



Two new and one known deep-sea Comesomatidae Filipjev, 1918 species (Nematoda: Araeolaimida) from New Zealand's continental margin

Sujing Fu¹ · Daniel Leduc² · Zeng Qi Zhao³

Received: 16 December 2018 / Revised: 17 March 2019 / Accepted: 19 March 2019 / Published online: 4 May 2019
© Senckenberg Gesellschaft für Naturforschung 2019

Abstract

One new *Sabatieria* Rouville, 1903 and one new *Hopperia* Vitiello, 1969 species are described from Conway Trough off the east coast of New Zealand's South Island based on light and scanning electron microscope observations and SSU and D2-D3 of LSU molecular sequences. Additional morphological observations and molecular sequence data are also provided for *Vasostoma hexodontium* Rosli, Leduc & Probert, 2014. *Sabatieria articulata* sp. nov. belongs to the Praedatrix group and differs from all other species of the genus in having jointed spicules. *Hopperia novazelandica* sp. nov. is characterized by a relatively stout body, cephalic setae 0.2–0.3 corresponding body diameter long, amphideal fovea with 3.0 turns in males and 2.2–2.5 turns in females, buccal cavity with three teeth, spicules 2.3–2.5 cloacal body diameters long, presence of 19 precloacal supplements, and conico-cylindrical tail with swollen tip and terminal setae. Scanning electron microscopy observations show that the outer cuticle surface of *V. hexodontium* is smooth and that the inner labial papillae are slightly larger than the outer labial papillae. The SSU consensus tree could not resolve relationships among most Comesomatidae Filipjev, 1918 species and genera included in our analysis. Analyses based on D2-D3 of LSU sequences suggest that *Sabatieria*, *Hopperia*, and *Dorylaimopsis* are not monophyletic; instead, species/genera from the same region tended to cluster together, suggesting that features that characterize the genera *Hopperia* and *Dorylaimopsis*, such as a buccal cavity with teeth and an expanded posterior portion, have evolved multiple times independently, possibly from a *Sabatieria*-like ancestor. This hypothesis, however, will have to be tested using more comprehensive molecular datasets.

Keywords Conway trough · D2-D3 region of large subunit (LSU) 28S rDNA gene · Morphology · Scanning electron microscopy · Small subunit (SSU) 18S rDNA gene · Taxonomy

Communicated by M. Schratzberger

This article is registered in ZooBank under urn:lsid:zoobank.org:pub:F427666F-D91C-43E3-B6BD-D2BE2C2475B0

Sabatieria articulata sp. nov. is registered under urn:lsid:zoobank.org:act:7D3EFE23-2BA0-4BED-A661-9CB85AEEEF62

Hopperia novazelandica sp. nov. is registered under urn:lsid:zoobank.org:act:0D32112A-9499-4217-A48F-03FA553EC97C

Communicated by M. Schratzberger

✉ Daniel Leduc
Daniel.Leduc@niwa.co.nz

¹ Key Laboratory of the Ministry of Education for Coastal and Wetland Ecosystems, Xiamen University, Xiamen 361102, China

² National Institute of Water and Atmospheric Research, Wellington 14-901, New Zealand

³ Landcare Research, Private Bag 92170, Auckland Mail Centre, Auckland 1142, New Zealand

Introduction

Comesomatids are a group of marine nematodes with a worldwide distribution and most commonly found in coastal, shelf, and continental margin sediments (Jensen 1979; Soetaert and Heip 1995; Muthumbi et al. 2011). The Comesomatidae Filipjev, 1918 are characterized by male and female reproductive systems with two opposed and outstretched gonads (except one *Hopperia* species) and multispiral amphids, a combination of characters not found in any other marine nematode family. The posterior portion of the buccal cavity (pharyngostoma) is either narrow and collapsed (subfamilies Comesomatinae Filipjev, 1918 and Sabatieriinae Filipjev, 1934) or expanded (*Dorylaimospinae* De Coninck, 1965), and the spicules are either long and slender (*Comesomatinae*), short and robust (*Sabatieriinae*), or variable (*Dorylaimospinae*). The taxonomy of the family was revised by Jensen (1979), Platt (1985), and Hope and Zhang (1995). The family comprised a total of 20

genera and 153 species in the latest overview by Fonseca and Bezerra (2014). In recent years, most of the new Comesomatidae species have been described from the northwestern and southwestern Pacific Ocean, in particular the coasts and continental margins of China and New Zealand (e.g., Guo et al. 2015; Leduc 2017). To our knowledge, none of the Comesomatidae species descriptions available to date include molecular sequence data, although a relatively limited number of molecular sequences have been obtained for a number of species and genera in phylogenetic and population genetic structure studies (e.g., Holterman et al. 2008; De Groote et al. 2017).

A total of 27 species, including two *Vasostoma* Wieser, 1954, two *Hopperia* Vitiello, 1969, and 12 *Sabatieria* Rouville, 1903 species, have been described/recorded from New Zealand waters over the last decade or so, 18 of which have not been recorded elsewhere (Leduc and Gwyther 2008; Rosli et al. 2014; Leduc 2017). Here, we describe one new *Sabatieria* and one new *Hopperia* species from Conway Trough off the east coast of New Zealand's South Island at depths of 490–590 m. Additional morphological observations based on scanning electron micrographs and SSU and D2-D3 of LSU molecular sequences are provided for *Vasostoma hexodontium* Rosli, Leduc & Probert, 2014 specimens from the same location.

Material and methods

Sampling and morphological analyses

The Conway Trough is located off the east coast of New Zealand's South Island.

It is a north-south-oriented sedimentary basin approximately 40 km long and up to 10 km wide, reaching to within 3 km from the shore (Carter et al. 1982). It is separated from the highly productive Kaikoura Canyon (De Leo et al. 2010; Leduc et al. 2014) to the north by a narrow sill.

Sampling was conducted using *RV Tangaroa* during National Institute of Water and Atmospheric Research (NIWA) voyage TAN1708 (September 2018). Sediment samples were collected using an Ocean Instrument MC-800A multicorer (internal diameter of core = 9.52 cm) at three sites along the axis of Conway Trough (TAN1708 sites 28, 29, and 30 at 570, 586, and 491 m water depth, respectively). At each site, a subcore was obtained by pushing a cutoff syringe (29 mm internal diameter) into the sediment of one core. The sediment was then sliced into 0–1- and 1–5-cm layers and fixed in 10% buffered formalin. The remaining 0–5-cm layer of sediment from the same core was transferred to a plastic bag and frozen at $-80\text{ }^{\circ}\text{C}$.

In the laboratory, frozen sediment samples were thawed overnight, then sieved through a 45- μm mesh to retain nematodes. Nematodes were extracted using the ludox flotation method (Somerfield and Warwick 1996) and sorted under a dissecting microscope. A single male specimen of each species was mounted in a drop of seawater on a temporary slide to confirm its identity, and images of key morphological features were taken to provide image vouchers. Nematodes from formalin-fixed sediment samples were extracted using the same method as for the frozen samples. Specimens for light microscopy were transferred to glycerol and mounted onto permanent slides (Somerfield and Warwick 1996). Specimens for scanning electron microscopy (SEM) were transferred to a 4% osmium tetroxide solution overnight, then gradually transferred to pure ethanol using a graded ethanol series, critical point dried, and mounted onto stubs before coating with gold using a sputter coater. Observations were made using a Hitachi TM3000 tabletop SEM at high vacuum mode.

Type specimens are held in the NIWA Invertebrate Collection (Wellington) and the National Nematode Collection of New Zealand (Auckland). All measurements are in micrometer, and all curved structures are measured along the arc. The terminology used for describing the arrangement of morphological features such as setae follows Coomans (1978). Abbreviations in the text and tables are as follows:

<i>a</i>	Body length/maximum body diameter
<i>b</i>	Body length/pharynx length
<i>c</i>	Body length/tail length
<i>c'</i>	Tail length/anal or cloacal body diameter
<i>cbd</i>	Corresponding body diameter
<i>L</i>	Total body length
<i>n</i>	Number of specimens
<i>V</i>	Vulva distance from anterior end of body
<i>%V</i>	$V/\text{total body length} \times 100$

DNA extraction, PCR, and sequencing

Following observation and digital imaging under a compound microscope, a male specimen of each species was transferred to lysis buffer and kept frozen at $-80\text{ }^{\circ}\text{C}$ prior to molecular analyses. DNA was extracted by the method of Zheng et al. (2002) with minor modifications. The DNA extract was stored at $-20\text{ }^{\circ}\text{C}$ until used as PCR template.

Primers for LSU amplification were forward primer D2A (5'-ACAAGTACCGTGAGGGAAAGT-3') and reverse primer D3B (5'-TGCGAAGGAACCAGCTACTA-3') (Nunn 1992). Primers for the rDNA small subunit (SSU) were the first fragment forward primer 1096F, 5'-GGTA ATTCTGGAGCTAATAC-3' and reverse primer 1912R, 5'-TTTACGGTCAGAACTAGGG-3', and the second fragment

Table 1 Morphometrics (μm , mean (range)) of *Sabatieria articulata* sp. nov. from Conway Trough

	Male holotype	Male paratypes	Female paratypes
<i>n</i>	1	3	2
<i>L</i>	1973	1956 (1758–2177)	1914, 2064
<i>a</i>	28	31 (27–34)	27, 32
<i>b</i>	8	8	8
<i>c</i>	11	12 (10–13)	9
<i>c'</i>	3.7	3.7 (3.6–3.7)	5.2, 5.3
Head diam. at cephalic setae	15	14 (12–15)	14, 15
Mouth diameter max	4	4	5
Length of cephalic setae	5	5 (4–5)	5, 6
Amphid height	12	11 (10–12)	10, 12
Amphid width	11	10 (9–11)	11
Amphid width/ <i>cbd</i> (%)	72	67 (55–73)	62, 66
Amphid from anterior end	6	6 (5–6)	5, 6
Nerve ring from anterior end	129	122 (118–125)	102, 124
Nerve ring <i>cbd</i>	47	45 (43–47)	42, 47
Excretory pore from anterior end	154	154 (151–157)	139, 158
Pharynx length	262	245 (230–263)	230, 266
Pharyngeal bulb diam.	38	37 (35–39)	40
Pharynx bulb <i>cbd</i>	59	55 (53–56)	58, 62
Max. body diam.	69	63 (61–65)	61, 77
Spicule length	126	126 (121–136)	–
Gubernacular apophyses length	24	27 (24–32)	–
Cloacal/anal body diam.	47	45 (43–46)	41, 44
Tail length	175	165 (154–171)	213, 238
<i>V</i>	–	–	878, 933
% <i>V</i>	–	–	45, 46
Vulva body diam.	–	–	60, 73

forward primer 1813F, 5'-CTGCGTGAGAGGTGAAAT-3' and reverse 2646R, 5'-GCTACCTTGTTACGACTTTT-3', respectively (Holterman et al. 2006). For both SSU and LSU, the 20 μl PCR contained 10 μl Go Tag® Green Master Mix (Promega Corporation, Madison, WI, USA), 1 μl (5 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 35 cycles of denaturation at 94 °C for 15 s, annealing at 53 °C for 30 s, and extension at 72 °C for 45 s. A final extension was performed at 72 °C for 7 min. The amplicons were electrophoresed on 1% TAE-agarose gel stained with SYBR® Safe, observed under UV illumination using the Gel-Doc system (BioRad, Hercules, CA, USA), and images processed using the Quantity One 1-D analysis software (BioRad). The PCR products were diluted three to five times and sequenced bidirectionally using the amplification primers by EcoGene (Auckland, New Zealand). Sequences were obtained with a 3130xl Genetic Analyzer (Applied Biosystems, USA) and assembled and edited with Sequencher 4.8 (Gene Codes Corp.).

