A New Species of Deep-sea Nematode, *Micoletzkyia mawatarii* sp. nov. (Nematoda: Enoplida: Phanodermatidae) from Northern Japan

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A new species of free-living marine nematode, belonging to the genus *Micoletzkyia* Ditlevsen, 1926 (Phanodermatidae Filipjev, 1927), is described from the deep sea off the coast of Hokkaido, northern Japan. *Micoletzkyia mawatarii* sp. nov. resembles *M. magna* Vitiello, 1970 and *M. longispicula* Huang and Cheng, 2012 in the shape of the tail and gubernaculum, but differs from the former in body width and in length of the cephalic setae, and from the latter in body length, proportional length of the tail, position of the nerve ring, and shape of the preloacal supplement. *Micoletzkyia mawatarii* sp. nov. also resembles *M. parelegans* Allgén, 1954 in its large body size, but differs from it in esophagus length, shape of the gubernaculum, size and position of the preloacal supplement, and in having a longer tail.

Key Words: Nematoda, marine, deep sea, benthos, new species.

Introduction

The free-living marine nematode family Phanodermatidae Filipjev, 1927 is characterized mainly by the presence of a head capsule, a characteristic cellular structure in the esophageal region, and elongate spicules (cf. Filipjev 1927; Inglis 1964; Lorenzen 1981; Platonova 1984). The genus *Micoletzkyia* Ditlevsen, 1926 differs from the other genera of this family in having a large body size (most species are 5–10 mm long), a constriction separating the head region from the cervical region, a weak cephalic capsule, a tubular preloacal supplement, and a conico-cylindrical tail (cf. Ditlevsen 1926; Allgén 1954a; Huang and Cheng 2012). By 2012, nine valid species of *Micoletzkyia* [twelve nominal species are known, but three of them were recently judged invalid by Huang and Cheng (2012), whom we follow in the present paper] had been described from the bottom at depths of 25 to 580 m in European waters (Ditlevsen 1926; Stekhoven 1946; Allgén 1954b, c, 1959; Vitiello 1970; Gagarin 2009) and the Yellow and South China Seas (Huang and Cheng 2012).

During a cruise in 2009 to northern Japan by the R/V *Soyo-Maru* (National Research Institute of Fishery Science, NRIFS), several specimens of free-living nematode were collected. From this material, we here described a new species of *Micoletzkyia*.

Material and Methods

All specimens examined were collected at two deep-sea sites in the Sea of Okhotsk and the Sea of Japan off the

coast of Hokkaido, northern Japan, during a survey by R/V *Soyo-Maru* (31 July to 12 August 2009). Muddy sediment samples were floated from the bottom and caught with small plankton nets fixed inside a larger beam trawl (benthos net). Sediment samples were washed in seawater, then sieved with a plankton net (450 µm in mesh size) to collect small animals. Nematode specimens were sorted under a stereomicroscope and fixed in DESS solution (Yoder *et al.* 2006) containing DMSO (dimethyl sulphoxide), EDTA (ethylenediaminetetraacetic acid, disodium salt), and saturated sodium chloride. Nematodes were transferred into 10% glycerin in 30% ethanol, then placed in a thermostatic chamber for 48–72 hours at 45°C. After evaporation, nematodes were mounted individually in anhydrous glycerin on glass slides before observation with an Olympus BX51 differential interference contrast microscope. Other specimens were dried in a critical-point dryer, sputter-coated with Au, and observed and imaged with a Hitachi S-3000N scanning electron microscope (SEM). All specimens examined were deposited in the Hokkaido University Museum, Sapporo, Japan, catalogued with the acronym ZIHU representing the former Zoological Institute, Hokkaido University.

Abbreviations related to measurements presented in the descriptions are: L, total body length; abd, body diameter at anus (in females) or cloaca (in males); mbd, maximum body diameter.

De Man's ratios are: a, body length divided by maximum body diameter; b, body length divided by esophagus length; c, body length divided by tail length; V, position of vulva as a percentage of body length from anterior end [all after Hooper (1986)].

Fig. 1. *Micoletzkyia mawatarii* sp. nov., holotype, ZIHU 4073 (A–C, E–G) and paratypes, ZIHU 4075 (D) and ZIHU 4077 (H). A, anterior region of male body; B, head of male; C, posterior region of male body; D, posterior region of female body; E, reproductive system of male (testes and vas deferens); F, distal end of spicules and gubernaculum at cloacal region of male; G, precloacal supplement of male; H, reproductive system of female (ovaries, uterus, eggs, and vulva). Scale bars: A, E, H, 200 μ m; B, 10 μ m; C, D, 100 μ m; F, 10 μ m; G, 20 μ m.

