



A review of the genus *Tripylina* Brzeski, 1963 (Nematoda: Tripylidae), with descriptions of five new species from New Zealand

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Abstract

Tripylina contains six accepted species. Morphologically, five new species can be briefly characterized as follows: *T. tearoha* **sp. nov.**, *T. manurewa* **sp. nov.** and *T. tamaki* **sp. nov.** each have a single ventromedian seta and two pairs of lateral setae in the cervical region; *T. tearoha* **sp. nov.** differs from *T. tamaki* **sp. nov.** by de Man's Index *a* (25–30 vs 20–23), and differs from *T. manurewa* **sp. nov.** by the distance of the ventromedian cervical seta from the head end (62–77 vs 78–86 µm). *T. manurewa* **sp. nov.** differs from *T. tamaki* **sp. nov.** by de Man's Index *a* (24–29 vs 20–23). Molecularly, these three new species can be differentiated by SSU & LSU analysis. *Tripylina yeatesi* **sp. nov.** and *T. kaikoura* **sp. nov.** are characterised by relatively long bodies, and the absence of ventromedian cervical setae; *Tripylina yeatesi* **sp. nov.** differs from *T. kaikoura* **sp. nov.** by de Man's Indices: *a* (29–30 vs 22–23), *b* (5.8–6.0 vs 6.0–6.2), *c* (18–26 vs 14–16) and *c'* (2.0–2.6 vs 2.7–3.0), respectively. Among the six previously described species, the main distinguishing features for *T. arenicola* and *T. ursulae* are their subventral stoma denticles lying posterior to the dorsal tooth, which differentiates them from *T. longa*, *T. macroseta*, *T. sheri* and *T. stramenti*. All published species also can be differentiated by: body length; values of De Man's Indices *a*, *b*, *c*, *c'* and *V*; presence or absence of setae and setal positions. A key is provided for all eleven species of the genus.

Key words: New Zealand, genus review, new species, morphology, molecular, Nematoda, Tripylidae, *Tripylina*, key

Introduction

Nematodes of the genus *Tripylina* Brzeski, 1963 are found in soil and aquatic habitats. Proposed as a genus containing three species in the family Tripylidae de Man, 1876 by Brzeski (1963), the genus has since been discussed and reviewed, and three more species have been added (Tsalolikhin 1983; Andrassy 1985, 2007; Brzeski & Winiszewska-Ślipińska 1993; Zullini 2006). *Tripylina* currently consists of six published species recorded from Europe, Asia, North America, South America, Africa and New Zealand (Brzeski & Winiszewska-Ślipińska 1993; Tsalolikhin 1983; Andrassy 2007 & 2008; Yeates 1972), of which *T. stramenti* (Yeates, 1972) Tsalolikhin, 1983 was described from New Zealand. All these taxa are based solely on morphological characters. Since March 2007, the nematodes in the family Tripylidae have been sampled from various areas in New Zealand. Over 200 soil and litter samples from native forests and conservation parks have been examined.

The ribosomal DNA (rDNA) is a component of the middle repetitive DNA of the nuclear genome, and the presence of multiple copies of these genes in the genome facilitates PCR amplification from a single nematode (Powers *et al.* 1997). The large subunit ribosomal (LSU) rRNA gene has been useful for resolving closely related taxa (Al-Banna *et al.* 1997; Nadler and Hudspeth 1998; Duncan *et al.* 1999). The small subunit ribosomal (SSU) rRNA gene has been found to be useful for phylogenetic analysis across the Phylum Nematoda (Fitch *et al.* 1995; Aleshin *et al.* 1998; Blaxter *et al.* 1998; De Ley *et al.* 2002; Ye *et al.* 2007a,b; Zhao *et al.* 2008). Therefore, sequence analysis of PCR amplified from both the D2/D3 expansion segments

of LSU and the near full length of SSU were used for to analysis of the phylogenetic relationships of New Zealand tripylids.

In this paper, five new species of *Tripylina* are described from New Zealand, new morphological features are utilized, and molecular taxonomy was applied extensively to this group for the first time. A review of the genus is also presented, along with a key to species of all eleven species of the world.

TABLE 1. Nematode species voucher number, GenBank accession number and collection localities in New Zealand.

Species	Voucher number	GenBank Accession Number		Locality
		SSU	LSU	
<i>Tripyla</i> sp. long tail ST01	NE109	GQ503061	GQ503038	St Johns Bush, AK, NZ
<i>Tripyla</i> sp. long tail BOT2	NE164	GQ503062	GQ503039	Botanic Garden, AK, NZ
<i>Tripyla</i> sp. long tail SB03	NE201	GQ503063	GQ503040	Smith's Bush, AK, NZ
<i>Tripyla</i> sp. long setae CH09a	NE73a	GQ503064	GQ503041	Christchurch, NZ
<i>Tripyla</i> sp. long setae RA5	NE43	GQ503065	GQ503042	Rotorua, NZ
<i>Tripyla</i> sp. long setae AS03	NE155	GQ503066	GQ503043	South Island, NZ
<i>Tripyla</i> sp. long setae CH09b	NE73b	GQ503067	GQ503044	Christchurch, NZ
<i>Tripyla</i> sp. short setae T4T	NE16	GQ503068	GQ503045	Thames, NZ
<i>Tripyla</i> sp. short setae CH012	NE76	GQ503069	GQ503046	Christchurch, NZ
<i>Tripyla</i> sp. short setae WS	NE127	GQ503070	GQ503047	Western Springs Park, NZ
<i>Tripyla</i> sp. short setae Abbotts	NE128	GQ503071	GQ503048	Waiatarua Reserve, AK, NZ
<i>Tripyla</i> sp. short setae CH014	NE78	GQ503072	GQ503049	Christchurch, NZ
<i>Tobrilus</i> sp. WW1	NE45	GQ503073	GQ503050	Waiau Falls, NZ
<i>Tripyrella</i> sp. big chamber R10	NE88	GQ503074	GQ503051	Rauroa Bush Reserve, NZ
<i>Trischistoma</i> sp. Chris01	NE106	GQ503075	GQ503052	Waiotama, NZ
<i>Trischistoma</i> sp. BOT3	NE165	GQ503076	GQ503053	Botanic Garden, AK, NZ
<i>Geomonhystera</i> sp. Bruce Park	NE461V	GQ503077	GQ503054	Bruce Park, NZ
<i>Monhystera</i> sp. T4M	NE16	GQ503078	GQ503055	Thames, NZ
<i>Trischistoma</i> sp. CH09c	NE73c	GQ503079	-	Christchurch, NZ
<i>Tripylina tearoha</i> sp. nov.	NNCNZ 2535–2541	FJ480406	GQ503056	Te Aroha Domain, NZ
<i>Tripylina tearoha</i> sp. nov.	NNCNZ 2542–2545	FJ480407	GQ503057	Smith's Bush, AK, NZ
<i>Tripylina manurew</i> sp. nov.	NNCNZ 2546–2553	FJ480408	GQ503058	Botanic Garden, AK, NZ
<i>Tripylina tamaki</i> sp. nov.	NNCNZ 2546–2553	FJ480409	GQ503059	St Johns Bush, AK, NZ
<i>Tripylina tamaki</i> sp. nov.	NNCNZ 2546–2553	FJ480410	GQ503060	St Johns Bush, AK, NZ

Note: NNCNZ—Nematode National Collection New Zealand; NE—Nematode Extraction; AK—Auckland; NZ—New Zealand.

Material and methods

Nematode sampling, extraction & processing

Soil and litter mixtures were collected from several locations in New Zealand (Table 1). The 0–10 cm topsoil and litter mixtures were collected by a trowel from under trees or shrubs. The samples were placed in plastic bags, and transported back to the laboratory, where they were kept at 10°C until extraction. Nematodes were extracted using the Whitehead and Hemming tray method from sub-samples of 500 g material over 2 days, at room temperature. Using a 20-µm-mesh sieve the suspension was reduced to about 5 ml and left to stand for about one hour. The volume was reduced to 3 ml by aspiration of excess fluid. The nematodes were

then transferred to a glass block for examination by hand using a dissecting microscope at 8X to 35X magnification (Leica EZ4, Germany). Nematodes actively swimming through water by means of bursts of rapid oscillations of the head, with narrow stoma, pharynx a muscular tube, were selected for further study.

For morphological examination, nematodes were killed and fixed using hot 3% formaldehyde, and left to harden for at least 2 weeks. All nematodes were processed to glycerol, and mounted on glass slides, as described by Hooper (1986) and modified by Davies and Giblin-Davis (2004).

Drawings were made using an interference contrast microscope with a camera lucida (Nikon, Eclipse 90i). Measurements were made using NIS-Elements Basic Research (Nikon, Version 2.32). Head diameter was measured at the level of cephalic setae. Body length was measured along the mid-line. De Man's (1880) Indices were determined as follows: a = total body length divided by greatest body diameter; b = total body length divided by distance from anterior end to posterior of pharynx (excluding pharyngeal-intestinal glands); c = total body length divided by tail length; c' = tail length divided by body diameter at anus or cloaca; V = anterior end to vulva as percentage of total body length. The terminology for head sensillae was based on the latest revision of the family Tripylidae by Andr ssy (2007).

DNA extraction

Several nematodes of the same apparent species were hand-picked from living nematode suspension, and put in a tube containing 1M NaCl. This was stored at -20°C in a freezer for future DNA extraction by the nematode DNA extraction method of Zheng *et al.* (2002). Total genomic DNA was extracted from a single nematode using worm lysis buffer containing proteinase K (Williams *et al.* 1992). DNA extracts were stored at -4°C until used as PCR template.

Polymerase chain reaction (PCR) and PCR product purification

Primers for LSU amplification were forward primer D2A (5' ACAAGTACCGTGAGGGAAAGT 3') and reverse primer D3B (5' TGCGAAGGAACCAGCTACTA 3') (Nunn 1992). Primers for SSU amplification were forward primer 18S-G18S4 (5' GCTTGTCTCAAAGATTAAGCC 3') and reverse primer 18S-18P (5' TGATCCWKCYGCAGGTTTCAC 3') (De Ley *et al.* 2002; Dorris *et al.* 2002).

For both SSU and LSU, the 20 μl PCR contained 10 μL Go Taq[®] Green Master Mix (Promega Corporation, Madison, WI, USA), 1 μL (0.05 μM) each of forward and reverse primers, and 2 μl of DNA template. The thermal cycling program was as follows: denaturation at 95°C for 3 min, followed by 30 cycles of denaturation at 94°C for 60 sec, annealing at 55°C for 45 sec, and extension at 72°C for 45 sec. A final extension was performed at 72°C for 10 min.

PCR products were purified by Wizard[®] SV Gel and PCR Clean-Up System (Promega Corporation, Madison, WI, USA).

Sequencing

Purified DNA PCR products were cycle sequenced in both directions with the appropriate primers using BigDye Terminator Ready Reaction Mix v3.1 (Applied Biosystems, USA). Sequences were obtained with a 3130xl Genetic Analyzer (Applied Biosystems, USA) and assembled and edited with Sequencher 4.8 (Gene Codes Corp.). Each sequence was confirmed by double sequencing.

Sequence alignments and phylogenetic inferences

The SSU and LSU sequences of *Tripylina tearoha* **sp. nov.**, *T. manurewa* **sp. nov.**, *T. tamaki* **sp. nov.** and all other nematodes from New Zealand studied in the paper were deposited in the GenBank database (Table 1). DNA sequences were aligned by Clustal W (Thompson *et al.* 1994). The models of base substitution in both SSU and LSU data sets were evaluated using MODELTEST (Posada & Crandall 1998) and PAUP*4.0b10 (Swofford 1998). The resulting alignment was then analysed in MrBayes v3.1.2 (Ronquist & Huelsenbeck 2003) under the best fit-AIC model. Four heated chains with a temperature of 0.2 were run for 5

million generations under the GTR+I+ Γ model. Prior distributions were as follows: revmatpr (dirichlet = 1,2,1,1,2,1), statefreqpr (dirichlet = 1,1,1,1), brlenspr (exponential = 10), shapepr (exponential = 5), pinvarpr (uniform = 1–10), and topologypr (uniform). The thinning interval and burn in were 1000 and 500,000 respectively. This analysis was repeated twice and results compared between runs to ensure that convergence was reached. The posterior probabilities for nodes were taken from summarizing the output set of trees (Larget & Simon 1999).

