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## Five new species of the genus *Tripylella* (Nematoda: Enoplida: Tripylidae)

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### Abstract

Five new species of the genus *Tripylella* are described, two from México, one from Fátima, Portugal, one from Quito, Ecuador, and one from California, USA. *Tripylella mexicana* sp. n. is characterized by its short body (average 0.74 mm), short pharynx (average 161 µm), short tail (average 117 µm), presence of an excretory pore and small setae distributed sparsely along the body, the presence of body pores, the posterior position of the subventral teeth in relation to the small dorsal tooth with all teeth in contiguous stomal chambers, the finely-striated cuticle with many anastomoses, the non-protruding vulval lips, and the presence of sclerotized pieces in the vulval region. *Tripylella muscusi* sp. n. is characterized by its body length (average 0.94 mm), pharynx length (average 201 µm), tail length (average 140 µm), the anterior position of the subventral teeth in relation to the small dorsal tooth in a single stomal chamber, the presence of an excretory pore, the presence of body pores and sparse somatic setae, the finely-striated cuticle with sparse anastomoses, protruding vulval lips and sclerotized oval-shaped pieces present in the vulval region. *Tripylella quitoensis* sp. n. is characterized by the short body length (average 0.72 mm), the short outer labial setae, the short pharynx (average 175 µm), the location of the anterior subventral teeth and posterior dorsal tooth in the same stomal chamber, the short tail (average 98 µm), the apparent absence of an excretory pore, presence of body pores, presence of somatic setae, a finely-striated cuticle, non-protruding vulval lips, and very small oval sclerotized pieces in the vulva. *Tripylella fatimaensis* sp. n. is characterized by the short body, (average 0.74 mm) long, by the length of the pharynx (average 180 µm), the length of the tail (average 110 µm) and in the length of its reduced diameter portion, 45–58 µm, the presence of an excretory pore, body pores and three pairs of caudal setae (one pair each latero-ventral, latero-dorsal and ventral). *Tripylella dentata* sp. n. is characterized, and differs from all the species of the genus, by the presence of two adjacent stomal chambers, with two large teeth, one dorsal and one ventral, in the posterior stomal chamber and two subventral teeth in the anterior smaller chamber, short body (average 0.85 mm), pharynx length (average 209 µm), tail length (average 115 µm), the apparent absence of an excretory pore, the presence of two cervical setae in a lateral position, and by the presence of conspicuous pores along the body.

**Key words:** New species, *Tripylella*, moss, Tripylidae, Enoplida

### Introduction

The genera *Tripylella* Brzeski & Winiszewska-Ślipińska, 1993 and *Tripyla* Bastian, 1865 (Tripylinae, Tripylidae, Enoplida, Nematoda) are both diovarial and amphidelphic but are distinguished morphologically in the configuration of whorls of anterior sensillae. Both genera have six circumoral inner labial papillae, but in *Tripyla* the whorl of four cephalic setae is well-separated and posterior to the whorl of six outer labial setae while in *Tripylella*, the two whorls are very close together so that they appear as a single whorl of six, usually longer, and four, usually shorter, setae. The other genus of the subfamily, *Tripylina* Brzeski, 1963, also has the outer labial and cephalic setae in a single whorl but the females are monovarial, prodelphic (Andrássy, 2007; Cid del Prado *et al.*, 2010; and Cid del Prado *et al.*, in press).

The type species of the genus *Tripylella* is *T. intermedia* (Bütschli, 1873) Brzeski & Winiszewska-Ślipińska, 1993, which was originally described by Bütschli as a species of *Tripyla*. Only four other species of *Tripylella* have been described thus far: *T. iucunda* Andrássy, 2008, *T. maiuscula* Andrássy, 2006, *T. minuscula* Andrássy, 2006 and *T. subintermedia* Zhao, Xi & Xu, 2014.

The general configuration of the stoma of *Tripylella*, like other Tripylidae, is characterized by one dorsal and two subventral teeth. In different species of the genus, the subventral teeth are either anterior or posterior to the dorsal tooth and usually are in a separate stomal chamber from the dorsal tooth. The pharynx is uniformly cylindrical throughout its length with slight enlargement in the latter portion associated with the location of the pharyngeal glands. About midway down its length the pharynx is surrounded by the nerve ring. The cardia at the base of the pharynx is enlarged and comprised of six cells. The tail either tapers uniformly or is cylindrical in the anterior region and then narrows abruptly. The spinneret at the tail terminus emits secretions of three caudal glands which provides adherence to substrates. In species of *Tripylella* described thus far, males are extremely rare. When present, they have a row of ventral supplements extending anteriorly from the cloaca (Andrássy, 2007; Brzeski & Winiszewska-Ślipińska, 1993).

Species of the genus *Tripylella* are reported from moist soil, mosses and aquatic habitats. We sampled mosses growing on tree trunks in moist situations in the eastern region of México State, México, in northern California, USA, in Portugal and in Ecuador. We discovered five new species that are described here.

## Materials and Methods

**Specimen Preparation:** Nematodes were separated from the plant material either by a modified Baermann funnel technique (Barker, 1985), in which the moss was placed directly on a mesh screen and not separated from the water in the funnel by tissue paper, or the moss was placed in a jar of water and the suspension decanted through a 400-mesh sieve (38 µm aperture) after 24, 48 and 72 h. Nematodes of the genus *Tripylella* 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 °C) 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 sample was agitated and poured into a labelled Petri dish which was loosely covered and placed in a small desiccator over 95% ethanol and incubated at 40 °C. 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 under a dissecting microscope.

Samples were processed to glycerin using a modification of the Seinhorst (1959) method. An equal volume of Seinhorst A solution (1 part glycerin, 20 parts 96% ethanol, 79 parts water) was added to the dish and it was kept at 40°C with the cover slightly open. When the solution level evaporated to 2 mm, it was increased to 4 mm with additional Seinhorst A. When the solution level evaporated to 1 mm, Seinhorst B solution (95 parts 96% ethanol, 5 parts glycerin) was added to increase the depth to 4 mm. When the solution level again evaporated to 1 mm, the depth was increased to 2 mm with additional Seinhorst B. Three days later, 1 ml of pure glycerin was added to the dish.

Selected nematodes were hand-picked from the glycerin for mounting 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. Type specimens were deposited in the Colección Nacional de Helmintos, Instituto de Biología, Laboratorio de Helmintología, Universidad Nacional Autónoma de México (abbreviated herein as CNHE), the Colección Nematológica del Colegio de Postgraduados (abbreviated herein as CNCP) and the Nematode Collection Europe (NCE) at the National Plant Protection Organization of The Netherlands.

For scanning electron microscopy, six specimens of each species were placed in a pH 7.2 phosphate buffer for 15 minutes and dehydrated in an alcohol series (10 to 100%) for 15 minutes at each concentration. The specimens were critical-point dried and coated with gold-palladium before observation using a scanning electron microscope (Jeol JSM-6390) at 10 kv.

For molecular analysis, nucleic acids used for polymerase chain reaction (PCR) amplifications were extracted from individual adults stored in 95% ethanol. All individuals sequenced were collected from their type localities. Nucleic acids were extracted from individual nematodes and prepared using either a Chelex protocol (Cid del Prado Vera *et al.*, 2012) or the PrepGEM extraction kit (ZyGEM Ltd.) in a total volume of 25 mL. Next, 2–4 mL of

DNA extract was used to amplify a region of the SSU ribosomal DNA (3'-end). Primers used for PCR amplification were forward #648 (5'-GTATGGTTGCTGAAAC) and reverse #136 (5'-TGATCCTTCTGCAGGTTCACCTAC). Methods for amplification and sequencing were as described in Cid del Prado Vera *et al.* (2010).

A SSU dataset was prepared using sequences from three of the five new species, species of *Tripylella* available in GenBank, plus additional genera (e.g., *Alaimus*, *Paramphidelus*, *Paratrichodorus*, *Prismatolaimus*, *Trefusia*) 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 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 was the best-fit model for these taxa as determined using MrModelTest 2.3 (Nylander, 2004). The posterior probability distribution of trees was determined using MrBayes 3.2.3 (Ronquist & Huelsenbeck, 2003) as executed using the CIPRES Science Gateway and XSEDE (Miller *et al.*, 2010). The dataset, which consisted of 43 taxa and 659 aligned SSU characters, was run for 5 million generations and chains were sampled every 5,000 generations. Burn-in was estimated empirically as 20% by assessment of the convergence in the log likelihood values of the chains.

## Species Descriptions

### *Tripylella mexicana* sp. n.

(Fig. 1 A–D, Fig. 2 A–D)

*Measurements:* See Tables 1 and 2.

**TABLE 1.** Morphometric data for holotypes of females of *Tripylella* sp. n. (measurements in  $\mu\text{m} \pm \text{S.D}$  except where indicated).

Character	<i>T. mexicana</i> sp. n.	<i>T. muscisi</i> sp. n.	<i>T. quitoensis</i> sp. n.	<i>T. fatimaensis</i> sp. n.	<i>T. dentata</i> sp. n.
L	0.820 mm	0.994 mm	0.716 mm	0.773 mm	0.839 mm
a	22.8	24.9	23.9	22.1	22.7
b	4.5	4.8	4.0	4.2	4.2
c	6.2	6.8	7.5	6.9	7.7
c'	4.9	5.7	4.6	4.4	4.4
V%	48.6	48.3	52.6	49.9	51.7
Vulva to anus	287.5	367.6	240	273	292
Dorsal tooth from anterior end	15	16	13	13	17
Pharynx length	184	207.4	179	183.7	197.9
Excretory pore	88	-	absent	86	absent
Tail	132	146.1	96	111	109.5
Tail% body	16.1	14.7	13.4	14.3	13.1

*Female* n=14: Body C-shaped upon relaxation and fixation. Cuticle very thin, 1  $\mu\text{m}$ , with fine striations and anastomoses. Small setae and pores present around and along the body. Head region rounded, 13–15 (14 $\pm$ 0.2)  $\mu\text{m}$  wide. Inner labial papillae conoid; outer labial setae conoid 2–3 (2.7 $\pm$ 0.1)  $\mu\text{m}$  long; cephalic setae small, 1  $\mu\text{m}$  long, and separated from the outer labial setae by <1 $\mu\text{m}$  so that they appear as a single whorl of six longer and four shorter setae. The dorsal tooth is 13–18  $\mu\text{m}$  (14.8 $\pm$ 0.3) from the anterior end of the body and 2–3 (2.2 $\pm$ 0.1)  $\mu\text{m}$  behind the small subventral teeth. The dorsal tooth and subventral teeth are in adjacent stomal chambers; the anterior chamber is very small and sometimes appears to be contiguous with the posterior chamber. Amphids caliciform, 8–11 (9.7 $\pm$ 0.2)  $\mu\text{m}$  from anterior end of the body. Distance from base of pharynx to vulva is 141–283

( $205\pm10$ )  $\mu\text{m}$ . Cardia very conspicuous, 20–27 ( $25\pm0.6$ )  $\mu\text{m}$  long and 20–26 ( $24\pm0.6$ )  $\mu\text{m}$  wide, comprised of six cells, the anterior and posterior of medium size and the median cells larger. Excretory pore observed in ten of the 14 specimens.

**TABLE 2.** Morphometric data for paratypes of *Tripylella* spp. n. (measurements in  $\mu\text{m} \pm \text{S.D}$  followed by range, except where indicated).

