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Chordodes colchis (Nematomorpha, Gordiida), a new species from Georgia

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Abstract

A new species, *Chordodes colchis*, is described from several locations in Georgia. The species resembles some other *Chorodes* species, especially *C. anthophorus*, which has also been described from Georgia, but differs in conspicuous details. The new species possesses prominent thorn areoles, which were not described in *C. anthophorus*. The new species is the seventh species known from Georgia.

Key Words

Nematomorpha
Gordiida
Chordodes
new species
Georgia

Introduction

Horsehair worms (Nematomorpha) are parasites of arthropods, the majority of them (approximately 360 species in the taxon Gordiida) parasitizes terrestrial insects. Worms develop in their host, alter its behavior and drive them towards water, where they emerge (Hanelt et al. 2005). Mating, egg deposition and hatching of larvae take place in water. Aquatic invertebrates serve as paratenic host for larvae, but the exact pathways of transmission to the terrestrial final host are still not completely clear (Hanelt et al. 2005).

To date about 360 species are known and new species are described constantly. Scanning Electron Microscopy (SEM) has become the standard method to document the fine cuticular structures on the gordiid surface or on the posterior end, particular in males. This is very important in the genus *Chordodes*, which includes about 100 species, which mainly occur in tropical or subtropical regions. Compared to nematomorphs from other genera, the cuticle of *Chordodes* specimens is very rich in substructures and these are important for species determination (Schmidt-Rhaesa et al. 2008). In most cases it is not clear, how variable the cuticular characters are, but the first applications of DNA barcoding show that the morphological differences between species may be fine (Bolek et al. 2010, Chiu et al. 2011).

From Georgia, seven species of Gordiida, with additional undetermined records, have been described (Gorgadze and Kintsurashvili 2002, Gorgadze et al. 2008, 2012, Kintsurashvili and Schmidt-Rhaesa 2014a, b, Kintsurashvili et al. 2011). Species belong to the genera *Gordius*, *Gordionus*, *Chordodes* and *Spinochordodes*. From the genus *Chordodes*, two species have been described. *Chordodes oscillatus*, which was described by Kirjanova (1953), was later synonymized with *C. anthophorus* (De Villalobos et al. 2007). Kintsurashvili et al. (2011) described *C. parabipilus*, the second species. We report here specimens from several locations, which are close to *C. anthophorus*, but differ from these in some respect and are therefore described as a new species.

Material and methods

Specimens were collected in Tbilisi and several regions in western Georgia, namely Imereti and Adjara regions. Materials were collected in 2002 and 2013-2015, during September and October. Horsehair worms were collected from artificial water reservoirs (trout farm) and from insect hosts. All our specimens, which were collected from insect hosts, were from praying mantis, *Mantis religiosa*, a common species of *Mantis* in Georgia. We also collected another species of *Mantis* (*Mantis* sp.), however they have not been infested with horsehair worms.

Twentynine specimens of horsehair worms were collected in total. Two specimens were collected in Tbilisi, 12 in Imereti (village Kldeti and Mirontsminda), and 15 specimens in Adjara (village Charnali) (Fig. 1). From these, 16 were females and 13 males. It must be noted, that one of the praying mantis (in Imereti) "gave birth" to ten horsehair worms (5 males and 5 females), and another praying mantis (in Adjara) "gave birth" to seven specimens of horsehair worms (2 males and 5 female).

Specimens were conserved in 70% ethanol. Entire pieces from the midbody region (approximately 1 mm long) and in males additionally the posterior end (about 1–2 mm from the terminal tip), were prepared for Scanning Electron Microscopy (SEM). Pieces were dehydrated in an increasing ethanol series, critically point dried and coated with gold in a sputter coater. Observation took place using a LEO SEM 1524 at 10 kV. Digital images were taken.

Results

***Chordodes colchis* sp. n.**

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Holotype. Male from Imereti, deposited in the collection of Institute of Zoology, Ilia University, Tbilisi, Georgia under accession number 114.