Family **Phanodermatidae** Filipjev, 1927
 Subfamily **Crenopharynginae** Platonova, 1976
 Genus *Micoletzkyia* Ditlevsen, 1926
Micoletzkyia mawatarii sp. nov.
 (Figs 1–3)

Material examined. Four males and four females. *Holotype*. Male (ZIHU 4073), whole mount, collected on 5 August 2009 by K. Kakui. *Paratypes*. One male (ZIHU 4074) and three females (ZIHU 4075–4077), whole mounts; one male (ZIHU 4078) and one female (ZIHU 4079), Au-coated SEM specimens; same collection data as for holotype. One male (ZIHU 4080), whole mount, collected on 2 August 2009 by K. Kakui.

Type locality and habitat. In muddy sediment, west of Musashi Bank, Sea of Japan (44°41.5'N, 139°48.3'E), depth 528 m.

Other locality and habitat. In muddy sediment, Sea of Okhotsk (44°34.6'N, 144°41.7'E), depth 1,550 m (ZIHU 4080).

Etymology. The specific name *mawatarii*, a masculine noun in the genitive singular, honors Professor emeritus Shunsuke F. Mawatari (Hokkaido University) for his great

contributions to invertebrate taxonomy in Japan.

Measurements (Table 1). Holotype: L=11.0 mm, mbd=196 μ m, a=56.2, b=11.0, c=24.0. Males ($n=3$, including holotype): L=10.5–11.0 mm, mbd=196–200 μ m, a=52.4–56.2, b=9.8–11.0, c=24.0–27.5. Females ($n=3$): L=10.7–12.7 mm, mbd=214–227 μ m, a=47.2–58.6, b=10.5–11.0, c=24.5–27.7, V=50.2–53.1.

Description. Body long; anterior and posterior regions gradually tapering; anterior part of cervical region (Figs 1A, 2A) sharply tapering to anterior body end. Cuticle smooth; somatic setae sparse. Numerous large gland cells (Figs 1A, 3A) opening on cuticular surface around intestine, densely present particularly between anterior end of intestine and 30–40% from anterior end of body.

Head (Figs 1B, 2B, C) rounded at anterior end, set off by constriction just posterior to cephalic setae. Head diameter at level of cephalic setae 8–9% of mbd. Body diameter at constriction 0.5–1.3 μ m smaller than head diameter. Three low lips well developed. Labial and cephalic setae 6+6+4 pattern (observed with SEM, but appearing 6+10 under light microscopy); inner labial setae very short (2–3 μ m); outer labial setae 0.8–1.0 times head diameter; cephalic setae slightly shorter than outer labial setae, 0.6–0.9 times

Table 1. Measurements of *Micoletzkyia mawatarii* sp. nov. (in μm , except for a, b, c, and V).

	Males			Females		
	ZIHU			ZIHU		
	*4073	4074	4080	4075	4076	4077
L	11008	10670	10474	11765	10717	12720
a	56.2	54.4	52.4	55.0	47.2	58.6
b	11.0	10.8	9.8	10.9	10.5	11.0
c	24.0	25.9	27.5	24.5	27.7	26.4
V (%)				50.2	53.1	51.2
head diameter at cephalic setae	17.2	17.1	17.1	16.4	17.1	16.8
anterior end to head constriction	12.5	12.9	12.8	12.4	12.0	11.4
body diameter at head constriction	16.4	16.3	16.6	15.1	16.2	16.0
longest outer labial setae length	17.5	16.9	17.1	14.5	16.6	13.3
shortest outer labial setae length	16.9	16.3	16.6	14.2	16.3	12.8
longest cephalic setae length	14.4	13.9	12.3	12.3	15.3	12.7
shortest cephalic setae length	10.8	13.8	10.4	10.5	14.3	10.5
buccal cavity length	3.6	4.3	3.5	3.8	4.4	5.0
buccal cavity width	2.2	2.8	2.0	3.2	3.9	3.3
anterior end to amphid	13.5	13.7	13.9	9.9	13.2	10.0
amphidal aperture width	6.8	7.1	9.5	7.5	8.2	7.6
esophagus length	1002	985	1074	1084	1025	1156
body diameter at base of esophagus	156	152	175	175	186	181
anterior end to excretory pore	193	215	249	200	208	197
anterior end to nerve ring	413	383	447	451	463	509
body diameter at nerve ring	108	113	114	117	123	119
ventral gland width	85	89	92	37	56	32
anterior end to posterior end of ventral gland	1538	1505	1577	1621	1575	1610
mbd	196	196	200	214	227	217
abd	86	92	89	83	81	86
tail length	458	412	381	481	387	481
diameter at filiform part of tail	11.7	12.2	10.5	12.1	11.5	14.3
anterior end to anterior testis	3872	4617	4173			
anterior end to posterior end of posterior testis	5352	6224	6475			
spicule length on arc (left/right)	708/691	632/664				
gubernaculum length	63	61	62			
gubernaculum width at proximal end	38	34	33			
gubernaculum width at distal end	8	8	6			
precloacal supplement length	49	49	47			
cloaca to precloacal supplement	240	240	235			
anterior end to anterior ovary				4693	4305	4891
anterior end to posterior end of posterior ovary				7357	7003	8311
anterior end to vulva				5911	5689	6511
body diameter at vulva				166	184	171