Character	<i>T. mexicana</i> sp. n. n= 13	<i>T. muscusi</i> sp. n. n= 14	<i>T. quitoensis</i> sp. n. n= 10	<i>T. fatimaensis</i> sp. n. n= 12	<i>T. dentata</i> sp. n. n= 20
L	0.74 $\pm$ 0.16 mm 0.63–0.82	0.94 $\pm$ 0.02 mm 0.8–1.0	0.72 $\pm$ 12.9 mm 0.68–0.79	0.73 $\pm$ 0.02 mm 0.59–0.81	0.85 $\pm$ 0.1 mm 0.79–0.90
a	22 $\pm$ 0.34 20–25	25.8 $\pm$ 0.4 24–29	23.4 $\pm$ 1.8 16–36	23 $\pm$ 0.9 20–31	26 $\pm$ 0.4 23–30
b	4.6 $\pm$ 0.05 4.3–4.8	4.6 $\pm$ 0.06 4.3–4.9	4.1 $\pm$ 0.09 3.8–4.7	4.1 $\pm$ 0.11 3.4–4.8	4.1 $\pm$ 0.4 3.8–4.5
c	6.3 $\pm$ 0.1 5.5–7.5	6.7 $\pm$ 0.1 6.0–7.2	7.5 $\pm$ 0.3 6.9–10.1	6.7 $\pm$ 0.09 6.2–7.2	7.4 $\pm$ 0.1 6.6–8.5
c'	4.7 $\pm$ 0.08 4.1–5.1	5.3 $\pm$ 0.6 4.9–5.7	4.6 $\pm$ 0.2 3.9–6.2	4.7 $\pm$ 0.2 4.1–6.1	5.4 $\pm$ 0.3 4.2–8.6
V%	48.8 $\pm$ 0.39 48–53	49 $\pm$ 0.3 47–51	51 $\pm$ 0.6 49–55	49.5 $\pm$ 0.4 46–52	52.6 $\pm$ 0.2 51–54
Vulva to anus	255.7 $\pm$ 7.1 222–292	339 $\pm$ 7.8 273–380	253 $\pm$ 6.8 226–292	253 $\pm$ 8.7 189–287	283.6 $\pm$ 3.3 264–302
Dorsal tooth from anterior end	14.8 $\pm$ 0.34 13.0–18	15.3 $\pm$ 0.45 10.0–17	12.3 $\pm$ 0.65 9.0–16	14.6 $\pm$ 0.31 13–16	16.9 $\pm$ 0.18 15.0–18
Pharynx length	161 $\pm$ 3.6 141–184	201 $\pm$ 1.9 184–207	175 $\pm$ 3.2 160–189	180 $\pm$ 3.7 160–207	209 $\pm$ 2.2 198–226
Excretory pore	85.6 $\pm$ 1.4 80–93	91.3 $\pm$ 7.0 62–110	absent	73.7 $\pm$ 2.9 58–86	absent
Tail	117 $\pm$ 2.8 99–132	140 $\pm$ 2.9 123–160	98.1 $\pm$ 3.8 71–112	110 $\pm$ 2.8 90–128	115 $\pm$ 1.9 104–126
Tail % body	15.9 $\pm$ 0.5 13.4–18.1	14.9 $\pm$ 0.2 14–17	13.7 $\pm$ 0.2 12–14	15.1 $\pm$ 0.2 14–16	13.6 $\pm$ 0.2 12–15

Female gonads reflexed, the anterior 47–104 ( $81\pm5.4$ )  $\mu\text{m}$  long, 6.2–14.2 ( $10.9\pm0.7$ )% of the body length, and the posterior 19–132 ( $88\pm8.6$ )  $\mu\text{m}$  long, 2.4–16.2 ( $11.9\pm1.1$ )% of the body length (we consider the very short posterior gonad in one specimen to be a developmental anomaly). Vulva without protruding lips but with small oval cuticular structures. Rectum 18–25 ( $21\pm0.6$ )  $\mu\text{m}$  long or 0.7–1.0 ( $0.8\pm0.02$ ) times anal body diameter. Tail cylindroid, wide anteriorly for 22–27 ( $25\pm0.4$ )  $\mu\text{m}$  or 42–65 ( $56\pm1.86$ )% of its length and then narrowing abruptly, ending in a spinneret 2–4 ( $3.1\pm0.2$ )  $\mu\text{m}$  long. Two pairs of caudal setae present, one pair at seven annuli posterior to the anus, in a lateral position, and the second pair in a latero-dorsal position, also a single seta in a dorsal position, less than 20  $\mu\text{m}$  from the point at which the tail becomes reduced in diameter.

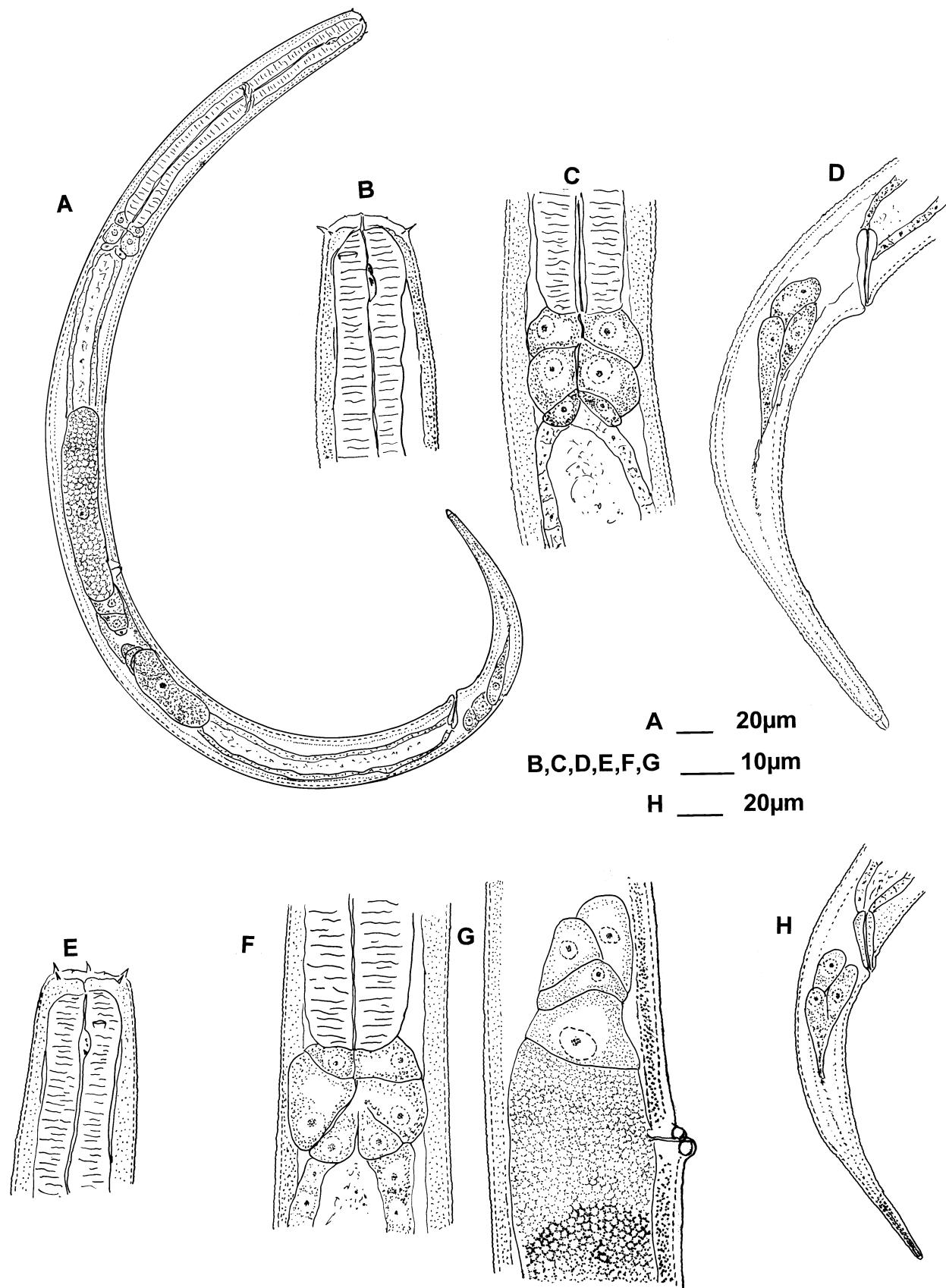
*Male:* Unknown.

*Type Locality and Habitat:* Moss on the trunk of a pirul tree, *Schinus molle* L., in La Purificación, Tepetitla, Texcoco, México State, México, N $19^{\circ}31'01.7''$ , W $98^{\circ}48'37.7''$ , 2421 m above sea level. Collector I. Cid del Prado-Vera on August 10, 2008.

*Type Specimens:* Holotype female (CNHE 9053) and paratypes (CNHE 9054) in CNHE; paratypes in CNCP and NCE.

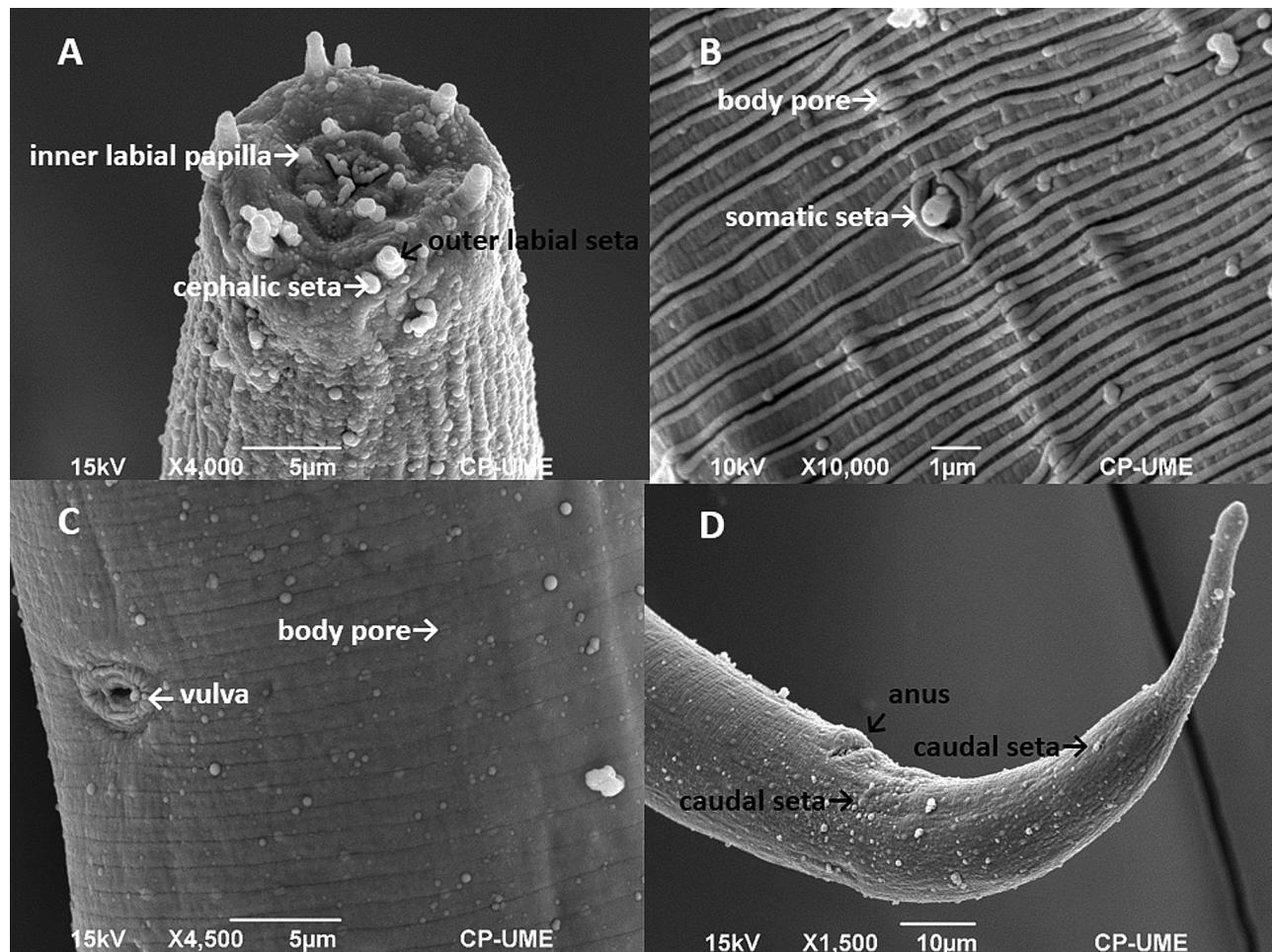
*Etymology:* The specific epithet refers to México, the type locality of the species. *Diagnosis and Relationships:* *Tripylella mexicana* sp. n. is characterized by the small size of the body, the finely-striated cuticle that has many anastomoses, pores and small setae along the body, the short pharynx, short tail, and the presence of a readily-visible excretory pore, the anterior position of the subventral teeth in relation to the small dorsal tooth and the teeth