Previously described, accepted species

The measurements and descriptions of previously described, accepted species are based on either the original descriptions or the revision of Brzeski & Winiszewska-Ślipińska (1993): specimens were not re-examined in this study. An attempt was made to re-examine type material of *Tripylina stramenti* (Yeates, 1972) Tsalolikhin 1983, but unfortunately both holotype and paratypes are in very bad condition.

Genus *Tripylina* Brzeski, 1963

Synonym. *Abunema* Khera, 1971 (see Andrásy 1985, 2007)

Description (after Brzeski 1963). Body length 0.8–1.8mm. Cuticle smooth with numerous pores, thin, and not annulated. Six longer and four shorter cephalic setae in a single whorl. Stoma with dorsal tooth in a stomatal chamber, with two subventral denticles anterior or posterior to it. Pharyngeal-intestinal valve composed of three glands around anterior portion of intestine. Female genital organ mono-prodelphic, without post-vulval uterine sac, reflexed; vagina with internal sclerotized pieces; *V* 60–80. Males very rare. Spicules narrow, sickle-shaped. Supplements confined to precloacal region. Tail in both sexes short, anteriorly conical and posteriorly cylindrical, strongly bent.

Type species. *Tripylina arenicola* (de Man, 1880) Brzeski, 1963.

Diagnosis and relationships. The mono-prodelphic female genital system characterizes this genus and differentiates it from both *Tripyla* Bastian, 1865 and *Tripylella* Brzeski & Winiszewska-Ślipińska, 1993.

Etymology. *Tripylina* is a diminutive of *Tripyla*.

Remarks. There is no consensus on generic level classification in the family Tripylidae based on morphological characters, despite many morphological studies in the last 20–30 years. Tsalolikhin (1983) included four genera: *Tripyla* Bastian, 1865, *Paratripyla* Brzeski, 1964, *Trischistoma* Cobb, 1913 and *Tripylina*. Brzeski and Winiszewska-Ślipińska (1993) partly accepted Tsalolikhin and assigned *Tripylina*, *Tripyla* Bastian, 1865 and *Tripylella* Brzeski & Winiszewska-Ślipińska, 1993 to the family. Zullini (2006) largely accepted the classification of Brzeski and Winiszewska-Ślipińska (1993), but provisionally included *Trischistoma* Cobb, 1913, *Tobrilia* Andrásy, 1967 and *Abunema* Khera, 1971 because those genera had no alternative placements. More recently, Andrásy (2007) considered the family Tripylidae to comprise three subfamilies (Tripylinae de Man, 1876, Trischistomatinae Andrásy, 2007 and Tobriliinae Andrásy, 2007), and proposed placing *Tripylina*, *Tripyla* and *Tripylella* into the subfamily Tripylinae; *Trischistoma* into Trischistomatinae and *Tobrilia* into Tobriliinae.

Molecular taxonomy has been applied in many areas of nematode systematics for more than a decade. However, this is the first attempt to study extensively in the family Tripylidae by molecular phylogenetic methods. This analysis, including the New Zealand tripylids and previously published nematode SSU and LSU sequences from GenBank indicated the following (Fig. 1 & 2).

1) Genetically *T. tearoha* **sp. nov.**, *T. tamaki* **sp. nov.** and *T. manurewa* **sp. nov.** are different species.

2) *T. tearoha* **sp. nov.** and *T. tamaki* **sp. nov.** are closely related and some evolutionary distance separates them from *T. manurewa* **sp. nov.**

3) The family Tripylidae may contain five to six groups (*Tripyla* with long tails, *Tripyla* with long cephalic setae, *Tripyla* with short cephalic setae, *Tripylina* and *Trischistoma*) (Zhao & Thomas 2009).

- 4) *Tripylina* and *Trischistoma* are not closely related to *Tripyla*, the type genus of the family Tripylidae.
 5) The relationships among genera within Tripylidae are not well resolved.
 6) The genus *Tripylina* appears monophyletic with respect to *Trischistoma* and more closely related to the Enoplida than to the Triplonchida, a result consistent with the results of Meldal *et al.* (2007).
 The systematic position of the genera in the family Tripylidae is discussed in Zhao & Thomas (2009).

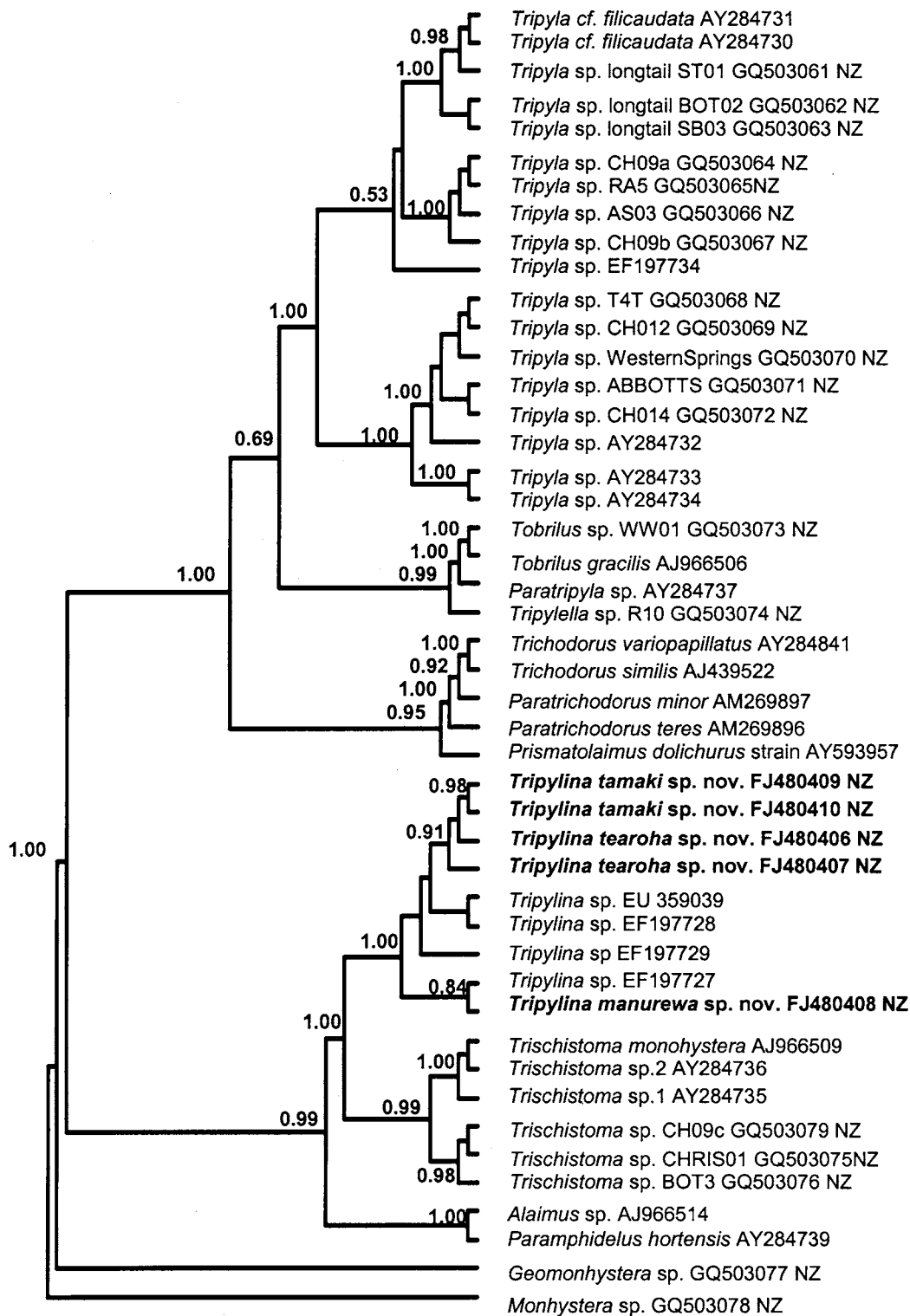


FIGURE 1. Bayesian tree inferred from SSU gene DNA sequences. Posterior probabilities exceeding 50% are given on appropriate clades. Nematode species, GenBank numbers, locations are listed for each taxon if known.

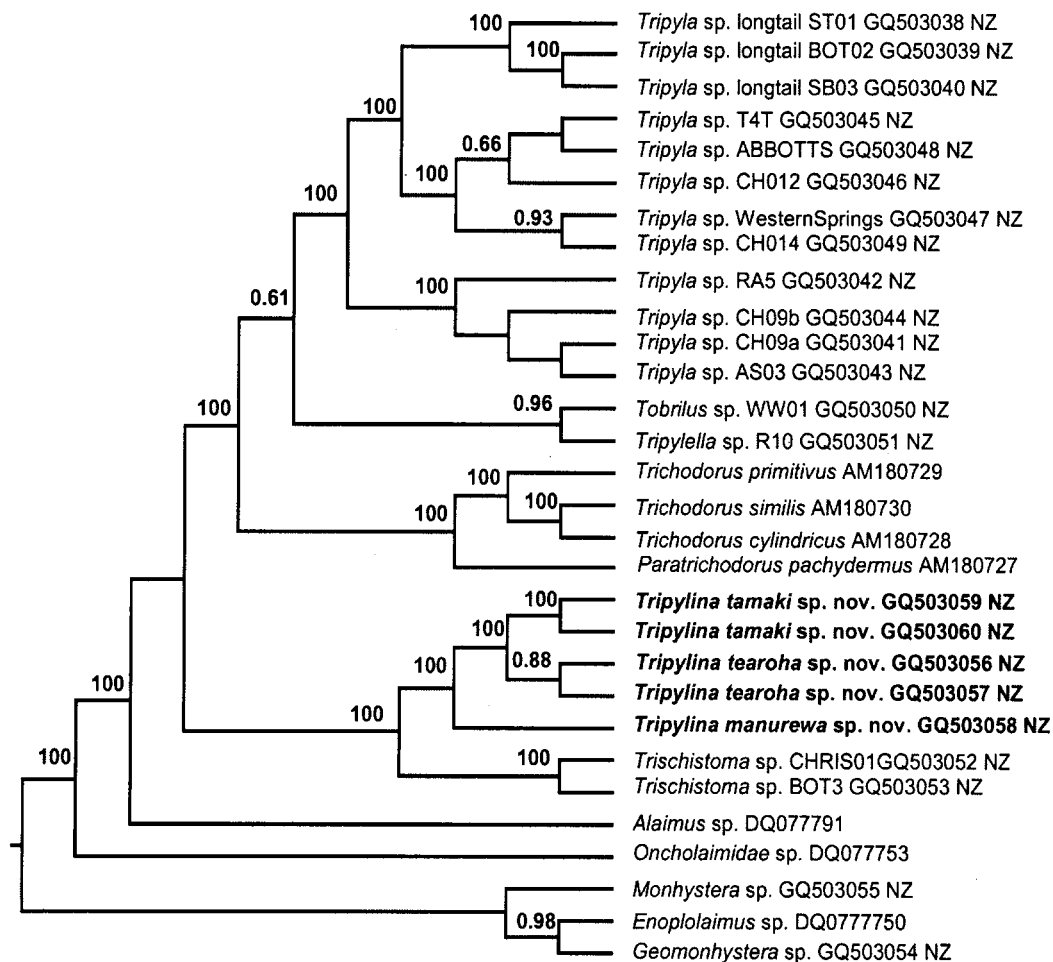


FIGURE 2. Bayesian tree inferred from LSU gene DNA sequences. Posterior probabilities exceeding 50% are given on appropriate clades. Nematode species, GenBank numbers, locations are listed for each taxon if known.

***Tripylina tearoha* sp. nov.**

(Fig. 3A–F)

Measurements. Table 2.

Material examined. Holotype: National Nematode Collection New Zealand (NNCNZ), slide No. 259.

Paratypes: eleven females. NNCNZ, slide Nos 2535–2545.

