A New Free-living Marine Nematode Species of the Genus Graphonema (Nematoda: Chromadorida: Chromadoridae) from Antarctica

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A new species of free-living marine nematode (Chromadorida: Chromadoridae), *Graphonema antarcticum* sp. nov., is described. The nematode was found in the shallow water of Kita-no-ura, off Syowa Station in Lützow-Holm Bay, Antarctica. *Graphonema antarcticum* sp. nov. is similar to *G. metuliferum* Kito, 1981 in the ratios of the spicules length to the body diameter at the cloaca, the well-developed gubernaculum with L-shaped lateral pieces bending at an obtuse angle and equipped with minute denticles at the distal end, and the tail length in both sexes. However, *G. antarcticum* sp. nov. differs from the latter in having a larger body size and presence of the lateral differentiation. The new diagnosis of the genus and the key to the species are provided. *Graphonema achaeta* Platonova, 1971 was transferred to *Endeolophos* due to the absence of the lateral pieces of gubernaculum that are present in the genus *Graphonema*.

Key Words: Systematics, taxonomy, new species, *Endeolophos achaeta*, new combination, meiobenthos, meiofauna, Syowa Station.

Introduction

Graphonema Cobb, 1898 is one of eleven genera in the chromadorid subfamily Euchromadorinae Gerlach and Riemann, 1973 (Inglis 1969; Gerlach and Riemann 1973; Warwick and Coles 1975; Blome 2002, 2005; Tchesunov 2014). Cobb (1898) established Graphonema as a monospecific genus based on a new species G. vulgare Cobb, 1898. Wieser (1954, 1959a) reviewed the genus and added 12 species that were transferred from other genera; however, his work was dismissed by Inglis (1969), who redescribed the genera of Euchromadorinae based on detailed observation of the cuticular ornamentations. Warwick and Coles (1975) treated Protochromadora Inglis, 1969 as a junior synonym of Graphonema because there was no distinction between the diagnoses of these genera by Inglis (1969). Currently, Graphonema consists of ten valid species, eight of which were listed by Warwick and Coles (1975) and two of which were added by Platonova (1971) and Kito (1981).

In this paper, a new species of *Graphonema* is described. The description is based on specimens collected from Antarctica during the 46th Japanese Antarctic Research Expedition (JARE 46). This is the second report on marine nematode species, following the one by Shimada *et al.* (2017). Both are outcomes of the National Institute of Polar Research (NIPR) workshops on the diversity of the Antarctic meiobenthos.

Materials and Methods

Macroalgal samples were collected by KW, by means of bait traps from 27 m depth in Kita-no-ura (68°59'55"S, 39°35'28"E), off Syowa Station in Lützow-Holm Bay, Antarctica on 16 December 2005, during JARE 46. Frozen samples were deposited in NIPR, Tachikawa, Japan. They were thawed on 2 November 2016, during the NIPR workshop on the diversity of the Antarctic meiobenthos. Nematodes were extracted by a decantation method, fixed in 70% ethanol, and then treated in one of two ways: either mounted in pure glycerin by a wax ring method (Hooper 1986a) for differential interference contrast microscope observation, or dried by a hexamethyldisilazane method (Nation 1983) and sputter-coated with Au to 200 Å thickness for scanning electron microscope (SEM) observation. All the specimens examined including type series were deposited in the Invertebrate Collection of Hokkaido University Museum (ICHUM), Sapporo, Japan.

For comparison, the holotype male, allotype female and a paratype male of *Graphonema metuliferum* Kito, 1981, which are kept in ICHUM, were also examined with a differential interference contrast microscope.

The terminology follows Maggenti (1981) and Tchesunov (2014). Abbreviations: abd, body diameter at the cloaca (male) or anus (female); mbd, maximum body diameter; vbd, body diameter at the vulva. De Man's ratios (*cf.* Hooper 1986b) are: a, ratio of the body length to the maximum body diameter; b, ratio of the body length to the pharyngeal length; c, ratio of the body length to the tail length on arc; and V, position of the vulva from the anterior body end, expressed as a percentage of body length.

