

## Scanning electron microscopy of *Chordodes moutoni* Camerano, 1895 (Gordiida, Nematomorpha)

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### Abstract

Taxonomic characters of both male and female horse-hair worms *Chordodes moutoni* Camerano, 1895 (Nematomorpha) are re-described using scanning electron microscopy. The features are compared with the original description. *C. moutoni* cuticle has five different areolar types in male while female have six types of areoles. The crowned areoles, characteristic of *Chordodes*, are surrounded by other areoles forming pairs or clusters. Sexual dimorphism was found in the cuticular pattern, the female showing two different types of crowned areoles with distinct distribution pattern while the male has only one type of crowned areoles. These data are compared to other *Chordodes* species that have been studied.

**Key words:** Gordiida, China, scanning electron microscopy, re-description

### Introduction

The genus *Chordodes* Creplin, 1874 is the largest genus of Nematomorpha with about 90 described species. The *Chordodes* species are distributed mainly in tropical and subtropical regions, with few exceptions (see Montgomery 1898, Kirjanova & Spiridonov 1989; Spiridonov 2000). The identification of a species of *Chordodes* is not easy because they have the highest diversity in cuticular structures (called areoles) among Nematomorpha. The diagnostic feature (and autapomorphy) (Schmidt-Rhaesa 2002a) of this genus is the presence of crowned areoles. The crowned areoles are a special type of areoles with one stem and a crown of apical filaments. Most of the original descriptions of *Chordodes* species 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. Therefore, detailed descriptions and reinvestigations with higher

magnifying microscopical techniques are extremely necessary. Scanning electron microscopy (SEM) has been applied only in some species of *Chordodes* (see Miralles 1989, Kirjanova and Spiridonov 1989, Baeck 1993, Miralles and De Villalobos 1994, 1997a, 1997b; De Villalobos and Camino 1999; Schmidt-Rhaesa 2002b, 2004; Schmidt-Rhaesa and Yadav 2004; Schmidt-Rhaesa and Menzel 2005; Schmidt-Rhaesa *et al.* 2003; De Villalobos and Zanca 2001, 2005, De Villalobos *et al.* 2004a, De Villalobos *et al.* 2004b).

About 300 species of freshwater nematomorph are known worldwide (Schmidt-Rhaesa 1997). At present, 10 species of Gordiida have been described from China: *Chordodes moutoni* Camerano, 1895 (Camerano 1895, Wu and Tang 1933); *C. wangi* Wu and Tang, 1933 (Wu and Tang 1933), *Gordius semilunaris* (Linstow, 1906) (Camerano 1915, Wu and Tang 1933); *G. fulgur* Baird, 1861 (Camerano 1897, 1915, Wu and Tang 1933); *G. omensis* Wu and Tang, 1933 (Wu and Tang 1933); *Gordionus wolterstorffii* (Camerano, 1888) (as *Parachordodes wolterstorffii* Camerano 1904, 1915); *G. violaceus* (Baird, 1853) (as *Parachordodes violaceus* Camerano 1915, Wu and Tang 1933); *Parachordodes kaschgaricus* Camerano, 1897 (Camerano 1897, 1915, Wu and Tang 1933); *P. pustulosus* (Baird, 1853) (Camerano 1897, 1915, Wu and Tang 1933) and *P. coreanus* (Linstow, 1906) (Camerano 1915, Wu and Tang 1933) Also another 2 specimen, reported as parasites of human in mainland China, were assigned to *Neochordodes* sp. (Mao 1991) and *Parachordodes* sp. (Wei & Yang 1978; Mao 1991).

To date no photographic documentation (neither light microscopy nor SEM) has been made of *Chordodes moutoni*. In the present study we re-described *C. moutoni* from China by SEM. This is the first ultrastructurally investigation of a nematomorph species from China.