* Holotype.

head diameter. Cephalic capsule weak. Buccal cavity small, cylindrical, without teeth or denticles. Amphids pocket-like, just posterior to constriction; amphidal foveae deep, cup shaped; apertures oval, width 0.4–0.6 times head diameter.

Esophagus (Fig. 1A) gradually tapering to anterior end, length 9.1–10.3% of L. Excretory pore at 17–23% of esophagus from anterior end. Nerve ring at 39–45% of esophagus from anterior end. Cervical setae sparse. Ventral gland (Figs 1A, 3B) larger in males than in females; width 0.4–0.5 times corresponding body diameter in males, 0.2–0.3 times in females. Posterior end of ventral gland situated at 1.4–1.5

times esophagus length from anterior end.

Tail (Figs 1C, D, 2D) conical in anterior third (27–36%), filiform in posterior two-third; length 4.3–5.3 times abd in males, 4.8–5.8 times in females; 3.6–4.2% of L. Abd 44–47% of mbd in males, 36–40% in females. Width of filiform part of tail 12–14% of abd in males, 14–17% in females. Several short setae in caudal and anal (cloacal) regions, but their arrangement varying among individuals. Tail acute at tip, with spinneret, but without terminal setae. Caudal gland inconspicuous.

Males. Reproductive system (Fig. 1E) diorchic. Testes

Fig. 2. SEM images of *Micoletzkyia mawatarii* sp. nov., paratypes, ZIHU 4079 (A–C) and ZIHU 4078 (D). A, lateral view of anterior region of female body; B, anterior view of female head; C, ventrolateral view of female head; D, lateral view of posterior region of male body. Scale bars: A, D, 100 μ m; B, C, 10 μ m.

Fig. 3. Light photomicrographs of *Micoletzkyia mawatarii* sp. nov., holotype, ZIHU 4073. A, gland cells surrounding intestine; B, ventral gland. Scale bars: 100 μ m.

equal, opposed, outstretched; anterior testis beginning at 35–43% of L, posterior testis ending at 49–62% of L from anterior body end. Spicules (Fig. 1C, F) paired, equal, proximally expanded, bent ventralwards, distally acute (spicules broken in ZIHU 4080); length 6.9–8.2 times abd, 1.5–1.6

times tail length, 16–17% of L. Gubernaculum (Fig. 1C, F) surrounding spicules, conical, strongly tapering to distal end, without apophysis; 9–10% of spicule length. Precloacal supplement (Fig. 1C, G) tubular, without accessory structure; length 7–8% of spicule; situated at 2.6–2.8 times abd

anterior to cloaca.

Females. Reproductive system (Fig. 1H) didelphic. Ovaries equal, opposed, reflexed; anterior ovary beginning at 38–40% of L, posterior ovary ending at 63–65% of L from anterior body end. Eggs (found only in ZIHU 4077) oval, 317–362 µm long, 137–139 µm wide, numbering 2 in uterus. Vulva situated at 50–53% of L from anterior body end; corresponding body diameter 78–81% of mbd.

