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Ultrastructural redescription of *Chordodes moraisi* (Carvalho, 1942) and *Chordodes straviarskii* Carvalho and Feio, 1950, and re-interpretation of *Chordodes gestri* Camerano, 1904 and *Pseudochordodes griffinii* (Camerano, 1898) (Gordiida, Nematomorpha)

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Abstract
The Gordiida species *Chordodes moraisi* (Carvalho, 1942) and *C. staviarskii* Carvalho and Feio, 1950 are re-described using scanning electron microscopy (SEM). *Chordodes moraisi* is characterized by four areole types, two of which correspond to tubercle areoles, the crown areoles appear as single structures and occur on both sides along the ventral midline. *Chordodes staviarskii* is characterized by a polymorphism of crowned areoles which appear as two types with distinct distribution patterns. Two species, *Chordodes gestri* Camerano, 1904 and *Pseudochordodes griffinii* (Camerano, 1898), are synonymized with *Neochordodes occidentalis* (Montgomery, 1898) and *Chordodes peraccae* (Camerano, 1894).

Keywords: Chordodes, *Nematomorpha, re-description, ultrastructure*

Introduction

The Gordiida (Nematomorpha) possess either a smooth cuticle or one with cuticular structures, called areoles. The cuticle of genus *Chordodes* Creplin, 1847 displays a high diversity of areole types but is characterized by the existence of a specialized type of areole called crowned areoles (De Villalobos and Zanca 2001; Schmidt-Rhaesa 2002a). *Chordodes* is mainly distributed throughout tropical and subtropical regions, with a few exceptions such as *Chordodes morgani* Montgomery, 1898 which is only found in North America (Schmidt-Rhaesa and Ehrmann 2001; Schmidt-Rhaesa 2002b). Twenty-five species of *Chordodes* are recorded in the literature for South America. The majority of the original descriptions were based upon light microscopy of small areas of the cuticle and give scarce information on the differences in shape and distribution of the areoles along the whole body. At present, the scanning electron microscope allows a perfect documentation of the cuticular features. This technique has been applied only to seven species of *Chordodes* from

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South America: *C. carmelitanus* (Miralles, 1989), *C. lotus* (Miralles and De Villalobos, 1997), *C. matensis* (De Villalobos and Miralles, 1997), *C. cornuta* (De Villalobos and Camino, 1999), *C. festae* Camerano, 1897 and *C. peraccae* (Camerano, 1894) (De Villalobos and Zanca 2001), and *C. balzani* Camerano, 1896 (De Villalobos et al. 2004).

The aim of this paper is: (1) to redescribe by scanning electron microscopy (SEM) two South American species, *Chordodes moraisi* and *Chordodes staviarskii*, and (2) to re-interpret the taxonomic position of *Chorodes gestri* and *Pseudochordodes griffinii*.

Material and methods

We investigated the holotypes of *Chordodes moraisi*, *Chordodes staviarskii* (Museu Nacional of Rio de Janeiro, Brazil) and the holotypes and the paratypes of *Chordodes gestri* (Museum Regionale di Science Naturali, Torino, Italy; MZUT G34) and *Pseudochordodes griffinii* (Museum Regionale di Science Naturali, Torino, Italy; MZUT G39). Body measurements of *Chordodes moraisi* and *Chordodes staviarskii* were made with outstretched worms using a ruler. Diameters were measured under dissecting microscope using a calliper ruler. Fragments of all the worms studied were dehydrated in an increasing ethanol series, critical point-dried, mounted on bronze blocks and gold sputter-coated. Observations were performed using a JEOL SLM 1000 scanning electron microscope.

Chordodes moraisi (Carvalho, 1942)

(Figure 1)

Neochordodes moraisi Carvalho 1942, p 219.
Chordodes moraisi: Carvalho 1944, p 488.

Holotype: one female, Viçosa Minas Gerais, Brazil (deposited in the Museu Nacional of Rio de Janeiro, Brazil).

Additional specimen. One male, Viçosa, Minas Gerais, Brazil. (This specimen is not in the collection of the Museu Nacional do Rio de Janeiro as indicated by Carvalho 1944. Unfortunately, it seems to be lost.)

Material examined. Holotype: SEM of posterior end and midbody.

