External ultrastructure of *Manayunkia speciosa* (Fabriciidae) from Uruguay River, Argentina

Ultraestructura externa de *Manayunkia speciosa* (Fabriciidae) del Río Uruguay, Argentina

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Abstract. The external ultrastructure of *Manayunkia speciosa* Leidy, 1858 from specimens collected at the lower Uruguay River, South America is described using scanning-electron-microscopy. The branchial crown has a pair of semicircular lateral lobes, a pair of mediodorsal radioles and a pair of medioventral ones. Radiolar surfaces are ciliated. The faecal groove is observed on peristomium middorsally. The anterior margin of the anterior peristomial ring formed a rectangular lobe, with a ciliated band. Chaetal shape exemplifies the basic type of tapering cylinder. Two types of thoracic notochaetae: one wider distally, with a smooth handle, and covered on the surface by denticles, and other are significantly finer at the base and covered by thin structures with a free distal end. These structures were not previously recorded in Fabriciidae. Rows of 3 aligned uncini anteriorly projected are found in the thorax. Each uncinus presents a long manubrium which connects with the crest, extending to a main fang. The teeth of the crest are equal in size. The abdomen present 4 types of neurochaetae: one of small size than the other 3. In the abdomen 10-14 uncini form dense dorsal-transverse-lines. Each uncinus presents a short manubrium. The crest is covered with teeth of similar size.

Key words: *Manayunkia speciosa*, Polychaeta, ultramorphology, external characters, scanning-electron microscopy.

Resumen. Se describe la ultraestructura externa mediante el uso de SEM de especímenes de *Manayunkia speciosa* Leidy, 1858, colectados en el Río Uruguay, Sudamérica. La corona branquial presenta un par de lóbulos laterales semicirculares, un par de radioles en posición mediodorsal y otro medioventral. La superficie de las pinulas es ciliada. Sobre el peristomio se observa un surco fecal mediodorsal. El margen anterior del anillo peristomial anterior está formado por un lóbulo rectangular con una banda ciliada. Las setas son de tipo cilíndrico ahusado. Existen 2 tipos de notosetas torácicas: unas anchas distalmente con mango liso, cubiertas por denticúlos sobre su superficie; y otras significativamente más delgadas en la base, cubiertas por extensiones delgadas con extremo distal libre. Estas estructuras son registradas por primera vez en Fabriciidae. El tórax presenta grupos de 3 uncinos alineados, proyectados anteriormente, cada uno presenta un manubrio largo, cresta y diente principal. Los dientes de la cresta son similares en tamaño. En el abdomen se registraron 4 tipos de neurosetas: una pequeña y 3 de mayor tamaño. El abdomen presenta de 10 a 14 uncinos agrupados en una línea transversal dorsal. Cada uncino posee un manubrio corto. La cresta está cubierta por dientes de tamaño similar.

Palabras clave: *Manayunkia speciosa*, Polychaeta, ultramorfología, caracteres externos, microscopía electrónica de barrido.

Introduction

The freshwater polychaete *Manayunkia speciosa* (placed within the recently named Fabriciidae, Huang et al. (2011) and Kupriyanova and Rouse [2008]) was first named by Leidy (1858) from specimens collected in the Schuylkill river (Pennsylvania, USA). The species was later described by the same author in the first complete record of a strictly fresh-water polychaete (Leidy, 1883). Posteriorly, Potts (1884), Foulke (1884) and Meehean (1929) provided additional information to the original description of this species. In 1939 Krecker found specimens of *Manayunkia* in Lake Erie and gave the name of *M. eriensis* to those specimens collected by him and Meehean 10 years before. In a review of the subfamily

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Fabriciinae, Hartman (1951) indicated that *M. eriensis* Krecker might be the same as *M. speciosa* Leidy. Pettibone (1953) redescribed the species and placed *M. eriensis* in synonymy with *M. speciosa*. Years later, *M. speciosa* was recorded in different freshwater environments of North America, extending from the Great Lakes to the west coast and from Alaska to the Gulf of Mexico (Britt, 1965; Hiltunen, 1965; Hazel, 1966; Holmequist, 1967; Mackie and Qadri, 1971; Spencer, 1976; Brehm, 1978; Croskery, 1978). Recently, the presence of *M. speciosa* in the Uruguay River, Argentina, was documented by Armendáriz et al. (2011). This is its first record for the Neotropical region and so far, the southernmost location of this species.