Description. Female. Body ventrally arcuate when fixed (Fig. 3A), posterior more curved than anterior. Cuticle smooth, about 1–2 μm thick anteriorly, no distinct layering observed. Maximum body width generally near ovary, occasionally near vulva or base of pharynx. Body pores small and numerous, evenly distributed on both ventro-lateral and dorso-lateral aspects.

Head rounded, continuous with body contour. Labial papillae short, conical. Six long and four short cephalic setae in a single whorl; the six setae 11–13 μm long or 52–60% of head diameter, more or less arcuate and directed anteriorly; the four setae 5.0–5.8 μm long, thinner than the six long cephalic setae, more or less arcuate. A single ventromedian cervical seta, thin, located 62–77 μm , or 37–38% of pharyngeal length, from anterior end of body. Two pairs of lateral cervical setae, one anterior and one posterior to nerve ring (Fig. 3C). Stoma walls thickened; dorsal tooth large, triangular; two small subventral denticles in stomatal chamber 2–3 μm anterior to dorsal tooth (Fig. 3B). Amphids cup-like with transverse oval opening, 23–26 μm from anterior end of body (Fig. 3B).

Excretory pore 93–116 μm , or 49–59% of pharyngeal length, from anterior end of body (Fig. 3B). Nerve ring 77–91 μm , or 43–47% of pharyngeal length, from anterior end of body. Three prominent cells located at the pharyngo-intestinal junction. In intestinal region, 4–5 oval to fusiform ventro-lateral coelomocytes each 6–8 μm x 26–32 μm ; the first oval shaped, near pharyngo-intestinal glands; the rest fusiform, spread almost evenly from mid-body to anus.

Female genital system mono-prodelphic, ventral or lateral to intestine, 111–183 μm long, or 11–18% of body length from vulva to point of flexure (Fig. 3D). Ovary reflexed 2/3–3/4 of the way back to vulva. No eggs observed. Vulva simple, without protuberant lips, pore-shaped in lateral view, no sclerotised pieces seen in vaginal area.

No distinct prerectum. Rectum slightly shorter than anal body diameter (18 vs 23 μm). Tail usually bent ventrad, narrowing evenly (Fig. 3E). One pair of subdorsal caudal thick, short setae on anterior part of tail (Fig. 3E). Three tandem caudal glands (Fig. 3A & E); spinneret terminal, 2–3 μm long (Fig. 3E & F).

Male. Not known.

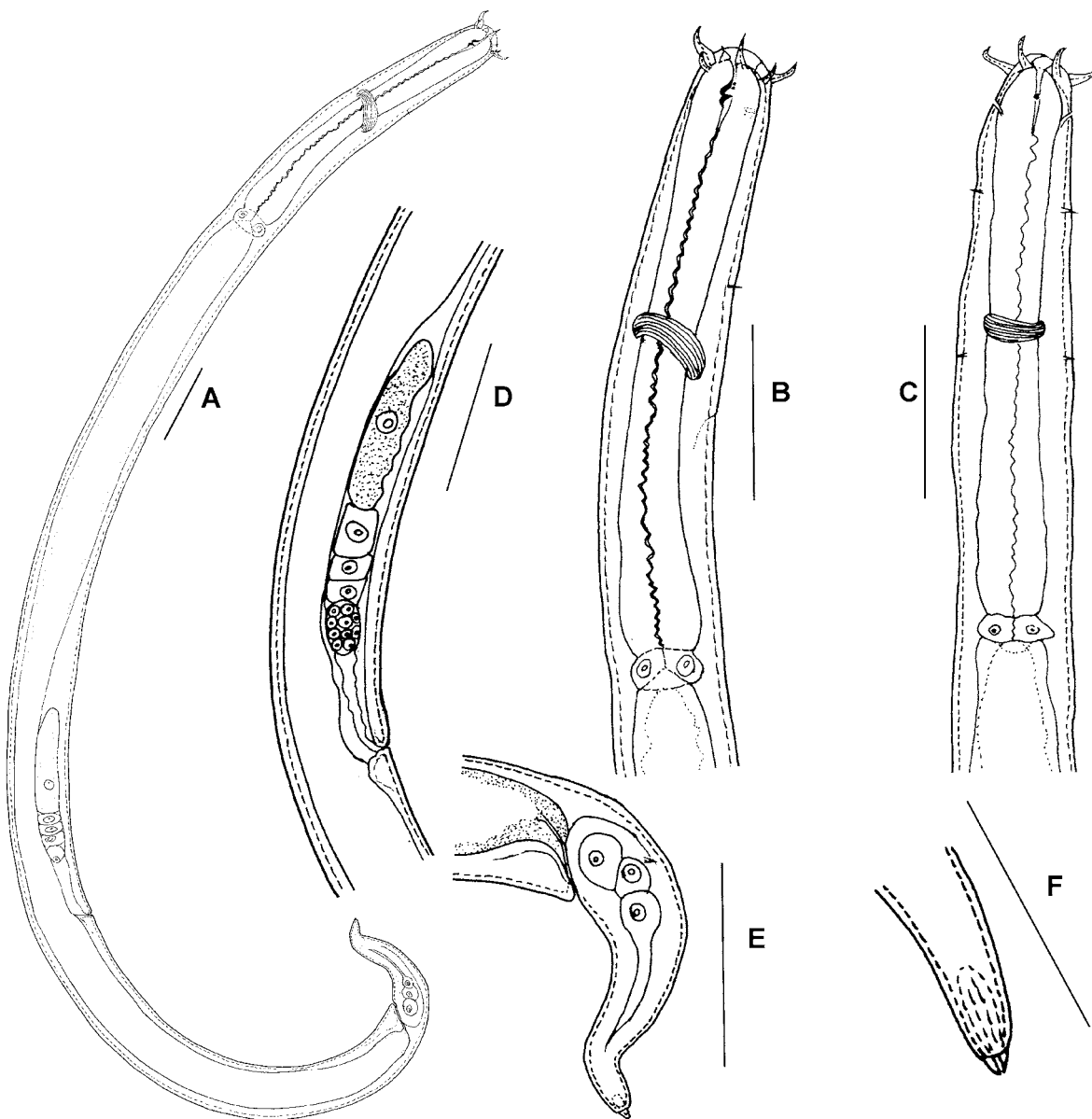


FIGURE 3. *Tripylina tearoha* sp. nov. female. A: Entire body. B: Pharyngeal region, lateral, showing the single cervical seta. C: Pharyngeal region, ventral, showing two pairs cervical setae. D: Genital region, lateral. E: Tail. F: Spinneret. Scale bars: A, B, C, D, E = 50 μm ; F = 25 μm .

TABLE 2. Morphometric data for *Tripylina tearoha* sp. nov., *T. manurewa* sp. nov., *T. yeatesi* sp. nov., *T. tamaki* sp. nov., *T. yeatesi* sp. nov. and *T. kaikou*

	Holotype female					Paratype females (Mean ± S.D.)				
	<i>T. tearoha</i> sp. nov.	<i>T. manurewa</i> sp. nov.	<i>T. tamaki</i> sp. nov.	<i>T. yeatesi</i> sp. nov.	<i>T. kaikoura</i> sp. nov.	<i>T. tearoha</i> sp. nov. (Te Aroha)	<i>T. tearoha</i> sp. nov. (Smith's Bush)	<i>T. manurewa</i> sp. nov.	<i>T. tamaki</i> sp. nov.	<i>T. tamaki</i> sp. nov.
n	1	1	1	1	1	7	4	8	3	3
a	28.3	24.8	23.5	30.1	22.3	27.8 ± 1.8 (25.1–30.0)	28.0 ± 1.1 (27.1–29.5)	26.4 ± 2.3 (24.0–29.2)	21.0 ± 1 (19.9–22)	21.0 ± 1 (19.9–22)
b	5.4	6.1	5.6	5.8	6.0	5.5 ± 0.2 (5.3–5.9)	6.2 ± 0.2 (6.0–6.4)	5.8 ± 0.3 (5.4–6.1)	6.3 ± 0. (5.8–6.4)	6.3 ± 0. (5.8–6.4)
c	13.8	14.9	15.2	25.7	14.3	15.0 ± 1.7 (13.6–17.7)	13.1 ± 0.5 (12.4–13.6)	13.8 ± 2.0 (10.7–17.8)	14.9 ± 0 (14.5–15)	14.9 ± 0 (14.5–15)
c'	3.3	3.0	2.3	2.0	3.1	3.0 ± 0.2 (2.9–3.3)	3.9 ± 0.1 (3.7–3.9)	3.0 ± 0.5 (2.3–3.6)	3.0 ± 0. (2.9–3.4)	3.0 ± 0. (2.9–3.4)
V	64.7	66.5	67.0	67.1	66.0	65.8 ± 1.8 (63.5–68.6)	63.3 ± 0.5 (62.8–63.7)	65.7 ± 1.1 (63.8–67.1)	65.9 ± 2 (63.5–67)	65.9 ± 2 (63.5–67)
Body length µm	1031	1164	955	1473	1574	1047 ± 66.9 (961–1149)	1151 ± 61.1 (1103–1240)	1090 ± 46.8 (1032–1164)	1171 ± 2 (1146–11)	1171 ± 2 (1146–11)
Head diam µm	22.0	25.0	21.5	26.0	29	21.1 ± 1.6 (19–23)	22.1 ± 1.5 (21–24)	23.2 ± 1.3 (21–25)	23.6 ± 1 (22–24)	23.6 ± 1 (22–24)
Body diam µm	36.4	46.9	40.6	49.0	71	37.7 ± 1.3 (36–40)	41.1 ± 2.5 (38–44)	41.5 ± 3.4 (37–43)	56.1 ± 4 (61–60)	56.1 ± 4 (61–60)
Dorsal tooth- anterior µm	14.8	18.3	17.4	17.0	19	14.9 ± 0.7 (14–16)	15.3 ± 0.3 (15–16)	17.3 ± 1.0 (17–19)	16.8 ± 1 (15–18)	16.8 ± 1 (15–18)
Excretory pore-anterior µm	114	110	100	114	120	105.4 ± 9.8 (93–116)	107.4 ± 2.8 (105–111)	108.0 ± 3.2 (103–113)	105.2 ± 1 (99–113)	105.2 ± 1 (99–113)
Pharynx length µm	666.7	190	171.6	251	264	688.4 ± 49.5 (610–716)	728.5 ± 35.1 (703–779)	187.7 ± 7.9 (177–203)	187.6 ± 1 (176–20)	187.6 ± 1 (176–20)
Vulva-anterior µm	196	775.0	639.6	989.0	1038	189.8 ± 10.9 (175–202)	185.2 ± 9.9 (172–195)	716.0 ± 12.1 (618–775)	772.7 ± 3 (728–80)	772.7 ± 3 (728–80)
Amphid-anterior µm	26.1	19.9	19.1			24.0 ± 1.1 (23–26)	18.0 ± 2.0 (16–20)	16.8 ± 1.8 (15–20)	20.2 ± 1 (19–22)	20.2 ± 1 (19–22)
Nerve ring-anterior µm	90.5	87.9	79.0			86.3 ± 5.6 (77–91)	86.5 ± 4.1 (82–91)	89.2 ± 1.5 (88–91)	83.8 ± 7 (77–91)	83.8 ± 7 (77–91)
Ventromedian cervical seta-anterior µm	71.4	86.1	74.1			71.5 ± 4.0 (66–77)	71.0 ± 7.9 (62–77)	80.9 ± 2.7 (78–86)	81.8 ± 3 (79–86)	81.8 ± 3 (79–86)
Tail length µm	74.5	77.9	62.9			70.3 ± 6.7 (62–77)	88.2 ± 6.1 (81–95)	80.3 ± 13.1 (71–105)	78.9 ± 4 (74–82)	78.9 ± 4 (74–82)

TABLE 3. Comparative mean morphometrics of *Tripylina* species with (range).

