

TAXONOMY AND NOMENCLATURE

A new species of *Trileptium* (Nematoda: Thoracostomopsidae) from Bahia, Brazil

Ana C. Vilas-Boas^{1*}, Maria C. da Silva², Orane F. de Souza Alves¹,
 Francisco J.V. de Castro² & Eraldo P. Pinheiro-Junior¹

¹Museu de História Natural da Bahia, Universidade Federal da Bahia. Rua Barão de Geremoabo, 40170-290 Salvador, BA, Brazil.

²Centro de Educação e Saúde, Universidade Federal de Campina Grande. Olho d'água da Bica, 58175-000 Cuité, PB, Brazil.

*Corresponding Author E-mail: ananinanina@hotmail.com

ABSTRACT. A new species of a free-living marine nematode found in Brazil is described: *Trileptium ribeirensis* sp. nov. The type material is deposited in the Nematoda collection of the Museu de História Natural da Bahia, located at the Universidade Federal da Bahia. *Trileptium* includes eleven valid species of nematodes that have teeth and jaws positioned forward. The species was collected with the help of corers (3.5 cm in diameter and 30 cm long) on a sandy beach in Bahia. This species is distinguished from its congeners based on a combination of characters of the male (cuticle, buccal cavity, supplement and gubernaculum). An updated taxonomic key to the genus is presented.

KEY WORDS. Nematode, Salvador, sandy beach, Trileptiinae.

Enoplida are aquatic free-living nematodes, most of which occur in marine environments. LORENZEN (1994) considered this order as monophyletic in view of the presence of metanemes. However, molecular analysis did not corroborate the monophyly of Enoplida (SMOL & COOMANS 2006). Enoplida is currently subdivided into seven suborders: Enoplina Chitwood & Chitwood, 1937; Trefusiina Siddiqi, 1983; Oncholaimina De Ley & Blaxter, 2002; Ironina Siddiqi, 1983; Tripyloidina De Coninck, 1965; Campyodorina Jairajpuri, 1983; and Alaimina Clark, 1961. Enoplina comprises only the superfamily Enoploidea Dujardin, 1845, in which Thoracostomopsidae Filipjev, 1927, is placed. LORENZEN (1994) and SMOL & COOMANS (2006) stated that Enoploidea includes the families Enoplidae Dujardin, 1845; Anoplostomatidae Gerlach & Riemann, 1974; Phanodermatidae Filipjev, 1927; and Anticomidae Filipjev, 1918, in addition to Thoracostomopsidae. However, BIK et al. (2010) showed that the group is paraphyletic, based on morphological and molecular analyses, and proposed the inclusion of Leptosomatidae Filipjev, 1916 (Iroñoidea de Man, 1876) in the clade formed by these five families.

Thoracostomopsidae consists of marine and freshwater animals (Enoplolaiminae). These marine species can be found on beaches with intensive hydrodynamics and shallow sublittoral areas, and their abundances vary seasonally (GREENSLADE & NICHOLAS 1991, NICHOLAS 2007). This family forms a monophyletic clade with Enoplidae and Phanodermatidae (BIK et al. 2010).

Thoracostomopsidae consists of three subfamilies: Thoracostomopsinae Filipjev, 1927 (1 genus – *Thoracostomopsis* Ditlevsen, 1918); Trileptiinae Gerlach & Riemann, 1974 (1 genus – *Trileptium* Cobb, 1933); and Enoplolaiminae De Coninck, 1965 (18 genera) (LORENZEN 1981, NICHOLAS 2007, LO RUSSO et al. 2013). The main distinguishing characteristic in this family is the oral cavity, which can be large or small, with or without jaws, with teeth or spear. The presence of cervical setae and the length of the spicules are also diagnostic characters among genera (SMOL & COOMANS 2006).

Trileptium was originally mentioned as *Trilepta* by COBB (1920), when he described the type species, *Trilepta guttata*. COBB (1933) renamed the genus to *Trileptium*. It consists of marine nematodes that have teeth and jaws positioned forward, though it is occasionally difficult to see the jaws (PLAIT & WARWICK 1983). Currently, this genus has 11 valid species, with records on the coast of subtropical and temperate countries. VENEKEY et al. (2010) reported only two species of *Trileptium* in Brazil: *T. stylum* Gerlach, 1956, and *T. subterraneum* (Gerlach, 1952).

MATERIAL AND METHODS

Ribeira beach is located on the Itapagipe Peninsula (12°55'S, 38°30'W), east coast of Todos os Santos Bay (TSB), west coast of Salvador, state of Bahia, Brazilian northeast (Fig. 1). This area has been greatly impacted by human activities, such