Relationships amongst the various clades of fabriciid are largely unresolved. Some studies on the external ultrastructure of species of Fabriciidae revealed the meaning of the observed characters from the systematic (Fitzhugh et al., 1990; Fitzhugh, 1996; 2005; Rouse, 1996; Bick, 2005), adaptive (Bartolomaeus, 2002), and phylogenetic points of view (Bick, 2004). One potential solution to this problem is to introduce further characters or the details of known species in the analysis, as well as to describe new species (Rouse, 1996). Although different works describe in detail the external structure of some species of Fabriciidae (Fitzhugh 1983; 1998; Fitzhugh et al. 1990; 1994; Rouse 1996; Bartolomaeus 2002; Bick 2004) among which species of the genus *Manayunkia* can be found, to date the external ultrastructure of *M. speciosa* is not known.

The purpose of this report is to contribute to the description of these representatives of the species collected in South America, and particularly to provide specific details regarding certain external ultramorphological characters by scanning-electron microscopy (SEM).

**Materials and methods**

*Manayunkia speciosa* was collected in the Uruguay River, one of the longest South American Rivers. The Uruguay River drainage basin lies along the eastern margin of the continent and joins the Paraná River to form Del Plata basin (Fig. 1). Along its 1,770-km length it serves as the border between Argentina and Brazil, and further downstream between Argentina and Uruguay.

Specimens were collected seasonally between November 2007 and March 2009 in the lower Uruguay River. During the study were conducted 5 samplings in an area located at 33° 5’ S - 58° 12’ W, 33° 5.9’ S - 58° 25’ W, covering the main channel, the bays, and the reed beds along the banks of the river (Fig. 1). The material was collected with van Veen dredges and fixed *in situ* with 5% formalin.

In the laboratory the samples were washed on a sieve of 0.5-mm mesh size and stained with erythrosin. The specimens were picked out of the sediment under a stereoscopic microscope, identified by light microscopy, and preserved in 70% aqueous alcohol. Some of the individuals were measured (total length, maximum width, thorax and abdomen length, and length of the radioles) under an Olympus model CX31 compound microscope.

For observation in the scanning electron microscope, the organisms were dehydrated by three 15-min washes at each of 4 successive concentrations of aqueous ethanol: 70, 80, 96, and 100%. The material was finally critical point dried, then mounted on stubs and precoated with gold and palladium in a Sputter Model Thermo VG Scientific SC 7620 Coater. The chaetae and the details of the anterior and posterior ends were then photographed under a PHILIPS XL30 TMP scanning electron microscope.

Specimens were deposited at the Colección Argentina de Invertebrados, Museo Argentino de Ciencias Naturales Bernardino Rivadavia (MACN-In 37788), and in the División Zoología Invertebrados, Anelidos, Museo de Ciencias Naturales de La Plata (DZI-MLP 6800), Argentina.

**Terminology.** The terminology used in this paper follows the proposal given by Huang et al. (2011). The branchial crown is also known as branchial plume Pettibone (1953); the semicircular lateral lobes sensu lateral lophophores of Leidy and the radioles are also denominated ciliated barbules Leidy (1883) or tentacles Pettibone (1953). The long-handled ventral uncini are also recognized as podal hooks or neuropodial hooks Pettibone (1953).
long-handled uncini, podal hooks or notopodial hooks consisting of numerous small hooks are arranged in a torus. Uncini and hooked chaetae consists of a main fang Fitzhugh (1989), rostrum sensu Bartolomaeus (2002), Hauptzahn sensu Hartmann-Schröder (1971), Basalzahn sensu Banse (1957)) which is surmounted by a series of smaller teeth. Both structures are bent at an arc of almost 80° to 90° towards the manubrium also denominated shaft or handle sensu Pettibone, (1953), shaft sensu Hartmann-Schröder (1971).

Description

Manayunkia speciosa Leidy (1858: 90), Leidy (1883: 204-212, Pl. 9, Figs. 1-13), Potts (1884: 21-22), Foulke (1884: 48-49), Zenkevitsch (1925: 36-38), Meehean (1929: 479-480), Hartman (1951: 389).


