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Five new species of the family Trischistomatidae (Nematoda: Enoplida) from North and Central America, with keys to the species of *Trischistoma* and *Tripylina*

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Abstract

Three new species of *Trischistoma* and two new species of *Tripylina* (Trischistomatidae) are described. *Trischistoma ripariana* **n. sp.** was collected in the surface organic material and upper rhizosphere soil on a stream bank in Oakville, California, USA. It is characterized by a short, thin body, the vulva at 79–83%, a small index c (17–29) and a short tail, 34–57 µm. *Trischistoma corticulensis* **n. sp.** was found in moss on tree bark in a tropical forest at the La Mancha Ecological Institute, Veracruz State, México. The vulva is at 67–73% and the tail is elongate conoid (51–84 µm). *Trischistoma helicoformis* **n. sp.** was collected on lichen growing on tree bark near the Carretera Interamericana in Costa Rica. It is characterized by its spiral shape after fixation, the tail length (76–101 µm), a very small index c (10–14.5) and very small sclerotised pieces around the vagina. *Tripylina rorkabanarum* **n. sp.** was collected from moss on tree bark in a tropical forest at the La Mancha Ecological Institute. It is characterized by the presence of two cervical setae, the position of the subventral teeth posterior to the dorsal tooth, the absence of sclerotized pieces around the vagina and the distance of the dorsal tooth from the anterior, 10–15 µm. *Tripylina iandrassyi* **n. sp.** was collected from soil around a banana tree at the La Mancha Ecological Institute. It is characterized by the presence of a post-uterine sac, well-developed buccal lips, subventral teeth located posterior to the dorsal tooth, one cervical seta in females and two in males, and by spicules not completely surrounded by a muscular sheath.

Key words: new species, systematics, taxonomy, Trischistomatidae, Tripylidae

Introduction

In recent classifications of the Nematoda, the three subfamilies recognized within the family Tripylidae de Man, 1876, order Enoplida Filipjev, 1929 were Tripylinae de Man, 1876, Trischistomatinae Andrassy, 2007 and Tobriliinae Andrassy, 2007 (Andrassy 2007) The subfamily Tripylinae included the genera *Tripyla* Bastian, 1865, *Tripylina* Brzeski, 1963 and *Tripylella* Brzeski & Winiszewska-Ślipińska, 1993. The Trischistomatinae had only the genus, *Trischistoma* Cobb, 1913, and the Tobriliinae had only one genus, *Tobrilia* Andrassy, 1967.

Phylogenetic analysis of small subunit (SSU) ribosomal DNA sequences distinguished the clade of *Tripylina* plus *Trischistoma* from the monophyletic group represented by species of *Tripyla*. Reconstruction of the evolution of ovary number on the phylogenetic tree indicates that the monovarial condition envolved in the most recent common ancestor of the *Tripylina* plus *Trischistoma* clade (Cid del Prado-Vera *et al.* 2010). Other molecular phylogenies have separated *Trischistoma* from the other genera and suggested that it is closely related to the family Trefusiidae in the order Enoplida (Holterman *et al.* 2006; Holterman & Holovachov 2007; Meldal *et al.* 2007; Zhao & Buckley 2009; van Megen *et al.* 2009), which has affinities in spicule characteristics, intestinal tract and muscle arrangement (O. Holovachov, personal communication).

Based on the morphological and molecular evidence that *Tripyla*, the type genus of the Tripylidae, is not closely related to *Trischistoma* and *Tripylina*, Zhao (2011) elevated the subfamily Trischistomatinae to family rank and transferred *Tripylina* into that family. Consequently, the family Tripylidae includes the genera *Tripyla* and

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Tripylella while the Trischistomatidae includes the genera *Trischistoma* and *Tripylina* (Zhao, 2011; Zhao *et al.* 2012).

Morphologically and anatomically, the number of gonads, the position, shape and size of the stomatal teeth, and the proximity of whorls of labial and cephalic setae are used to distinguish genera in the Tripylidae and Trischistomatidae. In the Tripylidae, *Tripyla* is diovarial, has a striated cuticle, and the outer labial setae and cephalic setae are well separated; *Tripylella* females are diovarial, amphidelphic, and the whorls of outer labial and cephalic setae are very close together so that they appear as a single whorl. In the Trichistomatidae, *Trischistoma* has a single ovary and the outer labial and cephalic setae well separated; *Tripylina* has a single ovary, the outer labial and cephalic whorls of setae are close together (Tsalolikhin 1983; Zullini 2006; Cid del Prado-Vera *et al.* 2010; 2012). Males of Tripylidae have the spicules surrounded by a muscular sheath, which has been used to support the suggestion that the family has greater affinity with the Triplonchida than with the Enoplida (De Ley & Blaxter 2004). However, Andrassy (2007) retained the family in the Enoplida and molecular phylogenetic analysis of SSU sequences strongly supports the relationship with the Enoplida (Zhao & Buckley 2009; Cid del Prado-Vera *et al.* 2010). Spicules in males in the Trischistomatidae are not surrounded by a muscular sheath (Brzeski 1965; Andrássy 1985).

Herein we report morphometric and molecular characteristics in descriptions of new species of *Trischistoma* and *Tripylina*. To avoid confusion with abbreviations of the generic names, we use *T.* for *Trischistoma* and *Tp*. for *Tripylina*.

Material and methods

The specimens described herein were collected in different localitions in the USA, Costa Rica and México by the first and second authors in 2012 and 2013. Nematodes were separated from moss or surface organic material after soaking or gently shaking the substrate in water for up to 1 hour then decanted through 60 (250 µm aperture) and 325 mesh (43 µm aperture) sieves. Nematodes collected on the 325 mesh sieve were washed into glass vials. Nematodes from soil were extracted from 200 cm and processed using a decanting, sieving and centrifugal-flotation method (Jenkins 1964). They were collected on a 500-mesh (25 µm aperture) sieve. Subsamples of the collected suspension were examined under the dissecting microscope for selection of nematodes.

Nematodes of the Trischistomatidae were distinguished on the bases of their morphology, activity and behavior. For each species, at least 10 nematodes were hand-picked and placed in 95% ethanol for molecular analysis. Other specimens were killed by heating (to 40 ºC) in about 7 ml of water in a small vial. An equal volume of 8% formalin was added to the suspension to achieve a final fixative concentration of 4% formalin; the vial was stored at room temperature for 10 days. Then 50% of the fixative was carefully removed from the surface by pipette without disturbing the nematodes. The vial was agitated and the sample poured into a labelled Petri dish that was loosely covered and placed in a small desiccator over 95% ethanol and incubated at 40 ºC. After 3 days, when the odor of formalin was no longer detectable, the volume of liquid in the dish was reduced to half, without disturbing the nematodes, by removing liquid with a pipette while observing the sample with a stereomicrocope.

Samples were processed to glycerin using a modification of the Seinhorst (1959) method as described by Cid del Prado-Vera & Subbotin (2012) and mounted on glass slides using the paraffin wax ring method (De Maeseneer & d´Herde 1963). Measurements and drawings were made using a drawing tube mounted on an American Optical compound microscope.

For scanning electron microscopy, specimens were immersed in a pH 7.2 phosphate buffer for 15 minutes and dehydrated in a graded ethanol series (10 to 100%) for 15 minutes at each concentration. The specimens were critical-point dried and coated with gold-palladium before observation under the scanning electron microscope (Jeol JSM-6390) at 10 kv.

Nucleic acids were prepared from individual nematodes using either the Chelex protocol (Cid del Prado-Vera *et al.* 2012) or the PrepGEM extraction kit (ZyGem) in a total volume of 25 mL. For both nucleic acid preparation methods, 2–4 mL of nucleic acid extract was used to amplify and sequence a region of the SSU ribosomal DNA (3'-end) as described in Cid del Prado-Vera *et al.* (2010).

A SSU dataset was prepared using sequences from the new taxa, species analyzed in previous investigations (Cid del Prado-Vera *et al.* 2010; 2012) and additional genera (e.g., *Paratrichodorus, Prismatolaimus, Trefusia, Alaimus*)

obtained from GenBank. These additional genera were added to provide phylogenetic context and selected based on the analysis of Holterman et al. (2006). Outgroup taxa (*Dorylaimus* species) were chosen to root the trees based on the SSU phylogeny of *Tripyla* (Zhao, 2009). Sequences were aligned using ProAlign Version 0.5 (Loytynoja & Milinkovitch 2003). The GTR+I+G substitution model was used for Bayesian phylogenetic inference because it is the best-fit model for these taxa as determined using MrModeltest 2.3 (Nylander, 2004).

Descriptions

Trischistoma ripariana n. sp.

Measurements. Table 1.

Trischistoma ripariana **n. sp.** was collected by the second author in July 2012 from the surface litter layer along a stream bank near Oakville, Napa County, California, USA.

Female (n = 18). Body slender, curved and dorsally bent posteriorly; some specimens with the tail dorsally reflexed after fixation. Cuticle thin (1.0 m) and finely striated under SEM. Lip region rounded, 7–11 (9.5±0.23) m wide, continuous with the body contour, consisting of three lips, each with two small labial papillae at the base. Head 10–12 (11±0.2) m wide at the level of the outer labial setae (Figs. 1,2).

Body with a few scattered somatic setae close to the vulva region, cervical setae absent; anterior sensillae in three whorls, the inner whorl consisting of two small papillae at the base of each lip (inner labial papillae); the second of six outer labial setae, oriented slightly anteriorly and 4–6 (5±0.1) m long, the length almost 50% of the labial diameter. The third whorl of four cephalic setae, 4–6 (4.9±0.1) m long, are much thinner than the outer labial setae and 7–12 (10±0.3) m from the anterior end. Amphid apertures rather obscure, posterior to the cephalic setae 15–28 (20.7± 0.7) m from the anterior end. Dorsal tooth small, 13–19 (15.8±0.42) m from the anterior end; small subventral teeth 3–7 (4.1±0.85) m posterior to the dorsal tooth. Pharynx cylindrical, strongly muscular, 189–236 (213.5±2.7) µm long and 21–25 (22.4 ±0.26)% of total body length, terminating in a small cardia, 4–8 (5.7±0.2) m long and 6–12 (9.4±0.4) m wide (Fig. 1).

Genital system monovarial prodelphic without a post-uterine sac, the ovary in most of the specimens is reflexed; but outstretched in one specimen. Vulva width 19 m, with small, slightly protruding lips and with small rod-like sclerotized structures. Distance between posterior of pharynx and vulva 499–660 (559±9.1) m. Vulva to anus distance 108–161 (133±2.7) m. Tail elongate conoid, 34–57 (46±1.8) µm long, occupying 2.5–5.9 (4.7±0.21)% of total body length. A pair of setae in latero-dorsal position on the tail posterior to the anus. Spinneret small, 1–4 (1.6±0.31) µm long.

Male: Unknown.

Type habitat and locality. Surface organic material and rhizosphere soil surrounding a California Bay tree on stream bank, next to Mondavi Vineyard, Oakville, California, N 38°25'50.3" W 122°25'13.2", 86 m above sea level.

Type specimens. Holotype female CNHE 9258 and paratypes CNHE 9259 of *T. ripariana* **n. sp.** are deposited in the CNHE (Coleccion Nacional de Helmintos, Instituto de Biologia, Universidad Nacional Autónoma de México); paratypes A-079-2 in CNCP (Colección Nematologica del Colegio de Postgraduados, Texcoco, México, UCRNC (Nematode Collection of the University of California, Riverside), and USDANC USDA Nematode Collection, Nematology Investigations, Beltville, MD, USA).

Etymology. The specific epithet is derived from the Latin *riparius*, referring to the stream bank locality of the type habitat.

Diagnosis and relationships. *Trischistoma ripariana* **n. sp.** is characterized by its small, very thin, body 0.8–1.0 (0.95±0.01) mm; thin cuticle, small index c, 17–29 (21±1.3) and short tail, 34–57 (46.2±1.8) µm which is 2.5–5.9 (4.7±0.21)% of body length.

Trischistoma ripariana **n. sp.** is similar at *T. pellucidum* Cobb, 1913 in the index b and in the position of the vulva; it can be distinguished by the body length, 0.8–1.1 vs. 0.6–0.8 mm, by the short tail 34–57 (46.2±1.8) vs. 50–60 µm long, the indices a = 45–59 vs. 36–43 and c = 17–29 vs. 13–14. It resembles *T. waiotoma* Zhao, 2011, in the index b and in the position of vulva, but it can be distinguished by the length of the tail 34–57(46±1.8) vs 50–64, in the indices a and c, 45–59 and 17–29 vs 32–41 and 10.9–15.6, respectively.

Molecular characteristics. *Trischistoma ripariana* was distinct in nucleotide sequence when compared to

other *Trischistoma* congeners. *Trischistoma ripariana* showed 15 or more differences in pairwise congeneric comparisons.

TABLE 1. Morphometrics of holotypes and paratypes of *Trischistoma ripariana* **n. sp.**, *T. corticulensis* **n. sp.** and *T. helicoformis* **n. sp.** females. All measurements are in µm, except for L (mm); mean±standard deviation, and ranges.

