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Revision of the genus Chordodes (Gordiida, Nematomorpha) from Africa—I. Ultrastructural redescription of Chordodes gariazzi Camerano, 1902, C. heinzei Sciacchitano, , C. kolensis Sciacchitano, , C. muelleri Sciacchitano, , and C. ruandensis Sciacchitano,

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# Revision of the genus *Chordodes* (Gordiida, Nematomorpha) from Africa—I. Ultrastructural redescription of *Chordodes gariazzi* Camerano, 1902, *C. heinzei* Sciacchitano, 1937, *C. kolensis* Sciacchitano, 1933, *C. muelleri* Sciacchitano, 1937, and *C. ruandensis* Sciacchitano, 1937

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#### Abstract

Thirty-five species of *Chordodes* have been reported from Africa. The original description of several of these species is incomplete and needs reinvestigation by scanning electron microscopy (SEM) which allows the observation of fine structural details. We reinvestigated five of these species: *Chordodes gariazzi*, *C. heinzei*, *C. kolensis*, *C. muelleri*, and *C. ruandensis*. All of them show crowned areoles occurring in clusters. These clusters are formed by crowned areoles that are surrounded by another areolar type. The females of *C. kolensis* and *C. muelleri* have polymorphic crowned areoles with both short and long filaments; these areoles are only observed along the ventral groove. *Chordodes gariazzi*, *C. heinzei*, and *C. muelleri* have five areole types, *C. ruandensis* has three different types of areole, and *C. kolensis* displays a sexual dimorphism where males have six types of areole and females have seven areole types. *Chordodes gariazzi*, *C heinzei*, and *C. muelleri* have tubercle areoles. Host data and a new distribution record of *C. kolensis* are reported.

Keywords: Africa, Gordiida, Nematomorpha, scanning electron microscopy

#### Introduction

The genus *Chordodes* was created by Creplin (1847) when he found that a specimen of freshwater Nematomorpha from Brazil showed different characteristics in comparison to *Gordius*, the only nematomorph genus then known. Camerano (1897) was the first to give a characterization of the genus *Chordodes*. Species of *Chordodes* are distributed worldwide, mainly in tropical and subtropical regions with few exceptions (Montgomery 1898;

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Kirjanova and Spiridonov 1989; Spiridonov 2000). The cuticle of *Chordodes* species contains a higher diversity of structures compared to other genera of Nematomorpha, and up to six different types have been described in one species (Schmidt-Rhaesa 2002; De Villalobos et al. 2004a). Characteristic for the genus are crowned areoles which occur, according to the species, isolated, in pairs or in clusters with another areolar type.

To date about 90 species of *Chordodes* have been described around the world, 35 of which occur in Africa (Table I), but no photographic documentation (neither light microscopy (LM) nor SEM) has been made for any species of *Chordodes* from Africa. Also, the original description of several of these species is incomplete and needs reinvestigation by modern methods (SEM) which allow the observation of fine structural details. In this paper we will describe by SEM five African species, while the others will be studied in separate investigations.

The cuticle in all *Chordodes* species is rich in more or less elevated structures called areoles. Areoles are the most important characters for species determination in this genus, because size and coloration seem to be variable, and the posterior ends (which are important for determination in other genera) are very similar among species. The diversity of areoles in *Chordodes* makes it desirable to standardize the terminology in order to have a better basis for a comparison of the patterns in different species. We employ a terminology which will make it easier to compare homologous structures among *Chordodes* species.

#### Material and methods

We investigated the holotypes of *Chordodes gariazzi*, *C. heinzei*, *C. kolensis*, *C. muelleri*, and *C. ruandensis* from the Museo Regionale di Science Naturali, Torino, Italy (MRSNT) and the Africa Museum Tervuren, Belgium (Koninklijk Museum voor Midden-Afrika) (AMT). Six further specimens, from The Natural History Museum, London (NHM) and Muséum National d'Histoire Naturelle, Paris (MNHNP) were also included in the analysis.