Family **Chromadoridae** Filipjev, 1917 Subfamily **Euchromadorinae** Gerlach and Riemann, 1973

Diagnosis [after Tchesunov (2014)]. Chromadoridae. Body cuticle usually with complex heterogeneous ornamentation along the body, with or without lateral differentiation. The six outer labial and four cephalic sensilla setiform may be arranged in a single circle. Amphideal fovea transverse slit-like or oval, located posterior to the cephalic setae. Buccal cavity with large dorsal tooth, with or without denticles or smaller ventrosublateral teeth. Pharynx with or without defined terminal bulb. Gubernaculum usually with hammer- or L-shaped lateral pieces [sometimes mistakenly called as "telamon," which indicates a thickened immovable plate of ventral cloacal wall in Strongylida (*cf.* Maggenti 1981; Tchesunov 2014)]. Precloacal supplements absent, but a precloacal differentiation of body cuticle may be present.

Type genus. *Euchromadora* de Man, 1886. **Other genera.** Ten genera:

- Actinonuma Cobb 1020-Da
- Actinonema Cobb, 1920=Pareuchromadora Schuurmans Stekhoven and Adam, 1931 [synonymized by Wieser (1954)];
- 2. Adeuchromadora Boucher and de Bovée, 1971;
- 3. Crestanema Pastor de Ward, 1985;
- 4. Endeolophos Boucher, 1976;
- Graphonema Cobb, 1898=Protochromadora Inglis, 1969 [synonymized by Warwick and Coles (1975)];
- Parapinnanema Inglis, 1969=Austranema Inglis, 1969 [synonymized by Warwick and Coles (1975)];
- Portmacquaria Blome, 2005=Macquaria Blome, 2002 (junior homonym of Macquaria Cuvier in Cuvier and Valenciennes, 1830);
- 8. Rhips Cobb, 1920;
- 9. *Steineridora* Inglis, 1969; and
- 10. Trochamus Boucher and de Bovée, 1971.

Remarks. Eleven genera, including the type genus, are valid (Inglis 1969; Gerlach and Riemann 1973; Warwick and Coles 1975; Blome 2002, 2005; Tchesunov 2014). Four other genera—*Dasylaimus* Cobb, 1933, *Dicriconema* Steiner and Hoeppli, 1926, *Nygmatonchus* Cobb, 1933, and *Odontocricus* Steiner, 1918—were placed in Euchromadorinae before, but recently, these are now treated as *genera inquirendae* (Coles 1965; Inglis 1969; Hope and Murphy 1972; Warwick and Coles 1975).

Key to genera of Euchromadorinae

- 1 Spicules jointed Rhips
- Spicules not jointed2

_	Amphideal fovea without thickened walls4
3	Gubernaculum without lateral pieces · Adeuchromadora
_	Gubernaculum with lateral pieces Actinonema
4	Pharynx with posterior terminal bulb5
_	Pharynx without posterior terminal bulb7
5	Gubernaculum without lateral pieces Trochamus
_	Gubernaculum with lateral pieces
6	Precloacal cuticular elevation present Portmacquaria
_	Precloacal cuticular elevation absent Steineridora
7	Gubernaculum without lateral pieces Endeolophos
_	Gubernaculum with lateral pieces
8	Buccal cavity with rows of denticlesEuchromadora
_	Buccal cavity without denticles9
9	Precloacal cuticular elevation present Parapinnanema
_	Precloacal cuticular elevation absent
10	Amphideal fovea oval Crestanema
_	Amphideal fovea either transverse slit-like or indistinct
	Graphonema

Genus Graphonema Cobb, 1898

Protochromadora Inglis, 1969: 177.

Diagnosis [modified from Inglis (1969), Warwick and Coles (1975) and Tchesunov (2014)]. Euchromadorinae. Body cuticle with complex heterogeneous ornamentation. Lateral differentiation either present or absent. Amphideal fovea either transverse slit-like without a double contour, or not observed. Buccal cavity with a hollow dorsal onchium and two lateral or ventrosublateral onchia, without rows of denticles. Pharynx without posterior terminal bulb. Spicules not jointed. Gubernaculum containing a dorsal piece and a pair of hammer- or L-shaped lateral pieces. Precloacal supplement absent. Pre- and postcloacal cuticular elevation also absent.

Type species. *Graphonema vulgare* Cobb, 1898 (originally spelled *G. vulgaris*).

