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Contribution of Scanning Electron Microscope to the Study of Morphology, Biology, Reproduction, and Phylogeny of the Family Syllidae (Polychaeta)

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1. Introduction

Syllidae is a highly diverse family of the polychaetes (Annelida, Phyllodocida), with 72 described genera and almost 700 species (San Martín, 2003; Aguado & San Martín, 2009; Aguado et al., in press), and continuously new taxa are being described. They are small marine worms, usually of few mm long, although some species can reach up to 90 mm. Contrariwise to their small size, they are very complex, with a body exhibiting numerous structures, external and internal, some of them difficult to examine properly under light microscope, even using higher magnifications and Nomarsky system of polarized light. Description of most species before around the year 2000 was based only on examinations and drawings made with camera lucida under light microscopes. The use of SEM to study syllids is relatively recent, but produced the discover and descriptions of a number of new, unknown structures, or only incompletely known before, whose physiology and significance open a new field of research. The oldest SEM picture of a syllid is from 1980 in which Heacox showed the head of a *Chaetosyllis* stolon and a larval compound chaeta in a study of the life cycle of *Syllis pulchra* Berkeley & Berkeley, 1948 (Heacox, 1980); somewhat later, Pocklington & Hutchenson (1983) reported the viviparity of the interstitial species *Parexogone hebes* (Webster & Benedict, 1884) showing excellent and surprising SEM photographs of juveniles emerging through segmental apertures (probably nephridial pores) and also some characteristic crenulations of the ventral surface of female's body after releasing juveniles; in the same year, Pawlick published the first SEM photos of general aspect of body, chaetae, and details of ciliation on the basis of dorsal cirri on the species *Branchiosyllis oculata* Ehlers, 1887. This is the first paper, in our knowledge, in which some previously overlooked structures were showed and described thanks to SEM; finally, Sardá & San Martín (1992) redescribed one species of syllid from East coast of USA, with some SEM pictures. However, few descriptions of new taxa with SEM and only few papers with SEM pictures of syllids were published during next years. Most of examinations came from our own relatively recent papers; one is the book "Fauna Ibérica. Syllidae", published in 2003, in which the 161 recorded species of the Iberian Peninsula are described and figured,

with 122 SEM plates, showing morphology, and also details of the reproduction; the SEM study revealed some new, tiny structures, unknown before (San Martín, 2003). However, since that book was published in Spanish, several of these discoveries could be overlooked by some non-Spanish speakers. Same kind of study and descriptions, with both light microscope and SEM photos was followed since then for descriptions of syllids from other parts of the world (Aguado et al., 2006 and others), especially in the still unfinished series on Australian Syllidae (San Martín, 2005; San Martín & Hutchings, 2006; San Martín et al., 2008a, b; 2010, and others) and revisions of genera (Aguado & San Martín, 2008). In these papers, and others published by other authors profusely cited below, more unknown structures and new details on chaetae, ciliation pattern, pharyngeal armature, etc., were described, as well as important observations on the reproductive biology of many species. A review of these discoveries and their implications on some aspects of the knowledge of the family Syllidae, with especial relevance in taxonomy and systematics, will be analyzed in detail in this chapter.

Detailed morphological and reproductive observations under SEM had important relevance to the knowledge of the family Syllidae for two reasons.

- The first one is related with the phylogeny of the family; discoverment and descriptions of these structures and details of the reproductive modes provided more morphological features to analyze and, consequently, more robust hypothesis about the relationships among the different genera of the family.
- The second one is the great help to differentiate sibling complexes of species; species apparently identical morphologically can be differentiated by minute details, only perceptible under SEM.

Two books about polychaetes also includes excellent SEM pictures of Syllidae (as well as many others of different families of polychaetes): Rouse & Pleijel (2001), which shows 6 photos with details of morphology and reproduction, and Beesley et al. (2000) in which a couple of SEM photos about syllid reproduction are shown.

2. Material and methods

Syllids are generally of small size and hence, the process of preparation is usually complex and sometimes difficult, so, it is desirable to prepare several specimens for examination. At least, one to be examined dorsally and another one ventrally; specimen with exerted pharynx is also strongly recommended to examine and take photos of details of the anterior end of the pharynx and their armature, which are important characters for identification to genus level.

Minute specimens are very easily lost during the process of preparation, so it is important to be extremely careful, especially having a short number of specimens.

Fixation of the specimens is a very important process for taking good and sharp pictures. Specimens of syllids are usually dirty, especially those of the genera with dorsal papillae, which produce a sticky secretion which agglutinate debris. However, details of papillation is an important taxonomic trait for identification of species and therefore, it is necessary to clean up them, using a brush of a single, slender hair.

Techniques could be different depending upon the authors and the type of SEM used. The specimens for taxonomic studies are usually fixed in formalin, examined in pure water and finally stored in 70% ethanol. To be prepared for SEM, the selected specimens experience a series of progressive baths in more concentrated alcohol (80%, 90%, and pure ethanol), then dried on critical point and covered of a coat of gold. Rouse & Pleijel (2001) recommend Osmium tetroxide (OsO_4) as preferred fixative, although its high toxicity (see Rouse & Pleijel, 2001, p. 7).

