

First Report of Five *Tobrilus* Species (Nematoda: Triplonchida) from Korea

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ABSTRACT

Member of the genus *Tobrilus* Andr ssy, 1959, which belongs to Tobrilidae Filipjev, 1918, are known as free-living nematodes in freshwater habitat. This genus was previously unknown from Korea. Five *Tobrilus* species are here reported for the first time from Korea: *Tobrilus aberrans* (Schneider, 1925), *Tobrilus diversipapillatus* (Daday, 1905), *Tobrilus gracilis* (Bastian, 1865), *Tobrilus longus* (Leidy, 1851), and *Tobrilus wesenbergi* (Micoletzky, 1925). Specimens were collected from sediments of the Nakdong River in Korea. Morphological characters and measurements of the specimens generally agree with the original descriptions of *Tobrilus* species, except for some differences that can be attributed to intraspecific variation among populations (e.g., nerve ring position [% pharynx] and reproductive length). Each species can be distinguished from other members of the genus by specific characters (e.g., cephalic setae length and position, buccal cavity and pocket shape, vulva position, degree of development of reproductive system, and tail length and shape). Here, five species in the genus *Tobrilus* are fully redescribed and illustrated using optical microscopy images. DNA barcode sequence information (the D2–D3 region of 28S rDNA) is also provided for molecular species identification.

Keywords: nematode, Triplonchida, *Tobrilus*, freshwater, Korea

INTRODUCTION

Tobrilidae Filipjev, 1918 are free-living nematodes and exist in almost all soil and aquatic habitats. Member of the genus *Tobrilus* Andr ssy, 1959, which belongs to Tobrilidae Filipjev, 1918, are known as free-living nematodes with a worldwide freshwater distribution. About 80 species have been described in this genus, but *Tobrilus* species have never previously been reported from Korea. In this study, we report five *Tobrilus* species (*Tobrilus aberrans* [Schneider, 1925], *Tobrilus diversipapillatus* [Daday, 1905], *Tobrilus gracilis* [Bastian, 1865], *Tobrilus longus* [Leidy, 1851], and *Tobrilus wesenbergi* [Micoletzky, 1925]) for the first time from Korea and describe their morphological characters and morphometrics. In addition, molecular sequences of the D2–D3 region of 28S rDNA of this species are provided as DNA barcode sequence information.

Specimens were collected live from freshwater and sediment of the Nakdong River and were isolated using sieving

and the Baermann funnel method (Baermann, 1917). Specimens were placed in 2 mL water in a 15 mL tube and then fixed by the addition of 4 mL of 80°C TAF (2% triethanolamine and 7% formaldehyde) solution. Fixed nematodes were processed into glycerin (Seinhorst, 1959), mounted in glycerin on HS-slides (Shirayama et al., 1993), and examined under an optical microscope (Olympus BX-51, Tokyo, Japan) with differential interference contrast (DIC). Morphometrics were measured from digital photographs (taken with a Cool-Snap Photometrics color CCD camera) using the program QCapture Pro 5.

DNA extraction from single individuals was done with a nematode lysis buffer (Holterman et al., 2006) following the manufacturer's directions. One nematode was placed in a 0.2 mL tube with 25 μ L sterile water, and an equal volume of lysis buffer was added, consisting of 0.2 M NaCl, 0.2 M Tris-HCl (pH 8.0), 1% (v/v) β -mercaptoethanol, and 800 μ g/mL proteinase-K. Lysis was done by putting each PCR tube in a 65°C heating block for 2 h, and then incubating for 5 min at

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100°C. The lysate was then either used right away or stored at -20°C. The D2-D3 regions of the 28S rDNA gene were PCR-amplified with universal primer sets (D2A [5'-ACAAG-TACCGTGAGGGAAAGTTG-3']/D3B [5'-TCGGAAG-GAACCAGCTACTA-3']) (De Ley et al., 1999). PCR reactions took place in 50 µL including 2 µL template DNA, 10 pmol of each primer, 10× Ex Taq buffer, 0.2 mM dNTP mixture, and 1.25 U of Taq polymerase (TaKaRa Ex Taq, Japan). PCR amplification conditions were an initial denaturing 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 a final extension at 72°C for 10 min. A QIAquick Gel Extraction Kit (Qiagen, Hilden, Germany) was used to purify the PCR products according to the manufacturer's instructions. Fragments were sequenced on a Big Dye Terminator Cycle-Sequencer (Applied Biosystems, Waltham, MA, USA). The resulting DNA sequences were deposited in GenBank. Sequence analyses were done in Geneious v11.0.5 (Biomatters, Auckland, New Zealand) (Kearse et al., 2012). Sequences from the specimens were aligned in Clustal X with default options (Thompson et al., 1997) together with sequences of other nematode species of the same genus from GenBank. MEGA 5.2.2 was used to reconstruct a molecular phylogenetic tree using the maximum likelihood (ML) method with 1,000 bootstrap replications (Tamura et al., 2011). Genetic distances were calculated with the Kimura-2-parameter model (Kimura, 1980).

SYSTEMATIC ACCOUNTS

Order Triplonchida Cobb, 1920
Suborder Tobrilina Tsalolikhin, 1922
Superfamily Tobrioloidea Filipjev, 1918
Family Tobrilidae Filipjev, 1918
Genus *Tobrilus* Andrassy, 1959

¹**Tobrilus aberrans* (Schneider, 1925) (Table 1, Fig. 1)

Trilobus gracilis var. *aberrans* Schneider, 1925: 544–555, figs. 1–5.

Tobrilus aberrans: Andrassy, 1959: 224.

