

A redescription and ultrastructural study of *Diploscapter coronatus* (Cobb, 1893) Cobb, 1913 from Ethiopia and Iran

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Summary. Populations of *Diploscapter coronatus* (Cobb, 1893) Cobb, 1913 are described from Ethiopia and Iran. Scanning electron microscope pictures of this species and genus are provided for the first time. The lip region of females bears six labial papillae and lacks cephalic papillae. Both populations have very prominent annulation between the anus and mid-tail, a character not mentioned previously in descriptions of *Diploscapter* species.

Key words: *Diploscapter coronatus*, taxonomy, morphology, free-living nematodes, SEM, tropics.

Soil samples collected in Ethiopia from the rhizosphere of *Ensete ventricosum* (Welw.) Cheesman and in North Iran from the rhizosphere of *Musa paradisiaca* L. both contained very similar specimens of *Diploscapter* species. We identified these as *Diploscapter coronatus* (Cobb, 1893) Cobb, 1913. Culturing of the Ethiopian population gave us the opportunity to study the species under the light (LM) and scanning electron microscope (SEM). In this paper, we therefore present a detailed redescription of *Diploscapter coronatus*.

The genus *Diploscapter* is a cosmopolitan genus with most of its species reported from the southern hemisphere. *D. coronatus* is the most widely reported species (Andrássy, 1983).

MATERIAL AND METHODS

The soil sample from Ethiopia was collected by Ato Zewdie Abera in January 1995 from the rhizosphere of *Ensete ventricosum*, Wolaita Sodo, southern-central Ethiopia. The altitude of this site is approximately 1900–2100 m above sea level. The soil is red (Lectosols) and the agro-ecological climate could be identified as mid-land (Woina-Dega according to local classification). The sample from North Iran was collected by the second author from the rhizosphere of *Musa paradisiaca* in August 1995.

The Ethiopian population is kept in continuous

culture, and has been assigned *Caenorhabditis* Genetics Centre strain code PDL0010. It was extracted by the Baermann funnel method and cultured in two systems on agar in 9 cm Petri plates. "Dry plates" consisted of 1% nutrient agar (OXOID 'LabLemco' agar) and cholesterol (final concentration 5 µg/ml). After setting, 0.3 ml was added from a liquid *Escherichia coli* 9001 culture. "Wet plates" consisted of 1% pure agar (OXOID bacteriological agar No.1) and cholesterol (5 µg/ml). After setting, 5 ml autoclaved water buffered with 0.05 M K₂HPO₄/KH₂PO₄ at pH 7.3 was added, together with 5 droplets of liquid *E. coli* 9001 culture from a Pasteur pipette. The plates were left overnight at room temperature before being inoculated with nematodes. At room temperature, dry plates tended to be overgrown more quickly by contaminants, and nematode populations climaxed and collapsed after only two or three weeks. For longterm maintenance of the strain, wet plates kept at 17 °C were more efficient, as they had to be subcultured only once every four to six weeks. For morphological study; nematodes were washed from one wet plate and transferred to a glass vial, which was plunged in a water bath at 85 °C for 30 seconds. The killed and stretched specimens were subsequently fixed in 4% formaldehyde: 1% glycerin at 65 °C and left for one day to allow the fixative to penetrate and act thoroughly. The Iranian population was extracted by centrifugal-flotation and fixed in 4%

formaldehyde: 1% propionic acid.

After fixation, nematodes from both populations were transferred to anhydrous glycerine using Seinhorst's rapid method (Seinhorst, 1959) as modified by De Grisse (1969). Permanent slides were prepared according to Cobb (1918). For SEM, nematodes in permanent slides were first transferred into a small embryo dish filled with a drop of glycerine. Distilled water was added drop by drop until the nematodes were in pure distilled water, and thereafter they were initially dehydrated by passing them through a gradual ethanol concentration gradient. This was followed by an overnight dehydration in 100% ethanol, after which the dried nematodes were coated with gold and examined using a JEOL JSM-840.

DESCRIPTION

Diploscapter coronatus (Cobb, 1893)

Cobb, 1913
(Figs. 1 & 2)

Measurements: see Table 1

Female. Body after fixation almost straight or slightly undulated, tapering towards the posterior end, maximum body width occurring near the vulva. Cuticle annulated along most of the body starting from the base of the lip region until mid-tail; annule width and clarity variable: about 1.0 μm wide and conspicuous just behind lip region, less than 0.5 μm wide and inconspicuous along the rest of the body till anus, then increasing again to about 1.5-2.0 μm wide and very prominent between anus and mid-tail, and finally posterior half of tail quite smooth. Under LM, the lateral field appears to be marked by four longitudinal lines, which resolve under SEM as two separated longitudinal cuticular wings.

