Soil inhabiting nematodes of the genera *Trischistoma*, *Tripylina* and *Tripyla* from México and the USA with descriptions of new species

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Summary - Five species of the family Tripylidae were collected; two from the Biosphere Reserve at San Fernando, Los Tuxtlas, Veracruz, México: *Trischistoma veracruzense* sp. n., and *Tripyla tropica* sp. n.; two from soil in the Napa Valley, California, USA: *Tripyla alaecaudata* sp. n., and *Tripyla napaensis* sp. n., and one from the University of California, Davis, Student Farm: *Tripylina arenicola*. In all cases the habitats are moist soils from either natural seepage (Napa), irrigation (Davis) or rainfall (México). *Trischistoma veracruzense* sp. n. is characterised by females that are slightly longer than males, average 1.2 *vs* 1.0 mm, the absence of cervical setae, the long, wide post-uterine sac, abundant males without pre-cloacal papillae and producing oval sperm cells. In *Tripylina arenicola* the subventral teeth are posterior to the dorsal tooth and there is a small ventromedian cervical seta close to the anterior end. This population differs from type material in having a pre-anal ventral papilla. *Tripyla tropica* sp. n. is characterised by a stomatal chamber, a posteriorly-hooked dorsal tooth with subventral teeth posterior to it, a large cardia, the absence of sclerotised structures in the vulva and the heavily muscular vagina. The male lacks ventromedian papillae, has curved spicules and a straight gubernaculum. *Tripyla alaecaudata* sp. n. is distinguishable from all other species of the genus by the lateral alae in the juvenile, female and male tails, and a pair of papillae in the tail region. *Tripyla napaensis* is characterised by tiny subventral denticles anterior to the wedge-shaped dorsal tooth, vulva with slightly protruding lips, spermatheca oval with oval-shaped sperm cells, male with 14 ventral papillae, and tail in both sexes tapering in anterior region and becoming narrowly cylindrical in posterior third, ending in a long spinneret. Keys to species of *Trischistoma* and *Tripyla* are provided. Representatives of the new *Tripyla* and *Trischistoma* species are each distinct in pairwise comparisons of small subunit (SSU) ribosomal DNA sequences, supporting their morphologically-based differential diagnosis. Phylogenetic analyses of the sequences place the new *Tripyla* and *Trischistoma* species within clades representing greater species diversity of their respective genera. The molecular data support taxonomic reorganization of Tripylidae into at least two clades, one including *Tripylina*, *Trischistoma* and *Trefusia* and another including *Tripyla*, *Tripylella*, *Paratripyla* and *Tobrilus*.

Key words: New species, phylogeny, SSU rDNA, systematic, taxonomy, Tripylidae.

Resumen - Cinco especies de la familia Tripylidae fueron colectadas; dos de la Reserva de la Biosfera en San Fernando, Los Tuxtlas, Estado de Veracruz, México: *Trischistoma veracruzense* sp. n., y *Tripyla tropica* sp. n.; dos del suelo del Valle de Napa, California, E.U.: *Tripyla alaecaudata* sp. n., y *Tripyla napaensis* sp. n.; y una del campo experimental de prácticas de la Universidad de California, Davis: *Tripylina arenicola*. En todos los casos el hábitat es suelo húmedo en forma natural (Napa), irrigación (Davis) o lluvia (México). *Trischistoma veracruzense* sp. n. se caracteriza porque las hembras son más largas que los machos, en promedio 1.2 *vs* 1.0 mm, la ausencia de setas cervicales, el largo y ancho saco postuterino, abundantes machos sin papilas precloacales y espermatozoides ovales. En *Tripylina arenicola* los dientes subventrales son posteriores al diente dorsal, y hay una pequeña seta cervical ventromediana cerca del extremo anterior. Esta población difiere del material tipo en tener una papila preanal ventral. *Tripyla tropica* sp. n. se caracteriza por tener una cámara estomática, un diente dorsal en forma de gancho, los dientes subventrales posteriores a éste, un cardia grande, ausencia de estructuras esclerotizadas en la vulva, vagina fuertemente muscular, macho sin papilas ventromedianas, espículas curvadas y gubernáculo recto. *Tripyla alaecaudata* sp. n. se diferencia de las demás especies del género por la presencia de ala caudal en los juveniles, hembras y machos, y un par de papilas en la región de la cola. *Tripyla napaensis* sp. n. se caracteriza por los pequeños dientes subventrales y el diente dorsal en forma de cuña, vulva con los labios ligeramente sobresaliendo del contorno del cuerpo, espermateca y espermatozoides ovales, macho con 14 papilas ventrales, y cola en ambos sexos que adelgaza progresivamente, es cilíndrica en el último tercio y termina en una espinereta. Se presentan claves para identificar las especies de *Trischistoma* y *Tripyla*. Las nuevas especies de *Tripyla* y *Trischistoma* son distintas cuando se comparan las secuencias de las subunidades pequeñas (SSU) del ADN ribosómico, lo que apoya la diagnosis diferencial hecha a partir de estudios morfológicos. El análisis filogenético de las secuencias sitúa las nuevas especies de *Tripyla* y *Trischistoma* dentro de clados que representan mayor diversidad que sus respectivos géneros. Los datos moleculares apoyan la reorganización taxonómica de la familia Tripylidae en dos clados, uno que incluye a *Tripylina*, *Trischistoma* y *Trefusia*, y el otro a *Tripyla*, *Tripylella*, *Paratripyla* y *Tobrilus*.

Palabras clave: Especie nuevas, filogenia, sistemática, SSU rDNA, taxonomía, Tripylidae.

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29

J. Nematode Morphol. Syst., 13 (1): 29-49 (2010)

Introduction

The genera *Tripyla* (Bastian, 1865), *Trischistoma* (Cobb, 1913) and *Tripylina* (Brzeski, 1963) are in the family Tripylidae (De Man, 1876), which is assigned to either the order Triplonchida (de Ley & Blaxter, 2004) or Enoplida (Andrássy, 2007). Recent molecular phylogenies separate *Trischistoma* from the other genera and suggest that it is closely related to the family Trefusiidae in the order Enoplida (Holterman *et al.*, 2006; Holterman & Holovachov, 2007), which has affinities in spicule characteristics, intestinal tract and muscle arrangement (O. Holovachov, personal communication). Interestingly, Andrássy (2007) considered *Trefusia monodelphis* Bussau, 1990 identical to, and a synonym of, *Trischistoma gracile* Andrássy, 1985.

Andrássy (2007) recognizes three subfamilies within the Tripylidae: subfamily Tripylinae that includes *Tripyla* Bastian, 1865, *Tripylina* Brzeski, 1963, and *Tripylella* Brzeski & Winiszewska-Slipinska,1993; subfamily Trischistomatinae with one genus, *Trischistoma* Cobb, 1913; and subfamily Tobriliinae with one genus, *Tobrilia* Andrássy, 1967a. The number of gonads, the position, shape and size of the stomatal teeth, and the striation of the cuticle distinguish genera in the family. *Tripyla* has paired gonads in the female, striated cuticle, a stomatal tooth and two small subventral denticles, and three whorls of sensillae (circumoral inner labial papillae, outer labial setae, and cephalic setae) that are well separated; *Tripylina* has a single gonad in the female, the outer labial and cephalic whorls of setae are close together, and the two subventral teeth may be either anterior or posterior to the dorsal tooth (Tsalolikhin, 1983; Zullini, 2006). *Trischistoma* has a single ovary, a smooth cuticle, and three well-separated whorls of setae. In *Trischistoma*, the body is thinner than most Tripylidae and bent dorsad, the spicules do not have a muscular sheath, males are monorchic, the oesophago-intestinal valve consists of relatively small cells, and there are no coelomocytes near the oesophago-intestinal junction (Andrássy, 1985; Brzeski, 1965).

The pharynx in the Tripylidae is, in general, uniformly cylindrical throughout its length with slight enlargement in the latter portion associated with the location of the oesophageal glands. There are five oesophageal gland nuclei, the dorsal oesophageal gland reportedly opening through the dorsal tooth, the first pair of subventral glands open slightly posterior to that, and the second pair of subventral glands open near the nerve ring (Chitwood & Chitwood, 1937). At the base of the pharynx is a tri-lobed structure variously termed the cardia or cardiac valve, except in *Trischistoma* as noted above.

Herein we report morphometric and, where possible, molecular characteristics in a redescription of *Tripylina arenicola* (De Man, 1880) Brzeski,1963 and for descriptions of new species of *Trischistoma* and *Tripyla*. We use 18s rDNA sequences to examine relationships within the Tripylidae. We present keys for all species of *Trischistoma* and *Tripyla* that are based on valid descriptions.

Materials and Methods

Sources, specimen processing and morphometric characterisation.- Soil samples were collected from Napa Valley, California, USA in July 2005, and from San Fernando, Municipio de Soteapan, Los Tuxtlas, Estado de Veracruz, México in September 2006. *Tripyla alaecaudata* sp. n. and *T. napaensis* sp. n. occurred in sandy soil of relatively undisturbed oak (*Quercus* spp.) / California bay (*Umbellularia californica*) / toyon (*Heteromeles arbutifolia)* woodland in hills bordering the Napa Valley. *Tripylina arenicola* was found in samples from the campus of the University of California, Davis. The samples from Los Tuxtlas, Estado de Veracruz, México, contained *Tripyla tropica* sp. n. and *Trischistoma veracruzense* sp. n. The California samples are all from locations with a Mediterranean climate consisting of hot dry summer and rainfall in winter. The climate of the Mexican locations is tropical.

Nematodes were separated from samples of 200 cm³ of soil by decanting and sieving followed by Baermann funnel extraction (Barker, 1985). Sub-samples of each extract were examined with the nematodes either alive or heat-killed to distinguish specimens on the bases of gross morphology, activity and behavior. The specimens in bulk samples of the nematode extract were killed by heating (to 40 °C) in about 7 ml water in a 5-cm diam. Petri dish, until movement ceased. An equal volume of 8% formalin was added to the suspension to achieve a final fixative concentration of 4%; the covered dish was stored at room temperature for 10 days. The fixative was then carefully removed from the surface, by pipette under a microscope, without disturbing the nematodes, until the depth was reduced to ~4 mm. The covered Petri dish was placed over a container of 95% ethanol in a small desiccator 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 while observing under a stereomicroscope.

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 95% ethanol, 79 parts water) was added to the dish and it was incubated at 40°C with the cover slightly open. When the solution level dropped to 2 mm, it was increased to 4 mm with additional Seinhorst A. When the solution level dropped to 1 mm, Seinhorst B solution (95 parts 95% ethanol, 5 parts glycerin) was added and the dish incubated at 40°C. When the solution level again dropped to 1 mm, it was increased to 2 mm with additional Seinhorst B. Three days later, 1 ml of pure glycerin was added to the dish.

J. Nematode Morphol. Syst., 13 (1): 29-49 (2010)

30

Selected nematodes were hand-picked from the dish for mounting on Cobb and 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.

