; ss, sub-acrosomal space; n, nucleus; m, mitochondria; f, flagellum. Scale bar: 4.5 μm.

In the wide opening of the subacrosomal space an accumulation of electron-dense granular material from which thin filaments radiated was visible (Fig. 3C). Two centrioles were placed below the nucleus; two thin electron-dense areas were observable laterally to the distal centriole, probably part of a tore-shaped structure located below the mitochondria (Fig. 3D). Two or three mitochondria were visible in longitudinal section, but up to six in transversal sections. The flagellum begins immediately behind the distal centriole (Fig. 4) and has a normal structure (9+2x2 microtubes).

DISCUSSION

Odontosyllis ctenostoma is epigamous and like other species in the genus is probably characterised by a synchronised sexual swarm strategy. Wilkens and Wolken (1981) reported that *O. enopla* eyes have maximum sensitivity in the green part of the visible spectrum, which corresponds to that of the green bioluminescence emitted by these "fireworms" during spawning. No remarkable epitokal modifications similar to those observed in *O. enopla* (Fischer and Fischer, 1995) were visible in the specimens examined herein. However, male and female specimens of *O. ctenostoma* differed in colour pattern (orange males and green females). In spite of possible chemical signals between polychaete sexes (e.g. Hardege *et al.*, 1998), this kind of provisional sexual dimorphism might help reciprocal recognition during swarming or account for a differential, sex-linked cryptic strategy. Due to the low density

of the sampled population, the complete series of events leading to mature sperms and eggs in *O. ctenostoma* was not fully sequenced. Only large oocytes, which appear morphologically similar to the oocytes of *Syllis pulchra* described by Heacox and Schroeder (1981b), were observed in females. Moreover, only some phases of spermatogenesis in males were described. However, the data are sufficient for interspecific comparisons and some general phylogenetic inferences.

The sperm structure observed herein, resembling the ect-aquasperm type, does not completely fit the reproductive strategy reported for *O. ctenostoma*. A particular brood protection with temporary maintenance of fertilised eggs in a mass on the ventrum of swarming females is in fact traditionally reported (Gravier and Dantan, 1928). A similar reproductive strategy has been confirmed in *Myrianida* (= *Autolytus*) *edwarsi* (Schiedges, 1979), and the sole examined sperm morphology from an autolytine showed an ent-aquasperm type (Franzén, 1982). Brood protection is also common in other Eusyllinae species, such as some small *Pionosyllis* (Pierantoni, 1903), but it is typical of small-sized epigamic Exogoninae having ent-aquasperm (Mastrodonato *et al*., 2003). Thus, it can be hypothesised that brood protection is an adaptive convergence in several small-sized species. By contrast, *O. ctenostoma* is a large syllid. Therefore, it can be assumed that the *O. ctenostoma* specimens described by Gravier and Dantan (1928) as brooders belonged to a different and possibly smaller species. On the other hand, on the basis of the intrafamiliar phylogenetic constraints observed in polychaetes (Giangrande, 1997), if compared to the egg size range in Syllidae, the size of *O. ctenostoma* eggs (c.a 100 μm) could support direct development and a brood protection strategy. Further studies are therefore needed.

As stated before, the sperm structure of *O. ctenostoma* fits the ect-aquasperm type, with a classical mid-piece with rounded mitochondria, and a fairly elongate barrel-shaped nucleus. The acrosome ultrastructure and its internal organisation are interesting, particularly because of the presence of fibrillar electron-dense material in the sub-acrosomal space. The basal invagination of the acrosomal vesicle forming the sub-acrosomal space is common in polychaete sperms (Jamieson and Rouse, 1989; Rice, 1992). In *S. krohni* (Lepore *et al*., 2006), as well as in the ect-aquasperm of *Syllis* sp (Jamieson and Rouse, 1989) and *S. pigmentata* (Heacox and Schroeder, 1981a), only a small opening is retained between the sub-acrosomal space and the underlying nucleus, and the sub-acrosomal material within the enclosed cavity is differentiated as an ellipsoid with a number of electron-dense thick filaments. This space is more developed in *S. krohni* (Lepore *et al*., 2006), as in the case of *O. ctenostoma*. However, the mature acrosome of the currently investigated species particularly resembled the up-side-down cup-shaped acrosome of the late spermatids of *Exogone naidina* and *E. dispar* (Giangrande *et al*., 2002), albeit with a reduced apical region that made it appear bell-shaped rather than cup-shaped. With maturation, however, the sub-acrosomal space in *Exogone* species becomes narrower and the sub-acrosomal material condenses and finally transforms into an electron-opaque meshwork within the acrosome. This has been seen as a simplification and possibly indicative of evolutionary derivation from the Syllinae ect-aquasperm type (Giangrande *et al.*, 2002). The similarity between the *Odontosyllis* mature sperm and *Exogone* spermatids could be indicative of a close phylogenetic relationship. Completely different late spermatids have up to now been found in the genus *Syllis*, which in the phylogenetic hypothesis of Nygren (1999) is more closely related to *Odontosyllis* than Exogoninae are. In fact, the subfamily Eusyllinae is considered paraphyletic in Nygren's (1999) cladistic analysis, and the genus *Odontosyllis* in particular is closer to the Syllinae but resembles the Exogoninae in the shared epigamy.

It is well known that Syllinae and Autolytinae are generally characterised by schizogamy and Eusyllinae and Exogoninae by epigamy; however, as stressed by Nygren (1999), it is uncertain which of the two is the plesiomorphic reproductive mode. Following our hypothesis, if the exogonine sperm was derived from simplification of a sylline sperm through a gradual passage from an *Odontosyllis*-like eusylline ancestor, it could be equally possible that epigamy is derived from schizogamy, *Odontosyllis* (or an *Odontosyllis*-like ancestor) representing the linkage between the two. Further studies on sperm morphology will likely contribute to a better understanding of syllid phylogeny.

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