A new nematode species, *Chromadorina tangaroa* sp. nov. (Chromadorida:Chromadoridae) from the hull of a research vessel, New Zealand (#47101)

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A new nematode species, *Chromadorina tangaroa* sp. nov. (Chromadorida:Chromadoridae) from the hull of a research vessel, New Zealand

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Chromadorina is a globally distributed, largely marine nematode genus frequently found on a variety of organisms, including macro- and microalgae and crustaceans, as well as artificial substrates such as settlement plates and ship hulls. Here, Chromadorina tangaroa sp. nov. is described from filamentous seaweed growing on the hull of RV Tangaroa anchored in Wellington, North Island of New Zealand. It is characterized by body length 763-1086 microns, and pore of secretory-excretory system located at or near level of teeth. Males have spicules gradually tapering distally and with narrow, rounded capitulum, a gubernaculum as long as the spicules, and three cup-shaped precloacal supplements, and females are characterized by a cuticularized prevulvar pad, vagina located at 46-48% of body length from anterior, and vagina pointing posteriorly. Chromadorina tangaroa sp. nov. is the first species of the genus to be described from New Zealand, but it is unclear whether it is native to the region because it may have dispersed as part of ship hull biofouling communities. Long-distance transport of nematodes through ship hull biofouling may be a common occurrence, but too little is known about the occurrence of nematodes on ship hulls to gauge the potential effect of shipping on nematode species distributions.

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Abstract

Chromadorina is a globally distributed, largely marine nematode genus frequently found on a variety of organisms, including macro- and microalgae and crustaceans, as well as artificial substrates such as settlement plates and ship hulls. Here, *Chromadorina tangaroa* sp. nov. is described from filamentous seaweed growing on the hull of *RV Tangaroa* anchored in Wellington, North Island of New Zealand. It is characterized by body length 763-1086 μm, and pore of secretory-excretory system located at or near level of teeth. Males have spicules gradually tapering distally and with narrow, rounded capitulum, a gubernaculum as long as the spicules, and three cup-shaped precloacal supplements, and females are characterized by a cuticularized prevulvar pad, vagina located at 46-48% of body length from anterior, and vagina pointing posteriorly. *Chromadorina tangaroa* sp. nov. is the first species of the genus to be described from New Zealand, but it is unclear whether it is native to the region because it may have dispersed as part of ship hull biofouling communities. Long-distance transport of nematodes through ship hull biofouling may be a common occurrence, but too little is known about the occurrence of nematodes on ship hulls to gauge the potential effect of shipping on nematode species distributions.

Introduction

Vessel hulls are colonized by a wide variety of sessile and mobile organisms ranging from microscopic prokaryotes and unicellular eukaryotes to large invertebrates and macroalgae. These biofouling communities are sometimes transported over large distances, which can lead to the introduction of non-indigenous organisms in new environments where they may establish new populations and potentially impact local ecological communities (Hewitt et al. 2009, Gallardo et al. 2016). Most of the literature on biofouling has so far focused on relatively large, macrofaunal-sized organisms, as well as microorganisms (Dang & Lovell 2000, Zardus et al. 2008, Farrapeira et al. 2011). Limited information is available on meiofaunal organisms such as nematodes (Fonseca-Genevois et al. 2006, Chan et al. 2016, von Ammon et al 2018), even though they are common epibionts and arguably the most numerous animals in shallow sedimentary environments worldwide (Giere 1993). Nematodes have very limited active dispersal abilities, but their small size allows them to be passively transported by currents into the water column (Shanks & Walters 1997, Boeckner et al. 2009), or as epibionts on sea turtle carapaces and drifting macroalgae (Arroyo et al. 2006, Corrêa et al. 2014). These transport pathways are thought to largely explain the cosmopolitan distribution of some nematode and other meiofaunal taxa (the so-called "meiofauna paradox"; Giere 1993). Long-distance transport of nematodes is also likely to occur through both biofouling and ballast slurry sediments (Radziejewska et al. 2006, Sutherland & Levings 2013), but little is known about the occurrence of nematodes on ship hulls and the potential effect of shipping on nematode species distributions.