Material examined. 5♀♀, Korea: Daegu, Dalseong-gun, Dasa-eup, Gangjeongbon-gil, 57, 35°50'15.69"N, 128°28'3.94"E, 17 Oct 2017. Voucher specimens are deposited in the Nakdonggang National Institute of Biological Resources (NNIBR), Korea.

Measurements. See Table 1.

Description. Female: Body slightly curved ventrad after

fixation; length 1,457.0–1,707.2 µm, width 40.6–49.5 µm (maximum value at a level of vulva). Cuticle thin and annulated, in total 1 µm thick at mid-body. Lip region with cephalic setae: longer cephalic setae 4.0–6.8 µm, shorter setae 1.8–2.1 µm long. Buccal cavity cylindrical, thick-walled, with length about 1.3–1.7 times the width, and with funnel-shaped base. Amphid cup-like with expressed filaments, its aperture 5.1–5.2 µm, located 9.5–12.3 µm from anterior end, situated at the level of the border of the buccal cavity and pockets. Teeth in two pockets, situated almost at the same level, 2.5–3.6 µm in length. Nerve ring located 149.3–187.9 µm from anterior end of body. Female reproductive system amphidelphic, weakly developed. Female anterior reproductive tract (Q1) 59.9–140.7 µm, posterior (Q2) 60.1–113.8 µm long. Vulva a transverse slit, located at 37.5–49.4% of body length. Vagina perpendicular to body axis, musculature weak. Rectum length 1.1–1.4 times anal body width. Tail tapering, terminus distinctively clavate. Subterminal seta absent. Male: Not found.

Habitat. Freshwater and sediment.

Distribution. Russia, Germany, Lithuania, Canada, Israel, Korea.

²**Tobrilus diversipapillatus* (Daday, 1905)

(Table 1, Fig. 2)

Trilobus diversipapillatus Daday, 1905: 54, Taf. II, figs. 18–23; Taf. III, fig. 1.

Tobrilus diversipapillatus: Andrassy, 1959: 224.

Material examined. 1♀, Korea: Daegu, Dalseong-gun, Dasa-eup, Gangjeongbon-gil, 57, 35°50'15.69"N, 128°28'3.94"E, 17 Oct 2017. Voucher specimen is deposited in the Nakdonggang National Institute of Biological Resources (NNIBR), Korea.

Measurements. See Table 1.

Description. Female: Body slightly curved ventrad after fixation; length 2381.6 µm, width 65.0 µm (maximum value at a level of vulva). Cuticle thin and annulated, in total 1 µm thick at mid-body. Lip region with cephalic setae: longer cephalic setae 7.6 µm, shorter setae 1.9 µm long. Buccal cavity cylindrical, thick-walled, with length about 1.2 times the width, and with funnel-shaped base. Amphid cup-like with expressed filaments, its aperture 4.8 µm, located 18.2 µm from anterior end, situated at the level of the anterior pocket. Teeth in two distinct pockets, separated by 11.1 µm. Nerve ring located 111.2 µm from anterior end of body. Female reproductive system amphidelphic, well developed. Female anterior reproductive tract (Q1) 289.6 µm, posterior (Q2) 270.4 µm long. Vulva a transverse slit, located at 39.4% of

Korean name: ¹*짧은털주머니선충 (신칭), ²*긴몸주머니선충 (신칭)

Table 1. Morphometrics of five *Tobrilus* species

| Character | <i>Tobrilus aberrans</i> (♀, n=5) | <i>Tobrilus diversipapillatus</i> (♀, n=1) | <i>Tobrilus gracilis</i> (♀, n=1) | <i>Tobrilus longus</i> (♀, n=1) | <i>Tobrilus wesenbergi</i> (♀, n=1) |
|-----------------------------------|--------------------------------------|---|--------------------------------------|------------------------------------|--|
| L | 1,599.9±112.9 (1,457.0-1,707.2) | 2,381.6 | 2,353.4 | 1,284.7 | 1,842.1 |
| Body width | 44.9±4.1 (40.6-49.5) | 65.0 | 71.8 | 48.2 | 48.9 |
| Pharynx length | 283.7±9.1 (274.8-297.3) | 350.5 | 383.7 | 239.1 | 316.7 |
| Tail length | 153.3±17.2 (135.0-174.1) | 240.6 | 225.7 | 162.7 | 272.9 |
| Anal region body width | 28.7±2.7 (26.5-33.5) | 33.5 | 31.0 | 26.5 | 31.1 |
| a | 35.9±4.2 (29.4-41.1) | 36.7 | 32.8 | 26.7 | 37.7 |
| b | 5.6±0.5 (5.1-6.2) | 6.8 | 6.1 | 5.4 | 5.8 |
| c | 10.6±1.6 (9.1-12.4) | 9.9 | 10.4 | 7.9 | 6.8 |
| c' | 5.4±0.6 (4.8-6.2) | 7.2 | 7.3 | 6.2 | 8.8 |
| Lip region width | 19.2±1.3 (17.9-21.2) | 13.7 | 18.1 | 18.2 | 14.4 |
| Longer cephalic seta | 4.9±1.1 (4-6.8) | 7.6 | 8.1 | 8.4 | 5.5 |
| Amphid location from anterior end | 10.7±1.3 (9.5-12.3) | 18.2 | 15.0 | 2.8 | 10.2 |
| Amphid aperture | 5.1±0.1 (5.1-5.2) | 4.8 | 5.8 | 2.9 | 4.2 |
| Buccal cavity length | 13.3±0.6 (12.8-14.3) | 11.4 | 14.4 | 11.6 | 12.5 |
| Buccal cavity width | 9.0±0.5 (8.3-9.5) | 8.9 | 11.5 | 8.9 | 9.9 |
| Stoma length | 23.7±0.9 (22.3-24.6) | 21.8 | 26.5 | 16.3 | 16.1 |
| Length between pocket teeth | 2.9±0.5 (2.5-3.6) | 1.6 | 2.9 | 10.1 | 4.5 |
| Nerve ring from anterior end | 164.1±16.6 (149.3-187.9) | 111.2 | 112.9 | 86.4 | 73.8 |
| Nerve ring (% pharynx) | 58.5±5.4 (54.3-66.3) | 31.7 | 29.4 | 36.1 | 23.3 |
| V (%) | 41.8±4.6 (37.6-49.4) | 39.4 | 42.2 | 45.1 | 43.7 |
| Anterior reproductive | 88.6±33.2 (59.9-140.7) | 289.6 | 413.0 | 177.5 | 180.8 |
| Posterior reproductive | 76.8±22.8 (60.1-113.8) | 270.4 | 453.1 | 188.4 | 169.1 |
| G1 (%) | 5.5±1.8 (3.8-8.2) | 12.2 | 17.5 | 13.8 | 9.8 |
| G2 (%) | 4.8±1.2 (3.6-6.7) | 11.4 | 19.3 | 14.7 | 9.2 |
| Rectum | 37.4±4.4 (32.7-44.1) | 42.0 | 44.2 | 33.2 | 32.0 |
| Rectum/anal region body width | 1.3±0.1 (1.2-1.4) | 1.3 | 1.4 | 1.3 | 1.0 |
| Vulva-anus distance | 793.9±109.8 (667-886.1) | 1,198.5 | 1,157.9 | 537.3 | 74.7 |