#### Material and Methods

Taking into account that Camerano (1895) described 4 specimens (type series) of *Chordodes moutoni* and no holotype and paratype exist, we designated as lectotype and lectoparatype the male and female specimens from the type series deposited in The Muséum National d'Histoire Naturelle, Paris ((MNHNP Boccal 41 N° 35). The other specimens, 1 male and 1 female are housed in the Museo Regionale di Scienze Naturali, Torino, Italy (MRSNT G49). We investigated the lectotype and lectoparatype of *Chordodes moutoni*.

Body measurements were made with outstretched worms using a ruler. Diameters were measured under dissecting microscope using a calliper ruler.

In order to preserve the lectotype and lectoparatype material, we removed a tangential section of the middle region of the body with a razor blade. Specimens were dehydrated in an increasing ethanol series, critical point-dried, mounted on bronze blocks and gold-sputter coated. Observations were performed using a JEOL JSM 6360 LV scanning

electron microscope (SEM). All preparations, i.e. SEM stubs, are stored together with the preserved specimen in the museum.

## Results

### *Chordodes moutoni* Camerano, 1895

(Figures 1, 2)

*Type series:* 1 male and 1 female (MNHNP Boccal 41 N° 35), 1 male and 1 female (MRSNT G49).

*Type locality:* China Henan province (Ho-Nan).

*Additional specimens:* Malaysia: 1 female Perak (Camerano 1899); 1 female Kedah (Camerano 1901) 1 male Foot of Gunong Inas, Perak (Camerano 1903). China: 4 males, 2 females Chekiang province, Yünhwo Hsien (Wu & Tang 1933).

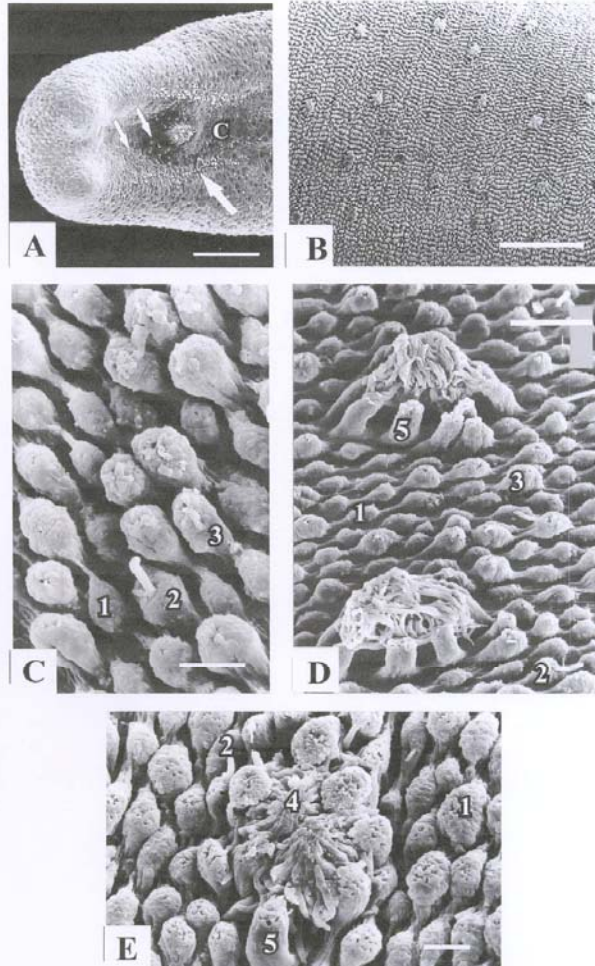
*Material examined:* Lectotype: 1 male. Lectoparatype: 1 female (MNHNP Boccal 41 N° 35), SEM midbody and posterior end.

*Host:* Undetermined mantid (Camerano 1901, 1903, Schmidt-Rhaesa & Ehermann 2001).