Character	*T. ripariana* Holotype	*T. ripariana* Paratypes n=19	*T. corticulensis* Holotype	*T. corticulensis* Paratypes n=10	*T. helicoformis* Holotype	*T. helicoformis* Paratypes n=18
L	1.008	0.96±0.1 0.85–1.07	0.985	1.0±0.04 0.8–1.18	1.008	1.0±0.02 0.9–1.2
a	53.1	51±0.9 45–59	41.0	42±0.9 34–45	42.0	42±1.4 33–53
b	4.5	4.5±0.04 4.1–4.8	5.8	5.7±0.2 4.7–6.9	4.0	4.4±01 3.7–6.5
c	17.7	21±0.89 17–29	19.5	16±0.6 14–20	12.0	11.7±0.15 10–14.5
ć	3.6	3.4±0.1 2.4–5	2.8	3–7±0.14 1.8–4.4	4.7	4.4±0.15 3.6–6.3
Outer labial setae	4	5.0±0.1 4–6	5	5.2±0.25 4–6	5	5.0±0.09 4–6
Cephalic setae	5	4.9±0.1 4–6	3	3.0±0.0 3.0	3	3.2±0.1 3–4
Cephalic setae to anterior	10	10±0.3 7–12	11	10.6±0.3 10–12	11	12.4±0.89 10–15
Max. body diam.	19	19±0.3 16–21	24	25±0.7 21–28	24	25±1.1 18–37
Pharynx length	226	213±2.7 189–236	169.7	182.4±5.9 151–217	24	235±5.7 170–263
Pharynx % body	22.4	22.4±0.3 21–25	17.2	18±0.5 15–21	25.2	23±0.6 15–27
Pharynx to vulva	579.6	560±9.2 500–660	508.9	564±26.3 396–698	527.8	541±20.6 394–707
Lip diam.	9		9		14	
Neck diam.	10		13		16	
Amphid to anterior	19	21±0.7 15–28	20	21±1.0 15–27	31	21±1.8 12–38
Cardia length	5	5.7±0.2 4–8	5	5.5±0.58 17–29	5	5.2±0.5 4–11
Cardia width	8	9.4±0.4 6–12	9	9.4±0.7 6–13	8	8.1±0.48 4–11
Tail length	56.8	46.2±1.8 34–57	50.5	65.3±2.9 51–84	84.2	87±2.0 76–101
Tail width	16	14±0.4 10–16	18	18±0.4 15–19	18	20±0.5 16–22
V%	79.9	81±0.2 79–83		70±0.48 67–73		78±0.8 67–85
Vulva to anus	136.7	133±2.7 108–161		247±8.9 194–286		131±5.0 94–179
Vulva to anterior	805.8	780±12 693–886		721±28.0 556–811		790±19.7 669–961

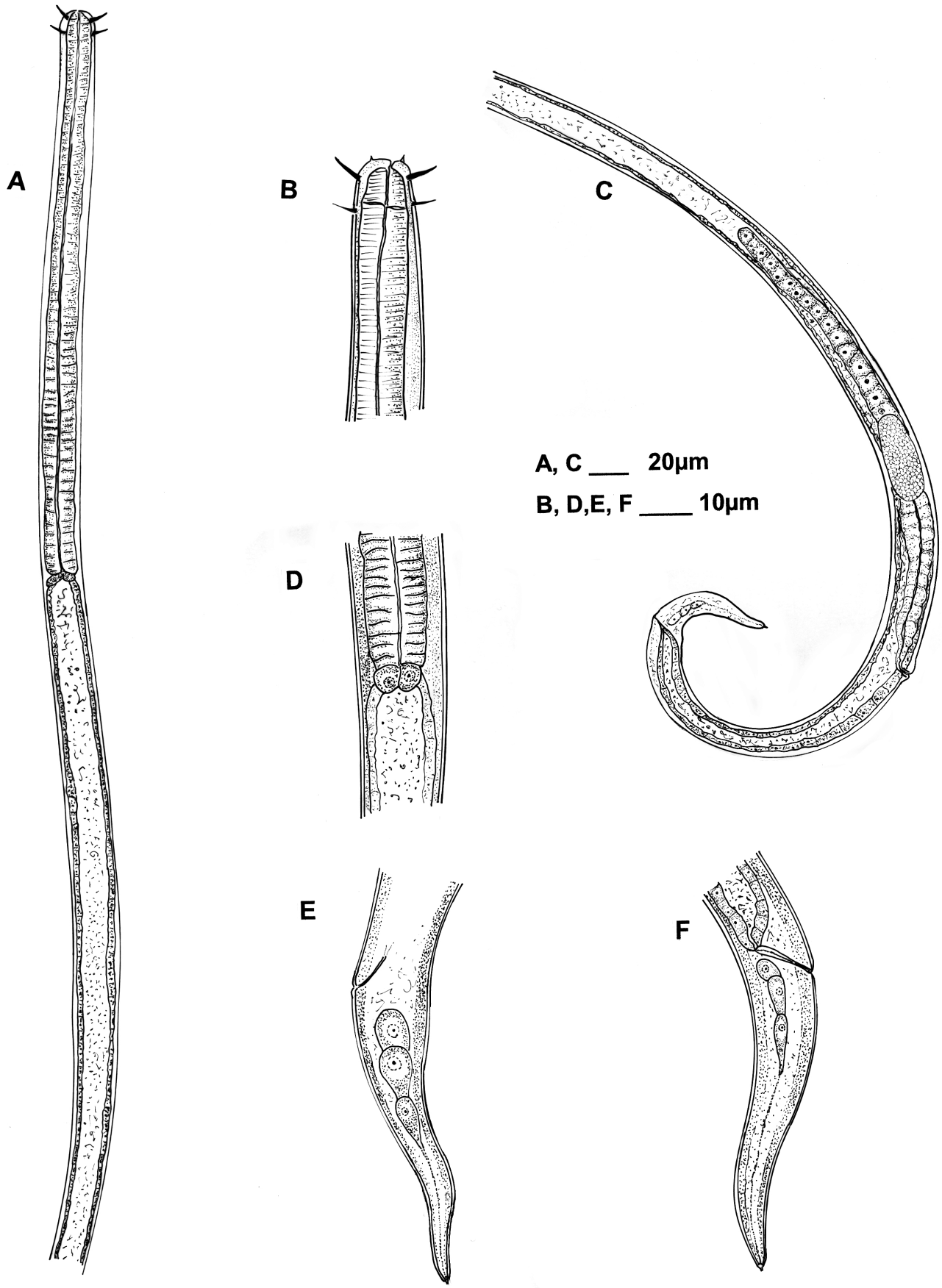

FIGURE 1. *Trischistoma ripariana* **n. sp.** Female A–F. A: Anterior half body; B: Anterior end;C: Posterior end body; D: Pharyngeo-intestinal junction; E,F: Tails.

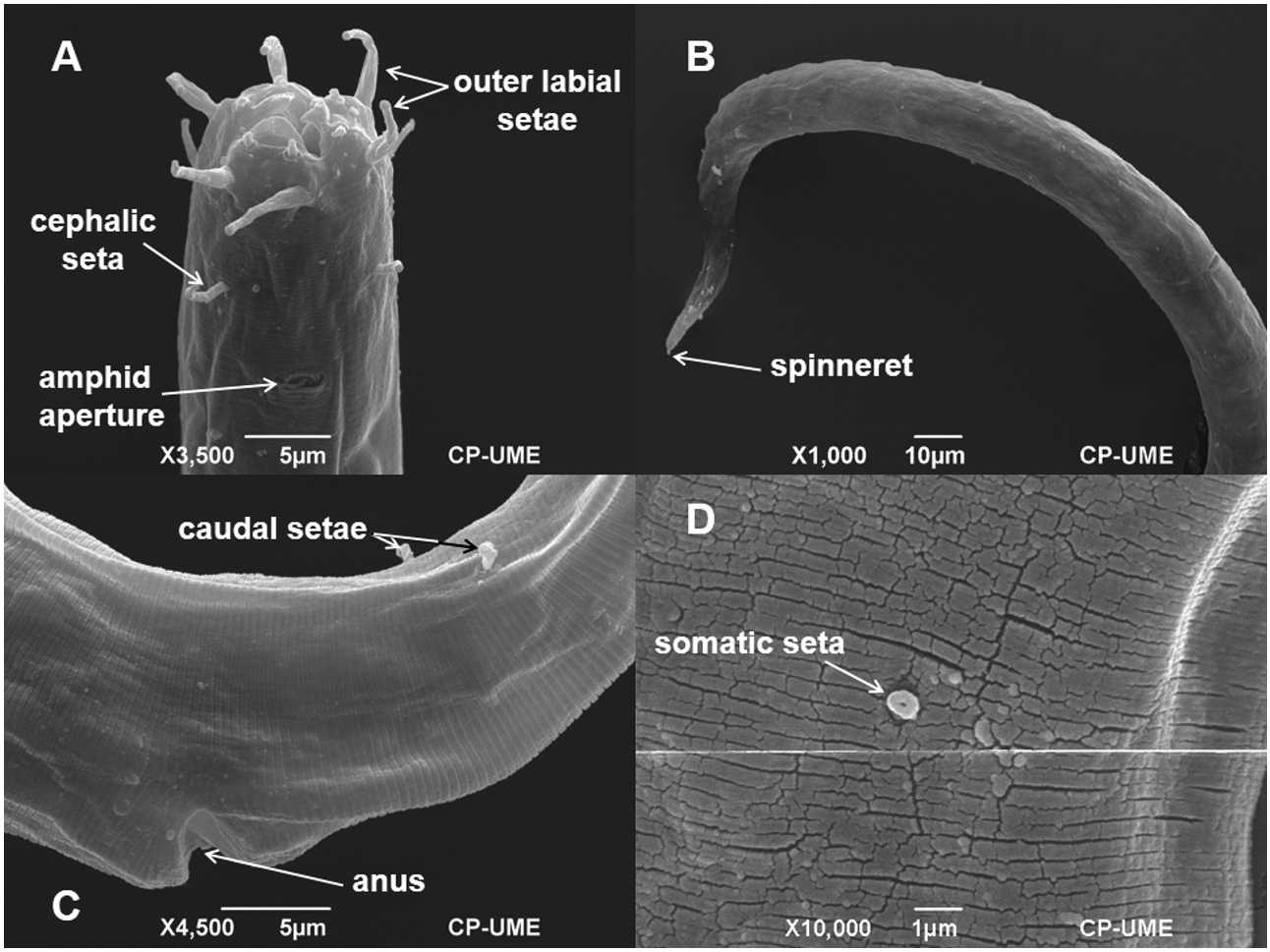

FIGURE 2. *Trischistoma ripariana* **n. sp.** Female A–D. A: Anterior end; B: Posterior end; C: Tail region lateral view; D: Somatic setae.

***Trischistoma corticulensis* n. sp.**

Measurements. Table 1.

Trischistoma corticulensis **n. sp.** was collected by the first author from moss on tree bark in an ecological reserve at the La Mancha Ecological Institute, Veracruz, México in August 2012.

Female (n= 10). Body slender, curved, bent dorsally at the posterior end, tail dorsally reflexed after fixation. Cuticle finely striated under SEM and thin, 1.0 m; lip region continuous with body contour, rounded and asymmetric, 7–12 (9.0±0.47) m wide and 10–14 (13±0.4) m at the level of the cephalic setae. There are a few somatic setae and one cervical seta. (Figs. 3,4).

Mouth with three lips, opening somewhat dorsally shifted, mouth cavity narrow, encircled by pharyngeal musculature. Cephalic sensillae in three whorls, the inner labial setae are very small and raised slightly above the cuticular surface at the base of each lip; the six outer labial setae are oriented slight anteriorly and 4–6 (5±0.25) m long, almost 50% of labial diameter; the four cephalic setae are 3.0 (3.0±0.0) m long, much thinner than outer labial setae and 10–12 (11±0.3) m from the anterior end. Amphid apertures posterior to cephalic setae, 15–27 (21± 1.0) m from the anterior end. One cervical seta present at 29 m from the anterior end, detected only by SEM (Figs. 3,4).