Host. Blatta orientalis Linnaeus, 1758 and *Miniblatta* sp.

Description

Body length 208 mm, diameter in the middle region 2 mm. Anterior end tapered, without dark ring. Mouth terminal. Terminal end with 484 µm maximum width, with a slightly pronounced furrow in the ventral surface (Figure 1A). The cloacal opening is situated terminally at the posterior end and is rounded. The cuticle surrounding the cloacal opening is free of areoles but with small bristles. The body colour is uniformly yellowish brown. Dorsal and ventral grooves are hardly visible.

The cuticle shows four types of areoles which are numbered consecutively for the purpose of description and orientation. Types 1, 2 and 3 are distributed all over the body surface while the fourth areolar type is found only in the longitudinal ventral groove. Type 1 areoles are the most abundant (Figure 1B, C), they are rounded (15–23 µm in

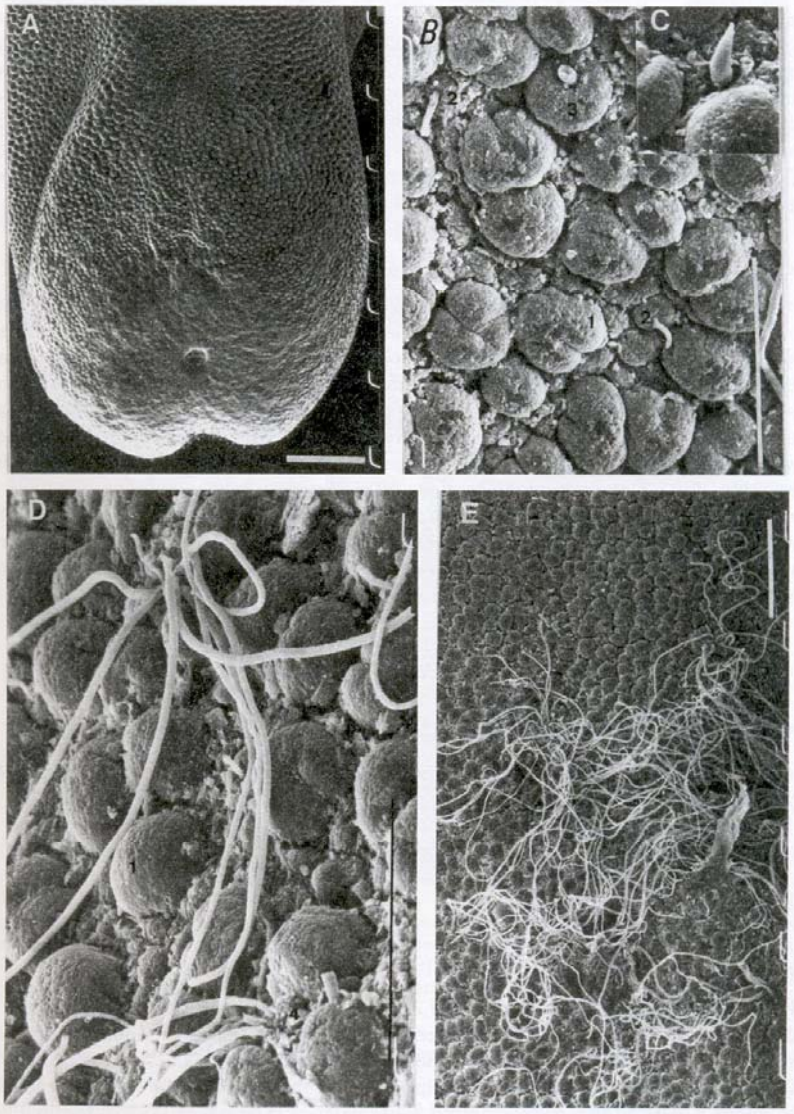

Figure 1. SEM of female of *Chordodes moraisi*, holotype. (A) Ventral view of posterior end with cloacal opening; (B) midbody cuticle, areoles types 1–3; (C) tubercle in the interareolar furrow; (D, E) areoles type 4 with long filaments in the longitudinal ventral groove. Scale bars: 100 μm (A, E); 50 μm (B–D).