All measurements are in µm and in the form mean±SD (range).

L, body length; a, body length/body diameter; b, body length/body diameter; c, body length/diameter at anus region; 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.

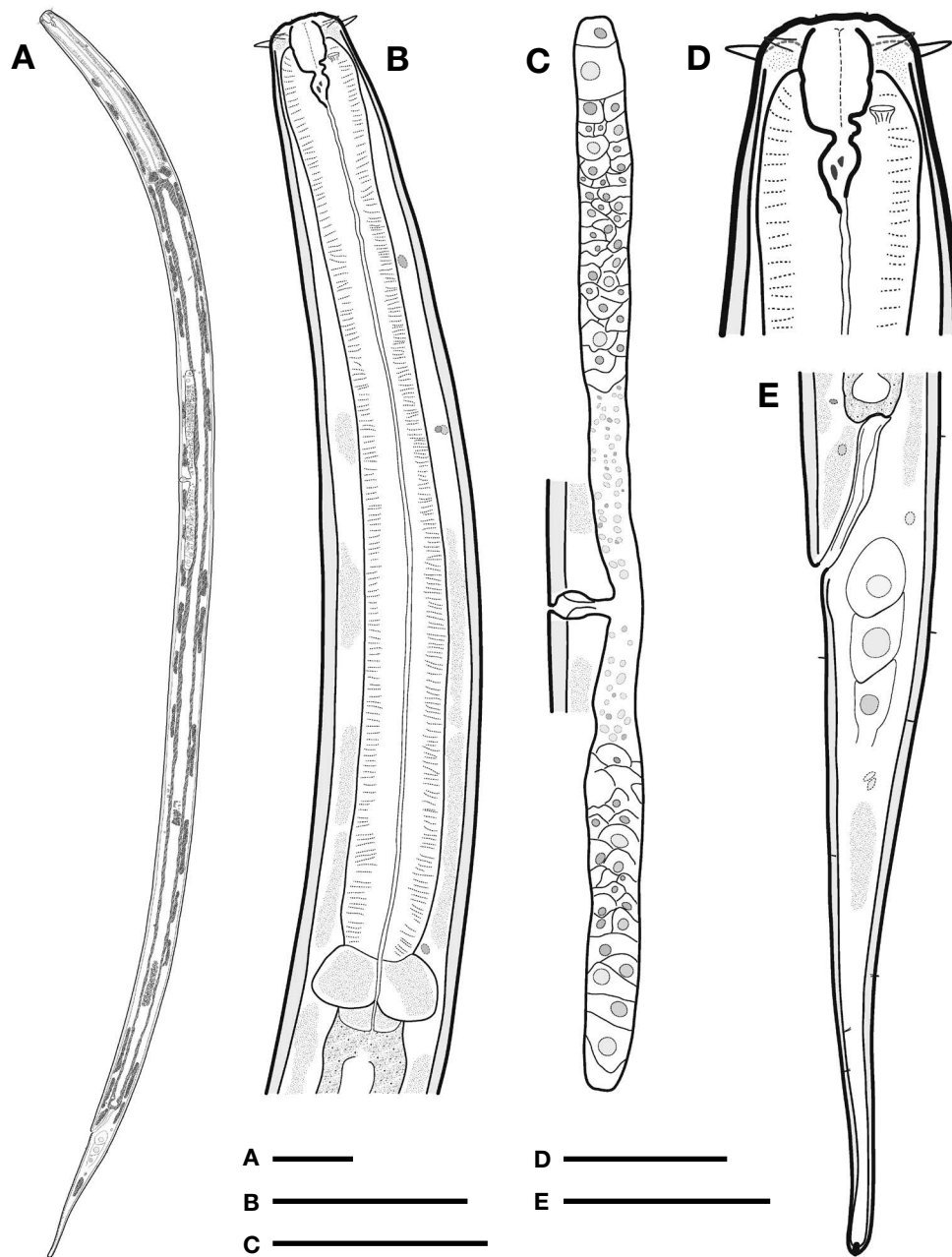


Fig. 1. *Tobrilus aberrans* (Schneider, 1925). A, Entire female; B, Pharyngeal region; C, Reproductive system; D, Head region; E, Posterior region. Scale bars: A=100 μm , B, C, E=50 μm , D=20 μm .

body length. Vagina distinctly oblique to body axis, conspicuous, musculature very strong. Rectum length 1.3 times anal body width. Tail tapering, terminus distinctively clavate, with dorsal subterminal seta. Male: Not found.