Dorsal tooth very small 9–12 (10.6±0.37) m from the anterior end and subventral teeth 8–11 (9.4±0.4) m posterior to the dorsal tooth. Pharynx cylindrical, strongly muscular, 151–217 (181±6.2) µm long and 14.5–20.5 (17.6 ±0.48)% of the total body length, terminating in a small cardia, 3–10 (5.5±0.58) m long and 6–13 (9.4±0.69) m wide. Genital system monovarial prodelphic without a post-uterine sac; the ovary in most specimens is outstretched. An egg, 88 x 18 m, was observed in only one specimen. Distance between posterior end of pharynx

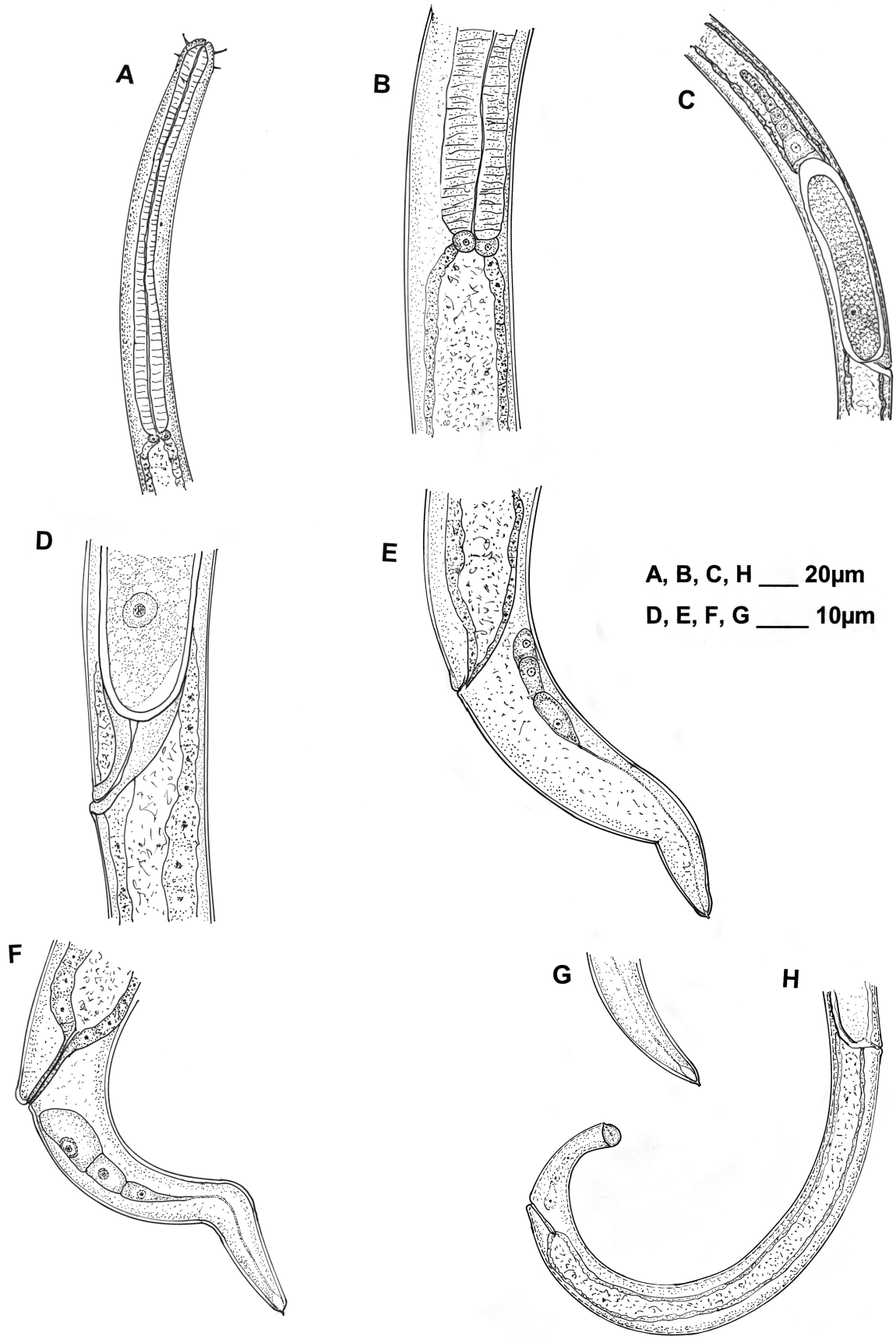

FIGURE 3. *Trischistoma corticulensis* **n. sp.** Female A–H. A: Anterior end; B: Pharyngeo-intestinal junction. C,D : Vulva region. E,F: Tails; G: Tail with spinneret; H: Posterior region lateral.

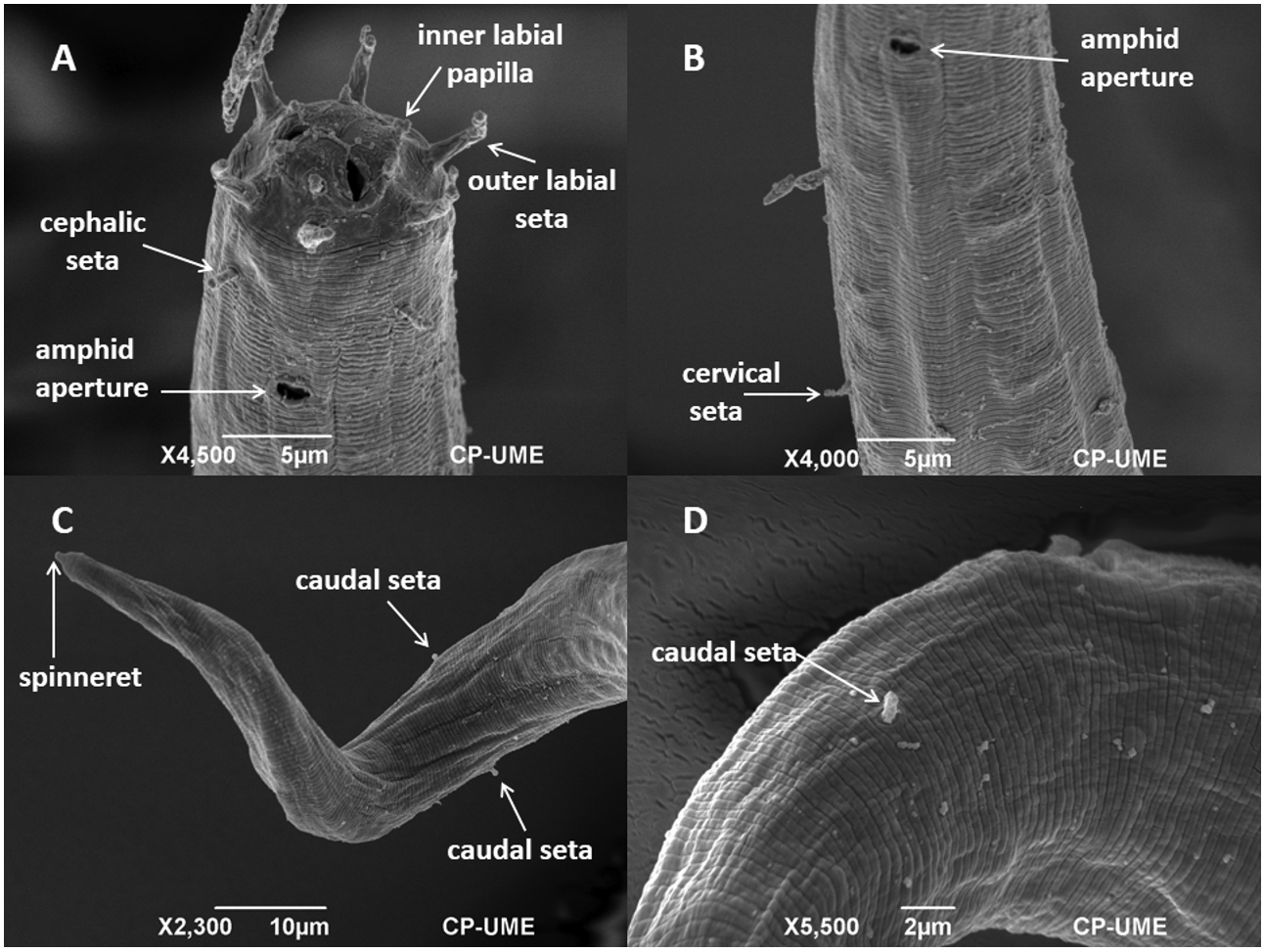

FIGURE 4. *Trischistoma corticulensis* **n. sp.** Female. A: Face view; B: Tail dorsal view; C: Tail lateral view; D: Tail with spinneret.

base and vulva 396–698 (564±26.3) m. Vulva with lips protruding and without sclerotised structures. Vulva to anus distance 194–286 (247±8.9) m. Tail elongate conoid, regularly tapering, dorsally bent in some specimens, 51–84 (65±2.9) µm long and 5.1–7.2 (6.3±0.21)% of total body length. A pair of setae in latero-dorsal position on the tail posterior to the anus. Rectum length almost equal to anal body diameter. Spinneret small, 1.0 µm long (Figs. 3,4).

Male: Unknown.

Type habitat and locality. Green moss growing on bark of tropical tree at the La Mancha Ecological Institute, Municipio de Actopan, Veracruz State, México, N 19°35'49.6" W 96°22'43.3", 24 m above sea level.

Type specimens. Holotype female CNHE 9256 and paratypes CNHE 9257 of *T. corticulensis* are deposited in CNHE; paratypes in CNCP (A-080-2) and UCRNC and USDANC.

Etymology. The specific epithet is derived from the Spanish word for bark: *corteza.*

Diagnosis and relationships. The new species is characterized by the very thin and short body 0.8–1.18 (1.0±0.04) mm; thin cuticle, the index c' = 2.8–4.4 (3.7±0.1), the position of vulva 67–73%, the relative length of the pharynx, 14.5–20.5 (17.6±0.48)% of body length, and the presence of one cervical seta.

Trischistoma corticulensis **n. sp.** is close to *T. ripariana* **n. sp.** in the size of the body, outer labial setae and the thin cuticle. It can be readily distinguished by the indices a = 34–45 (42±0.95) vs. 45–59 (51±0.95), c = 14–20 (16±0.56) vs. 17–29 (21±1.3), in the position of vulva 67–73 (69.6±0.5)% vs. 79–83 (81±0.2)%, in the length of the tail, 51–84 (65±2.9) vs. 34–57 (46±1.8) m and the distance vulva-anus 194–286 (247±8.9) vs. 108–161 (133±2.8). It resembles *T. otaika* Zhao, 2011, in the length of the body and in the indices a and b, but it can be distinguished by the vulva position, 67–73 (69.6±0.5)% vs. 79–83%, and the length of the tail, 51–84 (65±2.9) vs. 81–98 m.

Molecular characteristics. *Trischistoma corticulensis* was distinct in nucleotide sequence when compared to

other *Trischistoma* congeners. The new species exhibited five or more nucleotide differences in pairwise congeneric comparisons.

Trischistoma helicoformis n. sp.

Measurements. Table 1.

Trischistoma helicoformis **n. sp.** was collected by the second author from lichen on a shrub along the Carretera Interamericana in Costa Rica in August, 2012.

Female (n=18). Body slender without somatic setae, spiral shaped when relaxed, bent dorsally at the posterior end with tail dorsally reflexed after fixation. Cuticle thin, (1.0 µm) and finely striated under SEM; lip region continuous, rounded and asymmetric, 10–15 (12±0.35) µm wide and 13–16 (13±0.4) µm at the level of the cephalic setae which are 10–15 (12±0.37) µm from the anterior end. The vulva is at 67–79 (70±0.4)%; the index b = 3.9–4.6 (5.7±0.16) (Figs. 5,6).

Mouth with three lips, opening somewhat dorsally shifted, mouth cavity narrow, encircled by pharyngeal musculature. Cephalic sensillae in three whorls: the inner labial setae are very small and thin, raised slightly above the cuticular surface at the base of each lip; the six outer labial setae are oriented slightly anteriorly and 4–6 (5±0.25) µm long, almost 50% of labial diameter; the four cephalic setae are 3–4 (3.0±0.1) µm long, much thinner than outer labial setae and 11–14 (11±0.3) µm from the anterior end. Amphid apertures posterior to cephalic setae, 15–27 (21± 1.0) µm from the anterior end. One cervical seta at 24 µm from the anterior end. Dorsal tooth small 13–19 (15.8±0.42) µm from the anterior end and subventral teeth 10–14 (12.4±0.4) µm anterior to the dorsal tooth. Pharynx cylindrical, strongly muscular in its posterior part, 170–264 (235±5.65) µm long and 15–27 (23 ±0.58)% of the total body length, terminating in a small cardia, 3–8 (5.2±0.58) µm long and 4–11 (8.1±0.48) µm wide (Figs. 5,6).

Genital system monovarial prodelphic without a post-uterine sac. Ovary extending anteriorly, not reflexed, vulva to anus distance 94–179 (131±5.09) µm. Distance between pharynx base and vulva 452–594 (564±26.3) µm. Vulval lips do not protrude and have thin sclerotised structures. Tail elongate conoid, regularly tapering, dorsally bent, 76–101 (87±1.9) µm long, 16–22 (20±0.5) µm wide at anus level, and 6.9–10.0 (8.6±0.17)% of total body length. A pair of setae in latero-dorsal position on the tail posterior to the anus. Rectum 10–25 (16±0.97) µm long. Spinneret small, 2.0 µm long (Fig. 5).

Male: Unknown.

Type habitat and locality. Lichen growing on bark of shrub 20 m from the east side of the Carretera Interamericana, Costa Rica, N 9°34'22.6" W 83°45'26.8", 3341 m above sea level.

Type specimens. Holotype female CNHE 9260 and paratypes CNHE 9261 of T. *helicoformis* are deposited in CNHE; paratypes in CNCP (A-081-2), UCRNC and USDANC.

Etymology. The specific epithet come from the Greek ”helix” and refers to the spiral shape of the body when disturbed and after death.

Diagnosis and relationships. The new species is characterized by the spiral shape of the very thin body after death, the length of the tail 76–101 (87±1.9) µm, the indices c= 10–15 (12±0.25) and c’= 4.0–6.3 (3.7±0.1); thin cuticle, the position of the vulva 67–85 (78±0.8)%, and the length of the pharynx, 170–264 (235±5.6) µm long (Fig. 5, Table 1).

Trischistoma helicoformis **n. sp.** is close to *T. ripariana* **n. sp.**, and *T. corticulensis* **n. sp.** in the size of the body, outer labial setae, the thin cuticle and index b. It can be readily distinguished from them by the spiral habit of the dead body. From *T. ripariana* **n. sp.**, it differs in the length of the tail 76–101 (87±2.0) vs. 34–57 (46±1.8) µm, the size of the pharynx 170–264 (235±5.6) vs. 188–236 (213±2.6) µm, in the absence of a post-uterine sac and in the range of the position of the vulva 67–85 (78±0.8) vs. 79–83 (81±0.2)%. It differs from *T. corticulensis* **n. sp.** in the length of the tail 76–101 (87±2.0) vs. 51–84 (65±2.9) µm, in the length of the pharynx 170–264 (235±5.6) vs151–217 (181±6.2) µm and by the index c = 10–15 (12±0.3) vs. 14–19.5 (16±0.6), in the length of the tail, 76–101 (87±2.0) vs. 51–84 (65±2.9) µm and the vulva to anus distance, 94–179 (131±5.0) vs. 194–286 (247±8.9). It resembles *T. otaika* Zhao, 2011, in the length of the body, and in the indices a and b, but it can be distinguished by the absence of a post-uterine sac, length of the tail, 51–84 (65±2.9) vs. 81–98 (91±7.0) µm and the length of the body 0.88–1.2 (1.0±0.2) vs. 1.2–1.3 µm (Table 1).