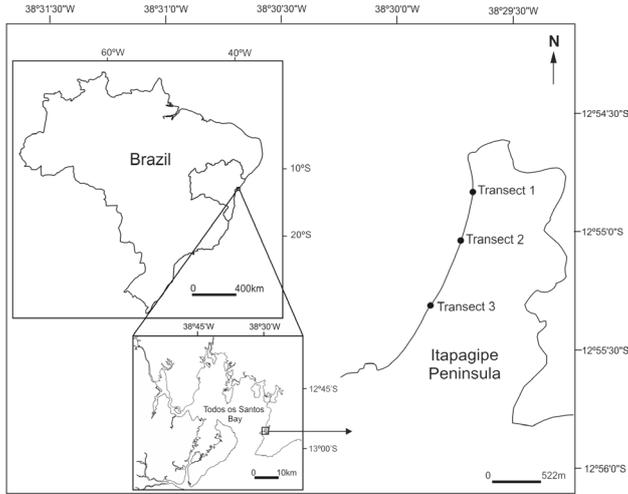


Figure 1. Study area and transects location on Ribeira beach (Bahia, Brazil).

as recreational and construction of habitations and commercial facilities. It consists of a low-energy wave coastline (less than 0.5 m height), dispersed to a distant part of the coast, and with the absence of rip currents (SILVA et al. 2010).

The beach sediment is characterized by quartz grains and fragments of carbonate skeletons of marine organisms', and mostly medium-sized sand grains (VILAS BOAS & BITTENCOURT 1992). The lack of fine fraction is due to turbulence, which conveys the material up to 0.25 mm in diameter. The slope ranges from 5 to 10 degrees, and the width varies from 20 m to 35 m at low tide (SILVA et al. 2010).

Nematode sampling was conducted in February, 2009, during a dry period with low spring tide. Samples were taken using corers of 3.5 cm in diameter and 30 cm long, divided into three strata: 0-10, 10-20, and 20-30 cm related to sediment depth. They were traced in three transects perpendicular to the waterline in the intertidal zone, with three points representing upper, middle and lower regions. Samples were fixed in saline buffered formalin (10%) and rinsed by hand in elutriation sieves with 0.5 mm and 0.045 mm. Nematodes were diaphanized using glycerol-ethanol solutions, according to the DE GRISSE (1969) protocol, and arranged on slides, following the method by COBB (1917).

The description and drawings of the animals were performed under an Olympus optical microscope (CX31), with attached drawing tube. The morphometry was performed using images taken by camera model QImaging Go-3, using the Q Capture software. All measurements are expressed in micrometers (μm). Images were imported into the Image-Pro Insight software for analysis. Photographs of the animals were taken under an Olympus microscope (BX53) with DIC.

Animals are deposited in the Nematode collection of the Museum of Zoology, a unit of the Museu de História Natural da Bahia (MHNBA), located at the Universidade Federal da Bahia. The institution is recognized by the Brazilian Ministry of Environment.

TAXONOMY

Phylum Nematoda Potts, 1932

Order Enoplida Filipjev, 1929

Suborder Enoplina Chitwood & Chitwood, 1937

Enoploidea Dujardin, 1845

Thoracostomopsidae Filipjev, 1927 (adapted from LORENZEN 1994, SMOL & COOMANS 2006, NICHOLAS 2007)

Diagnosis. Presence of three prominent lips. Setiform inner labial setae (papilliform only in *Fenestrolaimus*), whereas the outer labial and cephalic setae are robust and long. Cephalic organs are usually present, situated frontally or ventrofrontally in relation to the lateral cephalic setae exhibiting a variable form. Non-spiral and small amphidial fovea located posterior to the cephalic capsule are most likely absent. The inner layer of the cuticle forms a cephalic capsule, and the pharyngeal muscles are attached to it. Conical buccal cavity is tapered with three jaws and three conical teeth associated with the base of the jaws (one dorsal and two ventrosublateral) or a long eversible spear in Thoracostomopsinae members. Only dorsolateral orthometanemes with a robust scapulus occur but without caudal filament. The endings of the epidermal glands are particularly well differentiated. Female reproductive system is didelphic-amphidelphic, with antiodromously reflexed ovaries. Caudal glands can be found in the pre-caudal region.

Trileptium Cobb, 1933 (adapted from PLATT & WARWICK 1983, NICHOLAS 2007)

Type species. *Trileptium guttatum* (Cobb, 1920).

Diagnosis. Cuticle may be smooth or slightly striated. Three small lips, one dorsal and two subventral. The cephalic setae are arranged in three circles: six inner labial, six outer labial and four cephalic. Males also have a circle with subcephalic setae. The buccal cavity consists of one or three teeth, with similar sizes, well developed and positioned near the opening of the cavity. Subventral teeth may be located at the same level or more anterior to the dorsal tooth. Underdeveloped jaws may or may not be present. The genus has variable shapes and arrangements of spicules and gubernaculums. A pre-cloacal supplement may be present or absent.

Species included. This genus includes the 11 valid species listed below (modified from NICHOLAS 2007):

Trileptium americanum (Keppner, 1987)

Trileptium australis Nicholas, 2007

Trileptium ayum Inglis, 1964

Trileptium guttatum (Cobb, 1920)

Trileptium iacobinum Wieser, 1959

Trileptium longisetosum Inglis, 1966

Trileptium otti Jensen & Gerlach, 1976

Trileptium parisetum Warwick & Platt, 1973

Trileptium salvadoriense Gerlach, 1955

Trileptium stylum Gerlach, 1956

Trileptium subterraneum (Gerlach, 1952)

***Trileptium ribeirensis* sp. nov.**

Figs. 2-17, Table 1

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Diagnosis. Slightly striated cuticle, visible in the anterior region and through the tail. Three lips. Cephalic arrangement in three rings, outer-labial setae greater than cephalic setae. Males with two pairs of sub-cephalic setae. Narrow buccal cavity with three jaws and three isometric teeth, all on the same level and across the transverse mandibular bar. Males with two opposite testis left to the intestine. Simple shaped spicules. Laminar gubernaculum with a dorsal-caudal apophysis. Tubular pre-cloacal supplement. Conical tail tapering at the terminal portion.