Species	L of female (μm)	a	b	c	c'	V%	Cervical seta or setae	Setae on tail	References
Subdenticles anterior to dorsal tooth									
<i>tearoha</i>	1047 (961–1240)	27.9 (25–30)	5.9 (5.3–6.4)	14.1 (12.4–17.7)	3.5 (2.9–3.9)	64.6 (63–69)	1 single & 2 pairs	1 pair	This paper
<i>manurewa</i>	1164 (1031–1164)	26 (24–29)	5.8 (5.4–6.1)	13.8 (10.7–17.8)	3.0 (2.3–3.6)	65.7 (64–67)	1 single & 2 pairs	1 pair	This paper
<i>tamaki</i>	955 (955–1187)	21 (20–23)	6.3 (5.8–6.5)	14.9 (14.5–15.6)	3.0 (2.9–3.2)	65.9 (63–68)	1 single & 2 pairs	1 pair	This paper
<i>yeatesi</i>	1518 (1473–1563)	29.5 (29–30)	5.95 (5.9–6.0)	22 (18–26)	2.3 (2.0–2.6)	67.4 (67–68)	no	no	This paper
<i>kaikoura</i>	1581 (1574–1589)	22.5 (22–23)	6.1 (6.0–6.2)	15 (14.3–15.8)	2.9 (2.7–3.1)	65.5 (65–66)	no	no	This paper
<i>longa</i>	1560 (1480–1720)	36 (33–40)	7.0 (6.3–7.4)	28.2 (24.9–30.3)	2.0 (1.7–2.6)	79 (76–80)	2 single	1 pair	Brzeski & Winiszewska- Ślipińska 1993
<i>sheri</i>	1220 (870–1380)	30 (24–38)	5.9 (5.2–6.5)	17.7 (13.2–22.2)	3.2 (1.9–4.5)	66.5 (63–70)	1 single	unknown	Brzeski 1963
<i>stramenti</i>	1657 (1490–1690)	28.4 (26–30)	6.37 (5.7–6.4)	16.3 (14.4–19.3)	3.13 (2.5–3.5)	63.0 (61–65)	1 single	unknown	Yeates 1972; Tsalolikhin 1983
<i>macroseta</i>	870 (800–940)	22 (20–24)	5.05 (4.8–5.3)	12.7 (12–13.3)	3.2	63.5 (62–65)	no*	unknown	Vinciguerra & La Fauci 1978; Tsalolikhin 1983
Subdenticles posterior to dorsal tooth									
<i>arenicola</i>	1035 (800–1200)	24 (23–27)	5.2 (5.0–5.5)	17.7 (16.8–18.7)	2.2 (1.9–2.7)	69 (67–70)	1 single	unknown	de Man, 1880; Brzeski, 1963
<i>ursulae</i>	1050 (780–1170)	29 (22–26)	5.3 (4.8–5.7)	17.5 (13.6–27.2)	-	64 (61–67)	no	unknown	Argo & Heyns 1973; Tsalolikhin 1983

* Brzeski & Winiszewska-Ślipińska (1993) stated that although a single seta on the midventral line anterior to nerve ring was originally described, a ventral cervical seta could not be seen when they re-examined the specimens.

Locality and habitat. Holotype and seven paratypes (slide nos 2535–2541) from soil and litter mixture, 0–10 cm depth under a *Pinus radiata* tree, Te Aroha Domain, Thames Valley region, New Zealand (37°32.165 S, 175°42.911 E). Coll. Zeng Qi Zhao, 7. xi. 2007; four paratypes (slide nos 2542–2545) from soil, 0–10 cm depth under a native *Dacrycarpus dacrydioides* tree (common names: Kahikatea, white pine) surrounded by several *Geniostoma ligustrifolium* trees, Smith's Bush, North Shore City, Auckland, New Zealand (36°48.782 S, 174°45.026 E), Coll. Zeng Qi Zhao, 4. iv. 2008.

Diagnosis and relationships. *Tripylina tearoha* **sp. nov.** is characterised by having a single ventromedian cervical seta and two pairs of lateral cervical setae (Fig. 3B & C).

Females of *T. tearoha* **sp. nov.** are similar in total body length to *T. manurewa* **sp. nov.**, *T. tamaki* **sp. nov.**, *T. sheri* Brzeski, 1963, *T. arenicola* (de Man, 1880) Brzeski, 1963, *T. ursulae* (Argo & Heyns, 1973)

Tsalolikhin, 1983; they are longer than *T. macroseta* (Vinciguerra & La Fauci, 1978) Tsalolikhin, 1983 and shorter than all other species (*T. longa* Brzeski & Winiszewska-Ślipińska, 1993, *T. stramenti* (Yeates, 1972) Tsalolikhin, 1983, *T. yeatesi* **sp. nov.** and *T. kaikoura* **sp. nov.** (Table 3).

The genus of *Tripylina* contains two groups of species, one with subventral denticles anterior to the dorsal tooth, and one with them posterior (Table 3). *T. tearoha* **sp. nov.** belongs to the first group, with *T. manurewa* **sp. nov.**, *T. tamaki* **sp. nov.**, *T. sheri*, *T. macroseta*, *T. longa*, *T. stramenti*, *T. yeatesi* **sp. nov.**, and *T. kaikoura* **sp. nov.**.

T. tearoha **sp. nov.** is closest to *T. manurewa* **sp. nov.**, *T. tamaki* **sp. nov.** in that all have a single ventromedian seta and two pairs of lateral setae present in the cervical region. It differs from *T. sheri* and *T. macroseta* which lacks setae in the cervical region (Table 3).

T. tearoha **sp. nov.** differs from *T. tamaki* **sp. nov.** in body diameter (36–44 vs 61–60 µm), de Man's Index *a* (25–30 vs 20–23) and differs from *T. manurewa* **sp. nov.** by in the distance of ventromedian cervical seta from the anterior end of the body (62–77 vs 78–86 µm).

Based on the SSU and LSU molecular phylogenetic studies (Fig. 1 & 2), *T. tearoha* **sp. nov.** is genetically closer to *T. tamaki* **sp. nov.** than to *T. manurewa* **sp. nov.**. However, *T. tearoha* **sp. nov.** is clearly different from *T. manurewa* **sp. nov.** from both SSU and LSU trees, in which they were not in one clade, and different from *T. tamaki* **sp. nov.** by having a low posterior probabilities value support in SSU tree. Females of *T. tearoha* **sp. nov.** from Te Aroha and Smith's Bush show slight morphological differences but could not be distinguished using molecular techniques.

Etymology. The name 'Te Aroha' comes from the Maori name of the Mount Te Aroha. It is used here as a noun in apposition.

Tripylina manurewa **sp. nov.**

(Fig. 4A–G)

Measurements. Table 2.

Material examined. Holotype: NNCNZ, slide No. 260.

Paratypes: eight females. NNCNZ, slide Nos 2546–2553.

Description. Female. Body ventrally arcuate when fixed (Fig. 4A), posterior more curved than anterior. Cuticle smooth, about 1–2 µm thick anteriorly, no distinct layering observed. Maximum body diameter generally near ovary, occasionally near vulva or base of pharynx. Body pores small, numerous, indistinct.

Head rounded, continuous with body contour (Fig. 4A). Labial papillae short, conical. Six long and four short cephalic setae in a single whorl (Fig. 4F); the six setae 11–14 µm long, or 50–60% of head diameter, more or less arcuate, directed anteriorly; the four setae 6–8 µm long, thinner than the six setae, more or less arcuate. A single ventromedian cervical seta, thin, 78–86 µm, or 41–47% of pharyngeal length, from anterior end of body (Fig. 4B). Two pairs of lateral cervical setae, one anterior and one posterior to nerve ring (Fig. 4C). Stoma walls thickened, dorsal tooth relatively large, triangular; two small subventral denticles in stomatal chamber 2–4 µm anterior to dorsal tooth, difficult to observe (Fig. 4B). Amphids cup-like with transverse oval opening, 15–19 µm from anterior end of body (Fig. 4B).

Excretory pore 101–113 µm, or 54–62% of pharyngeal length, from anterior end of body (Fig. 4B). Nerve ring 88–91 µm, or 44–51% of pharyngeal length, from anterior end of body. Three prominent cells at pharyngo-intestinal junction. In intestinal region, 5–6 oval to fusiform ventrolateral coelomocytes, each 11–12 x 34–58 µm; positions variable, in holotype one coelomocyte near pharyngo-intestinal glands and the rest, fusiform, spread from mid-body to anus.

Female genital system mono-prodelphic, ventral to intestine, 138–172 µm long, or 13–16% of total body length from vulva to point of flexure (Fig. 4D), reflexed 1/2–3/4 of the way back to vulva. No eggs observed. Vulva simple, without protuberant lips, pore-shaped in lateral view, with weakly sclerotised pieces in vaginal area.

No distinct prerectum. Rectum length less than anal body diameter (20 vs 27 μm). Tail usually bent ventrad (Fig. 4E), narrowing evenly, with one pair of subdorsal caudal setae on anterior part (Fig. 4E). Three tandem caudal glands (Fig. 4E), spinneret terminal, 2.8–3.4 μm long (Fig. 4G).

Male. Not known.

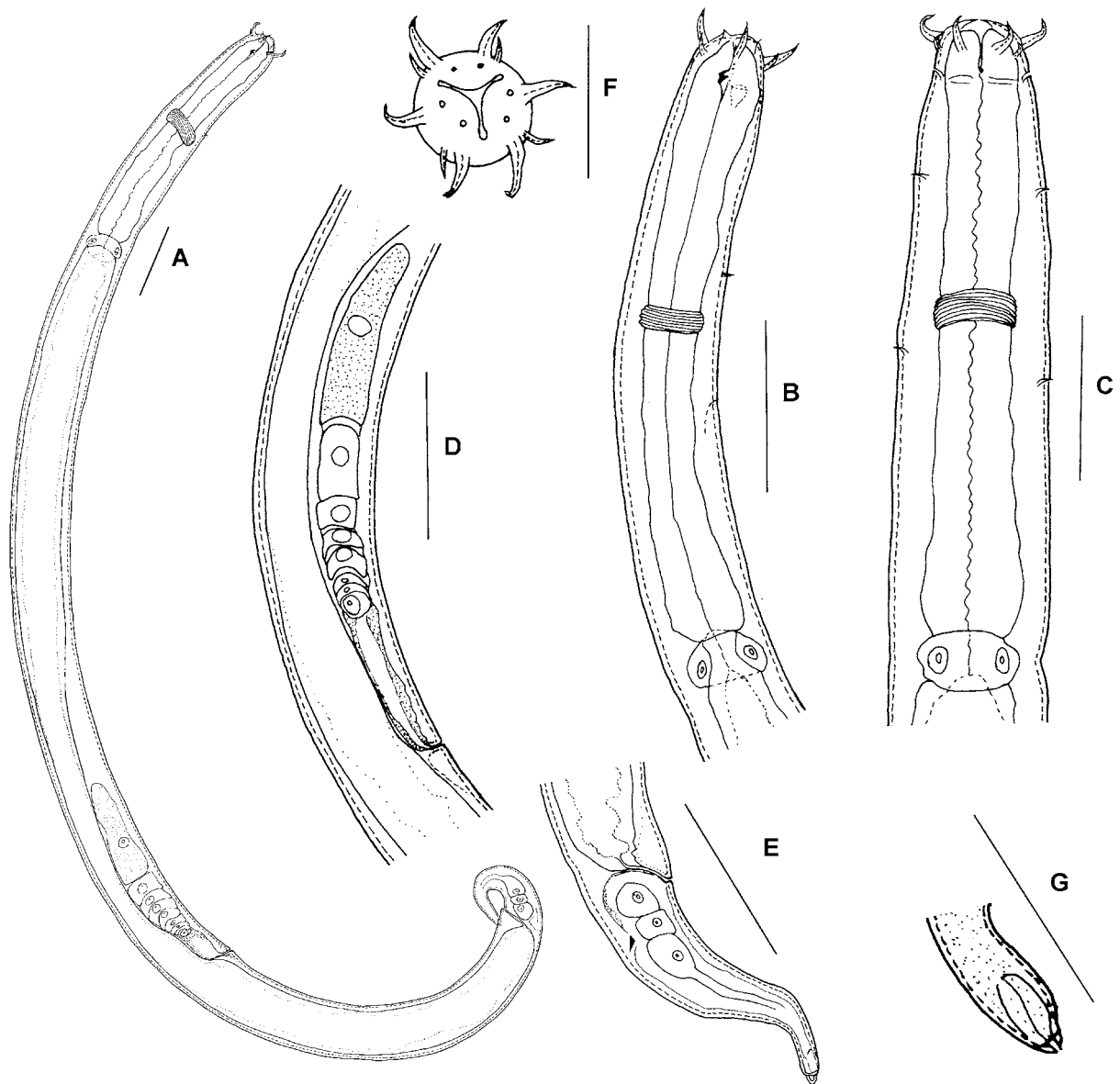


FIGURE 4. *Tripylina manurewa* sp. nov. female. A: Entire body. B: Pharyngeal region, lateral, showing the single cervical seta. C: Pharyngeal region, ventral, showing two pairs cervical setae. D: Genital region, lateral. E: Tail. F: *En face* view. G: Spinneret. Scale bars: A, B, C, D, E = 50 μm ; F, G = 25 μm .

