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Two new free-living nematode species (Trefusiina: Trefusiidae) from the Chatham Rise crest, Southwest Pacific Ocean

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Abstract. Two new species of the family Trefusiidae, viz., *Trefusia piperata* sp. nov. and *Trefusialaimus idrisi* sp. nov., are described from the crest of the Chatham Rise, Southwest Pacific Ocean (350 m water depth). The present study provides the first species records for this family in the region. *Trefusia* and *Trefusialaimus* comprise twenty and three valid species, respectively. A key to males of *Trefusia* is provided.

Keywords. *Trefusia piperata* sp. nov., *Trefusialaimus idrisi* sp. nov., New Zealand, continental slope, dichotomous key, sandy sediment.

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Introduction

Recent studies show high levels of nematode alpha (local) and beta (turnover) diversity on the continental slope of New Zealand (Leduc *et al*. 2012 a, b). The systematics of several free-living marine nematode taxa, however, remains unstudied in the region. One such taxon is the family Trefusiidae, for which no species records exist to date (Leduc & Gwyther 2008). Surveys of nematode communities on Chatham Rise and Challenger Plateau suggest the presence of at least twelve Trefusiidae morphospecies (Leduc *et al*. 2012a; D. Leduc unpublished data). The low density of most species in deep-sea habitats, however, often impedes the description of new species (e.g., Miljutin *et al*. 2010).

Most records of *Trefusia* De Man, 1893 and *Trefusialaimus* Riemann, 1974 are from relatively coarse (sandy) sediments (e.g., Riemann 1974; Keppner 1992; Van Gaever *et al*. 2004; Riera *et al*. 2010). *Trefusia* has been recorded in a variety of deep-sea habitats such as manganese nodule deposits (Bussau 1993; Miljutin *et al*. 2010), canyons (Vitiello 1970), and mounds (Van Gaever *et al*. 2004). *Trefusialaimus* is a rare genus and, until now, had only been recorded from the Arctic Ocean (Filipjev 1946) and Northeast Atlantic (Riemann 1974). Here, two new species, *Trefusia piperata* sp. nov. and *Trefusialaimus idrisi* sp. nov., are described from sandy sediments on the continental slope of New Zealand.

Methods

Sediment samples were obtained from Chatham Rise, a submarine ridge that extends eastwards from the South Island of New Zealand, from depths of *ca.* 250 to 3000 m. Samples were collected in February 2011 during National Institute of Water and Atmospheric Research (NIWA) cruise TAN1103, from a site near central Chatham Rise crest at a depth of 350 m (43.331° S, 178.288° E) (see Leduc & Pilditch 2013 for details of sampling site).

A total of 15 sediment samples was collected, using an Ocean Instruments MC-800A multicorer (MUC; core internal diameter = 9.5 cm). Samples were obtained from ten MUC deployments from the sampling site. Each sample consisted of one subcore of internal diameter 26 mm taken to a depth of 5 cm. Cores were split into 0-1, 1-3, and 3-5 cm sediment depth layers, fixed in 10% formalin and stained with Rose Bengal. Samples were subsequently rinsed on a 1 mm sieve to remove large particles and on a 45 µm sieve to retain nematodes. Nematodes were extracted from the remaining sediments by Ludox flotation and transferred to pure glycerol (Somerfield & Warwick 1996). Species descriptions were made from glycerol mounts using differential interference contrast microscopy (10-1000× magnification) and drawings were made with the aid of a camera lucida. Measurements (in µm) were made using image analysis software (cellSens Standard 1.6), and all curved structures are measured along the arc. Type specimens are held in the NIWA Invertebrate Collection (NIC), Wellington.

Abbreviations

a = body length/maximum body diameter
abd = anal body diameter
b = body length/pharynx length
c = body length/tail length
cbd = corresponding body diameter
L = body length
n = number of specimens
%V = vulva distance from anterior end of body × 100/total body length

Results

Family Trefusiidae Lorenzen, 1981

Diagnosis

Cuticle smooth or with faint striations. Metanemes absent. Amphid either spiral or non-spiral. Outer labial sensillae and cephalic setae usually in two separate circles (except in *Trefusialaimus*); jointed cephalic setae. Buccal cavity funnel- or barrel-shaped, teeth absent. Males usually with two testes (except *Trefusialaimus*) and females usually with two ovaries.

Genus *Trefusia* de Man 1893

Type species

Trefusia longicauda De Man, 1893

Diagnosis

Amphideal fovea cryptospiral, oval, or horse-shoe shaped. Funnel-shaped buccal cavity. Males with ventral (and sometimes subventral) longitudinal rows of cervical sensillae. Small papillose pre-cloacal supplements present, situated either in a single ventral row or two sub-ventral rows; post-cloacal papillae

absent. Spicules usually with handle-shaped proximal ends. Females with two ovaries. Tail conical, conico-cylindrical, or filiform.

