Article

New Zealand species of the genus *Tripyla* Bastian, 1865 (Nematoda:Triplonchida: Tripylidae). I : A new species, a new record and key to long-tailed species

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Abstract

This paper describes two species of the genus of *Tripyla* from New Zealand and also provides a key to species based on the morphology of females in eight long-tailed ($c < 5$) species in the genus of *Tripyla*. *Tripyla bioblitz* **sp. nov.** is characterized by its more anterior vulva position ($V = 43.7$–45.4%), relatively short body length (1150–1410 µm) and long tail ($c = 4.0$–4.4) in the group. *Tripyla filicaudata* de Man, 1880 is recorded for the first time from New Zealand and from the Southern Hemisphere. In addition, the phylogenetic relationships among species were analyzed using data from the near full length small subunit (SSU) and D2/D3 expansion segments of the large subunit ribosomal (LSU) rRNA genes, and these analyses revealed that *T. bioblitz* **sp. nov.** is close to but distinct from *T. filicaudata* de Man, 1880.

Key words: Description, new species, morphology, morphometrics, molecular, SSU, LSU, phylogeny, taxonomy, Nematoda, *Tripyla*

Introduction

Nematodes of the genus *Tripyla* Bastian, 1865 (family Tripylidae de Man, 1876) are found in soil and limnetic habitats (Brzeski 1963; Brzeski & Winiszewska-Ślipińska 1993; Tsalolikhin 1983; Zullini 2006; Andrássy 1985, 2006, 2007, 2008). The genus currently consists of twenty-five valid species *sensu* Andrássy (2007, 2008). These include:

Type species:

Tripyla glomerans Bastian, 1865

Other species:

T. affinis de Man, 1880

T. aquatica Brzeski & Winiszewska-Ślipińska, 1993

T. cornuta Skwarra, 1921

T. crassa Alekseev & Bestalannaya, 1990

T. dubia Gagarin, 1997

T. dybowskii Tsalolikhin, 1976

T. elegantula Brzeski & Winiszewska-Ślipińska, 1993

T. filicaudata de Man, 1880

T. glosaria (Gagarin, 1994) Andrássy, 2007

T. infa Brzeski & Winiszewska-Ślipińska, 1993

T. pulchella Andrássy, 2007

T. italica Tsalolikhin, 2003

T. koreana Winiszewska-Ślipińska, Brzeski, Choi & Kim, 2000

T. longicaudata Nesterov, 1979

T. magna Altherr & Delamare Deboutteville, 1972

T. minuta (Brzeski, 1963) Brzeski & Winiszewska-Ślipińska, 1993

T. pygmaea Micoletzky, 1922

T. scandinavica Andrássy, 2007

T. setifera Bütschli, 1873

T. sibirica Gagarin, 1993

T. subterranea Tsalolikhin, 1976

T. tenuis Brzeski, 1964

T. terricola Brzeski & Winiszewska-Ślipińska, 1993

T. vulvata Andrássy, 1977

Based on morphological grounds, *Tripyla* can be divided into two distinct groups: (1) with long tails (de Man's ratio $c<5$) and (2) with short-tails (de Man's ratio $c>5.9$) (Tsalolikhin 2003). Recent molecular phylogenetic studies suggest that the short-tailed group of *Tripyla* has two separate clades: (1) with long cephalic setae (the six long cephalic setae >5 μm), and (2) with short cephalic setae (the six long cephalic setae <5 μm) (Zhao 2009; Zhao & Buckley 2009).

Hitherto, only one species of the genus—*T. affinis*—has been recorded from New Zealand (Yeates 2006). Nematodes in the family Tripylidae were sampled from various areas in New Zealand from March 2007. Over 200 soil and litter samples from native forests and conservation parks were examined. One new and one known species belonging to the long-tailed group ($c < 5$) of *Tripyla* were discovered in four locations. This paper describes one species, re-describes *T. filicaudata* and also provides a key to long-tailed ($c < 5$) species based on the morphology of females in eight species of *Tripyla*. In addition, the phylogenetic relationships among sequenced species were analyzed using data from near full length small subunit (SSU) and large subunit ribosomal (LSU) rRNA gene.

The presence of multiple copies of rDNA in the genome facilitates PCR amplification from a single nematode (Powers *et al.* 1997). The 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) and the LSU rRNA gene has been useful for resolving closely related taxa (Al-Banna *et al.* 1997; Nadler and Hudspeth 1998; Duncan *et al.* 1999). Sequence analysis of PCR amplified from the near full length of SSU and the D2/D3 expansion segments of LSU of the ribosomal gene was therefore used to reconstruct the phylogenetic relationships of New Zealand tripylids.

Material and methods

Nematode sampling, extraction & processing specimens

Top-soil and litter mixtures (0–10cm) were collected from four locations in New Zealand. The nematodes were obtained from the samples using the Whitehead and Hemming (1965) tray method. The nematodes were then transferred to water in a glass block for examination using a dissecting microscope at 8–35× magnification (Leica EZ4, Germany).

For morphological study, 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 Southey (1986) and modified by Davies and Giblin-Davis (2004).

Drawings were made using an interference contrast microscopy (Nikon, Eclipse 90*i*) with a camera lucida. Measurements were made from material mounted in glycerol using NIS-Elements Basic Research (Nikon, Version 2.32). Head diameter was measured at the level of origin of the cephalic setae. Maximum body diameter was measured at the vulval region for females and at mid-length for males, respectively. Body length was measured along the mid-line. The following de Man's ratios were determined, respectively: $a =$ length divided by greatest body diameter; $b =$ length divided by distance from anterior end to posterior of pharynx (excluding pharyngeal-intestinal glands); $c =$ length divided by tail length; $c' =$ tail length divided by

diameter at anus or cloaca; *V* = anterior end to vulva as percentage of body length; *T* = length of testis from cloaca to end or flexure as percentage of body length. A microscopic attached camera (Nikon Camera Head DS-Fi1) was used to take a series of digital images of the specimens.

DNA extraction

A single nematode was hand-picked from a living nematode suspension, and several other nematodes of the same apparent species were put in a tube containing1M NaCl and stored at –20ºC freezer for future DNA extraction. The method of Zheng *et al.* (2002) was used to extract DNA from the nematodes. Total genomic DNA from a single nematode was extracted using worm lysis buffer containing proteinase K (Williams *et al.* 1992). DNA extracts were stored at –4ºC until used as PCR template. More details of preparation and photography of the nematodes can be found in Zhao and Buckley (2009).