Other species. Nine species:

- G. amokurae (Ditlevsen, 1921) Inglis, 1969=Spilophora amokurae Ditlevsen, 1921=Euchromadora amokurae Allgén, 1929;
- 2. *G. arcticum* (Filipjev, 1946) Warwick and Coles, 1975=*Euchromadora arctica* Filipjev, 1946;
- 3. G. georgei Inglis, 1969;
- G. mediterraneum (Allgén, 1942) Warwick and Coles, 1975=Euchromadora mediterranea Allgén, 1942=Protochromadora mediterranea Inglis, 1969;
- 5. G. metuliferum Kito, 1981;
- 6. G. northumbriae Warwick and Coles, 1975;
- G. parafricanum (Gerlach, 1958) Warwick and Coles, 1975=Euchromadora parafricana Gerlach, 1958=Protochromadora parafricana Inglis, 1969;
- 8. *G. scampae* (Coles, 1965) Warwick and Coles, 1975=*Euchromadora scampae* Coles, 1965=*Protochromadora scampae* Inglis, 1969; and
- 9. G. antarcticum sp. nov.

Remarks. Ten species, including the type species, are valid (Cobb 1898; Wieser 1954, 1959a; Inglis 1969; Gerlach

and Riemann 1973; Warwick and Coles 1975; Kito 1981). *Graphonema achaeta* Platonova, 1971 should be transferred to *Endeolophos* because of the absence of the lateral pieces of gubernaculum (Platonova 1971). Two specific names, *G. pachyderma* nom. nud. by Cobb (1898), and *G. biseriale* nom. nud. by Wieser (1959b; originally spelled *G. biserialis*) are unavailable (Gerlach and Riemann 1973), because they were published without any description, definition, or bibliographic reference.

Graphonema antarcticum sp. nov. (Figs 1–4; Table 1)

Material examined. Holotype: adult male (ICHUM 5867), whole mount, 68°59′55″S, 39°35′28″E, Kita-noura, off Syowa Station in Lützow-Holm Bay, Antarctica, surface of macroalgae collected by means of bait traps at 27 m depth, 16 December 2005. Paratypes: six adult males (ICHUM 5868–5872, 5876) and five adult females (ICHUM 5873–5875, 5877, 5878), whole mounts, same collection data as with holotype.



Fig. 1. *Graphonema antacticum* sp. nov. A–G, holotype; H, paratype (ICHUM 5870). A, male body; B, cephalic region, outer ventral view; C, cuticular ornamentations and lateral differentiation at the anterior 1/3 of pharyngeal region; D, ornamentations and lateral differentiation at the posterior end of pharyngeal region; E, ornamentations and lateral differentiation at the middle of body; F, ornamentations in the caudal region; G, cephalic region, inner ventral view; H, cephalic region, inner lateral view. Scale bars: A, 500 µm; B–H, 10 µm.



Fig. 2. SEM photographs of *Graphonema antarcticum* sp. nov. A–D, non-type (ICHUM 5879). A, cephalic region, lateral view; B, cephalic region, antero-ventral view; C, cuticular surface at the middle of body; D, tail end. Scale bars: A, B, 10 µm; C, D, 20 µm.



Fig. 3. *Graphonema antarcticum* sp. nov. A–C, holotype; D, paratype (ICHUM 5877). A tail end, outer lateral view; B, male cloacal and caudal regions; C, spicule and gubernaculum; D, female body. Scale bars: A, C, 10 µm; B, 50 µm; D, 500 µm.

Non-type: an adult male (ICHUM 5879), Au-coated SEM specimen, same collection data as with holotype.

Diagnosis. Graphonema antarcticum sp. nov. is characterized by large body size (equal to or more than 2.0 mm), truncated cephalic end, presence of lateral differentiation, long spicules (80–90 μ m and 1.3–1.6 abd), without capitulum, well-developed gubernaculum with L-shaped lateral pieces bending at an obtuse angle with minute denticles at distal end, and long tail in both sexes (approximately 4–6 abd in males and 6–9 abd in females).

Measurements. See Table 1.

Description. *Males.* Body (Fig. 1A) almost cylindrical, tapering toward both ends. Epicuticle coarsely annulated (Fig. 2), except at anterior and posterior body ends (Figs. 1B, 2, 4A, G). Exocuticle with heterogeneous ornamentations (*cf.* Gourbault and Vincx 1994): tiny punctate at anterior half of cephalic region (Figs 1B, 4A); regularly hexagonal to posterior half of cephalic region to anterior 1/3 of pharynx (Figs 1B, C, 4A, B); longitudinally elongated hexagonal from anterior 1/3 of pharynx to cloacal region (Figs 1D, E, 4C, D), becoming longer toward posterior end; regularly hexagonal in caudal region (Figs 1F, 4F), becoming smaller toward posterior end; no ornamentation at tail end (Figs 3A, 4G). Lateral differentiation (Figs 1C–E, 4B, C)