Habitat. Freshwater and sediment.

Distribution. Paraguay, Germany, Australia, Korea.

¹*Tobrilus gracilis* (Bastian, 1865) (Table 1, Fig. 3)

Trilobus gracilis Bastian, 1865: 99, figs. 20–22.

Tobrilus gracilis: Andr assy, 1959: 224.

Material examined. 1 ♀, Korea: Gyeongsangbuk-do, Sangju-si, Jungdong-myeon, Osang-ri, 968-1, 36°26'24.24"

Korean name: ¹*긴털주머니선충 (신칭)

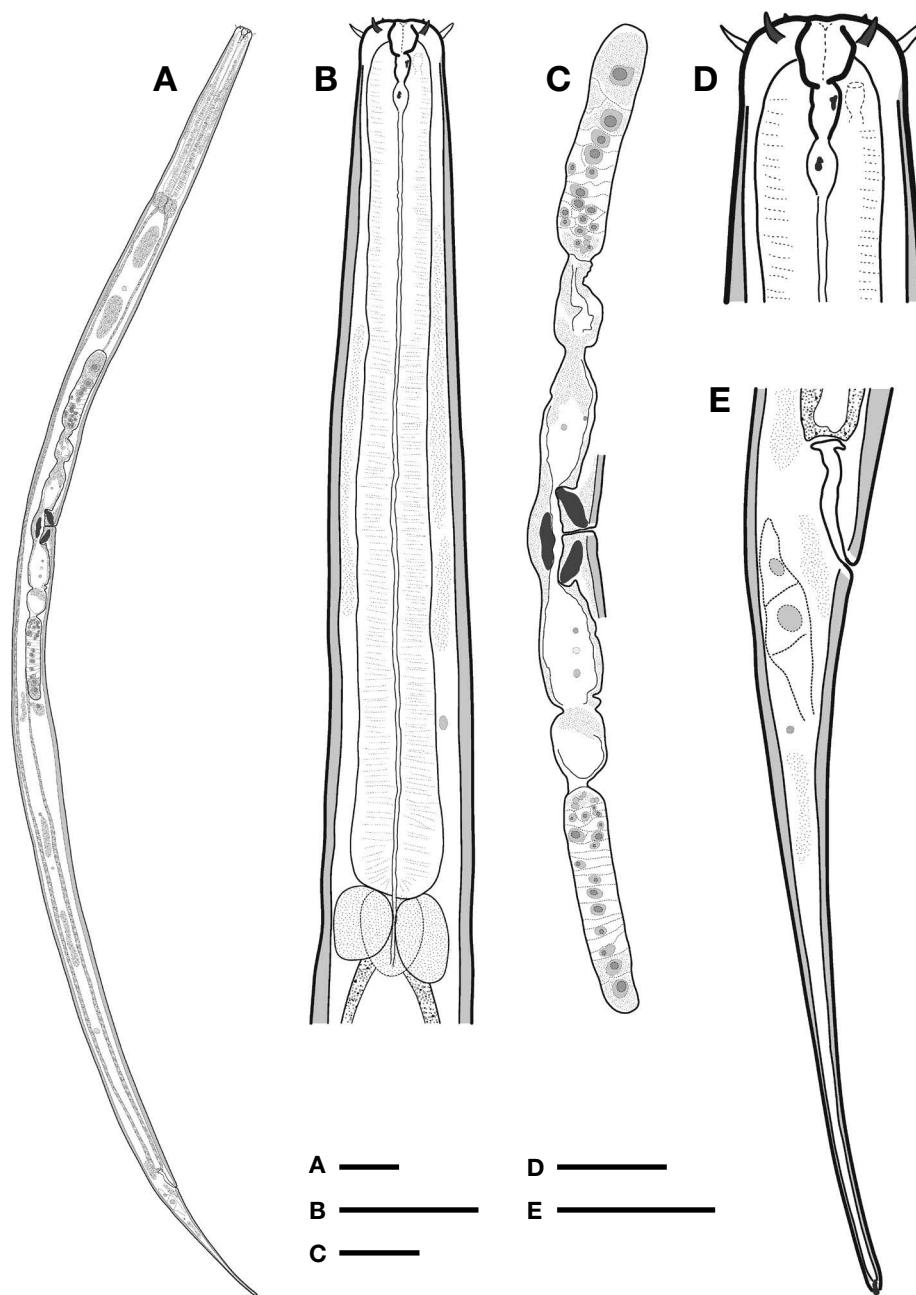


Fig. 2. *Tobrilus diversipapillatus* (Daday, 1905). A, Entire female; B, Pharyngeal region; C, Reproductive system; D, Head region; E, Posterior region. Scale bars: A=100 μ m, B, C, E=50 μ m, D=20 μ m.

N, 128°15'26.60"E, 17 Oct 2017. Voucher specimen is deposited in the Nakdonggang National Institute of Biological Resources (NNIBR), Korea.

Measurements. See Table 1.