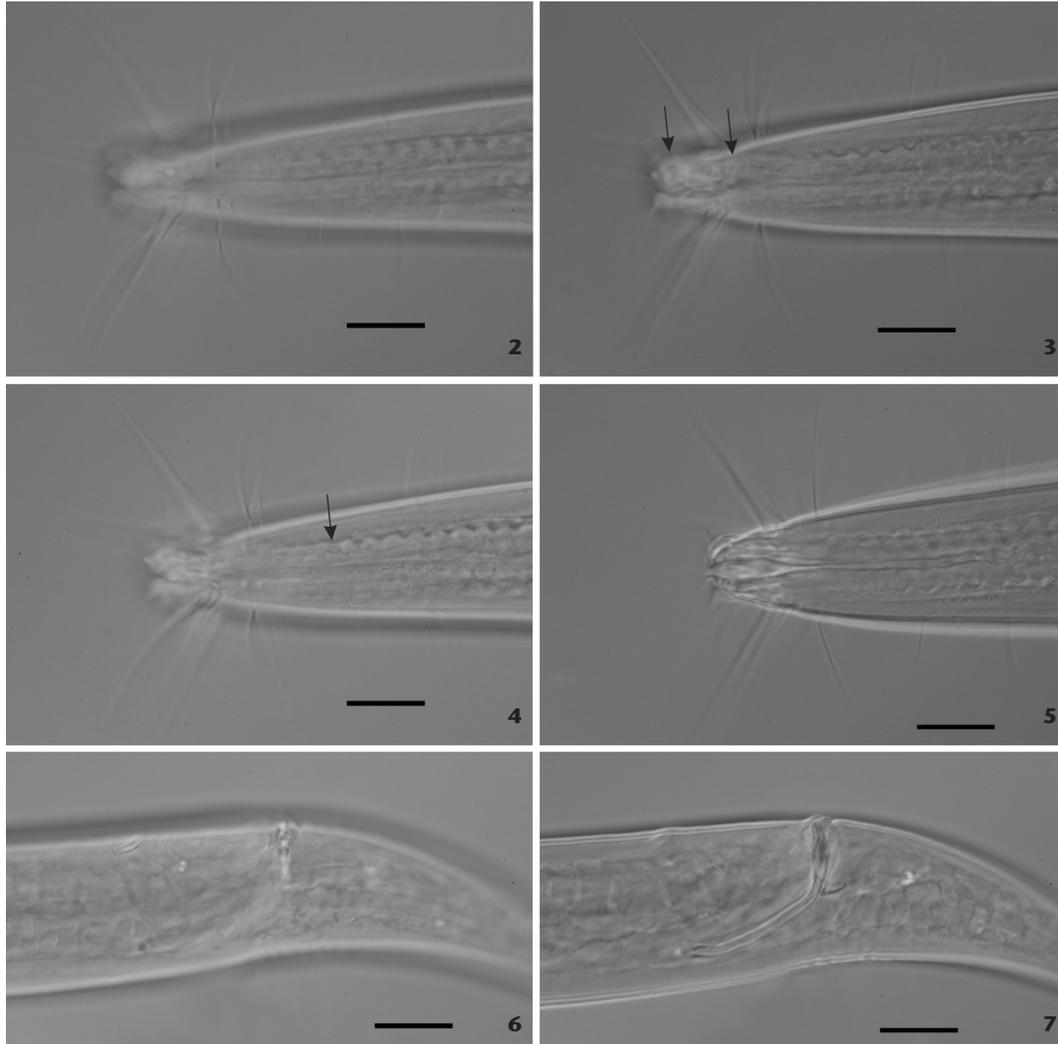
Type material. Holotype (male): Brazil, Bahia: Salvador (Ribeira Beach, 12°55'S, 38°30'W), 10-20 cm depth, collected with a corer, middle region, coarse sand, 45 µm sieve), 11.II.2009, Alves, O.F.S. et al. leg., Museu de História Natural da Bahia, Brazil (UFBA NEM01063). Allotype (female): Brazil, Bahia: Salvador (Ribeira Beach, 12°55'S, 38°30'W, 0-10 cm depth, collected with a corer, middle region, medium sand, 500 µm sieve), 09.II.2009, Alves, O.F.S. et al. leg., Museu de História Natural da Bahia, Brazil (UFBA NEM00371). Paratypes: Brazil, Bahia: Salvador (Ribeira Beach, 12°55'S, 38°30'W, 0-30 cm depth, collected with a corer, middle region, medium sand, 45 and 500 µm sieves), 9 males and 4 females, 11.II.2009; Alves, O.F.S. et al. leg., Museu de História Natural da Bahia, Brazil (UFBA NEM00509, UFBA NEM00963, UFBA NEM01036, UFBA NEM01055, UFBA NEM01166, UFBA NEM01171, UFBA NEM01217, UFBA NEM01224, UFBA NEM01351, UFBA NEM01357, UFBA NEM01358, UFBA NEM01366, UFBA NEM01388).