3. Results

3.1 Morphology

3.1.1 Ciliation

Many syllids are provided with numerous cilia, whose arrangement has taxonomic importance, but they are difficult to see under light microscope; sometimes, it is possible to observe the presence of tufts of cilia in some appropriate areas, but a detailed description of the arrangement of these cilia is almost impossible or extremely difficult. However, well prepared specimens show, under SEM, a system of transversal rows of cilia, which can be single (fig. 1A) or double (fig. 1B), sometimes some anterior segments with single and from one segment backwards being double (fig. 1B), sometimes only tufts of cilia on some areas; in some species only the peristomium is dorsally provided with a single band of cilia and remaining segments lack them. These details were not included in the descriptions until recently, and they are certainly useful for segregation of species and even similar genera. Descriptions arrangement of ciliary bands, based on SEM examinations are in San Martín (2003) for the species *Paraehlersia ferrugina* (Langerhans, 1881), *Odontosyllis fulgurans* (Audouin & Milne Edwards, 1834), *Myrianida benazzi* (Cognetti, 1953), *Myrianida convoluta* (Cognetti, 1953), *Myrianida edwardsi* (Saint-Joseph, 1887), and *Myrianida dentalia* (Imajima, 1966); San Martín & Hutchings (2006) for the species *Eusyllis kupfferi* Langerhans, 1879, *Odontosyllis polycera* Schmarda, 1863, *O. australiensis* Hartmann-Schröder, 1979; *Paraehlersia weissmannioides* (Augener, 1913), *P. ehlersiaeformis* (Augener, 1913), *Perkinsyllis koolalya* (San Martín & Hutchings, 2006), and *P. serrata* (Hartmann-Schröder, 1984); and Nogueira & Fukuda (2008) for *Trypanosyllis zebra* (Grube, 1860) and *T. aurantiacus* Nogueira & Fukuda, 2008. Also some ciliated areas on bases of dorsal cirri, lateral of segments, palps, ventral surface or in ventral cirri were described and figured by Pawlik (1983) for *B. oculata*; Licher & Kuper (1998) for *Syllis tyrrhena* (Licher & Kuper, 1998); López et al. (2001) for *Pionosyllis magnifica* Moore, 1906; San Martín (2003) for *Xenosyllis scabra* (Ehlers, 1864), and *Trypanosyllis aeolis* Langerhans, 1879; San Martín & Hutchings (2006) for *O. australiensis*; Aguado & San Martín (2008) for *Brachysyllis infusata* (Ehlers, 1901); Ramos et al. (2010) for *Streptodonta exsulis* Ramos, San Martín & Sikorski, 2010; and Salcedo-Oropeza et al. (2011) for *Trypanosyllis microdenticulata* Salcedo-Oropeza, San Martín & Solís-Weiss, 2011.

3.1.2 Nuchal organs

Nuchal organs are only present in polychaetes and are thought to be a synapomorphy for the group (Rose & Fauchald, 1997; Rouse & Pleijel, 2001), although they show different chemoreceptor structures and are different in shape among the families. The most typical shape appears as two semicircular, densely ciliated pits between prostomium and

peristomium; these are the kind of nuchal organs most common in the Syllidae, in fact most genera have the typical nuchal organs (fig. 2A), sometimes extending laterally to prostomium, forming two semicircular ciliated areas (fig. 2B) as in the genera *Eusyllis* Malmgren, 1867, *Odontosyllis* Claparède, 1863, and *Trypanosyllis* Claparède, 1864; however, some genera show other kind of nuchal organs, called nuchal lappets, forming two dorsal, longitudinal evaginations over a number of segments, sometimes only one, more or less spherical or elongate, straight (fig. 2C) or sinuous (fig. 2D), ciliated or lacking of cilia. Numerous descriptions of species include SEM photos of the nuchal organs, as those in Lanera et al. (1994); Lewbart & Riser (1996); Licher & Kuper (1998); Martín et al. (2002); Nogueira & San Martín (2002); San Martín (2003; 2005); San Martín & Hutchings (2006), Aguado & San Martín (2007, 2008); Lattig et al. (2007; 2010 a, b); Nogueira & Fukuda (2008); Lattig & Martin (2009); Ramos et al. (2010); San Martín et al. (2008 a, b; 2010); Salcedo-Oropeza et al. (2011); Lattig & Martín (in press a, b). San Martín & López (2003) described a new genus and species from Australia with nuchal organs laterally located, protected by two lips.

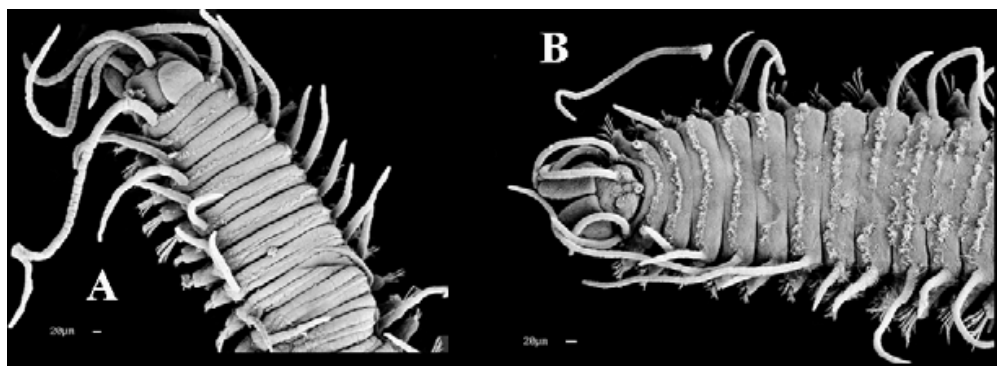


Fig. 1. A, Anterior end, dorsal view of *Odontosyllis australiensis*, showing single row of cilia on each segment. B, Anterior end, dorsal view of *Perkinsyllis serrata* showing single row of cilia on anterior segments and double row from chaetiger 9. San Martín & Hutchings (2006).

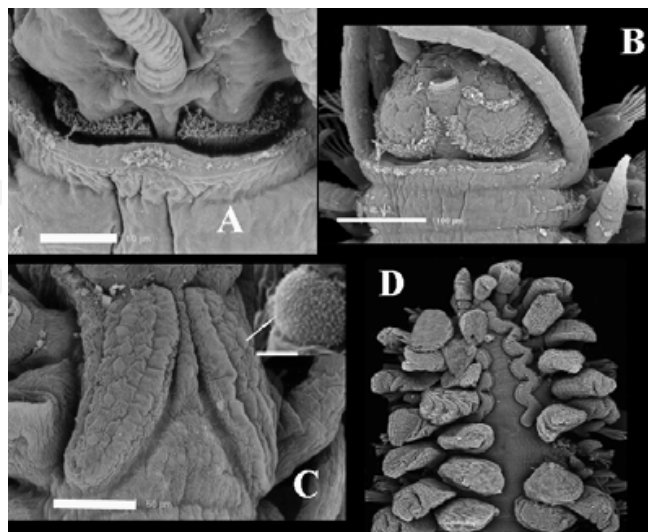


Fig. 2. Nuchal organs of: A *Syllis corallicola*, B *Eusyllis kupfferi*, showing the ciliation extending to lateral sides of prostomium; C, *Proceraea aurantiaca*, and detail (arrow, right) of the rugose bulks; D, *Clavisyllis alternata* Knox, 1957. A, C, San Martín (2003); B, San Martín & Hutchings (2006); D, Aguado & San Martín (2008).

