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Scanning electron microscopy and intraspecific variation of *Chordodes festae* Camerano, 1897 and *C. peraccae* (Camerano, 1894) (Nematomorpha: Gordioidea)

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Abstract

The nematomorph species *Chordodes festae* Camerano, 1897 and *C. peraccae* (Camerano, 1894) are redescribed by using scanning electron microscopy (SEM). *C. festae* has a cuticle with four different areolar types, the crown areoles being the most noticeable with long spiniform processes. The terminal end in the male specimen has two short lobe-like structures and a ventral groove. *C. peraccae* has three areolar types in which, as in *C. festae*, some areoles form groups surrounding the crown areoles. Intraspecific variations were found in body length and body colour in both species and, in *C. peraccae*, also in the cuticle.

Introduction

Nematomorpha or horsehair worms, as they are commonly called, have affinities with several 'pseudocoelomate' groups (Malakhov & Spiridonov, 1984; Wallace et al., 1996; Lorenzen, 1996; Nielsen et al., 1996; Schmidt-Rhaesa, 1998). They are parasites of invertebrates while in the larval stage, yet free-living as adults. The aquatic adults undergo sexual reproduction and the new-born larva re-initiates the life-cycle.

The taxonomy of hairworms is difficult due to the fact that most of the original descriptions were based on light microscopy (LM). These descriptions, in many cases, fail to give adequate diagnostic characters, leading to doubts on the real existence of some species. In order to develop a more complete description of the characters, ultrastructural analysis using SEM, which has proven to provide a far better assesment of cuticular details and to clarify the status of this group, is necessary. The study of the species of the genus *Chordodes* Creplin, 1842, which have more than two types of areoles, with complex areoles, crown areoles and different spiniform structures not only in the cuticle but also in the posterior end of males (Chandler & Wells, 1989; de Villalobos & Miralles, 1997; de Villalobos, 1995, 1999), is an example. In the present study holotypes of *Chordodes festae* Camerano, 1897 and *C. peraccae* (Camerano, 1894) are redescribed using both LM and SEM techniques. Data on morphological and meristic features are provided and the intraspecific differences found in each species are also noted.

Materials and methods

In the literature, 49 specimens of *Chordodes festae* have been recorded from Ecuador and Venezuela and one specimen from Central Africa. *C. peraccae* was described from one specimen from Argentina and one from Bolivia. We investigated 42 of these specimens from the Museum Regionale Di Scienze Naturali, Torino, Italy. The accesion numbers, sex, locality data and method of study of the investigated material are listed in Table 1.

Cuticular sections were made as tangential sections from the medial part of the body for examination using phase-contrast light microscopy. The cuticle fragments were washed in lactophenol for 24 hours, mounted and observed using a Wild M2 microscope, after cleaning and removing the remains of the muscular tissue. In order to examine specimens by scanning

Table 1. Specimens investigated.

Original detemination	Museum accession no.	Sex	Type	Locality	LM	SEM
C. festae	MZUT 64[a]	1♂	HT	Ecuador	X	X
C. festae	MZUT 64B	2♂	PT	Ecuador	X	
C. festae	MZUT 64C	3♂	PT	Ecuador		X
C. festae	MZUT 64D	1♀	PT	Ecuador	X	X
C. peracae	G43A	1♀	HT	Argentina	X	X
C. peracae	G 43B	1♀		Bolivia	X	X

Abbreviations: HT, holotype; PT, paratype; MZUT, Museo Regionali Di Sienze Naturali Torino, Italy.

electron microscopy (SEM), fragments of worms (anterior end, mid-body and posterior end) were fixed with formalin-propionic acid at 60% for 24 hours and washed with distilled water prior to their study. They were critical point dried and gold-sputter coated and observed and photographed using a JEOL SLM 1000 SEM.

Chordodes festae Camerano, 1897

Redescription (Figures 1, 2)

Holotype male

Body dark brown, cylindrical. Anterior end (Figure 1A) narrows slightly towards apex, 78.5 μm wide at this level. Mouth opens subterminally on cone at anterior extremity (see *Discussion*). Posterior end rounded (Figures 1B-D), 85.5 μm wide; extremity slightly bifid, having 2 small protuberant lobe-like structures which are separated by ventral groove. Cloacal opening (Figure 1B,C) subterminal, oval, 63 μm long and 300 μm distant from extremity, with no circum-cloacal spines, closely surrounded by circle of short bristles (11 μm long) with sharp ends, external to which are 2 rows of longer bristles (23 μm) anterolateral to cloaca and representing precloacal bristlefields. Cuticle, especially on dorsal side of posterior extremity (Figure 1D), has areoles with numerous bristles, giving velvet-like appearance, resembling areole type 1 (see below) but with longer tubercules, suggesting that they are modified at posterior end on body. In interareolar furrow are tubercles with narrow ends.