Description. Holotype: Slender and elongated body, particularly in the extremities (Length: 3713 µm). Slightly striated cuticle, visible in the anterior region, even after the nerve ring, and little less in the cloaca region extending through the tail. Narrow head with a sclerotized cephalic capsule. Presence of three lips. Cephalic arrangement in 6 + 6 + 4: six inner-labial thick and short setae (6 µm) and six outer-labial setae (50 µm) greater than cephalic setae (30 µm). Two pairs of sub-cephalic setae were also observed (31 µm). Somatic setae (13 µm) up to the nerve ring and absent in the rest of the body. Presence of orthometanemes, with one complete filament with 24 µm. Narrow buccal cavity with three jaws (20 µm) and three isometric teeth (13 µm), all on the same level and across the transverse mandibular bar. Pocket-shaped amphidial fovea, near the base of the cephalic capsule, which occupies 17% of the corresponding body diameter. Circular cephalic ring positioned between the inner labial setae circle and the outer labial setae circle (1 µm from the anterior end). Cylindrical pharynx with crenated contour and without a terminal bulb (742 µm). Triangular cardia inserted

Table 1. Males and females measurements (µm) of *Trileptium ribeirensis* sp. nov. (cbd) corresponding body diameter; (V%) position of vulva as a percentage of body length from the anterior end; (a) body length divided by maximum body diameter; (b) body length divided by pharyngeal length; (c) body length divided by tail length; (c') tail length divided by body width at the anus or cloacal opening.

	Holotype male	Paratypes (males = 9)	Allotype female	Paratypes (females = 4)
Body length	3713	2359-4415	4320	3489-4211
Head length	20	20-25	23	21-24
Head width at cephalic ring	17	14-18	15	15-20
Head to cephalic ring distance	1	2-3	2	2-3
Head width at lips	14	12-16	15	14-17
Maximum width at lips	18	16-18	19	19-21
Amphid width	4	4	4	3-5
Amphid cbd	24	20-28	25	23-30
Buccal cavity length	30	26-32	34	29-34
Buccal cavity cbd	19	18-21	21	19-22
Onchium length	13	10-12	12	10-15
Jaw length	20	9-13	16	13-16
Cephalic capsule length	22	19-24	23	21-25
Width at base of cephalic capsule	23	22-25	26	24-27
Inner labial setae length	6	4-7	6	4-6
Outer labial setae length	50	44-51	41	40-45
Cephalic setae length	30	16-28	17	11-15
Longest subcephalic setae length	31	23-31	–	–
Somatic setae length	13	10-14	13	10-13
Head to nerve ring distance	172	154-210	189	160-182
Nerve ring cbd	44	42-53	49	46-54
Pharynx length	742	713-895	782	692-766
Anterior testis length	138	50-143	–	–
Posterior testis length	123	55-118	–	–
Anterior ovary length	–	–	1984	771-1360
Posterior ovary length	–	–	1990	841-1878
Head to cloaca/anus distance	3524	2161-4192	4138	3307-4005
Head to vulva distance	–	–	2700	2296-2710
Tail length	189	177-223	181	178-206
Spicules, arc length	56	55-62	–	–
Spicules, chord length	48	47-56	–	–
Sppineret length	4	3-5	4	4-5
Gubernaculum length	13	9-16	–	–
Apophysis length	6	4-8	–	–
Supplement length	8	6-9	–	–
Supplement to cloaca distance	38	37-48	–	–
Maximum body width	46	44-56	62	53-65
Width at cloaca/anus	38	38-48	46	44-52
Orthometanemes length	24	28-39	36	24-45
V%	–	–	62.5	62.1-66.8
a	81.0	44.5-86.5	69.6	56.8-69.8
b	5.0	2.7-4.9	5.5	4.6-5.5
c	19.6	11.9-21.7	23.8	19.1-20.8
c'	4.9	3.7-5.2	4.0	3.0-4.0

in the intestine. No excretory-secretory system was observed. Two opposite testis (anterior 138 µm, and posterior 123 µm) left to the intestine. Simple shaped spicules lacking *capitulum*



Figures 2-7. Holotype of *Trileptium ribeirensis* sp. nov.: anterior region showing setae (2), cephalic ring and amphid (3), orthometaneme (4) and teeth and jaws (5); posterior region with emphasis on the supplement (6) and the spicules and gubernaculum (7). Scale bars = 20 μ m.

and *velum*, ventrally curved (56 μ m). Laminar gubernaculum (13 μ m) with a dorsal-caudal apophysis (6 μ m). Presence of a sclerotized and tubular pre-cloacal supplement (8 μ m) 38 μ m from the cloaca. Conical tail (189 μ m) tapering at the terminal portion, with irregularly distributed setae (5 to 8 μ m). No caudal glands were observed. Presence of spinneret (4 μ m).