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 holotype material, we removed a tangential section of the middle region of the body with a razor blade. The posterior end of specimens (AMT 1390, 14201, 1421, 1422, 1427) was only investigated under light microscopy and photographed under a stereomicroscope. 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. All preparations, i.e. SEM stubs, were stored together with the preserved specimen in the respective museums.

### Results

Several types of areole can be distinguished, but most of these types appear to be homologous between different species. Instead of numbering the different areoles and labelling them as type 1, type 2, etc. we suggest names for each type of areole. Such names may make comparisons between species more convenient.

Usually, the cuticular surface is covered by an abundant type of areole which we term a *simple areole*, and among which other areolar types are dispersed. Simple areoles are the least elevated areoles and their surface can vary from smooth to rough, carrying granules or short spines. Another regularly occurring type is the *tubercle areole* which resembles simple

Table I. Reports of Chordodes species (Nematomorpha) from A	Africa.
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Original determination	Type locality	Reference
C. aethiopicus Inoue, 1974	Ethiopia, Awash National Park	Inoue (1974)
C. africanus Sciacchitano, 1933	Democratic Republic of the Congo, Sankuru, Kondué	Sciacchitano (1933, 1937, 1958, 1961)
C. albibarbatus Montgomery, 1898	Democratic Republic of Ghana, Ogove, Gaboon River	Montgomery (1898), Camerano (1915) Spamer and Bogan (1992)
C. boulengerii Camerano, 1912	United Republic of Cameroon, Bitye, river Ja (=Dja)	Camerano (1912b, 1915), Sciacchitano (1958)
C. bukavuensis Sciacchitano, 1958	Democratic Republic of the Congo, Province Sud-Kivu, Costermansville (=Bukavu)	Sciacchitano (1958)
C. butensis Sciacchitano 1937	Democratic Republic of the Congo, Buta	Sciacchitano (1937, 1958)
C. capensis Camerano, 1895	Cape of Good Hope	Camerano (1895, 1897, 1915), De Beauchamp (1916), Baylis (1927), Sciacchitano (1932, 1933, 1937, 1955, 1958, 1961), Coombs and Crompton (1991)
C. capillatus Linstow 1901	Tanzania, Kilondo (=Langenburg), North of Lake Malawi	Linstow (1901)
C. clavatus Linstow, 1906	United Republic of Cameroon, Yaounde	Linstow (1906), Camerano (1915)
C. congolensis Sciacchitano, 1937	Democratic Republic of the Congo, Province Equateur, Flandria, South of Ingende	Sciacchitano (1933, 1958)
<i>C. digitatus</i> Linstow, 1901 <i>C. ferox</i> Camerano, 1897	Tanzania, Unyika Republic of the Congo (former French Congo)	Linstow (1901), Camerano (1915) Camerano (1897, 1915), Sciacchitano (1932, 1933, 1958)
C. gariazzi Camerano, 1902	Democratic Republic of the Congo (locality unknown)	Camerano (1902a, 1915), Sciacchitano (1958), this study
C. guineensis Spiridonov, 2002 C. hawkeri Camerano 1902	Republic of Guinea, Kankan Sudan	Spiridonov (2002) Camerano (1902b, 1908, 1912a, 1915), Sciacchitano (1958)
C. heinzei Sciacchitano, 1937	Democratic Republic of the Congo, Province Kasai, Luebo	Sciacchitano (1937, 1958), this study
C. ibembensis Sciacchitano, 1958	Democratic Republic of the Congo, Province Uele, Ibem	Sciacchitano (1958, 1961)
C. iturensis Sciacchitano, 1958	Democratic Republic of the Congo, Ituri, Nioka	Sciacchitano (1958)
C. kakandensis Sciacchitano, 1958	Democratic Republic of the Congo, Lualaba, Kakanda	Sciacchitano (1958)
C. kallstenii Jägerskiöld, 1897	United Republic of Cameroon, Ekundu	Jägerskiöld (1897), Camerano (1915)
C. kivuensis Sciacchitano, 1958	Democratic Republic of the Congo, Province Kivu, Tshibinda	Sciacchitano (1958)
C. kolensis Sciacchitano, 1933	Democratic Republic of the Congo, Province Kasai, Kole and Democrati Republic of the Congo, Province Equateur, Mbandaka (=Coquilhatville)	Sciacchitano (1933, 1958, 1961), c this study
C. lefeburei (orig. lefeburi (Sciacchitano, 1937) C. ligasiensis Sciacchitano,	Democratic Republic of the Congo, Province Katanga, Kanzenze Democratic Republic of the Congo,	Sciacchitano (1937, 1958) Sciacchitano (1933)

