

## New Records of Two *Dorylaimoides* Species (Nematoda: Dorylaimida: Mydonomidae) from Korea

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### ABSTRACT

The genus *Dorylaimoides* Thorne and Swanger, 1936 comprises over 70 species and predominantly inhabits soil; however, some species are found in semiaquatic or aquatic habitats. In this study, *D. elegans* (de Man, 1880) Thorne and Swanger, 1936 and *D. limnophilus* (de Man, 1880) Loof, 1964, collected from sediments in the Hangang River, are reported for the first time in Korea. These two *Dorylaimoides* species are distinguished from other *Dorylaimoides* species by their morphological characteristics: a didelphic-amphidelphic reproductive system, short tail, and digitate terminus in *D. elegans* and a mono-opisthodelphic reproductive system and long tail in *D. limnophilus*. Here, we provide detailed information on the morphological characteristic (description and illustration) and morphometrics of the two *Dorylaimoides* species, using optical microscopy. Additionally, 18S rDNA sequences of the two species from the Korean isolates are provided as molecular evidence.

**Keywords:** Nematode, Dorylaimida, *Dorylaimoides elegans*, *Dorylaimoides limnophilus*, new record, freshwater, Korea

### INTRODUCTION

The genus *Dorylaimoides* Thorne and Swanger, 1936 belongs to the family Mydonomidae Thorne, 1964. To date, the genus comprises over 70 species; however, only three species have previously been reported in Korea: *D. leptus* Husain and Khan, 1968; *D. micoletzkyi* (de Man, 1921) Thorne and Swanger, 1936; *D. punctatus* Khan and Park, 1999 (de Man, 1921; Husain and Khan, 1968; Khan and Park, 1999). In this study, we report *D. elegans* (de Man, 1880) Thorne and Swanger, 1936 and *D. limnophilus* (de Man, 1880) Loof, 1964 collected from Korea for the first time and provide detailed descriptions of their morphological characters, morphometrics, and 18S rDNA sequences.

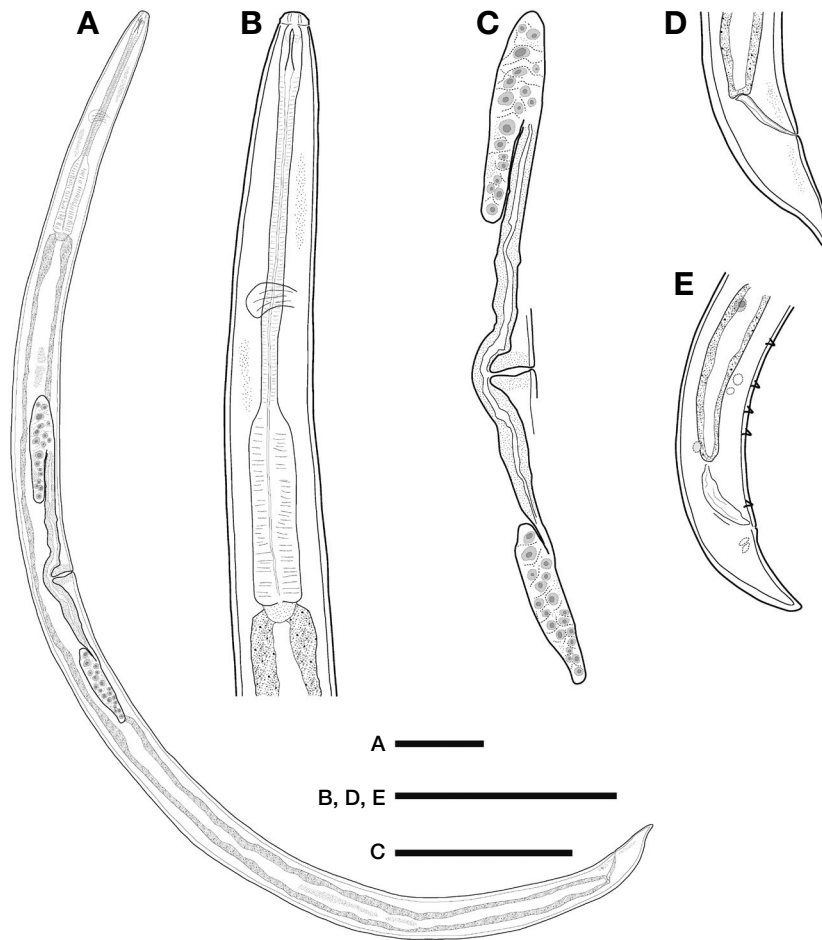
Live specimens were collected from freshwater and sediment samples (Wangdae-ri, Neungseo-myeon, Yeosu-si, Gyeonggi-do, Korea [GPS coordinates: 37°19'43.82"N, 127°36'15.40"E]) from the Hangang River and then isolated by sieving and the Baermann funnel method (Baermann, 1917).