Remarks

The last treatment of the genus *Trefusia* was by Keppner (1992), who provided an updated key to the males of the 17 species of the genus based on Riemann (1966). *T. varians* Gerlach, 1955 was not included in the key because the original description was based on female specimens only. Since then, *Trefusia monodelphis* Bussau, 1990 was synonymised with *Trichistoma gracile* Andrássy, 1985 by Andrássy (2007), and two new species were described by Bussau (1993). There are currently 20 valid *Trefusia* species (including *T. piperata* sp. nov.).

Trefusia piperata sp. nov.
Figs 1-2, Table 1
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Diagnosis and relationships

Trefusia piperata sp. nov. is characterised by six double-jointed outer labial setae with conspicuous clusters of dark granules at their base, four cephalic setae at level of amphid, one seta posterior to each amphid, and long filiform tail. Male is characterised by six cervical papillae, eight papillose pre-cloacal supplements, slightly bent spicules, and gubernaculum with funnel-shaped distal portion and pointed projections.

Trefusia piperata sp. nov. most closely resembles *T. helgolandica* Riemann, 1966, described from subtidal sediments in the German Bight. The two species are similar in the shape and position of the outer labial setae and cephalic setae, as well as in the structure of the spicules and gubernaculum. The new species, however, differs from *T. helgolandica* in head diameter (14-15 *vs.* 22-25), maximum body width (21-22 *vs.* ≥ 33), spicule length (24 *vs.* 38), number of setae posterior to the amphids (one *vs.* two or three), the number and shape of cervical papillae (six papillae with wide base *vs.* thirteen papillae without wide base), and number of pre-cloacal supplements (eight *vs.* fourteen). *Trefusia piperata* sp. nov. also differs from *T. helgolandica* in the presence of conspicuous clusters of dark granules at the base of the outer labial setae.

Etymology

The species name is derived from the latin word *piperatus* (= peppered), and refers to the conspicuous clusters of dark granules at the base of the cephalic setae.

Material examined

Holotype

♂, collected on 20 Feb. 2011 (NIWA cruise TAN1103, station 69), central Chatham Rise (43.331° S, 178.288° E), water depth 350 m, sediment depth 1-5 cm, mean grain size 55-59 µm, %sand 55-57%, particle sorting (geometric) 4.1-4.3 (NIWA 88347).

Paratype

1 ♀, same data as holotype (NIWA 88348).

Description

Male

Body cylindrical, slender, tapering slightly towards anterior extremity (Fig. 2C). Cuticle thin, < 1 µm thick, with very fine striations, difficult to observe. Head blunt, slightly rounded, with three lips; six