Locality and habitat. Holotype and paratypes from soil and litter, 0–10 cm depth under a native *Dacrycarpus dacrydioides* tree, Auckland Botanic Garden, South Auckland, New Zealand (37°0.657 S, 174°54.491 E). Coll. Zeng Qi Zhao, 23. iv. 2008.

Diagnosis and relationships. *Tripylina manurewa* sp. nov. is characterised by having a single ventromedian seta and two pairs of lateral setae in the cervical region (Fig. 4B & C), thickened stoma walls, large triangular dorsal tooth, two small subventral denticles in stomatal chamber 2–4 μm anterior to the dorsal tooth, and cup-like amphids with transverse oval opening located 15–19 μm from the anterior end of the body.

Females of *T. manurewa* **sp. nov.** are close to those of *T. tearoha* **sp. nov.**, *T. tamaki* **sp. nov.**, *T. sheri*, *T. arenicola* and *T. ursulae* in body length. However, they are longer than *T. macroseta* and shorter than all other species (*T. longa*, *T. stramenti*, *T. yeatesi* **sp. nov.** and *T. kaikoura* **sp. nov.**) (Table 3).

T. manurewa **sp. nov.** is similar to *T. tearoha* **sp. nov.**, *T. tamaki* **sp. nov.**, *T. sheri*, and *T. macroseta* in having two subventral denticles anterior to dorsal tooth, but differs from *T. arenicola* and *T. ursulae* which have two subventral denticles posterior to dorsal tooth (Table 3).

T. manurewa **sp. nov.** is more similar to *T. tearoha* **sp. nov.** and *T. tamaki* **sp. nov.** in having a single ventromedian seta and two pairs of lateral setae in the cervical region. It differs from *T. sheri* and *T. macroseta* which has no setae present in the cervical region (Table 3).

T. manurewa **sp. nov.** differs from *T. tamaki* **sp. nov.** in body diameter (37–43 vs 51–60 μm), de Man's Index *a* (24–29 vs 20–23). It differs from *T. tearoha* **sp. nov.** in the distance of ventromedian seta from the anterior end of the body (78–86 vs 62–77 μm).

Based on SSU and LSU molecular phylogenetic studies, *T. manurewa* **sp. nov.** is different from *T. tearoha* **sp. nov.** and *T. tamaki* **sp. nov.** (Fig. 1 & 2).

Etymology. Manurewa refers to the type locality. It is used here as a noun in apposition.

Tripylina tamaki **sp. nov.**

(Fig. 5A–F)

Measurements. Table 2.

Material examined. Holotype: NNCNZ, slide No. 261.

Paratype: three females. NNCNZ, slide Nos 2554–2556.

Description. Female. Body ventrally arcuate when fixed (Fig. 5A), posterior more curved than anterior. Cuticle smooth, about 1–2 μm thick anteriorly part, distinctly layered in pharyngeal region. Maximum body diameter generally near ovary, occasionally near vulva or posterior of pharynx. Body pores small and numerous, not clear.

Head rounded, continuous with body contour (Fig. 5A). Labial papillae short, conical. Six long and four short cephalic setae in a single whorl; the six setae 12–13 μm long, or 54–57% of head diameter, more or less arcuate, directed anteriorly; the four short setae 6–7 μm long, thinner than the six long cephalic setae, more or less arcuate. A single ventromedian cervical seta, thin, 79–86 μm , or 39–49% of pharyngeal length, from anterior end (Fig. 5B). Two pairs of lateral cervical setae, one anterior and one posterior to nerve ring (Fig. 5C). Stoma walls thickened; dorsal tooth large, triangular; two small subventral denticles in stomatal chamber 3–4 μm anterior to dorsal tooth (Fig. 5B). Amphids cup-like with transverse oval opening, 17–22 μm from anterior end.

Excretory pore 99–113 μm , or 56–58% of pharyngeal length, from anterior end of body (Fig. 5B). Nerve ring 77–91 μm , or 44–46% of pharyngeal length, from anterior end of body. Three prominent cells at pharyngo-intestinal junction (Fig. 5B). More than 6 oval to fusiform ventrolateral coelomocytes each (6–9 x 23–31 μm) in intestinal region; the first nearly circular, near pharyngo-intestinal glands, and the rest fusiform, spread between two body diameters posterior to pharyngo-intestinal glands and anus.

Female genital system mono-prodelfic, ventral to intestine, 169–250 μm long, or 18–29% of body length from vulva to flexure (Fig. 5D), reflexed 2/3–4/5 of the way back to vulva. Eggs present in all specimens (Fig. 5D). Vulva simple, without protuberant lips, pore-shaped in lateral view, weakly sclerotised pieces in the vaginal area.

No distinct prerectum. Rectum length less than of anal body diameter (21 vs 27 μm). Tail usually bent ventrad, narrowing evenly (Fig. 5E). One pair of subdorsal caudal setae on anterior part of tail (Fig. 5E). Three tandem caudal glands (Fig. 5E), spinneret terminal, 2–3 μm long (Fig. 5F).

Male. Not known.

Locality and habitat. Holotype and paratypes from soil and litter from 0–10 cm depth under a group of native tree fern *Cyathea medullaris* (common name: Mamaku), St Johns Bush, Auckland, New Zealand (36°52.354 S, 174°50.531 E). Coll. Zeng Qi Zhao, 14. iii. 2008.

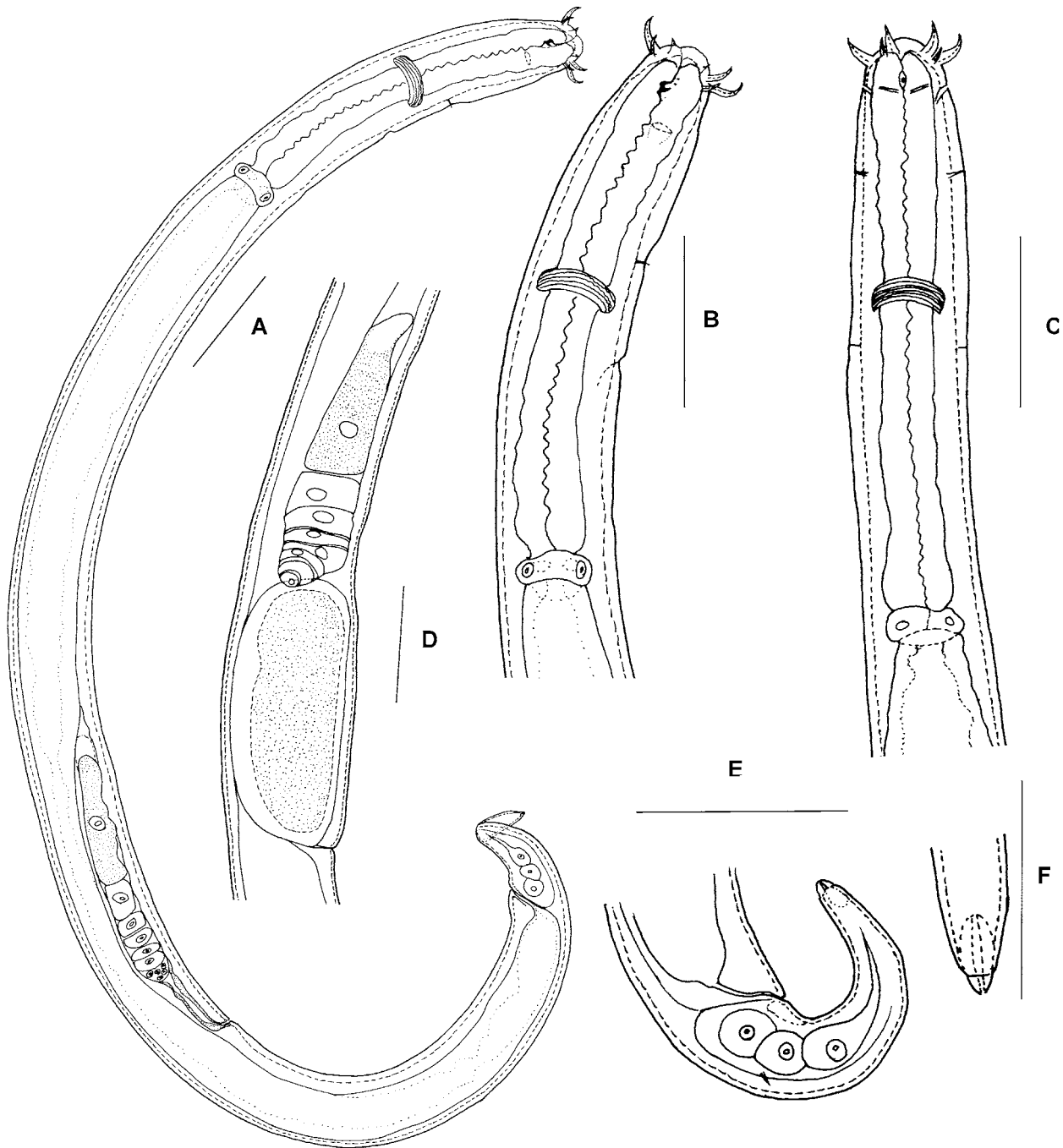


FIGURE 5. *Tripylina tamaki* sp. nov. female. A: Entire body. B: Pharyngeal region, lateral, showing a single cervical seta. C: Pharyngeal region, ventral, showing two pairs cervical setae. D: Genital region, lateral. E: Tail. F: Spinneret. Scale bars: A, B, C, D, E = 50 µm; F = 25 µm.

Diagnosis and relationships. *Tripylina tamaki* sp. nov. is characterised by a single ventromedian seta and two pairs of lateral setae in the cervical region (Fig. 5B & C), thickened stoma walls, large triangular dorsal tooth, two small subventral denticles in stomatal chamber 3–4 µm anterior to the dorsal tooth, and cup-like amphids with transverse oval opening 17–22 µm from anterior end of body.

Females of *T. tamaki* **sp. nov.** are similar to those of *T. tearoha* **sp. nov.**, *T. manurewa* **sp. nov.**, *T. sheri*, *T. arenicola* and *T. ursulae* in body length. However, they are longer than *T. macroseta* and shorter than all other species (*T. longa*, *T. stramenti*, *T. yeatesi* **sp. nov.** and *T. kaikoura* **sp. nov.**) (Table 3).

T. tamaki **sp. nov.** is similar to *T. tearoha* **sp. nov.**, *T. manurewa* **sp. nov.**, *T. sheri*, and *T. macroseta* in having two subventral denticles anterior to the dorsal tooth, but differs from *T. arenicola* and *T. ursulae* which have two subventral denticles posterior to the dorsal tooth (Table 3).

T. tamaki **sp. nov.** is similar to *T. manurewa* **sp. nov.** and *T. tearoha* **sp. nov.** in having a single ventromedian seta and two pairs of lateral setae in the cervical region. It differs from *T. sheri* and *T. macroseta* which has no setae present in the cervical region (Table 3).

T. tamaki **sp. nov.** differs from *T. tearoha* **sp. nov.** and *T. manurewa* **sp. nov.** in body diameter (51–60 vs 36–44 and 37–43 μm), de Man's Index *a* (20–23 vs 25–30 and 24–29). It also differs from *T. tearoha* **sp. nov.** in the distance of ventromedian cervical seta from the head end (79–86 vs 62–77 μm).