The general description of the specimens collected from the Uruguay river agree with Leidy (1858; 1883) and Pettibone (1953). The body is formed by 9 thoracic segments and 3 abdominal segments. The lengths of the thoracic segments increase toward the posterior region and are greater than the widths (Fig 2A). The abdominal segments are much shorter and narrower than the thoracic ones. Table 1 shows the body measurements of some specimens collected at the sampling site.

The branchial crown has a pair of semicircular lateral lobes, a pair of mediodorsal radioles and a pair of medioventral ones (Fig. 2B). The radioles on both semicircular lateral lobes are longer ventrally but gradually become shorter at more dorsal positions (Fig. 2C). The anterior appendages are clearly wrinkled but the pinnules surface is ciliated (Fig. 2D). In this figure the faecal groove is observed on the peristomium middorsally. The prostomium is fused with the first achaetous segment. The peristomial ring in Fabriciidae is divided in an anterior and a posterior ring with an anterior and a posterior margin in each of them. The anterior margin of the anterior peristomial ring of Manayunkia speciosa is higher ventrally, developed as a rectangular lobe (Fig. 2E). A trilobated structure is observed at the side of one of the radioles in lateral position (Fig. 2E). According to the bibliography, this structure is not comparable with any other known structure in Sabellidae. Therefore, it could be interpreted as a neoformation or as a developing lobe, produced as a response to an accidental cut of the radiolar lobe.

The chaetal shape in Manayunkia speciosa is essentially in the form of a tapering cylinder (flexible capillary chaetae, Fig. 2F). The thoracic limbate notochaetae consist of 2 types of chaetae: one much wider distally, with a smooth handle, and covered on the rest of the surface by denticles or little spines with a broad base and tapered end (Fig. 2G); the other likewise wider at the base but thinner distally. The handle is smooth and the blade is wider and covered with rigid denticles or spines fully adhered to the surface, similar to that observed in the above-mentioned chaetae. In the distal region, at about three-fourths of its length, the chaetae are significantly finer and are covered by thin structures that are attached at their region proximal to the surface of the chaetae, thus being observed as free extensions (Fig. 3A). Along the segments, the number of bigger chaetae presented a medium value of 3 per fascicle, with a tendency to increase from 2 to 4 towards the last segments. The smaller chaetae averaged 3 per fascicle with no marked tendency to increase in number along the segments.

In the ventral thoracic region are tori of 3 uncini, clearly separated from each other. Each uncinus presents a long manubrium (Fig. 3B) continuing with the crest (basal region), and prolonging into a main fang (distal region). The teeth of the crest are almost equal in size, except one large teeth above the main fang.

The abdominal region consists of 3 segments (Fig. 3C). The neurochaetae are found in groups of 4. In general, they present a wide base, which becomes thinner towards the end (Fig 3D). The smaller neurochaetae are thin with a curved end and a surface completely covered by small denticles, similar to those observed in the notochaetae (Fig. 3E). Among the biggest neurochaetae 3 types can be distinguished: one covered by denticles adhered to the whole surface (Fig 3E), another with slightly irregular surface and a third completely covered by small denticles with free distal end (Fig. 3F).

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<th>Table 1. Body measurements (in µm) of Manayunkia speciosa specimens (n= 15) collected from the Uruguay River, Argentina</th>
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Figure 3. A: detail of the thinnest notochaetae. B: view of thoracic uncini (6500x). C: general view of the abdomen (385x). D: general view of neurochaetae (2300x). E: detail of little neurochaetae with curved end and large neurochaetae both covered by denticles (10000x). F: detail of rugose neurochaetae and setae with free distal end (10000x). G: overall view of uncini on abdomen (2200x). H: detail of uncini on abdomen (9500x).
The arrangement and morphology of the abdominal uncini (Fig. 3G) are different to the ones observed in the thoracic uncini. In the abdomen there are dense dorsal transverse tori formed by 10-14 uncini. Each torus is found on an elevation of the body surface as observed in Fig. 3C.

Each uncinus presents a short manubrium; the crest is covered with numerous teeth of similar size over the apical surface in several vertical rows. The teeth of the crest are wider towards the distal end. The central structure is the basal tooth (Figs. 3G, H).

The pygidium is short, rounded, and without eyespots (Fig. 3C). Both males and females were present during the study, and 2 females were found with oocytes in the abdomen.