contained in adjacent stomal chambers, the absence of cervical setae, the vulval lips not protruding, and the vulva with small oval sclerotized pieces.



**FIGURE 1.** *Tripylella mexicana* sp. n. Female (A.D). A. Entire body; B: Anterior end; C: Pharyngo-intestinal junction; D: Tail. *Tripylella muscusi* sp. n. Female (E.H) E: Anterior end; F: Pharyngo-intestinal junction; G: Vulva region; H: Tail.

*Tripylella mexicana* sp. n. is close to *T. iucunda* Andrassy, 2008 in the lengths of the body, setae, pharynx, and tail. It differs in having a finely-striated cuticle vs. the smooth cuticle of *T. iucunda*, in having a smaller dorsal tooth, in the presence of an excretory pore and of abundant body pores, in the spherical (20–27 x 20–26  $\mu\text{m}$ ) vs. discoid (17–20 x 9–12  $\mu\text{m}$ ) cardia, and in the small oval vs. larger heart-shaped sclerotized pieces in the vulva. As a caveat, some of the minute surface characters described are only evident by SEM which was not used in the Andrassy (2008) descriptions.



**FIGURE 2.** *Tripylella mexicana* sp. n. Female (A.D). A: Cephalic region, dorsal view; B: Annuli with anastomoses, somatic setae and body pores; C: Vulva, latero-ventral view; D: Tail, lateral view.

*Tripylella mexicana* sp. n. is similar to *T. intermedia* (Bütschli, 1873) Brzeski & Winiszewska-Ślipińska, 1993 in the shape of the tail, the size of the outer labial and cephalic setae, the short gonads, and the finely-striated cuticle. It differs in the presence of body pores, the distance of the excretory pore from the anterior end, 80–93 ( $86 \pm 1.4$ )  $\mu\text{m}$  vs. 94–101  $\mu\text{m}$ , in the length of the body 0.63–0.82 ( $0.74 \pm 0.02$ ) mm vs. 0.81–0.96 mm, in the c ratio, 5.5–7.5 ( $6.3 \pm 0.1$ ) vs. 7.3–8.3, in the relative length of the cylindrical anterior part of the tail 42–65 vs. 60–67% of tail length, and in the absence of setae on the tail of *T. intermedia*.

Also, *T. mexicana* is close to *T. muscisi* in the size of the outer labial and cephalic setae, in the presence of abundant body pores, the position of the vulva, the subventral teeth anterior to the dorsal tooth, and the finely-striated cuticle. It differs in that *T. mexicana* is a smaller nematode than *T. muscisi*, with body length 0.63–0.82 ( $0.74 \pm 0.02$ ) mm vs. 0.80–1.02 ( $0.94 \pm 0.02$ ) mm, pharynx length 141–184 ( $160 \pm 3.6$ )  $\mu\text{m}$  vs. 184–207 ( $201 \pm 1.8$ )  $\mu\text{m}$ , in the length of the tail, 99–132 ( $117 \pm 2.8$ ) vs. 123–160 ( $140 \pm 2.9$ )  $\mu\text{m}$ , in the distance from vulva to anus 222–292 ( $256 \pm 7.1$ ) vs. 273–380 ( $339 \pm 7.8$ )  $\mu\text{m}$ , in smaller values of indices a and c 20.25 ( $22 \pm 0.3$ ) and 4.1–5.1 ( $4.7 \pm 0.08$ ) vs. 24–29 ( $26 \pm 0.4$ ) and 4.9–5.7 ( $5.3 \pm 0.06$ ), respectively, and in the oval shape of sclerotized pieces around the vagina vs. pear shaped pieces in *T. muscisi*.

*Molecular Characteristics:* See Molecular Diagnostics section.

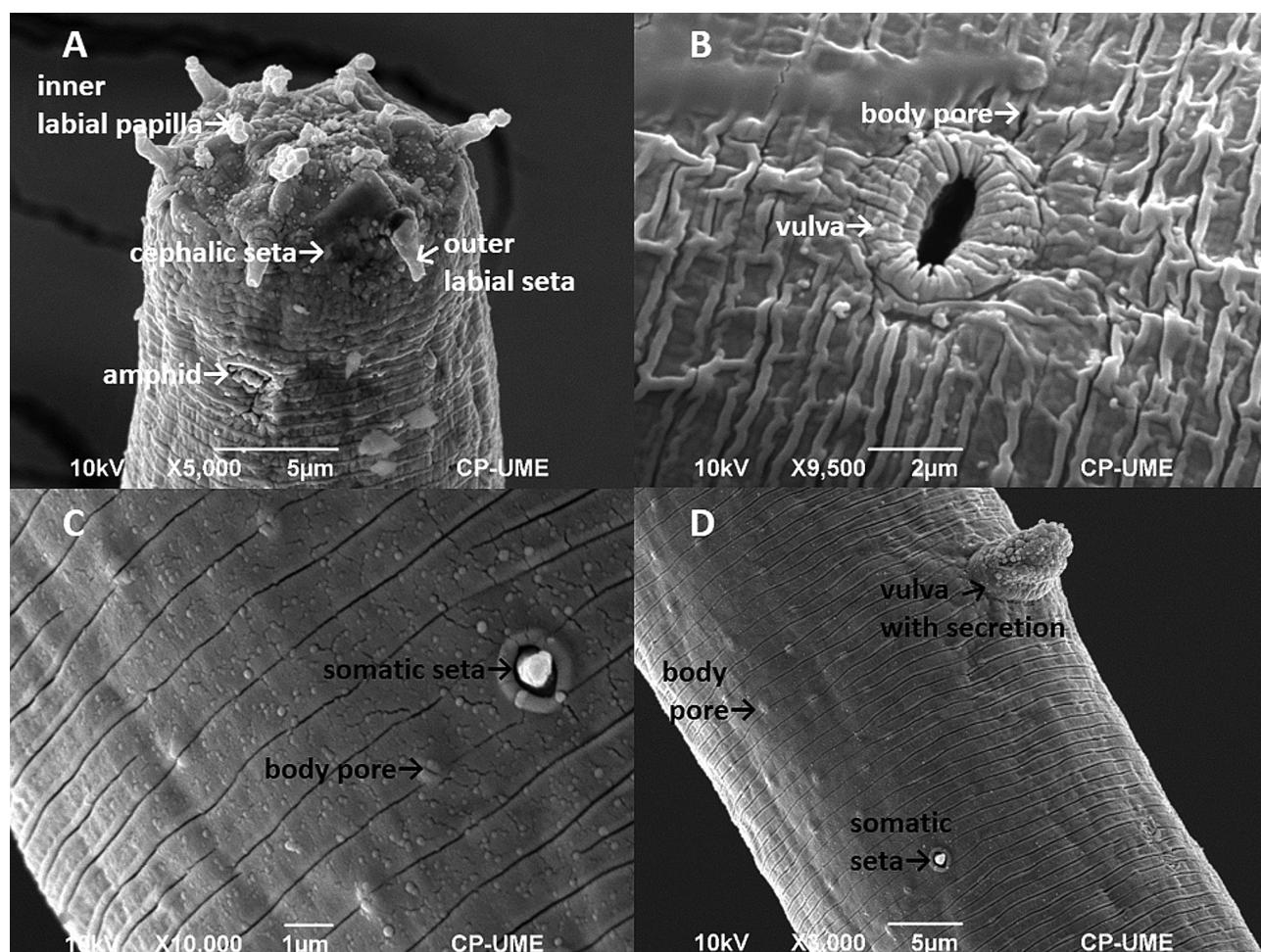
*Comments:* We have examined specimens of a population of *Tripylella* sp. from Pueblo Nuevo, Hidalgo State, México. *Tripylella mexicana* sp. n. is very close to that population in the size of the body, size of the pharynx and tail, the position of the vulva, and in that the subventral teeth are usually posterior to the dorsal tooth although in some specimens they appear to be anterior. It differs in the size of the cardia 20–27 vs. 12–21 µm long and 20–26 vs. 17–24 µm wide, in the length of the narrower portion of the tail 38–57 vs. 40–70 µm, and in having a more finely-striated cuticle. We consider the Pueblo Nuevo population to be a variant of *T. mexicana* sp. n..

### *Tripylella muscusi* sp. n.

(Fig. 1. E–H, Fig. 3 A–D)

*Measurements:* See Tables 1 and 2.

*Female* (n=14): Body an open C shape after relaxation. Cuticle 1 µm thick, with fine striations and sparse anastomoses along the body. Abundant pores and a few small setae present along and around the body. Labial region rounded 14–15 (14±0.1) µm wide; inner labial papillae conical; outer labial setae conical, 3 µm long; cephalic setae small, 1 µm long, and separated from outer labial setae by <1 µm so that there appears to be a single whorl of six longer and four shorter setae. The single stomal chamber contains two subventral teeth 1–2 (1.9±0.07) µm anterior to the small dorsal tooth, which is 10–17 (15±0.4) µm from the head end; amphids caliciform, 8–15 (10.9±0.5) µm from anterior end of the body; pharynx-vulva distance 212–292 (258±5.9) µm; cardia very conspicuous, 16–29 (24±1.0) µm long and 20–29 (24±1.0) µm wide, composed of six cells with the median cells largest; excretory pore observed in six specimens at 21–110 (82±9.3) µm from the anterior end.



**FIGURE 3.** *Tripylella muscusi* sp. n. Female (A.D). A: Cephalic region, frontal view. B: Vulva and body pores, ventral view. C: Annuli with anastomoses and body pores, lateral view. D: Vulva, somatic setae and body pores, latero-ventral view.

