

Three Species of the Genus *Nygolaimus* (Nematoda: Dorylaimida: Nygolaimidae) Newly Recorded from Korea

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ABSTRACT

Three species of the genus *Nygolaimus* – *N. brachyuris* (De Man, 1880) Thorne, 1930, *N. obtusus* Thorne, 1930, and *N. parvus* Thorne, 1974 – are newly recorded in Korea. These three species are distinguished from their congeners by the following characteristics: short tail, wider body, and weak mural tooth in *N. brachyuris*; wider lip region, long tail, and long body in *N. obtusus*; all characteristics (such as body, pharynx, and tail length) are generally smaller in *N. parvus*. All specimens were collected from freshwater and sediment in Han River. In this study, detailed descriptions and morphometric values of these three species are provided. Additionally, DNA barcode sequence information from the 18S rDNA gene is also provided for molecular species identification.

Keywords: nematode, Dorylaimida, *Nygolaimus*, new record, freshwater, Korea

INTRODUCTION

The genus *Nygolaimus* Cobb, 1913 belongs to the family Nygolaimidae Thorne, 1935, which consists of 11 genera. *Nygolaimus* comprises approximately 24 species, which occur in terrestrial and aquatic habitats and generally have a global distribution (Baker, 1962; Jairajpuri and Ahmad, 1992). Only three Nygolaimidae species have been previously reported from Korea – namely, *Aquatides intermedius* (de Man, 1880), *Paravulvulus kyeryongensis* Choi & Jairajpuri, 1998, and *Paravulvulus papillatus* Ahmad & Jairajpuri, 1982. In the present study, we report three *Nygolaimus* species from Korea for the first time – *N. brachyuris* (De Man, 1880) Thorne, 1930, *N. obtusus* Thorne, 1930, and *N. parvus* Thorne, 1974 – providing detailed descriptions of morphological characteristics, morphometric values, and 18S rDNA gene sequences for usage as barcodes.

The 300 g sediments with freshwater samples were collected from the bottom of the Han River. Nematode specimens were extracted by sieving (mesh pore size, 45 µm and 75 µm) and the Baermann funnel method (Baermann, 1917). Specimens were fixed with 4 mL of 80°C triethanolamine formalin (TAF,

2% triethanolamine and 7% formaldehyde) solution (water including specimens: TAF = 1 : 2), processed into glycerin (Seinhorst, 1959), and mounted in glycerin on HS-slides (Shirayama et al., 1993). Morphological characteristics of specimens were observed under an optical microscope (Zeiss Imager A2, Oberkochen, Germany) with differential interference contrast. Morphometric values were obtained from digital photographs (camera model: AxioCam 506 color) using Axiovision SE64 Rel. 4.9.1.

Total genomic DNA was extracted from individual specimens using the nematode lysis buffer (Holterman et al., 2006) and stored at –20°C if not used immediately. The 18S rDNA gene was amplified using universal primer sets (988-F [5'-CTCAAAGATTAAGCCATGC-3'], 1096-R [5'-GGTATTCTGGAGCTAATAC-3'], 1912R [5'-TTTACGGTCA GAAGTAGGG-3'], 1813-F [5'-CTGCGTGAGAGGTGA AAT-3'], 2646-R [5'-GCTACCTTGTTACGACTTTT-3']) (Holterman et al., 2006). PCR reaction mixtures (to a total of 50 µL) contained 2 µL template DNA, 10 pmol of each primer, 10 × Ex Taq buffer, 0.2 mM dNTP mixture, and 1.25 U of TaKaRa Ex Taq polymerase (TaKaRa, Otsu-Shiga, Japan).

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Amplification conditions were as follows: initial denaturation at 95°C for 1 min, followed by 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. 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). Sequence analyses were performed in Geneious v11.0.5 (Kearse et al., 2012); the obtained sequences were aligned with previously available sequences of congeneric species using Clustal X (Thompson et al., 1997) with default options. The 18S rDNA sequences of the three *Nygolaimus* species are deposited in GenBank.