present, beginning from anterior 1/3 of pharyngeal region to cloacal region, becoming wider toward posterior end. Cephalic region (Figs 1B, G, H, 2A, B) not set-off, truncated at anterior end. Cephalic diameter 25-30% of mbd. Inner labial sensilla inconspicuous. Six outer labial setae (2-3 µm) and four cephalic setae (4-6 µm) in single circle observed in the holotype and two paratype males (ICHUM 5869 and 5871), but inconspicuous in the other individuals including SEM specimen (Fig. 2A, B). Amphids not observed. Buccal cavity (Fig. 1G) divided into two sections: cheilostome-cup-shaped, with a circle of twelve tooth-like rugae; and esophastome-conical in shape, with a large dorsal onchium, two smaller ventrosublateral onchia, and four minute ventrosublateral teeth (Fig. 1G, H). Denticles absent. Pharynx (Fig. 1A, G) anteriorly surrounding esophastome, without posterior terminal bulb. Excretory pore and nerve ring indistinct. Ventral gland cell (Fig. 1A) located posterior to base of pharynx, posterior edge of gland at 1.5 times pharyngeal length from anterior body end. Testis (Fig. 1A) single, outstretched, beginning at anterior 20-25% of body length, located on right-hand side of intestine, posterior junction of vas deferens inconspicuous (complete testis observed only in the holotype and paratype ICHUM 5869, probably because of damage caused by freezing). Spicules



Fig. 4. Differential interference contrast micrographs of *Graphonema antarcticum* sp. nov. (A–G) and *G. metuliferum* Kito, 1981 (H–J). A, paratype (ICHUM 5870), B–F, holotype; G, paratype (ICHUM 5868); H–J, holotype (ICHUM, no accession number). A, cuticular ornamentations in the anterior region; B, ornamentations and lateral differentiation at the anterior 1/3 of pharyngeal region; C, ornamentations and lateral differentiations at the middle of body; E, cuticular wrinkles at the precloacal region; F, ornamentations at the cloacal region; G, ornamentations at the tail and; H, ornamentations in the anterior region; I, ornamentations at the anterior 1/3 of pharyngeal region; I, ornamentations at the anterior 1/3 of pharyngeal region; J, ornamentations at the middle of body. Scale bar: 10 µm.

Table 1.	Measurements of	Graphonema d	<i>antarcticum</i> sp.	nov. All mea	asurements a	are in μm,	except for	the de	e Man's :	ratios,	and in	the form:
mean±s.d	. (range). * ICHUN	И 5869.										

Character		Male	Female		
Character	Holotype Paratypes		Paratypes		
n	_	6	5		
L	2250	2315±193 (2030-2480)	2530±196 (2240-2750)		
a	25.9	27.1±1.2 (25.1-28.6)	23.3±1.2 (21.9-24.5)		
b	8.2	8.6±0.4 (8.3-9.4)	8.8±0.6 (8.2-9.5)		
c	9.7	9.2±0.4 (8.8-9.8)	8.4±1.1 (7.3-10.1)		
V	—	_	46.5±0.4 (45.9-47.1)		
Cephalic diameter	23	23±1.4 (22–26)	25±1.2 (23-26)		
mbd	87	86±7.7 (71-92)	109±9.1 (100-122)		
vbd	_	_	94±6.4 (87–104)		
abd	59	56±10 (38-68)	48±6.5 (39–57)		
Posterior end of pharynx from anterior body end	275	268±18 (244-290)	287±11 (270-302)		
Posterior end of ventral gland cell from anterior body end	391	399±22 (365-420)	457±59 (382–546)		
Tail length on arc	232	251±15 (226-270)	332±8.7 (326-347)		
Spicule length	82, 86	86±1.9 (83-89)	_		
Gubernaculum whole length	43	45±1.6 (43-47)	_		
Lateral piece length	35	34±1.1 (33-36)	—		
Testis from anterior body end	486	557*	_		
Vulva from anterior body end	_	_	1177±96 (1027-1280)		
Anterior end of anterior ovary from anterior body end	_	—	584±116 (428-705)		
Posterior end of posterior ovary from anterior body end	—	—	1870±27 (1843–1896)		

(Fig. 3B, C) equal, arcuate, without capitulum, gradually tapering distally and 1.3-1.6 abd or 30-40% of tail length. Gubernaculum (Fig. 3C) well-developed: dorsal piece thin, almost straight and parallel to spicules; lateral pieces paired, L-shaped and bending at an obtuse angle, with one or two minute denticles at distal end; whole length (from proximal end of dorsal piece to distal end of lateral piece) 50-55% of spicule length. Precloacal supplement or cuticular elevation absent. A longitudinal row of cuticular wrinkles (Figs 3B, 4E) present at both subventral sides of precloacal region in all males, however it possibly artefact of preservation in ethanol and/or freezing. One or two short ventral setae just anterior to cloaca either present or absent. Tail (Fig. 3B) conico-cylindrical, 3.9-6.0 abd long, without ventral cuticular elevation. Three caudal glands just anterior to cloaca. Distinct spinneret at tip of tail.