Molecular characterisation.- Nucleic acids used for polymerase chain reaction (PCR) amplifications were extracted from individual adults hand-picked from water and transferred to 95% ethanol. Nematodes were digested and nucleic acids prepared using the sodium hydroxide method (Floyd *et al.*, 2002). The PCR was used to amplify regions of the SSU ribosomal DNA in different taxa that ranged from near full-length (~1,700 bp) to a 686 bp fragment from the SSU 3'-end. Proofreading DNA polymerase (Finnzymes DyNAzyme EXT) was used for all amplifications. The SSU 3'-end region amplified by primers #648 (5'-GTATGGTTGCTGAAAC) and #136 (5'- TGATCCTTCTGCAGGTTCACCTAC) worked with all species, whereas primers designed for near full-length SSU regions were successful in only certain taxa, despite repeated attempts using different PCR conditions. For example, primers #652 (5'-GCAGCCGCGGTAATTCCAGCTC) and #653 (5'-CGTGTTGAGTCAAATTAAGCCGC) yielded a PCR product in all species except *T. tropica*, whereas 18S1A (5'-GGCGATCGAAAAGATTAAGCCATGCA) and #647 (5'-CATTCTTGGCAAATGCTTTCGC) worked only in *T. napaensis* sp. n. and *T. arenicola*. PCR products were sequenced directly following enzymatic treatment with exonuclease I and shrimp alkaline phosphatase (PCR product pre-sequencing kit, USB Corporation). Sequences were obtained from two individuals of *Tripyla alaecaudata* sp. n., two individuals of *Tripyla napaensis* sp. n., two individuals of *Tripyla tropica* sp. n., three individuals of *Tripylina arenicola*, and one individual *Trischistoma veracruzense* sp. n. All individuals sequenced were collected at their respective type localities.

A SSU dataset was prepared using newly-generated sequences plus taxa obtained from GenBank (GenBank accession numbers in Fig. 10). The dataset consisted of 57 taxa and 674 aligned SSU sites. Outgroup taxa were chosen and trees rooted based on the SSU phylogeny of New Zealand *Tripyla* (Zhao, 2009); other pertinent taxa were selected based on the analysis of Holterman *et al.* (2006). Sequences were aligned using ProAlign Version 0.5 (Loytynoja & Milinkovitch, 2003). Modeltest Version 3.7 (Posada & Crandall, 1998) was used to compare the fit of nucleotide substitution models for the aligned data using the Akaike information criterion; the best-fit model (GTR+I+G) was used for Bayesian phylogenetic inference to assess the posterior probability distribution of trees using MrBayes 3.1.2 (Ronquist & Huelsenbeck, 2003). The dataset was run for 1 million generations, and chains were sampled every 1000 generations. Burn-in was determined empirically by assessment of the convergence in the log likelihood values of the chains.

Descriptions

Trischistoma veracruzense sp. n.
(Figs 1 & 2)

Measurements: See Table I.

Female: Body slender, curved dorsally in the posterior part, with the tail tip dorsally reflexed. Cuticle smooth, varying in thickness but generally thin (1.0-1.5 µm). Lip region asymmetric and continuous with the body contour, 11-14 µm (13.0±0.4) wide. Cephalic sensillae in three whorls, the first composed of six very small inner labial papillae, the second of six outer labial setae 7-10 µm (9.3±0.4) long or 50-91% (71.8±4.6) of lip region width, the third of four slightly shorter cephalic setae 5-6 µm (5.2±0.2) long and separated from the second whorl by 5-7 µm (5.7±0.3). Amphid apertures vary from slightly posterior to slightly anterior to cephalic setae, 11-16 µm (13±0.8) from the anterior end. Ventromedian setae absent in cervical region. Stoma with small dorsal tooth, positioned within an invagination of the stoma wall, and two minute subventral denticles anterior to the dorsal tooth. Stoma walls not thickened. Pharynx cylindrical and muscular; sphincter muscle cells between pharynx and intestine are not enlarged into a cardia. Vulva with lips protruding very slightly and without sclerotised structures. One ovary, prodelphic and outstretched or reflexed; gonad length 16-57% of body length in females of differing maturity; most females with conspicuous oval-shaped sperm cells in the uterus and post-uterine sac (Figs 1 & 2). Post-uterine sac is almost as wide as the body and 94-170 µm (129±10.4) long; it terminates close to the anus in some specimens. Tail tip reflexed dorsally into a U-shape with a small spinneret; tail tip twisted in some specimens.

Male: Abundant, similar in general body shape to females but smaller and more slender. Lip region asymmetric and continuous with the body contour, 11-13 µm (12.0±0.4) wide. The inner labial papillae are very small, the outer labial setae are 9-10 µm (9.6±0.2) long or 64-91% (79±3.3) of lip region width; the cephalic setae are slightly smaller and thinner than the outer labial setae and 5-6 µm (5.7±0.2) long. The outer labial and cephalic whorls of setae are 5-7 µm (6.3±0.3) apart. Amphid apertures slightly posterior to cephalic setae, 10-14 µm (12.2±0.8) from the anterior end. Stoma walls not thickened. Sphincter cells between pharynx and intestine not enlarged into a cardia. The single testis is outstretched, occupying 29-90% (54±7.1) of body length, in one specimen it reached beyond the end of the pharynx; sperm cells 11-17 µm (12±0.4) long by 5-7 µm (6.2±0.2) wide, present throughout testis. Spicules slightly curved, not surrounded by a muscular pouch; gubernaculum very thin. Precloacal papillae or supplements absent. Tail twisted dorsally, strongly in most specimens, ending in a hyaline region with a small spinneret.

Diagnosis and relationships: Trischistoma veracruzense sp. n. is characterised by females that are

31

J. Nematode Morphol. Syst., 13 (1): 29-49 (2010)

FIGURE 1. *Trischistoma veracruzense* **sp. n.** Female. (A-E, G-H). A: Anterior region; B: Cervical region; C: Oesophago-intestinal valve; D: Posterior region; E: Lip region; G: Posterior region; H: Tail. Male. (F, I). F: Lip region; I: Tail.
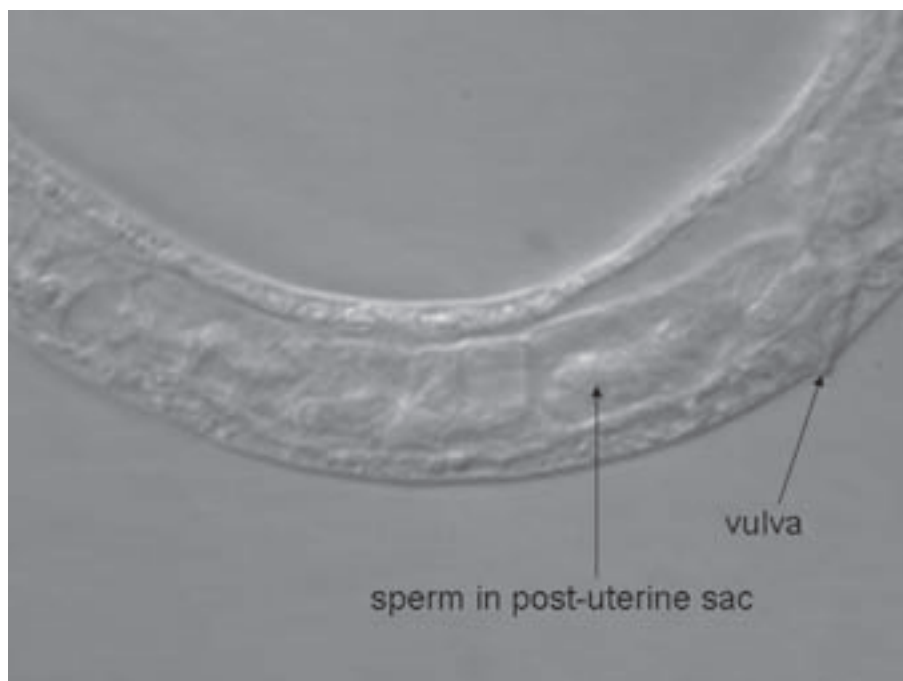
J. Nematode Morphol. Syst., 13 (1): 29-49 (2010)

32

FIGURE **2.** ***Trischistoma veracruzense* sp. n.** Female, Post-uterine sac.

slightly longer than males (average 1.2 *vs* 1.0 mm); absence of ventromedian cervical setae; the sizxe of the post-uterine sac, 129±10.4 μm long or 63±4.9% of the distance from vulva to anus; abundant males that lack pre-cloacal papillae; a slender body which, at the tail, is curved dorsally and reflexed into a U-shape in females and strongly twisted in males.

The new species is similar to *T. monohystera* (De Man,1880) Schuurmans Stekhoven, 1951 in the presence of a post-uterine sac but it differs in the length of the sac (45-79% *vs* 25%, respectively). The two species also differ in body length (1.0-1.3 *vs* 1.5-2.3 mm in females and 0.9-1.1 *vs* 1.5-2.2 mm in males). It resembles *T. pellucidum* Cobb, 1913 in the position of the vulva but can be distinguished by the greater size of the female body (1.0-1.3 *vs* 0.6-0.8 mm), in the presence a post-uterine sac, and in the absence of males in *T. pellucidum*. Finally, it is close to *T. equatoriale* Andrássy, 2006 in having a well-developed post-uterine sac, but differs in body length (1.0-1.3 *vs* 1.4-1.7 mm), absence of a precloacal supplement, shorter spicules (29-34 *vs* 40-44 μm), and shape of the sperm cells (oval *vs* spindle-shaped).

Type locality and habitat: Trischistoma veracruzense sp. n. was collected from soil around roots of trees in a tropical forest in San Fernando, Municipio de Soteapan, Los Tuxtlas, Estado de Veracruz, México (N 18° 18.58´; W 94° 53.45´), 1040 m above sea level.

Type specimens: Accession numbers of type specimens deposited in the University of California Davis Nematode Collection (UCDNC) are: holotype female (4973) paratype females (4974 and 4975), allotype male (4976), paratype male (4977), paratype juveniles (4978 and 4979). Other paratype material is deposited in the University of California Riverside Nematode Collection (UCRNC), Wageningen University Nematode Collection (WUNC), and the Colegio de Postgraduados Nematode Collection (CPNC).

Etymology: The specific epithet refers to geographical origin of the species, Estado de Veracruz, México.

Key to *Trischistoma* species
1. Post-uterine sac absent... 2
   Post-uterine sac present 3
2. Body 1.0-1.1 mm; c'=4.7-5.9... *gracile* Andrássy, 1985
   Body 0.6-0.8 mm; c'=3.5-4......*pellucidum* Cobb, 1913
3. Length of post-uterine sac under 110 μm, about 25% distance from vulva to anus .... *monohystera* (De Man, 1880) Schuurmans Stekhoven, 1951
   Post-uterine sac 130 μm long, more than 45% of distance from vulva to anus 4
   Female body 1.0-1.3 mm; V = 74-78; male without precloacal supplements .... *veracruzense* sp. n.
   Female body 1.4-1.7 mm, V = 81-83; male with one precloacal supplement .....*equatoriale* Andrássy, 2006

33

J. Nematode Morphol. Syst., 13 (1): 29-49 (2010)

Table I. Measurements and ratios of *Trischistoma veracruzense* sp. n. and *Tripylina arenicola* (De Man, 1880) Brzeski, 1963, in μm (except L, in mm) and in the form: mean ± standard deviation (range).