diameter) with a smooth surface or a surface partially traversed by superficial furrows. Scattered among these areoles, there are small areoles (type 2, Figure 1B) (6.5–8.9 μm), rounded and with a long (9.3 μm), thin (1.3 μm) and eccentrically situated tubercle (tubercle areole). Type 3 areoles are very similar to type 1 (Figure 1B, D) in shape and size (21 μm) but different in their short (6.6 μm) and wide (5.3 μm) tubercle with a lateral position (also tubercle areole) and they are scarce or rarely found. In the interareolar groove there are scattered tubercles (13 μm) with pointed ends (Figure 1C). Type 4 areoles, crowned areoles (Figure 1D, E), appear as single structures, they are short and sometimes difficult to distinguish, rounded (8.7 μm diameter) with long filiform projections arising from their centre (125 μm) covering the longitudinal ventral groove (Figure 1D, E).

Comments

Carvalho (1942), based on a female collected from a cockroach, *Blatta orientalis*, described a new species as *Neochordodes moraisi* with one type of areole. In 1944, Carvalho studied a male specimen collected from *Miniblatta* sp. and when comparing it with the female specimen of *N. moraisi* he noticed that both specimens possessed areoles with filiform projections (crowned areoles) which had not previously been observed in the female, and transferred them to the genus *Chordodes*. Carvalho (1944), when describing the cuticle of *C. moraisi*, considered that it displayed three areolar types distributed on the whole surface of the body. Our ultrastructural study allows us to increase the number of areolar types for *C. moraisi* to four, two of which correspond to tubercle areoles. Carvalho (1944) described crowned areoles as distributed in pairs between or on which filiform projections emerge, shorter in the male and longer in the female. We observed that in the female, crowned areoles never appear in pairs and are distributed only in the longitudinal ventral groove, the projections arising at the areolar apex and not between areoles.

Chordodes staviarskii Carvalho and Feio, 1950
(Figure 2)

Chordodes staviarskii Carvalho and Feio 1950, p 199.

Holotype: one female, Rio de Janeiro, Distrito Federal, Brazil, coll V. Staviarskii, 1949 (deposited in the Museu Nacional of Rio de Janeiro, Brazil).

Material examined. SEM posterior end and midbody.

Host. Unknown.

Description

Body length is 324 mm with a diameter of 2.1 mm. The anterior end is tapered, a white tip or dark collar is not present. The posterior end (Figure 2A) is rounded. The cloacal opening is circular, terminal and surrounded by radiating grooves. There are scattered fine bristles in the area between the radiating grooves and the cuticle of the terminal end. The body colour is dark brown with scattered white patches. The whole body is covered with protruding structures, the areoles. Six types of areole can be distinguished. Type 1 areoles (Figure 2B, C) are the most abundant and are slightly oval or circular in shape, they are structured apically (like a blackberry) with diameters of 8–11 μm and about 5 μm high.