Material investigated. Holotype, nine female paratypes (accession numbers 100, 113, 115-117, 119-120, 126-127) and five male paratypes (accession numbers 95, 121, 123-125), all deposited in the collection of Institute of Zoology, Ilia University, Tbilisi, Georgia; 14 specimens (7 females and 7 males) deposited in the collection of the Zoological Museum, Hamburg, Germany under accession numbers ZMH V13418-V13431.

Type locality. Imereti: village Kldeti, village Mirontsminda; Adjara: village Charnali; Tbilisi.

Etymology. The name *colchis* is an association with ancient kingdom Colchis, which includes modern West Georgia, where most of the specimens of the species were found.

Description of the male. The body length is 100–210 mm, the width is 1–1.9 mm (n=13). The body looks black with naked eye, however when observed under

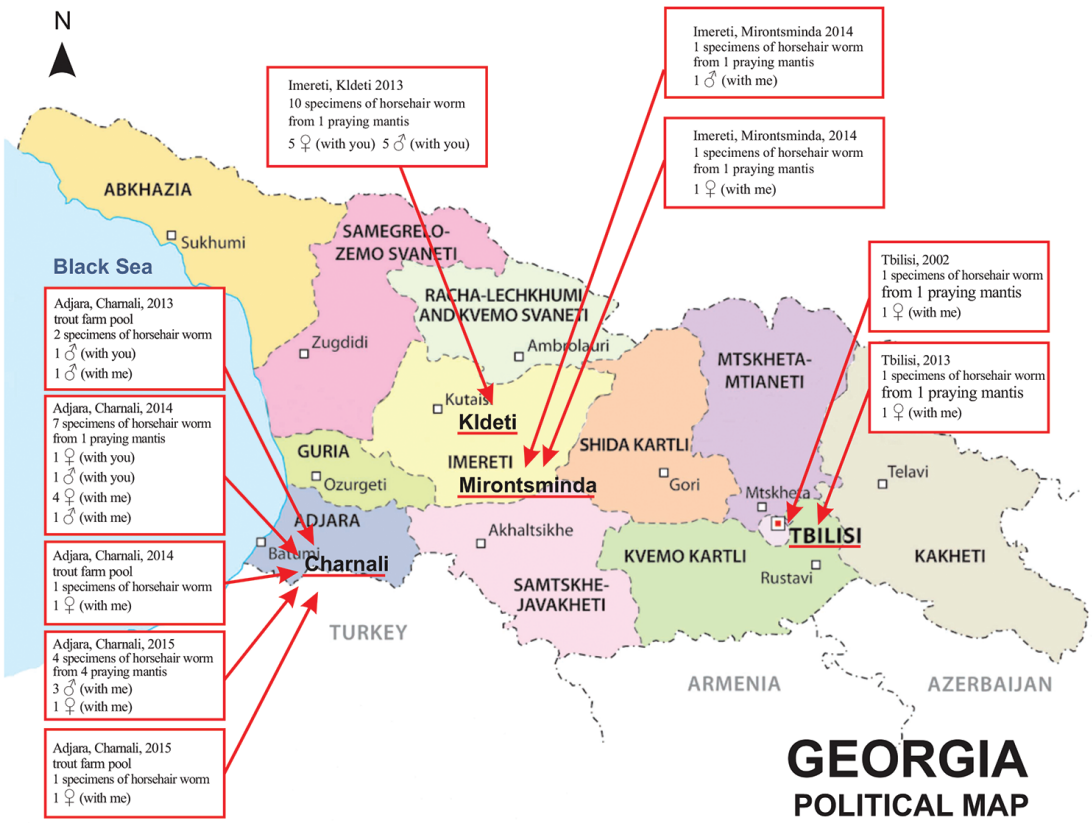

Figure 1. Distribution of *Chordodes colchis* in Georgia.