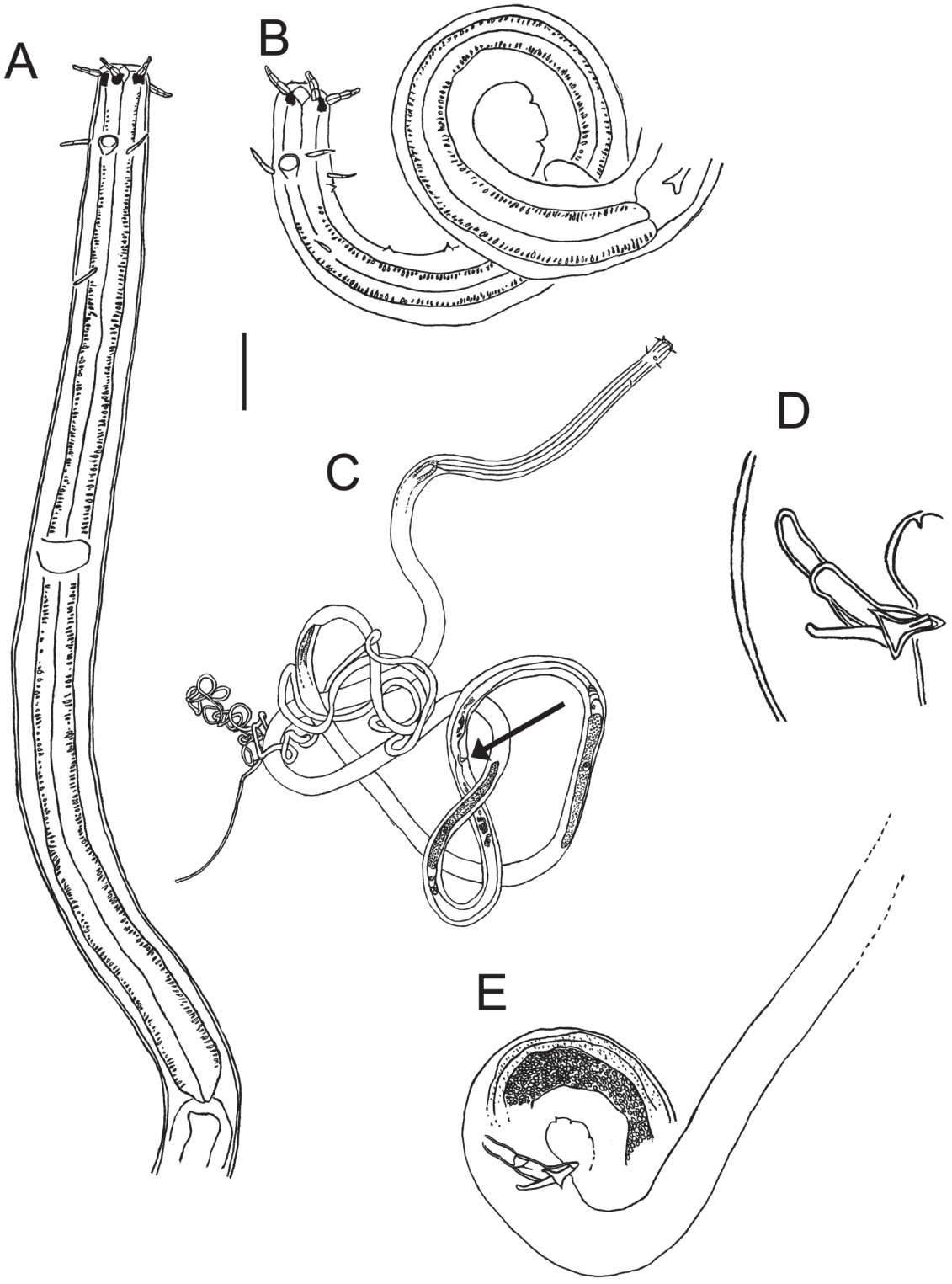

Fig. 1. *Trefusia piperata* sp. nov. **A**. Anterior body region of female. **B**. Anterior body region of male. **C**. Entire female. **D**. Right spicule and gubernaculum. **E**. Posterior body region of male. Arrow shows position of vulva. Scale bar: A-B, E = 20 µm; C = 75 µm; D = 8 µm.

small conical inner labial sensillae, ~1 µm long, six double-jointed outer labial setae with blunt ends, *ca.* 0.7 cbd (Fig. 1B). Conspicuous clusters of dark brown granules situated at base of each outer labial seta (Fig. 2A). Four cephalic setae at level of amphid, jointed, 5-7 µm long (Fig. 1B). Amphid pocket-shaped with oval aperture, *ca.* 4 µm wide by 2 µm high. One seta situated *ca.* 25 µm posterior to each amphid, 5 µm long. No other somatic setae observed. Six small cervical papillae with wide base situated ventrally in longitudinal row; row extends from posterior of amphid to about 60% of pharynx length from anterior (Fig. 1B). Buccal cavity funnel-shaped, without teeth. Pharynx cylindrical, slightly swollen at anterior