Sequence alignment and phylogenetic inference

The ribosomal DNA SSU and LSU D2-D3 sequences were deposited in GenBank (accession numbers MK161428-30 and MK161431-2, respectively). Sequences were compared with those of Comesomatidae species for which sequences were available in GenBank. Both SSU and LSU trees were rooted using sequences from the closely related Axonolaimidae Filipjev, 1918. DNA sequences were aligned using the MUSCLE (Edgar 2004a, b) and clustal alignment algorithms with default parameters, which yielded essentially identical SSU and LSU topologies. Initial SSU analyses could not clarify the placement of the three species from Conway Trough within the Comesomatidae. In an attempt to better define the placement of these species, regions of questionable alignment were removed using Gblocks (Castresana 2000; Talavera and Castresana 2007) with relaxed gap setting (only positions where 50% or more of the sequences have a gap are treated as a gap position) and the resulting fragments were concatenated. After removing sites of questionable alignment, Gblocks gave a 826-bp site alignment from the original SSU

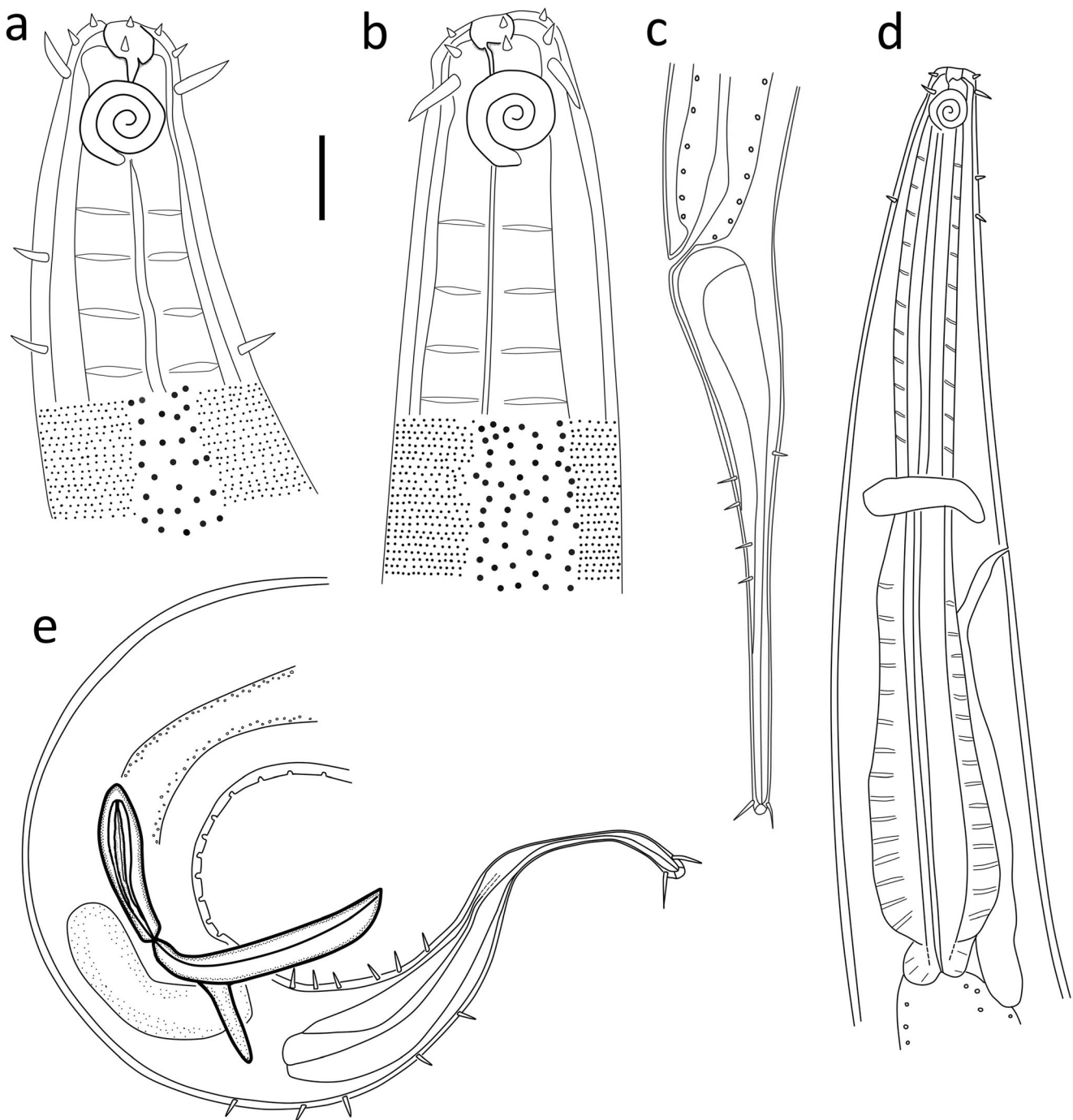


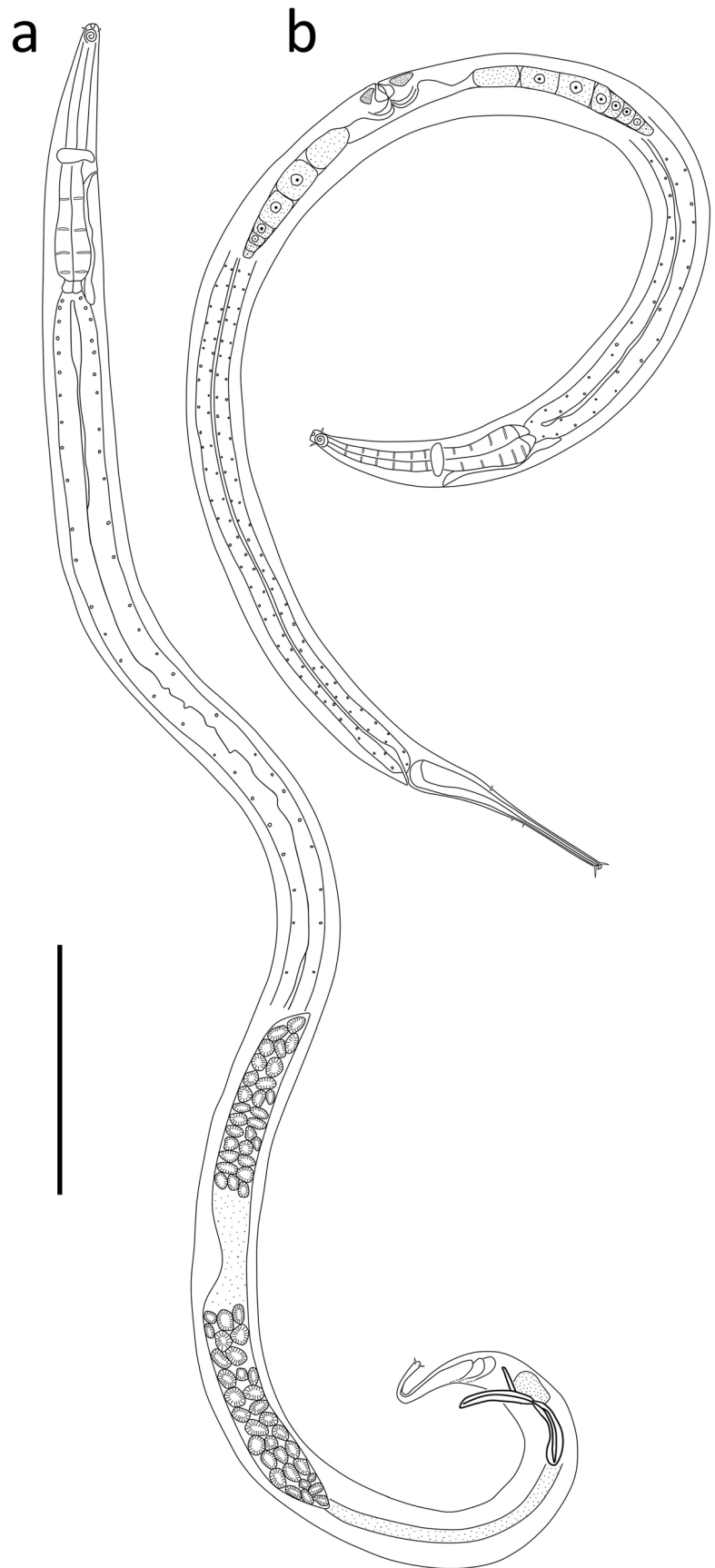
Fig. 1 *Sabatieria articulata* sp. nov. **a** Male anterior body region; **b** female anterior body region; **c** female posterior body region; **d** male pharyngeal body region; **e** male posterior body region. Scale bar: **a** and **b** = 10 μ m, **c** = 30 μ m, **d** and **e** = 24 μ m

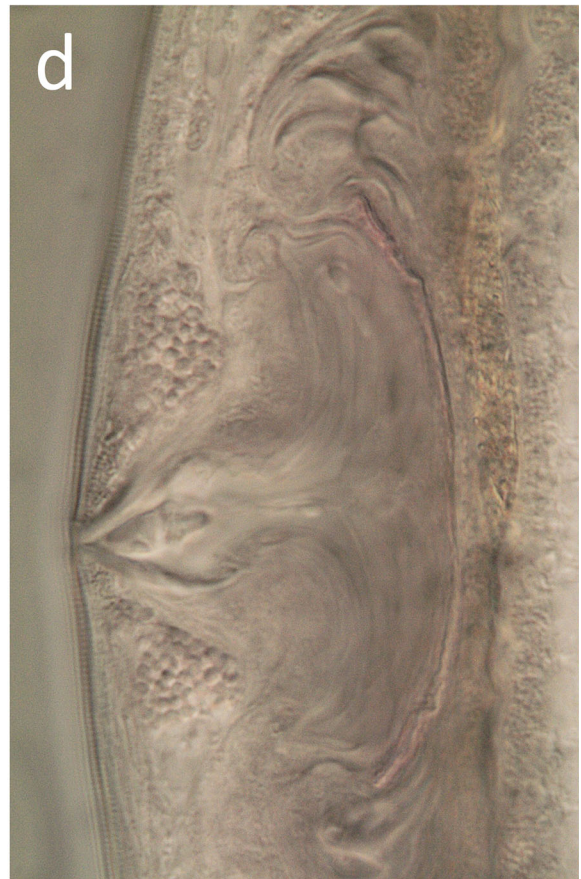
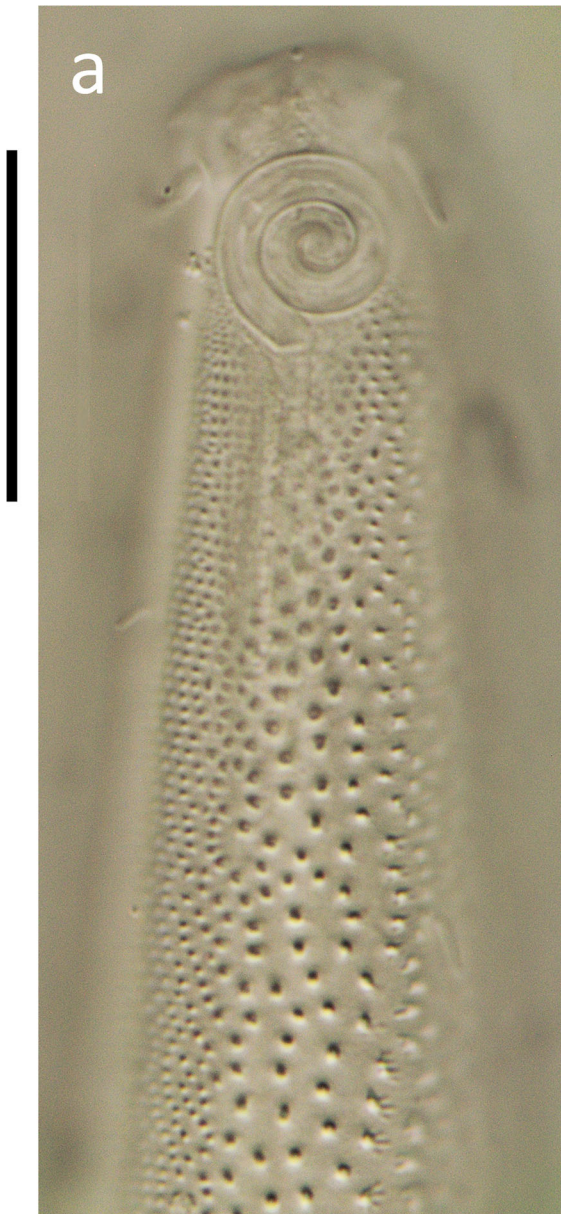
rDNA with 1578-bp sites concatenated alignment. The resulting SSU tree topology, however, was almost identical to SSU trees built without Gblocks.