Polymerase chain reaction (PCR), PCR product purification and DNA sequencing

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' TGATCCWKCYGCAGGTTCAC 3') (De Ley *et al.* 2002; Dorris *et al.* 2002). For both SSU and LSU, the 20 µl PCR contained 10 µl Go Tag® 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 95ºC for 3 min, followed by 30 cycles of denaturation at 94ºC for 60 s, annealing at 55ºC for 45 s, and extension at 72ºC for 45 seconds. A final extension was performed at 72ºC for 10 min. PCR products were purified by Wizard® SV Gel and PCR Clean-Up System (Promega Corporation, Madison, WI, USA). 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 alignment and phylogenetic inference

All sequences of New Zealand isolates used to compile the phylogenetic relationship of the group in this study have been deposited into GenBank (Zhao & Buckley 2009). Nematode taxa used in this study and their sequence accession numbers can be found from both phylogenetic trees (Figs 5; 6). DNA sequences were aligned by Clustal W (Thompson *et al.* 1994) using the multiple alignment method with default parameter values. The resulting alignment was checked by eye and corrected. The model of base substitution in the SSU, LSU sets was 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 'burnin' 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 outputted set of trees (Larget & Simon 1999).

Tripyla bioblitz **sp. nov.**
(Figs 1, 2)

Measurements. Table 1.

TABLE 1. Morphometric data for *Tripyla bioblitz* sp. nov. (measurements in µm ± S.D.)

	Holotype female	Paratype females (Smith's Bush)	Paratype females (Botanic Garden)
		Mean ± S.D. (range)	Mean ± S.D. (range)
n	1	3	4
a	30.3	30.8 ± 1.5 (29.4–32.4)	31.1 ± 0.7 (30.4–31.7)
b	5.4	5.2 ± 0.3 (4.9–5.5)	5.3 ± 0.2 (5.0–5.5)
c	4.3	4.2 ± 0.2 (4.0–4.4)	4.3 ± 0.1 (4.2–4.4)
c'	11.0	11.1 ± 0.5 (10.5–11.4)	11.5 ± 0.9 (10.1–12.3)
V	44.9	44.6 ± 0.9 (43.7–45.4)	44.7 ± 0.5 (44.1–45.2)
Body length	1301	1266.2 ± 103.3 (1150–1348)	1269.8 ± 99.9 (1174–1410)
Head diameter	19	20.2 ± 0.3 (19–20)	22.1 ± 1.5 (21–24)
Body diameter	43	41.2 ± 5.0 (35–44)	40.9 ± 4.0 (37–46)
Dorsal tooth from anterior	21	22.1 ± 1.3 (21–23)	20.7 ± 0.5 (20–21)
Excretory pore from anterior	74	73.0 ± 2.2 (70–76)	70.6 ± 0.9 (70–71)
Vulva from anterior	585	564.6 ± 36.6 (523–589)	569.9 ± 47.5 (522–635)
Pharynx length	239	242.7 ± 7.7 (234–248)	240.6 ± 9.6 (234–255)
Amphid from anterior	12	13.0 ± 5.0 (8–19)	10.9 ± 1.2 (9–12)
Nerve ring from anterior	88	90.0 ± 3.3 (87–93)	94.2 ± 3.2 (91–98)
Tail length	303	299.9 ± 15.2 (286–316)	296.9 ± 29.9 (271–340)

Material examined. Holotype: National Nematode Collection of New Zealand (NNCNZ), slide No. 264. Paratype: Seven females and seven juveniles. NNCNZ, slide Nos 2559–2572.

Description. Female. Body ventrally arcuate when fixed (Fig. 1A), posterior more curved than anterior. Cuticle distinct, about 2–3 µm thick at mid-body part, cuticular annules 2–3 µm wide. Maximum body diameter generally at level of vulva, occasionally at the level of base of pharynx. Body pores not seen.

Head rounded, smooth, continuous with body contour, narrower than adjacent body, cuticle 3–4 µm thick (Figs 1C, D; 2A). Labial papillae short and conical. Six long and four short cephalic setae arranged in two separate circles; six longer cephalic setae 6–7 µm long, or 28–35% of head diameter, more or less arcuate and directed anteriorly; four short setae 3–4 µm long and thinner compared with the six longer cephalic setae, more or less arcuate near tip, situated nearly one length of a longer seta from the anterior circle. Stoma walls thickened distinctively, dorsal tooth large, hook shaped, triangular; two tiny subventral teeth in stomatal chamber 4–5 µm anterior to dorsal tooth (Fig. 1C). Amphids cup-like with transverse oval opening, 8–19 µm from anterior end (Figs 1C; 2B).

Excretory pore 70–73 µm, or 27–30% of pharyngeal length, from anterior end (Fig. 1B). Nerve ring 87–98 µm, or 36–40% of pharyngeal length, from anterior end. Pharynx cylindrical, muscular, 233–254 µm long. Three prominent cells located at the pharyngeal-intestinal junction (Figs 1B; 2E). Coelomocytes not seen. Female genital system amphidelphic, gonad lying ventro-lateral to intestine, 233–337 µm long, or 19–25% of body length between points of flexure (Fig. 1E). Ovaries reflexed 1/3–1/2 of the way back to vulva. Eggs present in female reproductive systems (Fig. 1E). Vulva simple, without protuberant lips, vagina occupying one-fourth to one-third of corresponding body diameter, pore-shaped in lateral view (Figs 1E; 2F), in ventral view vulva appears to have an X-shaped opening (Fig. 1F), sclerotised pieces seen in the vaginal area.