Based on SSU and LSU molecular phylogenetic studies (Figs. 1 & 2), *T. tamaki* **sp. nov.** is closer to *T. tearoha* **sp. nov.** than to *T. manurewa* **sp. nov.**. However, *T. tamaki* **sp. nov.** is clearly different from *T. manurewa* **sp. nov.** from both SSU and LSU trees, in which they were not in one clade, and different from *T. tearoha* **sp. nov.** by having a low posterior probabilities value support in SSU tree (Fig. 1).

Etymology. Tamaki is a suburb of the city of Auckland, New Zealand where the St Johns Bush is situated. It is used here as a noun in apposition.

Remarks. St Johns Bush contains many different tree species. *T. tamaki* **sp. nov.** was isolated from two sites there. Two further collecting trips, made after *T. tamaki* **sp. nov.** was first found, failed to find more specimens.

Tripylina yeatesi **sp. nov.**

(Fig. 6A–D)

Measurements. Table 2.

Material examined. Holotype: NNCNZ, slide No. 262.

Paratype: NNCNZ, slide No. 2557.

Descriptions. Female. Body ventrally arcuate when fixed (Fig. 6A), posterior more curved than anterior. Cuticle smooth, about 1–1.5 μm thick anteriorly, without distinct layering. Maximum body diameter near vulva. Body pores present but obscure.

Head rounded, continuous with body contour. Labial papillae short, conical. Six long and four short cephalic setae in a single whorl; the six setae 11–13 μm long, or 43–47% of head diameter, more or less arcuate, directed anteriorly; the four setae 6–7 μm long, thinner than the six long cephalic setae, more or less arcuate. No setae in cervical region. Stoma walls thick; dorsal tooth large, triangular; two small subventral denticles in stomatal chamber 3–4 μm anterior to dorsal tooth. Amphids cup-like with transverse oval opening, 17–21 μm from anterior end of body.

Excretory pore 114–118 μm , or 45% of pharyngeal length, from anterior end of body (Fig. 6C). Nerve ring 114–116 μm , or 44–46% of pharyngeal length, from anterior end of body (Fig. 6B). Three prominent cells at the pharyngo-intestinal junction. Four oval to fusiform ventrolateral or lateral coelomocytes, each 10–12 x 42–57 μm ; the first oval, 16 x 31 μm , near pharyngo-intestinal glands; the rest fusiform, spread from middle body to anus, one normally near tip of gonad, two between vulva and anus.

Female genital system mono-prodelphic, ventral to intestine, 276–357 μm long, or 19–23% of total body length from vulva to point of flexure (Fig. 6C), reflexed to vulva. Eggs present (Fig. 6A). Vulva simple, without protuberant lips, pore-shaped in lateral view, slightly sclerotised pieces in vaginal area.

No distinct prerectum. Rectum length less than anal body diameter (21 vs 31 μm). Tail usually bent ventrad, narrowing evenly. No caudal setae. Three tandem caudal glands, spinneret terminal, 2.5 μm long (Fig. 6D).

Male. Not found.

Locality and habitat. Holotype and paratype collected from litter samples from native forest Bruce Park, Hunterville, New Zealand (39°57.650 S, 175°31.726 E). Coll. Gregor W. Yeates, 12. vi. 1989.

Diagnosis and relationships. *Tripylina yeatesi* **sp. nov.** is characterised by its long body, absence of ventromedian setae in the cervical region, thickened stomatal walls, large triangular dorsal tooth, two small subventral denticles in stomatal chamber 3.5–4 μm anterior to the dorsal tooth, and cup-like amphids with transverse oval opening, located 17–21 μm from the anterior end of the body.

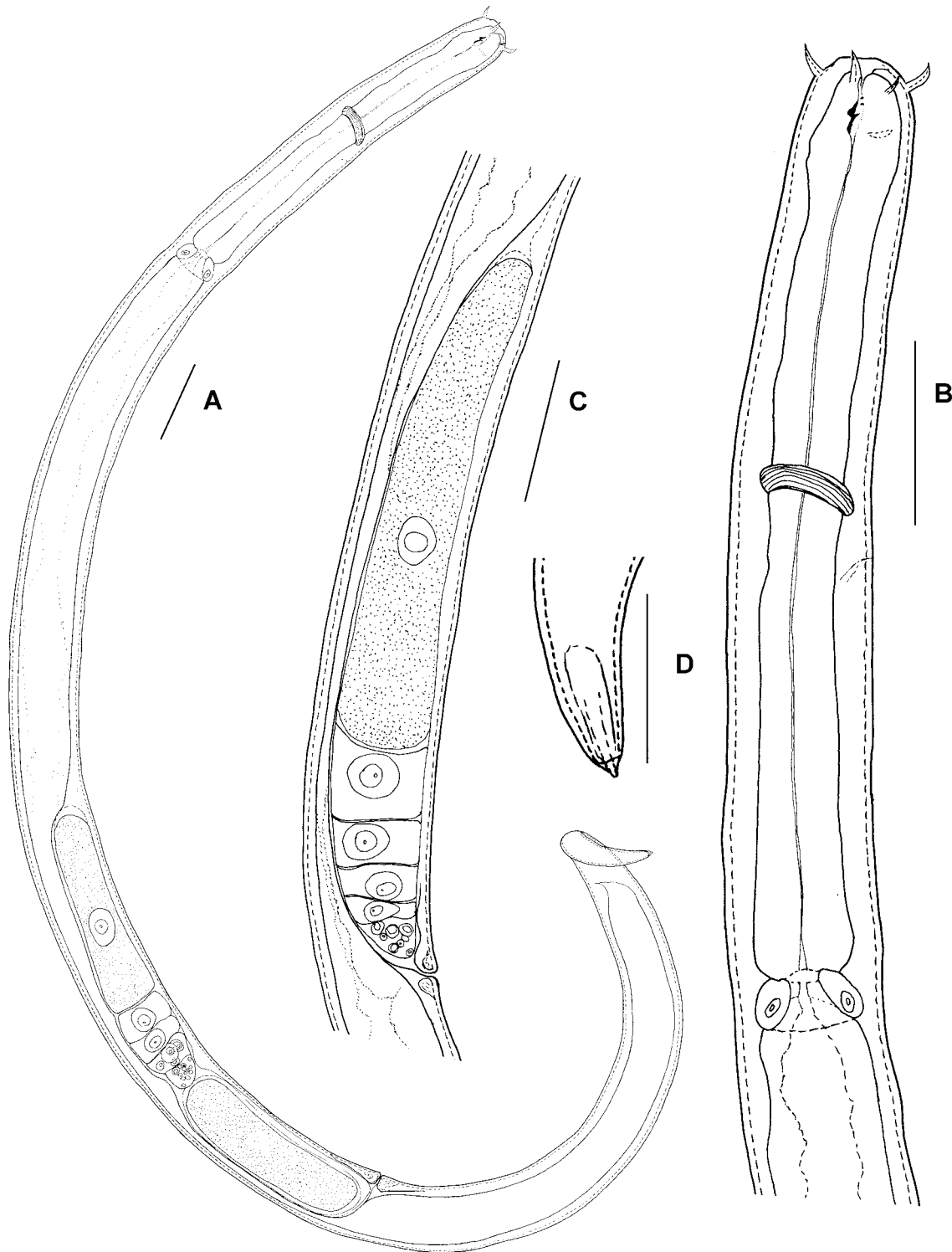


FIGURE 6. *Tripylina yeatesi* **sp. nov.** female. A: Entire body. B: Pharyngeal region, lateral. C: Genital region, lateral. D: Spinneret. Scale bars: A, B, C = 50 μm ; D = 25 μm .

Females of *T. yeatesi* **sp. nov.** are similar to those of *T. longa*, *T. stramenti* and *T. kaikoura* **sp. nov.** in body length. They are longer than all other species (*T. tearoha* **sp. nov.**, *T. manurewa* **sp. nov.**, *T. tamaki* **sp. nov.**, *T. sheri*, *T. arenicola*, *T. ursulae* and *T. macroseta*) (Table 3).

T. yeatesi **sp. nov.** is similar to *T. longa*, *T. stramenti* and *T. kaikoura* **sp. nov.** in having two subventral denticles anterior to dorsal tooth. It differs from *T. arenicola* and *T. ursulae* which have two subventral denticles posterior to dorsal tooth (Table 3).

T. yeatesi **sp. nov.** is similar to *T. kaikoura* **sp. nov.** in lacking a ventromedian seta and setae in the cervical region, but differs from *T. longa* which has two cervical setae and *T. stramenti* which has one cervical seta (Table 3).

T. yeatesi **sp. nov.** differs from *T. kaikoura* **sp. nov.** in having a narrower body (49–55 vs 68–71 μm) and different de Man's Indices *a* (29–30 vs 22–23), *b* (5.8–6.0 vs 6.0–6.2), *c* (18–26 vs 14–16) and *c'* (2.0–2.6 vs 2.7–3.0), respectively (Table 3).

Etymology. I have named the species *T. yeatesi* **sp. nov.** in honour of the great nematologist and zoologist Dr Gregor W. Yeates, Landcare Research, New Zealand.

Tripylina kaikoura **sp. nov.**

(Fig. 7A–D)

Measurements. Table 2.

Material examined. Holotype: NNCNZ, slide No. 263.

Paratype: NNCNZ, slide No. 2558.

Description. Female. Body ventrally arcuate when fixed (Fig. 7A), posterior more curved than anterior. Cuticle smooth, about 1–2 μm thick anteriorly, without distinct layers. Maximum body diameter one body width posterior to vulva. Body pores not seen. Glandular cells along body scattered, few in number (Fig. 7B).

Head rounded, slightly flat, continuous with body contour. Labial papillae short, conical. Six long and four short cephalic setae in a single whorl; the six setae 11 μm long, or 33–37% of head diameter, slightly arcuate, directed anteriorly; the four setae 6–8 μm long, thinner than the six long cephalic setae, more or less arcuate. No setae in cervical region. Stoma walls thickened; dorsal tooth large, triangular; two small subventral denticles in stomatal chamber 4 μm anterior to dorsal tooth. Amphids cup-like (?) with transverse oval opening, 20–22 μm from anterior end of body.

Excretory pore 120–140 μm , or 46–55% of pharyngeal length, from anterior end of body. Nerve ring 118–120 μm , or 46% of pharyngeal length, from anterior end of body. Prominent cells at pharyngo-intestinal junction, number difficult to determine. Coelomocytes difficult to distinguish.

Female genital system mono-prodelphic, ventral or lateral to the intestine, 303–322 μm long, or 19–21% of body length from vulva to flexure (Fig. 7C). Ovary reflexed to vulva. Eggs present. Vulva simple, without protuberant lips, pore-shaped in lateral view, no sclerotised pieces in vaginal area.

No distinct prerectum. Rectum length less than anal body diameter (20 vs 37 μm). Tail bent ventrad, narrowing evenly (Fig. 7D). No caudal setae. Three tandem caudal glands (Fig. 7A & D), spinneret terminal, 3 μm long (Fig. 7E).

Male. Not known.

Locality and habitat. Holotype and paratype from 0–5 cm soil depth from 10 random points under native forest in the Puhupuhi Scenic Reserve, Kaikoura, New Zealand (42°48.782 S, 173°38.996 E). Coll. Gregor W. Yeates, 28. v. 1991.

Diagnosis and Relationships. *Tripylina kaikoura* **sp. nov.** is characterised by a long body, absence of ventromedian setae in cervical region, thickened stomatal walls, large triangular dorsal tooth, two small subventral denticles in stomatal chamber 4 μm anterior to dorsal tooth, and cup-like amphids with transverse oval opening, located 20–22 μm from the anterior end of the body.

Females of *T. kaikoura* **sp. nov.** are close to those of *T. longa*, *T. stramenti* and *T. yeatesi* **sp. nov.** in body length. They are longer than all other species (*T. tearoha* **sp. nov.**, *T. manurewa* **sp. nov.**, *T. tamaki* **sp. nov.**, *T. sheri*, *T. arenicola*, *T. ursulae* and *T. macroseta*) (Table 3).