Females. Body (Fig. 3D) similar to males but thicker (mbd approximately $100-120 \,\mu$ m). Cephalic diameter 20–25% of mbd. In one specimen (ICHUM 5877), ventral gland cell reaches 1.9 times pharyngeal length from anterior body end. Female reproductive system didelphic and amphidelphic. Ovaries opposed and reflexed: anterior ovary beginning at 15–30% of body length, located on right-hand side of intestine; posterior ovary ending at 70% of body length, located on left-hand side of intestine. Eggs oval, 3–18 in uteri, 30–60 µm in diameter. Vulva situated slightly anterior to middle of body. Tail 6.1–8.4 abd long, longer and thinner than in males. Three caudal glands situated in anal region, one or two of them located preanally, and the others located postanally.

Etymology. The specific name *antarcticum* (Antarctic) is a Latin adjective taken from the type locality.

Remarks. Graphonema antarcticum sp. nov. is most

similar to G. metuliferum described from Japan in respect of the longer spicules (approximately 1.5 abd), the shape of the gubernaculum (whole length approximately 1/2 of spicule length, and with L-shaped lateral pieces bending at an obtuse angle and equipped with minute denticles at the distal end), and tail length in both sexes (approximately 4-6 abd in males and 6-9 abd in females). However, G. antarcticum sp. nov. differs from G. metuliferum by larger body size (L=2.0-2.5 mm in males, 2.2-2.7 mm in females of G. antarcticum sp. nov. vs. 1.0-1.4 mm in males, 1.2-1.4 mm in females of G. metuliferum) and the presence of the lateral differentiation (absent in G. metuliferum) (Fig. 4H-J). The presence or absence of lateral differentiation is a good diagnostic character used to distinguish species or genera in Chromadoridae (cf. Tchesunov 2014). The genus Graphonema contains five species reported to have lateral differentiation, viz., G. arcticum, G. northumbriae G. parafricanum, G. scampae, and G. antarcticum sp. nov., and only one species, G. metuliferum, reported to have no lateral differentiation (Filipjev 1946; Gerlach 1958; Coles 1965; Warwick and Coles 1975; Kito 1981). It is unknown that lateral differentiation is present or absent in the other four known species. In addition, following minor differences are found from the original description by Kito (1981) and our observation of the type series of G. metuliferum, but these may not be enough for the diagnostic characters: amphideal fovea (indistinct in G. antarcticum sp. nov. vs. distinct in G. metuliferum), ventral gland cell (rounded in G. antarcticum sp. nov. vs. elongated in G. metuliferum), and longitudinal rows of cuticular wrinkles in precloacal region (present in G. antarcticum sp. nov. vs. absent in G. metuliferum). The distinctions between the new species and all known congeners are shown in the following key.

Key to species of Graphonema

1	Gubernaculum (whole length) much longer than a half of spicule length2
-	Gubernaculum either almost equal to or shorter than a half of spicule length
2	Body shorter than 2.0 mm, spicules approximately 1.5 abd long G. mediterraneum
-	Body longer than 2.5 mm, spicule length equal to abdG. arcticum
3	Spicules shorter than 30 µm G. parafricanum
-	Spicules equal to or longer than 40 µm ······ 4
4	Tail 7–9 abd long in both sexes G. northumbriae
_	Male tail either equal to or shorter than 6 abd5
5	Gubernaculum thin, weakly developed
_	Gubernaculum and lateral pieces well-developed7
6	Spicule length almost equal to abd G. georgei
-	Spicules twice as long as abdG. vulgare
7	Proximal end of spicules with a set-off capitulumG. amokurae
-	Proximal end of spicules with a not set-off capitulum
8	Gubernaculum without tiny projections at distal end
-	Gubernaculum with tiny projections at distal end9
9	Body shorter than 1.5 mm, lateral differentiation absent
-	Body equal to or longer than 2.0 mm, lateral differentiation present

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