Species		*T. veracruzense* sp. n.		*T. arenicola*
	Holotype ♀	Paratypes 9♀♀	Paratypes 8♂♂	13♀♀
Character n				
L	1.06	1.2±1.6 (1.0-1.3)	1.0±0.03 (0.9-1.1)	1.2±0.05 (0.9-1.5)
a	37.5	45.9±0.2 (41-54)	41.5±1.3 (36-47)	28.3±1.3 (19.7-37.3)
b	6.2	5.5±0.2 (4.7-6.7)	4.9±0.2 (4.3-5.6)	5.5±0.1 (4.7-6.5)
c	13.3	14.8±0.6 (10-17.5)	12.1±0.6 (10-15)	17.0±0. 8 (14.3-21.3)
c'	5.2	4.4±0.4 (3-6.5)	3.5±0.9 (3.0-3.9)	3.1±0. 1 (2.3-3.5)
V	73.4	76±0.6 (74-78)	_	65.5±1.0 (59.5-69.2)
Body width	28	26.5±1.3 (20-32)	24±0.5 (23-27)	38.4±1.7 (29-48.7)
Anal body width	15.2	18.8±0.9 (15-21)	23.5±0.5 (22-26)	24.3±1.2 (20.3-34.5)
Dorsal tooth to anterior buccal cavity	12	13±0.8 (10-15)	13.7±0.6 (12-16)	11.4±0.5 (9.1-14.2)
Nerve ring	?	?	?	82±3.3 (67-93)
Pharynx	170	220±7.3 (189-255)	206±6.9 (170-236)	216±9.6 (163-287)
Tail	80	82±4.5 (63-104)	84±2.5 (70-93)	61.3±8.9 (50.9-90.5)
Spinneret	4.0	1.8±0.1 (1.0-2.0)	1.8±0.1 (1-2)	2.7±0.2 (2.2-3.9)
Spicules	-	-	30.5±0.5 (29-34)	-
Gubernaculum	-	-	12.1±1.1 (10-17)	-

Tripylina arenicola (De Man, 1880) Brzeski, 1963
(Fig. 3)

Measurements: See Table I.

Female: Habitus after fixation C-shaped, with the end of the tail bending first ventrally then dorsally. Cuticle thickness 2.5-4.5 μm (3.4) throughout body length, not separated from body on fixation, layers not evident and without striation. Lip region asymmetrically rounded and continuous with body contour, 15.5-18.5 μm (17.3±0.6) wide at level of base of outer labial setae. Amphid aperture 14.0-18.5 μm (16.1±0.7) from anterior end. Inner labial papillae small and conical, 2.0-4.0 μm (2.6±0.5) long; outer labial setae 12.0-15.5 μm (13.9±0.5) long or 66-88% (80.4±4) of lip region width; cephalic setae thin, conical, 5.0 μm long, 1.0-2.0 μm (1.4±0.2) behind outer labial setae. Single small ventromedian cervical seta 47-88 μm (65) from the anterior end. Dorsal tooth small, wedge-shaped; two small subventral teeth 3.1-5.1 μm (3.7±0.4) posterior to the dorsal tooth; stomal chamber small when not distended. Pharynx separated from intestine by a prominent cardia, 8.5-16.0 μm (11.7±0.83) long by 24-29 μm (26.1±0.46) wide. Vulva slit-shaped, without protruding lips but with two very small sclerotised structures which are 1.5 μm long; vagina short, about 8 μm. The single ovary is outstretched in young females and in mature females it is reflexed posteriorly over the prodelphic uterus and extends to the level of the vulva or slightly posterior to it. From the vulva to the point of reflex is 8.9-14.2% (12.3±1.2) of body length. Post-uterine sac absent. Tail lacking transverse striations, 71-91 μm long in live and 51-79 μm in preserved specimens, ending in a short spinneret. One ventral papilla present, 14-28 μm (22.8±2.03) anterior to the anus.

Male: Unknown.

Diagnosis and relationships: Using measurements provided by Brzeski and Winiszewska-Slipinska (1993), our population was identified as *T. arenicola* (De Man, 1880) Brzeski, 1963, based on the location of the subventral teeth posterior to the dorsal tooth, the small size of the dorsal tooth, the position of the vulva, the shape and size of the tail, and the position of the single ventromedian cervical seta. Most morphological characteristics of our population are in concurrence with the original description of *T. arenicola*. The only morphometric difference is the position of the ventromedian cervical seta, 47-88 *vs* 60-78 μm according to Brzeski and Winiszewska-Slipinska (*op. cit.*), and in the presence of a pre-anal ventral papilla.

Distribution: Ecological Garden of the Student Farm, University of California, Davis (N 38° 32.34'; W 121° 45.86'). Collected from irrigated soil around roots of various vegetables.

Voucher specimens: Accession numbers of specimens deposited in the UCDNC are 3969, 3970 and 3971. Other specimens are in UCRNC, WUNC, and CPNC.

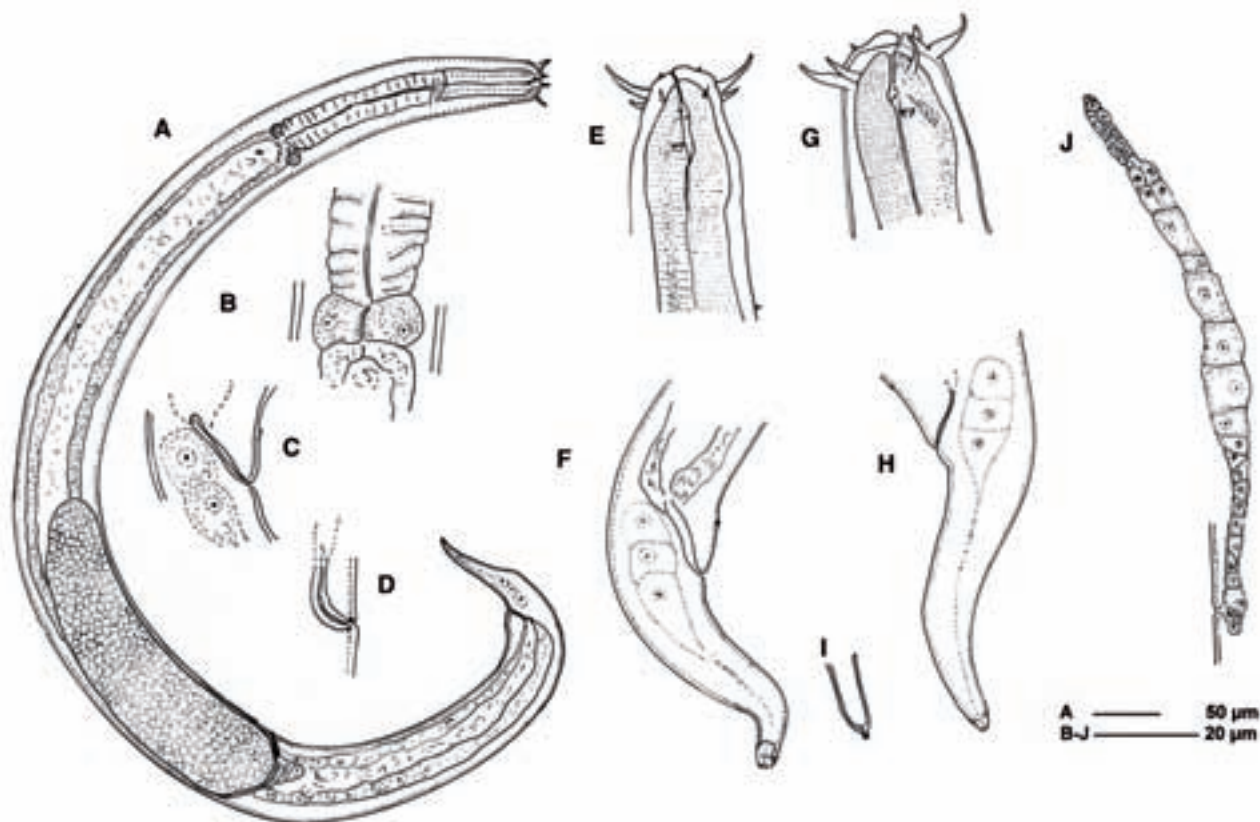*J. Nematode Morphol. Syst.*, 13 (1): 29-49 (2010)

34

FIGURE 3. *Tripylina arenicola* (De Man, 1880) Brzeski, 1963. Female. (A-J). A: Entire body; B: Cardia; C: Anal region; D: Vulva lateral view; E, G: Anterior end; F, H: Tail; I: Spinneret; J: Gonad of young female.

Tripyla tropica sp. n.
(Figs 4 & 5)

Measurements: See Tables II & III.

Female: Body J-shaped upon fixation. Cuticle 3.0-4.0 µm thick at lip region, 2.0 µm thick at cardia level, and 2.5 and 3.0 µm thick at vulva and anus level, respectively; cuticle with conspicuous transverse striations 1.0 µm wide. No body pores observed but a few very small setae are distributed irregularly along the body. Lip region symmetrical, lacking striations, flattened anteriorly, slightly constricted where cuticular striations begin, 19.5-24.5 µm (22.6±1.4) wide between the insides of the bases of the outer labial setae. Amphid apertures 10.0-16.5 µm (13.4±1.0) from the anterior end. Inner labial papillae small; outer labial setae crescent-shaped and directed anteriorly, 4.0-6.0 µm (4.8±0.3) long or 20-23% (21.0±0.4) of lip region width, cephalic setae thinner 2.0-3.0 µm (2.2±0.2) long and 4.0-7.0 µm (5.8±0.4) posterior to outer labial circle. Dorsal tooth wedge-shaped and hooked posteriorly at the tip, two small teeth 2.0-5.0 µm (4.5±0.6) posterior to the dorsal tooth, stomatal chamber

22.5 µm from anterior end. Pharynx with conspicuously strong muscles in the posterior half. Nerve ring at 32-50% (35.9±2.9) of length of pharynx. Large cardia 10-24 µm (14.6±4.5) long by 14-27 µm (21.1±4.2) wide between pharynx and intestine (Fig. 5A). Vulva without sclerotised structures. Vagina 10-22 µm (13.8± 4.3) long, heavily muscular along its length; there are two glands containing granules that have fine ducts opening into the vagina close to the vulva, the anterior is 5-9 µm (7.1) long and 6 µm wide, the posterior is 5-15 µm long and 6-9 µm wide (Fig. 5B). Eggs, 76-128 µm (102) long and 27-40 µm wide, were observed in mature females. Rectum 19-30 µm (23.4± 3.5) long. Tail curved ventrally and narrowing evenly. Spinneret conspicuous.

Male: Body size similar to female. Spicules curved. Gubernaculum straight, surrounded by a muscular spicule pouch. Precloacal ventromedian papillae absent (Fig. 5D). A few extremely small setae irregularly distributed along the body. Genital system diorchic, with opposite, outstretched testes that open into a common *vas deferens*. Tail tapering evenly, with conspicuous, terminal spinneret.