SYSTEMATIC ACCOUNTS

Order Dorylaimida Pearse, 1942
Superfamily Nygolaimoidea Thorne, 1935
Family Nygolaimidae Thorne, 1935
¹*Genus *Nygolaimus* Cobb, 1913

²**Nygolaimus brachyuris* (De Man, 1880) Thorne, 1930
(Table 1, Fig. 1)

Dorylaimus brachyuris De Man, 1880: 83.

Nygolaimus brachyuris: Thorne, 1930: 454–455, fig. 7.

Material examined. 3♀♀ and 1♂♂, Korea: Seoul, Mapo-gu, Hangangnang-ro, 22, 37°34'06.18"N, 126°52'18.94"E, 26 Feb 2019. Voucher specimens were deposited in the Nakdonggang National Institute of Biological Resources (NNIBRIV 57578–575780 for females and NNIBRIV575781 for males), Korea.

Measurements. See Table 1.

Description. Female: Body slender, ventrally curved after fixation, length 1,802.5–2,411.2 µm, width 41.3–49.2 µm (maximum value at level of vulva). Cuticle smooth with fine transverse striations, anterior region 1.8 µm thick, 2.5 µm at mid-body, and 3.0 µm at tail. Lip region rounded, set off by a distinct constriction, not wider than adjacent body, 14.5–17.6 µm wide, approximately 2.9–3.3 times wide as as high. Amphids obscure, aperture 5.1–6.1 µm wide, 0.4 times as wide as lip region diameter. Mural tooth deltoid, comparatively feeble, curved dorsally approximately 0.7 times lip region diameter, length 10.1–12.1 µm (ventral side) and 11.9–13.5 µm (dorsal side). Pharynx with slender muscular anterior part, narrow middle part, and posterior end expanding gradually into cylindrical bulb. Pharyngeal bulb without constriction, bulb length 221.5–230.1 µm, and approximately 33–43% of total neck length. Nerve ring located at 129.0–133.9 µm from anterior

end of body, at 19.5–25.2% of pharynx length. Excretory pore obscure. Female reproductive system didelphic-amphidelphic. Vulva transverse, located at 50.4–55.3% of body length. Vagina length 0.4 times body diameter. Uterus length 0.8–1.0 times body diameter. Oviduct length 1.5–1.8 times body diameter. Ovary reflexed, usually reaching and surpassing oviduct-uterus junction, anterior reproductive tract length (G1) 132.6–179.2 µm, posterior length (G2) 172.1–181.5 µm. Rectum length approximately 1.1 times anal body diameter. Tail dorsally convex. Male: General morphology similar to that of female. Body length 1,821.6 µm long. Cuticle annuli 1.8 µm thick at mid-body. Lip region 14.4 µm in diameter. Amphids aperture 4.5 µm wide. Mural tooth length 10.5 µm (ventral side) and 11.6 µm (dorsal side) long. Pharyngeal bulb length 222.1 µm long. Nerve ring located at 19.5% of pharynx length. Genital system diorchic with opposite testes. Testis reflexed dorsad anteriorly. Spicules strong, slightly arcuate, 42.5 µm, 1.5 times anal body diameter. Gubernaculum small, slightly curved ventrad. Pair of pre-anal subventral papillae present. Ventomedian supplements rudimentary, twice tail length, in front of tail papillae. Rectum length 29.9 µm long. Tail dorsally convex.

Habitat. Freshwater and sediment.

Distribution. Belarus, Bulgaria, Czech, Estonia, Hungary, Germany, Hungary, Italia, Korea (this study), Mauritius, Netherlands, New Guinea, Norway, Poland, Romania, Sardinia, Slovenia, South Africa, Spain, Switzerland, Sweden, United Kingdom, United States, Uzbekistan.