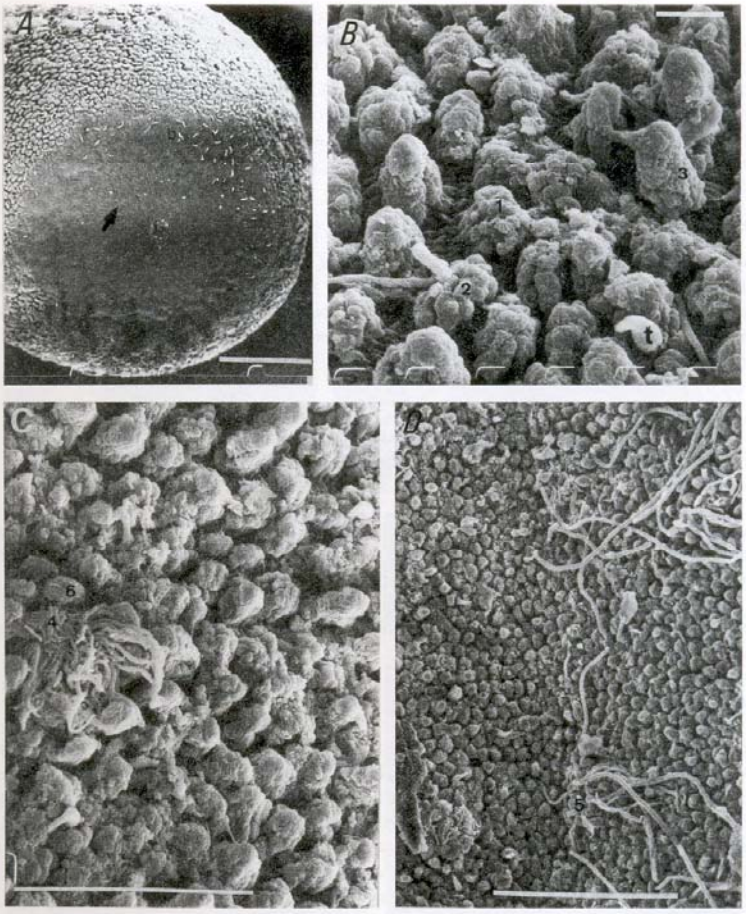

Figure 2. SEM of female of *Chordodes stavarskii* holotype. (A) Ventral view of posterior end with cloacal opening, bristles and radiating grooves (arrow). (B, C) Midbody cuticle: (B) areoles types 1–3 and interareolar tubercle; (C) areoles of type 1–3 and clusters containing types 4 and 6. (D) Areoles type 5 and 6 in the longitudinal ventral groove; b, bristles; t, tubercle. Scale bars: 100 µm (A, D); 10 µm (B); 50 µm (C).

Among these are scattered areoles with the same shape, but with a tubercle on the top (tubercle areole, type 2; Figure 2B, C). This tubercle is about 7 µm long and bears a distinct finger-like projection. Type 3 areoles are more elevated than the first two (14 µm), have a rounded apex and occur in clusters of two, three or more over the whole cuticle (Figure 2B, C). The areolar types 4 and 5 have filaments on the top and are termed crowned areoles. These crowned areoles occur in clusters of two and are surrounded by 10

or 12 elevated areoles (12–15 μm) of type 6 (Figure 2C, D). Type 4 crowned areoles bear moderately short filaments (20 μm) and are distributed over the whole body cuticle (Figure 2C). Type 5 crowned areoles have long filaments (108 μm), and occur only on each side along the dorsal and ventral midline (Figure 2D). Type 6 areoles have a slightly curved apex and a thin tubercle can be observed in some of them (Figure 2C). In the interareolar furrow separating areolar types 1, 2 and 3 there are scattered conical tubercles of up to 9 μm length (Figure 2B, C).

Comments

Chordodes staviarskii resembles *C. brasiliensis* Janda, 1894, but differs in the areolar groups formed by type 4 and 6 areoles distributed over the cuticle. Carvalho and Feio (1950) described the cuticle of *C. staviarskii* with four areolar types. Analysis using SEM shows with more precision the differences between areoles, which enables us to increase to six the number of recognizable areolar types.

[***Chordodes gestri*** Camerano, 1904]

(Figure 3)

Chordodes gestri Camerano 1904, p 93.

Holotype: one male, Quezaltenango Guatemala Museo Regionale di Science Naturali, Torino, Italy (MZUT G34). Paratype: one female, Museo Regionale di Science Naturali, Torino, Italy (MZUT G34).

Material examined. Holotype: SEM anterior end, posterior end and midbody. Paratype: SEM anterior end and midbody.

Host. Unknown.

Camerano (1904) described a male and a female from Guatemala with three types of areole. One type of short areole of variable dimensions; a second type of large areole arranged in groups of 7–10 between which a tubercle emerges, and a third type of areole distributed among type 1 areoles and with a small tubercle (see also Camerano 1915). These characters are not sufficient for the description of a new species belonging to the genus *Chordodes*, because the diagnostic feature of *Chordodes* is the existence of a specialized type of areole, called crowned areoles, which were not described for this species (De Villalobos and Zanca 2001; Schmidt-Rhaesa 2002a; De Villalobos et al. 2004). The reason why Camerano (1904) included this species in the genus *Chordodes* is seen to be unknown. In a SEM investigation the posterior end of the holotype is undivided, and a ventral groove is present. The cloacal opening is slit-like and surrounded by unbranched or apically branched circumcloacal spines (Figure 3A, B). The cuticle of the holotype and the paratype contains areoles of one type, which vary in size (Figure 3C). A megareolar pattern is present mainly in the midbody region. Among some of these large areoles a rounded apex tubercle emerges (Figure 3C). All the areoles are very close together. In the mid-anterior body region this areolar pattern changes and the areoles are of similar sizes, some with superficial furrows. The interareolar furrow is large with scattered small tubercles (Figure 3D).