*Description of male lectotype.* The body color is dark brown. The body length is 310 mm, diameter in the middle region 1.1 mm. Anterior end is tapered, without a white cap and dark collar. The mouth is terminal. The posterior end (Fig. 1A) is tapered at the apex (0.23 mm in diameter) and with two small protuberant lobe-like structures which are separated by ventral furrow. The cloacal opening is ventral and is situated 215  $\mu\text{m}$  anterior of the posterior margin of the worm. The cloacal opening is narrow, oval and is surrounded by circumcloacal spines. Anterolateral to the cloacal opening are two rows of bristles (bristlefields) that are 184.2  $\mu\text{m}$  long and 26.3  $\mu\text{m}$  wide. Bristles are undivided. The cuticle around the cloacal opening has short bristles (Fig. 1A). The ventral and dorsal longitudinal furrows of the body are not clearly marked.

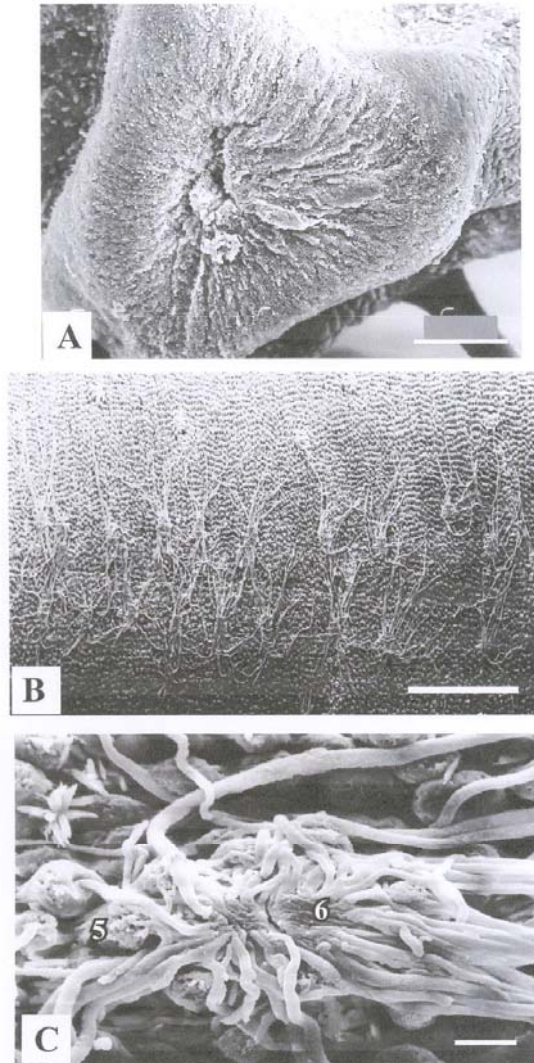
The whole body is covered with protruding structures called areoles (Fig. 1B). The cuticle between areoles is structured into cord-like folds (Fig. 1C, 1D). The body cuticle shows five different types of areoles which are numbered consecutively in the following for better orientation (Fig. 1D): Type 1 areoles (Figs. 1C, 1D) are the most abundant, they are oval or rounded (9.4- 10.6  $\mu\text{m}$  long and 4.3-5.9 wide), low (4.4 to 5.2  $\mu\text{m}$  high) with a roughly structured surface. Scattered among these areoles are areoles of type 2, they are rounded but with a tubercle on top (7.8  $\mu\text{m}$  high) (tubercle areoles) (Figs. 1C, 1D). These tubercles originate in a slight depression at the apex of the areole. The areolar type 3 are more elevated than the types 1 and 2 (7.9-11.9  $\mu\text{m}$  high) and carry on their apical surface projections (bristle-like) which are clustered. These areoles occur individually or in clusters of 3 to 4 areoles (Figs. 1C, 1D). Areoles of types 4 and 5 form groups (Figs. 1B, 1C, 1E). Type 4 are represented by crowned areoles (16.9  $\mu\text{m}$  high) that occur in pairs, have moderately short filaments (27.8  $\mu\text{m}$  long) on top and are surrounded by about 9-15

areoles of type 5. The areoles of type 5 are the highest (17.4-19.6 $\mu$ m), being conical, with a ring of short filaments at the apex and curved towards the center.