Each specimen was transferred to 2 mL of water, to which 4 mL of 80°C triethanolamine-formalin (TAF, 2% triethanolamine and 7% formaldehyde) solution was added for fixation. Fixed nematodes were dehydrated using glycerin (Seinhorst, 1959) and mounted on hydroglyceric slides (Shirayama et al., 1993). Under an Imager A2 optical microscope, with differential interference contrast, equipped with an AxioCam 506 color camera, morphological and morphometric characters of the specimens were observed and measured from digital photographs using the Axiovision SE64 Rel. 4.9.1 software (Zeiss, Oberkochen, Germany).

Total genomic DNA was extracted using a nematode lysis buffer (Holterman et al., 2006) consisting of 0.2 M NaCl, 0.2 M Tris-HCl (pH 8.0), 1% (v/v)  $\beta$ -mercaptoethanol, and 800  $\mu$ g/mL proteinase-K. DNA was stored at  $-20^{\circ}\text{C}$  if not used immediately. The 18S rDNA gene primer sets used in this study were 988-F (5'-CTCAAAGATTAAGCCATGC-3')/1096-R (5'-GGTAATTCTGGAGCTAATAC-3')/1912R (5'-TTTACGGTCAGAACTAGGG-3'), 1813-F (5'-CTGC

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**Fig. 1.** *Dorylaimoides elegans* (de Man, 1880) Thorne and Swanger, 1936. A, Entire female; B, Female neck region; C, Female reproductive system; D, Female posterior region; E, Male posterior region. Scale bars: A-E=100  $\mu$ m.

GTGAGAGGTGAAAT-3')/2646-R (5'-GCTACCTTGTTAC GACTTTT-3') (Holterman et al., 2006). PCR (50  $\mu$ L) was performed using 2  $\mu$ L template DNA, 10 pmol of each primer, 10  $\times$  Ex Taq buffer, 0.2 mM dNTP mixture, and 1.25 U of TaKaRa Ex Taq polymerase (TaKaRa, Otsu-Shiga, Japan). The conditions were as follows: initial denaturation at 95°C for 1 min, 40 cycles of denaturation at 95°C for 30 s, annealing at 50°C for 30 s, extension at 72°C for 1 min, and final extension at 72°C for 10 min. PCR products were purified with a QIAquick PCR Purification Kit (Qiagen, Hilden, Germany) and then sequenced using a 3730xl DNA Analyzer (Thermo Fisher Scientific, Waltham, MA, USA). The resulting 18S rDNA sequences were deposited in GenBank. Sequence analyses were performed using Geneious v11.0.5 (Biomatters, Auckland, New Zealand) (Kearse et al., 2012) and aligned with

sequences of available congeneric nematode species using Clustal X with default options (Thompson et al., 1997).