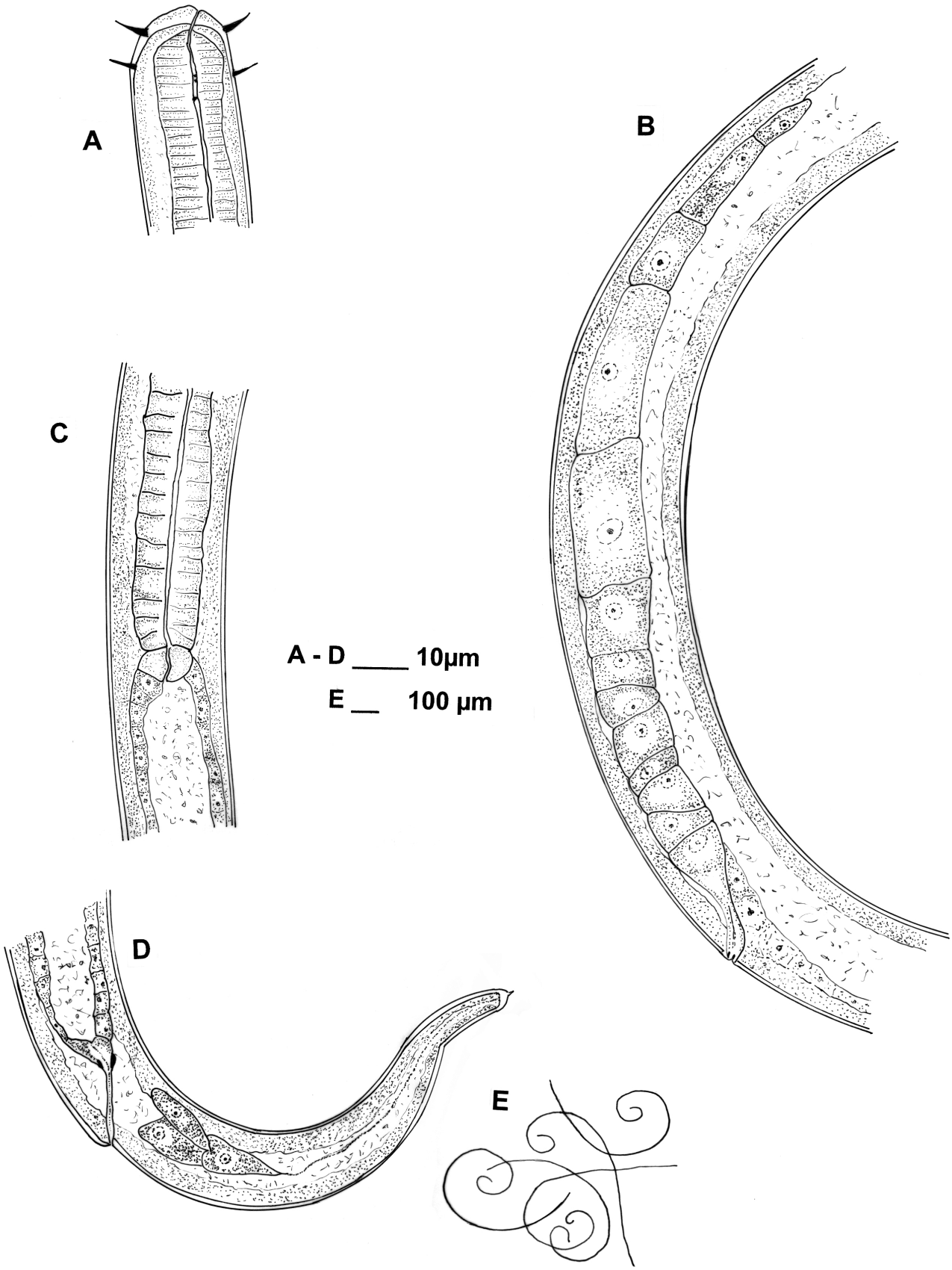

FIGURE 5. *Trischistoma helicoformis* **n. sp.** Female A–E. A: Anterior end; B: Vulva region; C: Pharyngeo-intestinal junction; D: Caudal setae lateral view; E: Body shape after death.

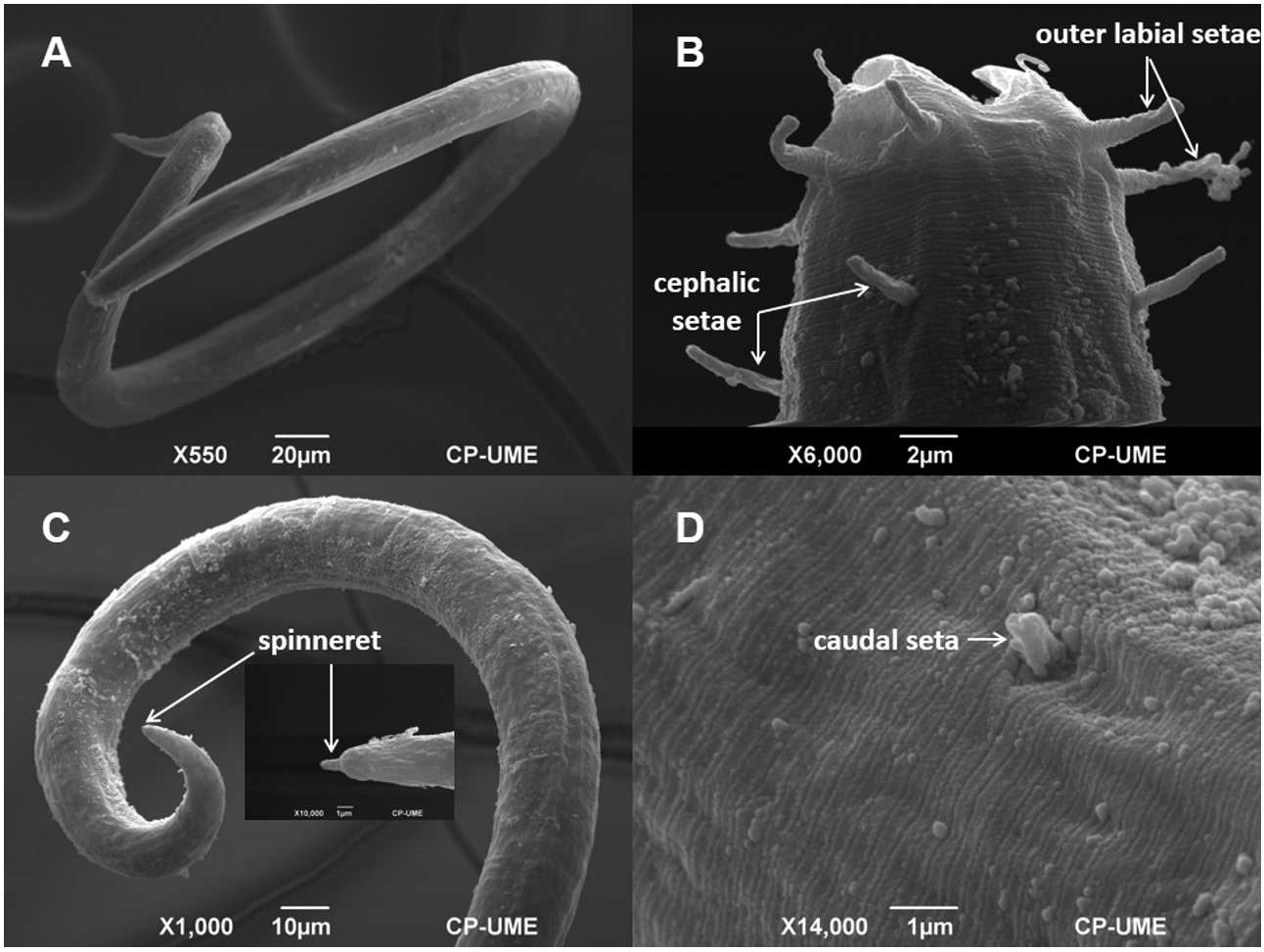

FIGURE 6. *Trischistoma helicoformis* **n. sp.** Female A–D. A: Spiral body form; B: Anterior end, lateral view; C: Posterior end, lateral view with caudal seta inset; D: Caudal seta lateral view.

Molecular characteristics. *Trischistoma helicoformis* was identical in sequence to *T. veracruzense*, but differed from other *Trischistoma* congeners by 13 or more nucleotide differences in pairwise congeneric comparisons. There are strong morphological differences that distinguish the two species: *T. veracruzense* has a post-uterine sac but *T. helicoformis* does not and the body form of *T. helicoformis*, when disturbed by touch or when relaxed by heat, is a tight spiral while that of *T. veracruzense* is an open C-shape with the posterior part curved dorsally.

Key to the species of *Trischistoma*

1.	Body a tight spiral shape after death	***T. helicoformis* n. sp.**
	Body not spiral-shaped after death	2
2.	Post-uterine sac present	3
	Post-uterine sac absent	7
3.	Body length > 2.0 mm	4
	Body length < 2.0 mm	5
4.	Body length 2.9–3.2 mm; tail length 220–242 µm	*T. subtillissimum* Andrássy, 2011
	Body length 4.0–4.1 mm; tail length 250–260 µm	*T. tenuissimum* Andrássy, 2011
5.	Male absent; V = 75%; tail length 81–98 µm	*T. otaika* Zhao, 2011
	Male present; body length 1.0–1.9 mm	6
6.	Body length 1.4–1.9 mm; a = 45–77; female tail length 115–150 µm, spicules 25–35 µm long; male tail with one supplement.	*T. monohystera* (de Man, 1880) Schuurmans-Stekhoven, 1951
	Body length 1.4–1.6 mm; a = 38–44; V = 81–83%; female tail length 80–96 µm; spicules 40–44 µm long; male tail with 2–3 supplements	*T. equatoriale* Andrássy, 2006

	Body length 1.0–1.3 mm; a = 41–54; female tail length 63–104 long; spicules 29–34 µm long	*T. veracruzense* Cid del Prado-Vera, Ferris & Nadler, 2010
	Body length 1.0–1.1 mm; a = 49–53; c = 12–13, female tail length 82–86 µm; spicules 25–32 µm long	*T. gracile* Andrássy, 1985
7.	Body length < 1.0 mm	.8
	Body length > 1.0 mm	10
8.	Body length 0.4–0.6 mm; female tail length 40–50 µm	*T. minor* Tahseen & Nusrat, 2010
	Body length 0.6–0.9 mm; V = 73–80%	9
9.	Cervical setae absent; pharynx length 155 µm; tail length 50–60 µm; b = 4.4–4.8	*T. pellucidum* Cobb, 1913
	Single latero-ventral cervical setapresent; pharynx length 171–198 µm; tail length 50–64 µm	*T. waiotama* Zhao, 2011
	Paired lateral cervical setae present; pharynx length 137–219 µm; tail length 44–72 µm	*T. triregius* Zhao, 2011
10.	Body length 0.80–1.2 mm; a = 34–45; V = 67–73%; female tail length 51–84 µm	***T. corticulensis* n. sp.**
	Body length 1.0–1.1 mm; a = 39–59; female tail length 34–92 µm	11
11.	a = 45–59; c = 17–29; b = 4.1–4.8; V = 67–73%; female tail length 34–57 µm	***T. ripariana* n. sp.**
	a = 39–47; c = 11–15; b = 3.8–4.1; V = 72–76%; female tail length 75–92 µm	*T. tukorehe* Zhao, 2011

Tripylina rorkabanarum n. sp.

Measurements. Table 2.

Tripylina rorkanbanarum **n. sp.** was collected by the first author from moss on tree bark ecological reserve at the La Mancha Ecological Institute, Veracruz, México in August 2012 and again in August, 2013.

Female: (n=19). Body slender, curved ventrally, posterior end of tail usually bent dorsally after fixation. Cuticle thin (1.0 m) with fine striations. Lip region asymmetric, 7–16 m (12±0.8) wide, continuous with the body contour. Three lips, each with two small labial papillae at the base. Outer labial setae plump and leaf-shaped with wide base and pointed apex. Cephalic setae in a whorl 1–2 µm behind the outer labial setae. Dorsal wall of stomal chamber thickened more strongly anteriorly (Figs. 7,8).

Head asymmetric, conoid, 16–20 µm wide. Relaxed specimens C-shaped, with tail curved ventrally and bent dorsally at posterior end. Body pores and a few somatic setae present, mainly in the anterior half of the body. Mouth with three small triangular-shaped lips, each with a pair of conical inner labial papillae at its base; outer labial setae longer 8–11 (10±0.33) µm long or 42–69% of head width; cephalic setae 5–6 (5.3±0.18) µm long, in a whorl 1–2 µm behind the outer labial setae. Amphid apertures almost circular, close to the level of the dorsal tooth 8–17 (11.6±1.0) µm or 0.4–1.0 (0.6±0.4) times the width of the head from the anterior end; dorsal tooth small, 10–15(13.6±0.63) µm from the anterior end and 1–2 µm anterior to the subventral teeth. Two cervical setae observed, the anterior 72–84 (80±1.5) µm, and the posterior 108–123 (115±4.4) µm, from the anterior end. Excretory pore not seen. Pharynx 151–185 (173±3.9) µm long. Base of pharynx to vulva 424–603 (508±14.7) µm and 669–867 (770±19.5) µm to anus. Cardia relatively small, 10–17 (13±0.9) µm long and 17–28 (23±1.5) µm wide, with two gland-like bodies. Nerve ring at 70–88 (82±5.8) µm from the anterior end (Figs. 7,8).