Features of cuticle surface can be seen using SEM (Figures 2A,B) to exhibt 4 types of areole. Type1 are most numerous and uniformly distributed on body. They are oval, 17–28.6 μm long, 11.6–18.6 μm and up to 9.4 μm in height. Their surface is irregular, having 6–12 small bristles, 2.5 μm long and narrowing distally, irregulary distributed on its apex. Type 2 (Figures 2A,B) are less numerous, occurring singly or in groups of 2, 3 or 4. They are taller than those of type 1 (13.3 μm in height), mainly rounded and 13.2 μm in diameter. Apically these areoles have central depressions or pores from which projections of different lengths (8.5–22.8 μm) emerge and no other type of tubercle. In interareolar furrow (Figure 2C), which is narrow (3.3 μm) and deep, are randomly distributed large, curved and sharp tubercles of 68.3 μm long and 11.6 μm wide. It is difficult to determine whether these tubercles emerge from different type of areole or from cuticular convolution. On body surface, there are also areolar groups (Figure 2D) separated from each other by distances of 100–233 μm. Each of these groups (Figure 2E) is formed by type 3 areoles; these surround pair of type 4 areoles or crown areoles, which are shorter (11.4 μm) and central in relation to group. Type 3 areoles are conical, with circular apex where there are small bristles with rounded ends. These areoles form 2 concentric circles of 8-10 areoles each, separated from each other by ca 91 μm. Pair of central areoles (crown areoles, type 4) are conical with truncated apex. From them issues long, filiform prolongations (120 μm), arising at centre of distal extemity and extending between type 3 areoles. Longitudinal ventral groove (Figure 2F) is slightly apparent, limited by areolar groups with emerging filiform structures which are probably very long in fresh material.

Analysis using LM agrees with results using SEM in relation to presence of different areolar types, but it does not reveal details or inter-areolar spinules.