Hard surfaces such as rocks and artificial structures do not provide adequate substrates for nematode colonization (Heip et al. 1985); once they are colonized by biofilm-forming microorganisms and/or habitat-forming macroalgae and invertebrates, however, they constitute an ideal substrate for a range of epibiotic nematodes (Jensen 1984, Kito & Nakamura 2001, Fonseca-Genevois et al. 2006, Majdi et al. 2011). Species of the mostly marine nematode genus *Chromadorina* Filipjev, 1918 are found living on a variety of other organisms, including macroalgae (*Chromadorina laeta* (de Man, 1876) Micoletzky, 1924, *C. obtusa* Filipjev, 1918, *C. berzicki* Andrássy, 1962) (Filipjev, 1918, Hopper & Meyers 1967), vascular plants (*C. epidemos* Hopper & Meyers, 1967*, C. erythrophthalma* (Schneider, 1906) Wieser, 1954) (Hopper & Meyers 1967, Jensen 1979, 1984), periphyton (*C. hiromii* Kito & Nakamura, 2001*, C. viridis* (Linstow, 1876) Wieser, 1954, *C. bioculata* (Schultze in Carus, 1857) Wieser, 1954) (Andrássy 1962, Kito & Nakamura 2001), and the gill cavity of spider crabs (*C. majae* Wieser, 1968) and crayfish (*C. astacicola* (Schneider, 1932) Wieser, 1954) (Wieser 1968, Schneider 1932). In addition, *Chromadorina* has been recorded on artificial settlement plates (Fonseca-Genevois et al. 2006, von Ammon et al. 2018) and ship hulls (Chan et al. 2016). Here, we describe a new *Chromadorina* species recovered from filamentous seaweed growing on the hull of *RV Tangaroa* anchored in Wellington, North Island of New Zealand.

Materials & Methods

Samples of macroalgae were collected by divers from the hull of *RV Tangaroa* while anchored at Burnham Wharf in Wellington Harbour on 10 November 2019. The macroalgal material containing nematodes was fixed in 10% buffered formalin. Nematodes were later transferred to glycerol and mounted onto permanent slides (Somerfield & Warwick, 1996).

All measurements are in µm, and all curved structures are measured along the arc. The terminology used for describing the arrangement of morphological features such as setae follows Coomans (1979). Type specimens are held in the NIWA Invertebrate Collection (Wellington), and the National Nematode Collection of New Zealand (Auckland).

Abbreviations:

A: body length/maximum body diameter

b: body length/pharynx length

c: body length/tail length

c': tail length/anal or cloacal body diameter

cbd: corresponding body diameter

L: total body length; n, number of specimens

V: vulva distance from anterior end of body

%V: V/total body length × 100

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Systematics

Order Chromadorida Filipjev, 1917

Family Chromadoridae Filipjev, 1917

Subfamily Filipjev, 1917

Genus *Chromadorina* Filipjev, 1918

Generic diagnosis: (Modified from Tchesunov (2014)) Homogeneous cuticle with transverse rows of punctations, without lateral differentiation. Amphideal fovea when visible located near level of cephalic setae, transverse slit-like, unispiral, spiral, cryptocircular or loop-shaped. Buccal cavity with three equal teeth or with dorsal tooth slightly larger than ventrosublateral teeth. Ocelli and cup-shaped precloacal supplements may be present. Tail conical or conico-cylindrical with conspicuous spinneret.

Type species: *Chromadorina obtusa* Filipjev, 1918

Remarks: Previous diagnoses state that when visible, the amphideal fovea is slit-like (Platt & Warwick 1988, Tchesunov 2014). However, several species, including *C. erythrophthalma* (Schneider, 1906) Wieser, 1954, *C. salina* Belogurov, 1978, *C. supralittoralis* Lorenzen, 1969, and *C. tangaroa* sp. nov., have a unispiral, spiral cryptocircular, or loop-shaped amphideal fovea. Venekey et al. (2019) et al. provided a list of 27 valid *Chromadorina* species.

***Chromadorina tangaroa* sp. nov.**

Figs. 1-3, Table 1

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Type locality: Hull of *RV Tangaroa* (stern), which at the time of sampling was berthed at Burnham wharf in Wellington Harbour, North Island of New Zealand (41.3135 °S, 174.8106

°E). Specimens found amongst filamentous brown (Ectocarpales) and green (*Chaetomorpha*) macroalgae.

Type material: Holotype male (NIWA 139243), three paratype males (NIWA 139244, NNCNZ 3331) and four paratype females (NIWA 139244, NNCNZ 3332), collected on 10 November 2019.

Measurements: See Table 1 for detailed measurements.