In the species *Proceraea aurantiaca* Claparède, 1868, San Martín (2003) found and described an enigmatic structure on the nuchal lappets, as semicircular, rugose bulks (fig. 2C, right, up, arrow) provided with pores and tiny hairs, whose function is totally unknown.

3.1.3 Papillation

Some genera of the family Syllidae, as *Sphaerosyllis* Claparède, 1863, *Prosphaerosyllis* San Martín, 1984, *Erinaceusyllis* San Martín, 2005, *Rhopalosyllis* Augener, 1913; and *Paraopisthosyllis* Hartmann-Schröder, 1991, as well as some species of other genera, as *Opisthosyllis* Langerhans, 1879, *Branchiosyllis* Ehlers, 1887, and *Trypanosyllis* Claparède, 1864, have the dorsal, and also sometimes the ventral, surface covered by conspicuous papillae (figure 3), sometimes also extending through parapodia, antennae, cirri and palps. These papillae can be scarce and scattered or densely distributed, in different sizes or all similar, hazardous distributed or arranged in rows. Information on size, shape and distribution of papillae has a very important taxonomic meaning, and it is very useful to differentiate species; although presence of papillae is easily perceptible under light microscope, the arrangement and sizes of papillae are much better evaluable under SEM. In *Sphaerosyllis*, *Prosphaerosyllis* and *Erinaceusyllis*, the papillae produce an adhesive secretion which sticks detritus for mask the individuals; so, it is sometimes difficult to see them because they are covered by detritus and the specimens requires a previous cleaning by a minute brush and washing of water. Numerous descriptions of species of these three genera are in San Martín (2003; 2005), Nogueira et al. (2004); Musco et al. (2005); Álvarez & San Martín (2009); and Olivier et al. (2011). Details of papillae of the species *Syllis papillosus* (Tovar-Hernández, Granados-Barba & Solís-Weiss, 2002) in Tovar-Hernández et al. (2002); *Trypanosyllis troll* Ramos, San Martín & Sikorski, 2010 are in Ramos et al. (2010), *Paraopisthosyllis alternocirra* San Martín & Hutchings, 2006 in San Martín & Hutchings (2006); *Opisthosyllis viridis* Langerhans, 1879 in San Martín et al. (2008 a); and *Branchiosyllis verruculosa* (Augener, 1913) in San Martín et al. (2008b).

3.1.4 Pharyngeal armature

The pharyngeal armature in syllids is an important diagnostic character at different taxonomic levels; for this reason, a careful examination of the pharyngeal armature is always necessary for identification. SEM photos help considerably in these observations, and also contributed greatly to the discover of some overlooked details, as ciliation and presence of pores on the pharyngeal papillae, coats of cilia on the pharyngeal opening, secondary crown of papillae, total absence of papillae, etc. The pharynx of syllids can be unarmed or provided with a single middorsal tooth (fig. 4A), usually surrounded by a crown of papillae, and sometimes with a complete or incomplete crown of teeth (trepan) (fig. 4B) sometimes there is a trepan but not a pharyngeal middorsal tooth; teeth of trepan are usually of the same size, but sometimes they are of different sizes (figs. 4C, D). Detailed observations under SEM of the pharyngeal armature, papillae and ciliation are in numerous papers: Capa et al. (2001); Tovar-Hernández et al. (2002); Nogueira & San Martín (2002); Martín et al. (2002; 2003; 2009); San Martín (2003; 2005); San Martín & Hutchings (2006); Aguado & San Martín (2007,2008); Nogueira & Fukuda (2008); San Martín et al. (2008a, b; 2010); Lattig & Martín (2009; in press a, b); Lattig et al. (2007; 2010 a, b); Ramos et al. (2010); Olivier et al. (2011); Salcedo-Oropeza et al. (2011).

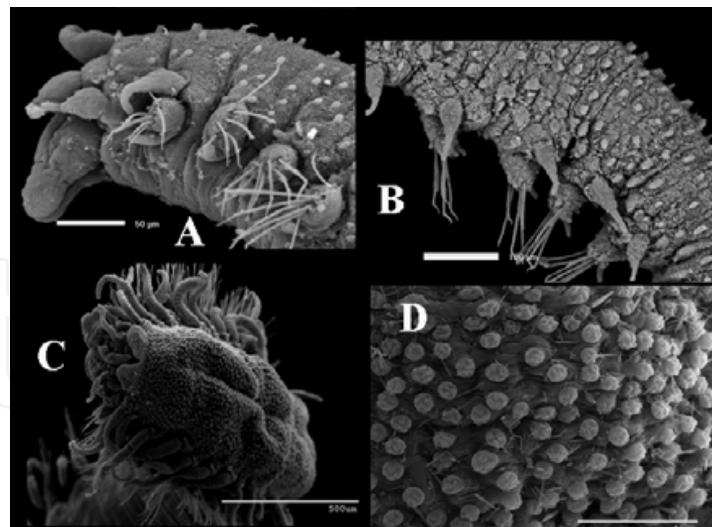


Fig. 3. Papillae: A, anterior end, lateral view, *Sphaerosyllis hirsuta*. B, midbody of the same. C, anterior end, dorsal view, *Opisthosyllis papillosa*. D, details of the papillae of the same. A, B, San Martín (2005). C, D, San Martín et al. (2008 a).