Lip region characteristic for the genus, the two subdorsal and two subventral lip pairs being modified to form well sclerotized, hook-like structures (labial hooks); lateral lips membranous, fan-shaped, bordered by 16-17 longitudinal finger-like projections which in some cases are linked to each other by transverse cross-bars to form a net-like structure. SEM observations show that the labial hooks are about 3.5 μm long and 1.5 μm wide in *en face* view, while they are about 1.5 μm high in lateral view (Fig. 2A & C). Observed with LM in lateral view, the lip region is 6.0-8.0 μm wide and equally long. Six labial sensillae seen with SEM, circular-papilloid with a central pore, positioned as: one on the outer lateral side of each lateral lip, and one on each side at the base of each labial hook. Cephalic sensillae appear to be absent. Amphids inconspicuous in most specimens, pore-like under the light microscope, minute lateral slits under SEM, about 0.5 μm wide (Fig. 2C)

and situated at about 2.0 μm behind base of lateral lip. *Fusus amphidialis* inconspicuous.

Mouth opening circular, about 1.7 μm in diameter. Stoma cylindrical (18-22 % of pharynx length for Ethiopian population, 27-32 % of pharynx length for Iranian population), its posterior part surrounded by pharyngeal tissue without glottoid valves or denticles. Pharynx muscular, with distinct procorpus, metacorpus, isthmus and basal bulb. The latter with well sclerotized serrated valve, usually with wide lumen. Nerve ring at three-quarter of neck length. Excretory pore and deirid slightly posterior to nerve ring. SEM pictures showed that the deirids do not open to the outside as a pore (Fig. 2E). Cardia often split at its posterior end, inconspicuous in some specimens. Cells of intestinal wall filled with brown granules, digestive tract posteriorly terminating in a 17.0-22.0 μm long rectum which is usually curved.

Reproductive system didelphic, amphidelphic. Each ovary reflexed near its tip and relatively short. Oocytes arranged in an irregular bunch rather than in single or multiple file. Position of reproductive system with respect to intestine constant, anterior branch to the right and posterior side to the left of the intestine. Vagina straight, extending over one-fourth of corresponding body width. Vulva transverse, a 7.0 μm wide slit with shallow comma-shaped grooves near either end (Fig. 2F), situated at or just posterior to mid-body. Tail straight, about 2-4 rectal lengths long, conoid anteriorly, filiform posteriorly, only supplied with somatic muscle tissue until mid-length, cuticular in the posterior half of its length. Phasmids pore-like, situated at 0.5-0.6 times anal body width posterior to anus.

Male. Not found.

DISCUSSION

In having a visibly annulated cuticle, pharyngeal corpus clearly separated from isthmus, vulva situated around mid-body, and stoma longer than 1.5 times lip region width, both these populations are similar to *D. coronatus* (Cobb, 1893) Cobb, 1913 and *D. pachys* Steiner, 1942.

Diploscapter pachys is one of the most comprehensively described species in the genus. Our populations differ from it in having: tail posteriorly filiform, excretory pore situated immediately anterior to bulbus, higher value for the ratio "a", semi-circular and distinctly crenate lateral lips, and labial hooks with sharp outer sides (*versus* tail terminus not filiform, excretory pore at the level of bulbus, a=12.4-13.7, lateral lips ovate and only faintly crenate, and labial hooks relatively blunt in *D. pachys* according to Steiner (1942).

Table 1. Measurements of females of *Diploscapter coronatus* from Ethiopia and Iran.