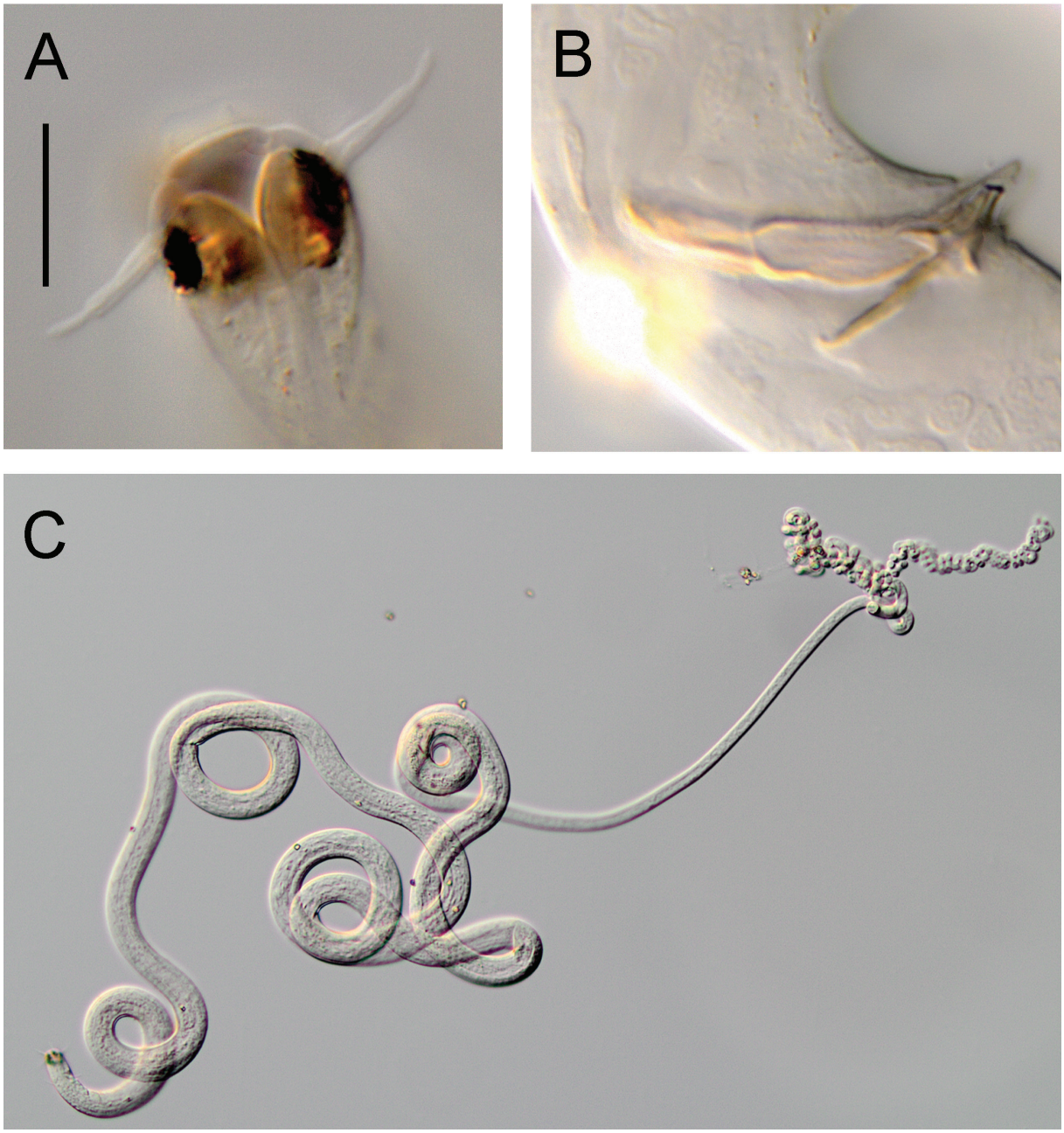

Fig. 2. *Trefusia piperata* sp. nov. Light micrographs. **A**. Head region of male, showing buccal cavity, cephalic setae, and clusters of dark granules at base of outer labial setae. **B**. Spicule and gubernaculum. **C**. Entire male. Scale bar: A-B = 10 µm; C = 100 µm.

Table 1. Morphometrics (µm) of *Trefusia piperata* n. sp. and *Trefusialaimus idrisi* n. sp. (a: body length/maximum body diameter, abd: anal body diameter, b: body length/pharynx length, c: body length/tail length, cbd: corresponding body diameter, V: vulva distance from anterior end of body, %V: V/total body length).

Species	***Trefusia piperata*** sp. nov.		***Trefusialaimus idrisi*** sp. nov.	
	Holotype ♂	Paratype ♀	Holotype ♂	Paratype juv.
n		1		1
L	3663	4492	4539	2826
a	167	214	130	97
b	14	16	13	9
c	2	2	7	5
Head diameter*	14	15	20	17
Mouth diameter	4	3	1	2
Length of sub-cephalic setae	5-7	6-7	-	-
Length of cephalic setae	9	7-8	8-10	6
Amphid height	5	4	2	2
Amphid width	4	3	6	5
Amphid width/cbd (%)	29	20	30	29
Amphid from anterior end	17	19	38	33
Nerve ring from anterior end	119	117	177	152
Nerve ring cbd	22	20	31	27
Pharynx length	260	281	357	314
Pharyngeal bulb diameter	13	19	20	17
Pharyngeal bulb cbd	21	14	34	28
Max. body diameter	22	21	35	29
Spicule length	24	-	39	-
Gubernaculum length	9	-	14	-
Anal body diameter	21	15	17	17
Tail length	1806	2330	650	590
Tail length/abd	86	155	38	35
V	-	1383	-	-
%V	-	31	-	-
Vulval body diameter	-	21	-	-

*at level of cephalic setae

and posterior extremities, completely surrounds buccal cavity. Nerve ring situated at ca. 45% of pharynx length from anterior extremity. Secretory-excretory system not observed. Cardia very small.

Reproductive system diorchic with outstretched testes. Position of testes relative to intestine difficult to ascertain. Sperm cells drop-shaped with rod-shaped nucleus; vas deferens *ca.* 445 µm long. Paired, equal spicules, slightly bent near distal one third, without obvious central cuticularised projection (i.e., lamella or median rib). Gubernaculum with narrow proximal region and funnel-shaped distal region with large anterior pointed projection and smaller posterior pointed projection (Figs 1D, 2B). Eight small papillose pre-cloacal supplements situated ventrally, 6-15 µm apart except for anterior-most supplement which is situated 40 µm from next supplement. Tail very long, *ca.* half of total body length, filiform, without setae, tightly coiled.