Phylogenies were built in Geneious 10.2.6 (<http://www.geneious.com>, Kearse et al. 2012). ModelTest 3.04 (Posada and Crandall 1998) in conjunction with PAUP*4.0b10 (Swofford 2002) and jModelTest 2.1.10 software (Darriba et al. 2012) were used to select the best model using the

Akaike Information Criterion. The substitution model [GTR (general time-reversible) + I (proportion of invariable sites) + G (gamma distribution)] was selected as the best-fit model for both SSU (1578 bp) and LSU (761 bp) alignments by ModelTest 3.04 in conjunction with PAUP*4.0b10. The models TIM2 + I + G and GTR + I + G were selected for SSU and LSU by the jModelTest 2.1.10., respectively. Because the model TIM2 + I + G cannot be implemented in Geneious 10.2.

Fig. 2 *Sabatieria articulata* sp. nov. **a** Entire male; **b** entire female. Scale bar = 240 μ m





◀ **Fig. 3** *Sabatieria articulata* sp. nov. Light micrographs. **a** Surface view of female anterior body region showing cuticle punctations, amphids and cephalic sensilla; **b** Optical cross-section of female anterior body region showing the buccal cavity; **c** sperm; **d** vulva. Scale bar: **a** and **b** = 20 μm , **c** = 70 μm , **d** = 37 μm

6, the most similar model GTR + I + G was used instead (Huelsenbeck and Ronquist 2001), which is presumed to have minimal impact on tree topology. The trees were run with a chain length of 1,100,000, and a burn-in length of 100,000. The perimeter files from multiple runs were inspected for chain convergence in Tracer 1.5 (Rambaut and Drummond 2007), and the trees were edited in FigTree v1.4.2 (<http://tree.bio.ed.ac.uk/software/figtree>) and PowerPoint. These analyses were also conducted with PhyML 3.0 using the default settings in Geneious 10.2.6. The substitution model GTR, the NNI (default, fast) topology search, and 1000 bootstrap replicates (Guindon et al. 2010) were selected for building the tree.

Results

Systematics

Family Comesomatidae Filipjev, 1918
 Subfamily Sabatieriinae Filipjev, 1934
 Genus *Sabatieria* Rouville, 1903

Diagnosis (modified from Jensen (1979) and Rosli et al. (2014)) Cuticle usually punctated, sometimes with lateral differentiation of larger or irregular punctations; in rare cases cuticle may appear striated or smooth. Four cephalic setae longer than the six outer labial setae. Anterior buccal cavity globular to cup-shaped, posterior buccal cavity narrow; small teeth-like structure sometimes present at the base of the anterior buccal cavity. Spicules usually short and arcuate; gubernaculum with dorso-caudal or caudal apophyses.

Type species *Sabatieria cettensis* Rouville, 1903

Remarks

The genus was originally described by Rouville (1903). A list of all 69 valid *Sabatieria* species was recently provided by Rosli et al. (2014). The latter authors also described *Sabatieria dispunctata* Rosli, Leduc & Probert, 2014. *Sabatieria megadena* Leduc, 2017 was subsequently described by Leduc (2017).

Sabatieria articulata sp. nov. Fu & Leduc

(Table 1, Figs. 1, 2, 3, 4, and 5)

Material examined Holotype male (NIWA 115471), two paratype males, and one paratype females (NIWA 115472 and 115473) deposited in the NIWA Invertebrate Collection (Wellington). One paratype male and one paratype female (NCCNZ 3319) deposited in the National Nematode

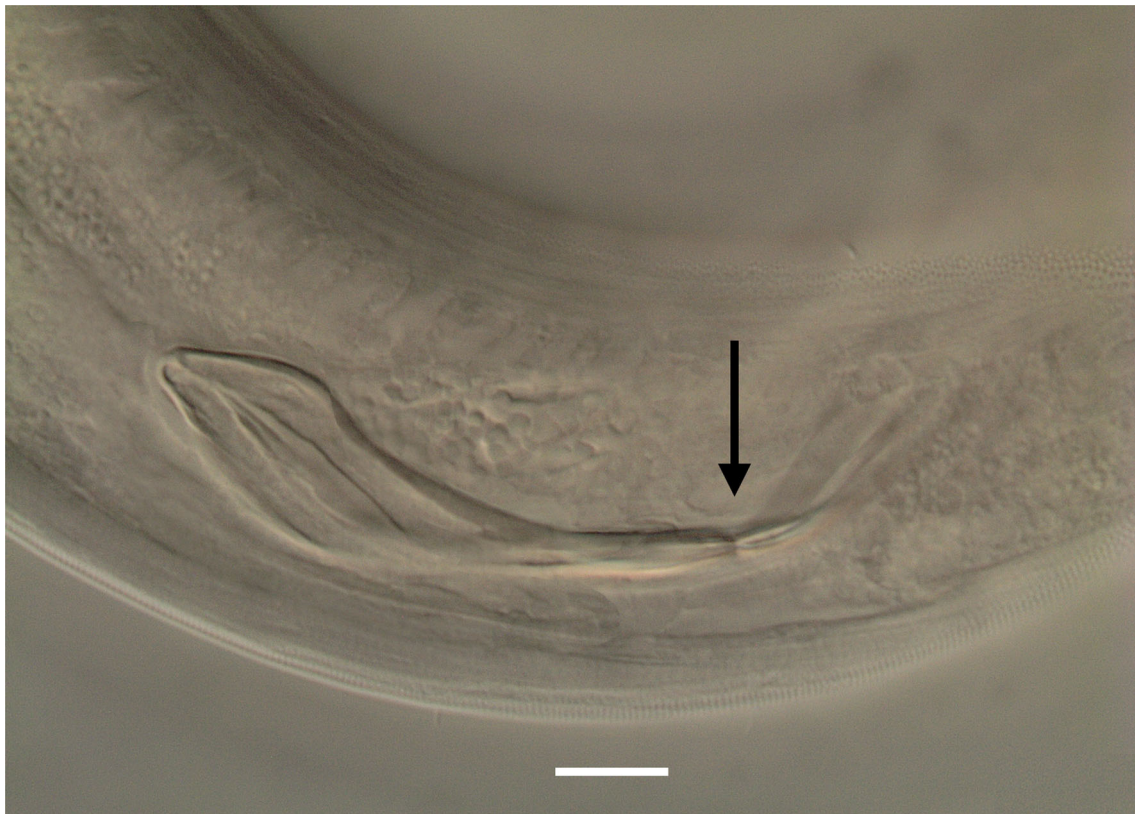


Fig. 4 *Sabatieria articulata* sp. nov. Light micrograph. Posterior half of right spicule showing position of joint (arrow). Scale bar = 10 μm

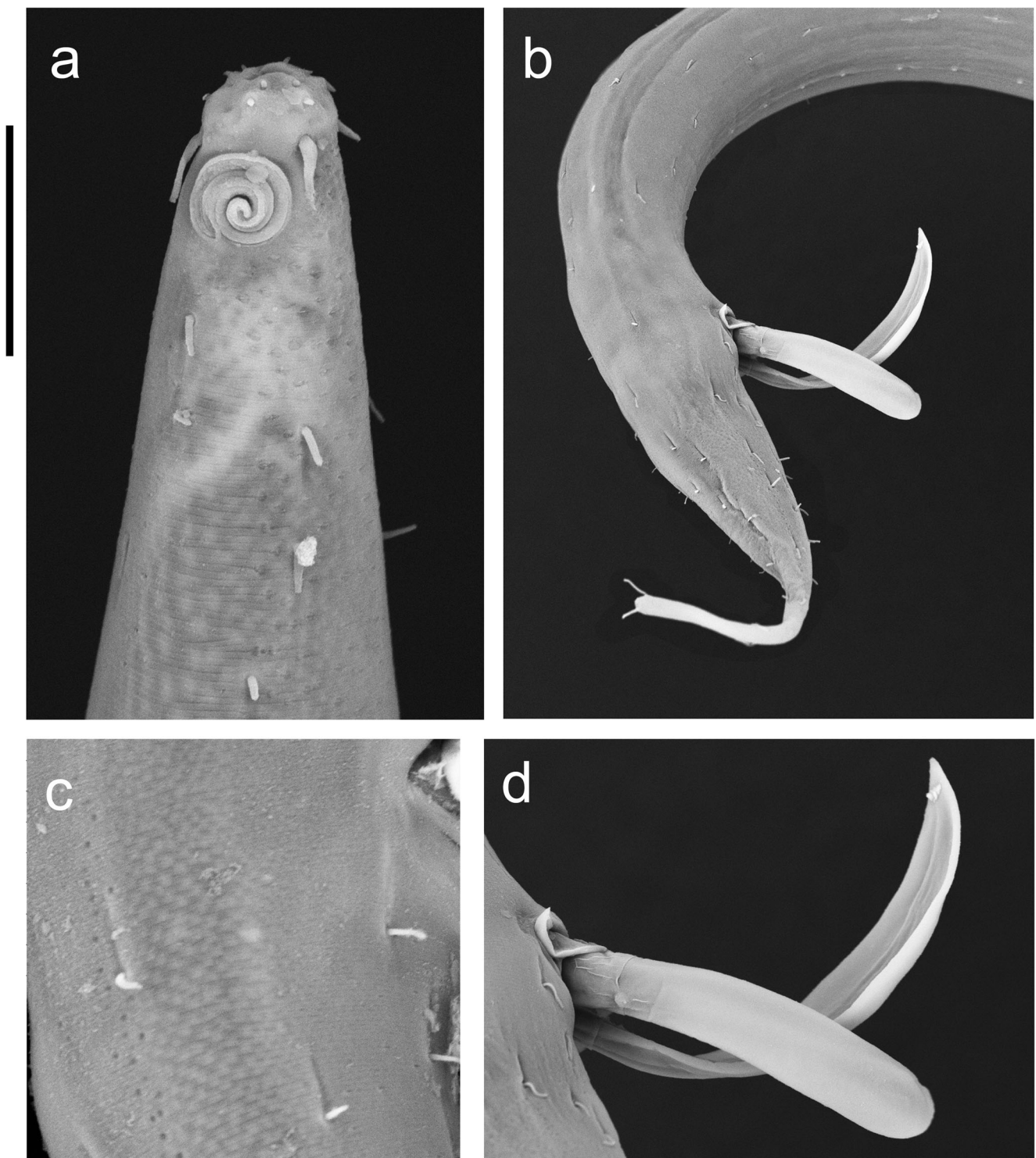


Fig. 5 *Sabatieria articulata* sp. nov. Scanning electron micrographs. **a** male anterior body region; **b** male posterior body region showing protruding spicules; **c** lateral fields at level of cloaca showing cuticle punctations; **d** detail of protruding spicules. Scale bar: **a** and **c** = 15 μ m, **b** = 50 μ m, **d** = 25 μ m

Collection of New Zealand (Auckland). All specimens collected on 11 September 2018.