3.1.5 Chaetae

Chaetae have a great value for taxonomic identifications; small details of chaetae can be crucial to assign one specimen to one species. Therefore, a careful examination of chaetae is decisive. They are usually the most photographed structures of syllids; together with the pharyngeal armature. The chaetae are the unique hard structures in syllids, so they are relatively easy to be examined and photographed by SEM; however, they are not always clean enough. Additionally, length and shape can vary from anterior to posterior part of body and from dorsal to ventral. In our knowledge, the oldest publications showing chaetae of any syllid are those of Heacox (1980) and Pawlik (1983) cited above. Later, Westheide (1990) showed the chaetae and also a special genital chaeta of a new species (*Sphaerosyllis hermaphrodita*) from Thailand. There are many variations on the chaetal types in syllids, and since then, numerous papers showing details of chaetae, many of them profusely cited in this chapter, and also others, like Licher et al (1995) or Martínez et al (2002), not mentioned in any other sections of this chapter. Relative length and orientation of spines on margin of blades of compound chaetae are difficult to see properly under light microscope but they are absolutely precise under SEM. In the figure 5, there is a selection of photos of different kinds of chaetae in syllids (see also figure 11). One interesting is the Australian species *Odontosyllis freycinetensis* Augener, 1913; compound chaetae of that species are apparently unidentate under light microscope, even examined with Normarsky system and high magnifications, but in fact they are bidentate, with a minute, spine-like distal tooth and a big, curved proximal tooth. The use of SEM for study of chaetae in syllids also clarify some other details; for instance, hyaline hoods on the margin of blades appear in few some species, but SEM examinations showed that in fact they are several rows of minute spines, instead of one row, with more or less well defined spines (fig. 6).

Images of the same chaeta under light microscope or SEM can be remarkably different, being the latter much more precise and useful for descriptions and phylogenetic inferences. Also study under SEM of special chaetae for reproduction and brooding of eggs produce interesting discoveries (see below).

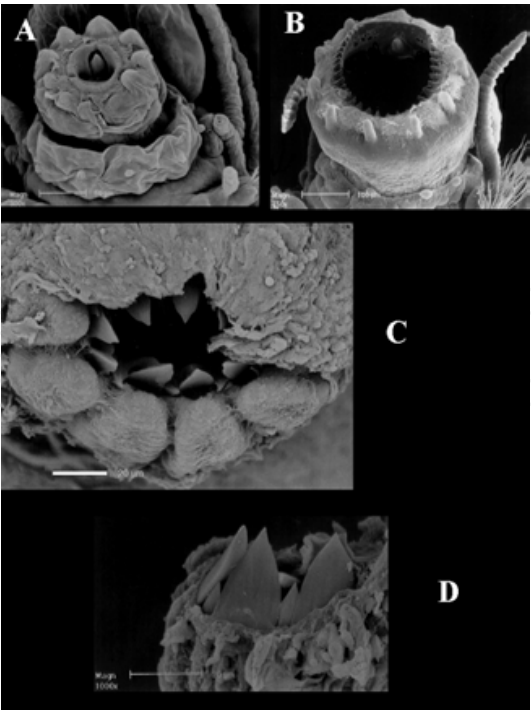


Fig. 4. Everted pharynx of: A, *Syllis gerundensis*, showing the pharyngeal tooth, and two crowns of papillae. B, *Eusyllis assimilis*, showing the middorsal tooth, incomplete trepan, and two crowns of papillae. C, D, frontal and lateral view of the trepan of *Proceraea picta*, showing the different sizes of teeth. San Martín (2003).

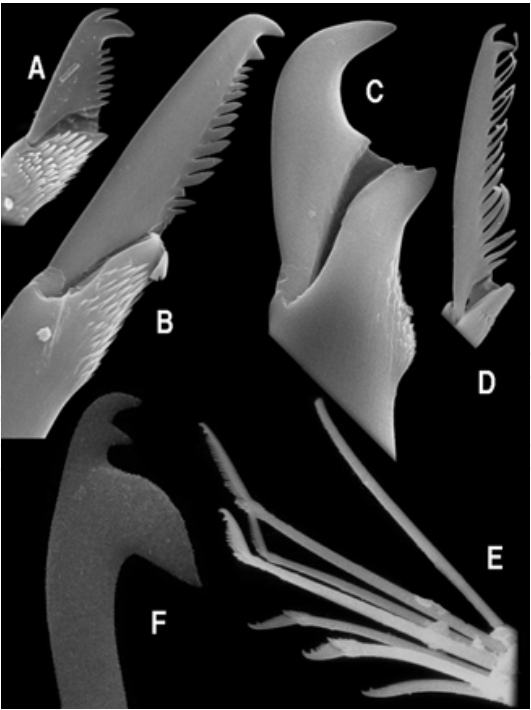


Fig. 5. Some examples of Syllid chaetae. A, *Paraehlersia elersiaeformis*; B, C, *Branchiosyllis maculata*; D, *Perkinsyllis heterochaetosa*; E, chaetal fascicle of *Syllis rosea*; F, *Parahaplosyllis brevicirra*. A, D, San Martín & Hutchings (2006); B, C, San Martín et al.(2008a); E, San Martín (2003); F, San Martín, et al. (2010).

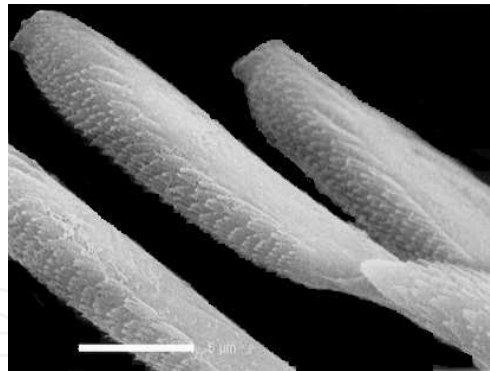


Fig. 6. Compound chaetae of *Streptodonta pterochaeta*, showing the rows of minute spines. San Martín (2003).