Juvenile: Body similar to females, C- or J-shaped upon fixation, Lip region lacking striations, anteriorly

35

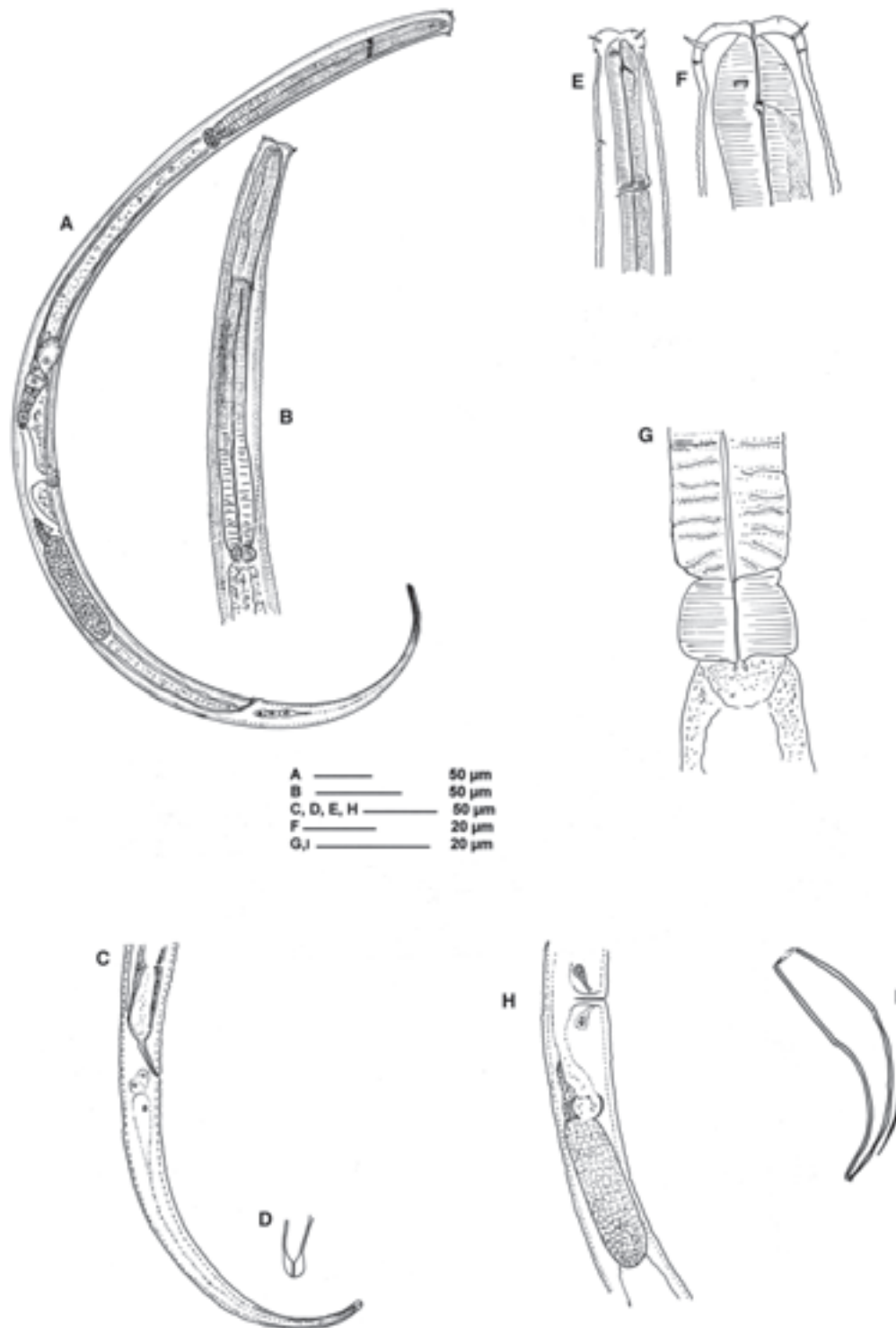*J. Nematode Morphol. Syst.,* 13 (1): 29-49 (2010)

FɪɢᴜʀE **4.** ***Tripyla tropica* sp. n.** Female. (A-H). A: Entire body; B: Anterior region; C: Posterior region; D: Spinneret: E: Cervical region; F: Lip region; G: Cardia; H: Posterior gonad; I: Spicules and gubernaculum.
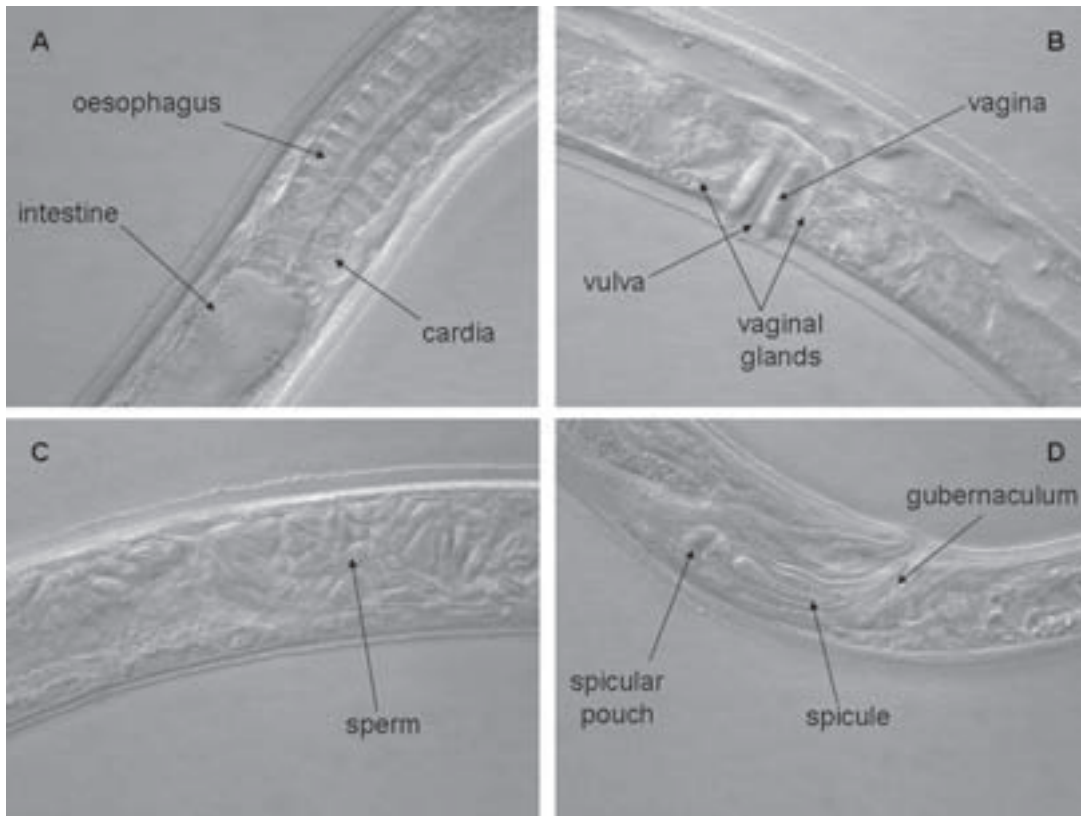
J. Nematode Morphol. Syst., 13 (1): 29-49 (2010)

36

FIGURE 5. *Tripyla tropica* **sp. n.** Female (A,B): A. Cardia; B. Vulva and vagina; Male (C,D): C. Testis; D. Spicules and gubernaculum.

flattened, cephalic setae visible, thick cuticle with conspicuous striations.

Diagnosis and relationships: Tripyla tropica sp. n. is characterised by the posteriorly-hooked tip of the wedge-shaped dorsal tooth, the posterior position of the subventral teeth, the large cardia, the absence of sclerotised structures in the vulva and the heavily muscular vagina. The male lacks ventromedian papillae, has curved spicules and a straight gubernaculum.

Based on measurements provided by Brzeski and Winiszewska-Slipinska (1993), the new species is similar to *T. setifera* Bütschli, 1873 in its body size and vulva position as well as in the lack of cuticular structures in the vulva and the muscular vagina. It can bedistinguished from *T. setifera* by its longer tail (c' = 6.9-10.4 *vs* c' = 4.1-6.8, respectively), the elongate shape of the posterior region of the tail, the hooked (*vs* triangular) tip of the dorsal tooth, the position of the subventral teeth (2.0-5.0 *vs* 7 μm posterior to the dorsal tooth), and the absence of ventromedian papillae anterior to the cloaca in males.

It also resembles *T. napaensis* sp. n. in body size and vulva position but differs in its longer tail (170-285 *vs* 153-173 μm) and shorter outer labial setae (4.0-6.0 *vs* 7-9 μm).

Type locality and habitat: Collected from soil around roots of trees in tropical forest in San Fernando, Los Tuxtlas, Municipio de Soteapan, Veracruz, México (N18° 18.58'; W 94° 53.45'). Paratypes were collected in tropical forests at López Mateos (N18° 19.11'; W 94° 52.89') and Venustiano Carranza (N18° 20.21'; W 94° 46.27'), Los Tuxtlas, Municipio de Catamaco, Veracruz, México.

Type specimens: Accession numbers of type specimens deposited in the UCDNC are: holotype female (3952), paratype females (3953 and 3954), paratype males (3955 and 3956), and paratype juvenile (3957). Other paratypes deposited in the CPNC.

Etymology: The specific epithet refers to the tropical habitat of type locality.

Tᴀʙʟᴇ **II.** Measurements and ratios of *Tripyla tropica* sp. n., *T. alaecaudata* sp. n., *and T. napaensis* sp. n. (females), in μm (except L, in mm) and in the form: mean ± standard deviation (range).

Species		*T. tropica* sp. n.		*T. alaecaudata* sp. n.		*T. napaensis* sp. n.	
		Holotype ♀	Paratypes 10♀♀	Holotype ♀	Paratypes 6♀♀	Holotype ♀	Paratypes 6♀♀
Character	n						
L		1.6	1.4±0.2 (1.1-1.8)	1.6	1.5 ± 0.02 (1.4-1.6)	1.5	1.47±0.01 (1.43-1.50)
a	36		34.2±4.4 (28.6-39.7)	33.9	29.5±1.3 (23.7-33.9)	32.1	30.6±0.7 (28.2-32.5)
b		4.7	4.9±0.3 (4.4-5.7)	4.4	4.8±0.1 (4.4-5.3)	4.9	4.9±0.1 (4.6-5.2)
c		6.7	6.2±0.3 (5.4-6.7)	8.4	8±0.3 (6.2-8.6)	9.3	8.8±0.3 (8.1-9.4)
c'		8.5	9.1±1.1 (6.9-10.4)	5.6	5.1±0.2 (4.7-6.1)	4.2	4.2±0.7 (4.0-4.7)
V%		54.1	53.2±1.0 (52-56)	54.9	53.19±0.9 (49-55)	54.2	52.5±0.7 (50-55)
Body width		43	43.1±6.3 (38-62)	48	55.2±3.7 (48-75)	46.7	48.2±0.7 (46.6-50.6)
Anal body width		25.6	26.2±3.4 (22-35)	34.7	35.9±0.6 (33.5-37.4)	37.9	37.8±0.8 (35.5-40.7)
Dorsal tooth to anterior buccal cavity		23.2	21±1.8 (18-26)	25.4	27±2.5 (24.3-39.9)	24.3	23.5±0.3 (22.6-24.3)
Nerve ring		100	101±13.4 (95-125)		95.7±4.2 (87-100)	86.6	93.4±5.7 (80-107)
Pharynx length		330	293±29.9 (240-350)	373.3	319±11.2 (297-373)	304	301±2.5 (293-307)
Tail		230	238±29.6 (170-285)	193.3	191±6.3 (177-227)	160	162±2.8 (153-173)
Spinneret		5.0	5.0±0.8 (4.0 – 6.0)	3.4	3.4±0.17 (2.8-3.9)	8.1	7.8±0.4 (6.5-9.2)

Tripyla alaecaudata sp. n.
(Figs 6 & 7)

Measurements: See Tables II & III.