Intestine showing undulating walls and a broad lumen. No distinct prerectum. Rectum less than anal body diameter (23 vs 26 μm). Tail tapering in anterior part reaching nearly to middle point, then becoming cylindrical (Fig. 1G), c = 4.0–4.4 (Table 1). Numerous tiny dots evenly distributed on posterior part of middle field of tails (Fig. 1G). Three tandem caudal glands (Fig. 1G); spinneret terminal, 4–6 μm long (Figs 1H; 2D).
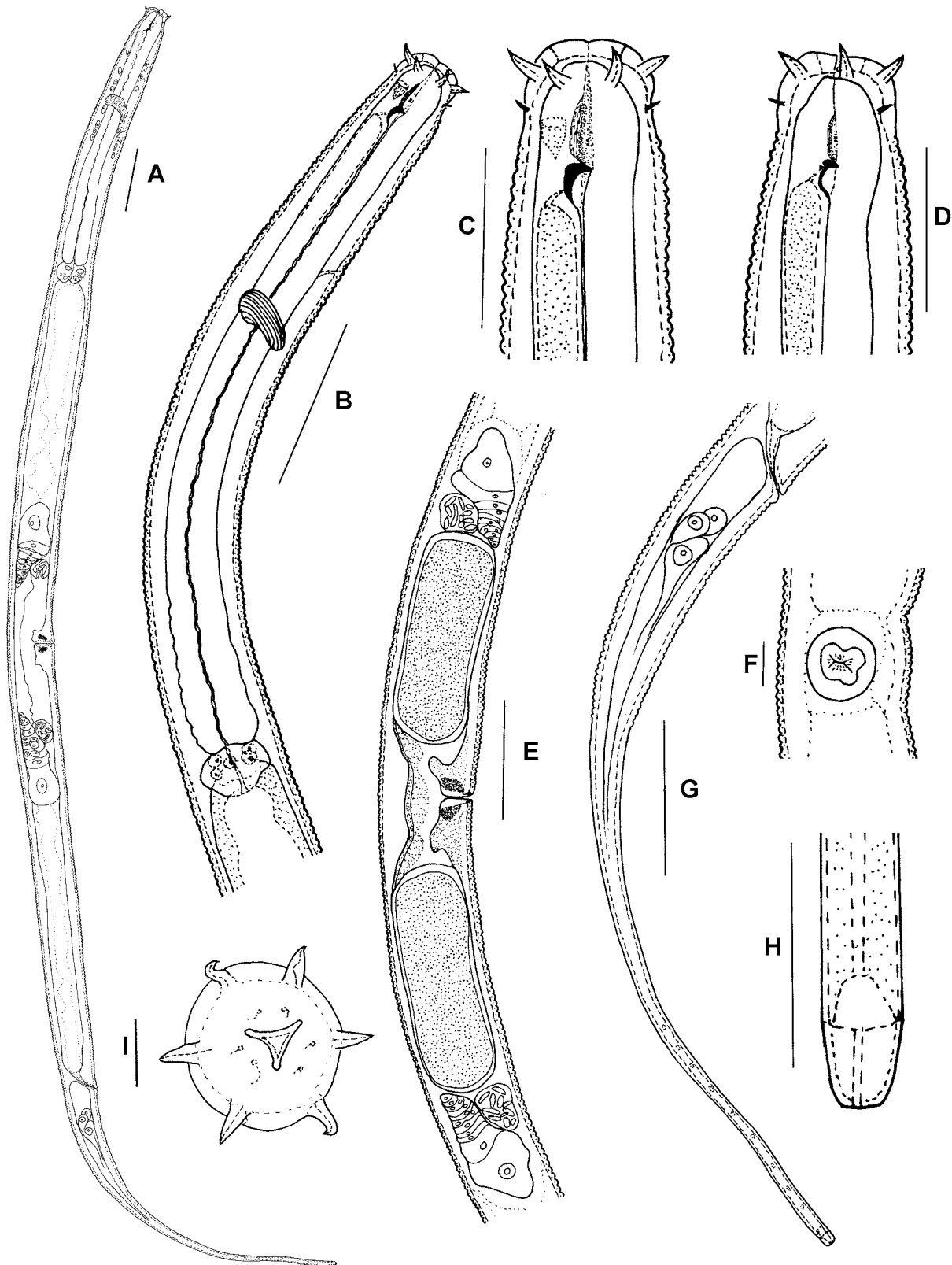

FIGURE 1 *Tripyla bioblitz* **sp. nov.** A: Female, entire. B: Pharyngeal region, lateral. C: Head, lateral. D: Sub-female head, lateral. E: Genital region, lateral. F: Ventral view of cross section through vagina. G: Female tail. H: Spinneret. I: *En face* view of female. Scale bars: A, B, E, G = 50 μm; C, D = 25 μm. F, H = 10 μm.
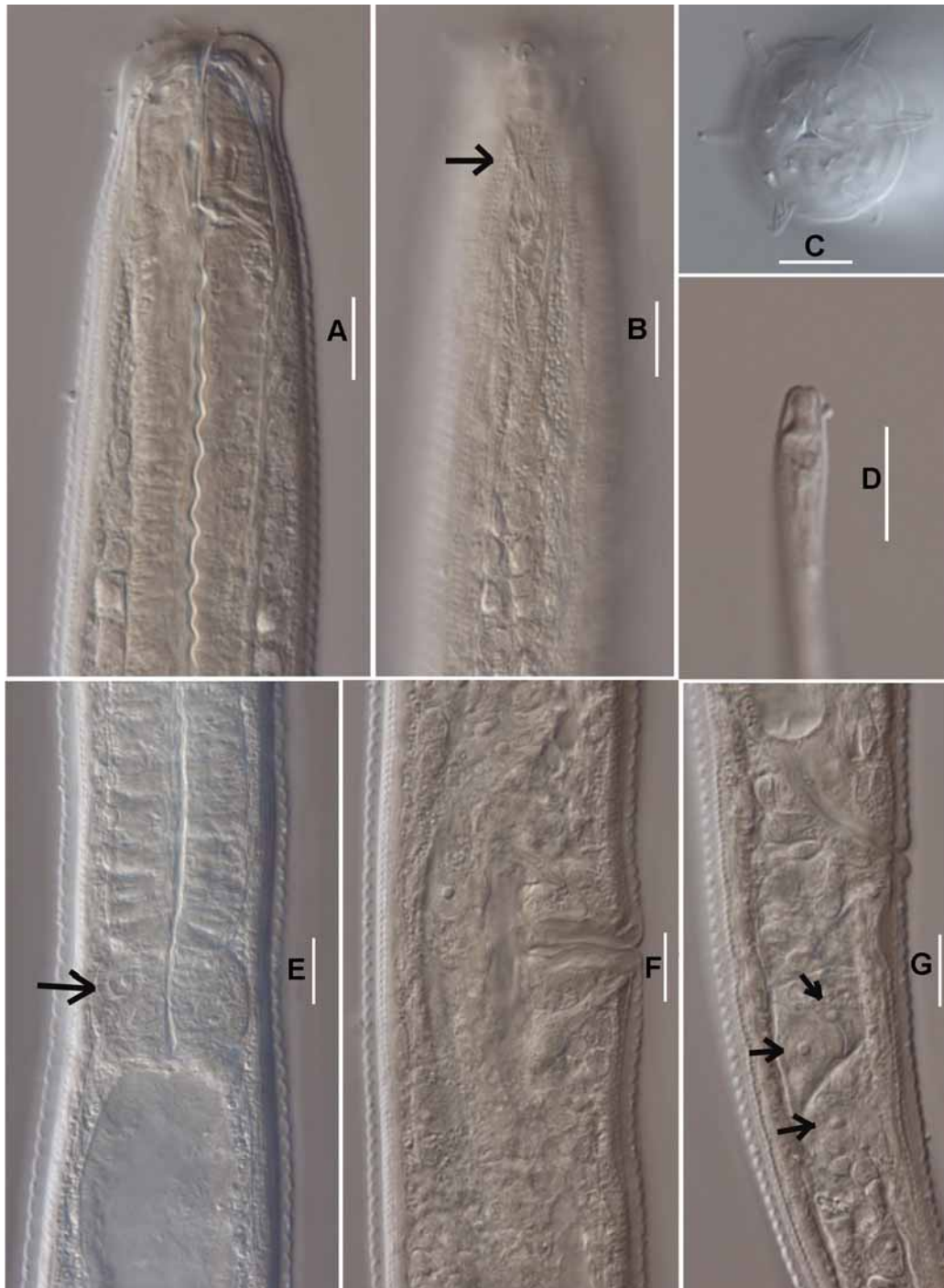
FIGURE 2 *Tripyla bioblitz* **sp. nov.** Photomicrographs. A: Female pharyngeal region, lateral. B: Amphid (arrowed). C: *En face* view. D: Spinneret. E: Cardia region (arrowed). F: Genital region, vulva, lateral. G: Anus & caudal glands (arrows indicate glands). Scale bars = 10 μm.