T. kaikoura **sp. nov.** is similar to *T. longa*, *T. stramenti* and *T. yeatesi* **sp. nov.** in having two subventral denticles anterior to dorsal tooth. It differs from *T. arenicola* and *T. ursulae* which have two subventral denticles posterior to dorsal tooth

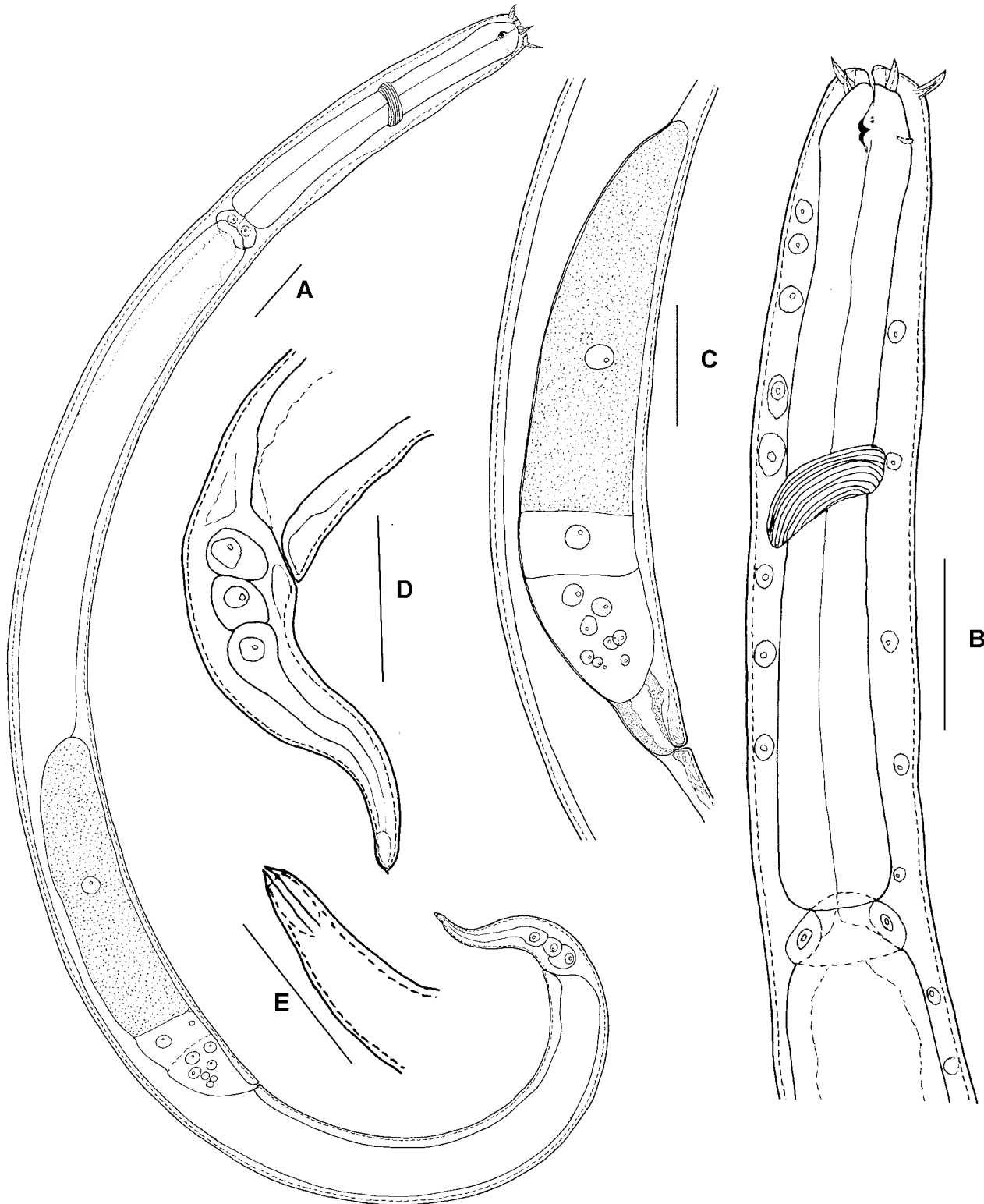


FIGURE 7. *Tripylina kaikoura* **sp. nov.** female. A: Entire body. B: Pharyngeal region, lateral. C: Genital region, lateral. D: Tail. E: Spinneret. Scale bars: A, B, C, D = 50 μ m; E = 25 μ m.

T. kaikoura **sp. nov.** is similar to *T. yeatesi* sp. nov in not having ventromedian seta or setae in the cervical region, but differs from *T. longa* which has two cervical setae and *T. stramenti* which has one cervical seta (Table 3).

T. kaikoura **sp. nov.** differs from *T. yeatesi* sp. nov in having a wider body (68–71 vs 49–55 μm) and de Man's Indices *a* (22–23 vs 29–30), *b* (6.0–6.2 vs 2.9–6.0), *c* (14–16 vs 18–26) and *c'* (2.7–3.0 vs 2.0–2.6), respectively (Table 3).

Etymology. Kaikoura is a locality name in New Zealand, it is used here as a noun in apposition.

Remarks. *Tripylina kaikoura* **sp. nov.** was collected and mounted by Dr Gregor W. Yeates in 1991. After nearly 20 years, the specimens are generally in good condition but as some of the nematode body contents were not clear for identification, some characters of the nematode are difficult to see. However, the body shape of this species is distinctively different from all other species of *Tripylina*.

Other species

T. arenicola (de Man, 1880) Brzeski, 1963 (Type species)

Synonyms. *Tripyla arenicola* de Man, 1880

Tripyla (*Trischistoma*) *arenicola* de Man, 1880 (Schneider, 1939)

Trischistoma arenicola (de Man, 1880) Schuurmans Stekhoven, 1951

Tripyla minor Cobb, 1893

Measurements (after Brzeski & Winiszewska-Ślipińska 1993). Females (n = 20): *L* = 810–1250 μm ; *a* = 20–30; *b* = 4.7–6.8; *c* = 13.1–18.3; *c'* = 1.9–3.2; *V* = 62–69%

Male: Brzeski & Winiszewska-Ślipińska (1993) stated that the only specimen was lost.

Description (after Brzeski & Winiszewska-Ślipińska 1993). Body ventrally arcuate when fixed, cuticle about 1 μm thick, not annulated. Head rounded, 18–22 μm diameter. Six long cephalic setae 10–14 μm long, or 52–74% of head diameter; four short cephalic setae 5–6 μm long. Stoma walls not thickened; dorsal tooth small, triangular; two subventral denticles posterior to dorsal tooth. A single ventromedian seta, thin, 60–78 μm , or 30–42% of pharyngeal length from anterior end of body. Vulva pore-shaped; sclerotized pieces small, comma-shaped with tips directed towards vulva. Male with six papillate ventromedian supplements. Other details of male missing, because the only specimen was lost.

Diagnosis and Relationships. The main distinguishing feature of *T. arenicola* is the subventral denticles posterior to the dorsal tooth.

Females of *T. arenicola* are similar to those of *T. macroseta*, *T. manurewa* **sp. nov.**, *T. tearoha* **sp. nov.**, *T. tamaki* **sp. nov.**, *T. sheri*, and *T. ursulae* in body length, and shorter than all other species (*T. longa*, *T. stramenti*, *T. yeatesi* **sp. nov.** and *T. kaikoura* **sp. nov.**) (Table 3).

T. arenicola is similar to *T. ursulae* in having two subventral denticles posterior to the dorsal tooth and differs from *T. tearoha* **sp. nov.**, *T. manurewa* **sp. nov.**, *T. tamaki* **sp. nov.**, *T. sheri*, and *T. macroseta*, which have the denticles anterior to the dorsal tooth (Table 3).

T. arenicola can be differentiated from *T. ursulae* by the absence of setae in the cervical region.

Habitat and distribution. Terrestrial. Type locality Katwijk, Holland. Recorded from more than 20 countries, including eight in Europe (Netherlands, Switzerland, Poland, Austria, Hungary, Italy, Lithuania, Russia), seven in Asia (Georgia, Uzbekistan, India, Vietnam, Indonesia, Korea,), three from North and Central America (Canada, Mexico, Costa Rica), one from South America (Paraguay) and one from South Pacific Ocean (Fiji Islands).

Etymology. The species epithet is derived from the Latin words *arena* = sand, and *incola* = an inhabitant.

Tripylina longa Brzeski & Winiszewska-Ślipińska, 1993

Measurements (after Winiszewska-Ślipińska 1993). Females (n = 8): $L = 1480\text{--}1720\ \mu\text{m}$; $a = 33\text{--}40$; $b = 6.3\text{--}7.4$; $c = 24.9\text{--}30.3$; $c' = 1.7\text{--}2.6$; $V = 76\text{--}80\%$; anterior cervical seta = $50\text{--}74\ \mu\text{m}$ from anterior end; posterior cervical seta = $49\text{--}63\ \mu\text{m}$ from anterior end.

Male (n = 1): $L = 1640\ \mu\text{m}$; $a = 19$; $b = 6.8$; $c = 26.6$; $c' = 1.8$; ventromedian papillae = 5; spicule along arc = $52\ \mu\text{m}$; gubernaculum = $19\ \mu\text{m}$; anterior cervical seta = $88\ \mu\text{m}$ from anterior end; posterior cervical seta = $175\ \mu\text{m}$ from anterior end.

Description (after Brzeski & Winiszewska-Ślipińska 1993). Anterior part of relaxed specimens straight, posterior spiral. Cuticle composed of two distinct layers, $1\text{--}1.5\ \mu\text{m}$ thick. Head $25\text{--}28\ \mu\text{m}$ wide. Six long cephalic setae $15\text{--}18.5\ \mu\text{m}$ long, or $59\text{--}71\%$ of head diameter; four short cephalic setae $5\text{--}7\ \mu\text{m}$ long. Dorsal part of stoma walls thickened; dorsal tooth relatively large, directed posteriad; two subventral denticles anterior to dorsal tooth,. Two ventromedian setae in cervical region, anterior $23\text{--}34\%$ of pharyngeal length from anterior end, posterior $57\text{--}68\%$. Vulva pore-shaped, sclerotized pieces large. A pair of subdorsal setae on anterior part of tail. Some females with another pair of subdorsal setae near junction of intestine and rectum.

Male similar to female. Five papillate ventromedian supplements near cloaca. Spicules narrow, sickle shaped. Gubernaculum U-shaped in cross section, surrounding spicules dorsally and laterally.

Diagnosis and Relationships. The main distinctive feature of *T. longa* is the large dorsal tooth directed posteriad. It is the longest recorded in the genus.

Females of *T. longa* are similar to those of *T. stramenti*, *T. kaikoura* **sp. nov.** and *T. yeatesi* **sp. nov.** in body length, and longer than all other species (*T. tearoha* **sp. nov.**, *T. manurewa* **sp. nov.**, *T. tamaki* **sp. nov.**, *T. sheri*, *T. arenicola*, *T. ursulae* and *T. macroseta*) (Table 3).

T. longa is also similar to *T. kaikoura* **sp. nov.**, *T. stramenti* and *T. yeatesi* **sp. nov.** in having two subventral denticles anterior to dorsal tooth, but its two cervical setae differ from *T. stramenti* which has one cervical seta, and from *T. kaikoura* **sp. nov.** and *T. yeatesi* **sp. nov.** which lack cervical setae (Table 3).

T. longa is similar to *T. tearoha* **sp. nov.**, *T. manurewa* **sp. nov.** and *T. tamaki* **sp. nov.** in having two subdorsal setae on tail, but its two cervical setae differ from *T. tearoha* **sp. nov.**, *T. manurewa* **sp. nov.** and *T. tamaki* **sp. nov.** which have a single ventromedian seta and two pairs of lateral setae in the cervical region, respectively.

Habitat and distribution. Found in clay soil around roots of grasses on the bank of Ema River, Italy.

Etymology. Not stated.