Description. Female: Body slightly curved ventrad after fixation; length 2,353.4 μ m, width 71.8 μ m (maximum value at a level of vulva). Cuticle thin and annulated, in total 1 μ m thick at mid-body. Lip region with cephalic setae: lon-

ger cephalic setae 8.1 μ m, shorter setae 2.0 μ m long. Buccal cavity cylindrical, thick-walled, with length about 1.2 times the width, and with funnel-shaped base. Amphid cup-like with expressed filaments, its aperture 5.8 μ m, located 15.0 μ m from anterior end, situated at the level of the border of the buccal cavity and pockets. Teeth in two pockets, situated almost at the same level, 2.9 μ m length. Nerve ring located 112.9 μ m from anterior end of body. Female repro-

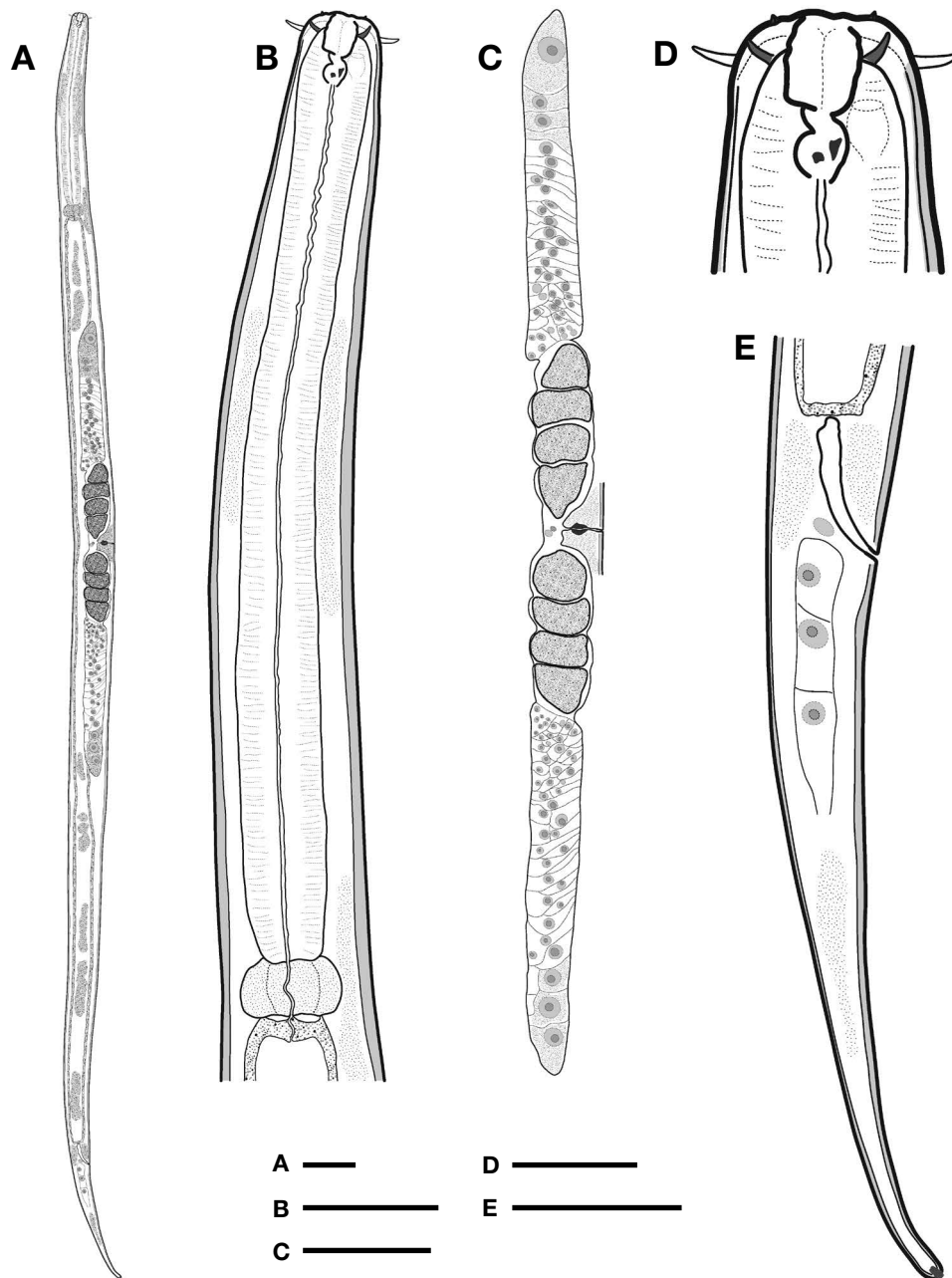


Fig. 3. *Tobrilus gracilis* (Bastian, 1865). A, Entire female; B, Pharyngeal region; C, Reproductive system; D, Head region; E, Posterior region. Scale bars: A, C=100 μ m, B, E=50 μ m, D=20 μ m.

ductive system amphidelphic, well developed. Female anterior reproductive tract (Q1) 413.0 μ m, posterior (Q2) 453.1 μ m long. Vulva a transverse slit, located at 42.2% of body length. Vagina perpendicular to body axis, musculature very strong, with concentric musculature. Rectum length 1.4 times anal body width. Tail tapering, terminus distinctively

clavate. Subterminal seta absent. Male: Not found.

Habitat. Freshwater and sediment.

Distribution. Canada, Russia, Germany, Hungary, Austria, India, Korea.