Male. Not known.

Locality and habitat. Holotype and six paratypes (NNCNZ slide nos 2559–2561 females; 2562–2564 juveniles) from soil and litter mixture, 0–10 cm depth under a native tree, *Dacrycarpus dacrydioides* (A. Rich.) de Laub. (common names: Kahikatea, white pine), surrounded by several trees, *Geniostoma ligustrifolium* A. Cunn., Smith's Bush, North Shore City, Auckland, New Zealand (36º48.782 S, 174º45.026 E), coll. Zeng Qi Zhao, 4. iv. 2008; eight paratypes (NNCNZ slide nos 2565–2568 females ; 2569–2572 juveniles) from soil and litter mixture, 0–10 cm depth under *D. dacrydioides*, Auckland Botanical Garden, South Auckland, New Zealand (37˚0.657 S, 174˚54.491 E), coll. Zeng Qi Zhao, 23. iv. 2008.

Diagnosis and relationships. The genus *Tripyla* is divided into two groups—some with "long-tails" (*c*<5) and others with "short-tails" (*c*>5.9) (Tsalolikhin 2003). *T. bioblitz* **sp. nov.** belongs to the former group, bringing its members to eight (Table 2).

TABLE 2. Comparative morphometrics of eight long-tailed *Tripyla* species.

Species	L of female (µm)	a	b	c	c'	V/T%	Spicule	Gubernaculum	References
Females									
dybowskyi	2700 2580–3000	24.6 20–30	5.6 4.9–6.4	5.0 4.4–6.0		50 46–53			Tsalolikhin 1976
filicaudata	1190–1930	28–41	5.0–6.0	3.6–5.5	7.2–14.3	47–53			De Man 1880; Brzeski & Winiszewska-Ślipińska 1993
	1360 1293–1428	32.7 29–36	5.5 5.1–5.8	4.4 3.6–5.2	11.0 8.3–13.5	48 46–50			This paper
italica	2402 1952–2686	55.2 40–64.8	6.2 5.4–6.8	4.3 3.6–4.8		48 45–51			Tsalolikhin 2003
longicaudata	1900 1500–2300	36.5 33–40	4.8 4.4–5.3	3.9 3.6–4.2		50 48–52			Nesterov 1979
subterranea	1830–1960	47–49	5.4–5.9	3.7–4.4		45–46			Tsalolikhin 1976
tenuis	1590 1490–1650	32 30–34	5.1 4.9–5.4	4.5 4.3–4.6	12.0 10.4–13.6	49 47–53			Brzeski 1964
***bioblitz* sp. nov.**	1266 1150–1410	31 29–32	5.2 4.9–5.5	4.3 4.0–4.4	11.3 10.1–12.3	45 44–45			This paper
scandinavica	1800–1900	38–43	5.2–5.5	6.3–6.9	8–11	55–56			Andrássy 2007
Males									
dybowskyi				Not Known					
filicaudata	1170–1810	34–46	4.8–6.0	4.2–5.2	8.4–11.8		31–45	10–14	De Man 1880; Brzeski & Winiszewska-Ślipińska 1993
	1329 1138–1457	35.0 31.0–39.0	5.4 4.4–6.0	4.4 3.8–5.3	9.7 9.0–11.7	76 72.9–78.6	37 27–40	13.6 12–16	This paper
italica	2396 2279–2580	54.6 44.6–64.5	6.2 5.8–6.7	4.4 4.2–5.7			47 44–48	20	Tsalolikhin 2003
longicaudata	1900 1600–2200	43 40–46	4.7 4.4–5.0	3.7 3.2–4.3			52		Nesterov 1979
subterranea	1700	43.3	5.1	3.6			48		Tsalolikhin 1976
tenuis	1970	44	6.4	5.2	11.8		43	14	Brzeski 1964
scandinavica	1820	56	4.8	6.3			40	13	Andrássy 2007
***bioblitz* sp. nov.**				Not Known					This paper

Tripyla bioblitz **sp. nov.** is characterized by its short body length (L = 1150–1410 μm) and anterior vulva (*V* = 44–45%) position. Its status as a distinct species is confirmed by molecular data from sequencing of SSU and LSU (Figs 5, 6).

Six species in the long-tails group of *Tripyla* have body lengths greater than 1490 μm, so that *T. bioblitz* **sp. nov.** (1150–1410 μm) is easily differentiated from them (Table 2). *T. bioblitz* **sp. nov.** overlaps the body length of *T. filicaudata* but differs from it in vulva position (44–45 *vs* 46–53%) (see Table 2).

Etymology. The name BioBlitz is applied to a series of public scientific events in New Zealand and other countries. It is used here as a noun in apposition.

Remarks. Some *T. bioblitz* **sp. nov.** were collected that looked like normal adult females except: 1) the vulval opening was covered by cuticle; 2) body length and diameter were smaller than in normal adult females; 3) the shape of the dorsal tooth differed from that of adults, i.e., a small tooth can be clearly observed at the dorsal part of dorsal tooth which is not found in normal adult female; and 4) the stoma wall was thinner than in normal adult females (Fig. 1D). The reproductive system of these nematodes appeared to be fully developed, but no eggs were observed. It is probable that these nematodes were mature fourth stage juveniles. Here, they are referred to as 'sub-females' (Fig. 1D), and were also seen in *T. filicaudata* (Fig. 3D).