³**Nygolaimus obtusus* Thorne, 1930 (Table 1, Fig. 2)
Nygolaimus obtusus Thorne, 1930: 454, fig. 6.

Material examined. 1♀, Korea: Gyeonggi-do, Yeosu-si, Yeosu-eup, Danyeon-ri, Kang Chun Bo, 37°16'27.91"N, 127°41'00.33"E, 4 Sep 2019. Voucher specimen was deposited in the Nakdonggang National Institute of Biological Resources (NNIBRIV57582), Korea.

Measurements. See Table 1.

Description. Female: Body slender, straight or slightly ventral-curved after fixation, length 2,890.5 µm, width 72.1 µm (maximum value at level of vulva). Cuticle smooth with fine transverse striations, 4.5 µm thick in anterior region, 5.1 µm at mid-body, and 5.8 µm at tail. Lip region rounded, set off by a distinct constriction, wider than adjacent body, 22.4 µm wide, approximately 3.9 times wide as high. Amphids obscure, aperture 7.5 µm wide, 0.3 times as wide as lip region diameter. Mural tooth deltoid, strong, curved dorsally approximately 0.6 times lip region diameter, length 12.9 µm (ventral side) and 13.5 µm (dorsal side). Pharynx with slender muscular anterior

Korean name: ¹*삼각창선충속 (신칭), ²*짧은꼬리삼각창선충 (신칭), ³*거대삼각창선충 (신칭)