3.1.6 Glands

Syllids are provided with numerous kinds of glands, whose function is mostly unknown; some of them were known from long time ago but others were discovered and described recently using SEM. Some of the previously known glands were studied under SEM, and some details of their structures were showed after SEM observations. Most of the different kinds of glands in Syllidae are present in the subfamilies Exogoninae Langerhans, 1879 and Syllinae Grube, 1850, especially in interstitial, minute species, but also in some large syllids. Members of the genus *Syllis* Lamarck, 1818 (including *Typosyllis* Langerhans, 1879) have convoluted, refringent glands within the articles of cirri. They are easily visible under light microscope, even under compound microscope. These glands are usually opened by means of minute pores, only perceptible under SEM (fig. 7A). The first picture of these pores are in Licher & Kuper (1998), for *Syllis thyrrena*, and later San Martín (2003) for *Trypanosyllis zebra*, *Trypanosyllis aeolis*, and *Syllis amica* Quatrefages, 1865, but they are probably present in many other species. San Martín et al. (2008b) showed these pores in the species *Parasphaerosyllis indica*. San Martín (2003) also discovered and published some photos of pores in some anterior ventral cirri of some species, as *Eurysyllis tuberculata*, *Plakosyllis brevipes*, *Xenosyllis scabra*, and *Trypanosyllis zebra*; especially interesting are those of *P. brevipes*, since they are half-moon like, with an arranged alveolar organization (fig. 7B). Since that species is strictly interstitial in sands, these glands could be adhesive; however, they are also present, although less developed, in other non-interstitial species, as *T. zebra*. Pores on dorsum of several species have been also described, as in *P. brevipes* (San Martín, 2003). San Martín et al. (2008b) described a species, *Branchiosyllis carmenroldanae*, from Australia, with the granular dorsum covered by numerous pores. Pores on the dorsal tubercles have been reported in *E. tuberculata* by San Martín (2003) and in the tips of dorsal crests (see below) in *Xenosyllis moloch* San Martín, Hutchings & Aguado, 2008 (San Martín et al., 2008b) (fig. 7C). Also dorsum densely provided of granules, opened by pores was described in the Anoplosyllinae *Syllides fulvus* (Marion & Bobretzky, 1875) by San Martín (2003). Dorsal pores have been reported in *P. ferrugina* by the same author, *P. ehlesiaeformis*, *P. weissmannioides*, by San Martín & Hutchings (2006). Especially interesting are the pores described and figured by the same authors for the species *Brevicirrosyllis mariae* (San Martín & Hutchings, 2006), located on bases of dorsal cirri, similar to the pores of parapodial glands of some Exogoninae (see below) (fig. 7D), and also at tips of dorsal cirri. Pores in ventral cirri have been also reported for other few species, as *Amblyosyllis madeirensis* Langerhans, 1879, and *Eusyllis lamelligera* Marion & Bobretzky, 1875.

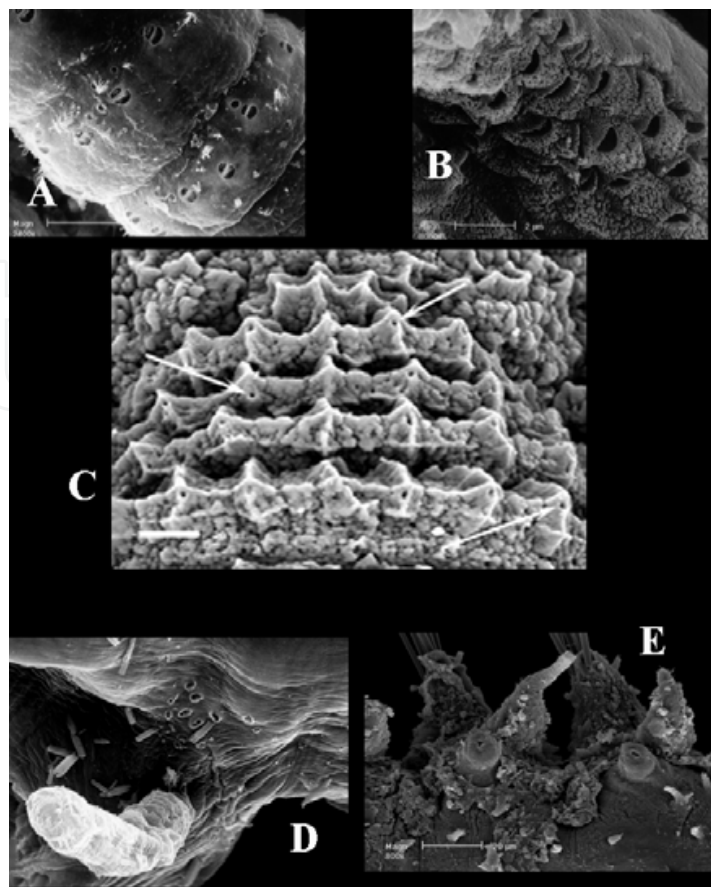


Fig. 7. Opening of different glands. A, articles of dorsal cirri, *Trypanosyllis zebra*; B, ventral cirri, *Plakosyllis brevipes*; C, dorsal crests, *Xenosyllis moloch*; D, base of dorsal cirri, *Brevicirrosyllis mariae*; E, parapodial glands, *Sphaerosyllis capensis*. A, B, San Martín (2003); C, San Martín et al. (2008a); D, San Martín & Hutchings (2006); E, San Martín (2005).