Species of *Tripyla* are thought to be predacious (Goodey 1963; Yeates *et al.* 1993). The biology of *T. bioblitz* **sp. nov.** is unknown. However, some prey debris (possibly nematode or rotifer) was observed in the intestine of some specimens examined here, confirming that it is a predator.

Tripyla filicaudata **de Man, 1880**
(Figs 3, 4)

Measurements. Table 3.

TABLE 3. Morphometric data for *Tripyla filicaudata* (measurements in μm ± S.D.)

	Females Mean ± S.D. (range)		Males Mean ± S.D. (range)	
n	6 (St Johns)	7 (Invercargill)	5 (St Johns)	1 (Invercargill)
a	31.1 ± 2.2 (28.8–33.8)	34.3 ± 3.0 (29.9–36.0)	34.2 ± 2.5 (31.0–36.4)	39.0
b	5.4 ± 0.2 (5.1–5.6)	5.6 ± 0.1 (5.4–5.8)	5.4 ± 0.6 (4.4–6.0)	5.8
c	4.9 ± 0.2 (4.8–5.2)	3.9 ± 0.2 (3.6–4.2)	4.6 ± 0.4 (4.3–5.3)	3.8
c'	9.3 ± 0.7 (8.3–10.2)	12.8 ± 0.7 (11.9–13.5)	9.4 ± 0.4 (9.0–10.1)	11.7
V/T	48.8 ± 1.6 (46.0–49.9)	46.5 ± 0.9 (45.6–48.3)	76.4 ± 1.5 (75.0–78.6)	72.9
Body length	1391.0± 39.8 (1317–1428)	1329.8 ± 38.0 (1293–1387)	1329.2 ± 127.6 (1138–1457)	1361
Head diameter	20.8 ± 1.5 (18–23)	20.07 ± (18.8–21.0)	20.9 ± 2.1 (19–23)	22.1
Body diameter	44.9 ± 3.6 (39–48)	38.9 ± 2.3 (37.0–43.8)	38.9 ± 3.3 (36–44)	34.9
Dorsal tooth from anterior	22.5 ± 1.3 (21–24)	20.2 ± 1.1 (18.5–21.7)	21.1 ± 1.3 (19–23)	20.6
Excretory pore from anterior	69.2 ± 3.7 (64–75)	62.7 ± 3.9 (58.0–67.7)	75.7 ± 8.8 (68–87)	69.0
Vulva from anterior	679.2 ± 24.9 (641–709)	617.6 ± 13.1 (600.9–632.9)		
Pharynx length	260.0 ± 8.9 (247–270)	237.1 ± 9.1 (221.5–248.0)	249.1 ± 31.9 (213–298)	235.4
Amphid from anterior	11.7 ± 0.8 (10–13)	12.2 ± .07 (11.5–12.8)	11.4 ± 1.3 (9–13)	12.1
Nerve ring from anterior	92.2 ± 2.3 (89–95)	86.6 ± 3.9 (78.9–89.9)	94.2 ± 3.2 (91–98)	90.9
Tail length	283.6 ± 19.0 (256–313)	340.9 ± 16.2 (340.9–16.2)	292.7 ± 42.3 (249–342)	362.1
Spicule			36.6 ± 4.4 (27–40)	38.3
Gubernaculum			13.6 ± 1.2 (12–16)	14.6
Testes extent			609.1± 95.5 (441.4–675.1)	549.4
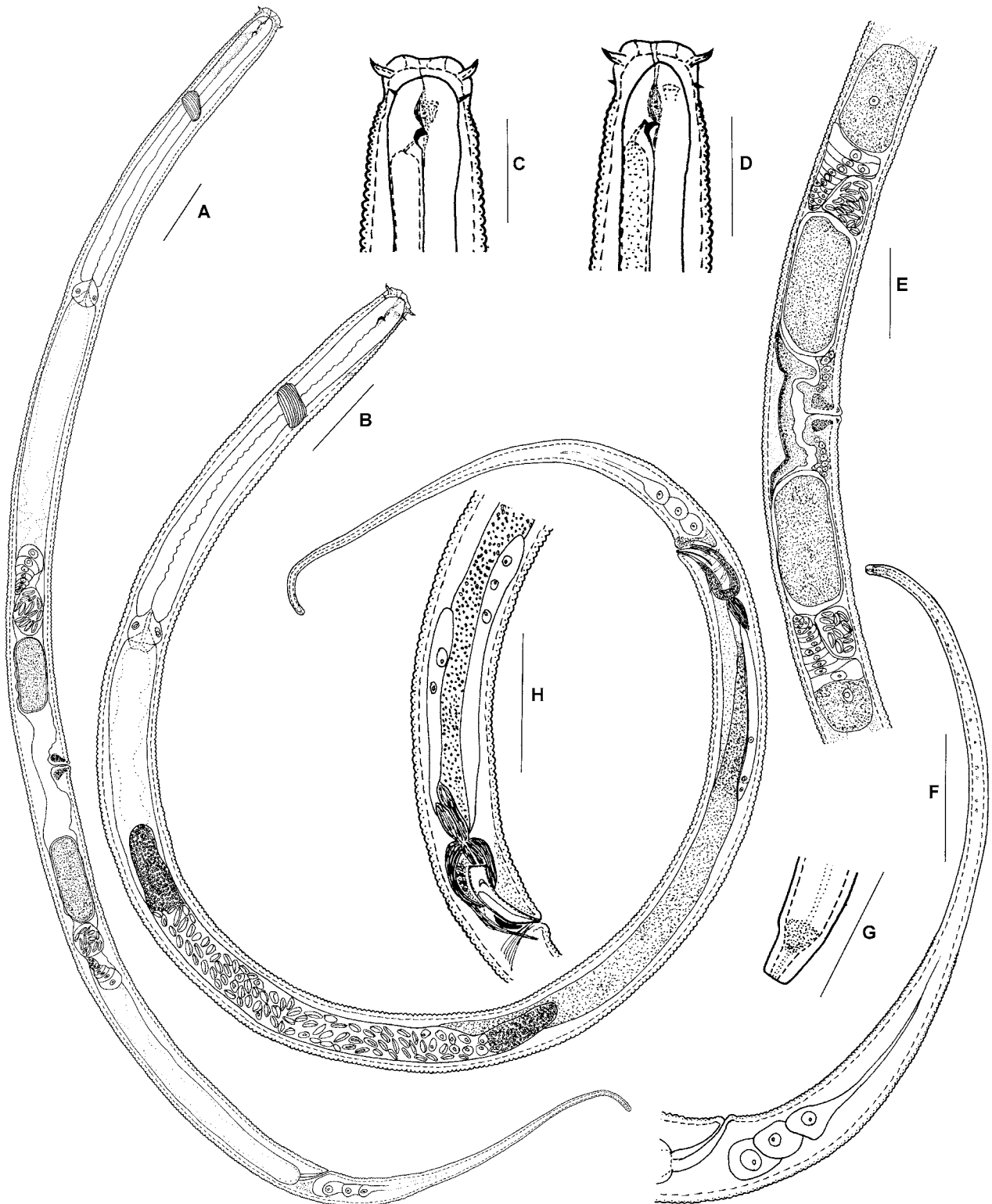
FIGURE 3 *Tripyla filicaudata*. A: Female entire, lateral. B: Male entire, lateral. C: Female head, lateral. D: Sub-female head, lateral. E: Genital region, lateral. F: Female tail. G: Spinneret. H: Male genital region. Scale bars: A, B, E, F, H = 50 μm; C, D = 25 μm. G = 10 μm.