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Table I. continued
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Original determination	Type locality	Reference
C. maculatus Sciacchitano, 1958	Democratic Republic of the Congo, Province Bandundu, Lake Mai-Ndombe and Republic of the Congo, Province Bandundu, Bolobo	Sciacchitano (1958)
C. madagascariensis Camerano, 1897	Madagascar, Antananarivo	Camerano (1897, 1915), Römer (1896), De Beauchamp (1916, 1923), Sciacchitano (1932, 1933, 1958, 1961)
C. mobensis Sciacchitano, 1958	Democratic Republic of the Congo, Province Shaba-Moba	Sciacchitano (1958)
C. montgomeryi Camerano, 1901	Madagascar, Fort Dauphin	Camerano (1901, 1915)
C. mulleri Sciacchitano, 1937	Republic of Rwanda, Gabiro	Sciacchitano (1937, 1958), this study
C. rigatus Sciacchitano, 1937	Democratic Republic of the Congo, Province Katanga, Elisabethville (=Lubumbashi)	Sciacchitano (1937, 1958)
C. ruandensis Sciacchitano, 1937	Republic of Rwanda, Gabiro	Sciacchitano (1937, 1958), this study
C. sandoensis Sciacchitano, 1937	Democratic Republic of the Congo, Lulua Sandoa	Sciacchitano (1937, 1958)
C. siamensis Sciacchitano, 1937	Democratic Republic of the Congo, Ibembo	Sciacchitano (1937)
C. schoutedeni Sciacchitano, 1933	Democratic Republic of the Congo, Province Katanga, Leopoldville (=Kinshasa)	Sciacchitano (1933, 1958, 1961)
C. uncinatus Sciacchitano, 1958	Democratic Republic of the Congo, Bidua, Lisala	Sciacchitano (1958)
C. tuberculatus Linstow, 1901	Langerburg (Lake Nyassa 160 m deep)	Linstow (1901), Camerano (1915)

areoles, but carries a finger-like projection on top, the tubercle. Several species have solid spines that appear to originate from an areole and can therefore be considered as another areolar type, the *thorn areole*. Thorns are distinguished from tubercles by their pointed tip and are usually much more solid. In some species, areoles closely resembling simple areoles are elevated distinctly more than these. They are termed *bulging areoles* and rarely occur singly, but often in clusters of two or more. The most conspicuous type of areole, and the one characteristic for the genus *Chordodes*, is termed the *crowned areole*. These areoles are composed of a stalk-like structure and a ring of apical filaments. Sometimes, two different types of crowned areole are present; then, one type with shorter filaments is distributed over most of the body surface while a second type with distinctly longer filaments is distributed along the ventral and sometimes also along the dorsal midline. Crowned areoles rarely occur singly, but often two or more are clustered. In many species, this cluster is surrounded by slender, slightly elevated areoles termed *circumcluster areoles*.

# Chordodes gariazzi Camerano, 1902

(Figure 1)

Chordodes gariazzi Camerano 1902a, p1.

Holotype: 13, Democratic Republic of the Congo. Specific locality unknown (Camerano 1902a) (MRSNT G36).