Female: Body J-shaped upon fixation. Cuticle at anterior varying in thickness from 3.0-5.5 μm in labial area, 1.0-2.5 μm at level of cardia, where it appears to consist of two layers, and 1.0-2.0 μm at vulva level; cuticle behind the lip region bearing transverse striations, which are 1.0 μm wide at the anterior end and 2.0 μm wide in the vulval region. Lip region symmetrical, width inside bases of outer labial setae is 22-30 μm (27.4±1.0) (Figure 7C). Amphid apertures 18-28 μm (22.4±1.2) from the anterior end. No body pores observed. Inner labial papillae very small; outer labial setae distinct, 5.0-6.0 μm (6.3±0.3) long or 18-27% (23.1±1.5) of lip region width, cephalic setae conical, thinner and smaller than outer labial setae, 2.0-3.0 μm (2.2±0.2) long and 3.0-5.0 μm (4.6±0.3) behind outer labial setae. Dorsal tooth wedge-shaped and tucked into an invagination in opposing stoma wall; two very small teeth, 1.0-6.0 μm (3.7±0.8) anterior to the dorsal tooth, stomatal chamber small when relaxed, cup-shaped when distended. Nerve ring at about one-third of pharynx length. Cardia between pharynx and intestine (Fig. 7D). Vulval lips protruding, 3.0-6.5 μm in live specimens but less after dehydration. Lateral to the vagina there are two granule-containing glands with fine ducts opening into the vagina close to the vulva, the anterior is 6.0-18.5 μm (10.5±2.8) long and 3.0-9.0 μm (5.4±1.3) wide, the posterior is

4.0-18.5 μm (8.9±3.2) long and 3.0-9.0 μm (5.4±1.3) wide (Fig. 7E). One egg was measured, 117 μm long and 34 μm wide. Spermatheca oval-shaped, 21-26 μm (24 ±0.8) long by 17-28 μm (21.0 ±1.9) wide. The terminal portion of the tail is straight or curved ventrally; lateral caudal alae observed both in live specimens and after fixation, extending anteriorly from tail tip, 137-240 μm (188 ±13.6) on the right and 139-253 μm (176±13.5) on the left (Fig. 7A,B); a pair of papillae are present in the caudal alae at approximately the same distance posterior to the anus, 120-140 μm (134±2.1) on the right and 107-140 μm (133±5.4) on the left. Anal aperture 16-20 μm wide. Spinneret short, terminal.

Male: Shape generally similar to females. Lip region width inside bases of outer labial setae is 22-28 μm (26.0±1.0). Amphid apertures 16.5-21.5 μm (19.0±1.2) from the anterior end. No body pores observed. Inner labial papillae very small; outer labial setae distinct, 5.0-7.0 μm (6.1±0.4) long or 19-32% (23.8±2.7) of lip region width, cephalic setae conical, thinner and smaller than outer labial setae, 3.0 μm long and 5.0 μm behind outer labial setae. Dorsal tooth wedge-shaped and tucked into an invagination in opposing stoma wall; two very small teeth, 4.0-5.0 μm (4.6±0.3) anterior to the dorsal tooth; stomatal chamber small when relaxed, cup-shaped when distended. Nerve ring at about one-third of pharynx length. Cardia between pharynx and intestine similar to female (Fig. 7D). At least fifteen ventral papillae were observed, approximately 68 μm apart, with the first 73 μm from the anterior end and the last 84 μm anterior to the cloacal opening. Spicules 9.6-13.0 μm wide at the manubrium, end of the shaft being bifid. Gubernaculum

J. Nematode Morphol. Syst., 13 (1): 29-49 (2010)

38

TABLE III. Measurements and ratios of *Tripyla tropica* sp. n., *T. alaecaudata* sp. n., *and T. napaensis* sp. n. (males), in µm (except L, in mm) and in the form: mean ± standard deviation (range).

Species		*T. tropica* sp. n.	*T. alaecaudata* sp. n.	*T. napaensis* sp. n.
		Paratypes	Paratypes	Paratypes
Character	n	5♂♂	7♂♂	4♂♂
L (mm)		1.2±0.1 (0.9-1.7)	1.6±0.03 (1.1-1.8)	1.49±0.02 (1.44-1.53)
a		34.4±2.6 (25.6-41.9)	30.3±0.3 (25.6-33)	35.7±0.5 (35.0-37.0)
b		5.1±0.1 (4.7-5.4)	4.8±0.1 (3.7-5.3)	5.1±0.05 (5.0-5.2)
c		5.2±0.1 (3.8-5.8)	7.9±0.1 (7.0-8.6)	8.8±0.3 (8.1-9.4)
c'		7.0±1.0 (8.6-10)	4.5±0.03 (4.3-4.7)	4.2±0.2 (4.0-4.7)
Body width		35.9±1.7 (31-41)	52±1.3 (35-64)	41.6±1.0 (40-44)
Anal body width		28±2.4 (25.6-32.8)	43.8±3.0 (32.2-53.3)	39.6±0.9 (37.4-41.9)
Dorsal tooth to anterior buccal cavity		23.8±2.3 (17.2-30)	27.8±2.4 (22.6-40.1)	22.9±0.3 (22.6-23.8)
Nerve ring		71.1±5.0 (67-80)	103±3.1 (93.3-111)	95.6±6.0 (87-107)
Pharynx length		242±23.7 (187-320)	319±1 (273-353)	288±6.9 (273-300)
Tail		225±27.7 (167-330)	194.3±9.7 (153-227)	168±7.8 (151-187)
Spinneret		4.12±0.2 (3.8-4.8)	3.0±0.2 (2.8-3.9)	6.7±0.6 (5.7-7.9)
Spicules		40.7±0.9 (34-46)	32.3±3.7 (28-42.4)	39.2±1.5 (35-42.4)
Gubernaculum		10±0.7 (8-12)	11.3±2.0 (9-15.3)	10.4±0.9 (8.5-11.3)

straight. Spicules surrounded by a muscular pouch (Fig. 7F). Cloacal aperture 7.5-18.5 µm wide. Lateral caudal alae extending anteriorly from tail tip, 146-180 µm (160±10.4) on the right and 120-177 µm (148±16.5) on the left (Fig. 7A). Paired papillae in the caudal alae, 127-131 µm (128±1.4) on the right and 131-140 µm (137±3.1) on the left posterior to the cloaca. Spinneret short, terminal.

Diagnosis and relationships: Tripyla alaecaudata sp. n. is easily recognized and differentiated from all other species of the genus by the lateral alae in the female, juvenile, and male tail (Fig. 7A,B), the paired papillae in the caudal alae of all stages, and the position of the subventral teeth immediately anterior to the dorsal tooth.

Type locality and habitat: Western hills of the Napa Valley, 0.75 km west of UC Oakville Experimental Vineyard, Oakville, California (N 38° 25.95'; W 122° 25.34'). Collected from moist soil in a grassy area (wild oats and native grasses).

Type specimens: Accession numbers of type specimens deposited in the UCDNC are: holotype female (3958), paratype female (3959), paratype males (3960 and 3961), paratype juveniles (3962 and 3963). Other paratypes deposited in UCRNC, WUNC, and CPNC.

Etymology: The specific epithet refers to the prominent lateral alae in the tail region of females, males and juveniles.

Tripyla napaensis sp. n.
(Figs 8 & 9)

Measurements: See Tables II & III.