The features observed in the cuticle along the body allow us to relate these two specimens of *Chordodes gestri* with the holotype and with the specimen from Nebraska of

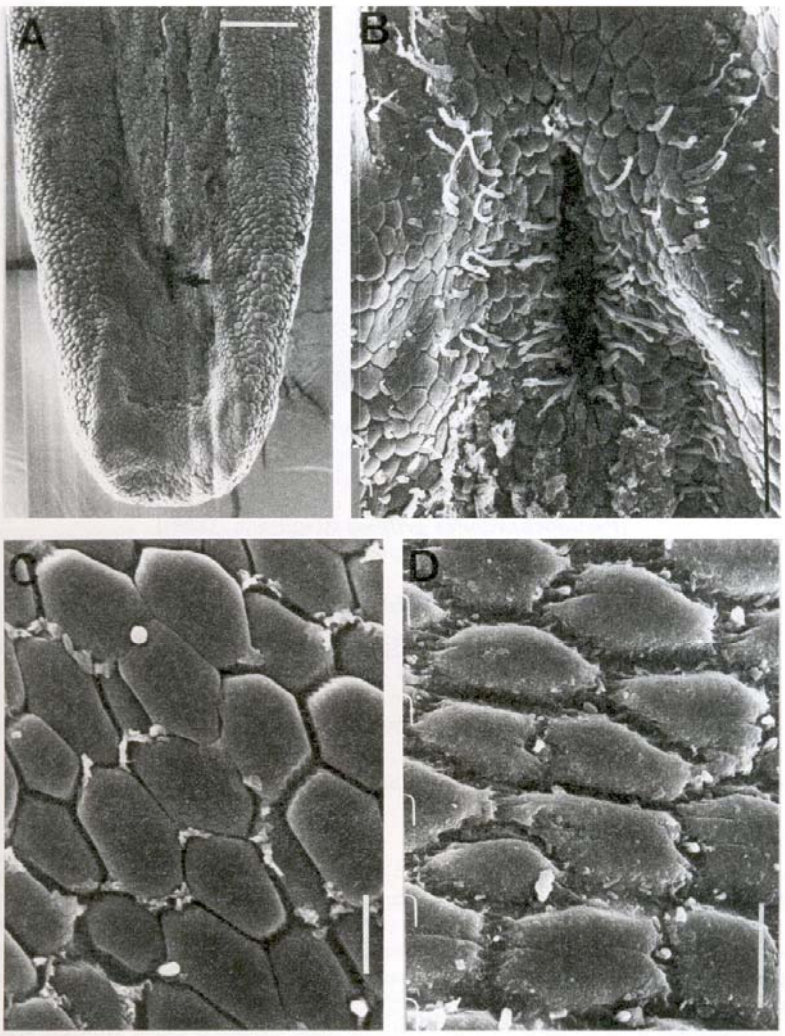

Figure 3. SEM of male of "*Chordodes gestri*" holotype. (A, B) Ventral view of posterior end: (A) whole posterior end with cloacal opening (arrow); (B) cloacal opening with circumcloacal bristles. (C) Cuticle at the midbody with megareolar pattern. (D) Areolar pattern in the anterior region of the body. Scale bars: 100 µm (A); 50 µm (B); 10 µm (C, D).

Neochordodes occidentalis (Montgomery, 1898) which were studied by Schmidt-Rhaesa et al. (2003, Figure 13A, F). Therefore, we regard *Chordodes gestri* as a synonym of *Neochordodes occidentalis*.