Figure 1. *Chordodes gariazzi*, SEM. (A) Ventral view of male posterior end with cloacal opening (c), arrow indicates position of the bristlefields; (B) general view of the midbody cuticle with simple (1), bulging (2), tubercle (3), crowned (4), and circumcluster areoles (5); (C) cuticle of the lateral side of the body with simple, bulging, and tubercle areoles (numbering as in B); (D) tubercle areole and areolar cluster of crowned and circumcluster areoles. Scale bars:  $100 \mu m$  (A);  $25 \mu m$  (B, C);  $10 \mu m$  (D).

## Material examined

Holotype, SEM midbody and posterior end.

Host

Unknown.

## Description

The body colour is medium brown with numerous darker brown patches all over the body. The body length is 240 mm, diameter in the middle region is 1.5 mm. Anterior end tapered, with a white cap, a dark collar is absent. The mouth is terminal. The posterior end (Figure 1A) is tapered at the apex (0.20 mm in diameter). The cloacal opening is ventral and is situated 266  $\mu$ m anterior of the posterior margin of the worm. The cloacal opening is narrow, oval (57  $\mu$ m long and 10.7  $\mu$ m wide), and without circumcloacal spines. Anterolateral to the cloacal opening are two rows of bristles (bristlefields) that are

 $221.4\,\mu\text{m}$  long and  $85.7\,\mu\text{m}$  wide. Bristles are undivided. The cuticle around the cloacal opening is smooth.

The cuticle contains all six types of areole as described above (Figure 1B). Simple areoles are the most abundant, they are low  $(4.5\,\mu\text{m})$ , oval or circular in shape, and have a structured surface ("blackberry-like"). Minute bristles are scattered on the apical surface (Figure 1B, C). Bulging areoles form groups of two to four, they are similar to simple areoles in shape although higher  $(9.7\,\mu\text{m})$ , and in the centre of the apex they have projections which are not scattered but clustered (Figure 1B, C). Occasionally, tubercle areoles with a long  $(7.6\,\mu\text{m})$  finger-like tubercle are present. These areoles are taller than the simple areoles  $(6.3\,\mu\text{m})$  (Figure 1B, C). Very rarely present are thorn areoles (Figure 1C). Two crowned areoles and 12–20 circumcluster areoles form clusters regularly distributed over the whole cuticle (Figure 1B, D). The filaments of the crowned areoles are approximately 40.6  $\mu$ m long. Circumcluster areoles are very slender, 20.2  $\mu$ m high, and curved towards the centre (Figure 1B, D). The cuticle between the areoles is structured into cord-like folds.

#### Comments

Only one male is known for this species, described by Camerano (1902a) and later mentioned by Camerano (1915) and Sciacchitano (1958). None of these descriptions contained any figures. Camerano (1902a) described the cuticle of *C. gariazzi* with five areolar types, corresponding to simple, bulging, tubercle, thorn, and crowned areoles from our investigation. Camerano (1902a) described crowned areoles occurring in clusters of 12–17, but our SEM investigation shows that there are only two crowned areoles, surrounded by circumcluster areoles.

#### Chordodes heinzei Sciacchitano, 1937

(Figure 2)

Chordodes henzei Sciacchitano 1937, p 141, Plate 142, Figure 2. Holotype: 13, Democratic Republic of the Congo, Province Kasai, Luebo. Coll. L. Achten, 1923 (Sciacchitano 1937) (AMT 1422).

#### Material examined

Holotype, SEM from midbody; LM from posterior end.

#### Host

Unknown.

#### Description

Body length is 186 mm, diameter in the middle region is 1.3 mm. The anterior end is tapered, with a dark ring. Mouth is terminal. The posterior end (Figure 2A) is rounded. The cloacal opening is subterminal, oval, and without circumcloacal spines. Two rows of bristles are present anterolateral of the cloacal opening. The body colour is light brown. A ventral midline is not clearly pigmented.



Figure 2. *Chordodes heinzei*, male. (A) Stereomicroscope, posterior end with subterminal cloacal opening (arrow). (B–F) SEM: (B) general view of the midbody cuticle; (C) simple and bulging areoles; (D) lateral view of the midbody cuticle; (E) lateral view of cluster with crowned and circumcluster areoles; (F) cluster as in (E) from top. Numbering: simple (1), tubercle (2), bulging (3), crowned (4), and circumcluster (5) areoles. Scale bars: 16.6  $\mu$ m (A); 100  $\mu$ m (B); 25  $\mu$ m (C, F); 12.5  $\mu$ m (D); 10  $\mu$ m (E).