In some genera of the subfamily Exogoninae (*Brania* Quatrefages, 1865, *Sphaerosyllis* Claparède, 1863, *Parapionosyllis* Fauvel, 1923) there is a typical kind of glands, the parapodial glands, which might show rod, granular, or hyaline material. In *Sphaerosyllis*, the parapodial glands are connected to a special papilla, opened by one pore (fig. 7E) described for *S. capensis* Day, 1953 by San Martín (2005). San Martín (2003) took some detailed SEM pictures of pores of parapodial glands in some species of *Parapionosyllis*, as *P. labronica* Cognetti, 1965, *P. brevicirra* Day, 1954, *P. elegans* (Pierantoni, 1903), *P. minuta* (Pierantoni, 1903), and *P. cabezali* (Parapar, Moreira & San Martín, 2000). These pores are very well defined, with a thickened, circular area around, and a digitiform, eversible and contractile structure inside, even one SEM image shows one of these digitiform structures with glandular material emerging from the body (fig. 8, below).

3.1.7 Other structures

Additionally, SEM, has provided the possibility to find some enigmatic structures, such as transversal lines of spines or papillae in some species of *Trypanosyllis* (San Martín, 2003; Nogueira & Fukuda, 2008; San Martín et al, 2008a; Ramos et al., 2010; Salcedo-Oropeza et al., 2011) (fig. 9A), a detailed arrangement of crests in *Xenosyllis* (San Martín, 2003; San Martín et

al., 2008b) (fig. 7C), dorsal glands in *Syllis pulvinata* (Langerhans, 1881), ciliated areas close to prostomium in *Sacconereis stolons* (San Martín, 2003) (fig. 9D), terminal papillae in palps of some species of *Syllides* and *Streptosyllis*, (San Martín, 2003; San Martín & Hutchings, 2006), subcirral papillae in some species of *Paraehlersia* (figs. 9B, C), etc. Furthermore, SEM has been used to take detailed photos of internal structures, as the proventricle, a distinct muscular and glandular structure of the gut, after dissection, formed by columns ending in hexagons (fig. 9E), which were photographed in SEM by San Martín (2003), San Martín et al., 2008a, 2010, Martín et al., 2009).

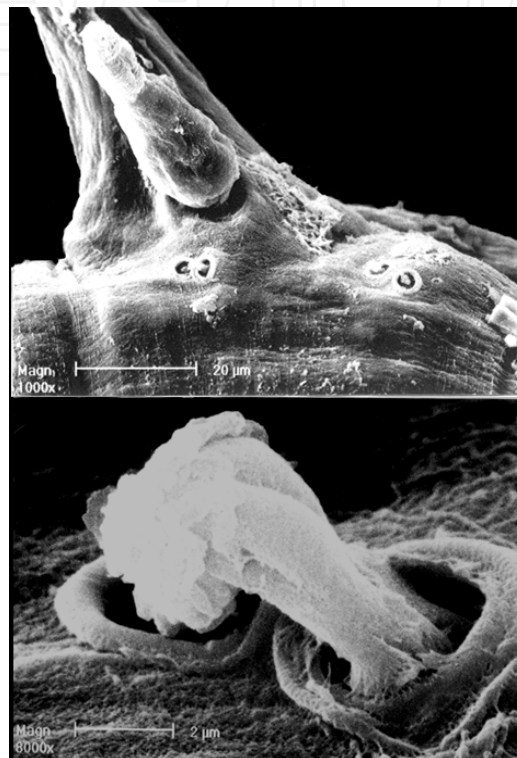


Fig. 8. Parapodial glands of *Parapionosyllis brevicirra* (above), detail of a papilla with glandular material (below). San Martín (2003).

3.2 Biology and reproduction

Some aspects of the biology has been also remarked using SEM, such is the case of specimens of the genus *Haplosyllis* crawling through the galleries inside sponges (Magnino et al., 1999 a, b), or specimens produced by asexual reproduction (Lattig et al., in press b), or in *Procerastea*. However, most important contributions of SEM to the knowledge of syllids are related with reproductive aspects, well documented by means of numerous pictures in several papers cited below. We have already mentioned the SEM photos of young *E. hebes* emerging from the mother's body (Pocklington & Hutchenson, 1983), stolons (Heacox, 1980), and genital especial chaetae (Westheide, 1990); after these papers, others also dealt with same or similar topics, as Qian & Chia (1989) for the larval development of one species of *Myrianida*.

Many interesting contributions were made on the external gestation of Exogoninae; Küper & Westheide (1998) demonstrated, including SEM photos, that in the genera *Prosphaerosyllis*

and *Salvatoria*, the eggs are attached by means of tiny, simple notochaetae which penetrate into the eggs, also examined posteriorly in other species of the same genera by ourselves, and in species of the genera *Erinaceusyllis* San Martín, 2005 (San Martín, 2003; 2005) (figs. 10 A, B). Especially interesting were the SEM photos taken to the new Australian genus and species *Nooralia bulgannabooyanga* San Martín, 2002, because it is up to date, the unique syllid which develops compound notochaetae for dorsal brooding of eggs (San Martín, 2002). Ventral brooding of eggs and development of juveniles attached to females in some species were recorded and photographed by San Martín (2003), Mastrodonato et al. (2003), Nogueira et al. (2004), and Böggemann & Purschke (2005) (figs. 10C, D). SEM pictures were also very useful to the study of reproductive stolons in the subfamily Syllinae, as in San Martín (2003), San Martín & Nishi (2003), San Martín et al. (2008a,b, 2010), and Nogueira & Fukuda (2008).