¹**Tobrilus longus* (Leidy, 1851) (Table 1, Fig. 4)

Korean name: ¹*넓은몸주머니선충 (신칭)

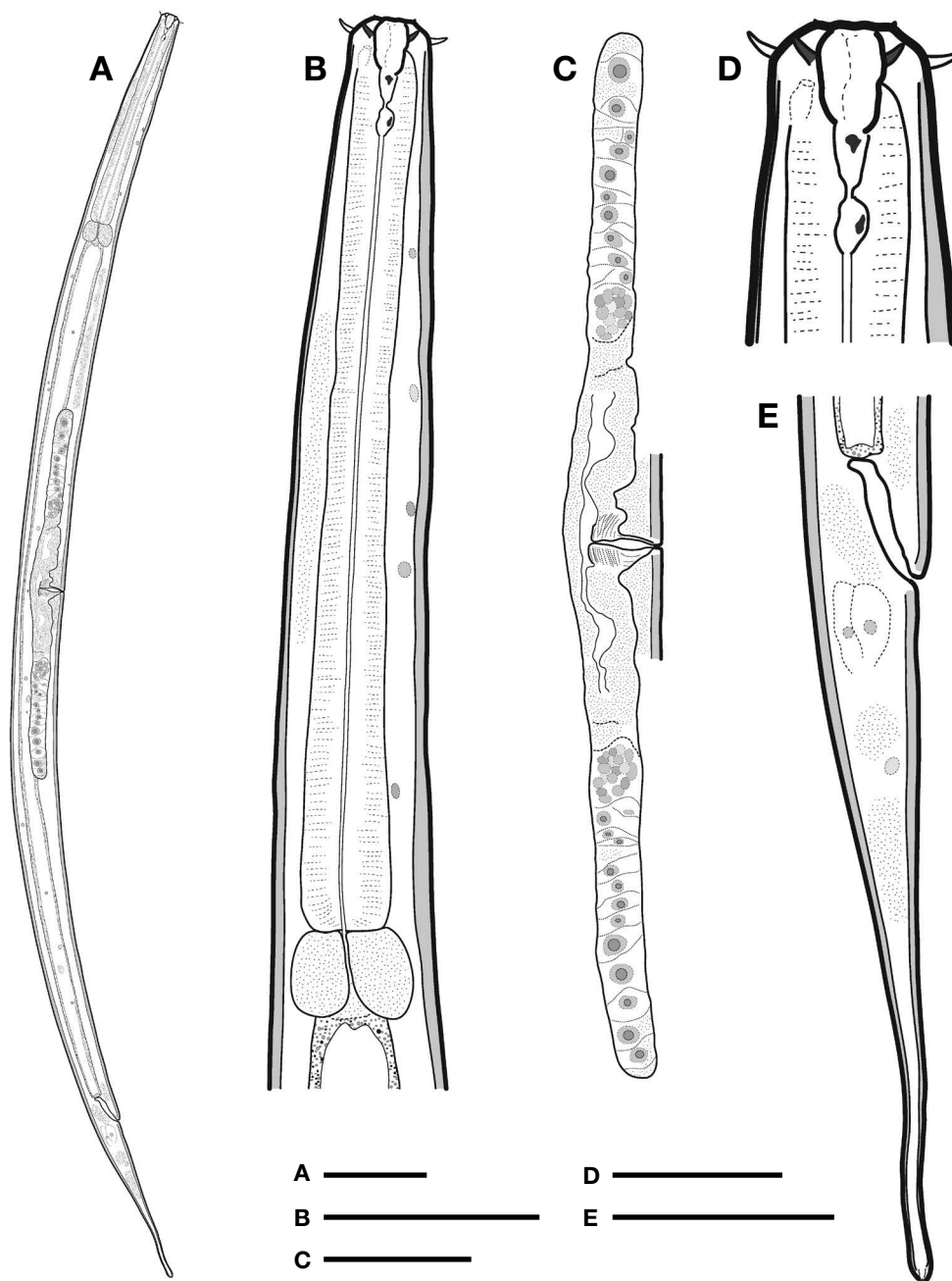


Fig. 4. *Tobrilus longus* (Leidy, 1851). A, Entire female; B, Pharyngeal region; C, Reproductive system; D, Head region; E, Posterior region. Scale bars: A=100 μm, B, C, E=50 μm, D=20 μm.

Anguillula longa Leidy, 1851: 225.

Tobrilus longus: Andrásy, 1959: 224.

Material examined. 1♀, Korea: Gyeongsangbuk-do, Chilgok-gun, Waegwan-eup, Waegwan-ri, 1330, 35°58'2.63"N, 128°23'41.04"E, 17 Oct 2017. Voucher specimen is deposited in the Nakdonggang National Institute of Biological Re-

sources (NNIBR), Korea.

Measurements. See Table 1.

Description. Female: Body slightly curved ventrad after fixation; length 1,284.7 μm, width 48.2 μm (maximum value at a level of vulva). Cuticle thin and annulated, in total 1.2 μm thick at mid-body. Lip region with cephalic setae: longer cephalic setae 8.4 μm, shorter setae 3.2 μm long. Buccal