Vulva with or without protruding lips and without sclerotized pieces around the vagina. Gonad 104–240 (171±13.8) µm long or 9.8–23.7 (17±1.3)% of the body length, ovary reflexed. Vulva anus distance 226–311 (263±9.5) µm. Tail ventrally curved 67–99 (81±2.7) µm long, 7–9 (8±0.2)% of the body length, with the posterior portion bent dorsally, terminated by a small spinneret 1–2 (1.7±0.1) µm long. A pair of latero-dorsal setae present posterior to the anus and another pair anterior to the reduction in tail width (Fig. 7, Table 2).

Male: Unknown.

Type locality and habitat. Black-colored moss growing on bark of a tropical tree at the La Mancha Ecological Institute, Municipio de Actopan, Veracruz State, México, N 19°35'50.3" W 96°22'36.8", 15 m above sea level.

Etymology. This new species is named in honor of Dr. Rodrigo Rodriguez Kabana, for his outstanding scientific productivity in Nematology and his great contributions to the Organization of Nematologists of Tropical America (ONTA).

Type specimens. Holotype female CNHE 9254 and paratypes CNHE 9253 of *Tp. rorkabanarum* are deposited in CNHE; paratypes in CNCP (A-082-2), UCRNC and USDANC.

Diagnosis and relationships. *Tripylina rorkabanarum* **n. sp.** is characterized by the presence of two cervical setae, the position of the subventral teeth posterior to the dorsal tooth, the absence of sclerotized pieces around the vagina, the thickened dorsal wall of the stomal chamber mainly in the anterior region, the length of the pharynx, 151–185 (173±3.9) µm, the distance of the dorsal tooth from the anterior end, 10–15 (13.6±0.63) µm and the presence of a few somatic setae (Figs. 7,8).

TABLE 2. Measurements of females of *Tripylina* spp. with a post–uterine sac (*Tripylina iandrassyi* n. sp., *Tp. bravoae* and *Tp. longa*) and of *Tp. rorkabanarum* n. sp. All measurements are in µm, except for L (mm); mean±standard deviation, and ranges.

Character	*Tp. iandrassyi* Holotype	*Tp. iandrassyi* Paratypes n= 14	*Tp. bravoae* Holotype	*Tp. bravoae* Paratypes n= 11	*Tp. longa* Holotype	*Tp. longa* Paratypes n= 8	*Tp. rorkabanarum* Holotype	*Tp. rorkabanarum* Paratypes n= 10
L	1.1 mm	1.1±0.04 mm 0.94–1.45	1.6 mm	1.6±0.6 mm 1.2–1.9	1.7 mm	1.56 mm 1.48–1.72	1.22 mm	1.0±0.03 mm 0.82–1.12
a	34.5	42.2±1.3 34.5–53	43	44±5.4 36–53	41	36 33–40	28	23.9±1.0 20–28
b	5.4	5.9±0.1 5.1–6.8	5.4	5.7±0.6 4.9–6.9	7.1	7.0 6.3–7.4	6.1	5.9±0.18 5.2–6.9
c	32.7	34±1.79 24–45	36.2	30±5.1 22–36	28.3	28 25–30	4.5	13.7±0.28 12.9–14.7
c’	1.7	1.8±0.1 1.4–2.4	1.7	2.1±0.4 1.7–3.1	2.0	2.0 1.7–2.6	4.5	2.7±0.21 1.9–3.4
V%	82	80±0.6 76–84	82	80±1.6 78–83	80	79 76–80	69.7	67.1±0.76 64.5–70.5
Max. body diam.	32	28±0.9 20–34	37	36±2.3 23–44			40	37.6±1.2 32–41
Anal body diam.	20	19±0.6 16–22	26	26±0.7 23–30			22	24±0.4 23–26
Dorsal tooth to anterior	17	17±0.6 16–22	25	15.8±0.5 18–35	28	27 26–28	14	13.6±0.63 10–15
Nerve ring	79	80±4.5 73–93	123	110±72 90–123			88	78±4.1 60–89
Pharynx length	204	195±4.5 164–226	297	280±8.0 236–320	247	225 216–242	183	153.7±5.7 123–179
Pharynx (% body length)	18.5	17±0.4 15–19.5	18.5	17.6±0.6 14–21			16.3	17±0.5 15–19
Tail length	33.7	34.3±1.4 29.5–46	44	53±1.6 44–56	62	56 49–63	98.9	64.6±4.2 51–80
Tail (% of body length)	3.1	2.9±0.2 2.2–4.1	2.8	3.4±0.2 2.8–4.5			8.8	7.3±0.14 6.8–7.7
Vulval lips	not protruding	not protruding	not protruding	protruding	not protruding	not protruding	slightly protruding	slightly protruding
Spinneret	2	2	2 1–2	1–2 2			2	1–2

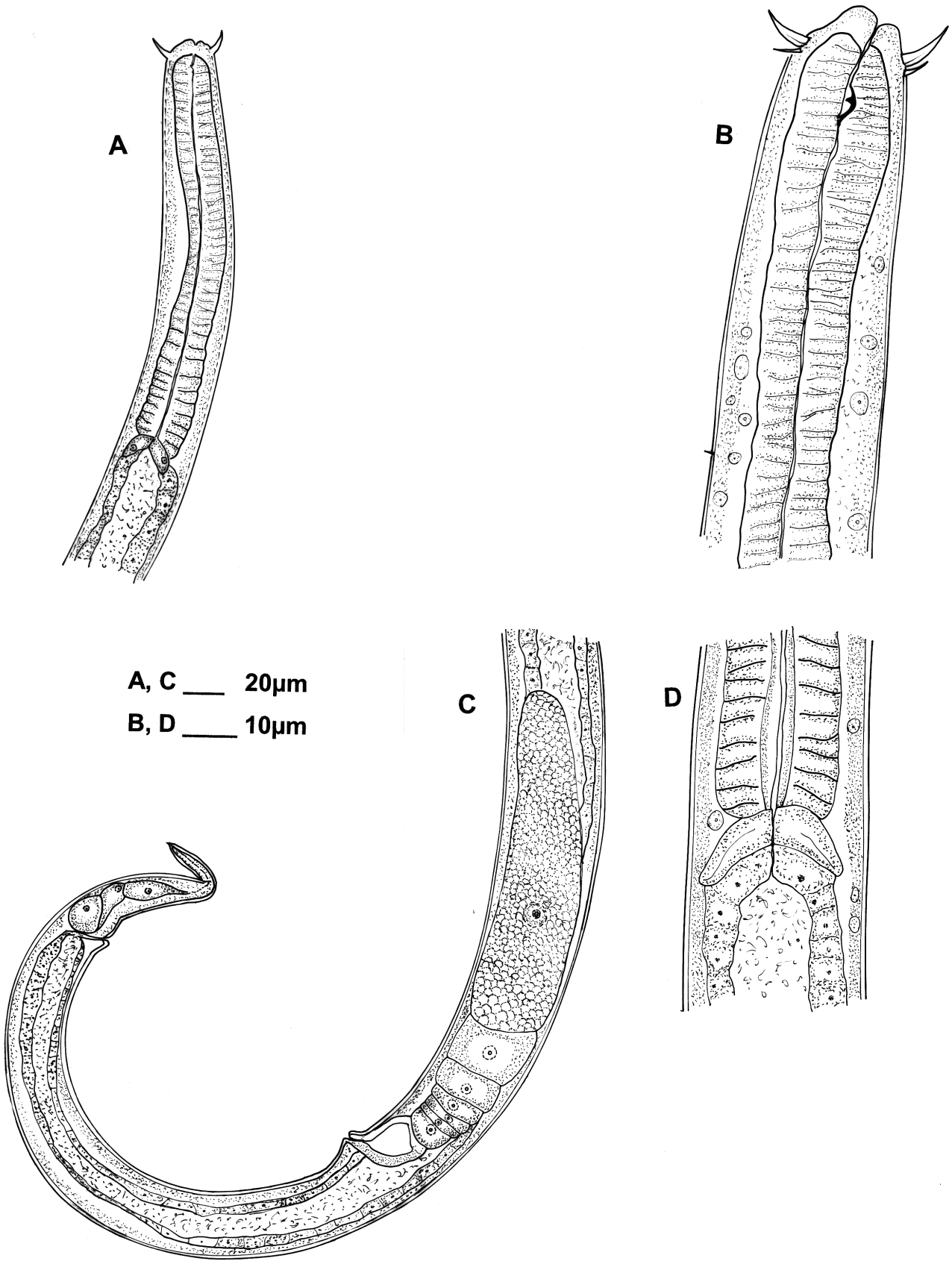

FIGURE 7. *Tripylina rorkabanarum* **n. sp.** Female. A–D. A, B: Anterior end; C: Posterior end body; D: Pharyngeo-intestinal junction.

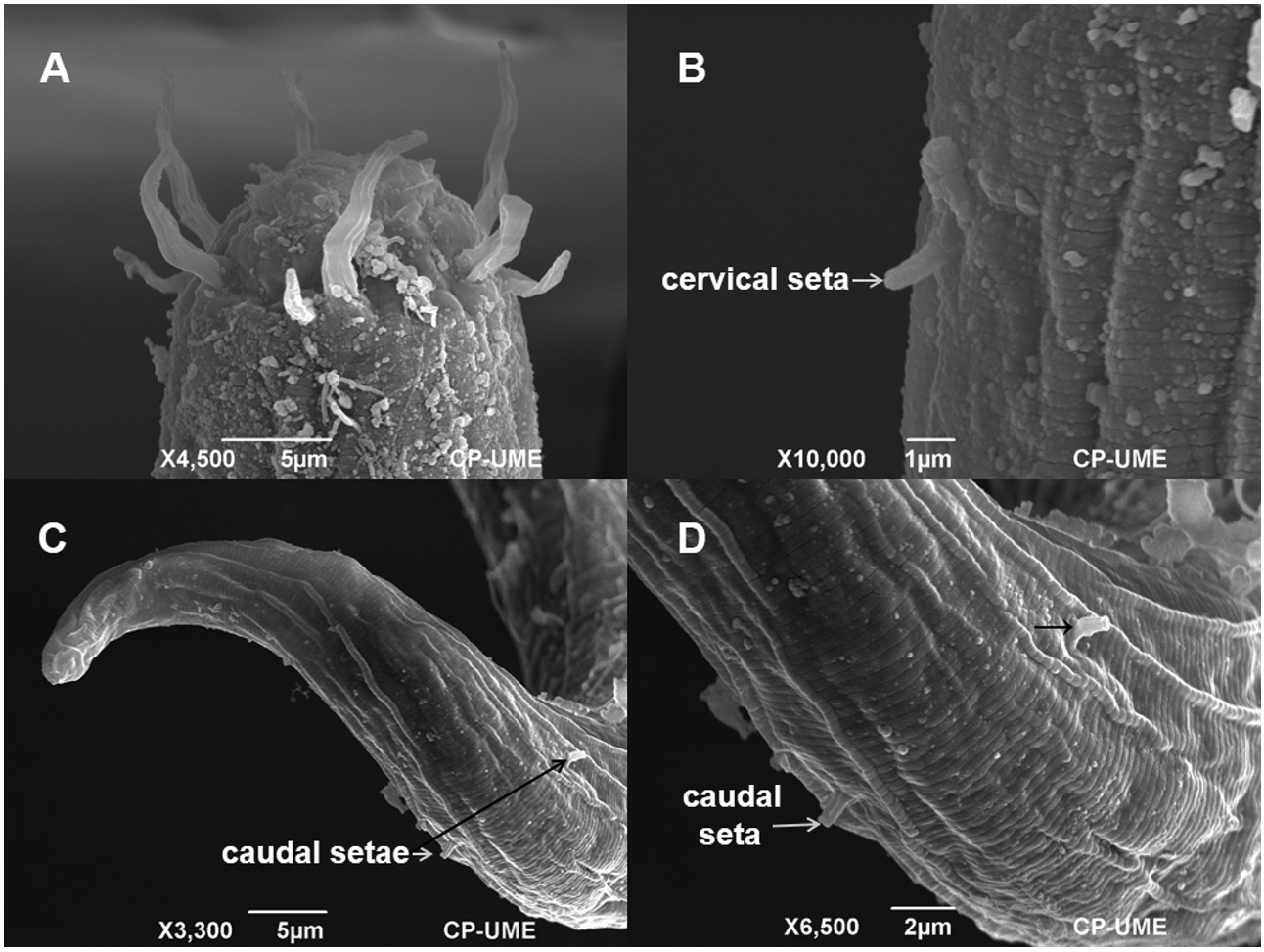

FIGURE 8. *Tripylina rorkabanarum* **n. sp.** Female A–D. A: Anterior end; B: A cervical seta; C: Tail; D: A caudal seta, dorso-lateral view.

Tripylina rorkabanarum **n. sp.** is similar to *Tp. montecilloensis* Cid del Prado-Vera, Ferris, Nadler & Lamothe-Argumedo, 2012, in body length, position of the vulva, absence of a visible excretory pore, and the presence of two cervical setae. It differs in the position of the dorsal tooth anterior vs. posterior to the subventral teeth, in the distance of the amphid to the anterior end 8.0–17 vs. 15–26, in the length of the tail 151–185 vs 141–207 µm, in the length of the outer labial setae 8–11 (10±0.3 vs. 10–15 (13±0.5), in the distance of the dorsal tooth from the anterior end, 10–15 (13.6±0.6) vs. 14–20 (16±0.5) µm and in the distance vulva-anus 226–311 (263±9.5) vs. 165–302 µm.