## SYSTEMATIC ACCOUNTS

Order Dorylaimida Pearse, 1942  
 Superfamily Tylencholaimoidea Filipjev, 1934  
 Family Mydonomidae Thorne, 1964  
 Genus *Dorylaimoides* Thorne and Swanger, 1936

<sup>1</sup>\**Dorylaimoides elegans* (de Man, 1880)  
**Thorne and Swanger, 1936 (Table 1, Fig. 1)**

*Dorylaimus elegans* de Man, 1880: 86.

*Dorylaimoides elegans*: Thorne and Swanger, 1936: 129–130, Pl. XXIX, fig. 174.

Korean name: <sup>1</sup>\*예쁜창선충 (신칭)

**Table 1.** Morphometrics of *Dorylaimoides elegans* and *D. limnophilus*

| Character                      | <i>D. elegans</i> |         | <i>D. limnophilus</i>           |
|--------------------------------|-------------------|---------|---------------------------------|
|                                | ♀, n=1            | ♂, n=1  | ♀, n=3                          |
| L                              | 1,318.2           | 1,241.2 | 1,207.3±154.0 (1,037.8–1,338.8) |
| Body width                     | 36.6              | 35.5    | 32.3±3.8 (28.6–36.1)            |
| Pharynx length                 | 192.7             | 209.1   | 209.1±16.4 (192.7–225.6)        |
| Tail length                    | 42.0              | 32.6    | 126.9±20.3 (105.0–145.1)        |
| Anal body diameter             | 25.3              | 25.1    | 19.5±2.5 (16.8–21.6)            |
| a                              | 36.1              | 35.0    | 37.4±1.3 (36.4–38.8)            |
| b                              | 6.8               | 5.9     | 5.8±0.3 (5.4–6.0)               |
| c                              | 31.4              | 38.1    | 9.5±0.3 (9.2–9.9)               |
| c'                             | 1.7               | 1.3     | 6.5±0.2 (6.3–6.7)               |
| V                              | 45.6              | –       | 31.0±0.9 (29.9–31.5)            |
| Lip region diameter            | 9.2               | 8.2     | 8.2±0.2 (8.0–8.3)               |
| Lip region height              | 4.1               | 3.9     | 3.4±0.4 (3.0–3.8)               |
| Amphid aperture                | 6.7               | 6.0     | 6.0±0.1 (6.0–6.1)               |
| Odontostyle (ventral side)     | 7.7               | 7.6     | 5.7±0.3 (5.4–6.0)               |
| Odontophore                    | 15.8              | 14.9    | 15.8±0.5 (15.2–16.2)            |
| Pharyngeal bulb length         | 63.7              | 58.6    | 65.2±2.8 (62.5–68.0)            |
| Cardia diameter                | 7.3               | 7.1     | 12.0±1.0 (11.1–13.0)            |
| Cardia length                  | 7.5               | 7.6     | 8.1±0.7 (7.5–8.8)               |
| Guiding ring from anterior end | 8.1               | 8.0     | 6.3±0.2 (6.1–6.5)               |
| Nerve ring from anterior end   | 105.4             | 96.2    | 99.3±4.4 (94.8–103.5)           |
| Nerve ring (% pharynx)         | 54.7              | 46.0    | 47.6±1.7 (45.9–49.2)            |
| Vulva from anterior end        | 601.7             | –       | 375.0±57.4 (311.1–422.2)        |
| Vulva to anus                  | 674.5             | –       | 705.5±76.4 (621.7–771.5)        |
| Vulva to anus/tail length      | 16.1              | –       | 5.6±0.3 (5.3–5.9)               |
| Vagina diameter                | 18.3              | –       | 14.5±1.7 (13.0–16.4)            |
| Vagina length                  | 20.9              | –       | 15.3±1.6 (13.9–17.0)            |
| Vagina/body diameter           | 0.6               | –       | 0.5                             |
| Reproductive tract length (G1) | 185.1             | –       | –                               |
| Reproductive tract length (G2) | 207.5             | –       | 210.7±58.0 (147.9–262.1)        |
| G1 (%)                         | 14.0              | –       | –                               |
| G2 (%)                         | 15.7              | –       | 17.2±2.7 (14.3–19.6)            |
| Spicules                       | –                 | 37.1    | –                               |
| Spicules/anal body diameter    | –                 | 1.5     | –                               |
| Spicules/tail length           | –                 | 1.2     | –                               |
| Ventromedian supplements       | –                 | 5.0     | –                               |
| Rectum                         | 33.2              | 32.2    | 22.6±2.2 (20.6–25.0)            |
| Rectum/anal body diameter      | 1.3               | 1.3     | 1.2±0.1 (1.1–1.2)               |