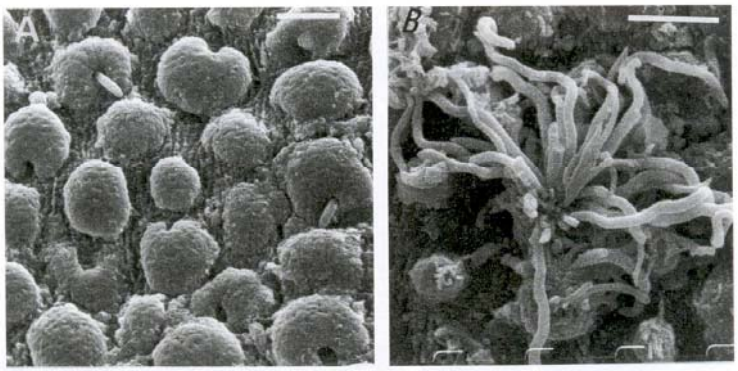

Figure 4. SEM of male of "*Pseudochordodes griffinii*" holotype. (A) Cuticle of midbody; (B) areolar group in the middle region of the body. Scale bars: 10 μm.

[***Pseudochordodes griffinii*** (Camerano, 1898)]
(Figure 4)

Chordodes griffinii Camerano 1898, p 74.
Pseudochordodes griffinii: Miralles and de Villalobos 1994, p 14.

Holotype: one male, Museo Regionale di Science Naturali, Torino, Italy (MZUT G39). Paratype: one female (MZUT G39).

Type locality. Altoyac, Vera Cruz Mexico.

Material examined. Holotype and paratype: SEM midbody.

Host. Unknown.

Camerano (1898) described the new species *Chordodes griffinii* from a male and a female from Mexico, having two types of areole. Miralles and De Villalobos (1994), basing their decision on Figure 5 and 5a of Camerano (1899), transferred this species to the genus *Pseudochordodes* Carvalho, 1942. In SEM investigation of the holotype (Figure 4A, B) and the paratype, the cuticle shows the same characteristics that were observed for *Chordodes peraccae* (see De Villalobos and Zanca 2001).

It is important to emphasize that although the male specimen shows crowned areoles with short filaments that occur in clusters of two and are surrounded by 8–10 elevated areoles (Figure 4B), it does not have crowned areoles with very long filaments distributed on both sides along the ventral midline. These types of crowned areole occur only in the females (De Villalobos and Zanca 2001) so their presence should be considered as a sexual dimorphism for this species.

On the other hand, the only possible explanation for the fact that Camerano (1898, 1899) did not notice the presence of crowned areoles in these specimens is that he probably based his analysis on a small portion of the cuticle. Therefore, we regard *Chordodes griffinii* as a synonym of *Chordodes peraccae.*

Conclusion

From this investigation we can confirm that *Chordodes moraisi* and *C. staviarskii* are valid species. *Chordodes staviarskii* shows two types of crowned areoles. This polymorphism of crowned areoles has been noted in *Chordodes queenslandi* Schmidt-Rhaesa, 2002 from Australia and in *Chordodes balzani* from Trinidad (De Villalobos et al. 2004). Likewise, this study by SEM allows us to confirm the observations previously made by Carvalho (1944) for *C. moraisi* and Carvalho and Feio (1950) for *C. staviarskii*: in females the crowned areoles with long filaments are limited to the ventral and/or dorsal and ventral groove. This particular feature in females has been mentioned for other South American species such as *C. brasiliensis* (Janda, 1894) (Camerano 1897; Carvalho 1946), *C. carmelitanus* Carvalho and Feio, 1950, *C. peraccae* (Camerano, 1894) (De Villalobos and Zanca 2001) and *C. balzani* Camerano, 1896 (Carvalho and Feio 1950; De Villalobos et al. 2004).

Although more studies are necessary on both sexes of species in the genus *Chordodes* in South America, there seems to be sexual dimorphism, as was considered by Carvalho (1946) for the species *C. brasiliensis* and by Camerano (1987), Carvalho and Feio (1950) and De Villalobos et al. (2004) for *C. balzani*, and in the present study for *C. peraccae*.

The ultrastructural studies of the holotypes and of the paratypes of *Chordodes gestri* and *Pseudochordodes griffinii* allow us to consider *C. gestri* as a synonym of *Neochordodes occidentalis*, enlarging its distribution to Guatemala, and *C. griffinii* as a synonym of *C. peraccae*, enlarging its distribution to Mexico.

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