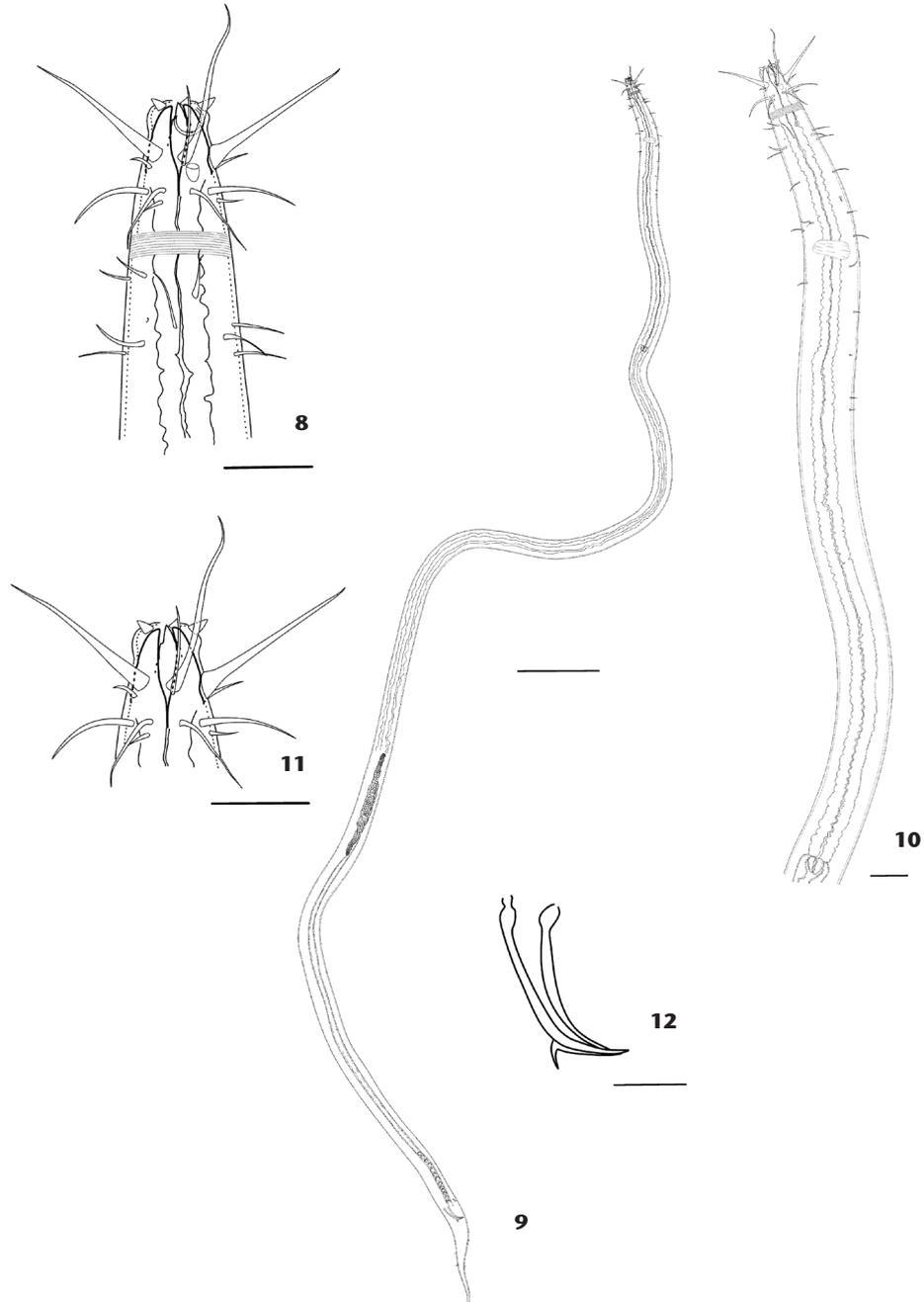
Allotype: Similar to the description of the male, except for the sub cephalic setae, which are present only in males. The sub cephalic setae is a sexual dimorphism in this species. Females had a didelphic reproductive system with opposite reflected ovaries (anterior 1984 μ m, and posterior 1990 μ m) left to the intestine. The vulva is located 2700 μ m from the anterior end, equivalent to 63% of the body length. Only non-gravid females were observed.

Etymology. The species' name honors the site where the animals were collected. This is the first description of a new species of Nematode for Ribeira Beach, Salvador, Bahia, Brazil.

DISCUSSION

The presence of sub-cephalic setae only in males indicates a type of sexual dimorphism. This dimorphism is constant in the genus, being also present in *T. australis*, *T. longisetosum*, *T. otti* and *T. salvadoriense*.