**FIGURE 1:** SEM. Lectotype male. **A:** complete view of the posterior end from the ventral side, cloacal opening (c) with circuncloacal spines, cuticle with short bristles (small arrow) and bristlefields anterolateral of the cloacal opening (large arrow), scale bar= 100 $\mu$ m; **B:** general view of midbody cuticle, scale bar= 250 $\mu$ m; **C:** midbody cuticle, areoles types 1-3, scale bar= 10 $\mu$ m; **D:** lateral view of midbody cuticle, areoles types 1-5, scale bar= 25 $\mu$ m; **E:** areolar clusters containing types 4 and 5 on lateral body, scale bar= 10 $\mu$ m.





**FIGURE 2:** SEM. Lectoparatype female. **A:** posterior end with terminal cloacal opening (large arrow) and short bristles (small arrow), scale bar= 50  $\mu$ m; **B:** crowned areoles (type 6) with long filaments in the longitudinal ventral groove, scale bar= 250 $\mu$ m; **C:** areolar clusters in detail containing types 5 and 6 in the ventral midline, scale bar= 10 $\mu$ m.

*Description of the female lectoparatype.* The body colour is dark brown. The female is 320mm long with a maximum diameter of 1.8mm. The posterior end (Fig. 2A) is rounded (0.30mm in diameter) and the cloacal opening is central and terminal. Scattered bristles are present around the cloacal opening. The ventral and dorsal sides of the body are marked by a very darkly pigmented line (Fig. 2B).

The body cuticle includes all five types of areoles as in males, but along the ventral and dorsal midline, one further type of crowned areoles is present (type 6) (Figs. 2B, 2C). This special type of crowned areoles occur with very long filaments on top (273 $\mu$ m long) and occur in clusters of two and are surrounded by about 10-11 elevated areoles of type 5 (Fig. 2C). These clusters are 150-163.9 $\mu$ m apart from each other. They have equal distances from either side of the midline and from each other. The other type of crowned areoles (type 4) with short filaments is distributed all over the cuticle as in the male.

*Dimensions.* According to the literature (Camerano 1895, 1899, 1901; Wu & Tang 1933) the males specimens of *C. moutoni* vary between 95 to 310 mm in length and between 0.5 to 1mm in diameter; females range from 145 to 312mm in length and from 1.2 to 2.5mm in diameter. The length of the specimen from the Foot of Gunong Inas could not be determinate by Camerano (1903) because the worm was in pieces.

## Discussion

In the original description, Camerano (1895) described the cuticle of *C. moutoni* with three areole types which would correspond to the types 1, 4 and 5 of our reinvestigation. Later, Camerano (1897) re-describes this species and enlarges the number of areolar types to five when describing tubercles areoles (our type 2) and areoles that occur in clusters (our type 3). Our observations by SEM, are coincident with the ones of Camerano (1897) but we can add crowned areoles with long filaments that occur in the ventral and dorsal midlines in females as an additional areolar type.

According to Wu & Tang (1933) the specimens of *C. moutoni* possess 4 areolar types that correspond in most respects with our description of the lectotype. But areoles of our third type and crowned areoles with long filaments along the ventral line in females are not described by Wu & Tang (1933).

The presence of crowned areoles with long filaments limited to the ventral or ventral and dorsal groove in females has been mentioned for South American species, *C. balzani* (see Carvalho & Feio 1950; de Villalobos *et al.* 2004a), *C. brasiliensis* (see Janda 1894, Camerano 1897, Carvalho 1946; de Villalobos *et al.* 2004b), *C. carmelitanus* (see Carvalho & Feio 1950; de Villalobos *et al.* 2004b), *C. nobilii* (see de Villalobos *et al.* 2004b), *C. staviarskii* (see Carvalho & Feio 1950; de Villalobos & Zanca 2005), *C. peraccae* (see De Villalobos & Zanca 2001). This particular feature in females was considered as sexual dimorphism in cuticular pattern by Carvalho (1946) for the species *C. brasiliensis* and by Camerano (1987) and Carvalho and Feio (1950) for *C. balzani*.