Female

Similar to male, but with slightly shorter cephalic setae and slightly smaller amphid (Fig. 1A). Female reproductive system didelphic, amphidelphic with reflexed ovaries. Position of ovaries relative to the intestine difficult to ascertain. Vulva situated at one third of body length (Fig. 1C).

Discussion

Trefusia piperata sp. nov. was rare at the study site, with only four specimens (the two type specimens and two juveniles in poor condition, each from a different subcore) recorded out of the 4412 individuals that were identified by Leduc & Pilditch (2013). Two individuals were found in the 1-3 cm sediment depth layers, and two were found in the 3-5 cm sediment depth layer (D. Leduc, unpublished data).

Key to the males of *Trefusia* (modified from Keppner 1992)**:**

1 Large complex sensillae present on ventral surface in cervical region ……2
- Large complex sensillae absent on ventral surface in cervical region; small papilliform sensillae may be present in cervical region ……3

2 Complex cervical sensillae pyriform; spicules narrow, arcuate; tail 7.4 abd long …… ……*T. honessi* Keppner, 1986
- Complex cervical sensillae circular; spicules broad with bladder-like structure on ventral side; tail 15-17 abd long ……*T. americana* Keppner, 1992

3 Circle of four cephalic sensillae distinctly anterior to amphid ……4
- Circle of four cephalic sensillae in region of, or posterior to amphid ……8

4 Spicules 6.8-7.4 abd long; gubernaculum present ……*T. filicauda* Allgén, 1933
- Spicules less than 2.5 cbd; gubernaculum present or absent ……5

5 Amphid situated ≥ 3 cbd from anterior extremity ……*T. attenuata* Bussau, 1993
- Amphid situated < 3 cbd from anterior extremity ……6

6 Amphid aperture circular ……*T. dominatrix* Bussau, 1993
- Amphid aperture oval ……7

7 Amphid aperture transversely oval; tail 4.0 abd long ……*T. cornea* Gerlach, 1958
- Amphid aperture longitudinally oval; tail 20 abd long ……*T. axonolaimoides* Allgén, 1953

8 Tail short, conical, 1.0 abd long ……*T. conica* Gerlach, 1957
- Tail long, conical to filiform, greater than 5.0 abd long ……9

9 Gubernaculum present ……10
- Gubernaculum absent ……14

10 Gubernaculum with lateral accessory piece ……11
- Gubernaculum without lateral accessory piece ……*T. spatulata* Keppner, 1992

11 Circle of four sub-cephalic sensillae posterior to amphid; spicules with strong arc …… ……*T. zostericola* Allgén, 1933
- Circle of four sub-cephalic sensillae at level of amphid; spicules without strong arc ……12

12 Lateral piece of gubernaculum distinct; spicules without distinct outer median rib (lamella) ……13
- Lateral piece of gubernaculum not distinct; spicules with distinct, outer, median rib; cervical region with single setiform postamphidial sensilla ……*T. longicauda* De Man, 1893

13 Cervical region with two or three setae posterior to each amphid; male with longitudinal row of 13 papillae in cervical region; no clusters of dark granules at base of cephalic setae ……*T. helgolandica* Riemann, 1966

- Cervical region with one seta posterior to each amphid; male with longitudinal row of six papillae on wide base in cervical region; clusters of dark granules at base of cephalic setae present ……*T. piperata* sp. nov.

14 Cervical and pre-cloacal papilliform supplements present on ventral surface; c greater than 5.1 ……15
- Cervical and pre-cloacal papilliform sensillae absent from ventral surface; c = 2.8 …… ……*T. pseudolittoralis* Vitiello, 1970

15 Spicules slender, gently curved, capitulum not broad and elongate ……16
- Spicules broad, acutely curved, capitulum broad and elongate ……17

16 Pre-cloacal papilliform sensillae 12-18 in number; a = 112-129 …… ……*T. multipapillatum* Bouwman, 1981
- Pre-cloacal papilliform sensillae 5-7 in number; a = 70-87 ……*T. littoralis* Allgén, 1932

17 Capitulum of spicules with elongate ventral projection …*T. curvispiculosa* Vincx & Vanreusel, 1989
- Capitulum of spicules without elongate ventral projection ……18

18 Circle of four sub-cephalic setae posterior to amphid; excretory pore heavily cuticularised; a = 88-117 ……*T. longicorpa* Keppner, 1986
- Circle of four sub-cephalic setae at level of amphid; excretory pore not observed; a = 53-57 …… ……*T. schiemeri* Ott, 1977

Genus *Trefusialaimus* Riemann, 1974

Type species

Trefusialaimus monorchis Riemann, 1974

Diagnosis

Sub-cephalic and somatic setae absent. Ten cephalic setae in one circle; amphid pocket-shaped. Male with one testis and peri-cloacal papillae. Elongated sperm cells with central rod and light-refractive nucleus at one extremity. Tail conico-cylindrical or filiform.