Female gonads short and reflexed, the anterior 61–118 ( $79\pm4.9$ )  $\mu\text{m}$  long and 6.5–13 (8.3±0.5)% of the body length and the posterior 33–123 ( $84\pm9.0$ )  $\mu\text{m}$  long and 3.3–13 (7.8±1.1)% of the body length; vulva with slightly protruding lips ornamented with longitudinal striations, and conspicuous pear-shaped cuticular structures surrounding the vagina; vulva 274–379 ( $339\pm7.8$ )  $\mu\text{m}$  anterior to the anus; rectum 20–25 (22±0.5)  $\mu\text{m}$  long or 0.7–1.0 (0.9±0.02) times anal body diameter; tail cylindroid, 122–160 ( $140\pm2.9$ )  $\mu\text{m}$  long and 14–17 (15±0.2)% of body length, narrowing abruptly at 27–54 (39±1.8)% of its length, ending in a small spinneret, 2–3  $\mu\text{m}$  long.

*Male:* Unknown.

*Type Locality and Habitat:* Moss on trunk of oak tree, *Quercus peduncularis* Née, in a forested area of San Pablo Ixayo, Texcoco, México State, México N19°28'06.8", W98°47'15.2", 2587 m above sea level. Collector: I. Cid del Prado-Vera on October 10, 2008.

*Type Specimens:* Holotype female (CNHE 9055) and paratypes (CNHE 9056) in CNHE; paratypes in CNCP and NCE.

*Etymology:* The specific epithet indicates the type locality habitat of the species is moss.

*Diagnosis and Relationships:* *Tripylella muscusi* sp. n. is characterized by the body length (average 0.94 mm), abundant body pores and a few somatic setae along the body, pharynx length (average 201  $\mu\text{m}$ ), tail length (average 140  $\mu\text{m}$ ), the thin, finely-striated cuticle with anastomoses, the anterior position of the subventral teeth in relation to the small dorsal tooth, with all teeth in a single stomal chamber, the absence of cervical setae, the presence of an excretory pore, the slightly protruding vulval lips and pear-shaped sclerotized pieces in the vaginal region.

*Tripylella muscusi* sp. n. is similar to *T. intermedia* (Bütschli, 1873) Brzeski & Winiszewska-Ślipińska, 1993 in the length of the body, abundant body pores, the shape of the tail, the position of the excretory pore, and the sizes of the anterior and posterior cephalic setae. It differs in having a single stomal chamber, the anterior position of the subventral teeth, the position of the vulva 47–51 (49±0.3)% vs. 51–52%, in the c ratio, 6.0–7.2 (6.7±0.09) vs. 7.3–8.3 (7.7) and in the c ratio, 4.9–5.6 (5.3±0.06) vs. 3.6–4.5 (4.1). *Tripylella muscusi* sp. n. is close to *T. mexicana* n. sp. but distinguished as indicated in the description of that species.

*Molecular Characteristics:* See Molecular Diagnostics section.

*Comments:* We examined specimens of a population of *Tripylella* sp. from San Diego Huehuecalco, Amecameca, México State, México. They resemble *Tripylella muscusi* sp. n. in the body and pharynx length, position of the vulva, the anterior position of the subventral teeth in relation to the dorsal tooth, the length of the tail and the abrupt narrowing of the tail. The Amecameca population differed in the distance of amphid apertures to the anterior end of body, in the distance of posterior end of pharynx to anus, in the size of narrow part of the tail and the smaller protrusion of the vulval lips. We consider that the Amecameca population does not differ morphologically from *T. muscusi* sp. n..

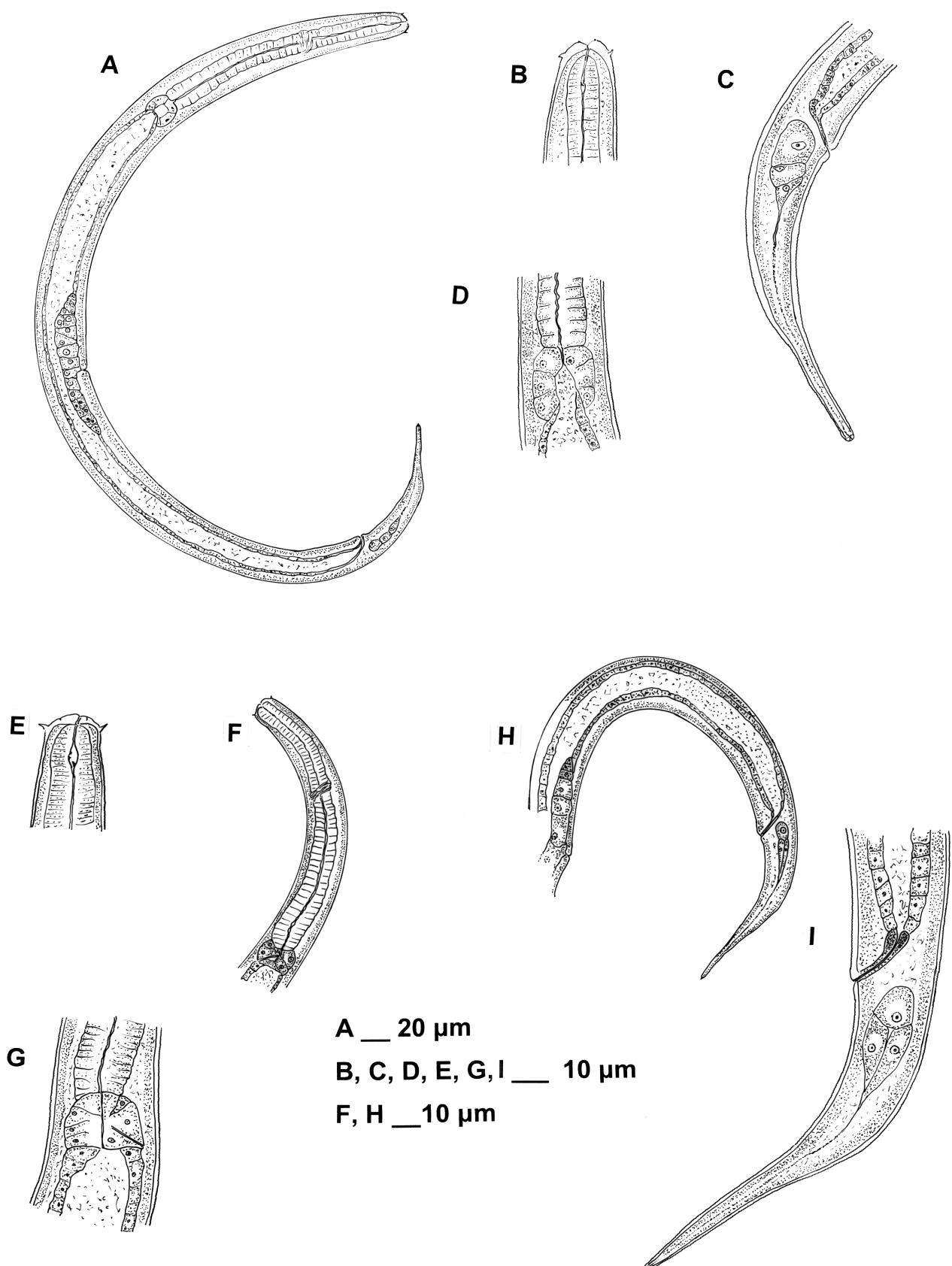
### *Tripylella quitoensis* sp. n.

(Fig. 4 A–D, Fig. 5 A–D)

*Measurements:* See Tables 1 and 2.

*Female* (n=10): Body C-shaped upon relaxation and fixation. Cuticle 2  $\mu\text{m}$  thick, with fine striations. Setae and pores present posterior to the cervical region, scattered along the body. Head region rounded, 12–16  $\mu\text{m}$  wide; inner labial papillae conical and very small; outer labial setae conical 1.8–2.9  $\mu\text{m}$  long; cephalic setae small, <1  $\mu\text{m}$  long and very difficult to see, separated from the outer labial setae by <1 $\mu\text{m}$  so that there appears to be a single whorl of six longer and four shorter setae. The dorsal tooth is 9–16 (12±0.7)  $\mu\text{m}$  from the anterior end and 1–3 (1.9±0.2)  $\mu\text{m}$  posterior to the two subventral teeth; the dorsal tooth and subventral teeth are in the same stomal chamber. Amphids caliciform, 7–8  $\mu\text{m}$  from anterior end of the body. Cervical setae absent. Distance between base of pharynx and vulva 160–238 (196±8.4)  $\mu\text{m}$ . Cardia very conspicuous, 15–25 (21±1.1)  $\mu\text{m}$  long and 15–26 (22±1.0)  $\mu\text{m}$  wide, comprised of six cells, the anterior and posterior of medium size and the median cells larger. Excretory pore not observed.

Female gonads reflexed, the anterior 36–118 ( $70\pm8.6$ )  $\mu\text{m}$  long and 5.1–15.6 (9.7±1.1)% of the body length and the posterior 47–80 (59±3.3)  $\mu\text{m}$  long and 7.1–11.3 (8.3±0.4)% of the body length. Vulva 226–292 (253±6.8)  $\mu\text{m}$  anterior to the anus, without protruding lips and with very small oval cuticular structures. Rectum 11–25 (16±1.6)  $\mu\text{m}$  long, 0.9–1.9 (1.4±0.1) times anal body diameter, anus with protruding lips. Tail cylindroid, narrowing abruptly at 54–64 (58±1.0)% of its length and ending in a spinneret 2–4  $\mu\text{m}$  long.