Female: Body J-shaped after fixation and strongly curved or in a slight ventral spiral posteriorly. Cuticle not separated from body after fixation, 2.0-3.0 µm thick at the lip region, 1.5 µm thick at vulva level and 3.0 µm thick at anus level; cuticle bearing fine transverse striations, which are more conspicuous at the anus level. Lip region symmetrical, continuous with body contour. Lip region width inside bases of outer labial setae is 21-26 µm (22.9±0.9). Amphid apertures 10.0-16.5 µm (12.2±1.4) from the anterior end. Inner labial sensillae papilliform. Outer labial setae 7.0-9.0 µm (7.8±0.3) long or 29-43% (38.2±1.9) of lip region width. Cephalic setae conical and 3.0-5.0 µm (3.6±0.5) long, 4.0-5.0 µm (4.8±0.3) posterior to the outer labial setae. Dorsal tooth small, wedge-shaped; stomatal chamber narrow when not distended. Two very small subventral denticles 4.0-5.0 µm anterior to the dorsal tooth (Fig. 9A). Excretory pore not observed. Nerve ring at about one-third of pharynx length. Prominent cardia between pharynx and intestine,17-23 µm (19.8±0.9) long by 22-27 µm (24.4±0.7) wide (Fig. 9B). Vulva with slightly protruding lips without sclerotised structures; vagina muscular, 15.5-19.0 µm (17.6±0.6) long. Anterior and posterior to the vagina are two oval-shaped glands filled with refractive material, anterior gland10.0-11.0 µm long and 6.0-9.5 µm wide, posterior gland 9.5-10.0 µm long and 5.0-7.0 µm wide. Spermatheca oval, 21-34 µm
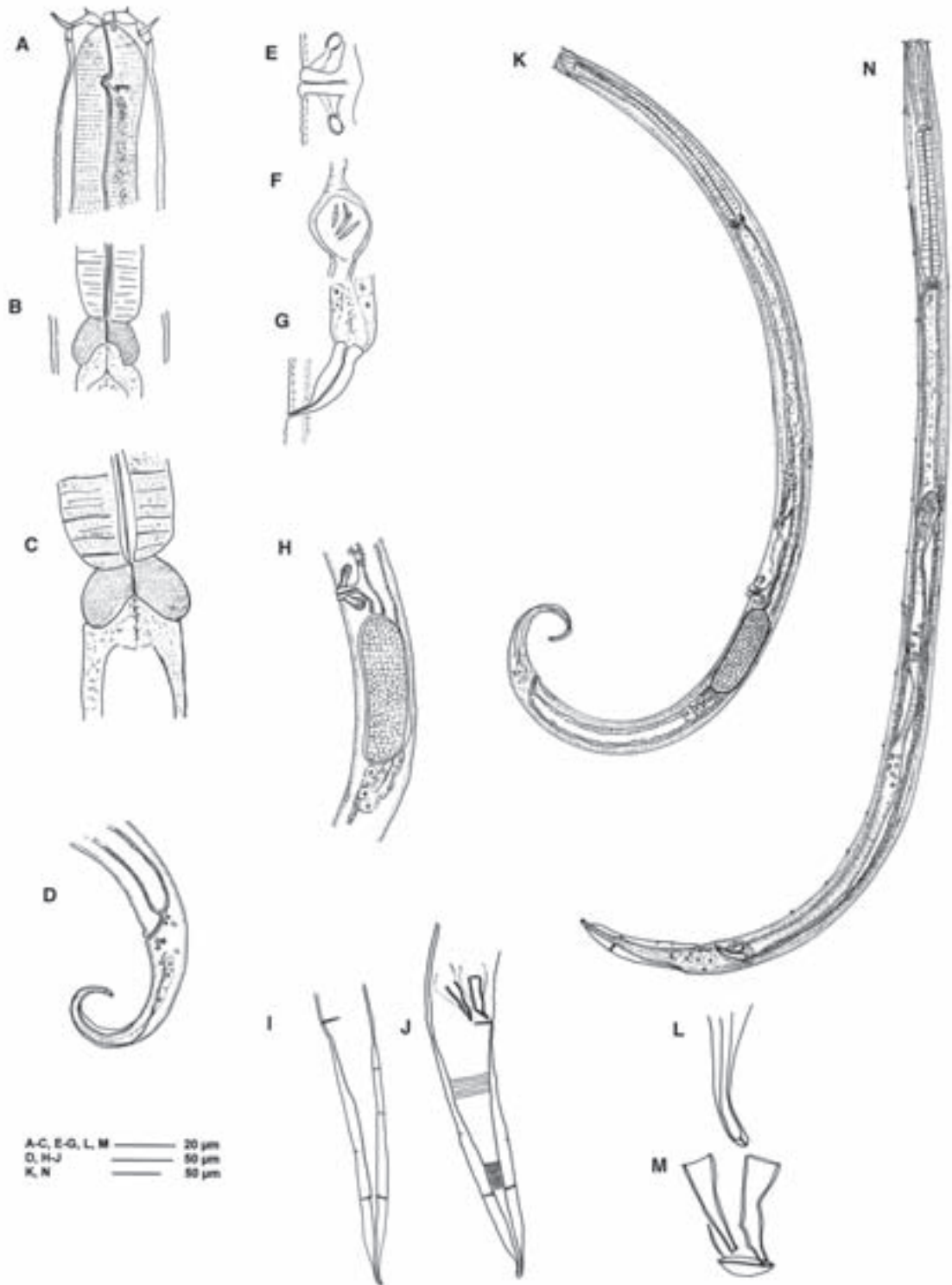
Figure 6. *Tripyla alaecaudata* sp. n. Female. (A-I). A: Anterior region; B, C: Cardia; D: Posterior region lateral; E: Vagina and glands; F: Spermatheca; G: Rectum; H: Posterior gonad lateral view: I: Tail latero-ventral view; K: Entire body: Male. (J, L-N). J: Tail, latero-ventral view; L: Spinneret; M: Spicules and gubernaculum; N: Entire body.
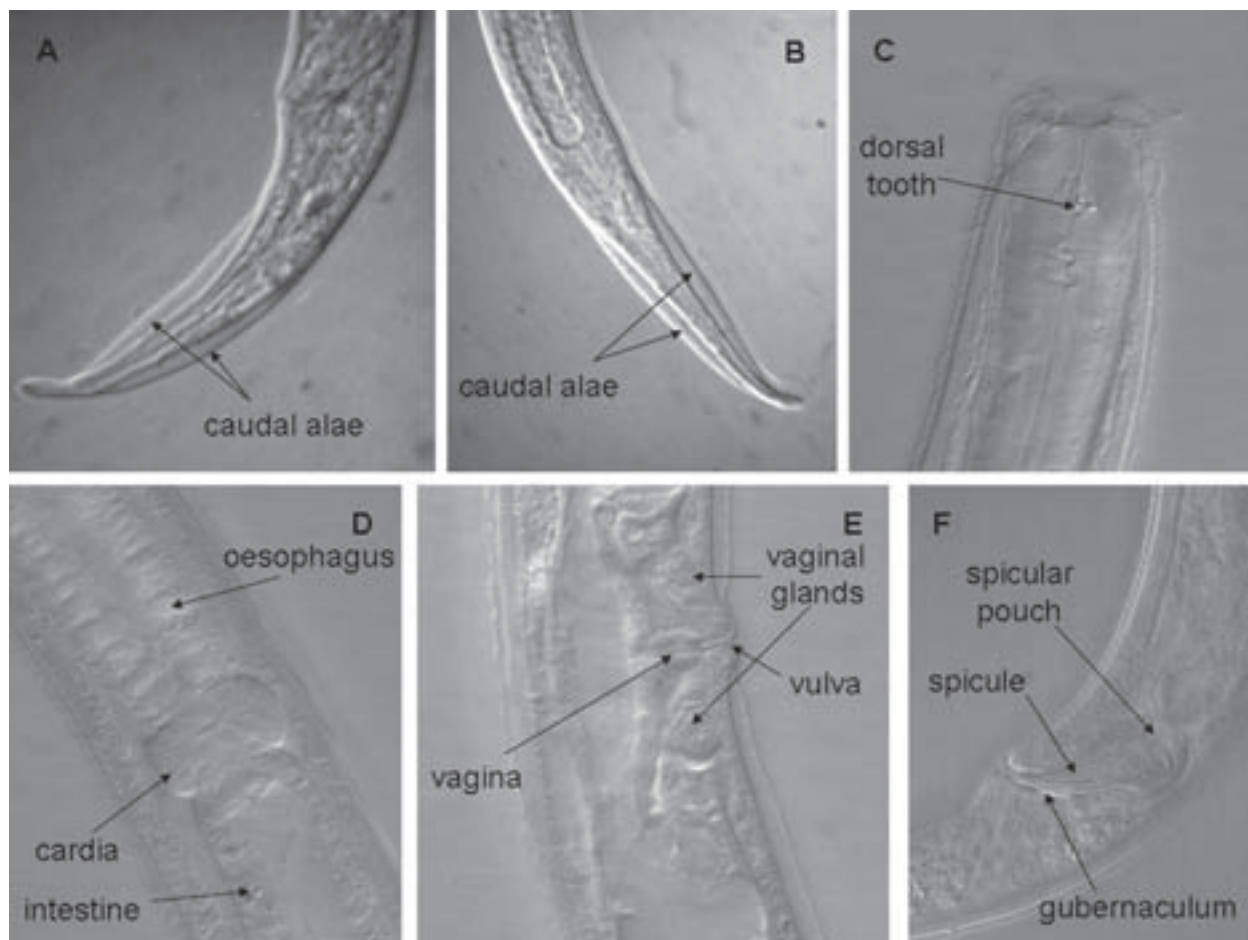
FIGURE 7. *Tripyla alaecaudata* **sp. n.** Male (A,D,F): A. Tail and caudal alae; D. Cardia; F. Spicules and gubernaculum; Female (B,C,E): B. Tail and caudal alae; C. Lip region; E. Vulva and vagina.

(29.1±2.9) long by 15-24 μm (20.3±2.1) wide, containing oval-shaped sperm cells. Rectum 23-37 μm (30.4±2) long. Tail curved ventrally, tapering in anterior region and becoming narrowly cylindrical in posterior third, ending in a long spinneret (Fig. 9D).

Male: Shape after fixation a simple spiral. Lip region symmetrical and continuous with body contour. Cuticle thickness on lip region 3.0 μm, and 1.5 μm along the rest of the body, not separated from body wall on fixation; with fine transverse striations over whole length of body, but bearing slightly coarser striations on caudal region. Lip region width inside bases of outer labial setae is 20-22 μm (21.4). Amphid apertures 10.0-11.5 μm (11.2) from the anterior end. Inner labial sensillae papilliform, outer labial setae conical, 9.0-10.5 μm (10.2) long or 41-50% (45.5) of lip region width. Cephalic setae conical and 5.0-6.0 μm (5.6) long, 3.0 μm posterior to the outer labial setae. Dorsal tooth small, wedge-shaped; very small subventral denticles 3.0-5.0 μm anterior to dorsal tooth. Nerve ring at about one-third of pharynx length. Fourteen ventral

papillae observed at varying distances apart, the first 70 μm from the anterior end and the last 50 μm anterior to the cloacal opening. Tail curved ventrally, with fine but distinct striation, narrowing to cylindrical in the posterior third of its length, and ending in a long spinneret. Oval-shaped sperm cells in *vas deferens* (Fig. 9C). Spicules with bifid end, surrounded by a muscular pouch; manubrium 7.0-8.5 μm (7.7±0.35) wide; gubernaculum slightly curved.

Diagnosis and relationships: Tripyla napaensis sp. n. is characterized by tiny subventral denticles anterior to the wedge-shaped dorsal tooth, the vulva with slightly protruding lips, the spermatheca oval with oval-shaped sperm cells, the 14 ventral papillae of the male, and the tail in both sexes tapering in the anterior region, becoming narrowly cylindrical in the posterior third and ending in a long spinneret.

Based on data provided by Andrássy (2007) and Brzeski and Winiszewska-Slipinska (1993), *T. napaensis*
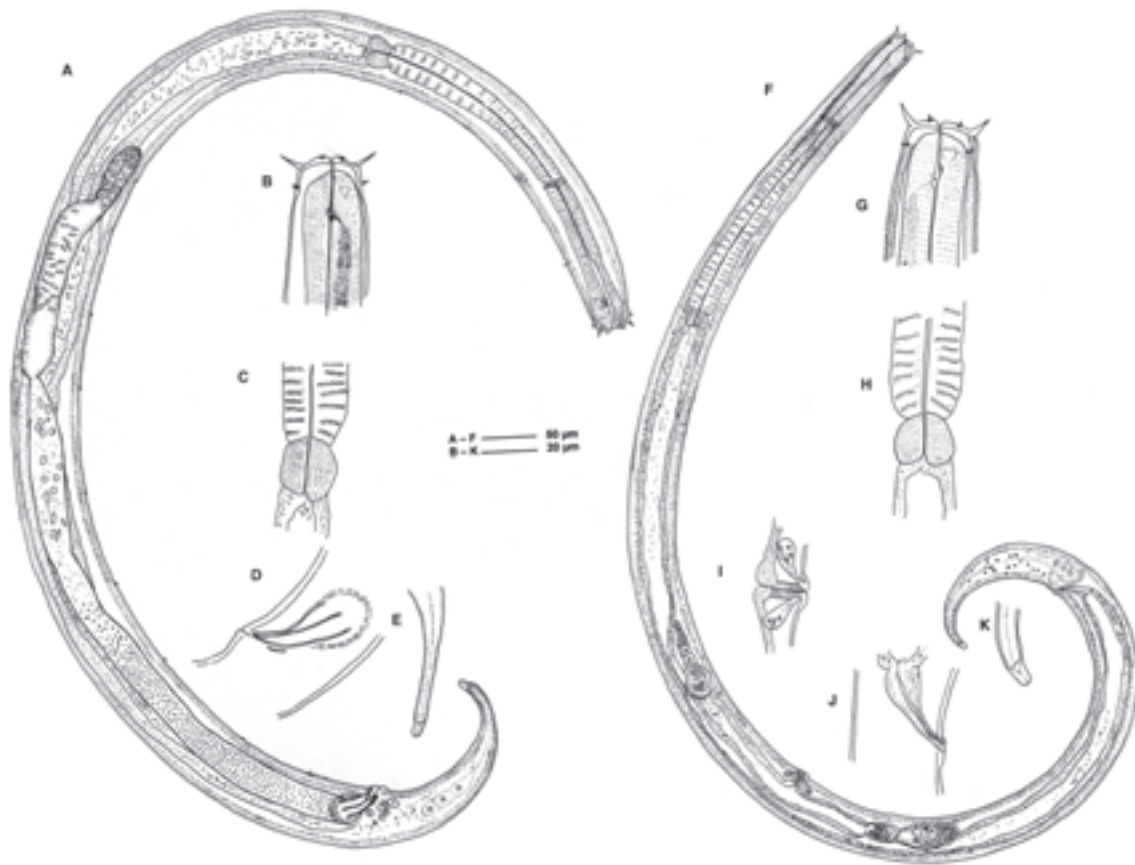
Figure 8. *Tripyla napaensis* Male (A-E). A: Entire body; B: Anterior region; C: Cardia; D: Spicules and gubernaculum, lateral view; E: Spinneret. Female. (F-K). F: Entire body; G: Anterior region; H: Cardia; I: Vagina and glands; J: Rectum; K: Spinneret.

sp. n. is similar to *T. setifera* Bütschli, 1873 in the size and shape of the outer labial setae (7.0-9.0 *vs* 5-10 µm) and the position of the vulva. We obtained slides of *T. setifera* from the USDA Nematode Collection in Beltsville, Maryland which contained six females and one male collected in Utah by Thorne. *Tripyla napaensis* sp. n. has longer outer labial setae than the Utah *T. setifera* (7.0-9.0 µm vs. 4.0-6.0 µm) but which do not differ from the 5-10 µm length reported by Brzeski and Winiszewska-Slipinska (*op. cit.*). Cervical setae are absent in *T. napaensis* sp. n. but present in *T. setifera*. Fourteen ventral papillae were observed in males of *T. napaensis* sp. n. but none in the male of *T. setifera* collected by Thorne. The new species has a narrow cylindrical posterior portion of the 150-168 µm (156.7±6.1) tail while that of *T. setifera* tapers uniformly and is variously reported as 115-250 µm by Brzeski and Winiszewska-Slipinska (*op. cit.*), 150-240 µm by Andrássy (*op. cit.*) and measured as 214-285 µm (250) in the specimens from Utah. *Tripyla napaensis* sp. n. differs from *T. setifera* in the size of the spinneret, 6.5-9.20

µm (7.8±0.4) *vs* 3.2-6.4 µm (4.8) in the material from Utah and 5-7 µm according to Brzeski and Winiszewska-Slipinska (*op. cit.*), and in the absence of cuticular structures in the vulva.