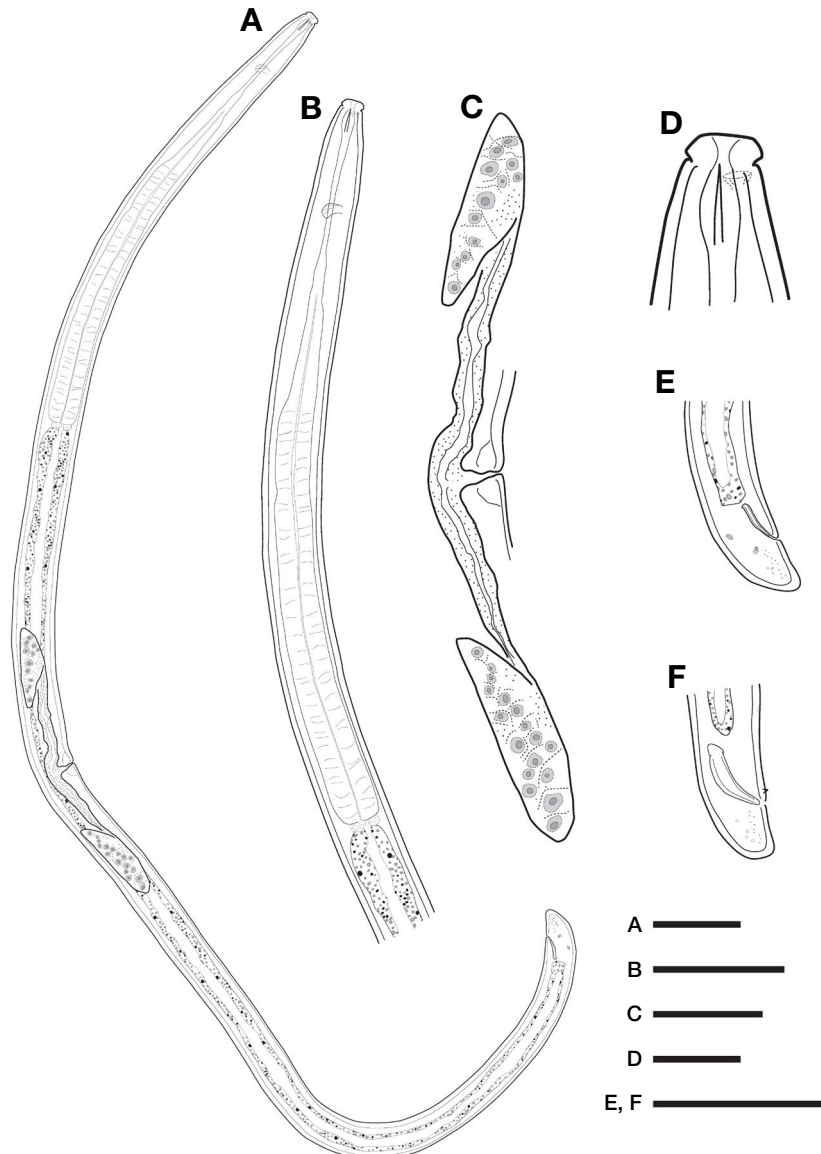


Fig. 1. *Nygolaimus brachyuris* (De Man, 1880) Thorne, 1930. A, Whole female; B, Female neck region; C, Female reproductive system; D, Female head region; E, Female posterior region; F, Male posterior region. Scale bars: A, B, E, F = 100 μm , C = 50 μm , D = 20 μm .

part, narrow middle part, and posterior end expanding gradually into cylindrical bulb. Pharyngeal bulb without constriction, bulb length 248.9 μm and approximately 40.7% of total neck length. Nerve ring located at 142.2 μm from anterior end of body, at 23.3% of pharynx length. Excretory pore obscure. Female reproductive system didelphic-amphidelphic. Vulva transverse, located at 52.3% of body length. Vagina length 0.3 times body diameter. Uterus length 1.0 times body diameter. Oviduct length 2.0 times body diameter. Ovary reflexed, usu-

ally reaching and surpassing oviduct-uterus junction, anterior reproductive tract length (G1) 185.4 μm , posterior length (G2) 203.9 μm . Rectum length approximately 1.0 times anal body diameter. Tail elongate-hemispherical. Male: Not found.

Habitat. Freshwater and sediment.

Distribution. Hungary, Korea (this study), Spain, United States.

^{1*}*Nygolaimus parvus* Thorne, 1974 (Table 1, Fig. 3)

Korean name: ^{1*}꼬마삼각창선충 (신칭)

Table 1. Morphometric values of three *Nygotaimus* species

Characteristic	<i>N. brachyuris</i>		<i>N. obtusus</i>	<i>N. parvus</i>
	♀, n=3	♂, n=1	♀, n=1	♀, n=1
L	2,073.9±309.7 (1,802.5-2,411.2)	1,821.6	2,890.5	1,448.5
Body width	45.4±4.0 (41.3-49.2)	42.2	72.1	38.1
Pharynx length	574.7±76.7 (511.7-660.1)	660.1	610.8	455.3
Tail length	33.5±2.7 (31.2-36.5)	31.6	59.1	22.1
Anal body diameter	27.0±1.6 (25.2-28.1)	28.1	58.9	22.8
a	45.5±3.0 (43.6-49.0)	43.2	40.1	38.0
b	3.6±0.1 (3.5-3.7)	2.8	4.7	3.2
c	61.6±4.2 (57.8-66.1)	57.6	48.9	65.5
c'	1.2±0.1 (1.2-1.3)	1.1	1.0	1.0
V	52.6±2.5 (50.4-55.3)	–	52.3	58.2
Lip region diameter	15.7±1.6 (14.5-17.6)	14.4	22.4	22.9
Lip region height	5.1±0.4 (4.7-5.5)	4.9	5.8	5.9
Amphid aperture	5.6±0.5 (5.1-6.1)	4.5	7.5	5.7
Mural tooth length (ventral side)	11.2±1.0 (10.1-12.1)	10.5	12.9	21.4
Mural tooth length (dorsal side)	12.6±0.8 (11.9-13.5)	11.6	13.5	22.0
Pharyngeal bulb length	226.9±4.7 (221.5-230.1)	222.1	248.9	198.1
Nerve ring from anterior end	131.5±2.4 (129.1-133.9)	129.0	142.2	133.5
Nerve ring (% pharynx)	23.1±2.5 (20.3-25.2)	19.5	23.3	29.3
Vulva from ant. End	1,088.3±144.5 (997.6-1254.9)	–	1512.4	842.6
Vulva to anus	952.1±173.3 (773.7-1119.8)	–	1319.0	583.8
Vulva to anus/tail length	28.2±3.1 (24.8-30.7)	–	22.3	26.4
Vagina length	16.5±1.2 (15.5-17.8)	–	18.1	15.2
Vagina/body diameter	0.4±0.0 (0.4-0.4)	–	0.3	0.4
Reproductive tract length (G1)	159.0±23.9 (132.6-179.2)	–	185.4	177.9
Reproductive tract length (G2)	178.2±5.3 (172.1-181.5)	–	203.9	180.2
G1 (%)	7.7±0.5 (7.4-8.2)	–	6.4	12.3
G2 (%)	8.7±1.1 (7.5-9.5)	–	7.1	12.4
Spicules	–	42.5	–	–
Spicules/anal body diameter	–	1.5	–	–
Spicules/tail length	–	1.3	–	–
Rectum	28.9±1.4 (27.4-30.2)	29.9	60.1	23.0
Rectum/anal body diameter	1.1±0.0 (1.0-1.1)	1.1	1.0	1.0