Type of habitat and locality Subsurface (1–5 cm) muddy sediments, Conway Trough, off east coast of New Zealand's

South Island, at three sampling stations: TAN1708/83 (586 m water depth; 42.6643 °S, 173.5857 °E), TAN1708/80 (570 m water depth; 42.6108 °S, 173.5822 °E), and TAN1708/87 (491 m water depth; 42.7250 °S, 173.6012 °E).

Table 2 Morphometrics (μm , mean (range)) of *Hopperia novazelandica* sp. nov. from Conway Trough

	Male holotype	Male paratype	Female paratypes
<i>n</i>	1	1	6
<i>L</i>	2648	2514	2414 (2146–2599)
<i>a</i>	28	26	26 (23–29)
<i>b</i>	8	8	8 (8–9)
<i>c</i>	16	17	14 (13–15)
<i>c'</i>	2.9	2.6	3.1 (2.8–3.3)
Head diam. at cephalic setae	14	13	15 (15–16)
Length of cephalic setae	3	3	4 (3–5)
Amphid height	13	12	11 (10–11)
Amphid width	11	10	10 (8–11)
Amphid width/ <i>cbd</i> (%)	61	56	49 (40–59)
Amphid from anterior end	6	6	7 (6–8)
Nerve ring from anterior end	150	150	131 (100–159)
Nerve ring <i>cbd</i>	67	66	64 (55–72)
Excretory pore from anterior end	156	155	149 (138–160)
Pharynx length	313	317	292 (276–314)
Pharynx diameter at the base	51	51	47 (40–55)
Pharynx <i>cbd</i>	83	82	82 (69–86)
Max. body diam.	95	95	93 (75–104)
Spicule length	140	129	–
Gubernacular apophyses length	33	26	–
Cloacal/anal body diam.	57	56	56 (52–60)
Tail length	164	145	172 (157–189)
<i>V</i>	–	–	1104 (993–1216)
% <i>V</i>	–	–	46 (44–48)
Vulva body diam.	–	–	86 (66–103)

Etymology The species name is derived from the latin term *articulus* (= joint, knuckle) and refers to the jointed spicules of this species.

Diagnosis

Sabatieria articulata sp. nov. is characterized by body length 1758–2177 μm , cuticle with lateral differentiation of irregularly spaced and conspicuously larger dots, walls of the anterior buccal cavity slightly cuticularized with small teeth-like projections at the base, spiral amphids with three turns, tail 3.6–3.7 cloacal body diameters long in males and 5.2–5.3 anal body diameters long in females. Males with slightly bent and jointed spicules, straight dorso-caudal apophyses, and 10–14 minute, pore-like precloacal supplements, and females with vulva located slightly anterior to mid-body.

Description

Males

Colorless cylindrical body, narrowing gradually towards both extremities. Cuticle with transverse rows of punctuations starting from level of amphid to near tail tip. Lateral differentiation consisting of irregularly spaced, conspicuously larger dots, most conspicuous in pharyngeal and cloacal regions. Cuticle striations visible on cuticle surface using scanning

electron microscopy but not light microscopy. Eight longitudinal rows of sparse somatic setae, 3–4 μm long. Cephalic region with distinct constriction at the level of cephalic setae. Anterior sensilla in three separate circles; six inner labial papillae on lip region, six outer labial papillae, and four cephalic setae, 0.3–0.4 *cbd* long. Subcephalic setae absent. Amphideal fovea and aperture spiral, with three turns, located immediately posterior to cephalic setae. Anterior buccal cavity cup-shaped, 3–4 μm deep, with lightly cuticularized walls and small, teeth-like projections at the base. The pharynx surrounds half of the anterior buccal cavity, broadens gradually posteriorly and forms a weak posterior bulb. Marginal tubes of the pharynx conspicuous, originating from the base of the buccal cavity and extending to the cardia. The cardia short, not surrounded by pharyngeal tissue. Nerve ring near middle of the pharynx. Excretory pore located slightly posterior to nerve ring. Ventral gland prominent, at level of the cardia.

Reproductive system diorchic, with anterior outstretched testis to the left of intestine and posterior outstretched testis to the right of the intestine. Sperm cells globular or irregular, 8–13 $\mu\text{m} \times 13$ –16 μm . Spicules slightly bent, 2.6–3.1 cloacal body diameters long, with joint present at 45% of spicules

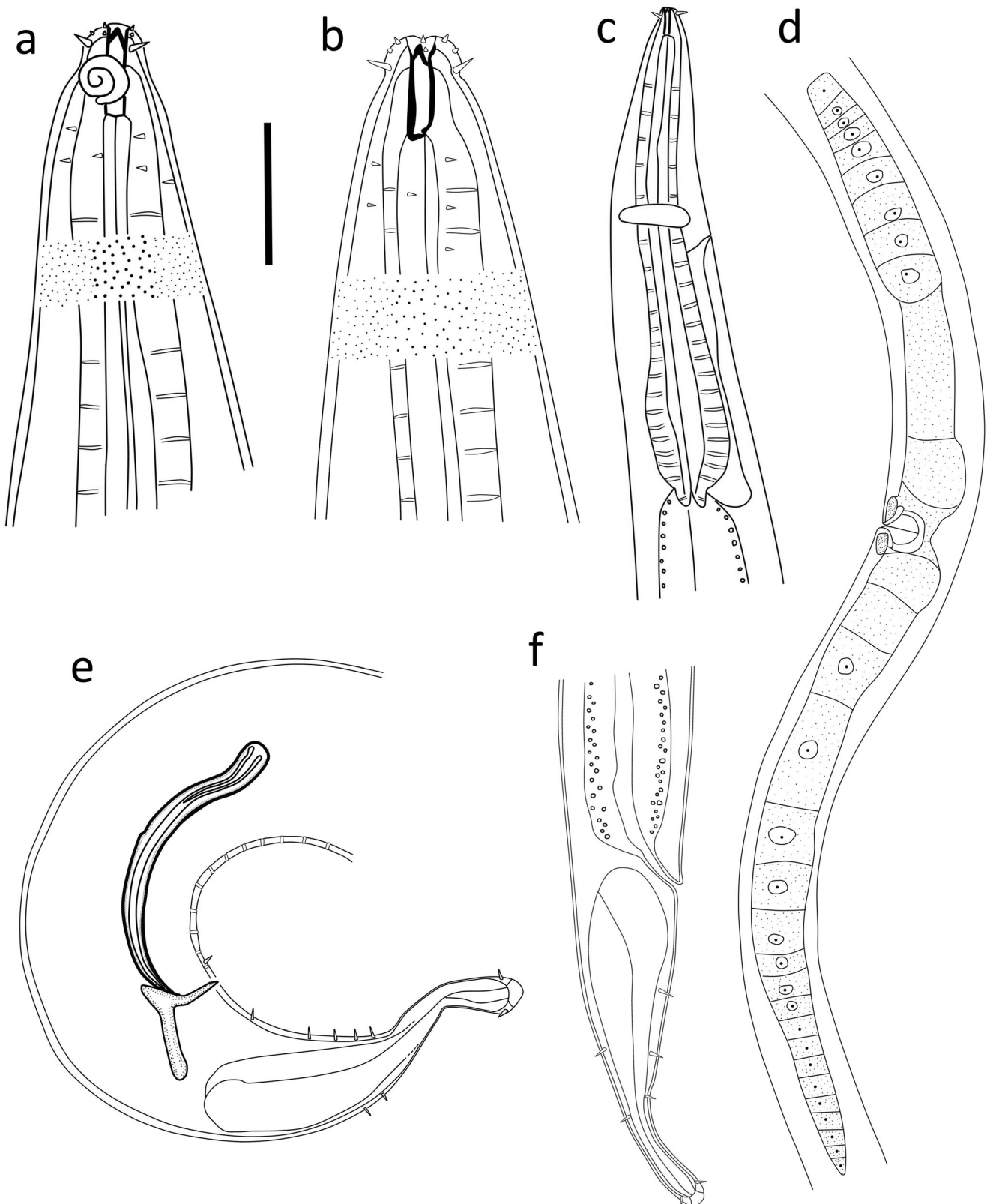


Fig. 6 *Hopperia novazelandica* sp. nov. **a** Male anterior body region; **b** female anterior body region; **c** male pharyngeal region; **d** female reproductive system; **e** male posterior body region; **f** female posterior body region. Scale bar: **a** and **b** = 30 μ m, **c** = 85 μ m, **d** = 140 μ m, **e** = 50 μ m, **f** = 75 μ m

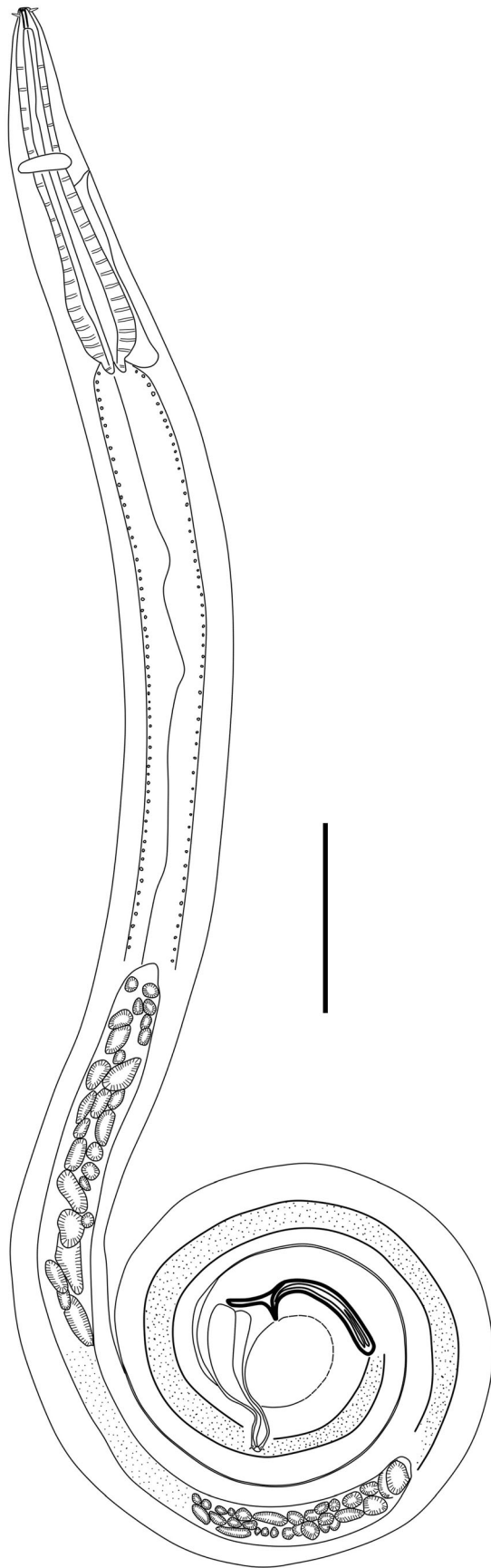


Fig. 7 *Hopperia novazelandica* sp. nov. Entire male. Scale bar = 150 μ m

length from the proximal end; central lamella present in proximal half of spicule. Gubernaculum with straight dorso-caudal apophyses. Ten to 14 minute, pore-shaped precloacal supplements; seven posterior-most supplements evenly spaced, then situated gradually further apart anteriorly. Precloacal seta not observed. Tail conico-cylindrical, with cylindrical part about one third of the total tail length; two subdorsal, two subventral, and four sublateral rows of short caudal setae, 4–6 μ m long, and three longer terminal setae, 6–8 μ m long.

Females

Similar to males, but with slightly longer tail. Reproductive system didelphic, with anterior ovary to the left of intestine and posterior ovary to the right of intestine. Spermatheca not observed. Vulva slightly anterior to mid-body. Granular vaginal glands present, proximal portion of *vagina uterina* surrounded by constrictor muscle.