Characters	<i>Diploscapter coronatus</i>	
	Ethiopian population (n=20)	Iranian population (n=14)
Length	427.5±25 (395-480)	350.9±23.4 (317-403)
LRW	9.5±1 (8-13)	9.3±0.8 (7.2-10.3)
Stoma length	21.8±0.9 (20-23)	20.9±1.2 (19-23)
Stoma width	3±0.2 (2-3)	2.3±0.1 (2-2.5)
Nerve ring	70±3 (65-77)	66.5±3.1 (60-71)
Pharyngeal length	108.5±5.2 (95-114)	67.5±2.7 (64-73)
Procorpus length	29.5±1.7 (27-35)	22.4±1.5 (20-25)
Metacarpus length	12±1 (11-14)	9.9±0.8 (9-12)
Isthmus length	24±2.2 (22-31)	19.4±1.3 (17-21)
Basal bulb length	20.7±2.6 (15-25)	9.8±8 (9-12)
Excretory pore	79±4.9 (70-89)	75.1±3.9 (67-82)
Deirid	79.5±6 (70-91)	77.7±5.2 (69-88)
Maximum width	24.3±1.8 (21-28)	21.7±1.5 (20-25)
Vaginal length	7.5±1 (5-8)	5.9±0.8 (5-7)
G1 length	60.7±11.3 (39-94)	51.8±7.2 (43-65)
G2 length	55.9±11.1 (38-72)	48.7±9.1 (31-65)
Lateral-field width	4.7±0.7 (4-6)	4.8±0.5 (4-5)
Vulva position	227.5±13.4 (205-255)	188.2±11.7 (168-206)
Anal body W	11.3±1 (10-14)	9.6±0.8 (9-12)
Rectal L	19.6±1.2 (17-22)	11.5±1.6 (8-14)
Tail L	55.5±6.3 (45-71)	51±9 (36-67)
a	17.7±1.2 (15.8-19.8)	16.2±0.4 (15.5-17)
b	4.0±0.2 (3.6-4.1)	4.0±0.2 (3.7-4.4)
c	7.8±0.7 (6.3-9.2)	7±1.1 (5.8-9.6)
c'	4.9±0.7 (4.1-6.5)	5.3±0.8 (3.5-6.1)
V %	53.2±1.5 (50-55.7)	53.7±2.1 (50.1-58.4)
NR %	64.7±3.1 (60.2-72)	75.2±2.8 (71.4-80.9)
Stoma %Ph L	20.1±0.8 (18.4-22.1)	29.1±1.2 (27.2-32.1)
Corpus/I+B	0.9±0 (0.9-1)	1.1±0.1 (1-1.2)

Diploscapter coronatus was originally described but under a different genus name (Cobb, 1893) from the rhizosphere of banana growing in Fiji. Cobb (1913) erected the genus *Diploscapter* to accommodate this species which, he suggested, could actually represent several species. The notion that many species may have been described under this name was also maintained by Steiner (1942). Cobb (1893) had reported the cuticle of this species to be striated but did not indicate whether the tail had a conspicuously wider annulation than the rest part of the body, as found in our populations. However, he described annulation on the anterior end. Also, subsequent reports of the species have never shown or mentioned the conspicuous character of an enlarged tail annulation (Cobb, 1913; Zimmerman, 1898; Kreis, 1929). Nevertheless, the absence of this character remains unproven in the original population, or in populations described subsequently, since original specimens could not be traced despite our enquiries with different collections. Therefore, despite the

unresolved condition of the tail annulation in the original population from Fiji, we identify our populations as *D. coronatus*. This is the first report of the species and genus from Ethiopia and Iran.

On the distribution of sensillae in the genus *Diploscapter*. Steiner (1942) made a notable contribution towards the understanding of the sensillae distribution on the anterior end of the genus. However, our SEM study did not verify his light microscopic observations on *D. pachys*. He reported twelve papillae on the anterior end: one on each lateral lip, one on each of the left and right lateral sides of each labial hook and lateral lip, and one anterior to each amphid (see Fig. 1A-C in Steiner, 1942). Our study shows the presence of six papillae on the anterior end: one on each lateral lip, and one on each side of each labial hook (Fig. 2A-D). Such a disparity in a single genus could be caused by our dealing with a different species, or by error of observation on his part. The latter is possible as