Trileptium ribeirensis sp. nov. was characterized as a new species based on the fact that males have a slightly annulated cuticle, a buccal cavity with three isometric teeth on the same level and across the transverse mandibular bar, a supplement

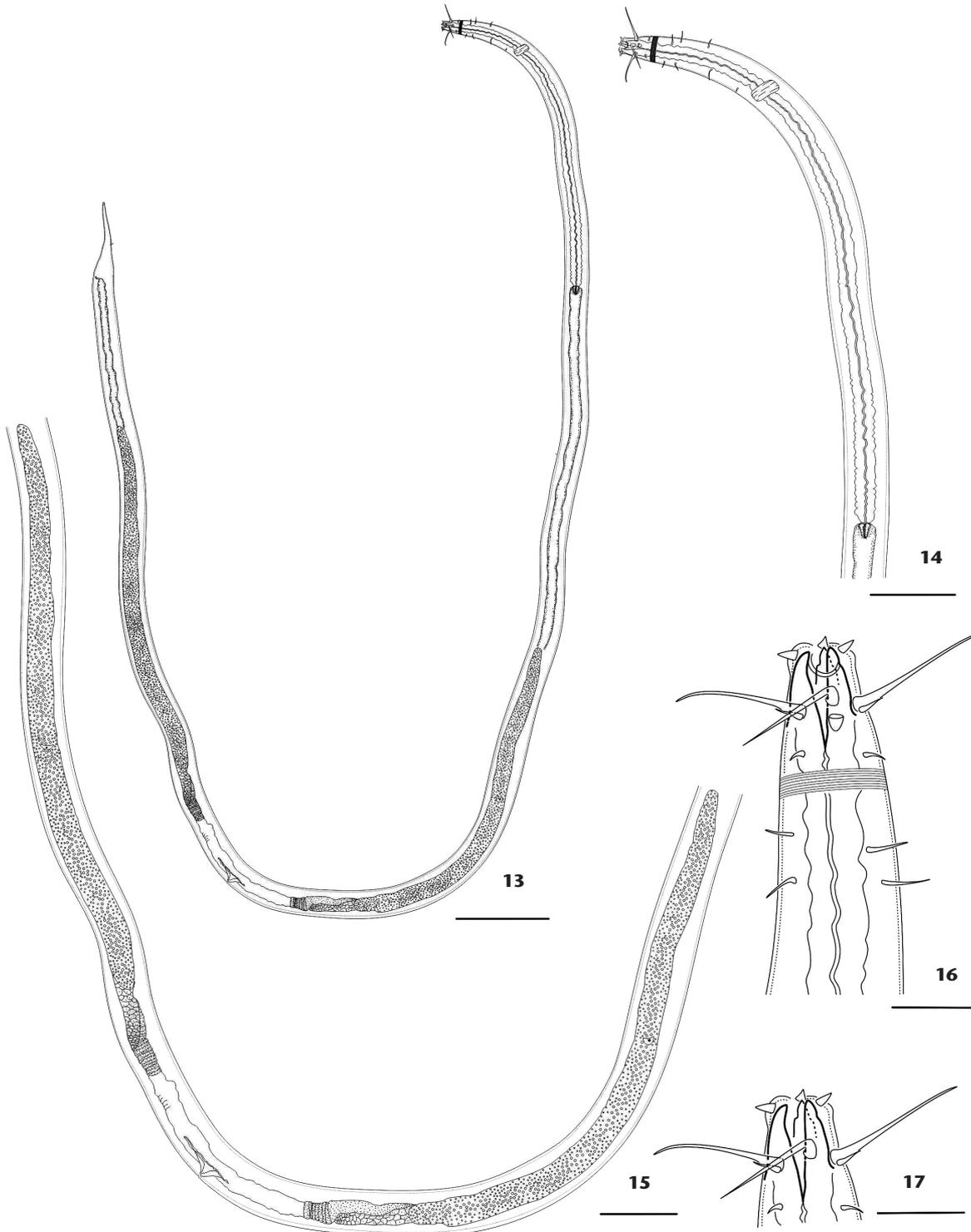


Figures 8-12. Holotype of *Trileptium ribeirensis* sp. nov.: (8) anterior region showing cephalic ring, amphid and cuticle; (9) total body; (10) body region with pharynx, nerve ring, somatic seta and cardia; (11) anterior region emphasizing setae and teeth; (12) spicules and gubernaculum. Scale bars: 8, 10, 11 = 50 μ m, 9 = 200 μ m, 12 = 20 μ m.

and a gubernaculum with dorsal-caudal apophysis that does not loop over spicules (Table 2).

Following GERLACH (1952), *T. subterraneum* has a gubernaculum with dorsal-caudal apophysis, similar to the new species.

However, *T. subterraneum* does not have an annulated cuticle, and its buccal cavity contains a single onchium. Differently, *T. ribeirensis* sp. nov. has a slightly annulated cuticle and three isometric teeth.



Figures 13-17. Allotype of *Trileptium ribeirensis* sp. nov.: (13) total body; (14) anterior region with pharynx, nerve ring, somatic seta and cardia; (15) body region showing the vulva and ovaries; (16-17) anterior region emphasizing the cephalic ring and amphid: (16), teeth and jaws (17). Scale bars: 13 = 200 μ m, 14, 15 = 100 μ m, 16, 17 = 50 μ m.

Table 2. Diagnostic characteristics of *Trileptium ribeirensis* sp. nov. compared with the other species of the genus. (Sup-clo) supplement to cloaca, (NP) none provided.