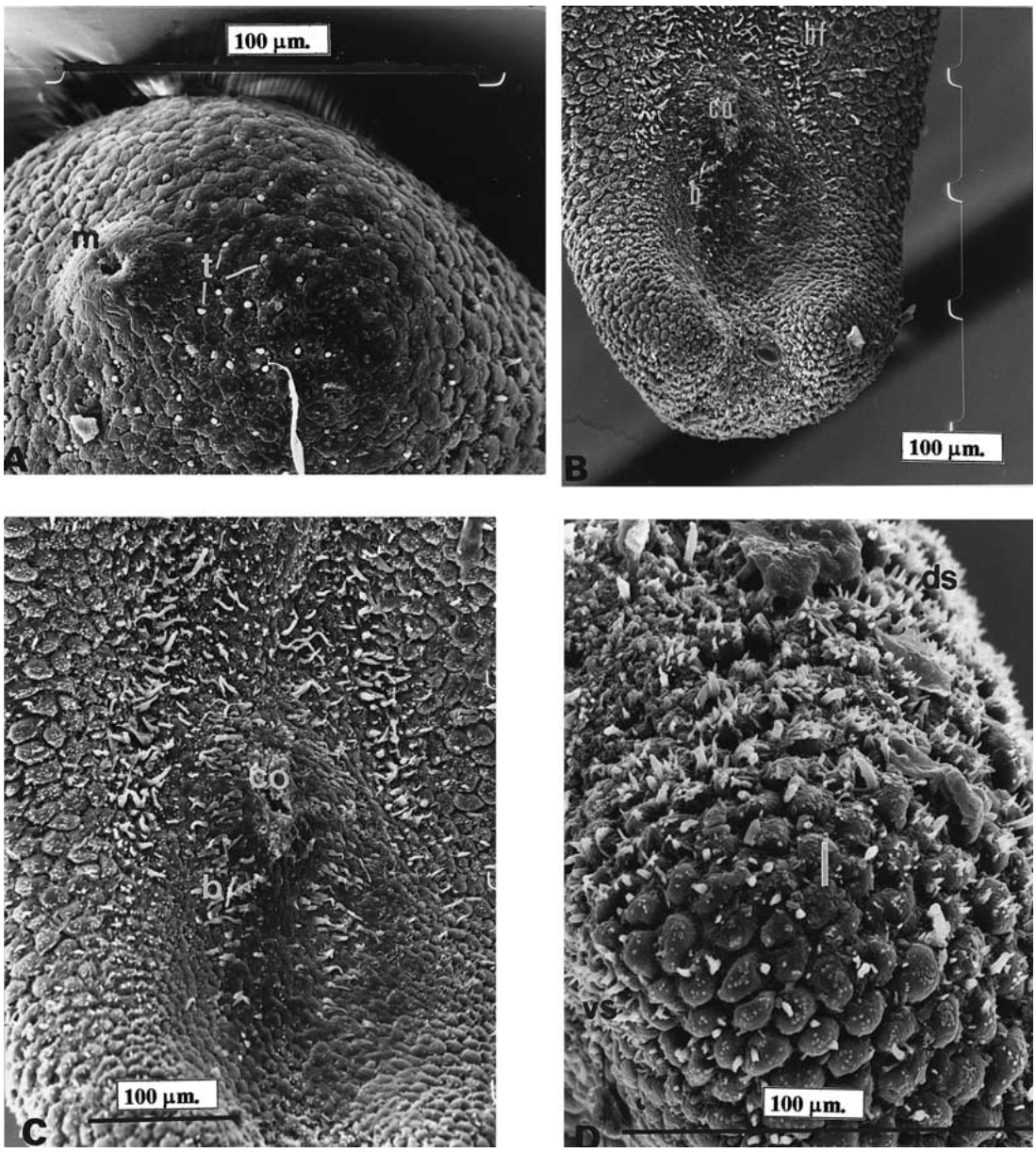

Figure 1. Chordodes festae. A. Anterior end. B. Ventral view of the posterior end of the male. C. Detail of the cuticular structures at the posterior end. D. View of the cuticular surface of one of the lobe-like structures at the posterior extremity. *Abbreviations*: b, bristles; bf, bristlefields; co, cloacal opening; ds, dorsal side; l, lobe-like structures; m, mouth; vs, ventral side. *Scale-bars*: 100 μm.

Material

The material examined is deposited in the Museo Regionale di Scienze Naturali di Torino, Italy (MZUT: G4).

Type-locality: Cuenca, Ecuador.

Holotype male: 120 × 0.7 mm; *Paratypes*: 33 males 60–113 (mean 82.75) mm long, 0.4 to 0.9 (0.7) mm wide and 7 females 73–110 (87.6) mm long, 0.6–1 (0.8) mm wide.

Other material (not examined): Merida, Venezuela, 9 males: length 84, 95, 105, 115, 120, 145, 165, 170, 175 mm; diameter 0.5–1 mm. Fiume Kalimabenge, Zaire, 1 male: 110 × 1 mm.