Description: Male. Body colourless in glycerin preparations, cylindrical, tapering slightly towards anterior extremity. Cuticle homogeneous, with striations approximately 1 µm apart and interspersed with transverse rows of punctations; lateral differentiation absent. Short somatic setae, 3 µm long, sparsely distributed throughout body. Cephalic region blunt, slightly rounded, with relatively well-developed lip region. Inner and outer labial sensilla inconspicuous. Four cephalic setae, 0.3-0.5 cbd long. One or two pairs of sublateral cervical setae present on each side of body, approximately 1.5 cephalic body diameters from anterior extremity. Pigment spots not observed. Amphid spiral, with 1.5 turns and transversely oval outline, at level of cephalic setae, sometimes difficult to distinguish. Buccal cavity with funnel-shaped pharyngostome; one solid dorsal tooth and two solid, slightly smaller ventrosublateral teeth. Pharynx muscular, not swollen anteriorly, lumen not cuticularized or only slightly cuticularized; conspicuous oval-shape posterior bulb with plasmatic interruptions. Nerve ring located at 55-60% of pharynx length from anterior. Cardia small, surrounded by intestine. Secretory-excretory system with elongated renette cell, 43-69 × 8-14 µm, and small accessory cell, 12-19 × 7-11 µm, both located well posterior to pharynx; pore located far anteriorly at or near level of teeth.

Reproductive system with single anterior outstretched testis located to the right of intestine. Mature sperm cells globular, 9-11 × 6-10 µm, with granular nuclei. Spicules paired, equal, gradually tapering distally and with narrow, rounded capitulum; gubernaculum about as long as spicules, with relatively wide, rounded proximal portion, narrow middle portion, and tapering distal portion. Three small, cup-shaped precloacal supplements present, beginning 5-8 µm anterior to cloaca and situated 8-13 µm apart. Precloacal seta not observed. Tail conical, curved ventrally, with few sparsely distributed sublateral setae, 3 µm long; three large caudal glands and well-developed spinneret.

Females. Similar to males, but with slightly lower values of a and longer tails. Reproductive system with two opposed, reflexed ovaries; anterior ovary situated to the right of intestine and posterior ovary situated to the left of intestine. Mature eggs 48-51 × 24-32 µm. Vulva not cuticularized, situated slightly anterior to mid-body, not at right angle with body surface but pointing posteriorly; constrictor muscle present. Prevulvar pad present, consisting of area of slightly thicker cuticle with coarse striations located on slightly to conspicuously raised ventral region immediately anterior to vulva. Vaginal glands not observed.

Diagnosis: *Chromadorina tangaroa* sp. nov. is characterized by body length 763-1086 µm, cephalic setae 0.3-0.5 cbd long, spiral amphid with 1.5 turns and 20-33% cbd wide, pore of secretory-excretory system located far anteriorly at or near level of teeth. Males with spicules gradually tapering distally and with narrow, rounded capitulum, gubernaculum as long as spicules, with wide, rounded proximal portion, narrow middle portion, and tapering distal portion, and three cup-shaped precloacal supplements. Females with prevulvar pad, vagina located at 46-48% of body length from anterior, and vagina pointing posteriorly.

Differential diagnosis: In addition to the new species, there are four *Chromadorina* species which possess two or three precloacal supplements: *C. obtusa* Filipjev, 1918, *C. paradoxa* Timm, 1961, *C. demani* Inglis, 1962, and *C. micoletzkyi* Inglis, 1962. The new species can be differentiated from *C. obtusa*, *C. paradoxa*, and *C. micoletzkyi* by the position of the secretory-excretory pore, which is located well posterior to the buccal cavity but anteriorly to the nerve ring in *C. paradoxa* and *C. micoletzkyi*, and posterior to the nerve ring in *C. obtusa* (*versus* at level of teeth in *C. tangaroa* sp. nov.). The new species can also be differentiated from *C. paradoxa* by the shorter body length (0.76-1.09 *versus* 1.3 mm), lower ratio of a (20-29 *versus* 37 in *C. paradoxa*), structure of the posterior pharyngeal bulb (simple *versus* double in *C.*