Trefusialaimus idrisi sp. nov.

Figs 3-4; Table 1

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Diagnosis and relationships

Trefusialaimus idrisi sp. nov. is characterised by relatively short body length, presence of numerous golden inclusions in the chords, cephalic setae 0.65-0.80 cbd long, spicules 2.3 abd long, 4 pairs of peri-cloacal papillae, and long, gradually tapering tail.

Until now, only two *Trefusialaimus* had been described, viz., *T. magnus* (Filipjev, 1946) and *T. monorchis* Riemann, 1974. *Trefusialaimus idrisi* sp. nov. is similar to *T. magnus* in the shape of the copulatory apparatus and tail, but can be differentiated from the latter by the shorter body length (4540 *vs.* 7700 µm), lower value of c (7 *vs.* 21), longer cephalic setae (0.65-0.80 *vs.* 0.4 cbd), longer spicules (2.3 *vs.* 1.7 abd), and longer tail (38 *vs.* 11 abd). *T. idrisi* sp. nov. can easily be differentiated from *T. monorchis* by the markedly longer cephalic setae (0.65-0.80 *vs.* 0.26 cbd), absence of pre- and post-cloacal papillae (present in *T. monorchis*), and tail shape (gradually tapering *vs.* conico-cylindrical).

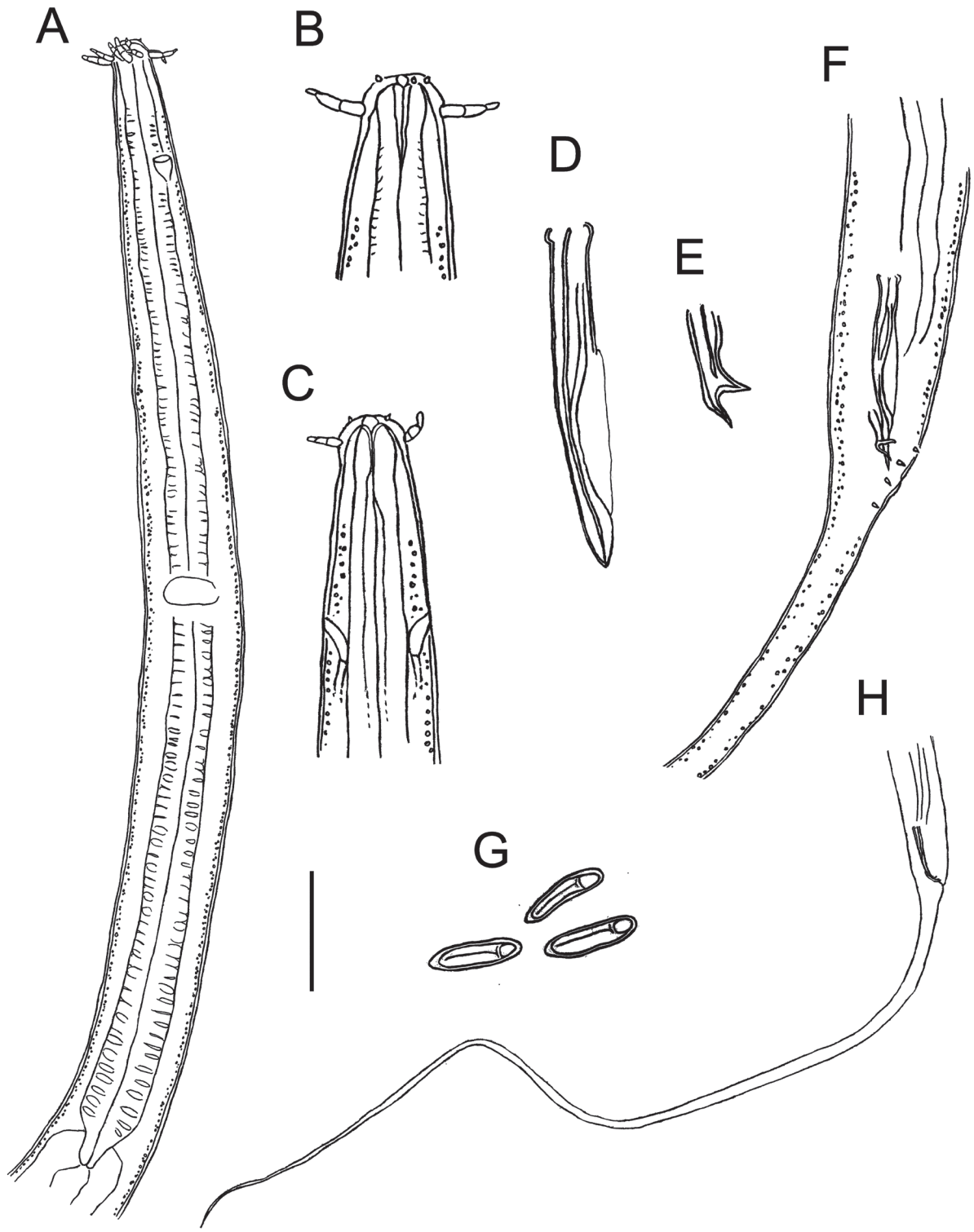

Fig. 3. *Trefusialaimus idrisi* sp. nov. **A**. Anterior body region of male. **B**. Head of male. **C**. Head of juvenile. **D**. Right spicule. **E**. Gubernaculum. **F**. Male copulatory apparatus. **G**. Mature sperm. **H**. Posterior body region of male. Scale bar: A = 40 µm; B-C, G = 20 µm; D-E = 14 µm; F = 28 µm; H = 75 µm.

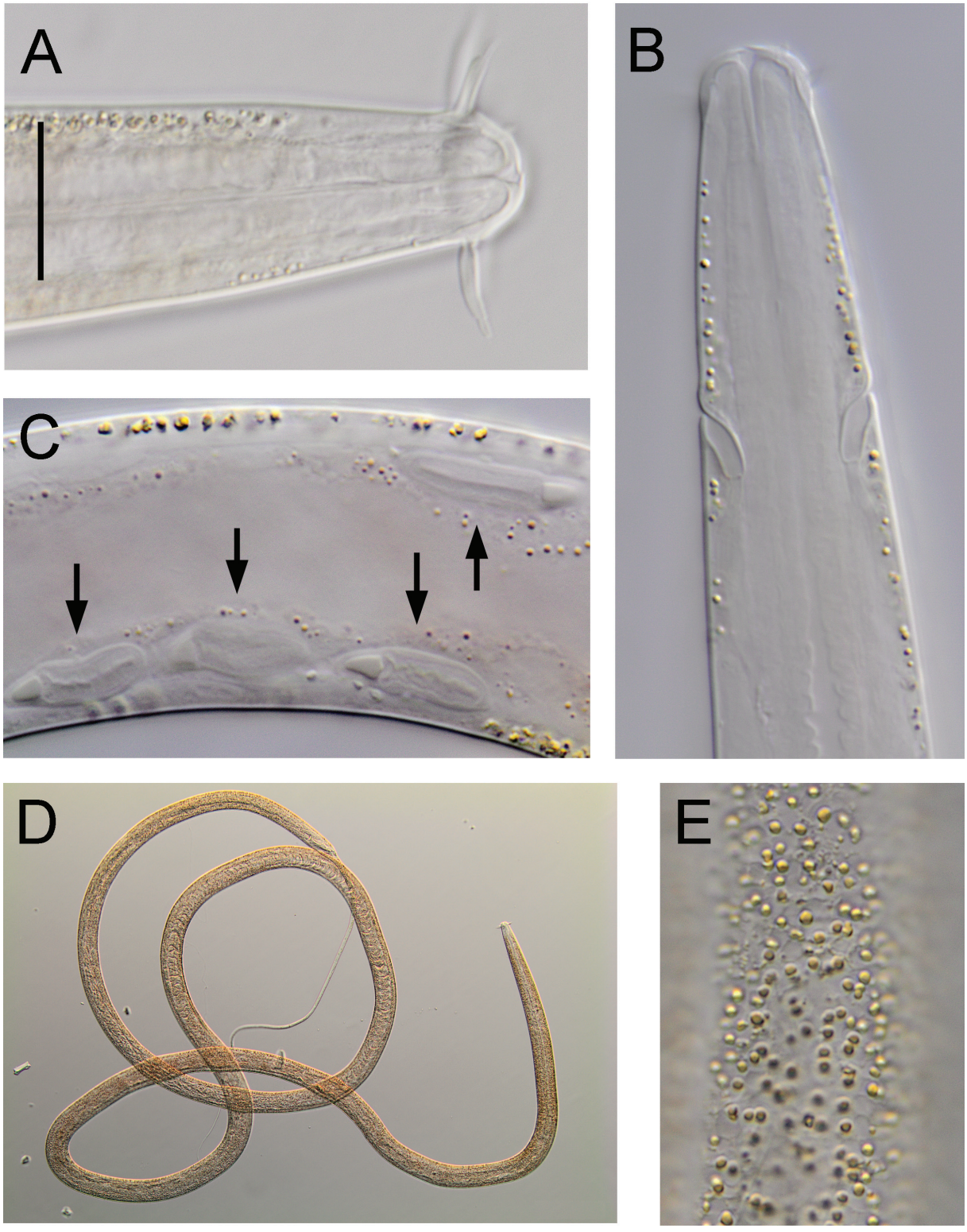

Fig. 4. *Trefusialaimus idrisi* sp. nov. Light micrographs. **A**. Anterior body region of male, lateral view. **B**. Anterior body region of juvenile, dorsal view. **C**. Mid-body region of juvenile, showing sperm cells in pseudocoelom. **D**. Entire male. **E**. Lateral chord of male, showing round golden inclusions. Arrows point to sperm cells. Scale bar: A-C, E = 15 µm; D = 260 µm.