Tripylina rorkabanarum **n. sp.** is also similar to *Tp. arenicola* (De Man, 1880) Brzeski, 1963 in the size of the body, in the position of vulva and in the posterior position of the subventral teeth relative to the dorsal tooth. It differs in the lack of a visible excretory pore, the length of the tail 67–99 (81±2.7) vs. 48–76 µm, the length of the outer labial setae 8.0–11 (10±0.3) vs. 10–14 µm, and in the indices c and c'= 11–15 (12.6± 0.3) and 3–4.5 (3.7±0.2) vs. 13–18.7 and 1.9–2.8, respectively.

Tripylina rorkabanarum **n. sp.** is also similar to *Tp. sheri* Brzeski, 1963, in the length of the body and in the thickened dorsal wall of the stoma. It differs in the lack of a visible excretory pore, in having two cervical setae, the lack of cuticular thickenings in the vaginal walls, in the position of the subventral teeth posterior to the dorsal tooth, in the length of the tail 67–99 (81± 2.7) vs. 46–93 and in the index c 11–14.7 (12.6± 0.3) vs. 13–22.

Molecular characteristics. Specimens of *Tp. rorkabanarum* did not yield PCR products to sequence.

***Tripylina iandrassyi* n. sp.**

Measurements. Tables 2,3.

TABLE 3. Males of *Tripylina* spp. in which females have a post-uterine sac (*Tripylina iandrassyi* **n. sp.**, *Tp. bravoae* and *Tp. longa*). All measurements are in µm, except for L (mm); mean±standard deviation, and ranges.

Character	*Tp. iandrassyi* Paratypes n=4	*Tp. bravoae* Paratypes n= 10	*Tp. longa* Paratypes n=1
L	1.25 ±0.07 1.1–1.4 mm	1.7±0.9 1.5–2.0 mm	1.7 mm
a	41±3.1 35–50	49±1.6 42–58	41
b	5.9±0.2 5.3–6.4	5.4±0.2 5.3–7.3	7.1
c	30.5±2.1 25.8–36	31±1.8 23–39	28.3
c’	1.7±0.16 1.4–2.0	1.9±0.1 1.5–2.8	2.0
T%	57±6.2 46–73	50 42–59	
Max. body diam.	30±1.0 29–33	36±1.0 30–40	
Anal body biam.	25	30±1.1 25–37	
Dorsal tooth to anterior	18.8±0.75 17–20	24±1.1 20–27	
Nerve ring	89.0	100±6.3 94–113	
Pharynx length	212±8.2 198–226	247±5.0 236–302	247
Pharynx (% of body length)	17±0.68 16–19	16±0.6 14–19	
Tail length	42±3.7 36–51	58±2.8 46–72	
Tail (% of body length)	3.4±0.2 3–3.9	5.2±0.3 2.5–4.2	
Spinneret	1.5±0.5 1.0–2.0		

Tripylina iandrassyi was collected by the first author from soil and litter layers at an ecological reserve in La Mancha, Veracruz, México in August 2012 and again in August, 2013.

Body long and slender in both females and males with the posterior end spiraling ventrally after fixation. Cuticle thin, 1 µm thick, with striations, a few anastomoses visible in SEM preparations but indiscernible under LM. Labial region asymmetric with three conspicuous, laterally expanded, triangular-shaped lips 15–21 (18±0.62) µm width, continuous with slender neck. Two small conical inner labial papillae, 2 µm long, in the base of each lip; outer labial setae strongly developed, 10–12 µm (11±0.2) long, or 50–63 (58±1.3)% of the head width; cephalic setae 3–5 µm (4.0±0.4) long, separated from the outer labial setae by 1 µm. Amphids oval shaped, 10–20 (16±0.8) µm from the anterior end. Mouth opening shifted dorsally; dorsal wall of mouth cavity thickened; dorsal tooth directed posteriad, large (2 µm long), lying 15–20 (17±0.4) µm from the anterior end; subventral teeth minute, 1–2 µm posterior to dorsal tooth (Figs. 9,10).

Pharynx heavily muscular in the posterior region, 164–226 (194±4.5) µm long. Distance from posterior end of pharynx to vulva 559–1005 (762±33.9) µm. Pharyngeal-intestinal junction with large cardiac glands and two pericardiac cells, 7–12 (8.9±0.4) µm long and 15–20 (17.6±0.5) wide. Two pairs of ventromedian setae in the cervical region in both females and males, the anterior pair 49–77 (60±5.0) µm and 62–79 (71±8.5) µm and the posterior pair 125–150 (134±8.0) µm and 95–163 (129±34) µm from the anterior end. A few somatic setae present

along the body. Rectum 9–19 (14±0.9) µm long and 0.43–1.1 (0.76±0.06) times as long as anal body diameter (Fig. 9).

Female (n=15). Monovarial, prodelphic, ovary reflexed and short, uterus in some specimens with some spermatozoa; gonad 198–330 (245±11.6) µm long and 15.6–26% of body length. Vulval lips not protruding, vagina short, 5–6 (5.8±0.2) µm long and with two conspicuous oval sclerotized plates. Post-uterine sac 65–198 (110±11.0) µm long with a few ovoid sperm, 8–13 (10±0.68) µm long and 4.6–9.0 (6.3±0.5) µm wide, one pole with a small protuberance. Vulva to anus distance 118–292 (188±12.5) µm. Tail curved ventrally, 29.5–46.3 (34.3±1.4) µm long, 2.2–4.1 (3.0±0.15)% of the body length, with the same width as anal body diameter, 16–22 (19±0.6), for the first one third and then tapering to a cylindrical shape through the posterior two thirds. One pair of small latero-dorsal papillae posterior to the anus. Caudal glands occupy almost the full width of the tail. Spinneret 2 µm long.

Male (n=4). Average body length similar to that of female (1.1–1.4 mm). The buccal lips strongly developed as in females, 16–22 (19±1.3) µm wide; two cervical setae were observed in two specimens, the anterior 62–79 µm and the posterior 95–163 µm from the anterior end. Genital tract 546–872 (709±92.5) µm long, occupying 46–73% of the body length. Spermatozoa ovoid in shape. Five precloacal supplements present, the first 4.2–8.4, second 16.8–25.3, third 37.9–54.7, fourth 63.2–92.6, and the fifth 80–117.9 µm from the cloaca.

Spicules sickle-shaped, 37.9–47.0 (42.5±2.4) µm long, with bifid terminus, not completely surrounded by muscle layer; gubernaculum distinct, an inverted U shape, 6–12 (8.3±1.4) µm long. Tail 35.8–50.5 (42±3.7) µm long, 3.0–3.9% of entire length of body; tapering to a cylindrical shape in the posterior two thirds. A pair of conspicuous glandular orifices present at the outer edges of the posterior cloaca lip, in one specimen there was a secretion from the left orifice. One pair of small latero-dorsal papillae posterior to the anus. Caudal glands and spinneret similar to those of the female Figs. 9,11).

Type locality and habitat. Surface soil collected from around a banana tree at the La Mancha Ecological Institute, Municipio de Actopan, Veracruz State, México, N 19°35'25.4" W 96°22'50.2", 5 m above sea level.

Etymology. This new species is named in honor of the eminent professor and systematist of the Phylum Nematoda, the late Dr. István Andrássy.

Type specimens. Holotype female CNHE 9252 and paratypes CNHE 9253 of *Tp. iandrassyi* are deposited in CNHE; paratypes in CNCP (A-083-2), UCRNC and USDANC.

Diagnosis and relationships. *Tripylina iandrassyi* **n. sp.** is characterized by the strong development of the buccal lips and the asymmetric head region, the dorsal tooth directed posteriad, the subventral teeth posterior to the dorsal tooth, the thickness of the dorsal stoma wall, two cervical setae in both females and males, and the presence of a post-uterine sac. Males have five papillate ventromedian supplements confined to the precloacal region. Spicules not clearly surrounded by a muscular sheath and glandular orifices in the lateral area of the posterior cloaca lip (Figs. 9,10,11).

Besides *Tp. iandrassyi* **n. sp.**, the only two other species of the genus *Tripylina* Brzeski, 1963 reported with a post-uterine sac, a thick dorsal wall of the stoma and males with sickle-shaped spicules are *Tp. longa* Brzeski & Winiszewska-Ślipińska, 1993, and *Tp. bravoae* Cid del Prado-Vera, Ferris, Nadler & Lamothe-Argumedo, 2012 (Zhao 2009, Cid del Prado-Vera *et al.* 2012). *Trischistoma iandrassyi* **n. sp.** and *Tp. bravoae* have distinct nucleotide sequences when compared to each other and to all *Tripylina* species sequences from GenBank (Cid del Prado-Vera *et al. 2*012).

Tripylina iandrassyi **n. sp.** is similar to *Tp. longa* in the posteriad orientation of the dorsal tooth, the thickness of the dorsal stoma wall, in the conspicuous sclerotised pieces of the vagina and in the position of vulva. Nevertheless, it can separated from that species in the length of the body 0.9–1.4 vs. 1.5–1.7 mm, the length of the outer labial setae 10–12 (11±0.2) vs. 15–18 µm, in the length of the pharynx 164–226 (194±4.5) vs. 216–242 (225) µm, the length of the tail 29–46 (34.3±1.4) vs. 49–63 (56) µm, in the posterior position of the minute subventral teeth and in strong development of buccal lips. Also *Tp. iandrassyi* **n. sp.** is similar to *Tp. bravoae* in the length of the body, the position of the vulva, the absence of body pores and excretory pore, but it differs, in females, in the length of the pharynx 164–226 (194±4.5) vs. 236–320 (280±8.0) µm, the length of the tail, 30–46 (34±1.4) vs. 44–56 (53±1.6) µm, the vulva to anus distance 117–292 (188±12.5) vs. 141–325 (264) µm, and in the strong development of the buccal lips. The males differ in the length of the body, 1.1–1.4 (1.25±0.07) vs. 1.5–2.0 (1.7±0.9) mm, length of the pharynx, 198–226 (212±8.2) vs. 236–302 (247±5.0) µm, in the tail length, 36–51 (42.1±3.7) vs. 46–72 (58±2.8) µm, and in the length of the outer labial setae, 11–15 (12.3±0.95) vs. 13–20 (17±1.0) µm (Tables 2,3).

Molecular characteristics. *Tripylina iandrassyi* was distinct in nucleotide sequence when compared to 15 other congeneric taxa, with 9 or more sequence differences in pairwise comparisons.

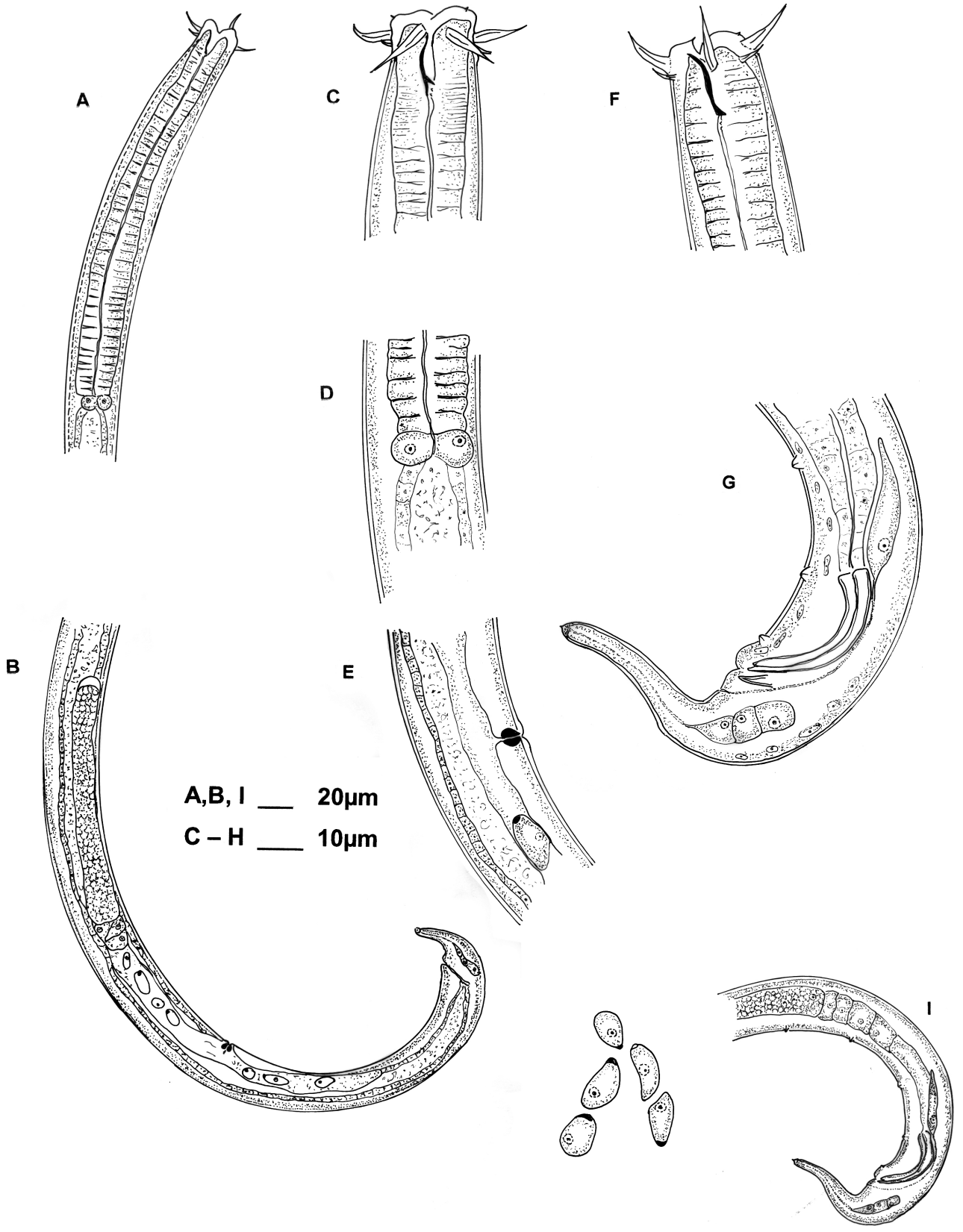

FIGURE 9. *Tripylina iandrassyi* **n. sp.** Female. A–E,H. A. Pharynx; B. Posterior end; C: Anterior end; D: Pharyngeo-intestinal junction; E: Vulva region; H: Spermatozoa; Male. F: Anterior end; G,I: Posterior region lateral.