Remarks

Sabatieria articulata sp. nov. belongs to the praedatrix group, which is characterized by simple tubular or pore-like precloacal supplements, straight gubernacular apophyses, cuticle usually with lateral differentiation of larger, more widely-spaced dots, and amphids usually with three turns (Platt 1985). The new species differs from all other species in this group, as well as all other species from the genus, in having jointed spicules. *Sabatieria lyonessa* Warwick, 1977 is the only other species of the genus with spicules of similar length to *S. articulata* sp. nov. (117 μ m and 2.1 cloacal body diameters long versus 121–136 μ m and 2.6–3.1 cloacal body diameters long in *S. articulata* sp. nov.) (Warwick 1977). *Sabatieria articulata* sp. nov. is also similar to *S. bubulba* Leduc, 2013 in having lateral differentiation of conspicuously larger dots and similar number of pore-like precloacal supplements (8–11 versus 10–14 in *S. articulata* sp. nov.). *S. articulata* sp. nov. differs from *S. bubulba* by lower values of a (38–59 versus 27–34 in *S. articulata* sp. nov.), much longer spicules (41–44 μ m versus 121–136 μ m in *S. articulata* sp. nov.), and in lacking a conspicuous, elongated posterior pharyngeal bulb (Leduc 2013).

Subfamily Dorylaimopsinae De Coninck, 1965.

Genus *Hopperia* Vitiello, 1969

Diagnosis (from Jensen (1979) and Leduc (2012)) Cuticle with lateral differentiation of larger dots, sometimes arranged irregularly or in transverse rows; posterior portion of the buccal cavity cylindrical, with three thorn-like teeth at border to anterior portion (except *H. hexadentata* Hope & Zhang, 1995 which has six teeth); ovaries usually outstretched, reflexed ovaries sometimes present; spicules arcuate; gubernaculum with caudal or dorso-caudal apophyses.

Type species *Hopperia massiliensis* Vitiello, 1969.

Remarks

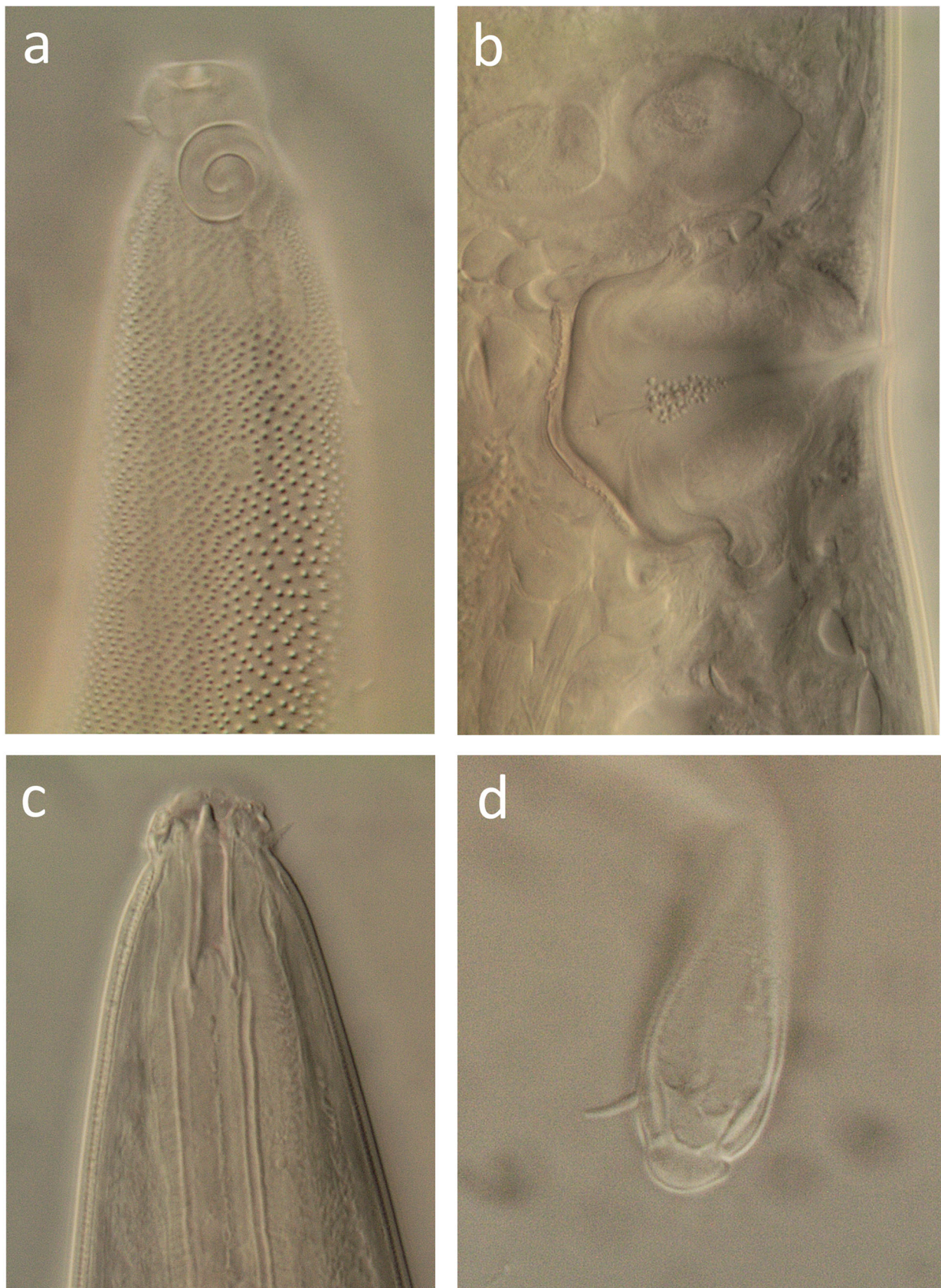


Fig. 8 *Hopperia novazelandica* sp. nov. Light micrographs. **a** Surface view of male anterior body region; **b** vulva; **c** optical cross-section of male anterior body region; **d** male spinneret. Scale bar: **a** and **c** = 25 μm , **b** = 28 μm , **d** = 18 μm

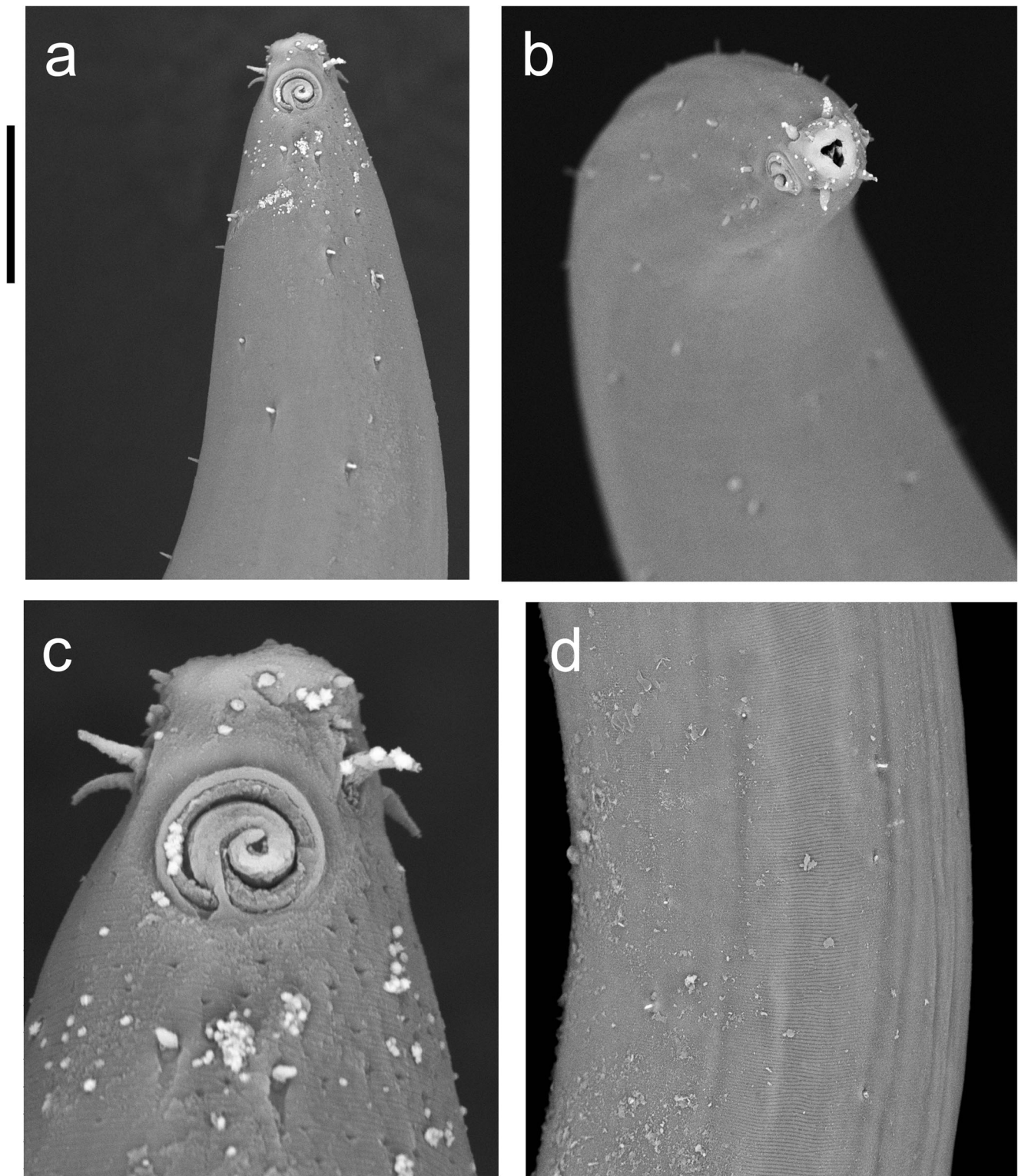


Fig. 9 *Hopperia novazelandica* sp. nov. Scanning electron micrographs. **a** Lateral view of male anterior body region; **b** male anterior body region showing mouth opening; **c** lateral view of female cephalic region; **d** mid-

body region of male showing cuticle striations. Scale bar: **a** and **b** = 20 μm , **c** = 6 μm , **d** = 22 μm

The genus was originally described by Vitiello (1969). A pictorial key to all valid species of the genus was recently provided by Guo et al. (2015); the key however provides the wrong

representation of *H. massiliensis*, which appears to have mistakenly been taken from drawings of the original description of *H. muscatensis* Warwick, 1973.

Table 3 Morphometrics (μm) of *Vasostoma hexodontium* Rosli, Leduc & Probert, 2014 from Conway Trough

	Male	Females
<i>n</i>	1	2
<i>L</i>	2318	2014, 2235
<i>a</i>	31	30, 32
<i>b</i>	7	6, 7
<i>c</i>	10	10
<i>c'</i>	3.7	4.5
Head diam. at cephalic setae	14	14
Length of cephalic setae	4	5
Amphid height	11	10, 12
Amphid width	10	8, 10
Amphid width/ <i>cbd</i> (%)	62	48, 67
Amphid from anterior end	7	6
Nerve ring from anterior end	154	122, 162
Nerve ring <i>cbd</i>	57	48, 52
Excretory pore from anterior end	171	184, 185
Pharynx length	337	309, 350
Pharyngeal diam. at base	37	34, 36
Pharynx <i>cbd</i>	72	53, 69
Max. body diam.	74	64, 74
Spicule length	98	–
Gubernacular apophyses length	41	–
Cloacal/anal body diam.	62	46–49
Tail length	226	207, 221
<i>V</i>	–	946, 1014
% <i>V</i>	–	45, 47
Vulva body diam.	–	66, 77

Hopperia novazelandica sp. nov. Fu & Leduc.