Etymology

The species is named after Idris Matai Kljucanin Brun, the author's godson.

Material examined

Holotype

♂, collected on 20 Feb. 2011 (NIWA cruise TAN1103, station 69), central Chatham Rise (43.331° S, 178.288° E), water depth 350 m, sediment depth 1-5 cm, mean grain size 55-59 µm, %sand 55-57%, particle sorting (geometric) 4.1-4.3 (NIWA 88349).

Paratype

1 juvenile, same data as holotype (NIWA 88350).

Description

Male

Body cylindrical, slender, tapering slightly towards anterior extremity (Fig. 4D), with slight golden colouration due to the presence of numerous round, *ca.* 1 µm diameter, golden inclusions in the chords (i.e., longitudinal thickenings of the hypodermis protruding internally between the sectors of the longitudinal muscles; Chitwood & Chitwood 1974) (Fig. 4E). Cuticle smooth, thin, *ca.* 0.7-0.9 µm thick, except in head region (anterior to cephalic setae) where it is slightly thicker, 1.0-1.6 µm. Head rounded, slightly set-off from body due to thickened cuticle, with three lips, each bearing two small, conical inner labial papillae 1.0-1.5 µm long (Fig. 3B). Six outer labial setae and four cephalic setae in one circle, double-jointed; cephalic setae slightly longer than outer labial setae (0.75-0.80 cbd *vs.* 0.65 cbd). Sub-cephalic and somatic setae absent. Amphid pocket-shaped with oval aperture, *ca.* 6 µm wide by 2 µm high (Fig. 3A). Buccal cavity funnel-shaped, without teeth. Pharynx cylindrical, slightly swollen at posterior extremity, completely surrounds buccal cavity. Pharyngeal lumen lightly but distinctly cuticularised at anterior extremity (Figs 3B, 4B). Nerve ring situated at *ca.* 50% of pharynx length. Secretory-excretory system not observed. Cardia small.

Reproductive system monorchic with single outstretched testis, *ca.* 1960 µm long. Position of testis relative to intestine difficult to ascertain. Elongated sperm cells, ca 3-5 µm wide by 13-16 µm long, with central rod and nucleus at one extremity (Fig. 3G); vas deferens *ca.* 520 µm long, without muscular ejaculatory duct. Paired, equal spicules, 2.3 abd long, slightly bent near distal one third, with broad proximal end and narrow pointed distal end; velum present (Fig. 3D). Gubernaculum with two pairs of narrow, pointed lateral crurae (Fig. 3E). Four pairs of small, conical peri-cloacal papillae (Fig. 3F). Pre-cloacal supplements absent. Tail long, *ca.* 14% of total body length, narrow, gradually tapering, without setae (Fig. 3H).

Juvenile

Similar to male, but with shorter and narrower body, shorter cephalic setae (Figs 3C, 4B), and smaller amphid. Numerous sperm cells are present throughout the pseudocoelom from *ca.* 90 µm posterior to pharynx to *ca.* 200 µm anterior to anus (Fig. 4C). Genital and copulatory (i.e., cloacal or vulval) primordia not observed.

Discussion

The presence of sperm cells in the pseudocoelom of the juvenile *Trefusialaimus idrisi* sp. nov. specimen is unusual. Some nematode species, such as *Oncholaimus oxyuris*, can transfer sperm through traumatic insemination (Coomans *et al*. 1988), a process whereby the male injects sperm directly into the body of a female (or potentially even a male or juvenile) by piercing the cuticle with the spicules. The presence of sperm cells in the juvenile specimen could be explained if a similar process occured in *T. idrisi* sp. nov.

The existence of such a reproductive strategy, however, is highly conjectural because no *Trefusialaimus* females have ever been observed and (to my knowledge) traumatic insemination has not been described in the suborder Trefusiina.

Trefusialaimus idrisi sp. nov. was rare at the study site, with only four specimens (the two type specimens and two juveniles in poor condition, each from a different subcore) recorded out of the 4412 individuals that were identified by Leduc & Pilditch (2013). All individuals were found in the surface (0-1 cm) sediment layer (D. Leduc, unpublished data). A single juvenile specimen (out of 4550 specimens identified from 30 locations on the New Zealand continental margin) was recorded from a site on the northern flank of Chatham Rise at a depth of 1000 m (178.500° E, 44.333° S; silt/clay content 95%) (Leduc *et al*. 2012a; D. Leduc unpublished data).

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