All measurements are in µm; mean±SD (range) are presented.

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.

Nygotaimus parvus Thorne, 1974: fig. 40.

Material examined. 1 ♀, Korea: Seoul, Songpa-gu, Cheonhodaero, Gwangnaru Hangang Park, 37°32'29.92"N, 127°06'54.56"E, 5 Sep 2019. Voucher specimen was deposited in the Nakdonggang National Institute of Biological Resources (NNIBRIV57583), Korea.

Measurements. See Table 1.

Description. Female: Body slender, ventrally curved after fixation, usually “G” or “J” shaped, length 1,448.5 µm, width 38.1 µm (maximum value at level of vulva). Cuticle smooth

with fine transverse striations, 1.6 µm thick in anterior region, 1.8 µm at mid-body, and 1.8 µm at tail. Lip region rounded, set off by a distinct constriction, not wider than adjacent body, 11.3 µm wide, approximately 3.8 times wide as high. Amphids obscure, aperture 5.7 µm wide, 0.5 times as wide as lip region diameter. Mural tooth deltoid, strong, curved dorsally, approximately 1.0 times of lip region diameter, length 21.4 (ventral side) µm and 22.0 (dorsal side) µm. Pharynx with slender muscular anterior part, narrow middle part, and posterior end expanding gradually into cylindrical bulb. Pharyngeal bulb without constriction, bulb length 198.1 µm and approxi-