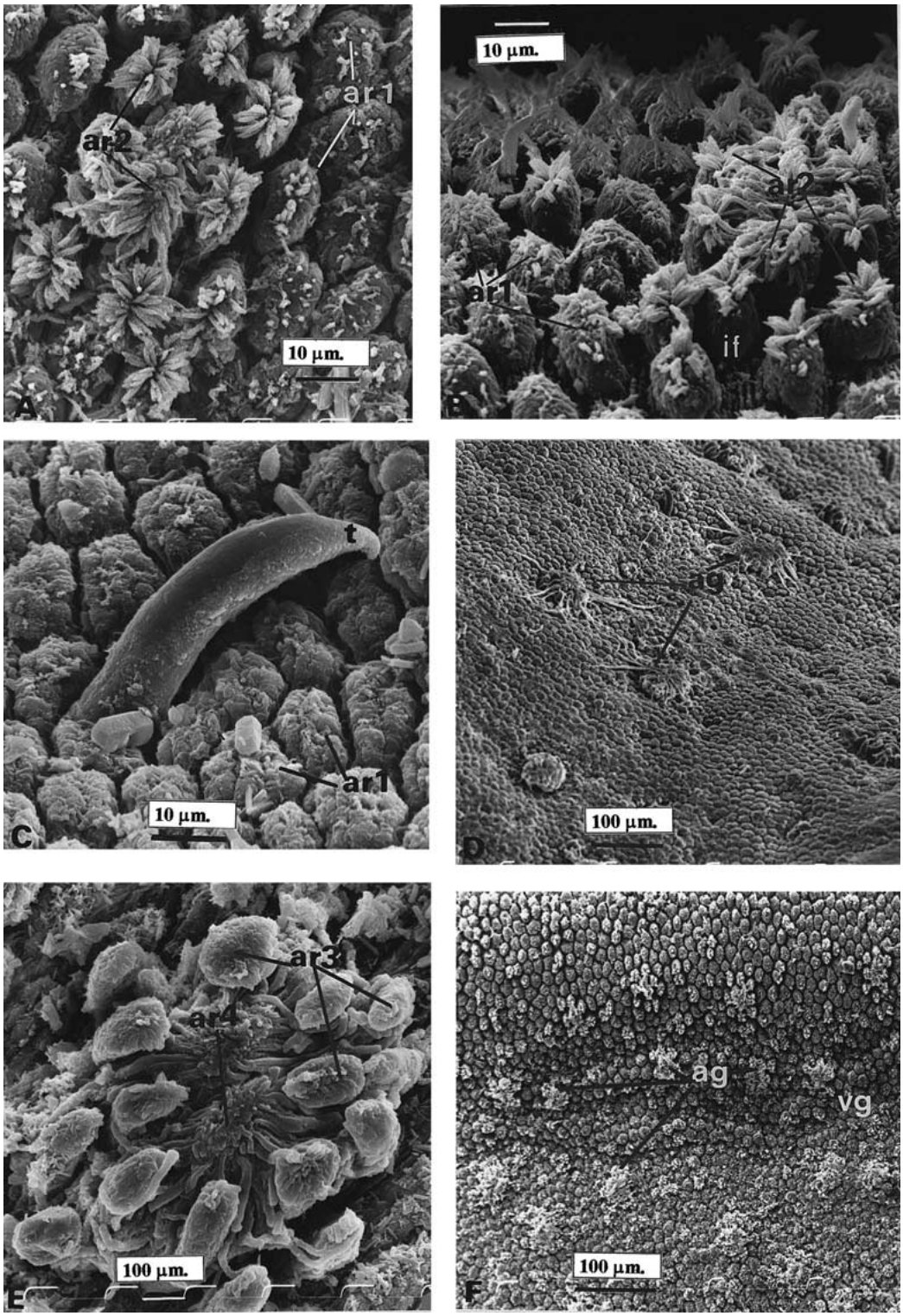

Figure 2. Chordodes festae. A-E. Cuticle of the middle region of the body. B. Lateral view. D. General view of the cuticle. F. Longitudinal ventral groove. *Abbreviations*: ar_1, areoles of type1; ar_2, areoles type 2; ar_3, areoles of type 3; ar_4, areoles of type 4; ag, areolar groups; if, interareolar furrow; t, tubercle; vg, ventral groove. *Scale-bars*: A-C, 10 μm; D-F, 100 μm.

***Chordodes peraccae* (Camerano, 1894)**

Redescription (Figures 3, 4)

Holotype female

Body light brown, cylindrical. Anterior end narrow. Posterior end rounded (Figure 3A), expanded at its extremity, 465 μm in diameter. Cloaca, terminal. Examination of cuticle with LM indicated presence of rounded areoles, some of them in shape of horseshoe, with irregular borders which may have short bristles, and areolar groups with long filiform projections terminally. Interareolar furrow wide, but no interareolar structures observed.

Using SEM cuticle of anterior and posterior ends (Figure 3A) has low areoles with scarce interareolar tubercles. Cuticle of middle region of body has 3 types of areoles (Figures 3B,C,D, 4A). Type 1 areoles (Figure 3B) are short (6.7 μm), have smooth surface and occur in variety of shapes: rounded, oval, half-moon or horseshoe. Rounded areoles have diameter of 7.8–11.1 (10.2) μm. Oval areoles were 15.5–21.1 μm in length, 10.0–12.2 μm in width. Those with horseshoe-shape have diameter of 13.3 μm. Areolar borders smooth. At surface of some areoles there is short, spiniform tubercle (7.8 $\times$ 3.3 μm long and wide respectively), somewhat curved, arising from central pore-like depression. Interareolar furrow wide (8.1 μm) and covered by cuticular cords. Types 2 and 3 areoles (Figures 3C,D) occur in groups. Those of type 2 are 9.2 μm high and 5.5 μm wide; their apex is rounded and single pore opens in centre from which arises crown of small tubercles not larger than 7.5 μm. They form simple circle of 8–10 areoles 7.5–10.0 μm apart and surrounding pair of central areoles, crown areoles, which represent type 3. These are 8.9 μm high and completely covered by long filiform projections, up to 169 μm long and 2.4 μm wide, which arise from middle of distal extremity in shape of crown. These projections are covered by light-coloured granules which glisten (Figures 3D, 4A).

Material examined: Female, San Pablo, Tucumán, Argentina. Dimensions: Length 126 mm, diameter 0.6 mm. *Host*: Unknown.

Chordodes peraccae from Bolivia

Female. Body dark brown, without white spots. Anterior end narrow terminally. Posterior end complete with central cloacal opening. Cuticle of middle region of body, under SEM (Figures 4B,C), has 3 areolar types. Areoles of type1 are oval, somewhat rounded and sometimes half-moon or horseshoe-shaped. Oval areoles vary, ranging from 10.8–19.2 μm in length and 12.5–15.8 μm in width. Horseshoe-shaped areoles are 17.9 μm in diameter. Some areoles have single spiniform tubercle arising from eccentric pore on areolar surface, which is 11.6 μm long, 2.5 μm wide and rarely observed. Areolar borders in these areoles (Figure 4C) are not smooth; finger-like structures emerge from them which connect areoles to each other. Interareolar furrow narrow, 2.1 μm. Types 2 and 3 areoles form groups exhibiting no obvious differences when compared with Tucuman specimes. Longitudinal ventral groove (Figure 4D) limited by areolar groups with long spiniform projections emerging from their centres. These areoles have same characteristics as type 3 areoles, differing only in their regular distribution.