(Table 2, Figs. 6, 7, 8, and 9)

Material examined Holotype male (NIWA 115474), one paratype male and three paratype females (NIWA 115475) deposited in the NIWA Invertebrate Collection (Wellington). One paratype female (NNCNZ 3320) deposited in the National Nematode Collection of New Zealand (Auckland). All specimens collected on 11 September 2018.

Type habitat and locality Subsurface (1–5 cm) muddy sediments, 586 m water depth, Conway Trough, off east coast of New Zealand's South Island (42.6643 °S, 173.5857 °E).

Etymology The species name is derived from the type locality.

Diagnosis

Hopperia novazelandica sp. nov. is characterized by relatively stout body 2146–2648 μm long, inner labial papillae slightly larger than the outer labial papillae, cephalic setae

0.2–0.3 *cbd* long, amphideal fovea with 3.0 turns in males and 2.25–2.5 turns in females, the buccal cavity with three teeth, spicules 129–140 μm (2.3–2.5 cloacal body diameters) long with central cuticular projection extending one third of spicule length from proximal end, precloacal seta and 19 precloacal supplements present, and conico-cylindrical tail with swollen tip and three terminal setae.

Description

Males

Body cylindrical, relatively stout, narrowing slightly towards anterior region. Cuticle with lateral rows of punctations from level of amphid to near tail tip; lateral differentiation consisting of larger, irregularly distributed dots from about 10 μm posterior to amphids. Cuticle striations visible on cuticle surface using scanning electron microscopy but not light microscopy. Somatic setae scarce, irregularly spaced, 2 μm long. Cephalic region set off by a constriction at level of cephalic setae. Anterior sensilla arranged in three circles; six inner labial papillae, six slightly shorter outer labial papillae, four cephalic setae, 0.2–0.3 *cbd* long. Amphideal fovea spiral, with 3.0 turns, located just posterior to cephalic setae. Anterior portion of the buccal cavity cup-shaped, with slightly cuticularized walls; posterior portion of the buccal cavity cylindrical, strongly cuticularized, ca. 20 μm deep, 3–5 μm wide. Three strongly cuticularized teeth situated at junction between the two portions of the buccal cavity; half to two thirds of the buccal cavity surrounded by pharyngeal tissue. The pharynx widens gradually posteriorly without forming a true bulb. Marginal tubes visible, extending from the base of the posterior buccal cavity to the cardia. The cardia small, surrounded by intestinal tissue. Nerve ring near middle of the pharynx. Secretory-excretory pore located slightly posterior to nerve ring; ventral gland situated at level of the cardia.

Reproductive system diorchic, with anterior outstretched testis to the left of intestine and posterior outstretched testis to the right of intestine. Sperm cells globular or oval shaped, 9–20 μm \times 6–18 μm . Spicules paired, equal, 2.3–2.5 cloacal body diameters long, curved, strongly cuticularized, slightly swollen proximally, with conspicuous central cuticular projection (lamella) extending one third of spicule length from proximal end. Gubernaculum weakly cuticularized with dorsal-caudally directed apophyses. One precloacal seta present, 2 μm long; approximately 19 fine tubular precloacal supplements present. Tail conico-cylindrical with distinctly swollen tip and three terminal setae, 3–5 μm long. Three caudal glands and spinneret present.

Females

Similar to males, but with slightly smaller ratio of *c* and slightly smaller amphids with fewer (2.2–2.5) turns. Reproductive system didelphic, with anterior ovary to the left of intestine and posterior ovary to the right of intestine. Spermatheca not observed. Vulva slightly anterior to mid-

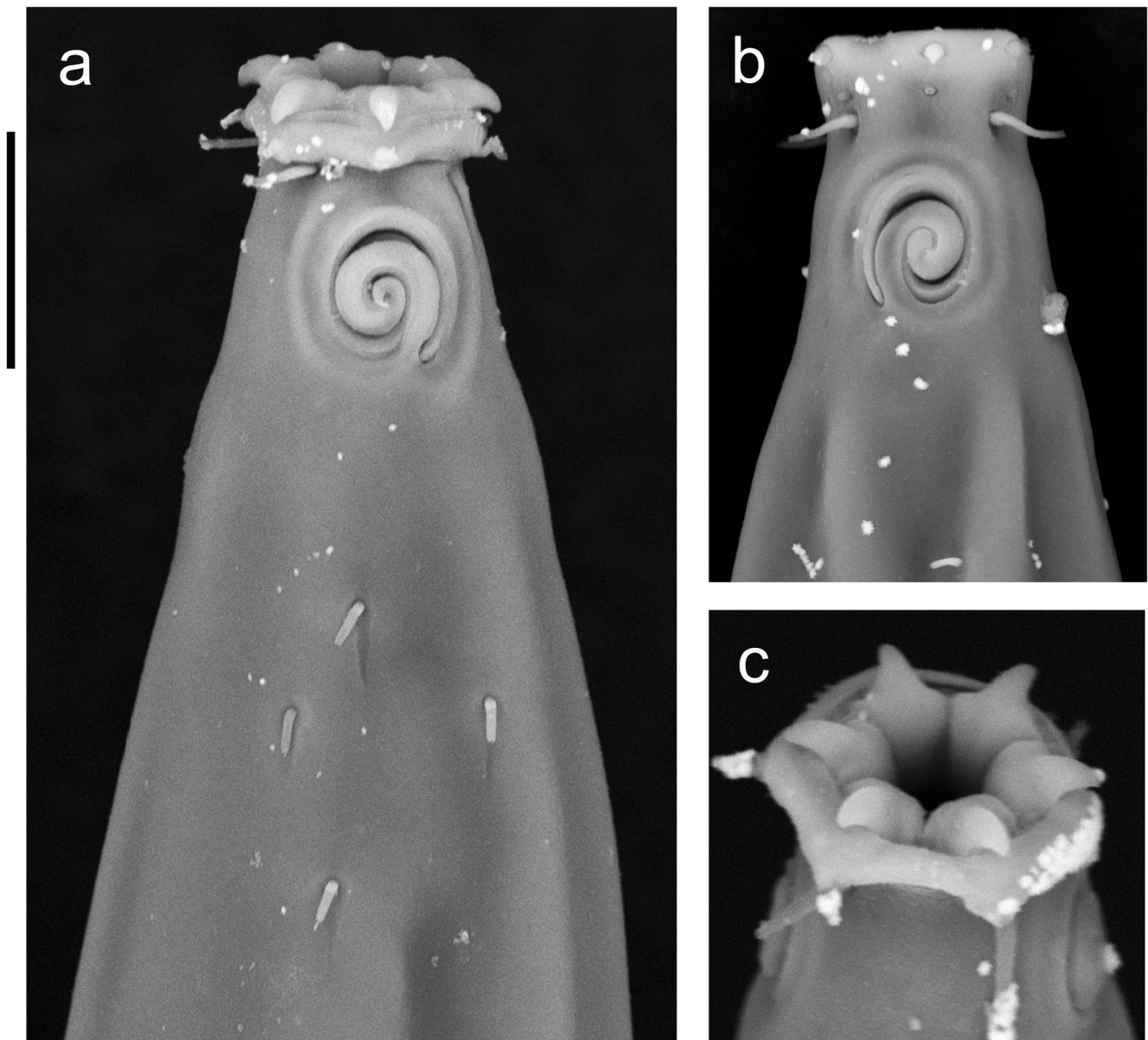


Fig. 10 *Vasostoma hexodontium* Rosli, Leduc & Probert, 2014. Scanning electron micrographs. **a** Anterior body region of male; **b** female cephalic region; **c** mouth opening of female showing protruding teeth. Scale bar: **a** and **b** = 10 μm , **c** = 6 μm

body. Granular vaginal glands present, proximal portion of *vagina uterina* surrounded by constrictor muscle.

Remarks

Hopperia novazelandica sp. nov. resembles *H. muscatensis* Warwick, 1973 and *H. hexadentata* Hope & Zhang, 1995 in having relatively long spicules (≥ 2.0 cloacal body diameters long) and a conico-cylindrical tail. The new species differs from *H. muscatensis* in having a lower ratio of a (31–44 versus 23–29 in *H. novazelandica* sp. nov.), a shorter tail (3.9–4.0 versus 2.6–3.3 anal/cloacal body diameters in *H. novazelandica* sp. nov.) with conspicuously swollen tip (versus tail tip not swollen in *H. muscatensis*), a longer pharynx (215–260 μm versus 276–317 μm in *H. novazelandica* sp. nov.), and longer spicules (87 μm versus 129–140 μm in *H. novazelandica* sp. nov.)

(Warwick 1973). *Hopperia novazelandica* sp. nov. can be most easily differentiated from *H. hexadentata* by the number of teeth in the buccal cavity (six versus three teeth in *H. novazelandica* sp. nov.) (Hope and Zhang 1995).

Genus *Vasostoma* Wieser, 1954

Diagnosis (from Wieser (1954) and Rosli et al. (2014)) Cuticle punctated, without lateral differentiation. Anterior sensilla in three distinct circles. Posterior portion of the buccal cavity cylindrical to conical, provided with three or six triangular teeth at border with anterior portion. Spicules curved or arcuate. Gubernaculum apophyses usually directed caudally, occasionally dorso-caudally. Supplements sometimes present.

Type species *Vasostoma spiratum* Wieser, 1954

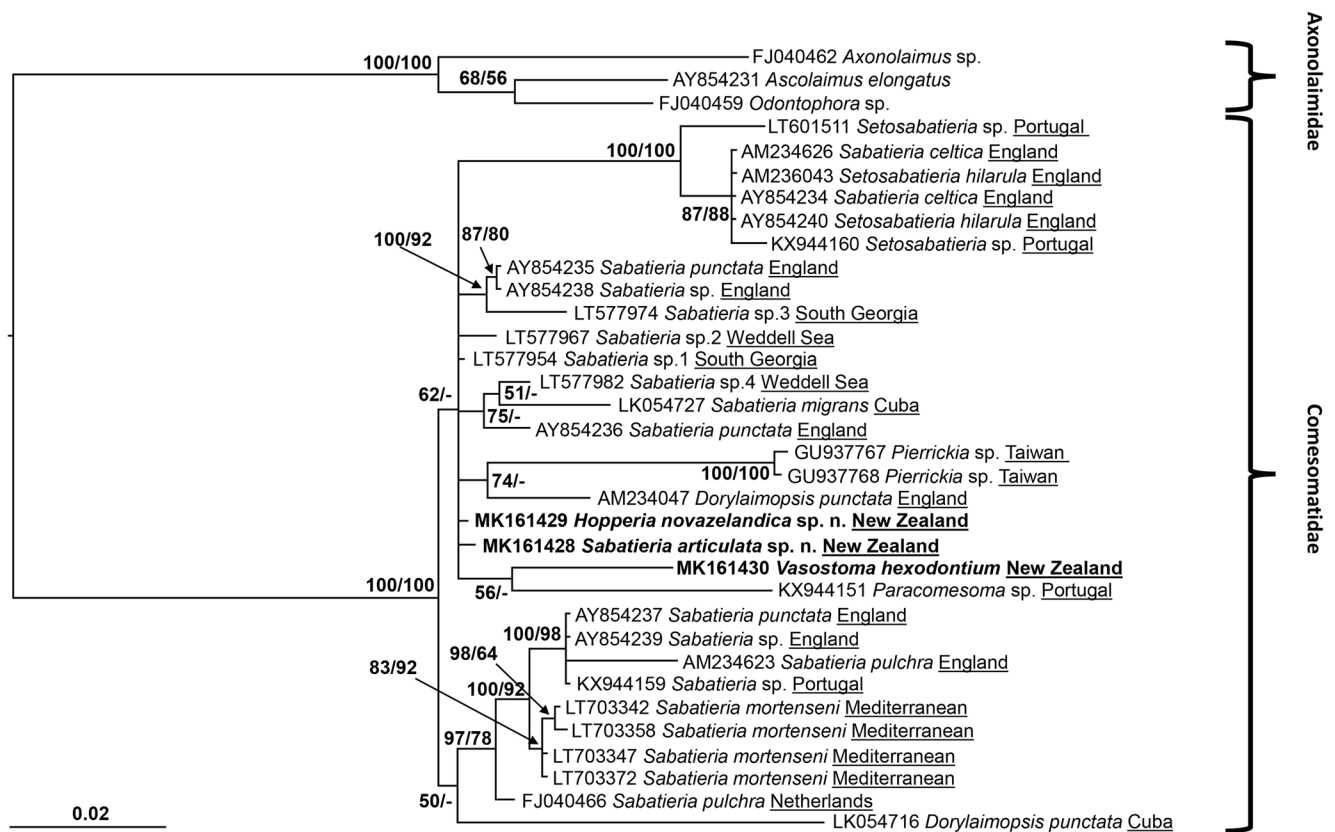


Fig. 11 Bayesian tree inferred from SSU sequences of the family Comesomatidae and Axonolaimidae (outgroup), aligned using the Clustal alignment algorithm under the general time-reversible (GTR)+gamma distribution (G) model. Posterior probabilities (left) and bootstrap

values (right) from the ML analyses greater than or equal to 50% are given on appropriate clades. Dashes (–) indicate less than 50% support. Localities of each sequence are underlined. The scale stands for substitutions per site