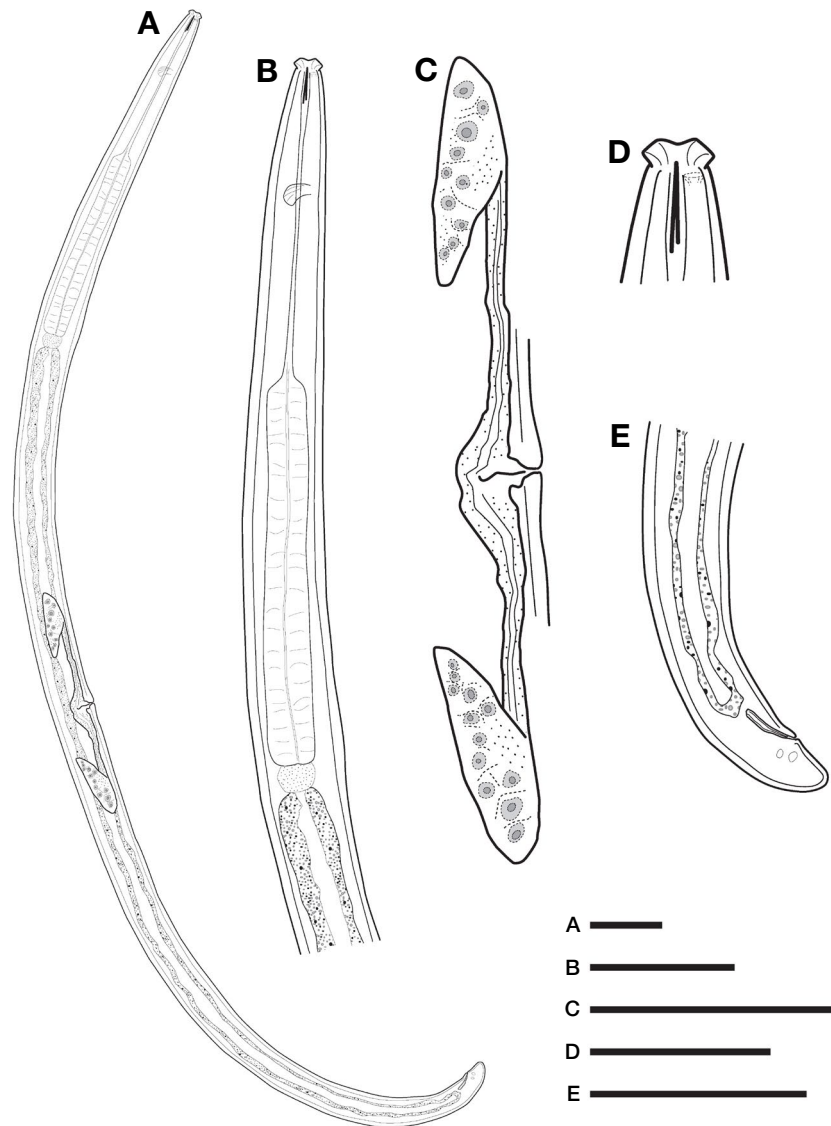


Fig. 2. *Nygolaimus obtusus* Thorne, 1930. A, Whole female; B, Female neck region; C, Female reproductive system; D, Female head region; E, Female posterior region. Scale bars: A–C, E=100 μ m, D=50 μ m.

mately 43.5% of total neck length. Nerve ring located at 133.5 μ m from anterior end of body, at 29.3% of pharynx length. Excretory pore obscure. Female reproductive system didelphic-amphidelphic. Vulva transverse, located at 58.2% of body length. Vagina length 0.4 times body diameter. Uterus length 0.8 times body diameter. Oviduct length 1.6 times body diameter. Ovary reflexed, usually reaching and surpassing oviduct-uterus junction, anterior reproductive tract length (G1) 177.9 μ m, posterior length (G2) 180.2 μ m. Rectum length approximately 1.0 times anal body diameter. Tail elongate-hemispherical. Male: Not found.

Habitat. Freshwater and sediment.

Distribution. Hungary, Korea (this study), Spain, United States.

Molecular sequence information. Molecular sequences (partial 18S rDNA sequences) deposited in GenBank: *N. brachyuris* (GenBank accession no: MN887605), *N. obtusus* (GenBank accession no: MN887604), and *N. parvus* (GenBank accession no: MN887602).

Diagnosis and molecular analysis. The morphological characteristics and morphometric values of the three *Nygolaimus* species herein studied were consistent with those of previous studies (Thorne, 1930 for *N. brachyuris* and *N. obtusus*; Thorne, 1974 for *N. parvus*). These three species can be distinguished