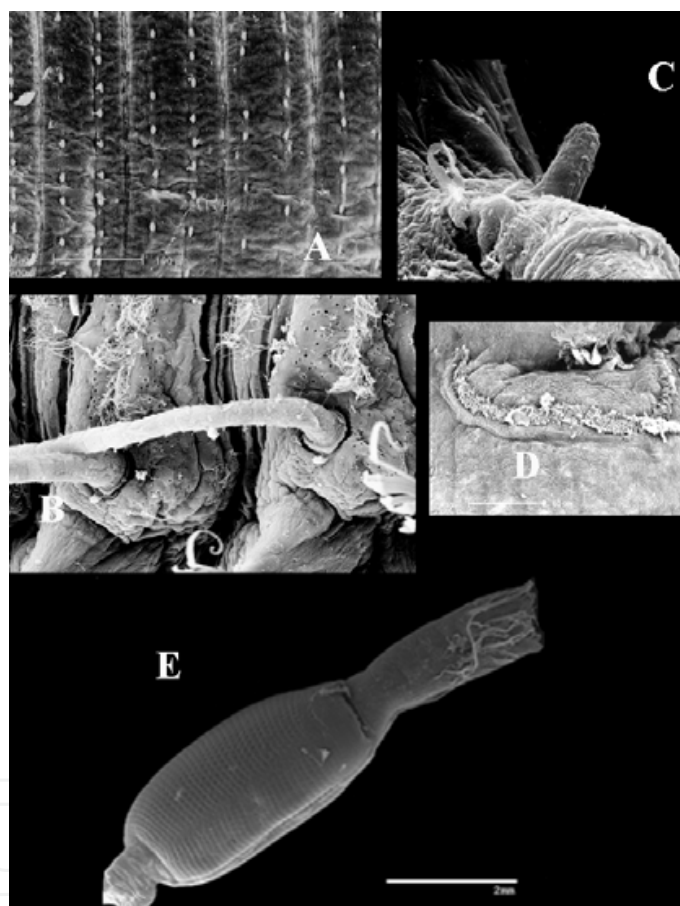


Fig. 9. A, transversal rows of spines, *Trypanosyllis aeolis*. B, subcirral papilla (under bases of dorsal cirri), *Paraehlersia ehlersiaeformis*. C, detail of subcirral papilla, *Paraehlersia weissmannioides*. D, ciliated area (nuchal organs?) of a *Sacconereis* stolon. E, pharynx and proventricle, *Megasyllis inflata*. A, D, San Martín (2003); B, C, San Martín & Hutchings (2006); E, San Martín et al. (2008a).

Especially interesting were some photos taken to stolons of the Australian species *Megasyllis corruscans* (Haswell, 1885), showing pores on ventral bases of parapodia, never reported before (fig. 10E). Also of great interest was the information taken on stolons in the complex of species of the genus *Haplosyllis* (see below).

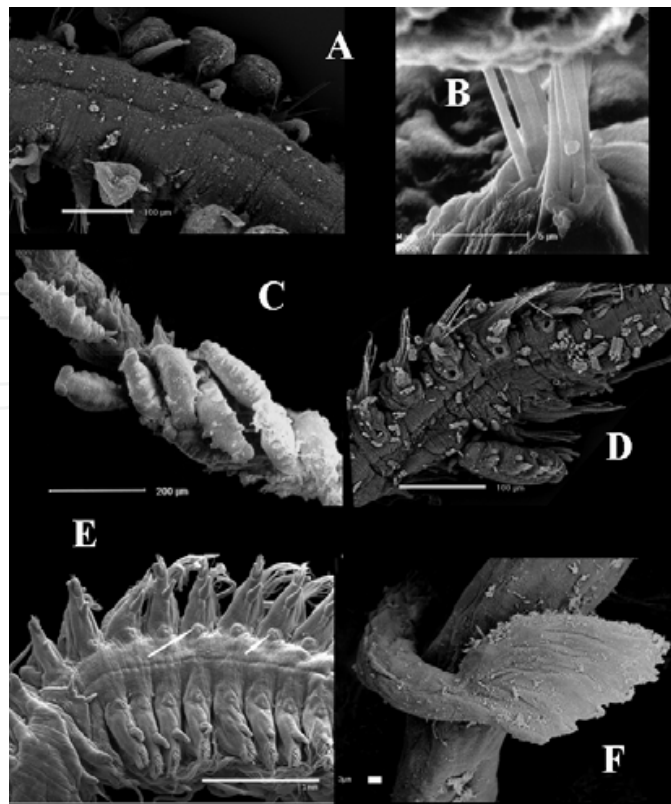


Fig. 10. A, dorsal brood of eggs by means of capillary notochaetae, *Prosphaerosyllis sexpapillata*. B, detail of capillary notochaetae inserting into an egg, *Salvatoria vieitezi*. C, juveniles attached to a female of *Exogone verugera*. D, juvenile attached to a female of *Exogone africana*, showing nephridial pores. E, ventral view of an stolon of *Megasyllis corruscans*, showing pores on parapodial bases. F, distal end of a dorsal cirri, *Murrindisyllis kooromundroola*. A, D, San Martín (2005); B, C, San Martín (2003); E, San Martín et al. (2008 a). F, San Martín et al. (2007).

3.3 Taxonomy

All these observations, discoveries, and more detailed descriptions of characters, thanks to SEM had important consequences, not only descriptions of new structures, opening new topics for future research, but also in the taxonomy and systematics of this complicate family of polychaetes. Detailed descriptions, using SEM, and comparison of different populations of supposed species of large distribution can help to differentiate cryptic species. The genus *Haplosyllis* has been recently revised, comparing specimens from all around the world, and using SEM techniques for all characters, especially the chaetae, apparently identical or very slightly different; however, the comparison of SEM photos show important differences (fig. 11). Before these studies, most of the reports all around the world of this genus were attributed to the species *Haplosyllis spongicola*, but that species is actually only present in the European seas, and with doubts in Australia. This study produced a number of papers (Martín et al., 2002, 2003; Lattig & Martín, 2009, in press a, b; Lattig et al., 2007, 2010 a, b) and the descriptions of numerous new species. Similarly, the genus *Haplosyllides* was also revised with similar results (Martín et al., 2009). Revisions of other genera of Syllidae are currently in process.