paradoxa), longer spicules (32-39 *versus* 29 µm in *C. paradoxa*), gubernaculum shape (rounded *versus* tapering proximal portion in *C. paradoxa*), and shorter male tail (3.4-3.7 *versus* 6.7 cloacal body diameters in *C. paradoxa*), from *C. micoletzkyi* by the longer body length (0.76-1.09 *versus* 0.57-0.66 mm in *C. micoletzkyi*) and higher ratio of b (6-8 *versus* <6 in *C. micoletzkyi*), and from *C. obtusa* by the longer spicules (32-39 *versus* 30 µm), higher ratio of a (20-29 *versus* 17-19 in *C. obtusa*), and number of precloacal supplements (three *versus* two supplements in *C. obtusa*). The position of the secretory-excretory pore in *C. demani* is not known, but the new species differs from the latter by the longer body length (0.76-1.10 versus 0.63-0.72 mm in *C. demani*), shorter cephalic setae (0.3-0.5 *versus* 0.7 cbd in *C. demani*), relative size of the ventrosulateral teeth (almost the same size as dorsal tooth *versus* conspicuously smaller than dorsal tooth in *C. demani*), position of the vulva (46-48 *versus* 51-55% in *C. demani*), and shape of the spicules (elongated and straight capitulum *versus* short and swollen capitulum in *C. demani*). *Chromadorina tangaroa* sp. nov. is the first species of the genus to possess a prevulvar pad, although it is conceivable that this feature may have been missed in previous species descriptions.

Etymology: The species is named after *RV Tangaroa*, on the hull of which the type specimens were found.

Remarks: During the survey of *RV Tangaroa* hull on 10 November 2019, the following invertebrate taxa were encountered: solitary tunicates, tubeworms, hydroids (*Ectopleura* and *Obelia* spp.), the native barnacle *Austrominius modestus*, the native bivalves *Perna canaliculus* and *Mytilus galloprovincialis*, and four bryozoans – the non-indigenous species *Cryptosula pallasiana* and *Watersipora subatra* and two species of uncertain identity, viz. *Electa oligopora* and *Celleporaria* sp.

Discussion

Chromadorina tangaroa sp. nov. is the first species of the genus to be described from New Zealand. Although the specimens were originally collected from Wellington Harbour, the population found on the hull of *RV Tangaroa* may have originated from another location in New Zealand, or even overseas. The vessel's movement in the four months prior to sampling include voyages to Hikurangi Margin off the east coast of New Zealand's North Island, off Campbell Island in the Southern Ocean, and included a three-day long anchorage in Dunedin on the southeast coast of New Zealand's South Island. However, during this four-month period the vessel spent over four weeks anchored in Wellington Harbour, which makes it the most likely origin for the hull population.

Chromadorina frequently occurs on a variety of organisms, including macro- and microalgae and crustaceans (Schneider 1932, Hopper & Meyers 1967, Wieser 1968, Kito & Nakamura 2001). In the present study, the presence of *Chromadorina tangaroa* sp. nov. appears to have been facilitated by the presence of filamentous brown seaweed on the ship's hull, among which numerous *C. tangaroa* sp. nov. specimens were found. Most samples obtained from the hull of *RV Tangaroa*, however, were not inspected for the presence of nematodes. Nematodes may therefore have been present on the surface of other organisms living on the hull such as tubeworms and bryozoans.

The present study provides the second record of *Chromadorina* on the hull of a vessel. Chan et al. (2016) demonstrated that *Chromadorina erythrophthalma* populations on the hull of military ships can survive long distance transits (1000s of kilometres covering over 10 degrees of latitude) from Halifax (eastern Canada) to the Arctic (Nanisivik). A study using high-throughput sequencing methods identified the presence of *Chromadorina* on settlement plates deployed in a New Zealand marina (von Ammon et al. 2018). Another study showed an unidentified *Chromadorina* species to be an early colonizer of artificial settlement plates off the coast of Brazil (Fonseca-Genevois et al. 2006). Experiments have shown that *Chromadorita tenuis* (Schneider, 1906) Filipjev, 1922 (family Chromadoridae) shows a marked preference for macroalgae over sediments, and actively swims several centimetres to colonise macroalgal substrates in response to chemical cues (Jensen 1981). It appears likely that similar habitat preference and behavior are present in *Chromadorina* species, but no evidence is yet available to test this hypothesis.