Material examined: Female, from Aguairenda Chaco Boliviano, Bolivia. Dimensions: length 198 mm, diameter 0.8 mm. 1 Female (not examined) from Urucum, Mato Grosso, Brazil. Dimensions: 60 $\times$ 0.5 mm. *Host*: Cockroach (Blattaria) *Ischnoptera brasiliensis* Br.

Discussion

Chordodes festae was described from South America (Cuenca, Ecuador) by Camerano (1897) and (Merida, Venezuela) by Camerano (1905). Another report, surprising for a South American species, was from Kalimabenge, Zaire, in Central Africa (Sciacchitano, 1958). We consider Sciacchitano's determination might be dubious and in need of confirmation.

The body lengths reported for the males of *Chordodes festae* vary from 84 to 175 mm with a diameter of 0.5 and 1 mm, and for females from 210 to 245 mm with a diameter of 1.5 and 1.7 mm. We agree with Camerano (1905) in relation to the differences in body colour: dark brown in males and light brown in females. In the original description, Camerano (1897) described the cuticle of *C. festae* with two areolar types. Analysis using SEM shows with more precision differences between areoles, which enables us to increase to four the number of recognisable areolar types. Future studies may allow us to determine whether the thick tubercles are connected to a different areolar type, and thus raising the number of areolar types to five, or whether they emerge from an interareolar cuticular convolution. The first areo-