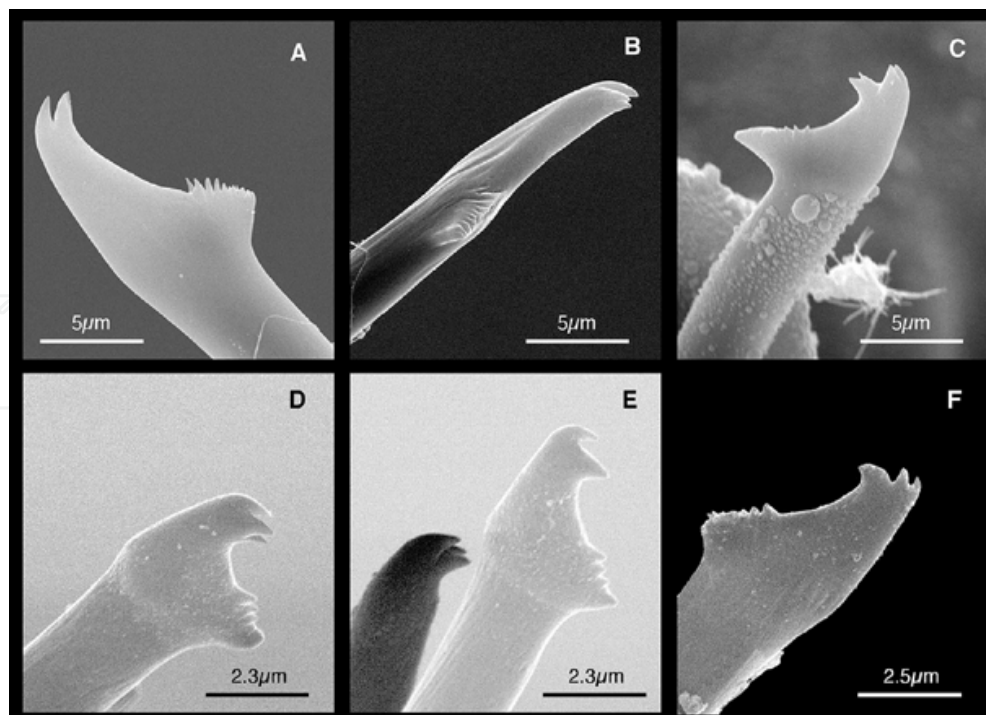


Fig. 11. Details of chaetae in different species of the genus *Haplosyllis*, showing the minute details, very difficult to see under light microscope. Martin et al. (2003).

3.4 Phylogeny

All these observations, above explained, under SEM on numerous species of syllids made possible the description of a number of new characters, previously unknown, as well as a better understanding of other features already known. This had important consequences in taxonomy, as already explained, and also on the phylogeny of the family. Sharp and detailed SEM photos are extremely useful to illustrate the characters used for phylogenetic analysis, as done by Nygren (1999), Aguado & San Martín (2009), and Aguado et al. (in press). Nygren (1999) used one plate with six SEM photos to illustrate 8 characters, and Aguado & San Martín (2009) 5 plates with a total of 33 SEM photos, in which ciliation of segments, body shape, ornamentation (papillae, tubercles, crests, among others) nuchal organs and details of nuchal lappets, details of peristomium and prostomium, size and shape of antennae and cirri, palps, parapodia, chaetae, several kinds of glands, pharyngeal armature, kinds of reproduction, etc., were profusely illustrated. The increase in the number of characters and detailed descriptions supported more robust hypotheses about evolutionary relationships of syllids. The information of new morphological traits or new details about well known structures has been extremely useful to provide evidence for synapomorphies of some clades. For instance, the glands in anterior ventral cirri highly contributed to support a clade with the genera *Eurysyllis*, *Plakosyllis* and *Trypanosyllis*; a character that is only perceptible under SEM.

4. Perspectives and conclusions

The use of SEM in the study of syllids has been proved of high usefulness, showing characters not previously described and for giving appropriate descriptions of other already

known. However, since it is also possible the discoverment of new taxa in wide unprospected areas of the world, and a detailed study under SEM still lacks for numerous syllid genera, new morphological structures may still be undescribed. For instance, San Martín et al. (2007) described a new genus and species from Australia (*Murrindisyllis kooromundroola*) with numerous autopomorphic characters. The most shocking characteristic is the tip of dorsal cirri, ending in a structure amazingly similar to a hand, with five “fingers” jointed by a membrane, giving a frog leg appearance (fig. 10F). These and other unusual characters of this genus were carefully detailed with SEM photos. At this point, the use of SEM is absolutely necessary for providing good and detailed descriptions of new taxa and numerous already known genera for which a detailed examination is still lacking. However, the use of SEM for the study of syllids is not the unique tool for improving the knowledge of the family; other techniques are absolutely necessary for phylogenetic studies in this family, such as molecular information, as shown by Aguado et al. (in press) who reorganizes the classification of the family using combined molecular and morphological data. Similarly, a worldwide catalogue for all the species of the family Syllidae is currently in process, in which SEM pictures acquire crucial importance.

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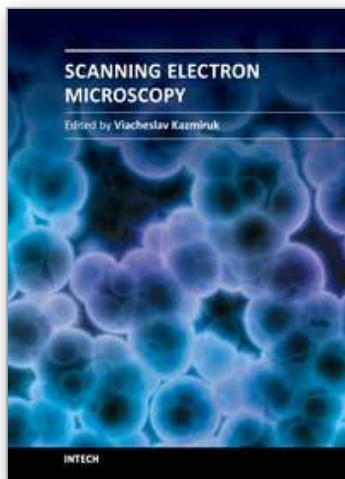
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Today, an individual would be hard-pressed to find any science field that does not employ methods and instruments based on the use of fine focused electron and ion beams. Well instrumented and supplemented with advanced methods and techniques, SEMs provide possibilities not only of surface imaging but quantitative measurement of object topologies, local electrophysical characteristics of semiconductor structures and performing elemental analysis. Moreover, a fine focused e-beam is widely used for the creation of micro and nanostructures. The book's approach covers both theoretical and practical issues related to scanning electron microscopy. The book has 41 chapters, divided into six sections: Instrumentation, Methodology, Biology, Medicine, Material Science, Nanostructured Materials for Electronic Industry, Thin Films, Membranes, Ceramic, Geoscience, and Mineralogy. Each chapter, written by different authors, is a complete work which presupposes that readers have some background knowledge on the subject.

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