Diagnosis. *Micoletzkyia mawatarii* sp. nov. is characterized by its large body size (L=10.5–11.0 mm, mbd=196–200 µm in males; L=10.7–12.7 mm, mbd=214–227 µm in females); particular ranges of De Man's ratios (a=52.4–56.2, b=9.8–11.0, c=24.0–27.5 in males; a=47.2–58.6, b=10.5–11.0, c=24.5–27.7, V=50.2–53.1 in females); long outer labial setae (length 0.8–1.0 times head diameter); short esophagus for the body length (9–10% of L); long spicules (632–708 µm, 6.9–8.2 times as long as abd); conical gubernaculum with no apophysis; simple, tubular precloacal supplement with no accessory appendage; and long tail (381–458 µm, 4.3–5.3 times as long as abd in males, 387–481 µm, 4.8–5.8 times abd in females).

Micoletzkyia mawatarii resembles *M. magna* Vitiello, 1970 and *M. longispicula* Huang and Cheng, 2012 in the shape of the tail (anterior ca. 30% conical, posterior 70% filiform), and the shape of the gubernaculum (conical and tapering to the distal end, without any apophysis), but differs from *M. magna* in the body diameter (maximally 196–200 µm, a=52.4–56.2 in males of *M. mawatarii* vs 113 µm, a=71.4 in *M. magna*), and the lengths of the outer labial and cephalic setae (10.4–17.5 µm, 0.6–1.0 times head diameter vs about 6 µm, less than 0.5 times head diameter) (cf. Vitiello 1970). *Micoletzkyia mawatarii* also differs from *M. longispicula* in body size (L=10.5–11.0 mm, mbd=196–200 µm vs 6.1 mm, 128 µm in *M. longispicula*), the proportional length of the tail (4.3–5.3 times as long as abd, c=24.0–27.5 vs 6.5 times abd, c=14.2), the position of the nerve ring (situated 39–42% of way from anterior end of the esophagus vs about mid-way along esophagus), and the simple tubular precloacal supplement (vs tubular with a head-like proximal end in *M. longispicula*) (cf. Huang and Cheng 2012). *Micoletzkyia mawatarii* also resembles *M. parelegans* Allgén, 1954 in its large body size (L>10 mm), but differs from the latter in esophagus length (b=9.8–11.0 vs 7.1), shape of the gubernaculum (cylindrical, surrounding the spicules vs tubular or plate-like in *M. parelegans*), size and the position of the precloacal supplement (47–49 µm long, 235–240 µm from the cloaca vs 68 µm long, 350 µm from the cloaca), and length of the tail (more than 4 times abd, c=24.0–27.5, vs about 2 times abd, c=45.6) (cf. Allgén 1954b, c).

Remarks. This is the 10th species known in *Micoletzkyia*, and the deepest record (ZIHU 4080 were collected from 1,550 m deep) for the genus. Although 12 nominal species have been described in the genus, Huang and Cheng (2012) considered *M. anomala* Wieser, 1953, *M. austrogeorgiae* Allgén, 1954, and *M. falklandiae* Allgén, 1954 to be invalid because of their incomplete descriptions based on immature female or juvenile specimens. Therefore, the genus *Micoletzkyia* contains 10 species, viz., *M. elegans* Ditlevsen, 1926

(type species), *M. parelegans*, *M. nudicapitata* Allgén, 1959, *M. magna*, *M. mucronata* Vitiello, 1970, *M. sedata* Gagarin, 2009, *M. filicaudata*, *M. longispicula*, *M. nanhaiensis* Huang and Cheng, 2012, and *M. mawatarii* sp. nov.

Referring to the identification key by Huang and Cheng (2012), *M. mawatarii* can be classified in the same category as *M. magna*, *M. elegans*, *M. longispicula*, and *M. sedata* (fifth choice in the key) by its having a tail with a long filiform part. The following key distinguishes these five species. Because measurements of *M. elegans* vary between the original description (Ditlevsen 1926) and redescrptions (Stekhoven 1946; Allgén 1954a), we only follow the original description.

1. Longest outer labial or cephalic setae much shorter than head diameter *M. magna* Vitiello
- Longest outer labial or cephalic setae almost equal to or longer than head diameter 2
2. Gubernaculum of peculiar, complex shape *M. elegans* Ditlevsen
- Gubernaculum simple 3
3. Spicule shorter than 5 abd, gubernaculum 23 µm. *M. sedata* Gagarin
- Spicule longer than 6 abd, gubernaculum 51–63 µm . . . 4
4. Nerve ring midway along esophagus, c=14.2, precloacal supplement with head-like proximal end. *M. longispicula* Huang and Cheng
- Nerve ring 39–42% of esophagus length, c=24.0–27.5, precloacal supplement unmodified tubular *M. mawatarii* sp. nov.