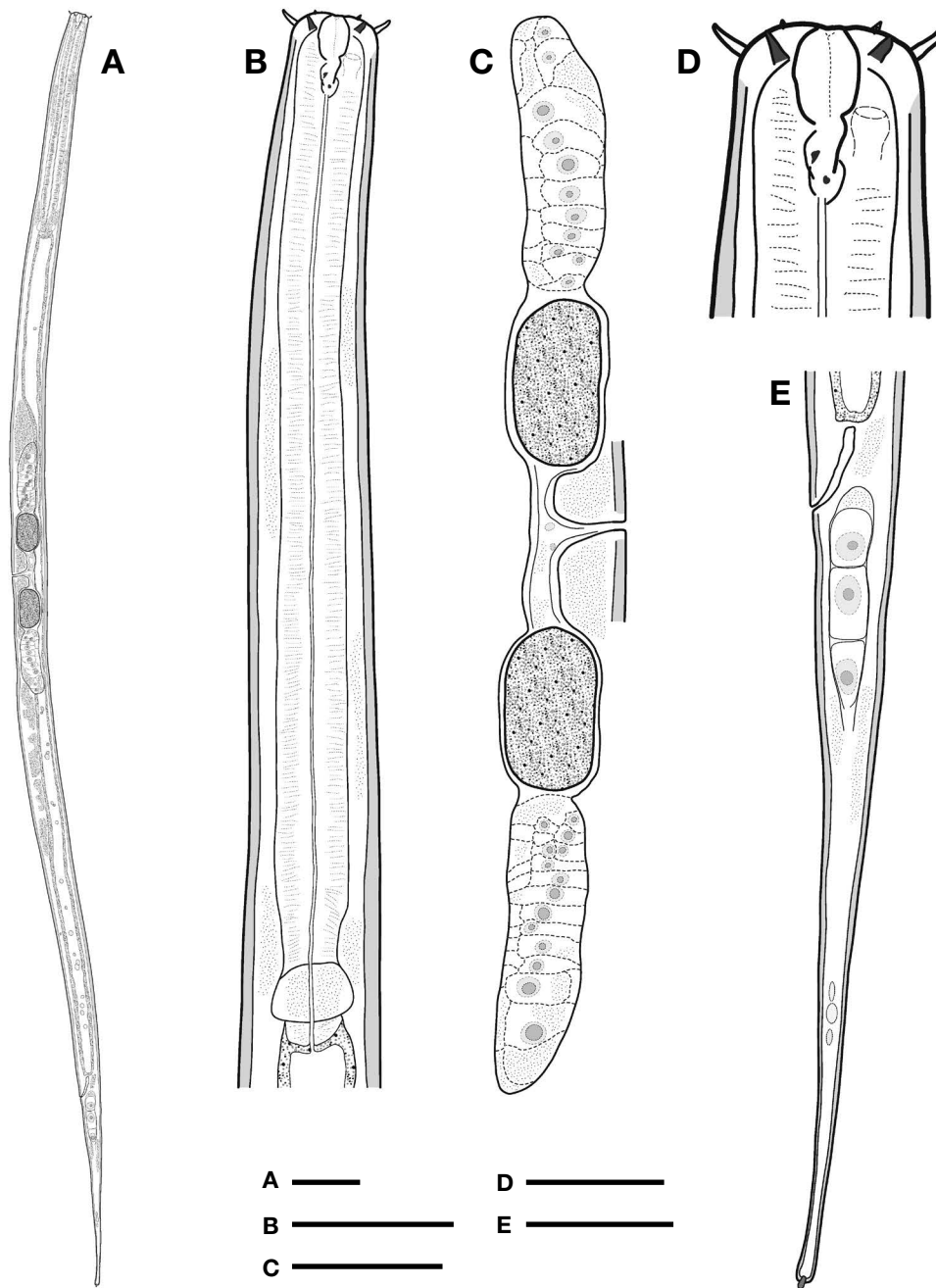


Fig. 5. *Tobrilus wesenbergi* (Micoletzky, 1925). A, Entire female; B, Pharyngeal region; C, Reproductive system; D, Head region; E, Posterior region. Scale bars: A=100 μm , B, C, E=50 μm , D=20 μm .

cavity cylindrical, thick-walled, with length about 1.3 times the width, and with funnel-shaped base. Amphid cup-like with expressed filaments, its aperture 2.9 μm , located 2.8 μm from anterior end, situated at the level of the anterior stoma. Teeth in two distinct pockets, separated by 10.1 μm . Nerve ring located 111.2 μm from anterior end of body. Female reproductive system amphidelphic, well developed. Female

anterior reproductive tract (Q1) 177.5 μm , posterior (Q2) 188.4 μm long. Vulva a transverse slit, located at 45.1% of body length. Vagina perpendicular to body axis, musculature very strong, with concentric musculature. Rectum length 1.3 times anal body width. Tail tapering, terminus distinctively clavate, with dorsal subterminal seta. Male: Not found.

Habitat. Freshwater and sediment.

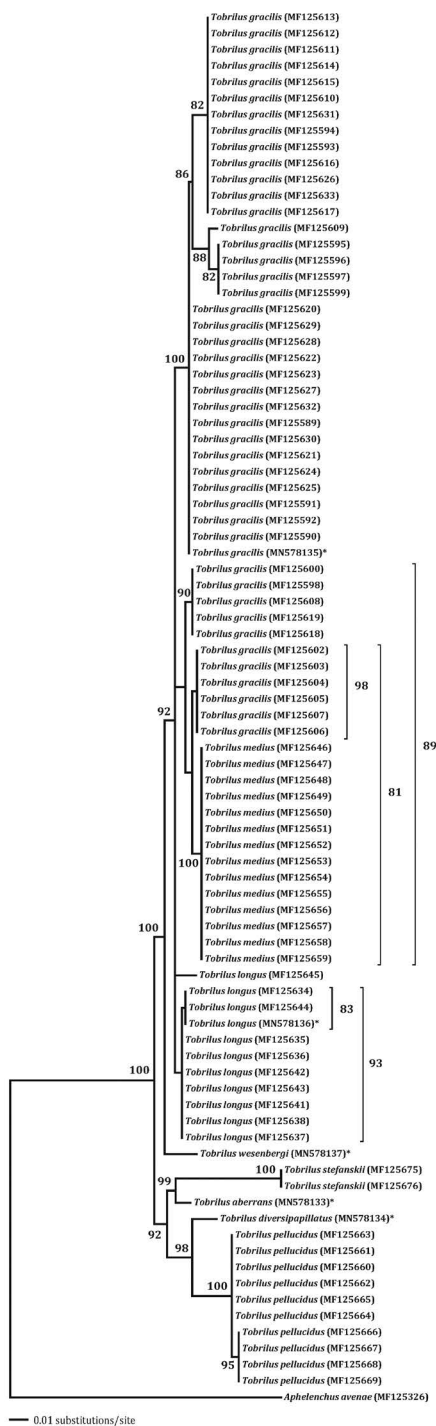


Fig. 6. Maximum likelihood phylogenetic tree based on the D2-D3 region of 28S sequences of eight *Tobrilus* species, including *Tobrilus aberrans*, *Tobrilus diversipapillatus*, *Tobrilus gracilis*, *Tobrilus longus*, and *Tobrilus wesenbergi* (*determined in this study) and outgroup (*Aphelenchus avenae*). Bootstrap values $\geq 70\%$ are shown above the branches. GenBank accession numbers are in parentheses after the name of the species.