The cuticle contains five types of areole distributed all over the cuticle (Figure 2B). Simple areoles (Figure 2B–D) are the most abundant, and are flat and rectangular in shape with a warty surface (blackberry-like). These areoles are transversal to the longitudinal axis of the body. Among these are scattered tubercle areoles of the same shape but with very long tubercles (12.6  $\mu$ m) on top (Figure 2D, E). Bulging areoles are isolated or form clusters of two, three, or four areoles (Figure 2C, D). These areoles are taller (5.2  $\mu$ m) than the simple areoles, with a pyramid form and small bristles on the top. Crowned areoles (Figure 2B, F) occur in pairs, have moderately short filaments on top, and are surrounded by about 17–24 circumcluster areoles. These circumcluster areoles are the tallest (20.5–23.8  $\mu$ m), being conical, with a ring of short filaments at the apex and curved towards the centre (Figure 2B, E, F).

#### Comments

Only one male specimen is known, described by Sciacchitano (1937). In the original description, Sciacchitano (1937) described the cuticle of *C. heinzei* with three areole types which would correspond to the simple, bulging and crowned areoles of our reinvestigation. Later, Sciacchitano (1958) redescribed this species and enlarged the number of areolar types to four when describing the high circumcluster areoles. Our observations by SEM are coincident with those of Sciacchitano (1958) but we can add tubercle areole as an

additional areolar type. Sciacchitano (1937) described and produced figures of areoles with long filaments (crowned areoles) but later (Sciacchitano 1958) modified this description by saying that these areoles have numerous but short projections. We could observe that along the whole body there are some crowned areoles in which the filaments are either broken or lost (see Figure 2B) probably due to manipulation or fixation of the specimen. Sciacchitano (1937) probably observed some of these damaged areoles when redescribing them.

Chordodes kolensis Sciacchitano, 1933 (Figure 3) Chordodes kolensis Sciacchitano 1933, p 53, Figure 3.

#### Type series

Democratic Republic of the Congo: 299, Province Bandundu, Bolobo (Borobo), (Uebi river) (AMT 1391, 1392); 19, Province Kasai, Kole (AMT 1394); 19, Province Equateur,



Figure 3. *Chordodes kolensis.* (A) Stereomicroscope, male posterior end, subterminal cloacal opening (arrow) (AMT 1400). (B–G) SEM: (B) female posterior end with terminal cloacal opening (arrow) (AMT 1390); (C–F) cuticle from male specimens; (C) general view of the midbody cuticle; (D) higher magnification from (C); (E) additional areolar types (labelled 3 and 4); (F) crowned areoles with short filaments from the lateral body sides; (G) female crowned areoles with long apical filaments from the ventral midline. Numbering: simple (1), bulging (2), special types (3, 4), crowned with short filaments (5), circumcluster (6), and crowned with long filaments (7) areoles. Scale bars:  $16.6 \mu m$  (A);  $100 \mu m$  (B, C);  $25 \mu m$  (D);  $10 \mu m$  (E, F);  $50 \mu m$  (G).

Mbandaka (=Coquilhatville) (AMT 1393); 19, Province Equateur (=Coquilhatville) Wangata (AMT 1396); 19, Province Katanga, Kanzenze (AMT 1395).

#### Additional specimens

Democratic Republic of the Congo: 233, Province Equateur, Tshuapa: Bokungu (AMT 27056–27057); 13, Province Equateur, Ubangi: Gemena (AMT 29564); 13 Province Orientale, Uele: Ibembo (AMT 29573); 13, 19, Province Equateur, Yokamba (AMT 29570); 492, 13, Province Orientale, Kisangani (=Stanleyville) (AMT 1390, 1397, 1398, 1399, 1420); 13, Gwange, Busu-Bodua (AMT 1400); Province Katanga, Leopoldville (=Kinshasa) (NHM 1989.793). Angola: 13, Tsharissoka, along the road from Dundo to Camissombo (AMT 12560). Ivory Coast: 19, Mont Nimba (Bocal H19 MNHNP 730). Kenya: 19, Nairobi, Karura Forest (NHM 1920.9.8.146).