Nevertheless, Schmidt-Rhaesa (2002b) observed crowned areoles with long filaments along the ventral and dorsal midline, and crown areoles with short filaments over the cuticle in both male and female specimens of *C. queenslandi* from Australia. Also Schmidt-Rhaesa & Yadav (2004) described two female specimens of *Chordodes* cf. *furnessi* from India with crowned areoles with long filaments along both side of the ventral midline. Although more studies are necessary on both sexes of this genus, there seems to be a feature of sexual dimorphism in the species of examined so far with modern methods.

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### References

- Baeck, K.M. (1993) Two species of genus *Chordodes* (Gordioidea: Nematomorpha) from Korea. *Korean Journal of Systematic Zoology*, 9, 221-228.
- Camerano L. (1895) Description d'une nouvelle espèce de Gordien de la Chine. *Bulletin de la Société Zoologique de France*, 20, 99-100.
- Camerano, L. (1897) Monografia dei Gordii. *Memoria Reale Accademia Scienze Torino*, 47, 339-419.
- Camerano, L. (1899) Gordii della Malesia e del Messico. *Atti delle Reale Accademia delle Scienze di Torino*, 34, 460-469.
- Camerano, L. (1901) Gordii raccolti dalla spedizione "Skeat" nella Penisola Malese 1899-1900. *Bollettino dei Musei di Zoologia ed Anatomia Comparata della Reale Università di Torino*, 16, 1-2.
- Camerano, L. (1903) On the entomo-parasites of the "Skeat expedition". *Proceedings of Zoological Society of London*, 2, 152-153.
- Camerano, L. (1904) Della identità specifica del *Parachordodes wolterstorffi* e del *Parachordodes plesskei*. *Bollettino dei Musei di Zoologia ed Anatomia Comparata della Reale Università di Torino*, 19, 1-4.
- Camerano, L. (1915), Revisione dei Gordii. *Memoria della Reale Accademia delle Scienze di Torino*, 66, 1-66.
- Carvalho, J.C. (1946) Gordiaceos do Museu de Historia Natural de Montevideo. *Comunicaciones Zoológicas del Museo de Historia Natural de Montevideo*, 2, 1-7.
- Carvalho, J.C. & Feio, J.L. (1950) Sobre alguns Gordiaceos do Brasil e da Republica Argentina (Nematomorpha, Gordioidea). *Anais da Academia Brasileira de Ciências*, 22, 193-206.
- De Villalobos, C. & Camino N. (1999) Dos nuevas especies de Gordiaceos (Nematomorpha), parásitos de *Stagmatoptera hyaloptera* (Mantidae) en la Argentina. *Iheringia Serie Zoologia*, 86, 71-76.
- De Villalobos, C. & Zanca, F. (2001) Scanning electron microscopy and intraspecific variation of *Chordodes festae* Camerano, 1897 and *C. peraccae* (Camerano, 1894) (Nematomorpha: Gordioidea). *Systematic Parasitology*, 50, 117-125.
- De Villalobos, C. & Zanca, F. (2005) Ultrastructural redescription of *Chordodes moraisi* (Carvalho, 1942) and *C. staviarskii* Carvalho & Feio, 1950 and reinterpretation of *C. gestri* Camerano, 1904 and *Pseudochordodes griffinii* (Camerano, 1898) (Gordiida Nematomorpha). *Journal of Natural History*, 39, 597-606.
- De Villalobos, C., Hancock E.G. & Zanca F. (2004a) Redescription and sexual dimorphism of *Chordodes balzani* Camerano, 1896 (Nematomorpha). *Journal of Natural History London*, 38, 2305-2313.