All measurements are in  $\mu\text{m}$  and in the form mean $\pm$ SD (range).

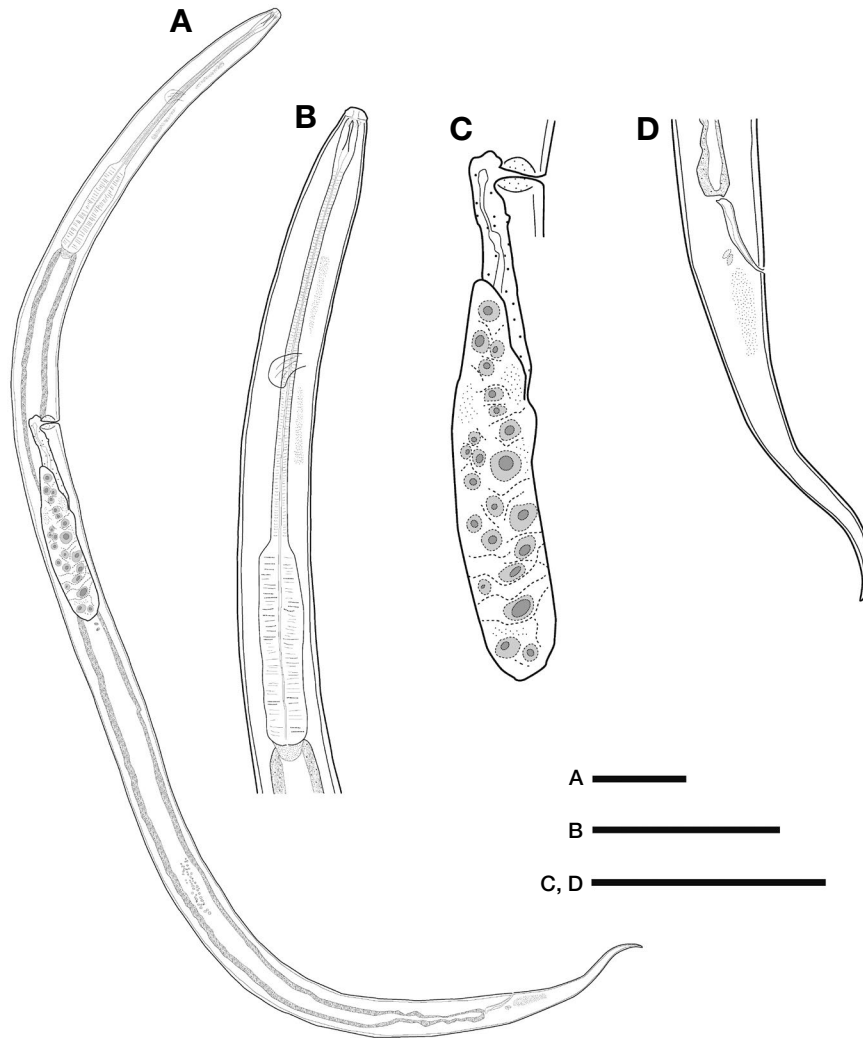
L, body length; a, body length/body diameter; b, body length/distance from anterior to base of esophageal glands; c, body length/tail length; c', tail length/diameter at anus region; V, % distance of vulva from anterior end/body length; G1, % length of anterior female gonad in relation to body length; G2, % length of posterior female gonad in relation to body length.