#### Material examined

SEM. Midbody from: 1 $\circ$ , 1 $\circ$  (AMT 1390, 1420), 1 $\circ$  (AMT 1400), 1 $\circ$  Province Equateur (=Coquilhatville) Wangata (AMT 1396), 1 $\circ$  (Boccal H19 MNHNP 730), 1 $\circ$  (NHML 1917.8.13.27), 1 $\circ$  (NHML 1920.9.8.146) (NHM 1989.793). Posterior end from: 1 $\circ$ ? (AMT 1390), 1 $\circ$  (AMT 1400).

#### Host

Undetermined mantis from specimens AMT 1390 and 1394 (Sciacchitano 1933). The mantis from specimen AMT 1394 is determined as *Tenodera* sp. (Sciacchitano 1958). Undetermined mantis from specimens AMT 1400 (Sciacchitano 1961) and NHM 1920.9.8.146 (new record).

#### Description

The body colour of males is dark brown, females are light brown. The body cuticle shows light spots. The anterior end is tapered, the anterior tip is white and a dark collar is lacking. The posterior end of males is undivided with a medial ventral groove (Figure 3A). There are two row of bristles (bristlefields) anterolateral to the cloacal opening. The female posterior end is rounded (Figure 3B).

The cuticle of males has six types of areole. Simple areoles (Figure 3C, D) are distributed over the whole cuticle with varying shapes, rounded, oval, or crescent-shaped. They are low (6.6  $\mu$ m), the apical surface is completely smooth or with minute bristles. Bulging areoles (Figure 3C, D) are conical and 14.7  $\mu$ m high. They are arranged among simple areoles, isolated or in groups of two or three areoles. Crowned areoles occur in pairs and are elevated 18.7–20.5  $\mu$ m above the surface (Figure 3C, F). They have an apical ring of very short filaments. Between 20 and 26 elevated (21.3  $\mu$ m) circumcluster areoles surround each pair of crowned areoles (Figure 3C, F). There are two additional types of areole which are unusual for *Chordodes* species. A conical, elevated areole is surrounded by three or four areoles with a half-moon shape (AMT 1390, 1396, 1420) (Figure 3E).

The cuticle of females contains the same areoles as the males, but along the ventral groove there is another type of crowned areole with very long filaments ( $107.2 \,\mu$ m) that also occurs in clusters of two and is surrounded by circumcluster areoles (Figure 3G).

#### Dimensions

Males are generally shorter and narrower than females. Length of the males varies between 57 and 127 mm, females between 110 and 352 mm (see Comments). Diameter in males is 1 mm and 0.5–2 mm in females (Sciacchitano 1933, 1958, 1961).

#### Comments

Sciacchitano (1933) described this species from six females (AMT 1391, 1392, 1393, 1394, 1395, 1396). These specimens were mentioned again by Sciacchitano (1958). Although the dimensions of the female from Kole (AMT 1394) (also noted by Schmidt-Rhaesa and Ehrmann 2001) and the sex of the specimen from Borobo (AMT 1392) differ in both publications, the collection date and collector's name correspond. It is assumed that these reports (Sciacchitano 1958) are a repetition of Sciacchitano's (1933) report. Also Sciacchitano (1958) considered the specimen from Province Orientale, Kisangani (=Stanleyville) (AMT 1390) to be a male, when it is really a female (see Figure 3B) Sciacchitano (1933), in the original description of C. kolensis, considered the cuticle to contain three areolar types. Taking into account that none of these areolar types corresponds to the crowned areoles which are the characteristic areole of the genus Chordodes (De Villalobos and Zanca 2001; Schmidt-Rhaesa and Ehrmann 2001; Schmidt-Rhaesa 2002; De Villalobos et al. 2004b), it is difficult to know on which characteristics Sciacchitano (1933) based his studies in order to include this species within Chordodes. Sciacchitano (1958) widened the description of the cuticle, describing four areolar types, among which he described areoles with short filaments (crowned areoles). These four areolar types are coincident with simple, bulging, circumcluster, and crowned areoles from our investigation. We can add two further areolar types, occurring in a cluster. Such clusters of a central and three or four surrounding areoles have not been described from any other species and have to be considered diagnostic for C. kolensis. The study of new specimens belonging to C. kolensis adds more localities and shows that this species is probably widespread in Central Africa.