The new species resembles *T. tropica* sp. n. in the size of the body and position of the vulva but differs in the tail length (153-173 *vs* 170-285 µm), the length of the outer labial setae (7.0-9.0 *vs* 4.1-6.1 µm in *T. tropica*), *c*-ratio (8.1-9.4 *vs* 5.4-6.7), *c´*-ratio (4.0-4.7 *vs* 6.9-10.4), and in the absence of ventral papillae in males of *T. tropica* sp. n.

Type locality and habitat: Western hills of the Napa Valley, 0.75 km west of UC Oakville Experimental Vineyard, Oakville, California (N 38° 25.99'; W 122° 25.31'). Collected from soil moisture seepage near California Black Oak (*Quercus kelloggii*).

Type specimens: Accession numbers of type specimens deposited in the UCDNC are: holotype female

J. Nematode Morphol. Syst., 13 (1): 29-49 (2010)

42

(3964), paratype females (3965 and 3966), paratype males (3967), paratype juveniles (3968). Other paratypes deposited in UCRNC, WUNC, and CPNC.

Etymology: The specific epithet refers to geographical origin of the species.

Key to species of *Tripyla*

1. Tail of females and males with lateral caudal alae .... *alaecaudata* sp. n.
   Tail of females and males without lateral caudal alae ..2
2. Outer labial sensillae < 3 µm long 3
   Outer labial sensillae > 3 µm long 11
3. Body length < 0.9 mm..4
   Body length > 0.9 mm..5
4. c' < 2 ....*pygmea* Micoletzky, 1922
   c' > 2 .... *minuta* (Brezeski, 1963) Brzeski & .... Winiszewska-Slipinska, 1993
5. c' < 6 6
   c' > 6 8
6. Body length < 1.25 mm..................*terricola* Brzeski & Winiszewska-Slipinska, 1993
   Body length > 1.25 mm...7
7. Body length < 2 mm.............. *cornuta* Skwarra, 1921
   Body length > 2 mm......................*crassa* Alekseev & .... Bestalannaja, 1990
8. Outer labial sensillae < 2 µm.... *affinis* De Man, 1880
   Outer labial sensillae > 2 µm 9
9. Body length < 1.3 mm .... *koreana* Winiszewska, ... Brzeski, Choi & Kim, 2000
   Body length > 1.3 mm 10
10. Male with < 25 supplements .... *glomerans* .... Bastian, 1865
    Male with > 25 supplements ...……...*infia* Brzeski & .... Winiszewska-Slipinska, 1993
11. Outer labial sensillae > 6 µm 22
    Outer labial sensillae 3-6 µm.................................12
12. Body length < 1.0 mm .... *pulchella* Andrássy, 2008
    Body length > 1.0 mm 13
13. c' > 20 ....*italica* Tsalolikhin, 2003
    c' < 20 14
14. c' 11-16 15
    c' < 11 16
15. a < 45................................. *filicaudata* De Man, 1880
    a > 45, c' > 12 .... *subterranea* Tsalolikhin, 1976
16. a < 25...17
    a > 25... 18
17. Body length < 1.5 mm.................... *glosaria* (Gagarin, ... 1994) Andrássy, 2007
    Body length > 1.5 mm .... *dubia* Gagarin, 1997
18. Body length > 2.3 mm................*elongatula* Brzeski & .... Winiszewska-Slipinska, 1993
    Body length < 2.3 mm...19
19. Outer labial sensillae > 4 µm 20
    Outer labial sensillae < 4 µm 21
20. c' < 6.9 ....*vulvata* Andrássy, 1977
    c' > 6.9 .... *tropica* sp. n.
21. c < 6.1 ....*tenuis* Brzeski, 1964
22. c > 6.1 ..... *aquatica* Brzeski & Winiszewska-Slipinska, ... 1993
23. Body length < 2.5 mm...23
    Body length > 2.5 mm...27
24. c' < 8 24
    c' > 8 25
25. c < 8 ....*setifera* Bütschli, 1973
    c > 8 .... *napaensis* sp. n.
26. Body length < 1.5 mm................ *bioblitz* Zhao, 2009
    Body length > 1.5 mm...26
27. c < 5 .... *longicaudata* Nesterov, 1979
    c > 5 . *scandinavica* (Andrássy, 1967b) Andrássy, 2007
28. c' > 8 .... *sibirica* Gagarin, 1993
    c' < 8 28
29. a > 30....... *magna* Altherr & Delamare Deboutteville, ... 1972
    a < 30...............................*dybowski* Tsalolikhin, 1976

Molecular study

Direct sequencing of SSU rDNA PCR amplicons from individual *Tripyla*, *Tripylina* and *Trischistoma* specimens yielded high-quality electropherograms with no sequence polymorphisms observed within individuals. The SSU sequences have been deposited in GenBank (see Fig. 10 for accession numbers). There were no nucleotide differences observed when multiple individuals (2-3) were sequenced for a species. Each of the three newly described *Tripyla* species was distinct in nucleotide sequence when compared to all other definitively diagnosed species represented by SSU sequences in Genbank (i.e., *T. filicaudata*, *T. bioblitz*, *T. tropica* sp. n., *T. alaecaudata* sp. n., and *T. napaensis* sp. n.). Similarly, *Trischistoma veracruzense* sp. n. had unique sequence differences when compared to *T. monohystera*, the only other definitively diagnosed *Trischistoma* species with a SSU rDNA sequence. In contrast, the partial SSU sequences of *Tripylina* species were not always unique in pairwise comparisons of the diagnosed species deposited in GenBank.

Bayesian posterior probabilities (BPP) were uniformly strong (100%) for the monophyly of *Tripyla* taxa (Fig. 10). One clade representing *Tripylina* and another including *Trischistoma* plus *Trefusia* both also received 100% BPP (Fig. 10). These two subclades also formed a monophyletic group with strong (100%) posterior probability. For the three newly described species of *Tripyla*, individuals representing conspecifics were each monophyletic with 100% BPP support. Similarly, *T. veracruzense* sp. n. was phylogenetically distinct from the other *Trischistoma* species. *Trefusia zostericola* was strongly supported (100% BPP) as part of the *Trischistoma* clade (Fig. 10). There was no phylogenetic resolution within the clade representing four described *Tripylina* species and two additional *Tripylina* isolates (Fig. 10). A clade represented by *Alaimus* and *Paramphidelus* was strongly supported as the sister group to *Tripylina* plus *Trischistoma* and *Trefusia*.
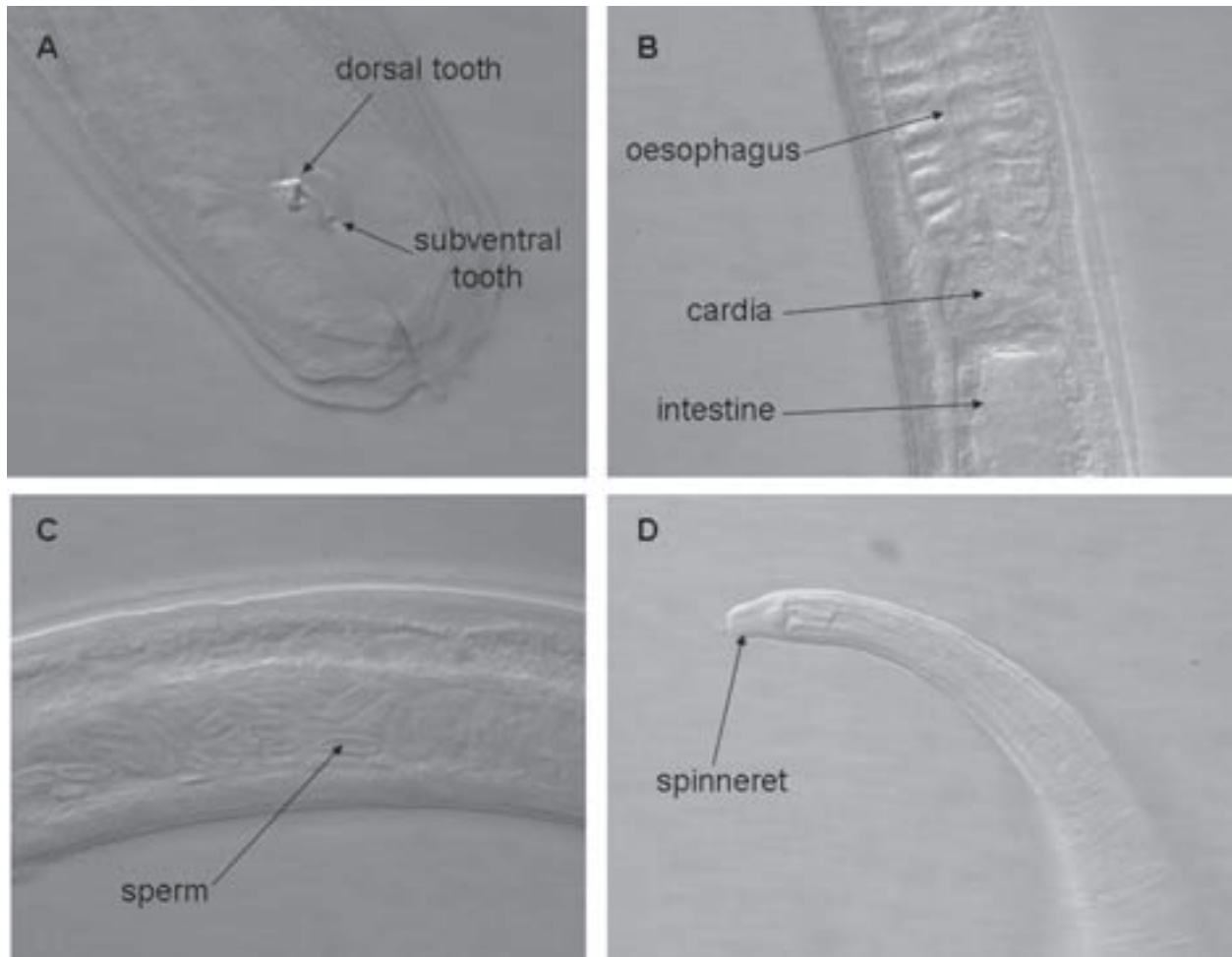
FIGURE 9. *Tripyla napaensis* sp. n. Male (A-C): A. Anterior end; B. Cardia; C. Testis. Female (D): Tail and spinneret.