Species	Cuticle	Buccal Cavity	Supplement	Sup-clo distance	Gubernaculum	Spicules	Tail
<i>T. americanum</i>	Smooth	3 jaws (15-19 µm); 3 isometric teeth, 2 beyond the transverse mandibular bar and 1 anterior	Present	6-11 µm	Complex, bipartite; without apophysis (23-25 µm)	Cephalate, curved, with constriction, striated at near tip, tip a blunt point (32-39 µm)	Conical, tip blunt (95-109 µm)
<i>T. australis</i>	Slightly striated	3 jaws (20 µm); 3 isometric teeth (13 µm), same level and beyond the transverse mandibular bar	Present	26-30 µm	Curved, loops over spicules; without apophysis (24-33 µm)	Strongly curved ventrally distally; thinner and less curved proximally; ventral pegs on mid sections (52-56 µm)	Conical (184-209 µm)
<i>T. ayum</i>	Smooth	3 jaws; 3 small teeth, same level and beyond the transverse mandibular bar	Present (13 µm)	46 µm	Without apophysis (58 µm)	Long and thin; without alae (650 µm)	Stout (213 µm)
<i>T. guttatum</i>	Slightly striated	1 dorsal tooth	Absent	-	Parallel to the spicula	Arculate and or somewhat bent	Conical-cylindrical
<i>T. iacobinum</i>	Slightly striated	Jaws weakly developed; 3 teeth, 2 beyond the transverse mandibular bar and 1 anterior	Present (Reduced to a cuticular disc)	30 µm	Absent	Long and curved (66 µm)	About 92.8 µm
<i>T. longisetosum</i>	Smooth	3 small jaws; 3 small teeth, same level and beyond the transverse mandibular bar	Present (6.5 µm)	38-40 µm	Simple; without apophysis (16-18 µm)	Equal and identical; slightly cephalate proximal ends; bluntly rounded distal tips (47-52 µm)	Long and narrow (200-210 µm)
<i>T. otti</i>	Smooth	3 teeth, 2 beyond the transverse mandibular bar and 1 anterior	Present (12 µm)	29 µm	Complex; distal lateral structures parallel to the spicules, proximally dilated; without apophysis	Equal; proximally cephalated; narrow area in the middle; distal part ventrally curved and dents on the ventral edge (50-54 µm)	About 180 to 190 µm
<i>T. parisetum</i>	Slightly striated	3 jaws; 3 isometric teeth, same level and beyond the transverse mandibular bar	Present (12 µm)	49 µm	2 halves, each with 2 lateral distally projections; without apophysis (14 µm)	Paired, equal, slender and non-cephalate (35 µm)	Anterior half conical and posterior half cylindrical (164 µm)
<i>T. ribeirensis</i> sp. nov.	Slightly striated	3 jaws (20 µm); 3 isometric teeth (13 µm), same level and beyond the transverse mandibular bar	Present (8 µm)	38 µm	Laminar gubernaculum (9-16 µm) with a dorsal-caudal apophysis (4-8 µm)	Simple, ventrally curved (56 µm)	Conical (189 µm)
<i>T. salvadoriense</i>	Slightly striated	3 jaws and 3 teeth	Absent	-	Weakly developed; inconspicuous cuticular rod	Steadily curved into na arch (39-42 µm)	Conical (99-105 µm)
<i>T. stylum</i>	Smooth	3 jaws and 3 teeth	Present	30 µm	2 simple long rod shaped pieces; without apophysis (20 µm)	Proximal end enlarged funnel-shaped; distal end narrow; ends pointed (90 µm)	Narrows in the anterior half and continues cylindrically (95 µm)
<i>T. subterraneum</i>	Smooth	1 dorsal tooth	Present	NP	Complex, 2 small pieces involve spicules tips; with a dorsal-caudal apophysis	Curved in the middle region and near the distal tip (47 µm)	Conical-cylindrical (156 µm)

Trileptium ribeirensis **sp. nov.** differed from *T. guttatum* in the supplement and spinneret, which were absent in the species described by COBB (1920), and the gubernaculum, which, according to NICHOLAS (2007), had no apophysis in *T. guttatum*. Compared with *T. parisetum*, both species have spinnerets and pre-cloacal supplements, but their gubernaculums have only one piece and lack lateral projections at their tip, though they present apophysis. The supplement of *T. ribeirensis* **sp. nov.** is smaller than that described by WARWICK & PLATT (1973), averaging 6 to 9 µm in length, while in *T. parisetum* it has 12 µm.

The main differences between *T. salvadoriense* and *T. ribeirensis* **sp. nov.** were the presence of the supplement and the morphology of the gubernaculums, simple-structured in *T. salvadoriense*. Besides, the spicules of *T. salvadoriense* were slightly smaller than those of the new species, ranging from 39 to 42 µm (GERLACH 1955), while in *T. ribeirensis* **sp. nov.** specimens the shortest spicules measured 55 µm and the longest 62 µm.