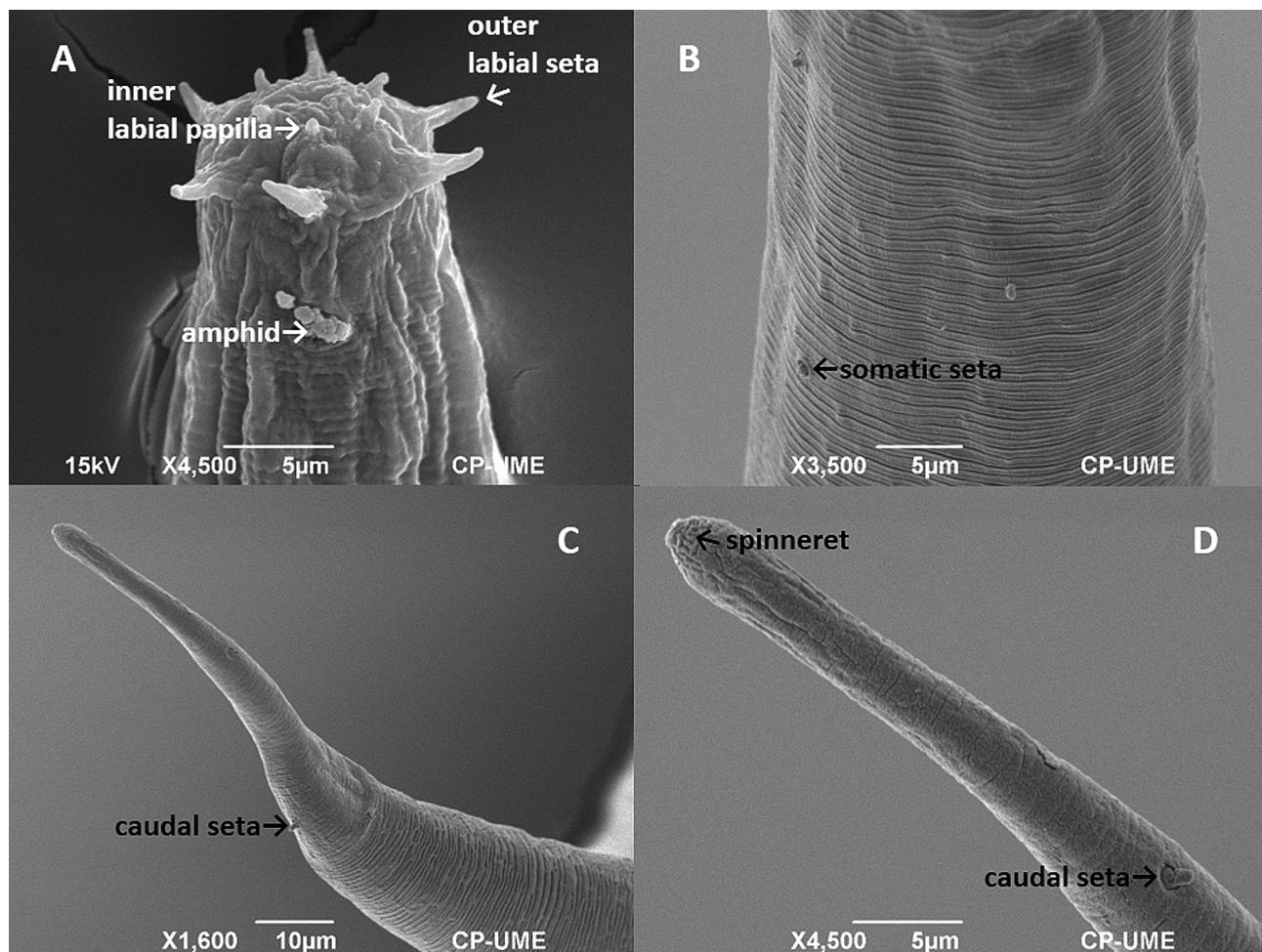


**FIGURE 4.** *Tripylella quitoensis* sp. n. Female (A.D). A. Entire body; B: Anterior end; C: Pharynx intestinal junction; D: Tail. *Tripylella fatimaensis* sp. n. Female (E.I) E: Anterior end; F: Anterior end; G: Pharyngo-intestinal junction; H: Posterior end; I: Tail.

*Male*: Unknown.

*Type Locality and Habitat*: Moss on trunk of tree, in the botanical garden, Parque La Carolina, Quito, Ecuador, S $0^{\circ}11'10.8''$ , W $78^{\circ}29'07.7''$ , 2779 m above sea level.

*Type Specimens*: Holotype female (CNHE 9057) and paratypes (CNHE 9058) in CNHE; paratypes in CNCP and NCE.



**FIGURE 5.** *Tripylella quitoensis* sp. n. Female (A.D). A: Anterior end lateral view. B: Annuli and somatic setae. C: Tail lateral view. D: Tapered region of tail and caudal setae.

*Etymology*: The specific epithet refers to the geographic location of the type species, Quito, Ecuador.

*Diagnosis and Relationships*: *Tripylella quitoensis* sp. n. is characterized by the short body length (0.7–0.8 mm), the small outer labial setae, 1.8–2.9 µm long, the dorsal tooth and subventral teeth in the same stomal chamber, the subventral teeth anterior to the dorsal tooth, the short tail (72–112 µm), the absence of an excretory pore, the presence of body pores, the finely-striated cuticle, the non-protruding vulval lips, and the vulva with very small oval sclerotized pieces.

*Tripylella quitoensis* sp. n. is close to *T. mexicana* sp. n. in the lengths of the body and pharynx, in the position of the vulva, the finely-striated cuticle and in the anterior position of the subventral teeth and their presence in the same stomal chamber as the dorsal tooth. It differs in having a thicker cuticle, the larger index c, 6.9–10.1 (7.5±0.3) vs. 5.5–7.5 (6.3±0.2), in the length of the tail 71–112 (98±3.8) vs. 99–132 (117±2.8) µm, and in the proportion of the tail that is reduced in diameter 36–60 (45±2.2)% vs. 28–48 (41±1.5)%.

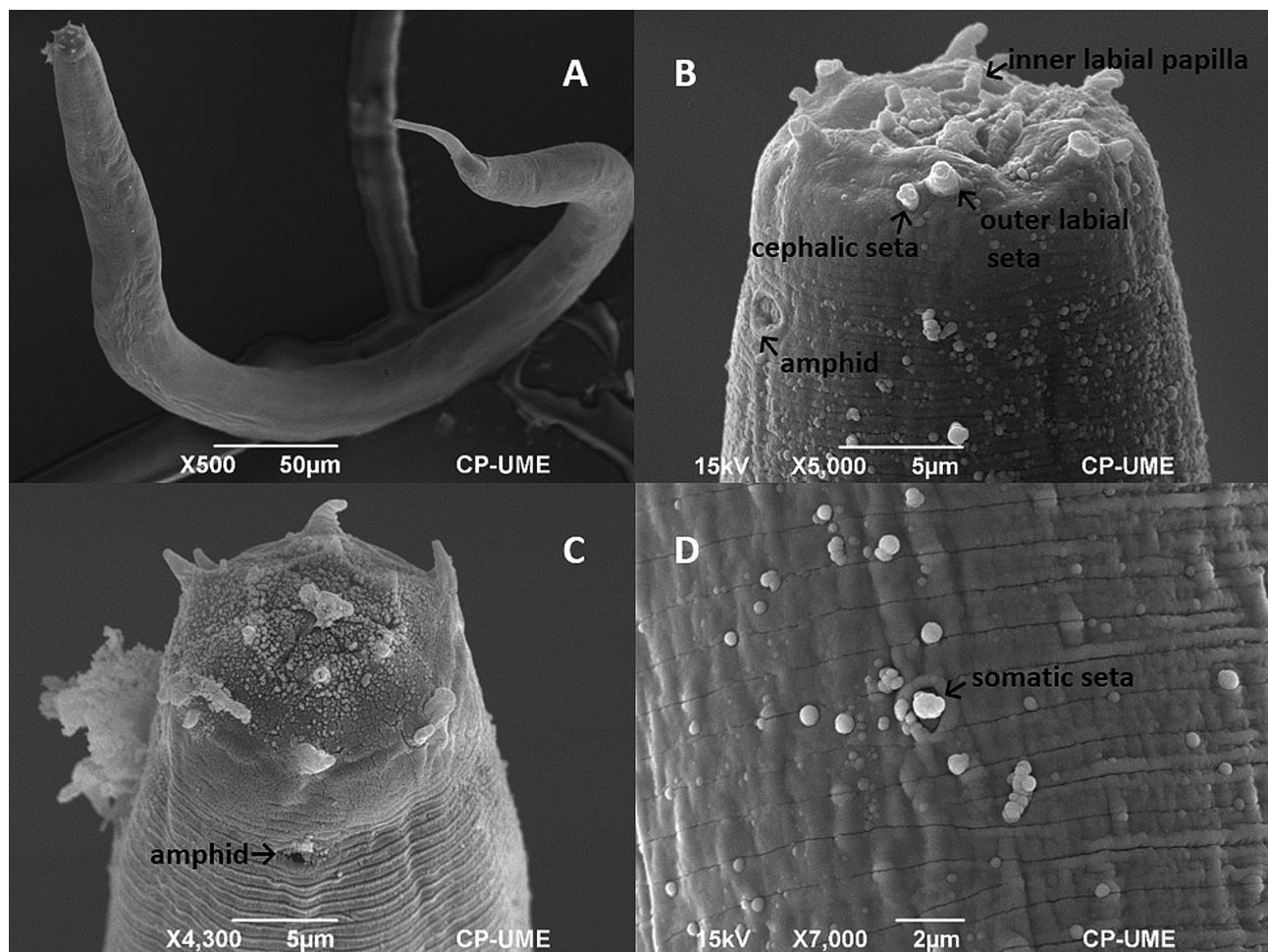
*Tripylella quitoensis* sp. n. is close to *T. iucunda* Andrassy, 2008 in the short pharynx and tail and small somatic setae, and in the posterior position of the subventral teeth. It differs in having a finely-striated cuticle vs. the smooth cuticle of *T. iucunda*, in the tail length 71–112 (98±3.8) vs. 105–120 µm, in the presence of body pores, in the absence of cervical setae vs. the two present in *T. iucunda*, in the length of the pharynx 160–189 (175±3.2) vs. 150–158 µm and in small oval vs. larger heart-shaped sclerotized pieces in the vulva.

*Molecular Characteristics*: There was no material available for molecular characterization of *T. quitoensis*.

*Tripylella fatimaensis* sp. n.  
 (Fig. 4 E–I, Fig. 6 A–D, Fig. 7 A–D)

*Measurements:* See Tables 1 and 2.

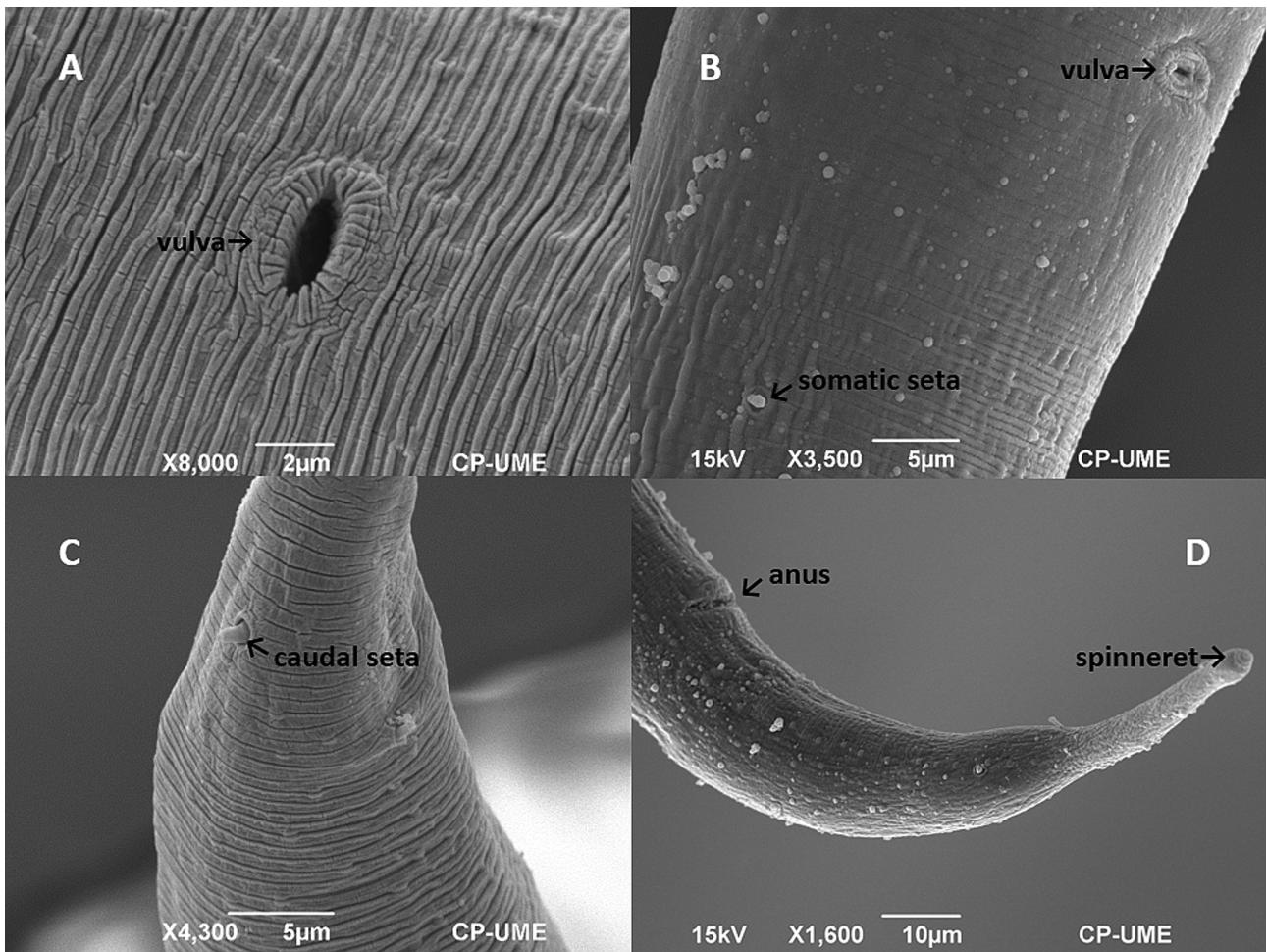
*Female* n=12: Body C-shaped upon relaxation and fixation. Cuticle thin, 1–2  $\mu\text{m}$ , with fine striations. Pores and setae present posterior to the cervical region, scattered along the body. Head region rounded, 14–16  $\mu\text{m}$  wide; inner labial papillae conical, very small; outer labial setae conical, 2–3  $\mu\text{m}$  long; cephalic setae small, 1  $\mu\text{m}$  long, and separated from the anterior cephalic setae by <1  $\mu\text{m}$  so that they appear as a single whorl of six longer and four shorter setae. The dorsal tooth is 13–16 ( $15 \pm 0.31$ )  $\mu\text{m}$  from the anterior end and 2–4 ( $3 \pm 0.1$ )  $\mu\text{m}$  posterior to the two subventral teeth. The dorsal tooth and subventral teeth are in the same stomal chamber. Amphids caliciform, 7–12  $\mu\text{m}$  from anterior end of the body. Pharynx-vulva distance 132–255 ( $190 \pm 9.4$ )  $\mu\text{m}$ . Cardia very conspicuous, discoid, 16–26 ( $19 \pm 0.8$ )  $\mu\text{m}$  long and 15–27 ( $22 \pm 1.1$ )  $\mu\text{m}$  wide, comprised of six cells, the anterior and posterior of medium size and the median cells larger. Excretory pore at 58–89  $\mu\text{m}$  from the anterior end.