# Chordodes muelleri Sciacchitano, 1937

(Figure 4)

Chordodes muelleri Sciacchitano 1937, p 143, Figure 3.

#### Holotype

19 (AMT 1421) (see Comments).

#### Type locality

Republic of Rwanda, Gabiro. Coll. Verhulst, 1934 (Sciacchitano 1937).

#### Material examined

Holotype, SEM from midbody; LM from posterior end.



Figure 4. *Chordodes muelleri*, female. (A) Stereomicroscope, posterior end with terminal cloacal opening (arrow); (B) body cuticle with simple (1) and tubercle areoles (2); (C) clusters containing crowned (3) and circumcluster (4) areoles; (D) cluster of crowned areoles with long filaments (5) along the ventral midline. Scale bars:  $16.6 \,\mu m$  (A);  $10 \,\mu m$  (B, C).

#### Host

Unknown.

#### Description

Body length is 223 mm, diameter in the middle region is 0.9 mm. Anterior end with white cap but a following dark collar is absent. The posterior end is rounded; the cloacal opening is terminal (Figure 4A). The body colour is light brown. The dorsal and ventral midline is darkly pigmented.

The cuticle contains five different types of areole. The most abundant are simple areoles, these occur in a variety of shapes: oval, rounded, or horseshoe-shaped. They are  $5.2 \,\mu m$  high. The surface of these areoles is rough or with fine lines and small bristles on top (Figure 4B). Simple areoles have lateral cuticular projections which continue in the interareolar groove and connect the areoles to one another. Among these areoles there are scattered tubercle areoles (Figure 4B). The tubercle originates in a central depression on top. The crowned areoles with short filaments occur along the whole body (Figure 4C), and on the ventral groove as a second type of crowned areole, with long filaments (about 80–95.3  $\mu$ m) (Figure 4D). The number of filaments per areole varies and the filaments can break leaving only short rudiments. Both types of crowned areoles are densely surrounded by a circle of circumcluster areoles. They are tall (12.6  $\mu$ m), conical with bristles or small

filaments apically. These areoles form clusters of 9–12 areoles that are curved towards the centre (Figure 4C, D).

#### Comments

Sciacchitano (1937) described *C. muelleri* from a specimen which he interpreted as a male. Sciacchitano (1958), when describing the terminal end of the same specimen, placed the cloacal opening ventrally and near the apex of the posterior end of the specimen. According to our studies the holotype corresponds to a female (see Figure 4A). As regards the cuticle, Sciacchitano (1937) described it with two areolar types, while later (Sciacchitano 1958) enlarging this description to three areolar types. Type 1 areoles (Sciacchitano 1937) correspond to our simple areoles, while types 2 and 3 (Sciacchitano 1958) correspond to our crowned and circumcluster areoles. By SEM we could describe in detail each one of the areolar types mentioned and we could add tubercle areoles and crowned areoles with long filaments as new types.

> Chordodes ruandensis Sciacchitano, 1937 (Figure 5)

Chordodes ruandensis Sciacchitano 1937, p 141, Figure 1. Holotype:  $\mathcal{Q}$  (AMT 1427).