**Habitat.** Uruguay River: Coordinates: 33°5.01'S, 58°12.64' W; 33°5.2'S, 58°22.41'S; 33°5.94'S, 58°25.2' W; 33°7.44'S, 58°22.96' W. Type of sediments: Plastic silty-clay with small proportions of scarcely sorted quartz sand (Iriondo and Kröhling, 2004). The physicochemical variables of the water in the study site varied according to the following values: water temperature fluctuated from 9.7 to 30.1; depth, from 0.9 to 9.6 m; turbidity, from 13 to 185.2 UTN; conductivity, from 46.7 to 177 µS cm⁻¹; pH, from 7.3 to 9.3; and dissolved oxygen, from 7.2 to 12.8 mg l⁻¹. The fauna accompanying the study was mainly represented by the following taxa: Nematoda; Oligochaeta; Bivalvia and Gastropoda mollusces; Ostracoda; Caenidae; Chironomidae, and Elmidae insects.

**Remarks.** The mean total length and maximum width coincided with those observed by Leidy (1883) and were lower than those obtained by Pettibone (1953). Leidy (1858), in the original description, indicated that the lateral radioles were simple, not bilobate; this point was questioned by Zenkevitsch (1925), since in *Manayunkia aestuarina* (Bourne, 1883) and *M. baicalensis* (Nusbaum, 1901) each radio is bilobed; close examination in our study shows that this is also the case in *M. speciosa*. The surface of the pinnules and ventral filamentous appendages in *M. speciosa* are clearly wrinkled similar to *M. aestuarina*, in contrast to all other Fabriciniae as *Fabricia stellaris stellaris* (Müller, 1774), *Fabriciola tonerella* Banse, 1959, *F. baltica* Friedrich, 1939 and *Pseudoaugeneriella nigra* (Langerhans, 1880) investigated by Bick (2004). There may be 6 to 10 brownish pigment spots on each side near the basis of the radioles, not visible when preserved (according to Meehean, 1929), in younger individuals there are few or none (Leidy, 1883). In coincidence with these records, in *M. speciosa* specimens no brownish pigment spots were observed.

The faecal groove is developed middorsal similarly to other species of Fabriciidae (*P. nigra, F. stellaris stellaris, M. aestuarina and F. tonerella*) observed by Bick (2004). The annulation between the anterior and posterior peristomial ring is distinct in this species, at least ventrally (Bick, 2004). In *M. speciosa* the anterior margin of the anterior peristomial ring is higher ventrally, and the ventral collar is developed as a rectangular lobe similar to *M. aestuarina*.

In contrast with some species of Fabriciniae such as *F. stellaris stellaris, F. tonerella* and *P. nigra*, observed by Bick (2004), there is no midventral ciliated patch in *M. speciosa*, there is instead a ciliated band in the ventral region of the anterior margin of the anterior peristomial ring. This characteristic was also observed in the posterior peristomial ring of *M. aestuarina*.

The chaetal shape in *M. speciosa* is essentially in the form of a tapering cylinder (flexible capillary chaetae) similar to that observed by Meaders and Hendrickson (2008). The shape of the thin abdominal neurochaetae coincides with those described by Pettibone (1953). The small denticles or spines adhered to the surface of the notochaetae and neurochaetae of *M. speciosa* coincide with observations made in other fabricids. However, the extensions with a free distal end found in *M. speciosa* had not been recorded previously in species of Fabriciidae.

We observed 3 uncini per torus on the thorax, these coinciding in number with those reported by Pettibone (1953), though Leidy (1883) had described some 4 or 5 on each fascicle. Unlike the observations of Pettibone (1953), we were able to discern by SEM the presence of a main fang and teeth of the crest equal in size, except one large spine above the main fang. Moreover, a long manubrium is observed in the thoracic uncini in agreement with what was observed in particular in Fabricidae by Bartolomaeus (2002).

The number of uncini on the abdominal tori was 11 to 14 in contrast with the descriptions of Leidy (1883), who had reported 9 to 14, and of Pettibone (1953), some 14 to 30. The linear orientation of the abdominal uncini on the crest of the surface of the body coincides with similar observations for other sabellids by SEM (Wu-Baoling, 1984).