**FIGURE 6.** *Tripylella fatimaensis* sp. n. Female (A.D). A: Entire body; B: Cephalic region, dorsal-lateral view; C: Face view, cephalic region; D: Somatic seta.

Female gonads reflexed, the anterior 40–115 ( $76 \pm 7.4$ )  $\mu\text{m}$  long and 6–22.9 ( $11 \pm 1.39$ )% of the body length and the posterior 39–86 ( $66 \pm 5.3$ )  $\mu\text{m}$  long and 6.5–11.4 ( $8.7 \pm 0.6$ ) % of the body length. Vulva 189–292 ( $254 \pm 8.9$ )  $\mu\text{m}$  anterior to the anus, with slightly protruding lips and with very small oval cuticular structures. Rectum 17–22 ( $20 \pm 0.5$ )  $\mu\text{m}$  long, 0.8–1.4 ( $1.2 \pm 0.1$ ) times anal body diameter. Tail cylindroid, narrowing abruptly at 50–60 ( $56 \pm 0.9$ )% of its length, ending in a spinneret 2–4  $\mu\text{m}$  long; three pairs of postanal caudal setae present: one pair latero-ventral, one pair latero-dorsal and one pair ventral.

*Male:* Unknown.



**FIGURE 7.** *Tripylella fatimaensis* sp. n. Female (A.D). A: Vulva and body pores. Ventral view; B: Vulva and body setae. Latero-ventral view; C: Caudal setae. Lateral view; D: Tail. Lateral view.

**Type Locality and Habitat:** Moss on a tree trunk in the garden of the Santuário de Fátima, Fátima, Portugal, N39°37'52.0", W8°40'29.9", 350 m above sea level. Collector I. Cid del Prado-Vera on September 10, 2011.

**Type Specimens:** Holotype female (CNHE 9059) and paratypes (CNHE 9060) in CNHE; paratypes in CNCP and NCE.

**Etymology:** The specific epithet refers to the geographic location of the type species, Fátima, Portugal.

**Diagnosis and Relationships:** *Tripylella fatimaensis* sp. n. is characterized by the short body (0.59–0.81) mm, by the length of the pharynx, length of the tail (90–128  $\mu$ m), length of the narrower portion of the tail (45–58  $\mu$ m), the presence of very small oval cuticular structures in the vulva region and by the slightly protruding vulval lips.

*Tripylella fatimaensis* sp. n. is very close to *T. quitoensis* sp. n. in the length of the tail, in the proportional length of the wider region of the tail, 53–60 vs. 54–64%, in the position of the vulva and in the absence of cervical setae. It differs from *T. quitoensis* sp. n. in the length of the tail (90–128 (110 $\pm$ 2.8)  $\mu$ m vs. 71–112 (98 $\pm$ 3.8)  $\mu$ m), in the length of the narrower portion of the tail (40–58 (49 $\pm$ 1.1)  $\mu$ m vs. 35–45 (42 $\pm$ 1.2)  $\mu$ m) and in the index c (6.2–7.2 (6.7 $\pm$ 0.1) vs. 6.9–10.1 (7.5 $\pm$ 0.3). Also, *T. fatimaensis* sp. n. is close to *T. iucunda* Andrassy, 2008, in the width of the head, the shape of the dorsal tooth and its distance from the anterior end of the body, the position of the vulva and the shape of the tail. It differs in the length of the body 0.59–0.80 (0.73 $\pm$ 19.9) vs. 0.68–0.75, pharynx 160–207 (180 $\pm$ 3.7)  $\mu$ m vs. 150–158  $\mu$ m, in the index c 6.2–7.2 (6.7 $\pm$ 0.1) vs 6.0–6.5, in the length of tail 90–128 (110 $\pm$ 2.8) vs. 105–120  $\mu$ m and the annulated vs. smooth cuticle in *T. iucunda*.

**Molecular Characteristics:** There was no material available for molecular characterization of *T. fatimaensis*.

***Tripylella dentata* sp. n.**

(Fig. 8 A–E, Fig. 9 A–D)

*Measurements:* See Tables 1 and 2.

*Female* (n=20): Body C-shaped after heat relaxation and fixation. Cuticle 2–3  $\mu\text{m}$  thick, with fine striations and without anastomoses. Many pores and scattered setae present posterior to the cervical region along body. Head region rounded, slightly flattened anteriorly, 14–17 ( $15\pm0.2$ )  $\mu\text{m}$  wide, smooth and slightly offset from the body contour. Six lips, each with one conical inner labial papilla at the base; six conical outer labial setae, 2–3 ( $2.9\pm0.1$ )  $\mu\text{m}$  long; four small cephalic setae, 1  $\mu\text{m}$  long, appearing to be in a single whorl of six longer and four shorter setae. Mouth cavity with two adjacent stomal chambers, the anterior smaller than the posterior. The posterior chamber has two teeth, one larger dorsal and one subventral and the anterior small stomal chamber has two small subventral teeth. The subventral tooth and dorsal tooth in the posterior stomal chamber are, respectively, 14–16 ( $15\pm0.2$ ) and 15–18 ( $16.9\pm0.2$ )  $\mu\text{m}$  from the stomal aperture. The subventral teeth from the anterior stomal chamber are 2.0–8.0 ( $4.3\pm0.42$ )  $\mu\text{m}$ , anterior to the dorsal tooth in the posterior chamber. Amphids caliciform, 10–18 ( $11.8\pm0.4$ )  $\mu\text{m}$  from anterior end of the body. Pharynx to vulva distance 217–291 ( $240\pm4.1$ )  $\mu\text{m}$  and pharynx to anus distance 481–566 ( $519\pm5.9$ )  $\mu\text{m}$ . Cardia very large, 18–25 ( $21\pm0.5$ )  $\mu\text{m}$  long and 21–27 ( $24\pm0.4$ )  $\mu\text{m}$  wide, comprised of six cells, the anterior and posterior of medium size and the median cells larger. Two cervical setae are present in lateral positions. Excretory pore not observed.

Gonads reflexed, the anterior 75–113 ( $95\pm2.3$ )  $\mu\text{m}$  long and 6.7–13 (10.8±0.4)% of the body length and the posterior 28–132 ( $77\pm5.5$ )  $\mu\text{m}$  long and 0.7–14.9 (8.7±0.8)% of the body length; in one specimen only the anterior gonad was present, 94  $\mu\text{m}$  long. Vulva with slightly protruding lips and small oval cuticular structures. Rectum 12–26 ( $19\pm0.9$ )  $\mu\text{m}$  long and 0.8–1.7 (1.2±0.06) times anal body diameter which is 12–22.5 ( $22.1\pm0.8$ )  $\mu\text{m}$ . Tail 104–126 ( $115\pm1.9$ )  $\mu\text{m}$  long, the posterior narrower portion of the tail is 42–55 ( $49.5\pm1.1$ )  $\mu\text{m}$  long and 34.4–52.7 (43.3±1.1)% of the tail length, ending in a spinneret 1–3 ( $2.2\pm0.1$ )  $\mu\text{m}$  long. Three pairs of caudal setae present, one pair in a lateral position posterior to the anus position, the second pair ventral and close to the end of the wide portion of the tail, and the third pair latero-dorsal at 50% of the length of the narrower part of tail.

*Male:* Unknown.

*Type Locality and Habitat:* Moss on trunk of California buckeye tree, *Aesculus californica* Nutt, at Bobcat Ranch, Winters, California USA, N38°32'35.6", W122°03'04.6", 147 m above sea level. Collector: Howard Ferris on November 4, 2013.