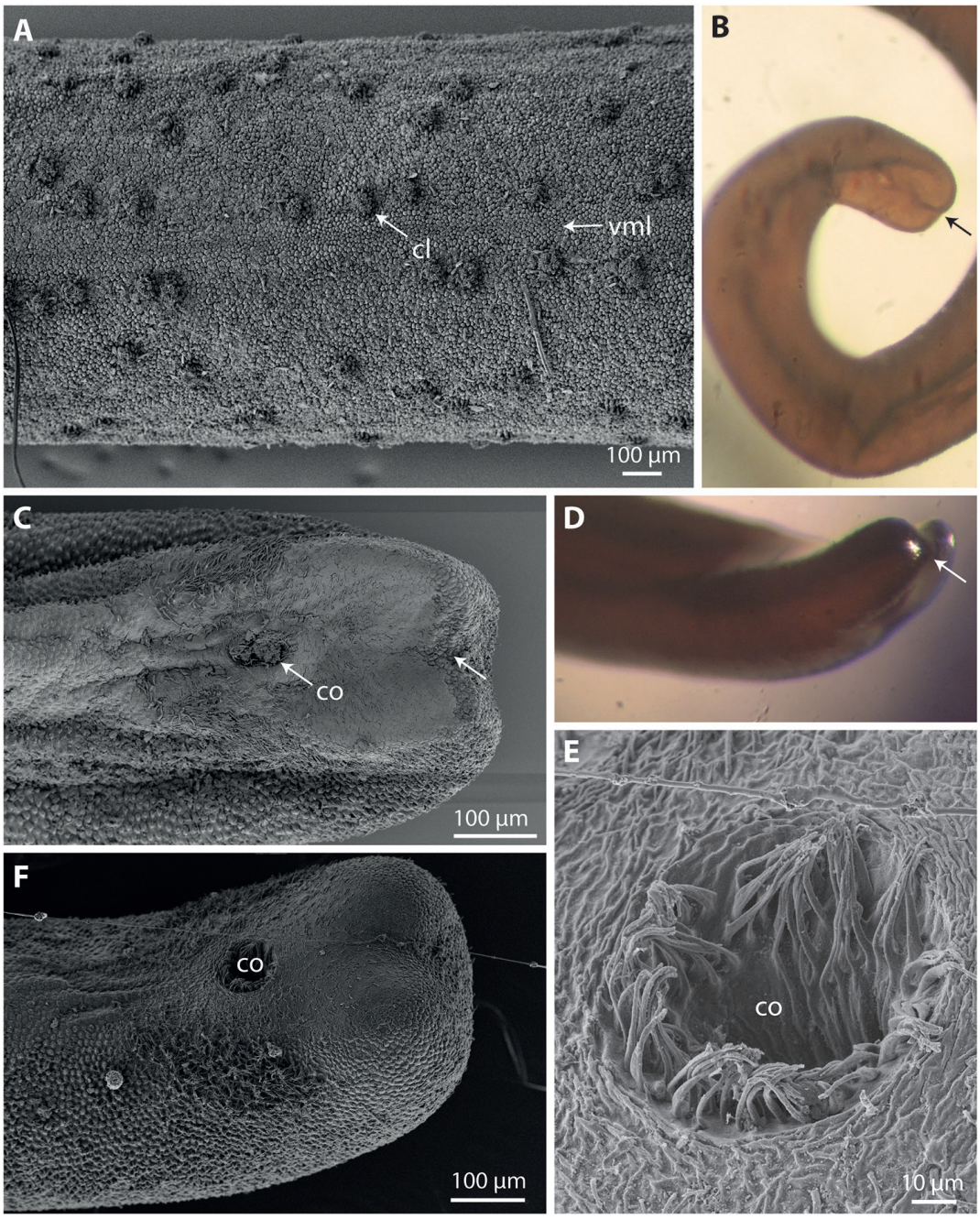

Figure 2. *Chordodes colchis*, male. **A.** Cuticle of the ventral side (vml = ventral midline) with the clusters of crowned areoles (cl). **B–D, F.** Posterior end with median longitudinal furrow (arrows in B–D) different degrees of partially divided posterior end. **E.** Cloacal opening (co) surrounded by branched circumcloacal bristles. A,C,E,F by SEM, B and D taken with Nicon Coolpix P 7700.