**Material examined.** 1♀, 1♂, Korea: Gyeonggi-do, Yeosu, Neungseo-myeon, Wangdae-ri, 37°19'43.82"N, 127°36'15.40"E, 25 Feb 2019. Voucher specimens were deposited in the Nakdonggang National Institute of Biological Resources (NNIBR), Korea.

**Measurements.** See Table 1.

**Description.** Female: Body slender, ventrally curved after

fixation, length 1,318.2  $\mu\text{m}$ , width 36.6  $\mu\text{m}$  (maximum width at level of vulva). Cuticle with fine transverse striations, 1.2  $\mu\text{m}$  thick at anterior region, 2.0  $\mu\text{m}$  at mid-body, and 5.1  $\mu\text{m}$  at tail. Lip region rounded, slightly offset, about 2.2 times as wide as high. Amphid funnel-shaped aperture 6.7  $\mu\text{m}$ , about 0.7 times as wide as lip region width. Odontostyle asymmetrical and slightly arched, ventral side length 7.7  $\mu\text{m}$ . Odon-



**Fig. 2.** *Dorylaimoides limnophilus* (de Man, 1880) Loof, 1964. A, Entire female; B, Female neck region; C, Female reproductive system; D, Female posterior region. Scale bars: A-D=100  $\mu$ m.

tophore arcuated, narrowing posteriorly, about 2.1 times as long as odontostyle. Guiding ring distinct and single, located 8.1  $\mu$ m from anterior end. Pharynx with a slender anterior and cylindrical bulb, 63.7  $\mu$ m in length, and about 28% of total neck length. Cardia rounded and distally enclosed by intestinal tissue, about 2.2 times as long as body width. Nerve ring located 105.4  $\mu$ m from anterior end, 54.7% of pharynx length. Excretory pore obscure. Female reproductive system didelphic-amphidelphic. Vulva transverse, located at 45.6% of body length. Vagina length 0.5 times body diameter. Uterus length 2.3–3.3 times body diameter. Oviduct length 1.8–3.0 times body diameter. Ovary reflexed, usually reaching junction of oviduct and uterus, anterior reproductive tract (Q1) 185.1  $\mu$ m, posterior (Q2) 207.5  $\mu$ m long. Rectum length about 1.3 times anal body diameter. Tail dorsally convex conoid,

with digitate tip. Male: General morphology similar to female, except for posterior region. Body ventrally hooked after fixation, length 1,241.2  $\mu$ m, width 35.5  $\mu$ m (maximum width at mid-body). Cuticle annuli 1.0  $\mu$ m thick at anterior region, 1.5  $\mu$ m at mid-body, and 4.9  $\mu$ m at tail. Lip region width 8.2  $\mu$ m. Amphid aperture 6.0  $\mu$ m. Odontostyle ventral side length 7.6  $\mu$ m. Odontophore 1.9 times odontostyle length. Nerve ring located at 46% of pharynx length. Genital system opposite. Spicules dorylaimoid, 37.1  $\mu$ m long, 1.1 times anal body diameter, with 11.0  $\mu$ m long lateral accessory pieces. Supplements mammiform, five ventromedians. Tail similar to female, with digitate terminus.

**Habitat.** Freshwater and sediment.

**Distribution.** America, Canada, Estonia, India, Korea (this study), the Netherlands, Romania.

**<sup>1</sup>\**Dorylaimoides limnophilus* (de Man, 1880)****Loof, 1964 (Table 1, Fig. 2)***Dorylaimus limnophilus* de Man, 1880: 96.*Thornenema limnophilum* Andrásy, 1959b: 195.*Dorylaimoides riparius* Andrásy, 1962: 6–8, Abb. 3.*Dorylaimoides* (*Tarjania*) *limnophilus*: Loof, 1964: 287.