When comparing *T. ribeirensis* **sp. nov.** with *T. stylum*, the cuticle of *T. stylum* is smooth. Furthermore, the buccal cavity *T. stylum* is simple, small and the structures are difficult to view (GERLACH 1956). The spicules are large (90 µm length), and the gubernaculum is simple. The buccal cavity of *T. ribeirensis* **sp. nov.** has teeth and jaws. The largest spicules measured 61 µm and the gubernaculum had an apophysis.

Trileptium iacobinum is different from the new species and the congeners in having no gubernaculum. The males have a cuticular plate, just above the anus, which WIESER (1959) believed to be a transformed tubular pre-cloacal supplement.

Unlike *T. ribeirensis* **sp. nov.** and the other species of the genus, *T. americanum* has three similar post-cloacal supplements near the tail tip (KEPPNER 1987). As in *T. ribeirensis* **sp. nov.**, it has three teeth and three jaws, but in different positions, not across the mandibular transverse bar. In the new species, teeth and jaws are at the same level and lie beyond the mandibular bar. Its spicules are smaller (32 to 39 µm) than in the species under study, with a constriction at approximately one third of the distance from the proximal region, and with a striated area on the ventral side of the distal region. In addition, the gubernaculum has no apophysis.

The measurements of the spicules of *T. otti* are similar to those of *T. ribeirensis* **sp. nov.** However, their morphology is more complex, with a median curvature and a serrated distal tip (JENSEN & GERLACH 1976). Furthermore, the shape of the gubernaculum is different, as is the size of the pre-cloacal supplement measuring 12 µm in *T. otti* and ranging from 6 to 9 µm in *T. ribeirensis* **sp. nov.** Another difference is the distance of the supplement to the cloaca, which is 29 µm in *T. otti* and ranges from 37 to 48 µm in the new species.

The spicules of *T. longisetosum* and *T. ribeirensis* **sp. nov.** are similar but in the former species they are slightly smaller (47 µm and 52µm), and the gubernaculum is simpler, without an apophysis, according to INGLIS (1966). *Trileptium ayum*, as described by INGLIS (1964), differs from all *Trileptium* and *T. ribeirensis* **sp.**

nov. species in the length of its slender spicules and gubernacula, 650 µm and 58 µm, respectively. Finally, *T. australis* has peculiar spicules, having median expansions, and the curved gubernaculum contours the spicules (NICHOLAS 2007).

According to the NICHOLAS (2007), the first step in the identification of *Trileptium* is to group them based on type of cuticle (smooth or annulated) and presence of gubernaculum. The cuticle of *T. ribeirensis* **sp. nov.** showed a similar pattern to that found in *T. guttatum*, *T. parisetum* and *T. salvadoriense* (i.e., not completely smooth), and all those species have a gubernaculum. Therefore, we suggested an amendment in step 1 to modify the proposal by NICHOLAS (2007).

Below is a dichotomous key, proposed in this work, modified from NICHOLAS (2007). The main changes were as follows: (1) in step 1, we inserted the variation annulated cuticle absent in the original key; and (2) we included *T. ribeirensis* **sp. nov.** as a species of the genus.

Taxonomic key to male *Trileptium* (modified from NICHOLAS 2007)

1. Cuticle annulated, gubernaculum absent
..... *T. iacobinum* Wieser, 1959
- 1'. Cuticle smooth or annulated, gubernaculum present2
2. Buccal cavity with a single forwardly-directed dorsal onchium.....3
- 2'. Buccal cavity with dorsal and two sub-ventral onchia4
3. Supplement absent, gubernaculum without apophysis.....
..... *T. guttatum* (Cobb, 1920)
- 3'. Supplement present, gubernaculum with a dorso-caudal apophysis *T. subterraneum* (Gerlach, 1952)
4. Supplement absent *T. salvadoriense* Gerlach, 1955
- 4'. Supplement present5
5. Dorsal onchium does not reach the mandibular transverse bar, sub-ventral onchia extend beyond bar.....6
- 5'. Dorsal and subventral onchia at same level, extend beyond mandibular bar7
6. Pre-cloacal supplement close to cloaca (6-11 µm)
..... *T. americanum* (Keppner, 1987)
- 6'. Pre-cloacal supplement not close to cloaca (29 µm)
..... *T. otti* Jensen & Gerlach, 1976
7. Spicules shallow arcs, gubernaculum does not loop over spicules8
- 7'. Spicules strongly curved distally, gubernaculum with ventral peg, loops over both spicules..... *T. australis* Nicholas, 2007
8. Gubernaculum with dorso-caudal apophysis.....
..... *T. ribeirensis* **sp. nov.**
- 8'. Gubernaculum without apophysis9
9. Tail long and thin, 5 times body width at cloaca.....
..... *T. longisetosum* Inglis, 1966

- 9'. Tail 4 times or less than body width at cloaca 10
 10. Gubernacula terminate in small plates each, with two lateral projections *T. parisetum* Warwick & Platt, 1973
 10'. Spicules cylindrical tapering to point, gubernaculum reduced to knob obscuring spicule tip
 *T. stylum* Gerlach, 1956
 10". Gubernaculum without terminal projections; does not obscure spicule tip *T. ayum* Inglis, 1964

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