the microscope, it is very dark brown. The apical part of the body is white and gradually changes into darker color towards the end of the body. There are numerous dark structures on the body surface, these are the clusters of crowned areoles (Fig. 2A). The shape of the posterior end varies to some degree. In some specimens, when observed from the ventral side, the posterior end has a median longitudinal furrow and therefore both sides look partially divided (Fig. 2B–D). In other specimens, the posterior end is less strongly divided (Fig. 2F). The cloaca is located 300 µm anterior from the apex (Fig. 2C, F). Short bristles, lying flat on the ventral surface, are present posterior of the cloacal opening while longer bristles are present lateral of the cloacal opening (Fig. 2C). The cloacal opening itself is surrounded by bristles which originate inside the opening, are attached to the lateral wall of the cloacal duct and then stand free around the opeing (Fig. 2E). Bristles are flat and wide at their base and then divide into 4, 5, 6 or more branches (Fig. 2E).

There are five types of areoles in the cuticle. Simple areoles are spread on entire surface of horsehair worm body. They are numerous. These are cylindrical struc-

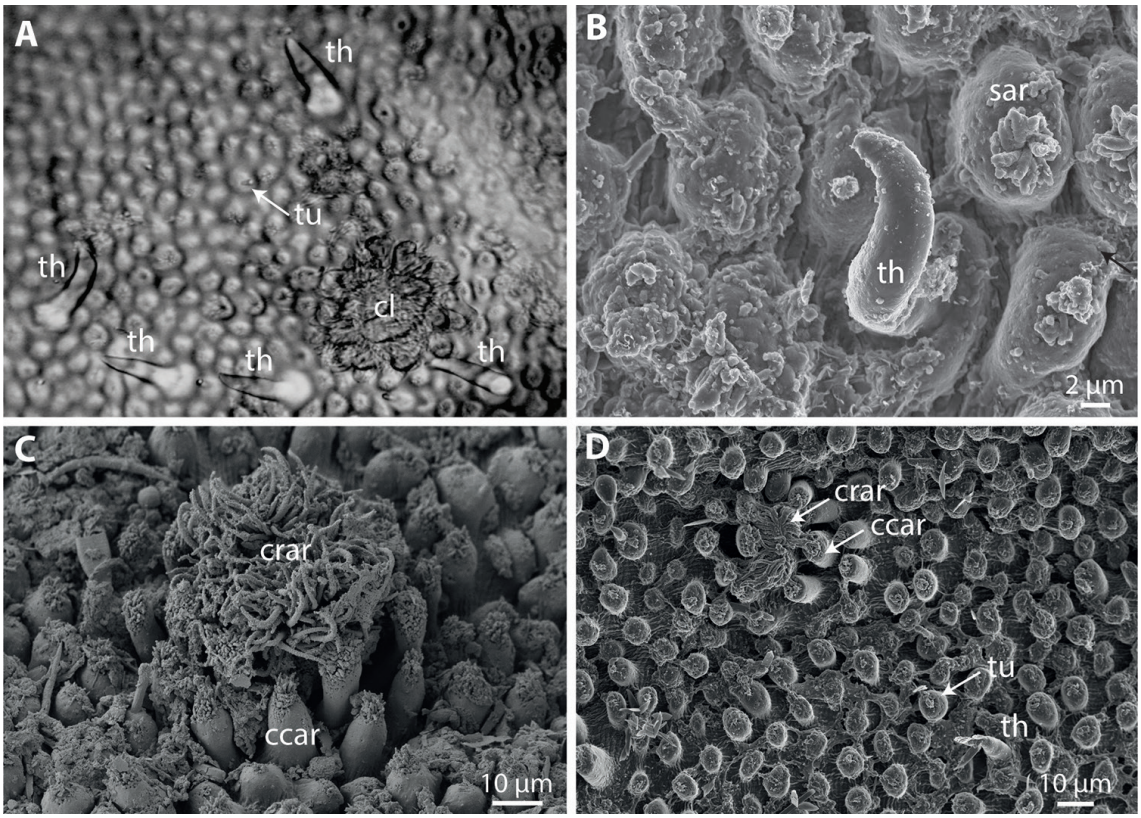

Figure 3. *Chordodes colchis*, male, cuticular structure. **A.** Cuticle of the ventral surface with simple, tubercle (tu), thorn (th) and crowned (cl) areoles. **B.** Magnification of twisted thorn surrounded by simple areoles (sar) and tubercle areoles. **C.** Cluster of crowned areoles (crar) with short filaments, surrounded by circumcluster areoles (ccar). **D.** Overview on the cuticle with all areolar types (for abbreviations see A–C). A by light microscopy (no scale taken), B–D by SEM.

tures, whose apical surface is spherical, oval or polygonal. Simple areoles measure 10-18 µm. Some simple areoles have small bristles on the apical surface (1–2,5 µm) (Fig. 3A, B, D). The cuticle between areoles is structured into cords (Fig. 3 B). Tubercle areoles are located between simple areoles (Fig. 3A, D). Tubercle areoles have finger-shaped apical projections (~10–20 µm). In some areoles these appendices are very small and difficult to measure. They look like big light-refracting dots. Thorn areoles are well distinguished