**Material examined.** 3♀♀, Korea: Gyeonggi-do, Yeosu-si, Neungseo-myeon, Wangdae-ri, 37°19'43.82"N, 127°36'15.40"E, 25 Feb 2019. Voucher specimens were deposited in the Nakdonggang National Institute of Biological Resources (NNIBR), Korea.

**Measurements.** See Table 1.

**Description.** Female: Body slender, ventrally curved after fixation, length 1,037.8–1,338.8 µm, width 28.6–36.1 µm (maximum width at level of vulva). Cuticle with fine transverse striations, 1.5–1.7 µm thick at anterior region, 2.0–2.3 µm at mid-body, and 4.2–4.6 µm at tail. Lip region angular, offset by a constriction, about 2.6 times as wide as high. Amphid cup-shaped, aperture 6.0–6.1 µm, about 0.7 times as wide as lip region width. Odontostyle asymmetrical and slightly arched, ventral side length 5.4–6.0 µm. Odontophore arcuated, narrowing posteriorly, about 2.8 times as long as odontostyle. Guiding ring distinct and single, located 6.1–6.5 µm from anterior end. Pharynx with a slender anterior and cylindrical bulb, length 62.5–68.0 µm and about 30% of total neck length. Cardia rounded and distally enclosed by intestinal tissue. Nerve ring located at 94.8–103.5 µm from anterior end, 45.9–49.2% of pharynx length. Excretory pore obscure. Reproductive system mono-opisthodelphic. Vulva transverse, located at 29.9–31.5% of body length. Vagina length 0.5 times body diameter. Uterus short. Oviduct short. Ovary reflexed, with numerous oocytes. Rectum length about 1.2 times anal body diameter. Tail long, filiform, tapering to terminus. Male: Not found.

**Habitat.** Freshwater and sediment.

**Distribution.** Belgium, Germany, Holland, Hungary, Italy, Japan, Korea (this study), the Netherlands, Romania, Slovakia, Spain, Sweden, Switzerland, Uzbekistan.

**Molecular sequence information.** Molecular sequences (partial 18S rDNA sequences) deposited in GenBank: *D. elegans* (GenBank accession no: MN880408) and *D. limnophilus* (GenBank accession no: MN880407).

**Diagnosis and molecular analysis.** Morphological and morphometric characters reported herein generally match those previously reported for these two *Dorylaimoides* species (de Man, 1880; Thorne and Swanger, 1936; Jairajpuri and Ahmad, 1992 for *D. elegans*; de Man, 1880; Andrásy, 1959a, 1959b, 1962; Loof, 1964; Peralta and Peña-Santiago, 1995b; Loof,

1990 for *D. limnophilus*), except for spicule length (37.1 vs. 30–32 µm) (Goseco et al., 1976) for *D. elegans*. The two *Dorylaimoides* species reported in this study were distinguished by their morphological characteristics: a didelphic-amphidelphic reproductive system, short tail, and digitate terminus in *D. elegans* and a mono-opisthodelphic reproductive system and long tail in *D. limnophilus* (Ahmad and Jairajpuri, 1983; Peralta and Peña-Santiago, 1995a, 1995b; Khan and Park, 1999; Ahmad et al., 2003; Ahmad and Mushtaq, 2004; Pedram et al., 2011; Gagarin and Gusakov, 2015). In addition, we obtained partial 18S rDNA sequences for the two *Dorylaimoides* species and assessed sequence similarity with other *Dorylaimoides* species from GenBank. The 18S sequences of our specimens are almost identical to those of *D. elegans* (AY146486.2, AY911976.1, AY911977.1; 99.8–100%) and *D. limnophilus* (AY284829.1, AY593950.1; 99.5–100%) deposited on GenBank.

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No potential conflict of interest relevant to this article was reported.

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