It seems likely that nematode taxa such as *Chromadorina* which preferentially occur on macroalgal substrates are transported between ports by shipping. It has been suggested that some cosmopolitan nematode species have been transported outside their native range following the accidental introduction of their macroalgal habitat in new environments (Kim et al. 2019). To date, the genera *Graphonema* Cobb, 1898, *Prochromadora* Filipjev, 1922 (family Chromadoridae Filipjev, 1917), and *Halomonhystera* Andrássy, 2006 (family Monhysteridae De Man, 1876) are the only other nematode genera (beside *Chromadorina*) that have so far been identified from ship biofouling assemblages (Chan et al. 2016). Like *Chromadorina*, these taxa are often associated with attached and drifting macroalgae or artificial substrates (Derycke et al. 2007, Perez-Garcia et al. 2015, Kim et al. 2019). Transport by ballast slurries may also occur; the limited data available to date suggest that they contain nematode assemblages more typical of sedimentary environments with genera such as *Desmodora* De Man 1889, *Sphaerolaimus* Bastian, 1865, and *Leptolaimus* De Man, 1876 having so far been identified (Radziejewska et al. 2006).

Conclusions

Chromadorina tangaroa sp. nov. is the first species of the genus to be described from New Zealand, but it is unclear whether it is native to the region because it may have dispersed as part of ship hull biofouling communities. Overall, the potential for shipping to act as dispersal vector for nematodes across ports and oceans remains largely unstudied. The accidental introduction of nematodes to new environments may be a relatively common occurrence, which could explain the cosmopolitan distribution of some species. Nematodes need to be included in studies of ship hull biofouling communities and in biosecurity surveys to gain further insights into the potential effect of shipping on the distribution of nematode species. Although environmental DNA metabarcoding offers a very useful tool for the identification of nematodes from hull samples, incomplete molecular reference datasets limit the ability to identify species or even genera (Holovachov et al. 2017). Furthermore, additional molecular methods will be required to accurately identify describe patterns of genetic connectivity (Darling et al. 2012).

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Figure captions

Figure 1. *Chromadorina tangaroa* sp. nov. (A) Female anterior body region. (B) & (C) Male cephalic region. (D) Female cephalic region. (E) female posterior body region. (F) Male posterior body region. Scale bar: 35 µm (A), 16 µm (B, C and D), 30 µm (E) and 25 µm (F).

Figure 2. *Chromadorina tangaroa* sp. nov. (A) Entire female. (B) Entire male. Scale bar: 125 µm (A and B).

Figure 3. *Chromadorina tangaroa* sp. nov. Light micrograph of female showing vulva (V), prevulvar pad (PVP) and posterior ovary (PO). Scale bar: 10 µm.

Table 1(on next page)

Table 1. Morphometrics of *Chromadorina tangaroa* sp. nov.

Table 1. Morphometrics (in microns , mean (range)) of *Chromadorina tangaroa* sp. nov. a, body length/maximum body diameter; b, body length/pharynx length; c, body length/tail length; c', tail length/anal or cloacal body diameter; cbd, corresponding body diameter; L, total body length; n, number of specimens; NO, not observed; V, vulva distance from anterior end of body; %V, V/total body length × 100

Table 1. Morphometrics (µm, mean (range)) of *Chromadorina tangaroa* sp. nov. a, body length/maximum body diameter; b, body length/pharynx length; c, body length/tail length; c', tail length/anal or cloacal body diameter; cbd, corresponding body diameter; L, total body length; n, number of specimens; NO, not observed; V, vulva distance from anterior end of body; %V, V/total body length × 100

	Males Holotype	Paratype 1	Paratype 2	Paratype 3	Females Paratype 1	Paratype 2	Paratype 3	Paratype 4
L	991	763	1002	847	1086	910	938	917
a	29	24	25	26	23	24	24	20
b	8	6	8	7	8	7	7	7
c	9	8	9	8	8	7	7	8
c'	3.4	3.6	3.7	3.5	5.0	5.1	5.1	4.5
Head diam. at cephalic setae	12	12	14	13	13	14	15	14
Head diam. at amphids	12	NO	NO	4	16	NO	3	15
Length of sub-cephalic setae	2-5	4-5	2-3	2-5	4-5	3-5	3-6	3-5
Length of cephalic setae	6	5-6	6	5	5	5	5	5
Amphid height	2	NO	NO	2	3	NO	2	2
Amphid width	4	NO	NO	4	4	NO	3	5
Amphid width/cbd (%)	33	NO	NO	31	25	NO	20	33
Amphid from anterior end	1	NO	NO	2	5	NO	3	2
Nerve ring from anterior end	70	72	70	68	77	74	73	69
Nerve ring cbd	27	26	27	27	31	28	27	29
Pharynx length	128	121	128	121	135	131	132	125
Pharyngeal bulb diam.	28	25	28	26	30	27	27	28
Pharynx cbd at base	31	29	33	31	36	33	32	33
Max. body diam.	34	32	40	33	47	38	39	45
Spicule length	36	32	36	32	-	-	-	-
Gubernaculum length	35	32	35	32	-	-	-	-
Cloacal/anal body diam.	33	28	33	30	29	24	25	27
Tail length	111	101	111	105	144	123	127	122
V	-	-	-	-	495	427	454	419
%V	-	-	-	-	46	47	48	46
Vulval body diam.	-	-	-	-	41	38	39	44