from the rest by their large size and location (Fig. 3A, B, D). They are located on the ventral surface of the body. Areoles have two parts: a spherical basal portion, with the diameter of 20–25 µm at its widest point and 15 µm at its narrower part. Each thorn is raised from the basal structure which resembles an areole. The size of thorns varies from 12.5 to 20–25 µm. The size of the entire thorn areoles varies distinctly from 15 µm to 42.5 µm. The shapes of thorns are also different: some are straight, some are slightly bent, and some are curved (Fig. 3B). Often, thorns are detached from the basal portion, which can be observed under the light microscope. In this case, the basal part of the areoles is well distinguished from simple areoles by its large size, and spherical light refracting structure on the top. The location of thorn areoles is especially interesting. They are situated on the ventral surface on both sides of the midline of the body, almost opposite to each other (Fig. 3A).

Scattered over the entire surface of the body are clusters of areoles, which are easily distinguished from the rest of the areoles (Figs 2A, 3A, C, D). There are two (rarely, one, relatively large) crowned areoles in the center, ~ 7–10 µm apart. There is one very small light refracting thorn between them. The surface of the crowned areoles is spherical or oval. The largest diameter of each areole is ~ 20–25 µm, the smallest diameter is ~ 15–17 µm. Crowned areoles have a cylindrical shape, which is raised from the surface of the body. There is a crown of filaments on top of the surface. Filaments are numerous and vary in size. The longest filaments are about 15–17 µm. The rest of the filaments are smaller, twisted and look like a bush (Fig. 3C). Crowned areoles are surrounded by two rows of circumcluster areoles. There are about 6-7 areoles in the inner row immediately surrounding the crowned areoles. The outer row comprises 12–13 areoles. However, sometimes circumcluster areoles are arranged around crowned areoles in one row. In those cases the number of circumcluster areoles is 20-21. The size of circumcluster areoles is much smaller than the crowned areoles. Circumcluster areoles look like simple areoles, but they are more projected from the surface of the body and have a dark color. They are