Vasostoma hexodontium Rosli, Leduc & Probert, 2014
(Table 3, Fig. 10)

Material examined One male and two females (NIWA 115476), collected on 11 September 2018. Subsurface (1–5 cm) muddy sediments, 586 m water depth, Conway Trough, off east coast of New Zealand’s South Island (42.6643 °S, 173.5857 °E).

Description

Males

Body cylindrical, narrowing gradually towards both extremities. Cuticle with transverse rows of punctations without lateral differentiation; surface of cuticle smooth when viewed using scanning electron microscopy. Somatic setae scattered. Six inner labial papillae, six slightly smaller outer labial papillae, and four cephalic setae, 0.3–0.4 cbd long. Cephalic region with slight constriction at level of cephalic setae. The buccal cavity ca. 30 µm deep, with cup-shaped anterior portion and heavily cuticularized conical posterior portion; six strongly cuticularized triangular teeth at junction between the two portions of the buccal cavity. Amphideal fovea spiral with 3.0 turns. The pharynx surrounds posterior half of the buccal cavity; junction of the pharynx and the buccal cavity wall sometimes visible as distinct line surrounding the buccal

cavity. The pharynx muscular, gradually widening posteriorly but not forming true bulb. The cardia small. Nerve ring near the middle of the pharynx. Excretory pore slightly posterior to nerve ring.

Male reproductive system diorchic with anterior outstretched testis to the left of intestine and posterior outstretched testis to the right of the intestine. Spermatozoa oval shaped, 7–19 µm × 6–9 µm. Three pairs of ejaculatory glands on either side of vas deferens; rectal gland present. Spicules paired, equal, arcuate, 1.6 cloacal body diameters long, strongly cuticularized. Gubernaculum strongly cuticularized with straight dorso-caudal apophyses. Fourteen tubular precloacal supplements. Three cloacal glands present; conico-cylindrical tail with three caudal glands and three terminal setae, 4 µm long.

Females

Similar to male. Reproductive system didelphic, with anterior ovary to the left or right of intestine and posterior ovary to the right of intestine. Spermatheca present. Vulva slightly anterior to mid-body. Granular vaginal glands present, proximal portion of *vagina uterina* surrounded by constrictor muscle.

Remarks

Our specimens are in good agreement with the original description of *V. hexodontium* by Rosli et al. (2014)

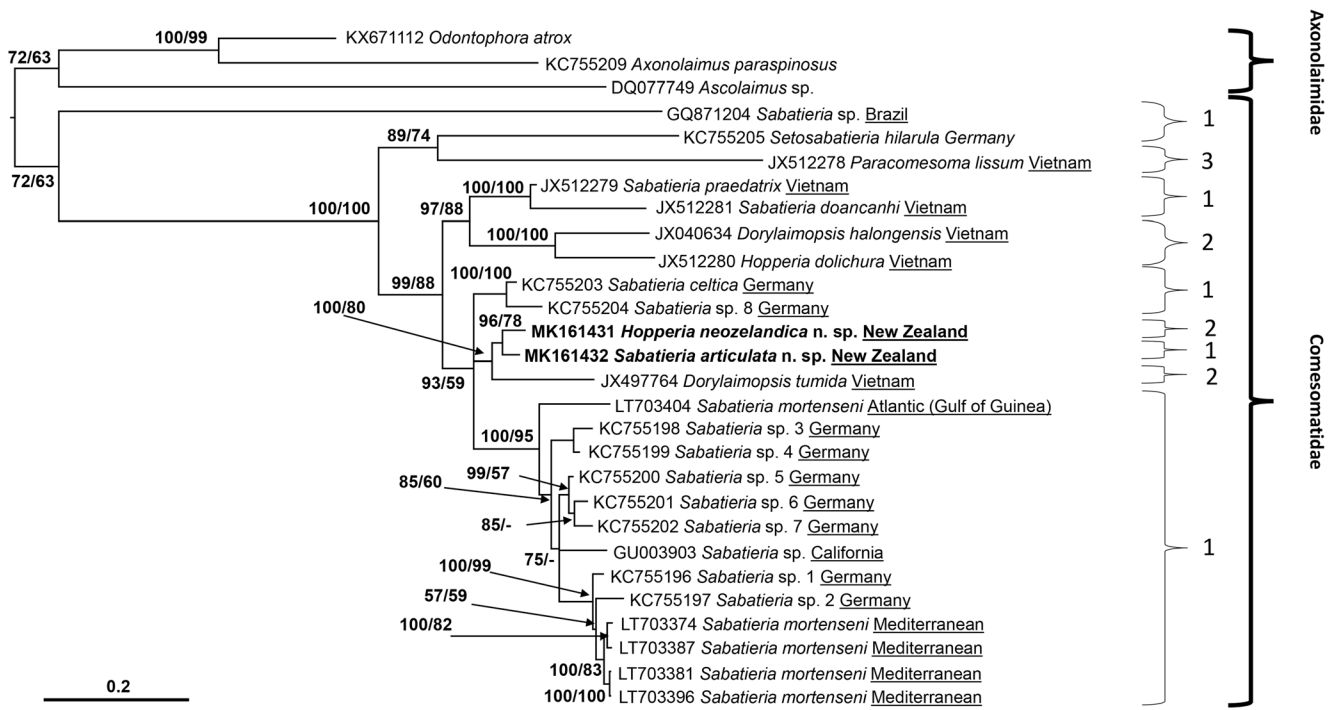


Fig. 12. Bayesian tree inferred from D2-D3 of LSU sequences of the family Comesomatidae and Axonolaimidae (outgroup), aligned using the Clustal alignment algorithm under the general time-reversible (GTR)+gamma distribution (G) model. Posterior probabilities (left) and bootstrap values (right) from the ML analyses greater than or equal to

50% are given on appropriate clades. Dashes (–) indicate less than 50% support. The locality of each Comesomatidae sequence is underlined. Comesomatid subfamilies are identified by the numbers 1 (Sabatieriinae), 2 (Dorylaimopsinae), and 3 (Comesomatinae) on the right-hand side of the tree. The scale stands for substitutions per site.

based on specimens from a submarine canyon on the Southern Hikurangi Margin, New Zealand. The main difference is that we did not observe a cuticularized pointed structure at the base of the cuneus. Our scanning electron microscopy observations show that the outer cuticle surface of this species is smooth, and that the inner labial papillae are slightly larger than the outer labial papillae.

Molecular phylogenetic relationships

The Comesomatidae formed a well-supported monophyletic group in the SSU consensus tree (100% posterior probability and bootstrap support; Fig. 11). One well-supported clade comprised a number of *Sabatieria* and *Setosabatieria* Platt, 1985 species from England and Portugal (100% posterior probability and bootstrap support), indicating that these two genera are not monophyletic. A moderately to well-supported clade comprised *Sabatieria* species from England, Portugal, the Netherlands, and the Mediterranean (97% posterior probability and 78% bootstrap support). Relationships among the remaining species and genera, including the species from Conway Trough, could not be resolved.

It was not possible to obtain a D2-D3 of LSU sequence for *Vasostoma hexodontium*; therefore, analyses

only included *Sabatieria articulata* sp. nov. and *Hopperia novazelandica* sp. nov. The Comesomatidae sequences formed a monophyletic clade with moderate support (72% posterior probability and 63% bootstrap support) in the consensus tree (Fig. 12). None of the three comesomatid subfamilies (Comesomatinae, Dorylaimopsinae and Sabatieriinae) and none of the genera represented by more than one sequence (i.e., *Sabatieria*, *Hopperia*, and *Dorylaimopsis* Ditlevsen, 1918) were monophyletic. *Sabatieria articulata* sp. nov. and *Hopperia novazelandica* sp. nov. sequences were grouped together (96% posterior probability and 78% bootstrap support) and formed a moderately to well-supported clade with *Dorylaimopsis tumida* Gagarin & Nguyen Vu Thanh, 2006a (100% posterior probability and 80% bootstrap support). There was a tendency for species and genera from the same geographical location to be grouped together, such as *Sabatieria praedatrix* de Man, 1907, *Sabatieria doancanhi* Nguyen Dinh Tu, Nguyen Vu Thanh, Smol & Vanreusel, 2008, *Dorylaimopsis halongensis* Nguyen Dinh Tu, Nguyen Vu Thanh, Smol & Vanreusel, 2008 and *Hopperia dolichura* Gagarin & Nguyen Vu Thanh, 2006b from the coast of Vietnam, and *S. articulata* sp. nov. and *H. novazelandica* sp. nov. from New Zealand.

Discussion

Sabatieria articulata sp. nov. is the only species of the genus with jointed spicules. Within the family, similar spicules are also found in some *Dorylaimopsis* species (Fonseca and Bezerra 2014). In the latter genus, spicule shape is highly variable and can be arcuate, sinusoid, slightly curved, or jointed. The occurrence of variable spicule shape in both *Dorylaimopsis* and *Sabatieria* indicates that this character is not informative for differentiating among genera of the family Comesomatidae.

Comesomatid species and genera from the same geographical location tended to be grouped together in our D2D3 of LSU phylogenetic analyses. The latter also suggested that the genera *Sabatieria*, *Dorylaimopsis*, and *Hopperia* are not monophyletic. Jensen (1979) hypothesized that *Sabatieria* (Sabatiieriinae) is the base group from which other comesomatids evolved. This hypothesis is consistent with the fact that *Sabatieria* is by far the most species-rich genus of the family (> 70 species), and is supported by the topology of the D2-D3 of LSU tree where a *Sabatieria* sequence is the most divergent sequence of the family. These findings suggest that features that characterize the genera *Hopperia* and *Dorylaimopsis*, such as a the buccal cavity with teeth and an expanded posterior portion, have evolved multiple times independently, possibly from a *Sabatieria*-like ancestor. This hypothesis, however, will have to be tested using more comprehensive molecular datasets.