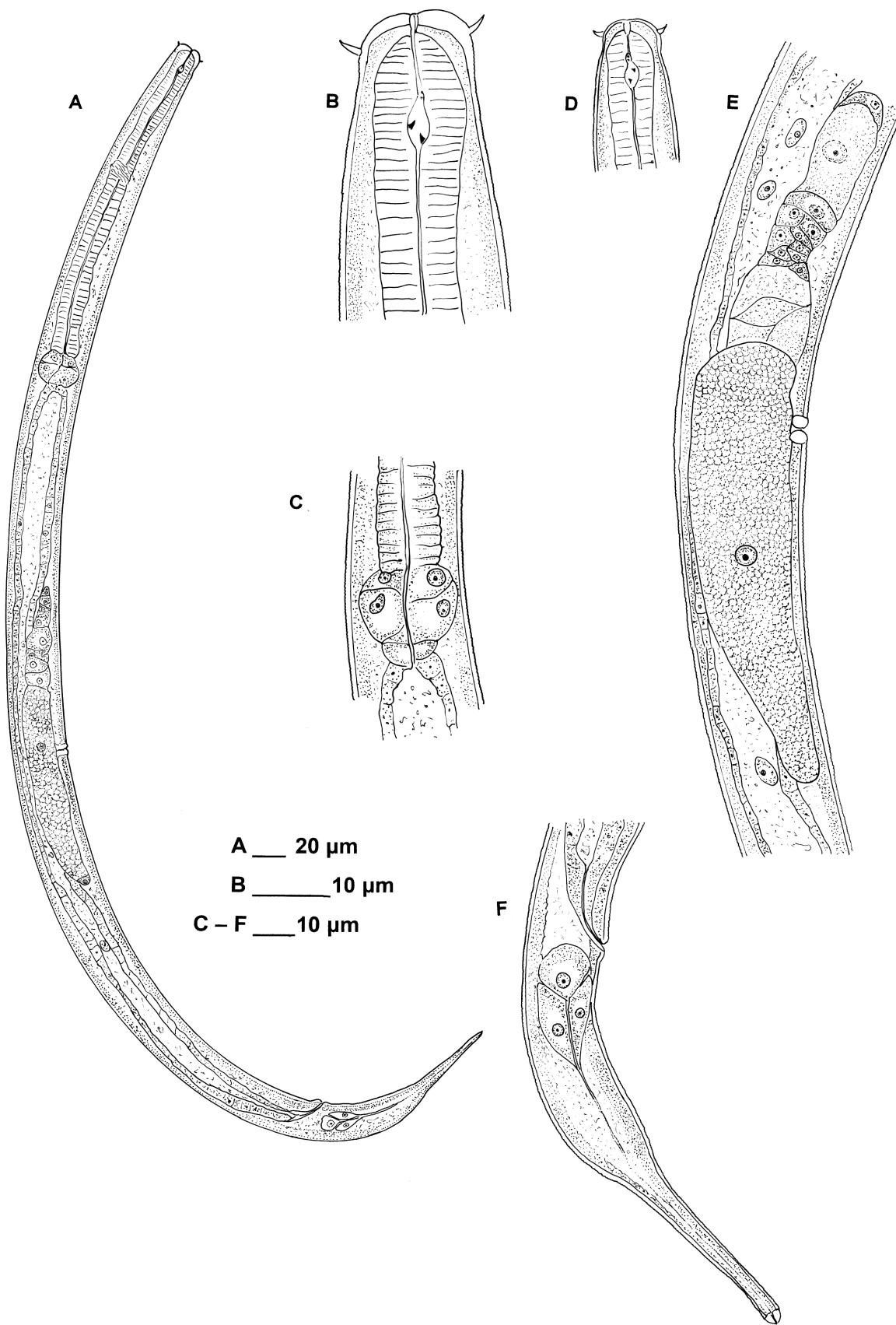
*Type Specimens:* Holotype female (CNHE 9061) and paratypes (CNHE 9062) in CNHE; paratypes in CNCP and NCE.

*Etymology:* The specific epithet refers to the presence of two conspicuous teeth in the posterior stomal chamber.

*Diagnosis and Relationships:* *Tripylella dentata* sp. n. is different from all species of the genus in the presence of four stomal teeth, two in a posterior stomal chamber, one dorsal and one ventral, and two subventral in an anterior chamber. It is characterized by the relatively thick cuticle (2–3  $\mu\text{m}$ ) with fine striations but without anastomoses, two lateral cervical setae, conspicuous body pores, the length of the pharynx, the slightly protruding vulval lips, and conspicuous oval sclerotized pieces in the vulva.

*Tripylella dentata* sp. n. is close to *T. minuscula* Andrássy, 2008 in the lengths of the body and pharynx, and in the presence of two cervical setae. It differs in the presence of two teeth in the posterior stomal chamber, in the length and shape of the tail which tapers abruptly vs. the uniform taper of *T. minuscula*, in having a finely-striated cuticle vs. a smooth cuticle. Also, *T. dentata* sp. n. is close to *T. mexicana* sp. n. in the length of the outer labial setae, in the indices  $a$  and  $\epsilon$  (20–25 and 4.1–5.1, respectively, in *T. mexicana* sp. n. vs. 23–30 and 4.2–8.6 in *T. dentata* sp. n.), and the finely-striated cuticle. It differs in the length of the pharynx, 198–226 ( $209\pm2.2$ )  $\mu\text{m}$  vs. 141–184 ( $161\pm3.6$ )  $\mu\text{m}$ , in the length of the tail 104–126 ( $115\pm1.9$ )  $\mu\text{m}$  vs 99–132 ( $117\pm2.8$ )  $\mu\text{m}$  and in the distance between the pharynx and anus, 481–566 ( $519\pm5.9$ ) vs. 382–509 ( $455\pm11.4$ )  $\mu\text{m}$ .

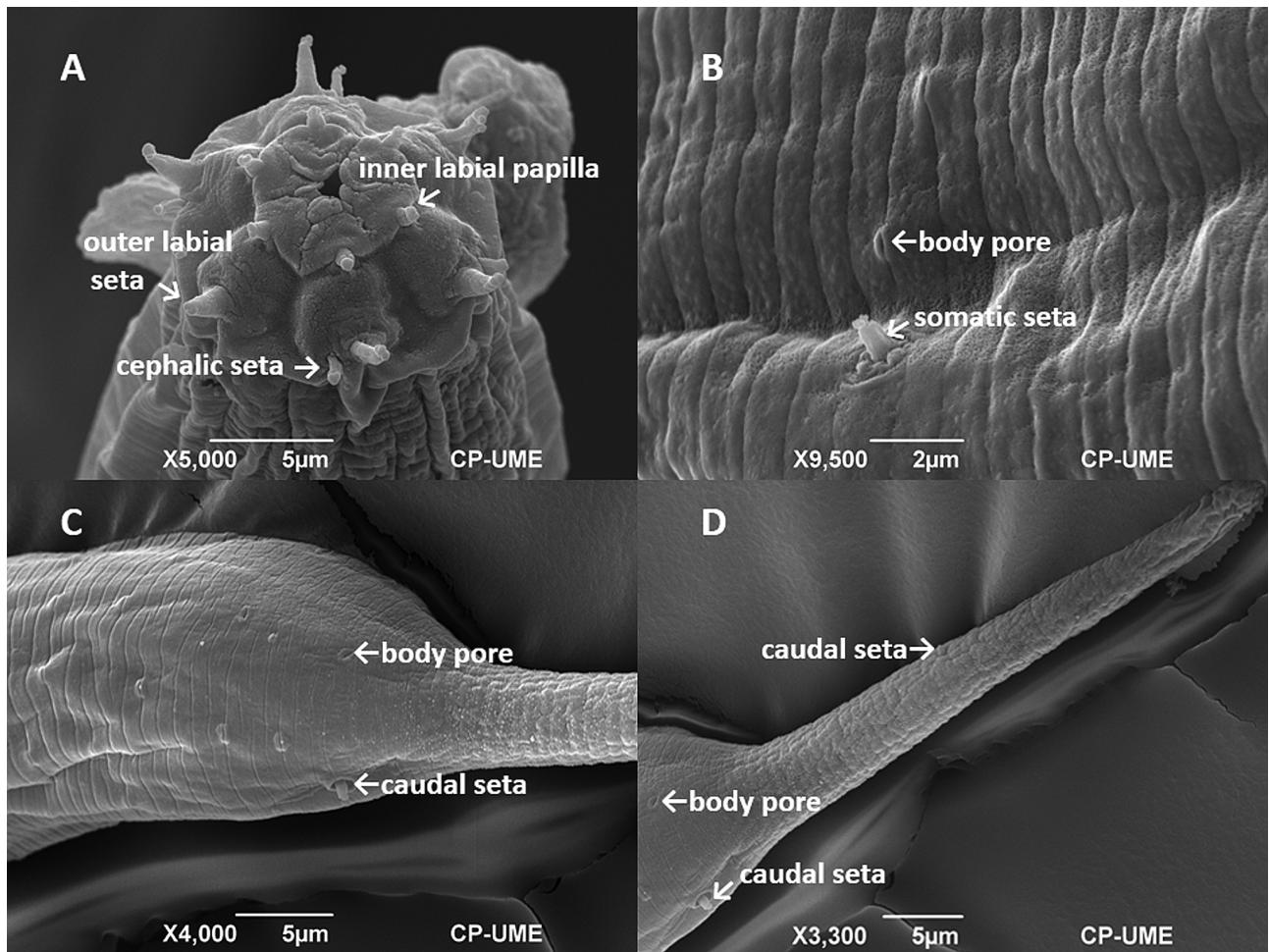
*Molecular Characteristics:* See Molecular Diagnostics section.



**FIGURE 8.** *Tripylella dentata* sp. n. Female (A-E). A. Entire body; B,D: Anterior end; C: Pharynx intestinal junction; E: Vulva region and gonads; F: Tail.

Table 3. Comparative morphometrics of *Tripyella* species. All measurements are in  $\mu\text{m}$ .

Species	L	a	b	c	$\epsilon$	V%	Cervical setae	Excretory pore	Subvent. teeth	References
Group 1: Tail tapers abruptly										
<i>incunda</i>	720 (680-750)	20 (20-21)	4.6 (4.5-4.7)	6.2 (6.0-6.5)	4.4 (4.2-4.6)	47 (47-49)	unknown	absent	anterior	Andrássy, 2008
<i>quitoensis</i>	723 (679-792)	23 (16-36)	4.2 (3.8-4.7)	7.5 (6.9-10)	4.6 (3.9-6.2)	51 (49-55)	absent	absent	anterior	This paper
<i>fatimensis</i>	733 (589-805)	23.5 (20-31)	4.1 (3.4-4.8)	6.7 (6.2-7.2)	4.7 (4.1-6.1)	50 (46-52)	absent	present	anterior	This paper
<i>mexicana</i>	740 (632-820)	22 (20-25)	4.6 (4.3-4.8)	6.3 (5.5-7.5)	4.7 (4.1-5.1)	49 (46-53)	absent	present	anterior	This paper
<i>subintermedia</i>	836 (789-903)	24.9 (22-27)	4.5 (4.3-4.7)	7.8 (6.9-8.2)	4.4 (3.7-5.1)	52 (50-53)	unknown	absent	anterior	Zeng et al 2014
<i>dentata</i>	850 (790-900)	26 (23-30)	4.1 (3.8-4.5)	7.4 (6.6-8.5)	5.4 (4.2-8.6)	53 (51-54)	2	absent	anterior	This paper
<i>muscusi</i>	940 (800-1000)	26 (24-29)	4.7 (4.3-4.9)	6.7 (6.0-7.2)	5.3 (4.9-5.7)	49 (47-51)	absent	present	anterior	This paper
<i>intermedia</i>	870 (810-960)	23 (21-24)	4.4 (4.3-4.6)	7.7 (7.3-8.3)	4.1 (3.6-4.5)	51 (51-52)	absent	present	posterior	Birzeski & W.S., 1993
Group 2: Tail tapers uniformly										
<i>maiuscula</i>	1080-1400	28-35	4.4-4.9	6.5-7.6	5.9-6.6	52-55	2	absent	anterior	Andrássy, 2006
<i>minuscula</i>	860-980	21-29	4.1-4.8	5.7-6.4	5.8-6.4	46-52	2	absent	posterior	Andrássy, 2006



**FIGURE 9.** *Tripylella dentata* sp. n. Female (A.D). A: Frontal view of cephalic region; B: Lateral cervical setae and body pores; C: Tail and body pores and setae; D: Taper area of tail and setae.