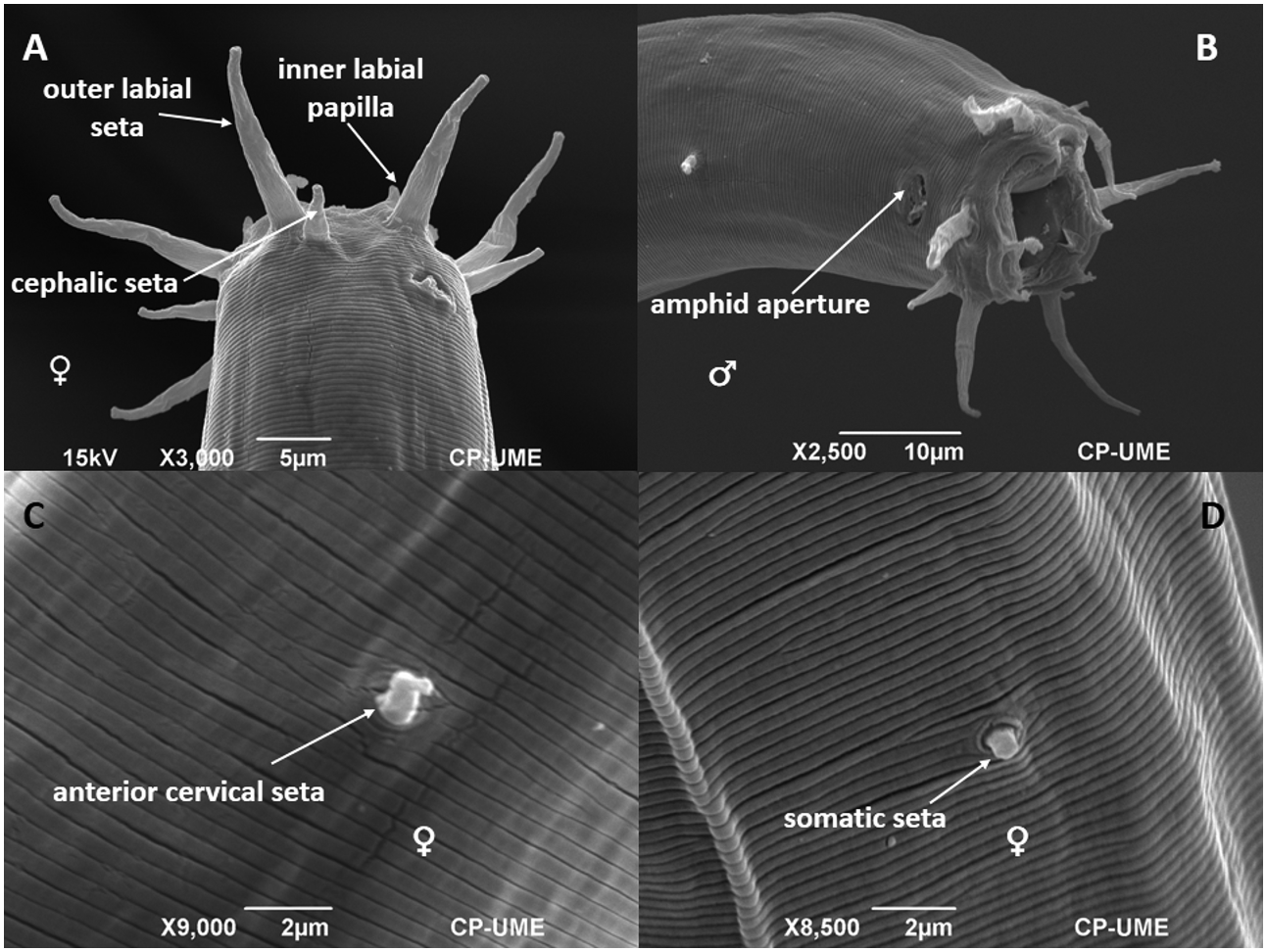

FIGURE 10. *Tripylina iandrassyi* **n. sp.** Female A, C, D. A: Anterior end; C: A cervical seta; D: A somatic seta; Male B: Lateral-face view anterior end.

Key to the species of Tripylina

1. V > 75%; post-uterine sac present or absent 2
   V < 75%; post-uterine sac absent 6
2. Post-uterine sac present 3
   Post-uterine sac absent 5
3. Body length <1.4 mm; outer labial setae <12 µm long; pharynx length 164–226 µm . . . . . ***Tp. iandrassyi* n. sp.**
   Body length >1.5 mm; outer labial setae >12 µm long; pharynx length 216–320 µm 4
4. Body length 1.5–2.0 mm; outer labial setae <13 µm long; pharynx length 236–320 µm; excretory pore absent . . . . . *Tp. bravoe* Cid del Prado-Vera, Ferris, Nadler & Lamothe-Argumedo, 2012
   Body length 1.5–1.7 mm; outer labial setae <15 µm long; pharynx length 216–242 µm; excretory pore present. . . . . *Tp. longa* Brzeski & Winiszewska-Ślipińska, 1993
5. Body length 1.5–1.9 mm; V = 78–81%; three single and two pairs of cervical setae . . . . . *Tp. gorganensis* Asghari, Pourjam, Heydari, Zhao & Ramaji, 2012
   Body length 0.8 to 1.6 mm , V = 62–68% cervical setae present or absent 6
6. Body pores present 7
   Body pores absent 12
7. Body length 0.8 to 1.2 mm 8
   Body length 1.3 to 1.6 mm 11
8. Body length 0.8–0.9 mm; cervical setae absent . . . . . *Tp. macroseta* Vinciguerra & La Fauci, 1978
   Body length 0.9–1.2 mm; cervical setae present. 9
9. One cervical seta present; tail length 71–105 µm . . . . . *Tp. manurewa* Zhao, 2009
   One ventro-median and two pairs of lateral cervical setae 10
10. Tail length 62–77 µm; index a = 25–30; index c = 12.4–17.7 . . . . . *Tp. tearoha* Zhao, 2009

Tail length 74–82 µm; index a = 20–23; index c = 14.5–15.6 . . . . . *Tp. tamaki* Zhao, 2009

11. Cervical setae absent, subventral teeth anterior to dorsal tooth; index c = 18–26 . . . . . *Tp. yeatesi* Zhao, 2009
Cervical setae present, subventral teeth posterior to dorsal tooth; index c = 14–19 . . . . . *Tp. zhejiagensis* Pham, Wang, Zhao & Zheng, 2013

12. Male absent; subventral teeth anterior or posterior to dorsal tooth; one or two cervical setae 13
Male present; subventral teeth posterior to dorsal tooth; one ventromedian cervical seta . . . . . *Tp. arenícola (*de Man, 1880) Brzeski, 1963

13. Vaginal sclerotized pieces present; excretory pore present 14
Vaginal sclerotized pieces absent; excretory pore present or absent 16

14. Subventral teeth anterior to dorsal tooth; dorsal wall of stomal chamber distinctly thickened . . . . . *Tp. sheri* Brzeski, 1963
Subventral teeth posterior or anterior to dorsal tooth; dorsal wall of stomatal chamber not thickened or only slightly thickened 15

15. Two ventromedian cervical setae; subventral teeth posterior to dorsal tooth; wall of stomatal chamber not thickened . . . . . ***Tp. rorkabanarum* n. sp.**
One ventromedian cervical seta; subventral teeth anterior to dorsal tooth; wall of stomatal chamber slightly thickened . . . . . *Tp. kaikoura* Zhao, 2009

16. Excretory pore present; subventral teeth anterior or posterior to dorsal tooth 17
Excretory pore absent; subventral teeth anterior to dorsal tooth 18

17. Body length 0.8–1.2 mm long; caudal setae absent; index c = 9.8–16; two unpaired cervical setae; subventral teeth anterior to dorsal tooth . . . . . *Tp.montecilloensis* Cid del Prado-Vera, Ferris, Nadler & Lamothe-Argumedo*,* 2012
Body length 1.3–1.6 mm; one pair of latero-dorsal caudal setae; index c = 15–23; two unpaired cervical setae, subventral teeth posterior to dorsal tooth . . . . . *Tp. ixayocensis* Cid del Prado-Vera, Ferris, Nadler & Lamothe-Argumedo*,* 2012

18. Body length 0.7–0.99 mm; index a = 18–39; subventral teeth anterior to dorsal tooth 19
Body length 0.8–1.3 mm; index a = 22–36; subventral teeth anterior or posterior to dorsal tooth 20

19. Body length 0.7–0.9. mm; two cervical setae; V = 63–73%; index a = 18–26. . . . . *Tp. tlamincaensis* Cid del Prado-Vera, Ferris, Nadler & Lamothe-Argumedo*,* 2012
Body length 0.9–1.0 mm; cervical setae absent; V = 66–68%; index a = 31–39 . . . . . *Tp. valiathani* Thaseen & Nusrat, 2010

20. Body length 0.8–1.2 mm; subventral teeth anterior to dorsal tooth; one cervical seta; index a = 22–26 . . . *Tp. ursulae* (Argo & Heyns, 1973) Tsalolikhin, 1983
Body length > 1.0 mm; a cervical seta present or absent; index a ≥ 26 21

21. Body length 1.0–1.1 mm; cervical setae absent; index a= 26–29; subventral teeth posterior to dorsal tooth . . . . . *Tp. ymyensis* Tahseen & Nusrat, 2010
Body length 1.0–1.3 mm; one or two cervical setae 22

22. Body length 1.0–1.2 mm; subventral teeth anterior to and smaller than dorsal tooth; two cervical setae; index a = 30–36 . . . . . *Tp. stramenti* (Yeates, 1972) Tsalolikhin, 1983.
Body length 1.0–1.3 mm; subventral teeth posterior to and similar in size to dorsal tooth; one cervical seta; index a = 26–34 . . . . . *Tp. puxianensis* Xu, Zhao, Wang & Zheng, 2013

Discussion

Morphology, morphometrics and keys. In assembling the keys for the species of *Trischistoma* and *Tripylina* we recognize that there is considerable overlap in characters of species that differ in appearance, habitat and on the basis of molecular characteristics. As more species of these genera are discovered we expect that these problems will be compounded and that there will be need for integrative taxonomic approaches using morphological matrices to delineate morphospecies groups in combination with molecular markers. Such methods have been applied to the species of *Xiphinema* (Gutiérrez-Gutiérrez et al., 2013; Archidona-Yuste *et al*., 2015) and probably will be important for identification of both morphologically defined and cryptic species within many nematode genera.

SEM has provided an important tool in revealing surface characteristics of the Trischistomatidae (Cid del Prado-Vera *et al*. 2010; 2012). Since this tool was either not available or were not used in earlier studies, minute body-surface details of many species of Trischistomatidae and Tripylidae are unknown. Application of SEM in recent studies reveals the fine striation of the cuticle, differences among species in the presence or absence of somatic pores, and in the number and location of minute cervical, somatic and caudal setae. Characteristics not easily seen by light microscopy are quite clear using SEM (Figs. 2,4,6,8,10).

Molecular characterization. Sequences for the new species, *T. corticulensis* **n. sp.**, *T. helicoformis* **n. sp.***, T. ripariana* **n. sp.** and *Tp. iandrassyi* **n. sp.**, were obtained from DNA extracts of single individuals. Direct sequencing of the PCR products yielded high-quality electropherograms with no sequence polymorphisms. Specimens of *Tp. rorkabanarum* **n. sp.** did not yield PCR products. New SSU sequences are deposited in GenBank (Accession numbers in Fig. 12). The Bayesian posterior probability (BPP) for the monophyly of *Tripylina* spp.

individuals was 100% (Fig. 12), and the sister-group to the *Tripylina* clade was composed of *Trischistoma* spp. plus *Trefusia zostericola* (100% BPP). Support for the clade of *Tripylina spp., Trefusia zostericola and Trischistoma spp.* was also high (100% BPP, Fig. 12).