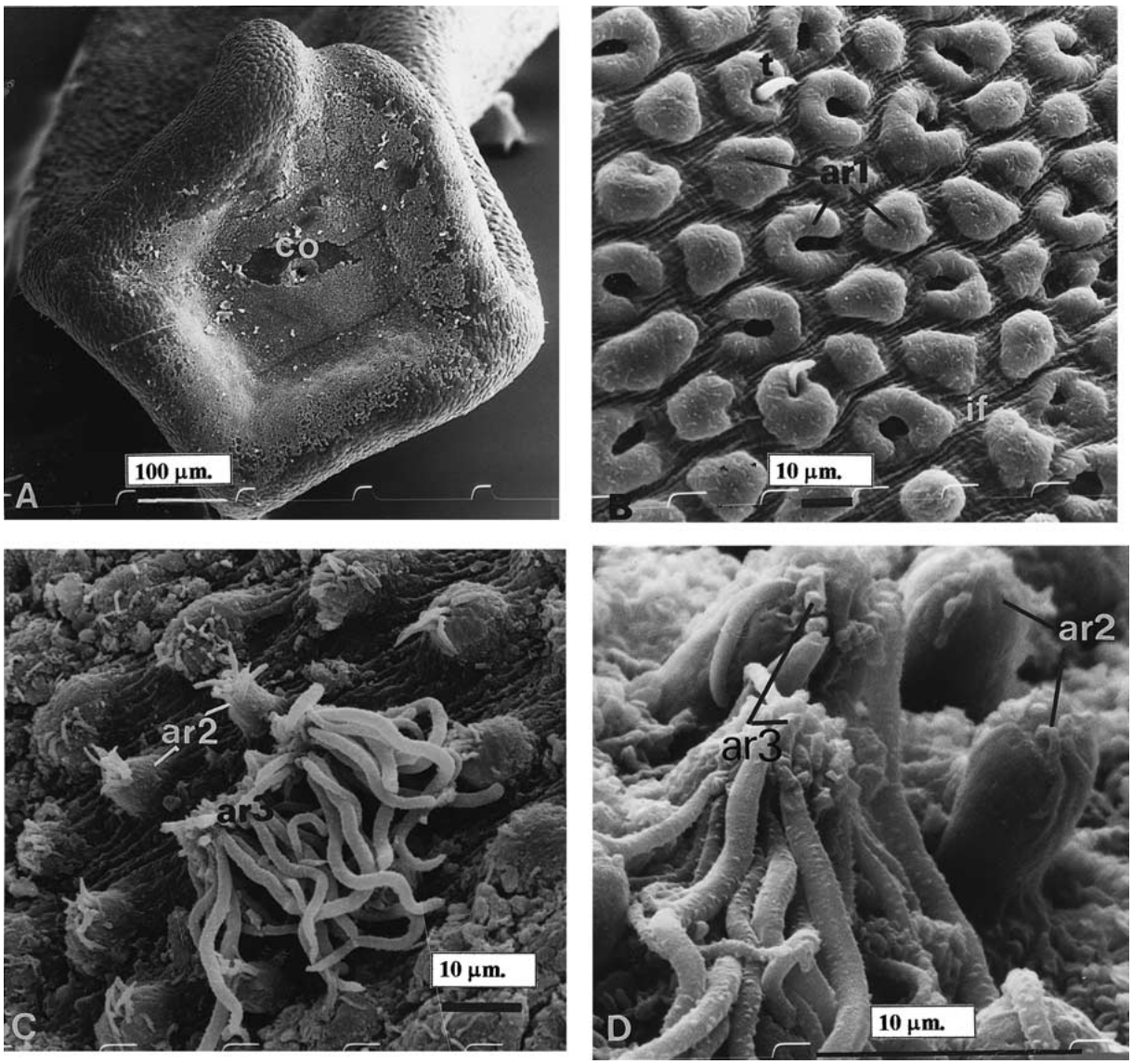

Figure 3. *Chordodes peraccae* from Argentina. A. Terminal end of the female. B. Cuticle of the middle region of the body. C. *En face* view of an areolar group. D. Lateral view of an areolar group. *Abbreviations*: ar_1, type 1 areoles; ar_2, type 2 areoles; ar_3, type 3 areoles; co, cloacal opening; if, interareolar furrow; p, projections; t, tubercles. *Scale-bars*: A, 100 μm; B-D, 10 μm.

lar type described by Camerano agrees with the SEM observations, but the second type does not. Camerano (1897, 1905, 1915) considered that the areoles with projections at their apex and present in isolated arrangements, forming groups of two, three or four or forming larger groups (25–30 areoles) around a central pair, all belong to the same areolar type. The analysis using SEM shows that the areoles in isolated arrangements and those forming small groups are characterised by features that make them different from the remainder. They are shorter with a pore at the apex from which a bunch of projections of different lengths emerges; these constitute, in our view, a second areolar type. On the other hand, those areoles forming more evident, compact groups are taller, do not have a central depression or pore and have small bristles at the apex and correspond to a third areolar type. The fourth type are the crown areoles, which form the central pair surrounded by the third type 3 areoles, have characteristics of their own, such as a truncated apex from which a bunch of large filiform projections emerge and extend over the body surface.