Figure 1

Drawings of body regions *Chromadorina tangaroa* sp. nov.

Figure 1. *Chromadorina tangaroa* sp. nov. (A) Female anterior body region. (B) & (C) Male cephalic region. (D) Female cephalic region. (E) female posterior body region. (F) Male posterior body region. Scale bar: 35 microns (A), 16 microns (B, C and D), 30 microns (E) and 25 microns (F).

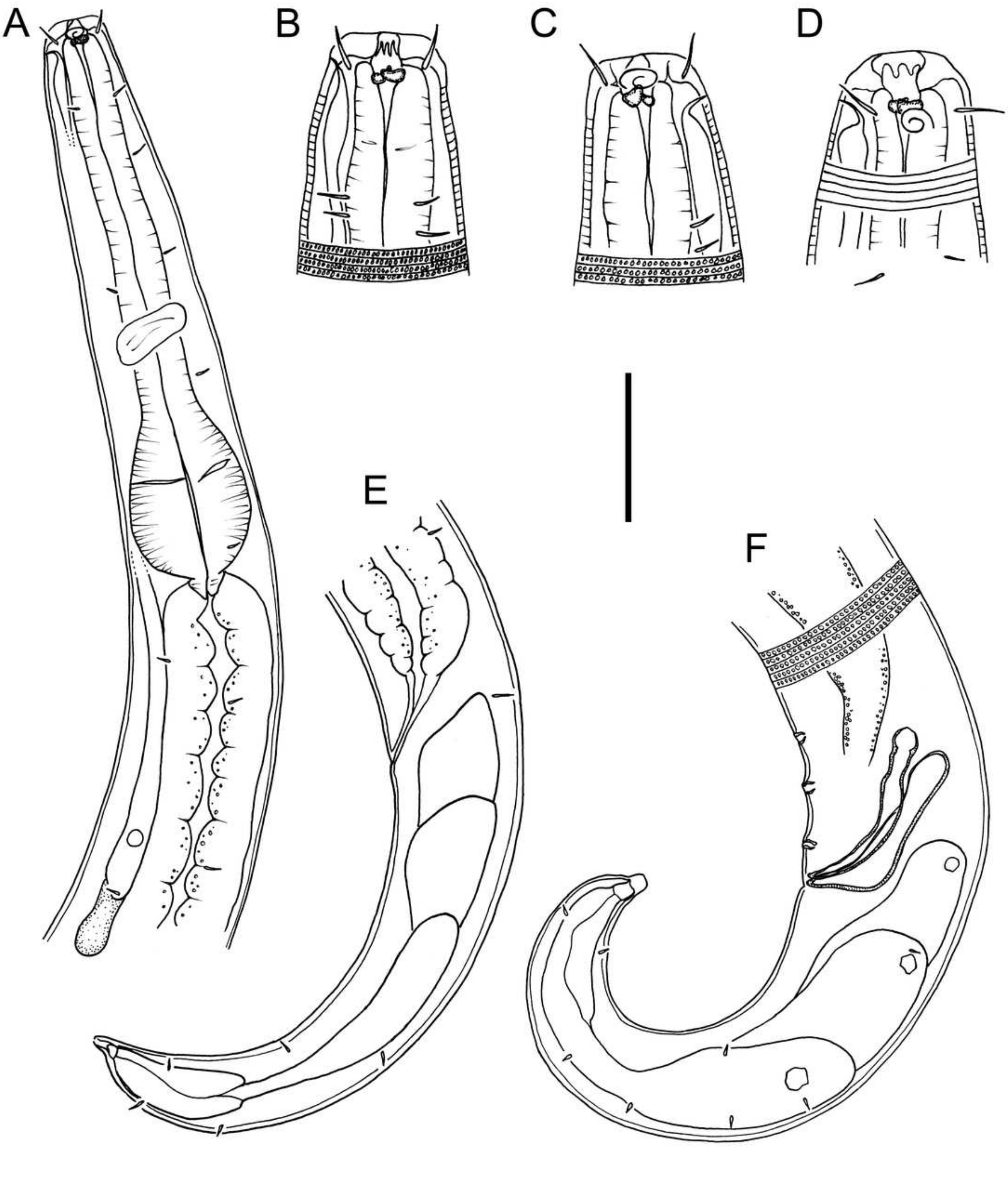

A
B
C
D
E
F

Figure 2

Drawings of entire male and female *Chromadorina tangaroa* sp. nov.