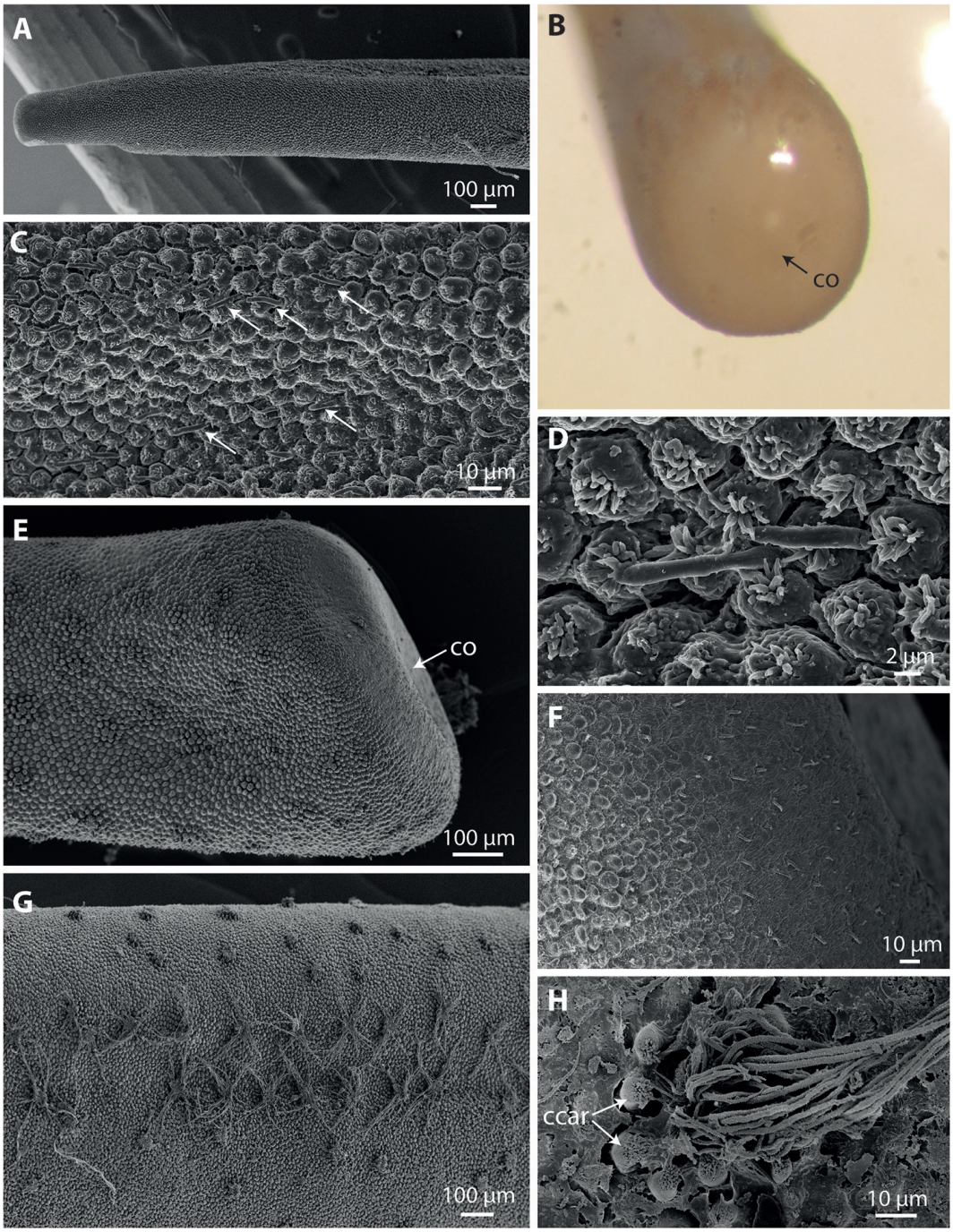

Figure 4. *Chordodes colchis*, female. **A**. Anterior end. **B.** Posterior end with central cloacal opening (co). **C, D**. Thin tubercles (arrows point at some of them) that appear to originate between areoles in the anterior end of the body. **E**. Lateral view on the posterior end with position of the cloacal opening (co). **F.** Some bristles behind the region with areoles in the posterior end. **G.** Clusters of crowned areoles with long filaments along the ventral midline. **H**. Magnification of a cluster ofcrowned areoles with long filaments and circumcluster areoles (ccar). A, C–H with SEM, B taken with Nicon Coolpix P 7700.

curved in the direction of crowned areoles. Circumcluster areoles have very thin, short bristles on the apical surface (Fig. 3C).