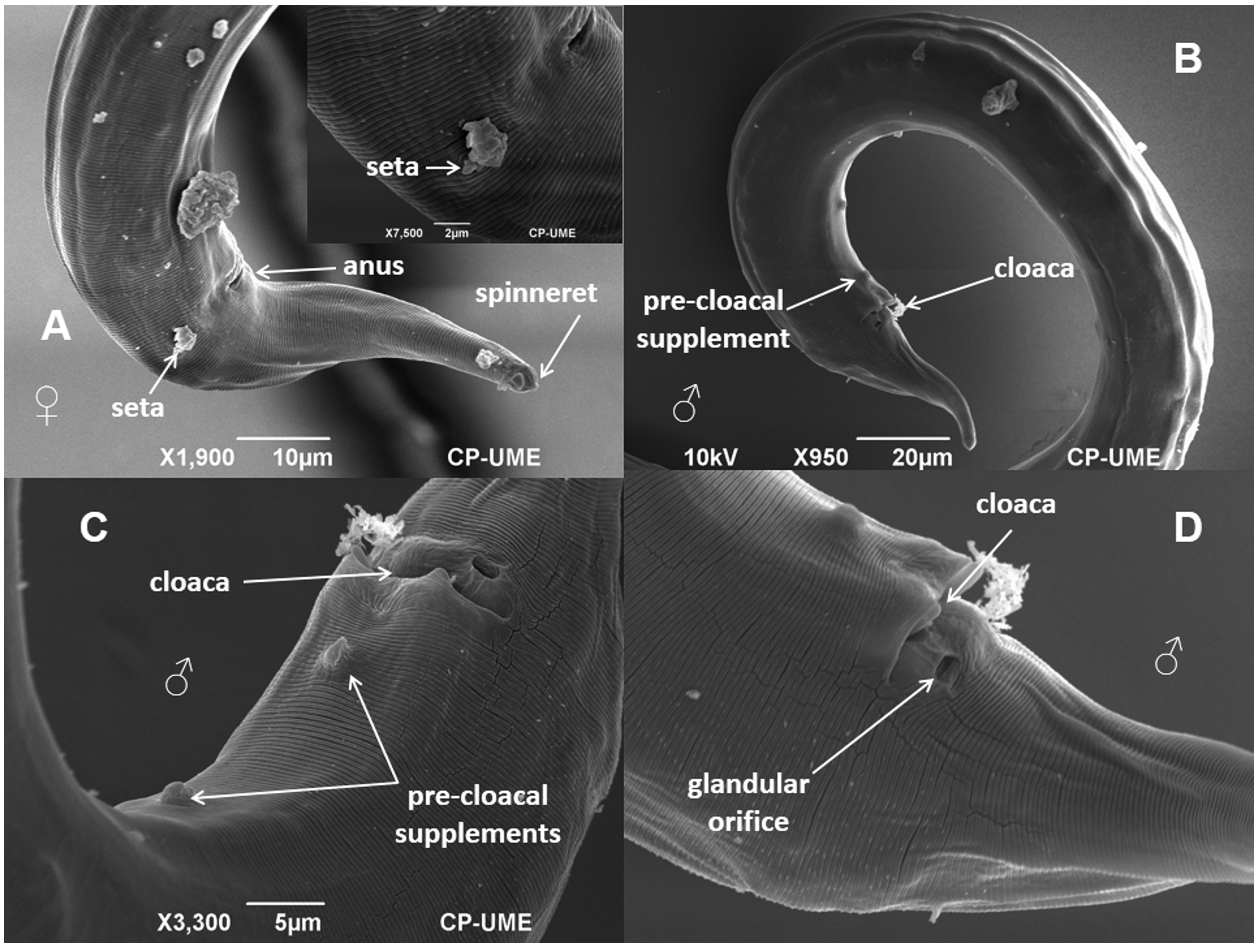

FIGURE 11. *Tripylina iandrassyi* **n. sp.** A: Female tail lateral view with caudal seta inset. Male B–D. B: Posterior end, lateral view; C: Supplements and cloaca; D. Cloaca, latero-ventral view.

There were two sub-clades within *Tripylina* spp., one of which consisted of *Tp*. *iandrassyi* **n. sp.** and *Tp. bravoae* as sister species. There was no resolution of species relationships within the second *Tripylina* sub-clade consisting of *Tp. tearoha, Tp. montecilloensis, Tp. tamaki, Tp. arenicola, Tp. ixayocensis, Tp. manurewa, Tp. tlamincasensis*, and *Tp.* sp. cf. *affinis*. We were unable to use the 18S of *Tp. puxianiensis* (Xu, Zhao, Wang & Zheng, 2013) from GenBank in the phylogenetic analysis because approximately 8% of the sites necessary for tree building were missing; consequently that species is not included in Fig. 12. However, Xu *et al*. (2013) reported that, based on their Bayesian analysis of the D2/D3 large subunit ribosomal DNA, *Tp*. *puxianesis* clustered with *T. tamaki* and *T. tearoha* in a clade having 99% BPP. *Tripylina iandrassyi* **n. sp.** was distinct in SSU sequence when compared to the other available *Tripylina* species. Phylogenetically it is the sister species to *Tp. bravoae* (Fig. 12). These two species form a separate sub-clade within *Tripylina*.

The two sub-clades of *Tripylina* are supported and demarcated by morphological differences. *Tripylina bravoae* and *Tp. iandrassyi* **n. sp.** both have a post-uterine sac in the female; the other species with this structure is *Tp. longa* (Cid del Prado-Vera *et al*. 2012*)* but unfortunately molecular data for *Tp. longa* are not yet available. The eight species forming the second sub-clade of *Tripylina* do not have the post-uterine sac.

Phylogenetic resolution within the genera *Tripylina* and *Trischistoma* based on SSU sequences is poor as also noted in Cid del Prado-Vera *et al.* (2012), however, there is strong support for monophyly of *Tripylina*. Within the *Trischistoma* clade, only the sister-species relationship between *T. helicoformis* **n. sp.** and *T. veracruzense* was strongly supported (Fig. 12). The species are separated by strong body morphology differences and the presence of a post-uterine sac in *T. veracruzense*.

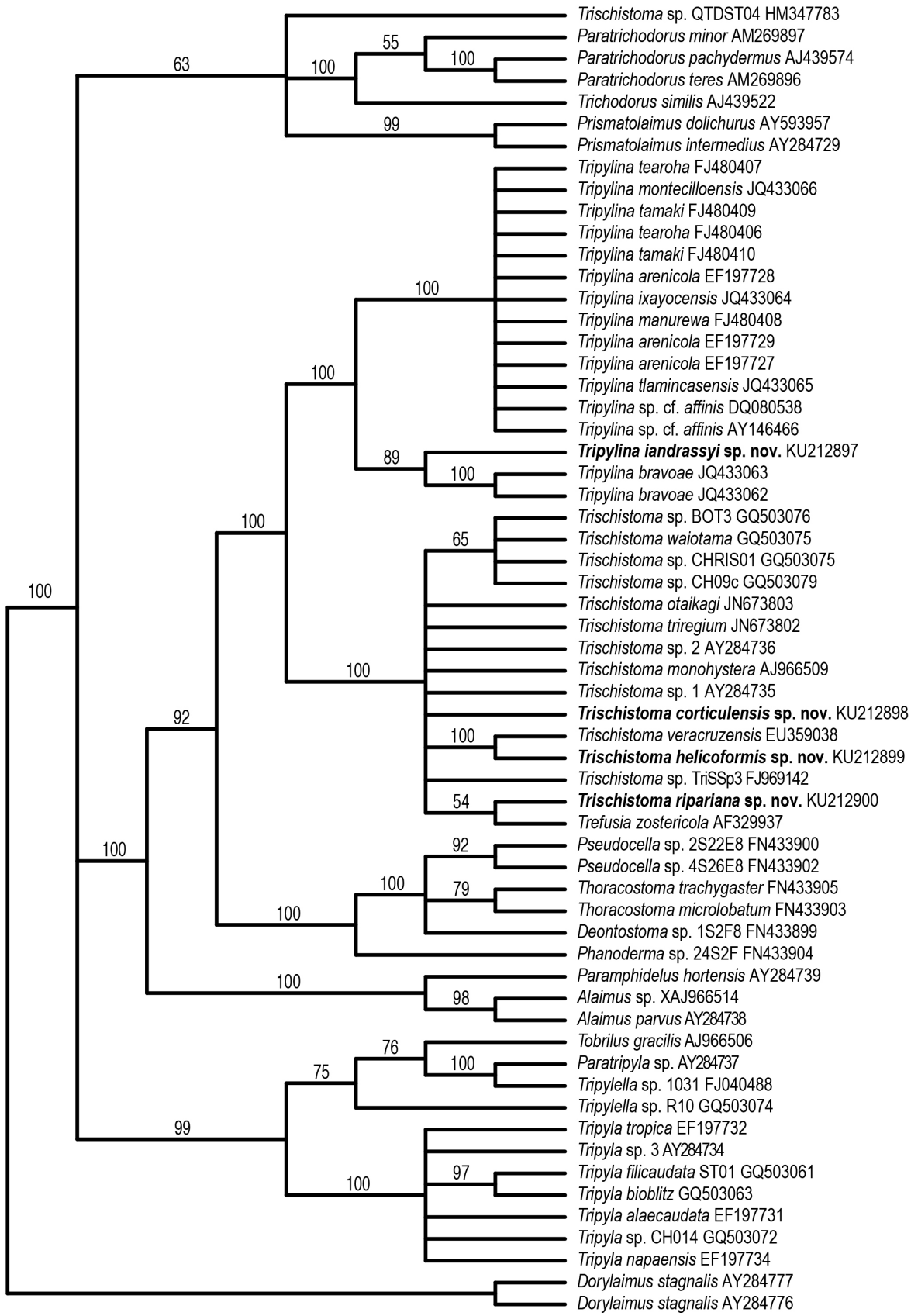

FIGURE 12. Bayesian posterior probability consensus tree inferred from the SSU rDNA sequence data. MCMC posterior probabilities are shown above branches. GenBank accession numbers are listed for each taxon and isolate designations are given when provided in the original publication or accession. Newly described species are indicated in boldface.

One *Trischistoma* sequence obtained from GenBank (HM347783) was unexpectedly recovered as part of a clade with *Trichodorus*, *Paratrichodorus*, and *Prismatolaimus*. Presumably this sequence represents a misidentification. The remaining *Trischistoma* spp., along with *T. zostericola*, were strongly supported as monophyletic. A molecule with a more rapid rate of change is needed to better resolve relationships within *Tripylina* and *Trischistoma.*

Biology and ecology. The evolutionary process of cephalization appears to have occurred in many taxa of the Nematoda. Assuming inferred evolutionary relationships are correct, the process is exhibited in the location of anterior sensillae in both the Tripylidae and Trischistomatidae. In the Tripylidae, *Tripyla* has the four cephalic setae posterior to the six outer labial setae whereas the cephalic setae in *Tripylella* are more anterior so that they appear almost as a single whorl with the outer labial setae. The same distinctions are apparent in the Trischistomatidae with greater apparent cephalization of the arrangement of the setae in *Tripylina* than in *Trischistoma*. It is tempting to speculate that such cephalization of tactile sensillae is associated with exploitation of terrestrial habitats that are more stable than the ephemeral conditions of marine and freshwater environments. However, we have found species of all four genera in terrestrial environments associated with mosses and surface litter although usually in moist conditions (Cid del Prado *et al*. 2010; 2012).

Particularly intriguing in SEM preparations are the gland orifices on the posterior lip of the cloaca of *Tp. iandrassyi* **n. sp.** Although the function of these glands is unknown, a secretion from one was visible in the SEM preparations (Fig. 11, C–D), prompting some speculation as to their function. The glands are not visible in females of *Tp. iandrassyi* **n. sp.** and have not been observed or reported for females of other Trischistomatidae or Tripylidae. We infer that they probably are not rectal or cloacal glands which usually, as in *Caenorhabditis elegans,* are reported to open into the nematode rectum (Lints & Hall, 2009; Goater *et al*. 2013). Similarly, ejaculatory glands in male nematodes, where reported, open into the rectum (Coomans & Lima 1965). There have been suggestions of attraction of females by males in some nematode species via chemically-mediated attractants, although in most reported cases of sex-attraction it appears that females attract males (Anya 1976). On the other hand, the attraction of females to males in *Trichinella spiralis* is stronger than that of males to females (Bonner & Etges 1967). Males are unknown in many species of Trischistomatidae and Tripylidae, but the observed glands in *Tp. iandrassyi* **n. sp.** suggest an interesting area of study of sexual attraction in species where males are known.

Very little is known of the biology, ecology and other aspects of the behavior of nematodes of the Trischistomatidae. They are classified as predators that ingest micro-invertebrates (Small 1987; Yeates *et al*. 1993; Traunspurger 2002) and we have observed partially-digested nematodes in the intestine of some specimens (Cid del Prado *et al*. 2010). In the laboratory, they may be observed to adhere to surfaces at the spinneret, by secretions of the caudal glands, and to intermittently thrash around actively with the remainder of the body. We infer that such action stirs up food resources in the substrate and perhaps creates currents that carry prey to the nematodes. The ability to adhere to surfaces may also contribute to their success in environments where water is moving (Traunspurger, 2000). Trischistomatidae and Tripylidae are frequently found in wet conditions (Traunspurger 2002) but also are associated with substrates and environments that are prone to drying as, for example, surface soil and leaf litter (*T. ripariana* **n. sp.**, *Tp. iandrassayi* **n. sp.**), lichens (*T. helicoformis* **n. sp.**) or moss on tree trunks (*T. corticulensis* **n. sp.**, *Tp. rorkabanarum* **n. sp.**). Nothing is known of their ability to enter anhydrobiosis or otherwise avoid adverse environmental conditions. We are unaware of any attempts to culture these nematodes on agar or gel surfaces that would facilitate behavioral studies and we suggest that this could be a fruitful and interesting area of research.

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