Figure 5. *Chordodes ruandensis*, female. (A) Stereomicroscope, posterior end with terminal cloacal opening (arrow); (B, C) SEM: (B) cuticle of the lateral side of the body with simple areoles; (C) cuticle with crowned (2) and circumcluster (3) areoles. Scale bars:  $16.6 \mu m$  (A);  $10 \mu m$  (B, C).

#### Type locality

Republic of Rwanda, Gabiro. Coll. Verhulst, 1934 (Sciacchitano 1937).

#### Material examined

Holotype, SEM from midbody; LM from posterior end.

#### Host

Unknown.

#### Description

The body length is 348 mm, diameter in the middle region is 1 mm. The body colour is dark brown. Anterior end without white cap. The posterior end is round with a terminal cloacal opening (Figure 5A).

The cuticle contains three types of areole distributed all over the cuticle. There are numerous simple areoles with varying shape. They are rounded or oval. The surface of these areoles is rough and carries on top small bristles (Figure 5B). Among the simple areoles are clusters of crowned and circumcluster areoles. Crowned areoles carry long filaments (43.7  $\mu$ m) on their apical surface and are surrounded by 16–19 circumcluster areoles. These are conical and with a ring of short bristles at the apex. In the interareolar furrow there are scarce high tubercles (20  $\mu$ m) with rounded apex (Figure 5B).

#### Comments

Sciacchitano (1937) described *C. ruandensis* from a female specimen but later (Sciacchitano 1958) referred to this specimen as a male. Taking into account that the measurements of this specimen, the collection date, and the collector's name correspond, it is assumed that Sciacchitano (1958) is a repetition of Sciacchitano's (1937) report. Our reinvestigation confirms that the holotype of *C. ruandensis* is a female (see Figure 5A). Sciacchitano (1937) described the cuticle of *C. ruandensis* with three areolar types, but the figure given is insufficient to distinguish the types properly from each other. Crowned areoles are not among the areoles described. Sciacchitano (1958) redescribed the cuticle, describing and producing figures of simple areoles and crowned areoles. Among the simple areoles, he distinguished a light and a dark type. From the SEM investigation, we cannot distinguish two types of simple areoles. The crowned areoles are clustered with circumcluster areoles. Therefore, although all three descriptions of *C. ruandensis* (Sciacchitano 1937, 1958; this investigation) count three types of areole, these types only correspond to each other in part.

#### Discussion

The cuticular characteristics shown by *Chordodes gariazzi*, *C. heinzei*, *C. kolensis*, *C. muelleri*, and *C. ruandensis* allow them to be considered as valid species. *Chordodes gariazzi*, *C. heinzei*, and *C. muelleri* show five areole types, *C. kolensis* shows a sexual dimorphism as males have six types of areole and females have seven areole types, and *C. ruandensis* shows three different types of areole. The females of *C. kolensis* and *C. muelleri* show two

types of crowned areole (as types 5 and 7 of *C. kolensis* and as types 3 and 5 of *C. muelleri*), crowned areoles with short filaments distributed over the whole cuticle and crowned areoles with long filaments that only occur on each side along the ventral midline. The polymorphism of crowned areoles limited to the dorsal or dorsal and ventral groove in females has been noted before for South American species of *Chordodes* (Camerano 1897; Carvalho 1946; Carvalho and Feio 1950; De Villalobos and Zanca 2001; De Villalobos et al. 2004a, 2004b; De Villalobos and Zanca 2005) and in two females of *C. furnessi* Montgomery, 1898 from India (Schmidt-Rhaesa and Yadav 2004). Nevertheless, Schmidt-Rhaesa (2002) observed polymorphism of crowned areoles in both male and female specimens of *C. queenslandi* Schmidt-Rhaesa, 2002 from Australia. *Chordodes gariazzi*, *C. heinzei*, *C. kolensis*, *C. muelleri*, and *C. ruandensis* have crowned areoles which occur in clusters. These clusters are formed by crowned areoles that are surrounded by another tall areolar type. These clusters have been described for a number of species of *Chordodes* and may indicate a monophyletic subtaxon of *Chordodes* (Schmidt-Rhaesa 2002).

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