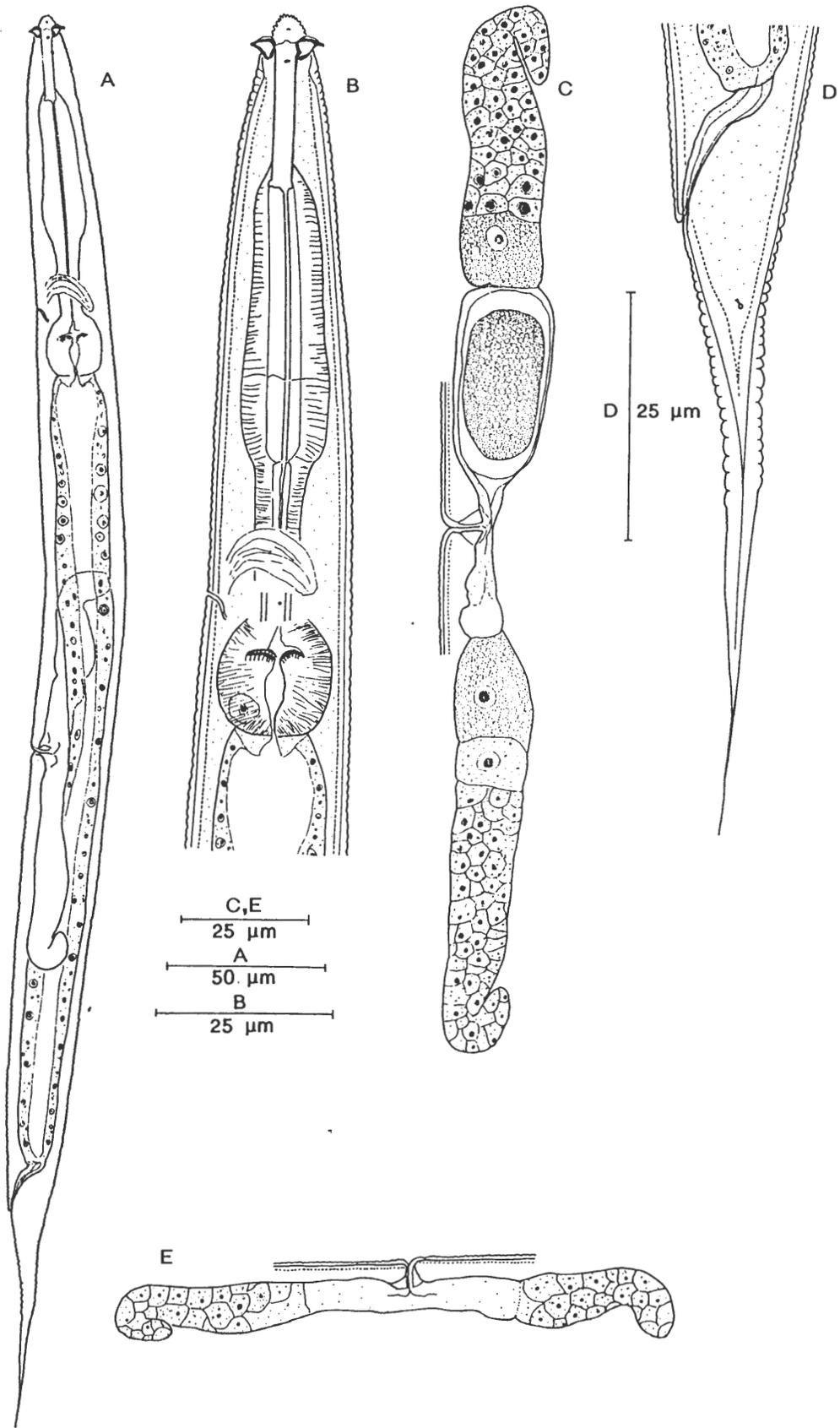


Fig. 1. *Diploscapter coronatus*, females from Iran, A: Entire female; B: Median view of pharyngeal region; C & E: Reproductive system of a mature and young female, respectively; D: Rectal region and tail.