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References

- Allgén, C. A. 1954a. *Micoletzkyia*, eine bemerkenswerte marine Nematodengattung. Zoologischer Anzeiger 152: 134–137.
- Allgén, C. A. 1954b. Freelifving marine nematodes from East Greenland and Jan Mayen. The Swedish Greenland Expedition 1899. Meddelelser om Grønland 107(6): 1–44.
- Allgén, C. A. 1954c. Kleine Mitteilungen über freilebende Nematoden I–IV. Zoologischer Anzeiger 153: 88–95.
- Allgén, C. A. 1959. Further Zoological Results of the Swedish Antarctic Expedition 1901–1903. Vol. 5. No. 2. Freelifving Marine Nematodes. Kungl. Boktryckeriet P. A. Norstedt & Söner, Stockholm, 293 pp.
- Ditlevsen, H. 1926. The Danish Ingolf-Expedition. Volume 4. No. 6. Free-living Nematodes. Bianco Luno, Copenhagen, 42 pp., 15 pls.
- Filipjev, I. N. 1927. Les nématodes libres des mers septentrionales appartenant à la famille des Enoplidae. Archiv für Naturgeschichte,

- Abteilung A, Original-Arbeiten 91 (6): 1–216, 7 pls.
- Gagarin, V. G. 2009. New species of enoplids (Nematoda: Enoplida) from Mediterranean Sea. *Zoologiya Bespozvonochnykh* 6: 117–127. [In Russian with English abstract]
- Hooper, D. J. 1986. Drawing and measuring nematodes. Pp. 87–94. In: Southey, J. F. (Ed.) *Laboratory Methods for Work with Plant and Soil Nematodes*. 6th Edition. Her Majesty's Stationery Office, London, 202pp.
- Huang, Y. and Cheng, B. 2012. Three new free-living marine nematode species of the genus *Micoletzkyia* (Phanodermatidae) from China Sea. *Journal of the Marine Biological Association of the United Kingdom* 92: 941–945.
- Inglis, W. G. 1964. The marine Enoplida (Nematoda): a comparative study of the head. *Bulletin of British Museum (Natural History)*. Zoology 11: 265–376.
- Lorenzen, S. 1981. Entwurf eines phylogenetischen Systems der freilebenden Nematoden. Veröffentlichungen des Instituts für Meeresforschung in Bremerhaven (Supplement 7): 1–472. [English translation by Platt, H. M.: Lorenzen, S. 1994. *The Phylogenetic Systematics of Freelifing Nematodes*. Ray Society, London, 383 pp.]
- Platonova, T. A. 1976. Nizshie Enoplida (svobodnozhivushchie morskije nematody) morei SSSR. Pp. 3–164. In: Platonova, T. A. and Gal'tsova, V. V. (Eds) *Nematody i ikh Rol v Meiobentose* [*Nematodes and their Role in the Meiobenthos*]. Nauka Publishers, Leningrad, 270 pp. [In Russian, English translation by Sharma, S.: Platonova, T. A. 1985. Lower Enoplida of the seas of the Soviet Union. Pp. 1–214. In: Platonova, T. A. and Gal'tsova, V. V. (Eds) *Nematodes and their Role in the Meiobenthos*. Amerind Publishing Co. Pvt. Ltd., New Delhi, v+366 pp.]
- Platonova, T. A. 1984. Materials for the revision of the family Phanodermatidae (Enoplida, Nematoda). *Zoologicheskii Zhurnal* 63: 507–516. [In Russian with English summary]
- Stekhoven, J. H. S. 1946. Freilebende marine Nematoden des Skageraks und der Umgebung von Stockholm. *Arkiv för Zoologi* 37A(16): 1–91.
- Vitiello, P. 1970. Nématodes libres marins des vases profondes du Golfe du Lion. I. Enoplida. *Tethys* (Marseille) 2: 139–210.
- Yoder, M., Tandingan De Lay, I., King, I. W., Mundo-Ocampo, M., Mann, J., Blaxter, M., Poiras, L. and De Ley, P. 2006. DESS: a versatile solution for preserving morphology and extractable DNA of nematodes. *Nematology* 8: 367–376.