FIGURE 4 *Tripyla filicaudata*. Photomicrographs. A & B: Female pharyngeal region. C: Amphid. D: Cardia region (arrowed). E: Male genital region. F: Male, sperm. G: Anus & caudal glands (arrows indicate glands). H: Genital region, vulva, lateral. I: Egg (arrowed) & Reproductive system. J: Spinneret. Scale bars = 10 μm.

Description. **Female**. Body ventrally arcuate when fixed (Fig. 3A), posterior more curved than anterior. Cuticle distinct, about 2–3 μm thick at mid-body part; cuticular annules 2–3 μm wide. Maximum body diameter generally at level of vulva, occasionally at the level of base of pharynx. Body pores not seen. Head rounded, smooth, continuous with body contour, narrower than adjacent body, cuticle 3–4 μm thick (Figs 3C, D; 4A, B). Labial papillae short and conical. Six long and four short cephalic setae arranged in two separate circles; six longer cephalic setae 5–6 μm long, or 24–30% of head diameter, more or less arcuate and directed anteriorly; four short setae 2–3 μm long and thinner in comparison to the six longer cephalic setae, more or less arcuate near tip, situated nearly one length of a longer seta from the anterior circle. Stoma walls thickened distinctively, dorsal tooth large, hook shaped, triangular; two tiny subventral teeth in stomatal chamber 5–6 μm anterior to dorsal tooth (Figs 3C, D; 4B). Amphids cup-like with transverse oval opening, located 10–13 μm from anterior end (Figs 3C; 4C).

Excretory pore 65–75 μm, or 25–28% of pharyngeal length, from anterior end (Fig. 3A). Nerve ring 89–95 μm, or 33–38% of pharyngeal length, from anterior end. Pharynx cylindrical, muscular, 247–270 μm long. Three prominent cells located at the pharyngeal-intestinal junction. Coelomocytes not seen.

Female genital system amphidelphic, gonad lying ventro-lateral to intestine, 245–366 μm long, or 17–27% of body length between anterior and posterior points of flexures (Fig. 3E). Ovaries reflexed 1/4–1/3 of the way back to vulva. Eggs were present in female reproductive systems (Figs 3E; 4I). Vulva simple, with protuberant lips, vagina occupying one-third of corresponding body diameter, in lateral view apparently pore-shaped (Figs 3E; 4H), sclerotised pieces seen in the vaginal area.

Intestine showing undulating walls and broad lumen. No distinct prerectum. Rectum less than anal body diameter (25 vs 30 μm). Tail tapering in anterior part (40%) reaching nearly up to middle point, then becoming cylindrical (Fig. 3F), $c = 4.5$–5.2 (Table 2). Numerous tiny dots evenly distributing on anterior part of middle field of tail (Fig. 3F). Three caudal glands arranged in tandem (Fig. 3F), spinneret terminal, 3–5 μm long (Figs 3G; 4J).

Male. Morphology similar to that of females. Body C- to spiral-shaped when fixed (Fig. 3B). Cuticle separated into two layers in some specimens. Testis outstretched; developing germ cells in single file (Fig. 3B). Spicules paired, 27–40 μm long along mid-line, horn-shaped, ventrally curved (Figs 3H; 4E). Gubernaculum 12–15 μm long, crescent-shaped. Four ventromedian supplementary papillae observed in pharyngeal region.

Locality and habitat. Specimens from soil and litter mixture, 0–10 cm depth from two different places in New Zealand: under a group of native tree fern *Cyathea medullaris* Swartz (common name: mamaku), St Johns Bush, Auckland (36°52.354 S, 174°50.531 E), Coll. Zeng Qi Zhao, 14. iii. 2008; under *Pinus radiata* D. Don, Queens Park, Invercargill (46°24.209 S, 168°21.264 E), Coll. Zeng Qi Zhao, 8. v. 2009.

Remarks. *Tripyla filicaudata* generally occurs in both terrestrial and limnetic habitats, mostly in soil and moss, but also in limnic biotopes, and in subterranean waters (Andrássy 2007). *Tripyla filicaudata* has been reported from three continents: Europe (Netherlands, Belgium, Germany, Poland, Switzerland, United Kingdom, Sweden, Faeroe Islands, the Baltic, Czech Republic, Slovakia, Hungary, Serbia, Bulgaria, Spain, France, Italy, Russia), Asia (Uzbekistan, Russian Far East) and North America (United States) (Andrássy 2007). This is the first report of this species in New Zealand and also in the Southern Hemisphere. In general, the present specimens (13 females, 6 males) correspond well to the descriptions given by Brzeski & Winiszewska-Ślipińska (1993) and Andrássy (2007). However, a big range in body length was mentioned in both descriptions (Brzeski & Winiszewska-Ślipińska 1993; Andrássy 2007). This was not observed in the New Zealand specimens.

Molecular phylogenetic analyses

The trees generated by Bayesian and MP analysis showed no significant conflict in branching order, so only Bayesian trees are shown.

Based on SSU and LSU molecular studies, *T. bioblitz* **sp. nov.** is a sister species to but distinct from *T. filicaudata* de Man, 1880 (Fig. 5). Molecular phylogenetic inference of SSU sequences using Bayesian analysis (Fig. 5) supported 1) monophyly of *T. bioblitz* **sp. nov.** with *Tripyla* cf. *filicaudata* (AY284730 and AY284731) and *Tripyla filicaudata* (GQ503031 and an isolate from Invercargill, New Zealand); 2) monophyly of the genus *Tripyla*. While only 56% posterior probability value supported *T. bioblitz* **sp. nov.** with *Tripyla* cf. *filicaudata* and *Tripyla filicaudata,* results with LSU support it as a sister species (Figs 5; 6).

FIGURE 5 Bayesian phylogenetic tree inferred from SSU gene DNA sequences. Posterior probabilities great than 50% are given on appropriate clades. Nematode species, GenBank numbers, locations are listed for each taxon if known.