#### Key to the species of *Tripylella* (see Table 3)

1. Two large teeth in the posterior stomal chamber and two subventral teeth in a smaller anterior chamber ..... *T. dentata* sp. n.  
Stoma with one dorsal and two subventral teeth..... 2
2. Tail cylindrical then narrowing abruptly; outer labial setae 2.0–3.0  $\mu\text{m}$  long ..... 3  
Tail tapering evenly; outer labial setae >3  $\mu\text{m}$  long; subventral teeth anterior or posterior to dorsal tooth ..... 9
3. Subventral teeth anterior to dorsal tooth ..... 4  
Subventral teeth posterior to dorsal tooth ..... 8
4. Dorsal tooth large; excretory pore absent ..... *T. subintermedia* Zhao et al., 2014  
Dorsal tooth small; excretory pore present or absent..... 5
5. Pharynx length 141–189  $\mu\text{m}$  ..... 6  
Pharynx length 160–207  $\mu\text{m}$  ..... 7
6. Pharynx length 141–184(161)  $\mu\text{m}$ ; posterior of pharynx to vulva 141–282(205)  $\mu\text{m}$ ; excretory pore present, 80–93(85.6)  $\mu\text{m}$  behind head ..... *T. mexicana* sp. n.  
Pharynx length 160–189(175)  $\mu\text{m}$ ; posterior of pharynx to vulva 160–238(196)  $\mu\text{m}$ ; excretory pore absent ..... *T. quitoensis* sp. n.
7. Pharynx length 160–207(180)  $\mu\text{m}$ ; posterior of pharynx to vulva 132–255(191)  $\mu\text{m}$ ; tail length 90–128(110)  $\mu\text{m}$ ; excretory pore 58–86(73.8)  $\mu\text{m}$  behind head ..... *T. fatimaensis* sp. n.  
Pharynx length 184–207(200)  $\mu\text{m}$ ; posterior of pharynx to vulva 212–292(258)  $\mu\text{m}$ ; tail length 122–160(140)  $\mu\text{m}$ ; excretory pore 95–110(82)  $\mu\text{m}$  behind head ..... *T. muscui* sp. n.
8. Body length 0.81–0.96 (0.87) mm; head width 18–19  $\mu\text{m}$  ..... *T. intermedia* (Bütschli, 1873) Brzeski & Winiszewska-Ślipińska, 1993  
Body length 0.68–0.75(0.72) mm; head width 15–16  $\mu\text{m}$  ..... *T. iucunda* Andrassy, 2008

9. Body length 1.1–1.4 mm; outer labial setae 7–9 µm long, excretory pore absent; subventral teeth anterior to dorsal tooth.....  
..... *T. maiuscula* Andrassy, 2006  
Body length 0.86–0.98 mm; outer labial setae 3.4 µm long; excretory pore present; subventral teeth posterior to dorsal tooth.....  
..... *T. minuscula* Andrassy, 2006

## Discussion

### Molecular Diagnostics

Sequences were obtained for three of the five new species: *T. mexicana* (two individuals), *T. dentata*, and *T. muscusi*. No specimens of *T. quitoensis* or *T. fatimaensis* were available that had been preserved for molecular study. Direct sequencing of the PCR products yielded high-quality electropherograms. There were three sequence site polymorphisms in *T. dentata*, one in *T. muscusi*, and one (in one individual) of *T. mexicana*. New SSU sequences were deposited in GenBank (Accession numbers in Fig. 10).

Seven of eight *Tripylella* sequences formed a monophyletic group with 100% posterior probability (Fig. 10). One taxon, *Tripylella* sp. isolate R10G was not a member of this clade, but was part of the larger clade that included *Tripylella*, *Tobrilus*, and *Tripyla*. This R10G sequence is represented in GenBank as *Tripylella*, but was not diagnosed to species. It is possible that the unexpected position of isolate R10G is due to sequencing errors, or perhaps misdiagnosis. The sister taxon to the clade of seven *Tripylella* taxa was *Tobrilus gracilis*. This sister-taxon grouping of *Tripylella* and *Tobrilus* was reported previously (Cid del Prado-Vera *et al.*, 2012; Zhao *et al.*, 2014). Lack of resolution within the *Tripylella* clade (seven taxa) appears to result from low sequence divergence between taxa.

*Tripylella dentata* was distinct in nucleotide sequence when compared to all seven other congeners (including R10G), with a mean of 4.30 sequence differences in pairwise comparisons. *Tripylella subintermedia* was also distinct from all other *Tripylella* taxa (mean of 9.32 differences). *Tripylella muscusi* was distinct from all taxa except *T. mexicana* and *Tripyla* sp. AY284737, thus, *T. muscusi*, *T. mexicana* and *Tripyla* sp. AY284737 cannot be distinguished by this region of SSU sequence. In this case, support for the separate species status of *T. muscusi* and *T. mexicana* comes from morphological features rather than the SSU sequences.

### Morphometrics and Morphological Features

With the description of new species, we can separate the genus into two groups (Table 3). In one group the tail tapers abruptly at 33–50% of its length:

*Tripylella intermedia* (Bütschli, 1873) Brzeski & Winiszewska-Ślipińska, 1993

Syn. *Tripyla intermedia* Bütschli, 1873

*Paratripyla intermedia* (Bütschli, 1873) Brzeski, 1964

*T. iucunda* Andrassy, 2008

*T. subintermedia* Zhao, Xi & Xu, 2014

*T. mexicana* sp. n.

*T. muscusi* sp. n.

*T. quitoensis* sp. n.

*T. fatimaensis* sp. n.

*T. dentata* sp. n.

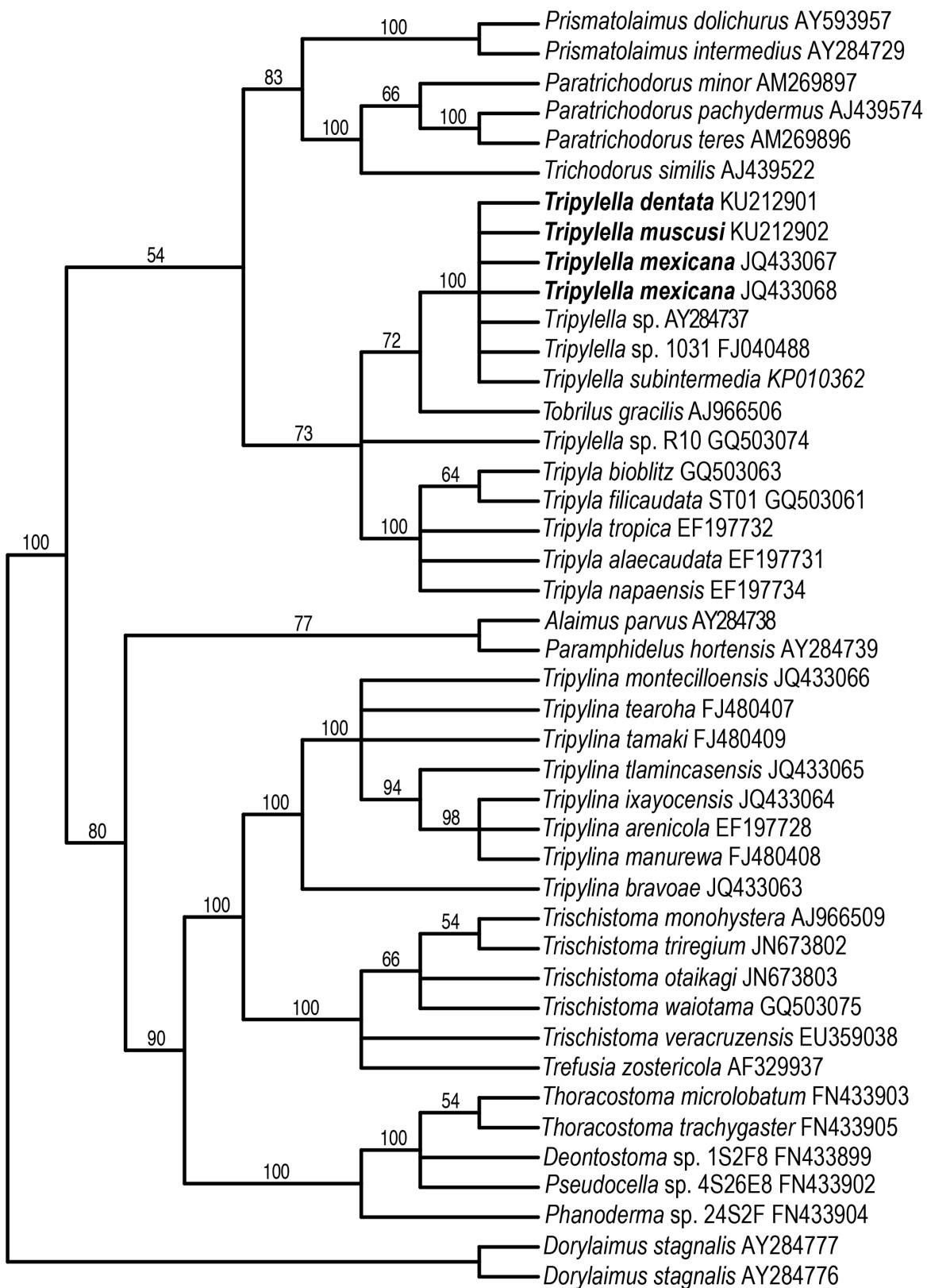
In the second group the tail tapers almost uniformly:

*T. maiuscula* Andrassy, 2006

*T. minuscula* Andrassy, 2006

Differentiation among the eight species with abruptly tapering tails and the two with uniformly tapering tails requires determination of the location of the subventral teeth in relation to the dorsal tooth and assessment of morphometric characteristics (see Table 3 and the key to the species of *Tripylella*). In our experience, detection and recognition of the subventral teeth is considerably easier in live or recently-killed nematodes than in those that have been fixed and preserved.

We concur with Professor Andrassy (personal communication) that when there is difficulty in pinpointing the location of the rather inconspicuous subventral teeth, the most definitive characters for genera and species in the Tripylidiae and Trichistomatidae are the size of body, length of the outer labial setae, position of the cephalic setae, position of the vulva and the number of gonads, male characters (when males are present), and the shape and size of the tail. In the genus *Tripylella*, although there are overlaps in their ranges, the morphometric characters that vary



**FIGURE 10.** 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. GenBank accession no. AY284737 was originally deposited as *Paratripyla* sp., but was subsequently used as *Tripylella* sp. by van Meegen *et al.* (2009), and Zhao *et al.* (2014) in their analyses of *Tripylella*.

most between the species are: L, a, c', vulva-to-anus distance, pharynx length and tail length. However, as we noted for the genera *Trischistoma* and *Tripylina* (Cid del Prado-Vera *et al.*, in press), as more species of *Tripylella* are discovered we expect that the problem of overlap in morphometric characteristics will be compounded. Integrative taxonomic approaches using morphological matrices to delineate morphospecies groups in combination with molecular markers and SEM determination of body-surface characteristics will be important for identification of both morphologically-defined and cryptic species.

SEM has provided an important tool in revealing surface characteristics of the Tripylidae and Trischistomatidae (Cid del Prado-Vera *et al.* 2010; 2012; in press). Since this tool was either not available or 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.

Molecular techniques provide an enormous advantage in assessing the validity of relationships inferred from morphometric studies. The 18S region confirms that the newly described species belong to the *Tripylella* clade. However, with regard to resolving relationships among congeneric species that appear to differ from a morphometric standpoint, the 18S region is too conserved. Further, thus far we have been unsuccessful in amplifying 28S rDNA, a more variable region that might resolve more about the evolutionary history of these species.

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