Description of the female: The body length is 140–340 mm, the width at the midpoint is 2-2.5 mm (n=16). The body is tapering towards the anterior end (Fig. 4A). The posterior end is spherical, with a cloaca in the center (Fig. 4B, E). The cuticle around the cloaca is smooth. Color of the body is light brown throughout the length of the body with the exception of the apical end, where ~1.5-2 mm of the body is white, gradually changing into brown. Also, the color of the posterior end is whitish.

There are six types of areoles in the cuticle. The cuticle consists of many small simple areoles. They are round

or oval shaped. Simple areoles have short, fine bristles, similar to males. The cuticle between the areoles is structured into cords. There are relatively large areoles with small finger-shaped projections situated among simple areoles. These are tubercle areoles, which are also found in males. However, in contrast of male specimens, there are a number of thin tubercles that appear to originate between the areoles in the anterior end of most specimens (Fig. 4C, D). In some specimens they are clearly oriented towards the anterior with their tip, in others the orientation is more irregular. In addition, female specimens have some bristles behind the region with areoles in the posterior end (Fig. 4E, F).

There are clusters of crowned areoles with short filaments on the surface of entire body. In contrast of males, females have clusters of crowned areoles with very long and relatively stout apical filaments on the dorsal and ventral central line (Fig. 4G, H). The structure of circumcluster areoles is identical to those of males (Fig. 4G). Thorn areoles, as described in males, are also present in females. Their structure and location is similar in both sexes. However, in females, the number of thorn areoles is lower. In addition, they are difficult to observe due to their location under the long apical filaments of crowned areoles.

Discussion

The specimens of *Chordodes* described here possess the "usual" complement of cuticular structures as found in many other *Chordodes* species. Simple areoles, tubercle and thorn areoles as well as clusters composed of crowned and circumcluster areoles are common features. Also the presence of very long filaments in crowned areoles on the ventral and sometimes also on the dorsal side is a common feature in females and very rare in males. Nevertheless, the specimens show some characters and character combinations that are distinct from other species described so far. The two other Georgian species known to date are *C. anthophorus* Kirjanova, 1950 and *C. parabipilus* Kintsurashvili, Schmidt-Rhaesa & Gorgadze, 2011. The latter species has apical filaments of the crowned areoles in two kinds of different thickness (Kintsurashvili et al. 2011), a character not present in the specimens described here. *Chordodes anthophorus* has been described from Tajikistan (Kirjanova 1950), later the species *C. oscillates* from Georgia (Kirjanova 1953), *C. aquaeductus* from Tajikistan (Kirjanova 1950) and *C. ferganensis* from Uzbekistan (Kirjanova and Spiridonov 1989) have been synonymized with *C. anthophorus* as result of an SEM reinvestigation of these species (De Villalobos et al. 2007). Thorn areoles were not described from *C. anthophorus*, which is the most significant difference between this species and the specimens from this investigation. By using the key in Schmidt-Rhaesa et al. (2008) for determination of *Chordodes* species the most close hit when using the characters of the new species is *Chordodes albibarbatus* Montgomery, 1898. This is a species from Gabon, but it differs in two aspects from the specimens from this investigation. First, circumcluster areoles occur in very high number in *C. albibarbatus* (20-30 per cluster) and thorn areoles are very rare. Due to these differences to the known species we here describe *C. colchis* as a new species.

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