FIGURE 6 Bayesian phylogenetic tree inferred from LSU gene DNA sequences. Posterior probabilities greater than 50% are given on appropriate clades. Nematode species, GenBank numbers, locations are listed for each taxon if known.

Although the Bayesian analysis of the D2/D3 LSU sequences involved a different subset of nematodes than the SSU analysis (Figs 5; 6), the results are generally similar to those using the more conservative SSU sequences (Fig. 5). In addition, D2/D3 analysis supports the monophyly of *T. bioblitz* **sp. nov.** with *T. filicaudata* (GQ503031 and an isolate from Invercargill, New Zealand), and the monophyly of the genus *Tripyla* (Fig. 6). These results from molecular analyses should be regarded with caution, as *T. bioblitz* **sp. nov.** and *T. filicaudata* are the only two members within the long-tailed group in the genus *Tripyla* to be sequenced. However, they do appear to be supported by the morphological similarity of the two species.

Key for identification of species of *Tripyla* from the long-tailed group (c < 5) (by females)

1. Tail length shorter than 430μm...2
 – Tail length longer than 480 μm...4
2. The six long cephalic setae (2–4 μm) are less than ¼ of a head diameter...*T. tenuis*
 – The six long cephalic setae (5–8 μm) are about ¼ of a head diameter...3
3. V = 46–53 ....*T. filicaudata*
 – V = 44–45 ....*T. bioblitz*
4. Pharynx longer than 450 μm..*T. dybowskii*

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References

Al-Banna, L., Williamson, L. & Gardner, S.L. (1997) Phylogenetic analysis of nematodes of the genus *Pratylenchus* using nuclear 26S r DNA. *Molecular Phylogenetic Evolution,* 7, 94–102.

Alekseev, V.M. & Bestalannaya, S.G. (1990) Aquatic nematodes of the genus *Tripyla* from Primorye and questions on the phylogeny of Tripylidae (Nematoda, Enoplida). *Trudy Instituta Biologii Vnutrennikh Vod*, 64, 129–144.

Aleshin, V.V., Kedrova, O.S., Milyutina, I.A., Vladychenskaya, N.S. & Petrov, N.B. (1998) Relationships among nematodes based on the analysis of 18S rRNA gene sequences, molecular evidence for monophyly of Chromadorian and Secernentian nematodes. *Russian Journal of Nematology,* 6, 175–184.

Altherr, E. & Delamare Deboutteville, C. (1972) Nematodes interstitiels des eaux douces des Etats-Unis d'Amerique (Etats de Washington, du Colorado et du Massachusetts) recoltes par Cl. Delamare Deboutteville. *Annales de Spéléologie*, 27, 683–689.

Andrássy, I. (1977) Ergëbnisse der zoologischen Forschungen von Dr. Z. Kaszab in der Mongolei. 356. Susswasser- und Bodennematoden aus den Jahren 1967–1968. *Opuscula Zoologca Budapest*, 13, 3–24.

Andrássy, I. (1985) A dozen new nematode species from Hungary. *Opuscula Zoologica Budapest*, 19, 3–39.

Andrássy, I. (2006) Three new species of the family Tripylidae (Penetrantia: Enoplida) from South America. *International Journal of Nematology*, 16, 208–216.

Andrássy, I. (2007) Pedozoologica Hungarica No. 4. *Free–living nematodes of Hungary*, II. Hungarian Natural History Museum, Budapest, 496 pp.

Andrássy, I. (2008) Two new and a known species of the family Tripylidae (Nematoda: Enoplida) from the tropics. *Opuscula Zoologica Budapest*, 37, 3–9.

Bastian, H.C. (1865) Monograph on the Anguillulidae, or free nematoids, marine, land, and freshwater; with descriptions of 100 new species. *Transactions of the Linnaean Society of London*, 25, 73–184.

Blaxter, M.L., De Ley, P., Garey, J.R., Liu, L.X., Scheldeman, P., Vierstraete, A., Vanfleteren, J.R., Machey, L.Y., Dorris, M., Frisse, L.M., Vida, J.T. & Thomas, W.K. (1998) A molecular evolutionary framework for the phylum Nematoda. *Nature*, 392, 71–75.

Brzeski, M.W. (1963) Nematode genera of the family Tripylidae (Nematoda, Enoplida). *Acta Zoologica Cracoviensia*, 8, 295–308.

Brzeski, M.W. (1964) Revision der Gattungen *Tripyla* Bastian und *Paratripyla* gen. n. (Nematoda, Tripylidae). *Annales Zoologici*, 22, 157–178.

Brzeski, M.W. & Winiszewska-Ślipińska, G. (1993) Taxonomy of Tripylidae (Nematoda: Enoplia). *Nematologica,* 39, 12–52.

Bütschli, O. (1873) Beiträge zur Kenntnis der freilebenden Nematoden. *Nova Acta Academiae Caesareae Leopoldino-Carolinae Germanicae naturae curiosorum*, 36, 1–144.

Davies, K.A. & Giblin-Davis, R.M. (2004) The biology and associations of *Fergusobia* (Nematoda) from the *Melaleuca leucadendra*-complex in eastern Australia. *Invertebrate Systematics,* 18, 91–319.

de Man, J.G. (1880) Die einheimischen, frei in der reinen Erde und im süssen Wasser lebenden Nematoden. *Tijdschrift*

van de nederlandse Dierkundige Vereeniging, 5, 1–104.

De Ley, I.T., De Ley, P., Vierstraete, A., Karssen, G., Moens, M. & Vanfleteren, J. (2002) Phylogenetic analysis of *Meloidogyne* small subunit rDNA. *Journal of Nematology,* 34, 319–327.

Dorris, M., Viney, M.E. & Blaxter, M.L. (2002) Molecular phylogenetic analysis of the genus *Strongyloides* and related nematodes. *International Journal of Parasitology,* 32, 1507–1517.

Duncan, L.W., Inserra, R.N., Thomas, W.K., Dunn, D., Mustika, I., Frisse, L.M., Mendes, M.L., Morris, K. & Kaplan, D.T. (1999) Molecular and morphological analysis of isolates of *Pratylenchus coffeae* and closely related species. *Nematropica,* 29, 61–80.

Fitch, D.H.A., Bugaj-Gaweda, B. & Emmons, S.W. (1995) 18S ribosomal rRNA gene phylogeny for some Rhabditidae related to *Caenorhabditis. Molecular Biology and Evolution,* 12, 346–358.

Gagarin, V.G. (1993) *Free-living nematodes from freshwater of Russia.* (In Russian) Sanit-Petersburg, 350pp.