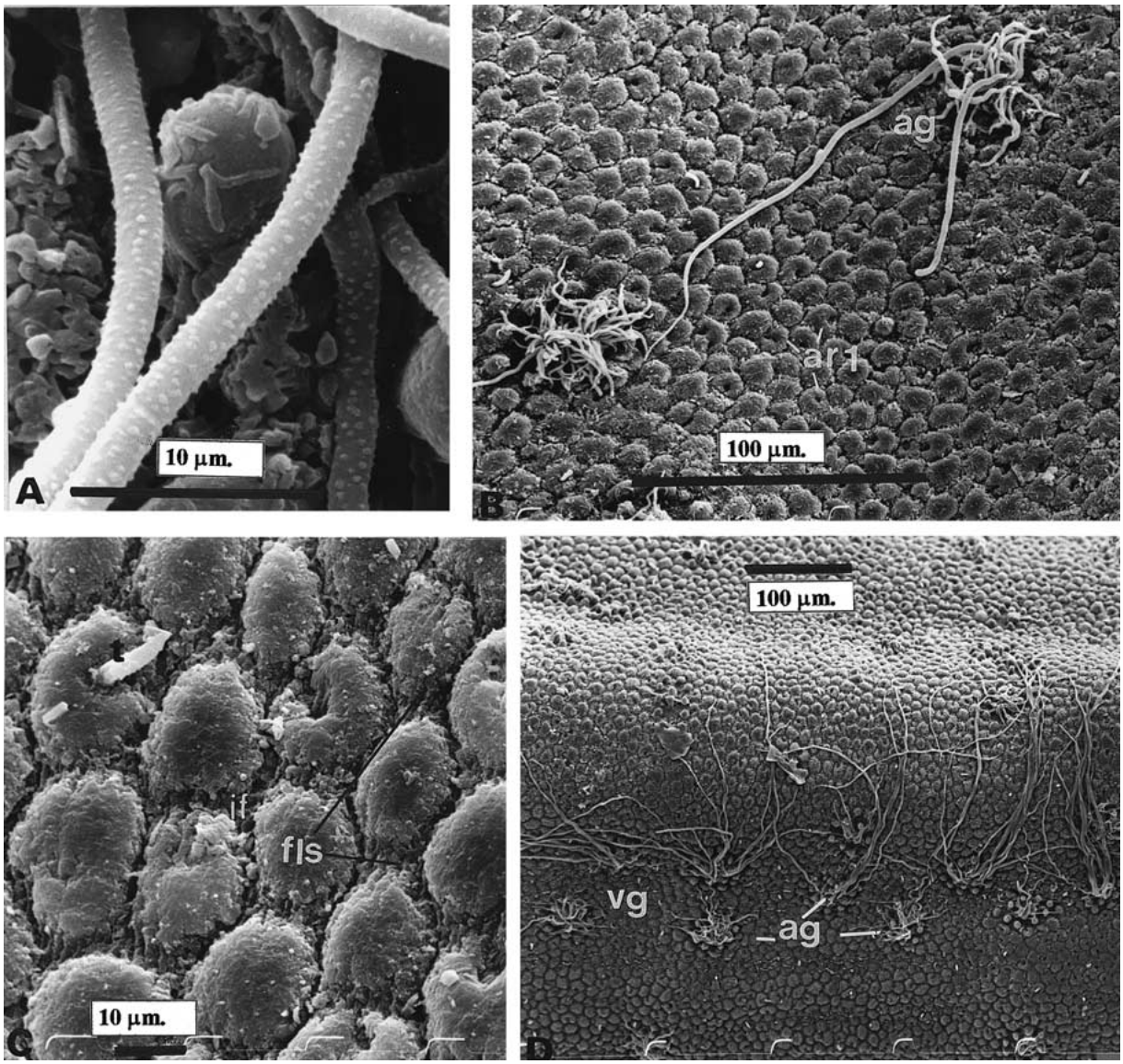

Figure 4. Chordodes peraccae. A. Detail of granulations covering the filiform projections. B-D. Specimen from Bolivia. B. General view of the cuticle. C. Detail of the cuticle. D. View of the longitudinal ventral groove. *Abbreviations*: ar_1, type 1 areoles; ar_2, type 2 areoles; ar_3, type 3 areoles; ag, areolar groups; if, interareolar furrow; fls, finger-like structure; t, tubercles; vg, ventral groove. *Scale-bars*: A,C, 10 μm; B,D, 100 μm.

No differences were found between the cuticular characteristics of males and females. All the males studied presented the posterior end slightly bifid, comprising two lobe-like structures. The SEM study allowed us to observe in detail the ultrastructure of the filiform prolongations of the crown areoles, which is not possible using LM.

Camerano (1905) observed, in male specimens from Merida, Venezuela, that in the medio-ventral groove there were long crests with filiform glistening prolongations emerging from the central pair of areolar groups. We were unable to observe this characteristic in the specimens from Cuenca, Ecuador, in which the areolar groups limiting the ventral groove have short projections. Although we cannot completely discount the possibility, in our material, of some damage to these filiform prolongations, it is interesting to note that, in both species of *Chordodes* studied, the apical filaments of crown areoles are extremely long throughout the length of the body in the dorsal, ventral and

lateral fields. Nevertheless, as we have already mentioned, it is usual that, due to a prolonged fixation or manipulation of the material, these filaments do break, especially in the less protected areas, such as the lateral region.

Chordodes peraccae was described from Tucuman, Argentina, and Aguairenda, Bolivia, by Camerano (1894), and from Urucum, Mato Grosso, Brazil, by Camerano (1901); all of them female specimens. The body lengths reported for *C. peraccae* range from 60 to 198 mm with a diameter of 0.5 and 0.8 mm. The Urucum specimen, collected while parasitising a cockroach (*Ischnoptera brasiliensis* Br.), is the smallest (60 × 0.5 mm). Camerano (1901) noted this in relation to the dimensions of the host's body, only 18 mm. Recently, in a study of a species of *Gordionus* Müller, 1927, from Scotland (de Villalobos et al., 1999), noted that there was no relationship between the length of the specimens and the length of the host, which agrees with Schmidt-Rhaesa (1997).

The cuticular characteristics of *C. peraccae*, analysed using SEM, are basically in accordance with the descriptions of Camerano (1894, 1897, 1901, 1915), although we noticed some differences in the cuticle between specimens from Argentina and Bolivia. The type 1 areoles on the specimen from Argentina present various shapes, with smooth borders, and they are well separated from each other because the interareolar furrow is broad. In the specimen from Bolivia, these areoles are more uniform, the horseshoe-shaped areoles being larger. The areolar borders are not smooth, most of them exhibiting finger-like projections which connect the areoles to each other and the interareolar furrow is narrow. The areolar tubercles are also longer and narrower than those observed in the specimen from Argentina.

Chordodes festae differs from *C. peraccae* as it has four areolar types and large, curved tubercles in the interareolar groove. The areolar groups are more compact and they are formed by two peripheral circles surrounding the central pair. Both species share one cuticular characteristic, bearing areolar groups with peripheral areoles surrounding two crown areoles which have a crown of long filiform projections at the centre of their apex. Both species also exhibit intraspecific variations, especially in relation to body dimensions and, in some cases, body colour. In *C. peraccae* intraspecific variations at the cuticular level were also observed. Such intraspecific variations have also been seen recently in other species of the Gordiacea, such as *Euchordodes nigromaculatus* Poinar, 1991 (see Schmidt-Rhaesa et al.,1998).