Distribution. United States, Canada, Korea.

1Tobrilus wesenbergi* (Micoletzky, 1925)**

(Table 1, Fig. 5)

Trilobus wesenbergi Micoletzky, 1925: 147, figs. 8a-f, table TV.

Tobrilus wesenbergi: Andr ssy, 1959: 225.

Material examined. 1♀, Korea: Gyeongsangbuk-do, Chilgok-gun, Waegwan-eup, Waegwan-ri, 1330, 35°58'2.63"N, 128°23'41.04"E, 17 Oct 2017. Voucher specimen is deposited in the Nakdonggang National Institute of Biological Resources (NNIBR), Korea.

Measurements. See Table 1.

Description. Female: Body slightly curved ventrad after fixation; length 1842.1 µm, width 48.9 µm (maximum value at a level of vulva). Cuticle thin and annulated, in total 1.5 µm thick at mid-body. Lip region with cephalic setae: longer cephalic setae 5.5 µm, shorter setae 1.3 µm long. Buccal cavity cylindrical, thick-walled, with length about 1.2 times the width, and with funnel-shaped base. Amphid cup-like with expressed filaments, its aperture 4.2 µm, located 10.2 µm from anterior end, situated at the level of the border of the buccal cavity and pockets. Teeth in two pockets, situated almost at the same level, 4.5 µm in length. Nerve ring located 73.8 µm from anterior end of body. Female reproductive system amphidelphic, well developed. Female anterior reproductive tract (Q1) 180.8 µm, posterior (Q2) 169.1 µm long. Vulva a transverse slit, located at 43.7% of body length. Vagina perpendicular to body axis, musculature weak. Rectum length 1.0 times anal body width. Tail tapering, terminus distinctively clavate. Subterminal seta absent. Male: Not found.

Habitat. Freshwater and sediment.

Distribution. Denmark, Korea.

Molecular sequence information. Molecular sequences (D2-D3 region of 28S rDNA) deposited in GenBank: *Tobrilus aberrans* (GenBank accession No. MN578133), *Tobrilus diversipapillatus* (GenBank accession No. MN578134), *Tobrilus gracilis* (GenBank accession No. MN578135), *Tobrilus longus* (GenBank accession No. MN578136), and *Tobrilus wesenbergi* (GenBank accession No. MN578137).

Diagnosis and molecular analysis. The morphological characters described here agree with those previously reported for five *Tobrilus* species, and the morphometric characters are also generally within the range of previous studies (McCall and Leidy, 1851; Bastian, 1865; Daday, 1905; Micoletzky, 1925; Schneider, 1925; Andr ssy, 1959). The five *Tobrilus* species are distinguishable from other *Tobrilus*

Korean name: 1*가는뫼주머니선충 (신칭)

species by specific characters: wide buccal cavity, short cephalic setae, amphid cup-like with expressed filaments, vagina perpendicular to body axis, vagina musculature weak, short and club-like tail for *T. aberrans*; longer and slenderer body, vulva position, vagina distinctly oblique to body axis, conspicuous, musculature very strong for *T. diversipapillatus*; longer body, longer cephalic setae, large amphid, vagina perpendicular to body axis, musculature very strong, with concentric musculature for *T. gracilis*; wider body, longer cephalic setae, vagina perpendicular to body axis, vagina musculature very strong, vagina with concentric musculature for *T. longus*; and shorter and slenderer body, short cephalic setae, vagina perpendicular to body axis, musculature weak, short and slender tail for *T. wesenbergi*.

In addition to morphological redescrptions, we obtained the sequence of the D2–D3 region of 28S rDNA from each species and reconstructed an ML phylogenetic tree with some *Tobrilus* species available on GenBank (Fig. 6). We also assessed sequence similarity among *Tobrilus* species. Since sequences of the D2–D3 regions of 28S rDNA are not yet available for three *Tobrilus* species (*T. aberrans*, *T. diversipapillatus*, *T. wesenbergi*), we compared sequences for the two *Tobrilus* species (*T. gracilis* and *T. longus*) with other *Tobrilus* species available on GenBank. The resulting tree depicted two distinct groups with 100% bootstrap supporting values (Fig. 6): one group contained *T. gracilis*, *T. medius*, *T. longus*, and *T. wesenbergi*, and the other group contained *T. stefanskii*, *T. aberrans*, *T. diversipapillatus*, and *T. pellucidus*. Within the first species group, *T. gracilis* and *T. longus* sequences (including the Korean isolates; MN578135 and MN578136) clustered together and were in turn sister to *T. wesenbergi* (Korean isolates; MN578137) with 100% bootstrap value. Within the second species group, *T. aberrans* (Korean isolates; MN578133) was sister to *T. stefanskii* and *T. diversipapillatus* (Korean isolates; MN578134) was sister to *T. pellucidus* with 99% and 98% bootstrap values, respectively. Intraspecific variation in the D2–D3 region of 28S rDNA within each species was lower ($\leq 0.9\%$) than interspecific variation among *Tobrilus* species ($\geq 1.9\%$). This suggests that the D2–D3 region of 28S rDNA can be useful for species identification in the genus *Tobrilus*.

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CONFLICTS OF INTEREST

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

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