Tripylina macroseta (Vinciguerra & La Fauci, 1978) Tsalolikhin, 1983

Synonym. *Trischistoma macroseta* Vinciguerra & La Fauci, 1978

Measurements (after Vinciguerra & La Fauci 1978; Tsalolikhin 1983). Females (n = 2): $L = 800, 940\ \mu\text{m}$; $a = 20\text{--}24$; $b = 4.8\text{--}5.3$; $c = 12\text{--}13.3$; $c' = 3.2$; $V = 62\text{--}65\%$.

Male: not known.

Description (after Brzeski & Winiszewska-Ślipińska 1993). Body curved ventrally. Cuticle smooth, thin. Lateral body pores distinct. Head $20\ \mu\text{m}$ diameter. Six long cephalic setae $13\ \mu\text{m}$ long, or 65% of head diameter; four short cephalic setae $5\ \mu\text{m}$ long. Cervical setae absent. Dorsal stoma wall not thickened; dorsal tooth small; two subventral denticles anterior to dorsal tooth,. Ventromedian cervical setae not seen. Vulva lips sclerotized. Tail short, ventrally curved.

Diagnosis and relationships. The distinctive feature for *T. macroseta* is the small body size, dorsal tooth and indistinct subventral denticles.

T. macroseta is among the shortest species in the genus, the female length overlapping with *T. arenicola*, *T. sheri* and *T. ursulae*, and being shorter than all other species (*T. longa*, *T. stramenti*, *T. yeatesi* **sp. nov.**, *T. kaikoura* **sp. nov.**, *T. tearoha* **sp. nov.**, *T. manurewa* **sp. nov.** and *T. tamaki* **sp. nov.**) (Table 3).

T. macroseta is similar to *T. sheri* in having two subventral denticles anterior to the dorsal tooth, but differs from *T. arenicola* and *T. ursulae* in which they are posterior (Table 3).

T. macroseta is differentiated from *T. sheri* by not having cervical seta, the small dorsal tooth, and indistinctive subventral denticles.

Habitat and distribution. Terrestrial. Type locality is the island of Lampedusa, Italy. Also reported from Hungary and the United States.

Etymology. The species epithet is derived from the Greek *macro* (μακρός) = long or large, and the Latin seta = a bristle.

Remarks. Brzeski & Winiszewska-Ślipińska (1993) stated that although a single seta on the midventral line anterior to nerve ring was originally described, a ventral cervical seta could not be seen when they re-examined the specimens.

Tripylina sheri Brzeski, 1963

Synonyms. *Trischistoma sheri* (Brzeski, 1963) Brzeski, 1965
Tripyla (Trischistoma) sheri (Brzeski, 1963) Khera, 1970

Measurements (after Brzeski 1963). Females (n = 18): $L = 870\text{--}1380\ \mu\text{m}$; $a = 25\text{--}38$; $b = 5.2\text{--}6.5$; $c = 13.2\text{--}22.2$; $c' = 1.9\text{--}4.5$; $V = 63\text{--}70\%$.

Male: not known.

Description (after Brzeski & Winiszewska-Ślipińska 1993). Body more or less C-shaped in relaxed specimens, with tail more tightly curved. A few glandular cells scattered along body. Head 19–27 μm wide. Six long cephalic setae 9–11 μm long, or 39–62% of head width (except for one female where they were 16 μm long); four short cephalic setae 4–5 μm long. Stomatal wall thickened, refractive; dorsal tooth relatively large; two subventral denticles anterior to dorsal tooth. Ventromedian cervical seta short, thin, located 40% (36–42%) of pharyngeal length from anterior end. Vaginal thickenings comma-shaped, small, lips sometimes protruding.

Diagnosis and relationships. The thickened stomatal wall of *T. sheri* is a distinctive feature for differentiating this species from other species of the genus.

Females of *T. sheri* are similar in total body length to *T. macroseta*, *T. manurewa* **sp. nov.**, *T. tearoha* **sp. nov.**, *T. tamaki* **sp. nov.**, *T. arenicola* and *T. ursulae*, but shorter than all other species (*T. longa*, *T. stramenti*, *T. yeatesi* **sp. nov.** and *T. kaikoura* **sp. nov.**) (Table 3).

T. sheri is similar to *T. tearoha* **sp. nov.**, *T. manurewa* **sp. nov.**, *T. tamaki* **sp. nov.** and *T. macroseta* in having two subventral denticles anterior to dorsal tooth, and differs from *T. arenicola* and *T. ursulae* which have them posterior (Table 3).

T. sheri can be differentiated from *T. tearoha* **sp. nov.**, *T. manurewa* **sp. nov.**, *T. tamaki* **sp. nov.** and *T. macroseta* by having one seta in the cervical region.

Habitat and distribution. Terrestrial. Type locality Berkeley, California, USA. Also collected from Mexico and Vietnam.

Etymology. The species was named in honour of the eminent systematist of nematode parasites of plants, Dr S. A. Sher.

Tripylina stramenti (Yeates, 1972) Tsalolikhin, 1983.

Synonym. *Trischistoma stramenti* Yeates, 1972

Measurements (after Yeates 1972; Tsalolikhin 1983). Females (n = 4): $L = 1490\text{--}1690\ \mu\text{m}$; $a = 26\text{--}36$; $b = 5.7\text{--}6.4$; $c = 13\text{--}19.3$; $c' = 2.5\text{--}3.5$; $V = 61\text{--}65\%$.

Male: not known.

Description (after Tsalolikhin 1983). Body relatively large, C- or G-shaped upon fixation, curved ventrally. Cuticle smooth, with fine circular markings, about 0.9 μm thick in vulval region. Six long and four short cephalic setae, 25–40% of head diameter long. Dorsal stomatal wall slightly thickened; dorsal tooth large; two subventral denticles anterior to dorsal tooth. A single ventromedian cervical seta about 1/2 body diameter anterior to nerve ring. Vulva simple, without protuberant lips.

Diagnosis and relationships. The main distinctive features of *T. stramenti* are great body length and the presence of a single cervical seta.

Seven species of *Tripylina* are less than 1300 μm in body length, so *T. stramenti* (1490–1690 μm) is easily differentiated from them. *T. stramenti* is similar to the remaining species in the genus (*T. longa*, *T. yeatesi* **sp. nov.** and *T. kaikouura* **sp. nov.**) in total body length. It differs from *T. longa* in vulva position (De Man's Index $V = 61\text{--}65$ vs $76\text{--}80\%$), and from *T. yeatesi* **sp. nov.** and *T. kaikoura* **sp. nov.** in having a single cervical seta (Table 3).

T. stramenti is similar to *T. tearoha* **sp. nov.**, *T. manurewa* **sp. nov.**, *T. tamaki* **sp. nov.**, *T. yeatesi* **sp. nov.**, *T. kaikoura* **sp. nov.**, *T. sheri*, *T. longa* and *T. macroseta* in having two subventral denticles anterior to dorsal tooth, but differs from *T. arenicola* and *T. ursulae* which have two subventral denticles posterior to dorsal tooth (Table 3).

T. stramenti is similar to *T. kaikoura* **sp. nov.**, *T. longa* and *T. yeatesi* **sp. nov.** in having two subventral denticles anterior to the dorsal tooth. However, it has a single cervical seta, while *T. longa* has two cervical setae, and *T. kaikoura* **sp. nov.**, and *T. yeatesi* **sp. nov.** have no cervical setae (Table 3).

Habitat and distribution. Litter and moss. Reported from New Zealand and Africa. Original collection from litter under a regrowth of bush dating from a fire in 1945–46, dominated by *Weinmannia racemosa* (Kamahi), *Cyathea medullaris* (black tree-fern) and *Brachyglottis repanda* (rangiora), Taita Experimental Station, Lower Hutt, New Zealand (Yeates 1972). Also collected in moss from rock, Lago Amelia, West Africa (Andrássy 2008).

Etymology. The species epithet is derived from the Latin *stramentum* (= straw or litter).

Remarks. The original description states that *T. stramenti* is a predacious species.

Tripylina ursulae (Argo & Heyns, 1973) Tsalolikhin, 1983

Synonym. *Trischistoma ursulae* Argo & Heyns, 1973

Measurements (after Argo & Heyns 1973; Tsalolikhin 1983). Females (n = 16): $L = 780\text{--}1170$ μm ; $a = 22\text{--}26$; $b = 4.8\text{--}5.7$; $c = 13.6\text{--}27.2$; $V = 61\text{--}67\%$.

Male: not known.

Description (after Tsalolikhin 1983). Body curved ventrally, C- shaped when relaxed. Cuticle smooth, about 0.6–1.2 μm thick. Head conical, diameter of lip region 19.8–23.4 μm . Six long cephalic setae 13–15 μm long, or 65–70% of head diameter. Dorsal stomatal wall slightly thickened; dorsal tooth similar in size to subventral denticles, which are anterior to the dorsal tooth. A single subdorsal cervical seta about one body diameter from anterior end of body. Vulva simple, uterus very long.

Diagnosis and relationships. *T. ursulae* differs from all species in the genus except *T. arenicola* in having subventral denticles posterior to the dorsal tooth: all other species have the denticles anterior to the dorsal tooth (Table 3).

T. ursulae is differentiated from *T. arenicola* by having the cervical seta located subdorsally about one body diameter from the anterior end of the body (midventral and more than two body diameters posterior in *T. arenicola*).

Females of *T. ursulae* are similar to *T. macroseta*, *T. manurewa* **sp. nov.**, *T. tearoha* **sp. nov.**, *T. tamaki* **sp. nov.**, *T. sheri* and *T. arenicola* in body length, but shorter than all other species (*T. longa*, *T. stramenti*, *T. yeatesi* **sp. nov.** and *T. kaikoura* **sp. nov.**) (Table 3).

Habitat and distribution. Holotype from soil bordering a furrow in a vineyard, in Cape Province, South Africa; paratypes from soils around ferns, bulbous plants, banana plants and cabbage plants.

Etymology. Not stated.

Key for identification of species of *Tripylina*

1. Subventral denticles posterior to dorsal tooth..... 2
 - Subventral denticles anterior to dorsal tooth 3
2. Dorsal tooth larger than subventral denticles, cervical seta 60–78 µm from anterior end *T. arenicola*
 - Dorsal and subventral denticles of similar size, cervical seta 22–28 µm from anterior end *T. ursulae*
3. Cervical setae absent..... 4
 - Cervical setae present 6
4. Body length less than 800– 940 µm, stomatal chamber walls of equal thickness..... *T. macroseta*
 - Body length more than 1400 µm 5
5. $a = 29-30$; $b = 2.9-6.0$; $c = 18-26$; $c' = 2.0-2.6$ *T. yeatesi* **sp. nov.**
 - $a = 22-23$; $b = 6.0-6.2$; $c = 14-16$; $c' = 2.7-3.0$ *T. kaikoura* **sp. nov.**
6. One ventromedian cervical seta..... 7
 - Two ventromedian cervical setae..... *T. longa*
7. No lateral cervical setae 8
 - Two pairs of lateral cervical setae..... 9
8. Body length 1.4 mm or less, V 64–70% *T. sheri*
 - Body length 1.6 mm or more, V 60–65% *T. stramenti*
9. $a < 23$ *T. tamaki* **sp. nov.**
 - $a > 24$ 10
10. Cervical seta 62–77 µm from anterior of body; dorsal tooth 13–16 µm from anterior of body..... *T. tearoha* **sp. nov.**
 - Cervical seta 78–86 µm from anterior of body; dorsal tooth 16–19 µm from anterior of body... *T. manurewa* **sp. nov.**

General remarks

The presence of a ventral cervical seta and its distance from anterior end have previously been considered as a specific character in *Tripylina* (Brzeski and Winiszewska-Ślipińska 1993). The presence of lateral cervical setae was not mentioned in any previous descriptions, perhaps because they are very difficult to observe when the nematode is lying laterally. The presently proposed new species have this character, which may be a very important character for discrimination among species within the genus *Tripylina*.

Tripylina is predacious, as is the related genus *Tripyla* (Goodey, 1963): some prey debris (such as nematodes or rotifers) was found in the intestine of some the specimens of the newly described species. Yeates (1972) found similar debris in *T. stramenti*.

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