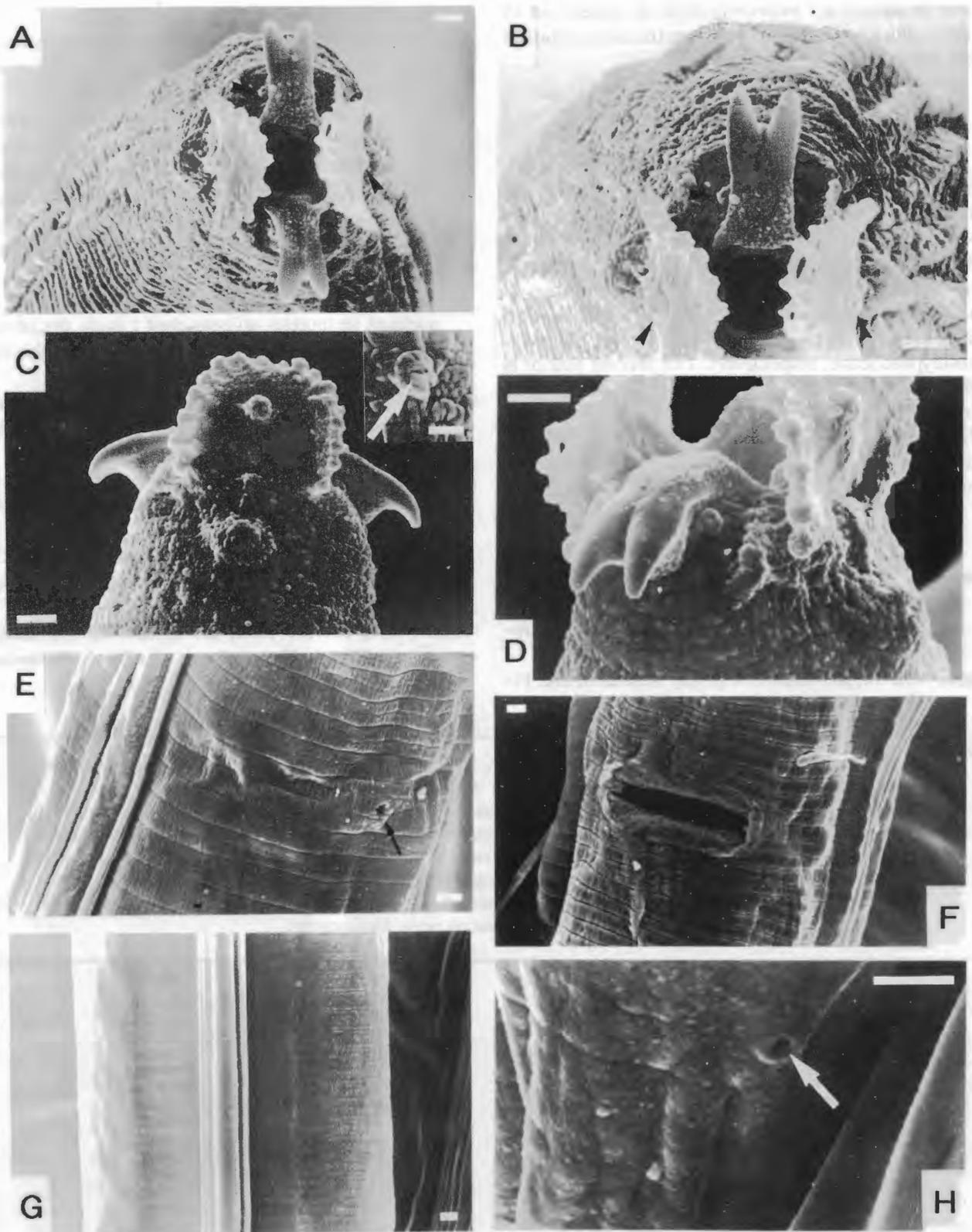


Fig. 2. SEM pictures of *D. coronatus*, females from Ethiopia, A & B: *En face* views (arrowheads indicate papilloid sensillae); C: lateral view of anterior end (arrow on in-set indicates amphidial opening); D: Dorsal view of anterior end; E: Ventral view of pharyngeal region showing excretory pore (arrow); F: Vulval region; G: Lateral view around mid-body showing lateral field and lateral lines; H: Lateral view of mid-tail showing phasmid pore (arrow). Scale bar - 1 μ m.

Steiner (1942) noted: "since in the diploscapters these structures are extremely difficult to see and to study, future work may still change the interpretation here presented".

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Eyualem A., Karegar A., Nabil H., De Ley P. Переописание и данные по ультраструктуре *Diploscapter coronatus* (Cobb, 1893) Cobb, 1913 из Эфиопии и Ирана.

Резюме. Описаны популяции *Diploscapter coronatus* (Cobb, 1893) Cobb, 1913 из Эфиопии и Ирана. Впервые проведено изучение представителей этого вида и рода в сканирующем электронном микроскопе. Головной конец самок несет шесть губных папилл и лишен головных папилл. Представители обеих популяций отличаются хорошо заметной кольчатостью кутикулы между анальным отверстием и средней частью хвостового отдела. Этот признак не был ранее отмечен при описании видов рода *Diploscapter*.