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References

Camerano, L. (1894) Viaggio del dottor Alfredo Borelli nella Repubblica Argentina e nel Paraguay. *Bollettino dei Musei di Zoologia ed Anatomia Comparata, Torino*, **9**, No. 175, 1–6.

Camerano, L. (1897a) Viaggio del Dr. Enrico Festa nella Repubblica dell¨Ecuador e regioni vicine. *Bollettino Musei di Zoologia ed Anatomia Comparata, Torino*, **12**, No. 293, 1–2.

Camerano, L. (1897b) Monografia dei Gordii. *Accademia Reale delle Scienze di Torino*, **47**, 339–419.

Camerano, L. (1897c) Viaggio del dottor Alfredo Borelli nel Chaco boliviano e nella Repubblica Argentina. *Bollettino Musei di Zoologia ed Anatomia Comparata*, Torino, **12**, No. 294, 1–2.

Camerano, L. (1901) Viaggio del Dr. A. Borelli nel Matto Grosso e nel Paraguay. Gordii. *Bollettino Musei di Zoologia ed Anatomia Comparata, Torino*, **16**, No. 411, 1–2.

Camerano, L. (1905) Osservazioni intorno al *Chordodes festae* Camerano. *Bollettino Musei di Zoologia ed Anatomia Comparata, Torino*, **20**, No. 504, 1–2.

Camerano, L. (1915) Revisione dei Gordii. Memorie della Reale Accademia delle Scienze di Torino, **66**, 1–66.

Chandler, C.M. & Wells, M.R. (1989) Cuticular features of *Chordodes morgani* (Nematomorpha) using scanning electron microscopy. *Transactions of the American Microscopical Society*, **108**, 152–158.

De Villalobos, L.C. (1995) Una Nueva Especie del Genero *Chordodes* Creplin, 1874, en la Argentina. *Neotropica*, **41**, 63–65.

De Villalobos, L.C. & Miralles, D.B. (1997) Una Nueva Especie de *Chordodes* (Gordiacea, Nematomorpha) Parasita de *Blaptica* sp. (Blaberidae, Blataria). *Comunicação. Musei de Ciências e Tecnologia da PUCRS, Série Zoologia, Porto Alegre*, **10**, 45–51.

De Villalobos, L.C. & Camino, N. (1999) Dos Nuevas Especies de Gordiaceos (Nematomorpha) parásitos de *Stagmatoptera hyaloptera* (Mantidae) en la Argentina. *Iheringia, Série Zoologia, Porto Alegre*, **86**, 71–76.

De Villalobos, L.C., Ribera I. & Downie I.S. (1999) Hairworms found in Scottish agricultural land, with descriptions of two new species of *Gordionus* Müller (Nematomorpha: Gordiidae). *Journal of Natural History*, **33**, 1767–1780.

Lorenzen, S. (1996) Nemathelminthes (Aschelminthes). In: Westheide, W. & Rieger, R. (Eds) *Spezielle Zoologie*. New York: Gustav Fischer Verlag, pp. 682–736.

Malakhov, V.V. & Spiridonov, S.E. (1984) The embryogenesis of *Gordius* sp. from Turkemia, with special reference to the position of the Nematomorpha in the animal kingdom. *Zoologicheskii Zhurnal*, **63**, 1,285–1,296. (In Russian).

Nielsen, C., Scharff, N. & Eibye-Jacobsen, D. (1996) Cladistic analyses of the animal kingdom. *Biological Journal of the Linnean Society*, **57**, 385–410.

Schmidt-Rhaesa, A. (1997) Nematomorpha. *In*: Schwoerbel, J. & Zwick, P. (Eds) *Süsswasserfauna von Mitteleuropa*. Germany: Gustav Fischer Verlag, 124 pp

Schmidt-Rhaesa, A., Thomas, F. & Poulin, R. (1998) Scanning electron microscopy and intraspecific variation in *Euchordodes nigromaculatus* from New Zealand. *Journal of Helminthology*, **72**, 65–70.

Schmidt-Rhaesa, A. (1998) Phylogenetic relationships of the Nematomorpha, a discussion of current hypotheses. *Zoologischer Anzeiger*, **236**, 203–216.

Sciacchitano, I. (1958) Gordioidea del Congo Belga. *Annales du Museé Royal du Congo Belge*, **67**, 8–110.

Wallace, R.L., Ricci, C. & Melone, G. (1996) A cladistic analysis of pseudocelomate (aschelminth) morphology. *Invertebrate Biology*, **115**, 104–112.