Other deeper nodes in the Bayesian tree generally had low posterior probabilities.

Discussion

The species described herein were discovered during the course of ecological studies. They were described first by morphometric characters and their differences confirmed by molecular analysis. In one study we characterised nematode fauna along a transect extending from a wooded hillside into a vineyard (Sánchez-Moreno & Ferris, 2006). Two distinct species of *Tripyla* were present on the hillside. Analysis of nematode faunal diversity in samples from an ecological study at the Los Tuxtlas Biosphere Reserve in México revealed *Tripyla tropica* sp. n. and *Trischistoma veracruzense* sp. n.

Excretory pores have been described for *Tripyla affinis, T. aquatica, T. elegantula* (Brzeski & Winiszewska-Slipinska, 1993). The character appears to be variable, or perhaps the excretory pore is difficult to see. We did not observe excretory pores in *T. tropica* sp. n., *T. alaecaudata* sp. n. or *T. napaensis* sp. n.

In some of the species we describe herein, we observe two structures that appear to have fine ducts opening into the vagina close to the vulva. The structures contain refractive granules and we characterize them as "vaginal glands". Chitwood and Chitwood (1950) suggest that the cell bodies of the vaginal muscle cells may be expanded in a bladder-like shape but that, in other cases, there are structures that may be actual glands. Vaginal glands are reported, e.g., for some Monhysteridae (Eyualem-Abebe & Coomans, 1996) and from marine nematodes such as *Halichoanolaimus* (Ditlevesen, 1921; Chitwood & Chitwood, 1950). Previous descriptions of

J. Nematode Morphol. Syst., 13 (1): 29-49 (2010)

44

Tripylidae do not mention vaginal glands although similar structures appear in .some drawings of the nematodes (*e.g.*, Zhao, 2009).

The Triplonchida assignment of the Tripylidae, excluding *Trischistoma*, based on molecular evidence is supported by the morphological association with the muscular pouch or "spicule protrusion capsule" surrounding the spicules. The pouch is involved, presumably, in spicule protraction since spicule protractor muscles are absent in the Triplonchida (De Ley & Blaxter, 2002; Zullini, 2006). Our male specimens of *Tripyla tropica* sp. n., *T. alaecaudata* sp. n. and *T. napaensis* sp. n. all exhibit the muscular pouch around the spicules. Males are unknown for *Tripylina arenicola*. Besides lacking the muscular pouch in males, *Trischistoma* spp. also differ from the other genera in that the oesophago-intestinal valve is a simple sphincter of small cells rather than a distinctive cardia structure (Fig. 1).

Molecular characterization.- Our purpose in analyzing sequence data was to compare the new species with relevant species already represented in GenBank, and to assess molecular phylogenetic relationships for these taxa. The SSU Bayesian phylogenetic hypothesis does not support monophyly of Tripylidae in the traditional sense (Zullini, 2006). For example, there is strong molecular support for most recent common ancestry of *Trischistoma*, *Tripylina* and a group of enoplean families (Alaimidae and Trefusiidae) not considered closely related to Tripylidae. The Bayesian analysis indicates that *Tripyla* shares a more recent common ancestry with taxa representing Tripyloidea, including *Tobrilus*, *Tripylella*, and *Prismatolaimus* plus two trichodorid genera (*Trichodorus* and *Paratrichodorus*). This result was previously reported based on both SSU and LSU rDNA sequences (Zhao, 2009). The strongly supported monophyly of *Trischistoma* and *Trefusia* was reported previously based on full-length SSU data (Holterman *et al.*, 2006). We extend this finding to include *Tripylina* in this clade, as sister to *Trefusia* and *Trischistoma*. Phylogenetic analyses of these partial SSU sequences resolved the newly described *Tripyla* species as distinct lineages, but these sequences did not resolve relationships among most *Tripyla* species. A similar result was reported by Zhao (*op. cit.*) for longer SSU sequences, whereas partial LSU sequences showed greater phylogenetic resolution among species of *Tripyla* and *Tripylina*. Sequencing multiple genes for unsampled representatives of Tripylidae (e.g., *Abunema*, *Tobrilia*) is needed to refine understanding of this group; however, results to date support the need for taxonomic reorganization of Tripylidae given that this family is not monophyletic. We agree with Holterman *et al.* (2006) and Holterman and Holovachov (2007) regarding the affinities of *Trischistoma* with *Trefusia* in the Enoplida, and we add *Tripylina* to that clade.

Individuals representing the same *Tripyla* morphospecies have identical SSU sequences. This result is consistent with the expectation of limited intra-individual ribosomal DNA polymorphism within species.

In contrast, pairwise comparisons of SSU sequences from different *Tripyla* species, including *T. alaecaudata* sp. n., *T. tropica* sp. n. and *T. napaensis* sp. n. reveals diagnostic sequence differences. That is, each of these species can be identified based on their characteristic SSU sequences. These molecular results provide independent evidence for the morphologically based differential diagnosis of these species. However, testing hypotheses of species using molecular data (species delimitation) is best accomplished by the more rigorous approach of phylogenetic (evolutionary) analysis rather than pairwise differences (Adams, 1998; Nadler, 2002). Phylogenetic analysis of these new SSU sequences supports the delimitation of *T. alaecaudata* sp. n., *T. napaensis* sp. n., and *T. tropica* sp. n. In contrast, phylogenetic analysis of the SSU sequences did not delimit the *Tripylina* species as distinct, and several of these taxa also lacked diagnostic sequence differences for this region of SSU rDNA. However, several of these same species show diagnostic sequence differences and are delimitation by phylogenetic analysis when compared using regions of D2D3 LSU rDNA (Zhao, 2009). Thus, interpreting the absence of SSU differences in *Tripylina* as evidence for conspecificity would not be prudent because more rapidly evolving regions of ribosomal DNA have been shown to distinguish *Tripylina* taxa as separate species even when more slowly evolving SSU regions do not (Zhao, *op. cit.*). In this case, there is no inconsistency in interpreting diagnostic sequence differences as corroborating a morphological differential diagnosis (i.e., *Tripyla* species), while conversely considering that a morphologically based species diagnosis is not invalidated by the absence of SSU molecular differences (i.e., *Tripylina*). For molecular species delimitation, best practices require sequencing genes representing multiple loci, and using genes with faster rates of substitution that should be more informative for the most recent speciation events (Nadler, 2002).

Biology and ecology.- Nematodes are represented by a rich and abundant diversity. The ecosystem services provided by the fungivore and bacterivore nematodes, including dispersal of bacteria and mineralization of important nutrients, are well-recognized (Ferris *et al.*, 1997; Fu *et al.*, 2005; Ingham *et al.*, 1985; Okada & Ferris, 2001). Those provided by nematodes that feed on other organisms are less well studied but are undoubtedly real. Predation by nematodes may regulate and stimulate turnover in their prey. However, in many cases we do not know the nature or extent of the prey (Yeates *et al.*, 1993) or the differences and relationships among the predators.

Based on observation of remains in the intestine of *Tripyla papillata* (since synonymised with *T. glomerans*) by Menzel (1920), amplified by subsequent reports, Tripylidae feed as generalist predators of protozoa, small nematodes, tardigrades and rotifers (Goodey, 1963; Yeates, 1971; Brzeski & Winiszewska-Slipinska, 1993; Yeates *et al.*, 1993; Traunspurger, 2002; Zullini, 2006). We have also seen nematode remains in intestines of the Tripylidae in this study. Although the stoma and pharyngeal lumen

45

J. Nematode Morphol. Syst., 13 (1): 29-49 (2010)

Figure 10. Bayesian posterior probability consensus tree inferred from the SSU rDNA sequence dataset. MCMC posterior clade 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. Taxa in boldface represent the newly described species.

J. Nematode Morphol. Syst., 13 (1): 29-49 (2010)

46

of these taxa appear to have a rather uniform, narrow diameter, contraction of the surrounding radial muscles causes the stoma to open into a cup-shaped aperture in which the tooth or teeth, normally tucked into a cavity in the stoma wall, become directed anteriorly to penetrate the cuticle of an ingested prey organism. The Tripylidae fall into the feeding category defined by Traunspurger (2002) as "chewers", which are characterised by a voluminous, sclerotised, buccal cavity armed with one or more teeth and denticles.

All known species of Tripylidae have a spinneret at the end of the tail for attachment to the substrate. In each case, three caudal glands supply the spinneret with mucoid secretions. The secretions allow temporary attachment to the substrate (Traunspurger, 2000). Nematodes of the Tripylidae move with whip-like motion in water or water films while remaining attached to the substrate. Periods of activity are followed by periods of bodily inactivity. The whip-like activity of these substrate-attaching nematodes results from rapid constrictions of the longitudinal muscles opposed by the hydrostatic pressure generated within the body cavity. Given the power of the muscular contractions and the consequent magnitude of the internal pressures, it is reasonable to expect well-developed sphincter muscles around canals to body apertures to prevent eversion of body contents. That seems to be the case in the Tripylidae.

It is interesting to consider the relationship between form and function. In moving water, the spinneret attachment would likely provide anchorage of the nematode in one location. However, these nematodes often are found in still water where sediments develop and those species living in soil do not encounter rapid water movement. Conceivably, therefore, one function of the rapid movements of the body is to stir the sediment and organisms within it. Prey organisms may then be consumed as they settle around the predator during the alternating periods of inactivity. The well-developed labial and cephalic setae presumably function as tactile sensillae in detecting the presence of prey. The bioperturbation associated with the body movement probably moves organisms to areas of unexploited substrate, increases microbial activity and the rate of carbon mineralization (Giere, 1993; Traunspurger, 2002).

As we are reminded by De Ley *et al.* (2006), all nematodes are aquatic, and the habitat distinction between a nematode in freshwater sediments and one in moist sandy soil is vague at best. The habitats occupied by *Tripyla*, *Trischistoma* and *Tripylina* range from the water-film thickness of wet sandy soil to the water films represented by streams or lakes, with the thickness limits at either end of the spectrum as yet undefined. Certainly, the attachment and bodily activity associated with feeding of these nematodes must require a water film thickness greater than that necessary for the movement of many soil-inhabiting nematodes.

In terms of trophic structure in the food web, *Tripyla*, *Trischistoma* and *Tripylina* are among the first of the predators that appear in a post-perturbation succession.

They are classified as early succession carnivores in the Ca-3 functional guild (Bongers & Bongers, 1998; Ferris *et al.*, 2001) which infers that, of the predators, they have a shorter generation time than many higher trophic level and larger-bodied species.

Many of the described species of *Tripyla* and other Tripylidae are from collections made in streams and lakes in Europe. Our studies and subsequent observations suggest that there is a rich diversity of the Tripylidae in the soil and aquatic habitats of the Americas.

Acknowledgements

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48

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49

J. Nematode Morphol. Syst., 13 (1): 29-49 (2010)