Gagarin, V.G. (1994) A new species of genus *Paratripyla* (Nematoda: Tripylidae). *Biologiya Vnutrennikh Vod Informatsionnyi Byulleten*, 96, 22–24.

Gagarin, V.G. (1997) Fauna of free-living nematodes of reservoirs from Archipelago Novaya Zemlya. *Biologiya Vnutrennikh Vod*, 3 33-40.

Goodey, T. revised by Goodey, J.B. (1963) *Soil and freshwater nematodes.* London, Methuen & Co Ltd, 544 pp.

Larget, B. & Simon, D.L. (1999) Markov chain Monte Carlo algorithms for the Bayesian analysis of phylogenetic trees. *Molecular Biology and Evolution,* 16, 750–759.

Micoletzky, H. (1922) Die freilebenden Erd-Nematoden. *Archiv Für Naturgeschichte,* 87A, 1–650.

Nadler, S.A. & Hudspeth, D.S.S. (1998) Ribosomal DNA and phylogeny of the Ascaridoidea (Nemata, Secernentea), implications for morphological evolution and classification. *Molecular Phylogenetic Evolution,* 10, 221–236.

Nesterov, P.I. (1979) *Plant-parasitic and free-living nematodes of south-west of USSR*. Kishinev, Shtiintza, 312 pp.

Posada, D. & Crandall, K.A. (1998) Modeltest: testing the model of DNA substitution. *Bioinformatics,* 14, 817–818.

Powers, T.O., Todd, T.C., Burnell, A.M., Murray, P.C.B., Fleming, C.C., Szalanski, A. L., Adams, B.A. & Harris, T.S. (1997) The rDNA interal transcribed spacer region as a taxonomic marker for nematodes. *Journal of Nematology,* 29, 441–450.

Ronquist, F. & Huelsenbeck, J. P. (2003) MR BAYES: Bayesian inference of phylogenetic tress. *Bioinformatics,* 19, 1572–1574.

Skwarra, E. (1921) Diagnosen neuerfreilebender Namatoden Ostpreussens. *Zoologischer Anzeiger,* 53, 66–74

Southey, J.F. (ed.) (1986) *Laboratory Methods for Work with Plant and Soil nematodes.* Reference Book 402, *Ministry of Agriculture, Fisheries and Food,* London, U.K.

Swofford, D.L. (1998) *PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods)*, Version 4. Sinauer Associates, Sunderland, Massachusetts.

Thompson, J.D., Higgins, D.G. & Gibson, T.J. (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignments through sequence weighting, position specific gap penalties and weight matrix choice. *Nucleic Acids Research,* 22, 4673–4680.

Tsalolikhin, S.J. (1976) New species of nematodes of the order Enoplida in the Baikal Lake (In Russian). *Zoologichesky Zhurnal*, 55, 346–353.

Tsalolikhin, S.J. (1983) *Nematodes of the families Tobrilidae and Tripylidae of the World Fauna.* Nauka, Leningrad, Russia, 232 pp. (in Russian)

Tsalolikhin, S.J. (2003) *Tripyla italica* sp. n. (Nematoda, Enoplida, Tripylidae). *Zoologičeskij žurnal*, 82, 1122–1125. (in Russian)

Whitehead, A.G. & Hemming, J.R. (1965) A comparision of some quantitative methods of extracting small vermiform nematodes from soil. *Annals of Applied Biology*, 55, 25–38.

Williams, B.D., Schrank, B., Huynh, C., Shownkeen, R. & Waterston, R.H. (1992) A genetic mapping system in *Caenorhabditis elegans* based on polymorphic sequence-tagged sites. *Genetics,* 131, 609–624.

Winiszewska-Ślipińska, G., Brzeski, M.W., Choi, Y.E. & Kim, J.I. (1993) Tripylidae (Nematoda: Enoplia) from Korea. *Annales Zoologici*, 50, 297–306.

Ye, W., Giblin-Davis, R.M., Braasch, H., Morris, K. & Thomas, W.K. (2007A) Phylogenetic relationships among *Bursaphelenchus* species (Nematoda: Parasitaphelenchidae) inferred from nuclear ribosomal and mitochondrial DNA sequence data. *Molecular Phylogenetics and Evolution*, 43, 1185–1197.

Ye, W., Giblin-Davis, R.M., Davies, K.A., Purcell, M., Scheffer, S.J., Taylor, G.S., Center, T.D., Morris, K. & Thomas, W.K. (2007B) Molecular phylogenetics and the evolution of host plant associations in the nematode genus *Fergusobia* (Tylenchida: Fergusobiinae). *Molecular Phylogenetics and Evolution*, 45, 123–141.

Yeates, G.W. (2006) Phylum Nematoda Roundworms and Eelworms. *In*: D. P. Gordon (ed) *The New Zealand Inventory of Biodiversity*: A Species 2000 Review. Canterbury University Press, Christchurch, (in press).

Yeates, G.W., Bongers, T., De Goede, R.G.M., Freckman, D.W. & Georgieva, S.S. (1993) Feeding habits in soil nematodes families and genera-An outline for soil ecologists. *Journal of Nematology*, 25, 315-331.

Zhao, Z.Q. (2009) A review of the genus *Tripylina* Brzeski, 1963 (Nematoda: Triplonchida), with descriptions of five

new species from New Zealand. *Zootaxa,* 2238, 1–24.

Zhao, Z.Q. & Buckley, T.R. (2009) Phylogenetic analysis of nematode nuclear 18S rDNA sequences indicates the genus *Tripylina* Brzeski, 1963 (Nematoda: Tripylidae de Man, 1876) should be placed in Enoplida. *Zootaxa,* 2238, 24–32.

Zhao, Z.Q., Ye, W.M., Giblin-Davis, R.M., Li, D.M., Davies, K., Riley, I.T. & Thomas, W.K. (2008) Morphological and molecular analysis of six aphelenchidoids from Australian conifers and their relationship to *Bursaphelenchus* (Fuchs, 1937). *Nematology,* 10, 663–678.

Zheng, J.W., Subbotin, S.A., He, S.S., Gu, J.F. & Moens, M. (2002) Molecular characterisation of some Asian isolates of *Bursaphelenchus xylophilus* and *B. mucronatus* using PCR-RFLPs and sequences of ribosomal DNA. *Russian Journal of Nematology,* 11, 17–22.

Zullini, A. (2006) Order Triplonchida. *In:* Eyualem, A., Andrássy, I. & Traunspurger, W. (Eds), *Freshwater Nematodes: Ecology and Taxonomy.* CABI, Wallingford, pp. 293–325.