**Discussion**

In many cases of Fabriciidae species, little is known about the size dependence or intraspecific variability of characters. The only example is Bick (1996), who described the significance of size/age-dependent characters (number of abdominal uncini) in an analysis of population dynamics in *Manayunkia aestuarina*. In *Pseudoaugeneriella nigra* the following characters have been found to be variable—dependent and independent of size—and should therefore be
used with caution in descriptions of species of Fabriciidae: number (and length) of chaetae and uncini, length of ventral filamentous appendages, manubrium to dentate region length ratio of abdominal uncini, and pigmentation (Bick, 2004). For this reason, in the present study the aspects of the external ultrastructure are emphasized. In other species of Sabellidae these aspects are considered characters more or less stable at a specific level.

In the external ultrastructure of *M. speciosa* observed, although the presence of numerous denticles or spines constituting the surface of the limbate chaetae has already been reported for other species of sabellids (Wu-Baoling, 1984), the structures with free distal end that cover the distal portion of those chaetae having wide bases in this species are relatively rare.

Moreover, the fabricids species present abdominal uncini without a main fang that corresponds with the ground pattern of the Fabriciidae (Fitzhugh, 1989, 1991; Fitzhugh et al., 1994). In some sabellids, an elongated basal tooth in the main fang is observed. Bartolomaeus (2002) suggests that the main fang of the abdominal uncini is plesiomorphic in Sabellidae and has been reduced in the stem lineage of the Fabriciidae. In order to determine if the observed structure of the abdominal uncini of *M. speciosa* is a real main fang, the number of microvilli present in the first stages of development should be analysed by transmission electron microscopy (TEM).

Structures on the anterior end of the body have been considered to be of particular significance in the taxonomy of Fabriciidae. The peristomial rings and collar have particularly been regarded as being of high value for taxonomy and cladistic analysis in this family (Bick, 2004). The anterior and posterior peristomial rings are well developed all the way around the body. The posterior margin of the ciliated patch marks the border between the anterior and posterior peristomial rings and may be helpful in detecting it (Bick, 2004). The occurrence of a ciliated patch on the ventral margin of the anterior peristomial ring is apparently a real character for Fabriciidae. It was described earlier in *Novafabricia* by Fitzhugh (1983) and Martin and Giangrande (1991) and the genera *Chone*, *Paradialychone* and *Dialychone* (Sabellidae) (Tovar-Hernández, 2008). Rouse (1993) found a ciliated flap as a projection of the ventral collar among species of *Fabriciola*, and Nishi (1996) described a ciliated, glandular patch on the anterior peristomial ring in *Fabricinuda bikini* (Hartman, 1954). This author assumed that a ciliated or glandular field was common among Fabriciinae. No similar structure has been observed in *M. speciosa*. Both *M. aestuarina* and *M. speciosa* possess a ciliated band rather than a ciliated patch. If this character is to be used in phylogenetic analyses, evidence must be furnished as to whether it also occurs in other species of Fabriciinae, particularly in the genus *Manayunkia* (Bick, 2004).

In reference to the presence of long manubria in thoracic uncini in *M. speciosa*, Knight-Jones (1981) assumes that long manubria in the thoracic neuropodial chaetae in fabriciins may also be related to their ability to migrate from the tubes if conditions deteriorate. Elastic tubes, rapid secretion of new tubes, and the ability to migrate from them when conditions deteriorate could be adaptations to instable environments. This hypothesis is supported by the observation that fabriciins are able to colonize unfavorable environments like silty estuaries or lagoons with limited substrata for settlement (Knight-Jones, 1981). The study area in Uruguay river presented clean, mainly silty-sand sediments in the main river channel, which were reducing their particle size and increasing their organic matter content to the shores and bays. In a recent study, Armendáriz et al. (2012) demonstrated that a positive relationship was found between the abundance of *M. speciosa* and the percentage of organic matter in the sediments. In addition, this species was present with greater frequency and abundance in areas of low waters, low current velocity and abundant littoral vegetation.

The present study describes the characters associated to the anterior region (the branchial crown and the anterior and posterior peristomial ring) as well as the external ultrastructure of notochaetae, neurochaetae and thoracic and abdominal uncini. The data obtained contribute to the advancement in the knowledge of morphofunctional and adaptive aspects of *M. speciosa* and provide information for the future study of the phylogenetic relationships between species of the genus *Manayunkia*.

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Literature cited