Phylogenetic analyses of SSU sequences could not resolve relationships among most of the comesomatid taxa, except for sequences of the *Sabatieria mortenseni* species complex from the Mediterranean and the Atlantic and other closely related species (De Groote et al. 2017). In addition, *Sabatieria punctata* sequences were located in different parts of the SSU tree, indicating the presence of unsuspected cryptic species or that the specimens were misidentified. Overall, our analyses indicate that SSU sequences cannot resolve relationships among genera and subfamilies of the family Comesomatidae. Phylum-wide phylogenetic analyses based on the small subunit 18S rDNA gene also tend to result in a poor separation between the Araeolaimida and Monhysterida (e.g., Meldal et al. 2007; Holterman et al. 2008), indicating a limited ability of SSU sequences to resolve deep relationships within and between these two orders.

Acknowledgments We thank the crew and scientific personnel of *RV Tangaroa* (voyage TAN1708) and three anonymous reviewers for providing constructive criticisms on the manuscript.

Funding This research was funded by NIWA under Coasts and Oceans Research programme 2 (2018/19 SCI) and supported by core funding for Crown Research Institutes from the Science and Innovation Group of the Ministry of Business, Innovation and Employment. This study was partially supported by the National Key R&D Program of China (Grant No.

2016YFC0502904) and a scholarship under the Graduate School of Xiamen University award to Sujing Fu.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All applicable international, national, and/or institutional guidelines for animal testing, animal care and use of animals were followed by the authors.

Sampling and field studies All necessary permits for sampling and observational field studies have been obtained by the authors from the competent authorities and are mentioned in the acknowledgements, if applicable. The study is compliant with CBD and Nagoya protocols.

Data availability statement All data sources on which this manuscript is based are either provided in the manuscript or available in GenBank.

References

- Carter L, Carter RM, Griggs GB (1982) Sedimentation in the Conway Trough, a deep-near-shore marine basin at the junction of the Alpine transform and Hikurangi subduction plate boundary, New Zealand. *Sedimentology* 29:475–497. <https://doi.org/10.1002/9781444304473.ch40>
- Castresana J (2000) Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Mol Biol Evol* 17:540–552. <https://doi.org/10.1093/oxfordjournals.molbev.a026334>
- Coomans A (1978) A proposal for a more precise terminology of the body regions in the nematode. *Ann Soc Roy Zool Bel* 108:115–117
- Darriba D, Taboada GL, Doallo R, Posada D (2012) jModelTest 2: more models, new heuristics and parallel computing. *Nat Methods* 9:772. <https://doi.org/10.1038/nmeth.2109>
- De Coninck LA (1965) Systématique des Nématodes. In: Grassé PP (Ed.), *Traité de Zoologie: Anatomie, Systématique, Biologie. Nématothelminthes (Nematodes)*. Paris: Masson et Cie
- De Groote A, Hauquier F, Vanreusel A, Derycke S (2017) Population genetic structure in *Sabatieria* (Nematoda) reveals intermediary gene flow and admixture between distant cold seeps from the Mediterranean Sea. *BMC Evol Biol* 17:154. <https://doi.org/10.1186/s12862-017-1003-2>
- De Leo FC, Smith CR, Rowden AA, Bowden DA, Clark MR (2010) Submarine canyons: hotspots of benthic biomass and productivity in the deep sea. *Proc R Soc B* 1695:2783–2792. <https://doi.org/10.1098/rspb.2010.0462>
- Ditlevsen H (1918) Marine freeliving nematodes from Danish waters. *Vidensk Meddr dansk naturh Foren* 70:147–214
- Edgar RC (2004a) MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res* 32:1792–1797. <https://doi.org/10.1093/nar/gkh340>
- Edgar RC (2004b) MUSCLE: a multiple sequence alignment method with reduced time and space complexity. *BMC Bioinformatics* 113. <https://doi.org/10.1186/1471-2105-5-113>
- Filipjev IN (1918) Free-living marine nematodes of the Sevastopol area. *Transactions of the Zoological Laboratory and the Sevastopol Biological Station of the Russian Academy of Sciences* 2:1–203
- Filipjev IN (1934) The classification of the free-living nematodes and their relation to the parasitic nematodes. *Smithson Misc Coll* 89:1–63

- Fonseca G, Bezerra TN (2014) Order Araeolaimida De Coninck & Schuurmans Stekhoven, 1933. In: A. S.-R. Editor (Ed.), Handbook of Zoology Gastrotricha, Cycloneuralia and Gnathifera. Volume 2: Nematoda. Hamburg: De Gruyter
- Gagarin VG, Thanh NV (2006a) Three new species of free-living nematodes of the family Comesomatidae from the delta of the Mekong River, Vietnam (Nematoda, Monhysterida). *Zoost Rossica* 15:221–228
- Gagarin VG, Thanh NV (2006b) Three new species of the genus *Hopperia* (Nematoda, Comesomatidae) from mangroves of the Mekong River Delta (Vietnam). *Zool Zhurnal* 85:18–27
- Guindon S, Dufayard JF, Lefort V, Anisimova M, Hordijk W, Gascuel O (2010) New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. *Syst Biol* 59:307–321. <https://doi.org/10.1093/sysbio/syq010>
- Guo Y, Chang Y, Chen Y, Li Y, Liu A (2015) Description of a marine nematode *Hopperia sinensis* sp. nov. (Comesomatidae) from mangrove forests of Quanzhou, China, with a pictorial key to *Hopperia* species. *J Ocean U China* 14:1111–1115. <https://doi.org/10.1007/s11802-015-2742-6>
- Holterman M, Van Der Wurff A, Van Den Elsen S, Van Megen H, Bongers T, Holovachov O, Bakker J, Helder J (2006) Phylum-wide analysis of SSU rDNA reveals deep phylogenetic relationships among nematodes and accelerated evolution toward crown clades. *Mol Biol Evol* 13:1792–1800. <https://doi.org/10.1093/molbev/msl044>
- Holterman M, Holovachov O, van den Elsen S, van Megen H, Bongers T, Bakker J, Helder J (2008) Small subunit ribosomal DNA-based phylogeny of basal Chromadoria (Nematoda) suggests that transitions from marine to terrestrial habitats (and vice versa) require relatively simple adaptations. *Mol Phylogenet Evol* 48:758–763. <https://doi.org/10.1016/j.ympev.2008.04.033>
- Hope WD, Zhang Z (1995) New nematodes from the Yellow Sea, *Hopperia hexadentata* n. sp. and *Cervonema deliensis* n. sp. (Chromadorida: Comesomatidae), with observations on morphology and systematics. *Invertebr Biol* 114:119–138
- Huelsenbeck JP, Ronquist F (2001) MR BAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* 17:1754–1755. <https://doi.org/10.1093/bioinformatics/17.8.754>
- Jensen P (1979) Revision of Comesomatidae (Nematoda). *Zool Scr* 8:81–105
- Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, Thierer T, Ashton B, Mentjies P, Drummond A (2012) Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* 28:1647–1649. <https://doi.org/10.1093/bioinformatics/bts199>
- Leduc D (2012) Deep-sea nematodes (Comesomatidae) from the Southwest Pacific Ocean: five new species and three new species records. *Eur J Taxon* 24:1–42. <https://doi.org/10.5852/ejt.2012.24>
- Leduc D (2013) Seven new species and one new species record of *Sabatieria* (Nematoda: Comesomatidae) from the continental slope of New Zealand. *Zootaxa* 3693:1–35. <https://doi.org/10.11646/zootaxa.3693.1.1>
- Leduc D (2017) Four new nematode species (Araeolaimida: Comesomatidae, Diplopeltidae) from the New Zealand continental slope. *Zootaxa* 4237:244–264. <https://doi.org/10.11646/zootaxa.4237.2.2>
- Leduc D, Gwyther J (2008) Description of new species of *Setosabatieria* and *Desmolaimus* (Nematoda: Monhysterida) and a checklist of New Zealand free-living marine nematode species. *N Z J Mar Freshw Res* 42:339–362. <https://doi.org/10.1080/00288330809509962>
- Leduc D, Rowden AA, Nodder SD, Berkenbusch K, Probert PK, Hadfield MG (2014) Unusually high food availability in Kaikoura Canyon linked to distinct deep-sea nematode community. *Deep-Sea Res Pt II* 104:310–318. <https://doi.org/10.1016/j.dsr2.2013.06.003>
- de Man JG (1907) Sur quelques espèces nouvelles ou peu connues de nématodes libres habitant les côtes de la Zélande. *Mém Soc Zool France* 20:33–90
- Meldal BHM, Debenham NJ, De Ley P, De Ley IT, Vanfleteren JR, Vierstraete AR, Bert W, Borgonie G, Moens T, Tyler PA, Austen MC, Blaxter ML, Rogers AD, Lamshead PJD (2007) An improved molecular phylogeny of the Nematoda with special emphasis on marine taxa. *Mol Phylogenet Evol* 42:622–636. <https://doi.org/10.1016/j.ympev.2006.08.025>
- Muthumbi WNA, Vanreusel A, Vincx M (2011) Taxon-related diversity patterns from the continental shelf to the slope: a case study on nematodes from the western Indian Ocean. *Mar Ecol* 32:453–467. <https://doi.org/10.1111/j.1439-0485.2011.00449.x>
- Nunn GB (1992) Nematode molecular evolution. Ph.D. Thesis, University of Nottingham, UK
- Platt HM (1985) The freelifving maine nematode genus *Sabatieria* (Nematoda: Comesomatidae): taxonomic revision and pictorial keys. *Zool J Linn Soc-Lond* 83:27–78
- Posada D, Crandall KA (1998) Modeltest: testing the model of DNA substitution. *Bioinformatics* 14:817–818. <https://doi.org/10.1093/bioinformatics/14.9.817>
- Rambaut A, Drummond AJ (2007) Tracer v 1.4. Available from <http://beast.bio.ed.ac.uk/Tracer>
- Rosli N, Leduc D, Probert PK (2014) Two new species and a new record of Comesomatidae (Nematoda, Araeolaimida) from Southern Hikurangi Margin. *Zootaxa* 3900:505–525. <https://doi.org/10.11646/zootaxa.3900.4.3>
- Rouville E (1903) Énumération des nématodes libres du canal de Bourdigues (Cette). *Comptes Rendus des Scéances de la Société de Biologie, Paris* 55:1527–1529
- Soetaert K, Heip C (1995) Nematode assemblages of deep-sea and shelf break sites in the North Atlantic and Mediterranean Sea. *Mar Ecol Prog Ser* 125:171–183
- Somerfield PJ, Warwick RM. 1996. Meiofauna in Marine Pollution Monitoring Programmes: a Laboratory Manual. Lowestoft: Ministry of Agriculture, Fisheries and Food
- Swofford DL (2002) PAUP*. Phylogenetic analysis using parsimony (and other methods), version 4. Sunderland, MA, USA. Sinauer Associates
- Talavera G, Castresana J (2007) Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. *Syst Biol* 56:564–577. <https://doi.org/10.1080/10635150701472164>
- Tu ND, Thanh NV, Smol N, Vanreusel A (2008) New genus *Asymmelaimus* gen. n., sp. n. and new marine nematode species of the subfamily Dorylaimopsinae de Coninck, 1965 (Comesomatidae Filipjev, 1918) from Halong Bay, Vietnam. *Russ J Nem* 16:7–16
- Vitiello P (1969) *Hopperia*, nouveau genre de nématode libre marin (Comesomatidae). *Thetys* 1:485–492
- Warwick RM (1973) Freelifving marine nematodes from the Indian Ocean. *B Brit Mus Nat Hist* 25:86–117
- Warwick RM (1977) Some free-living marine nematodes from the Isles of Scilly. *J Nat Hist* 11:381–392
- Wieser W (1954) Free-living marine nematodes II. Chromadoroidea. *Acta U Lunds* 50:1–148
- Zheng JW, Subbotin SA, He SS, Gu JF, Moens M (2002) Molecular characterisation of some Asian isolates of *Bursaphelenchus xylophilus* and *B. mucronatus* using PCR-RFLPs and sequences of ribosomal DNA. *Russ J Nematol* 11:17–22