Figure 2. *Chromadorina tangaroa* sp. nov. (A) Entire female. (B) Entire male. Scale bar: 125 microns (A and B).

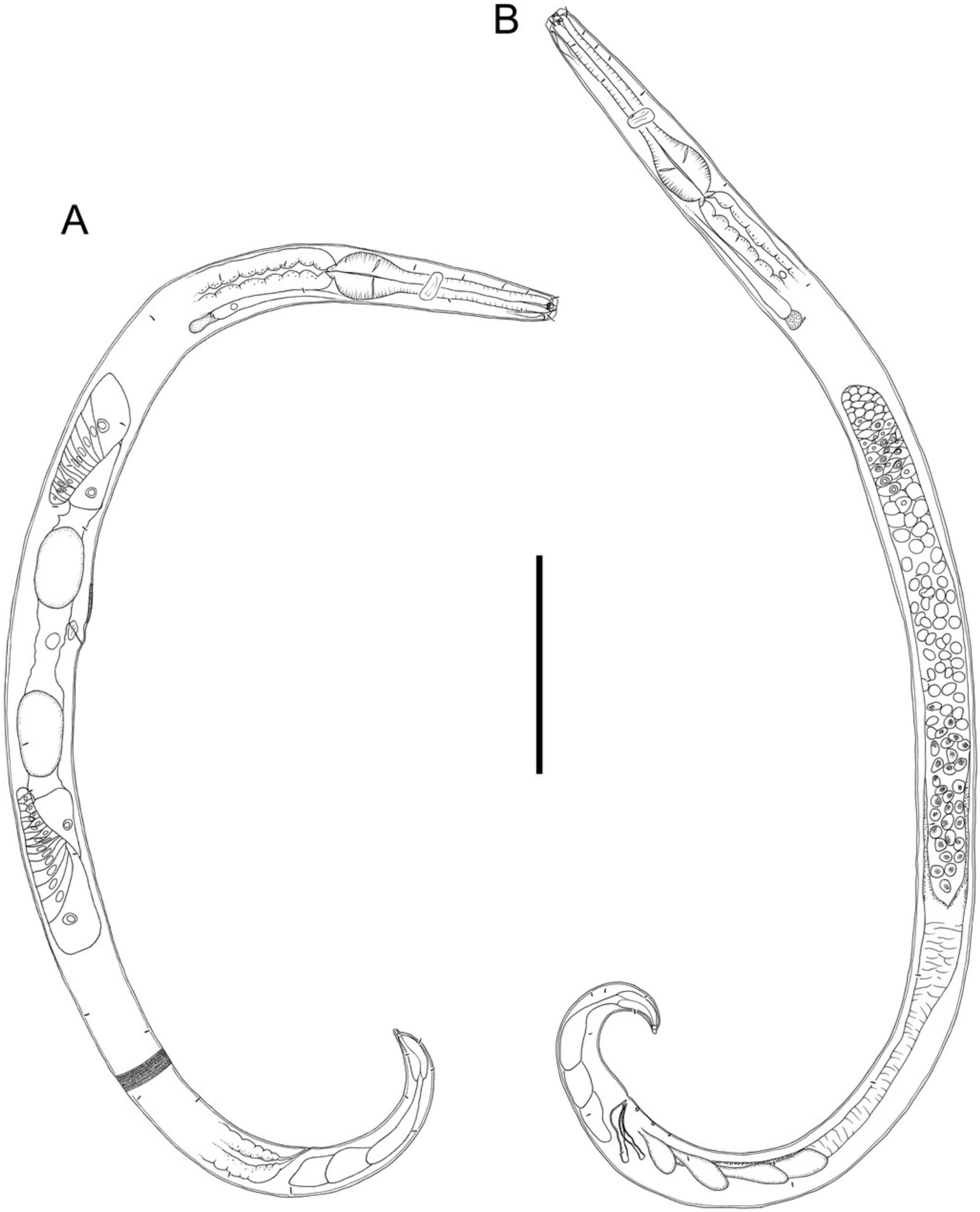
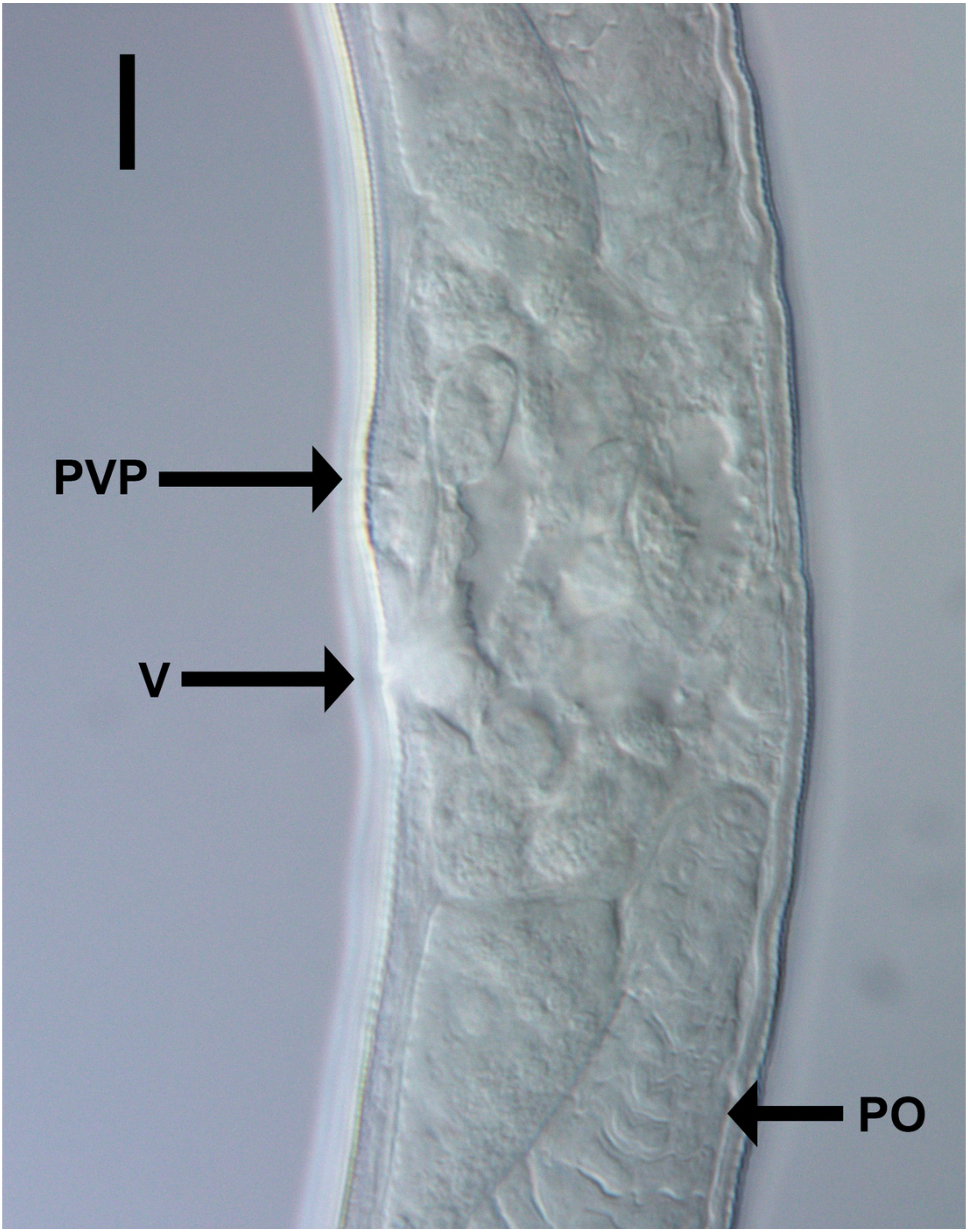

A
B

Figure 3

Light micrograph of *Chromadorina tangaroa* sp. nov.

Figure 3. *Chromadorina tangaroa* sp. nov. Light micrograph of female showing vulva (V), prevulvar pad (PVP) and posterior ovary (PO). Scale bar: 10 microns.


PVP
V
PO