- De Villalobos, C., Zanca F. & Schmidt-Rhaesa A. (2004b) New data on South American species of *Chordodes* (Nematomorpha). *Revista do Museu Nacional/UFRJ. Brasil*, 62, 375-386.
- Janda, J. (1894) Beiträge zur Systematik der Gordiiden. I. Die Gordiiden Galiziens. II Ueber das Genus *Chordodes*. *Zoologische Jahrbücher für Systematik, Geographie und Biologie der Tiere*, 7, 595-612.
- Kirjanova, E.S. & Spiridonov, S.E. (1989) Two new species of the nematomorph genus *Chordodes* from mantids. *Parazitologija*, 23, 358-352.
- Mao, S.P. (1991) Protozoan and Helminth parasites of human in Mainland China. *International Journal of Parasitology*, 21, 347-351.
- Miralles, D. (1989) Estructura cuticular de *Chordodes carmelitanus* Carvalho y Feio, 1950, nueva cita para la Argentina (Nematomorpha Chordodidae). *Neotropica*, 35, 95-99.
- Miralles, D.B. & De Villalobos, C. (1994) Redescription de *Chordodes jandae* Camerano, 1895 (Nematomorpha). *Comunicaciones do Museu de Ciencias y Tecnologia Brasil. Série Zoologia*, 7, 195-203.
- Miralles, D.B. & De Villalobos, C. (1997a) Una nueva especie de *Chordodes* (Gordiaceo Nematomorpha) parásita de *Blaptica* sp. (Blaberidae, Blataria). *Comunicaciones do Museu de Ciencias y Tecnologia. Série Zoologia*, 10, 45-51.
- Miralles, D.B. & De Villalobos, C. (1997b) Ultraestructura cuticular de una nueva especie de *Chordodes*, Creplin, 1874 para la Argentina (Nematomorpha, Gordioidea, Chordodidae). *Estudios de Biología, PUC-PR*, 42, 41-48.
- Montgomery, T.H. (1898) The gordiacea of certain American collections. *Bulletin of the Museum of Comparative Zoology at Harvard College*, 32, 23-59.
- Spiridonov, S.E. (2000) New species of gordian worms of the genus *Chordodes* (Nematomorpha: Chordodidae). *Helminthologia (Bratislava)*, 29, 193-196.
- Schmidt-Rhaesa, A. (1997) Nematomorpha. In: Schwoerbel, J. & Zwick, P. (Eds.), *Süßwasserfauna von Mitteleuropa*. Gustav Fisher, Stuttgart, pp. 1-117.
- Schmidt-Rhaesa, A. (2002a) Are the genera of Nematomorpha monophyletic taxa? *Zoologica Scripta*, 31, 185-200.
- Schmidt-Rhaesa, A. (2002b) Australian species of *Chordodes* (Nematomorpha) with a description of two new species, remarks on the genus *Chordodes* and its life history. *Journal of Natural History*, 36, 1569-1588.
- Schmidt-Rhaesa, A. (2004) Japanese horsehair worms (Nematomorpha) from the Lake Biwa Museum. *Species Diversity* 9, 97-107.
- Schmidt-Rhaesa, A. & Ehrmann R. (2001) Horsehair worms (Nematomorpha) as parasites of praying Mantids with a discussion of their life cycle. *Zoologischer Anzeiger*, 240, 167-179.
- Schmidt-Rhaesa, A. & Yadav, A.K. (2004) On the occurrence of *Chordodes* cf. *furnessi* (Nematomorpha) from praying mantis in India, and note on Indian Nematomorph species. *Current Science*, 86, 1023-1027.
- Schmidt-Rhaesa, A. & Menzel, L. (2005) Central American and Caribbean species of horsehair worms (Nematomorpha), with the description of three new species. *Journal of Natural History* 39, 515-529.
- Schmidt-Rhaesa, A., Hanelt, B. & Reeves, W.K. (2003) Redescription and compilation of Nearctic freshwater Nematomorpha (Gordiida), with the description of two new species. *Proceedings of the Academy of Natural Sciences of Philadelphia*, 153, 77-117.
- Wei, D.X. & Yang, W.Y. (1979) Case report on urinary infection of gordius worm. *Bulletin of Wuhan Medical College*, 8, 56-58.
- Wu, H.W. & Tang S.F. (1933) Notes on the Nematomorpha of China. *Sinensia*, 3, 173-178.