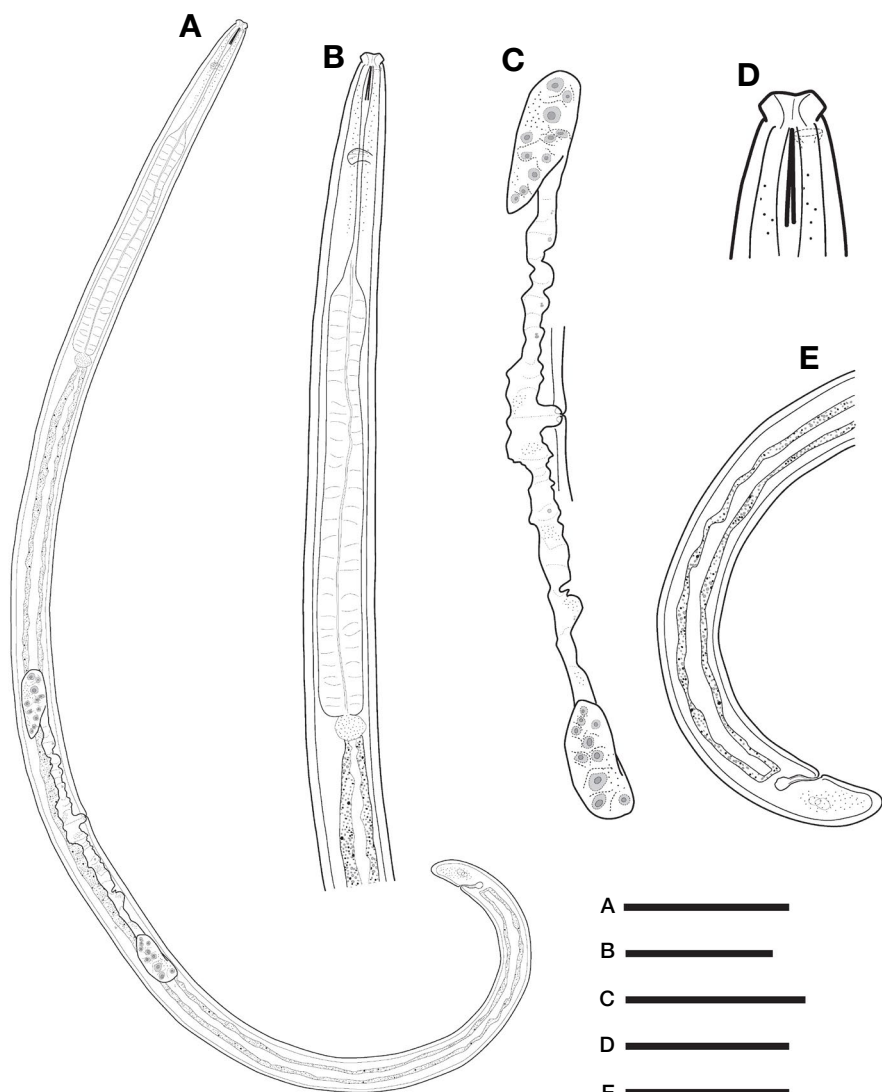


Fig. 3. *Nygolaimus parvus* Thorne, 1974. A, Whole female; B, Female neck region; C, Female reproductive system; D, Female head region; E, Female posterior region. Scale bars: A=100 μm, B–D=50 μm, E=20 μm.

from their congeners by the following characteristics: short tail, wider body, and weak mural tooth in *N. brachyuris*; wider lip region, long tail, and long body in *N. obtusus*; all characteristics (such as body, pharynx, and tail length) are generally smaller in *N. parvus* (Cobb, 1922; Andr assy, 1959; Das, 1963; Hunt, 1977; Ahmad and Jairajpuri, 1980; Santiago and Li banas, 1997; Shahina et al., 2005; Bohra and Sultana, 2008).

A molecular analysis of all three species was performed to test whether it supported the results of the morphological identification. For this, we assessed the similarity between the 18S rDNA sequences of the specimens herein studied and those of *N. brachyuris* and *N. parvus* from GenBank. A

high similarity was found between our sequences and those of *N. brachyuris* (AY284770.1; 99.9%) and *N. parvus* (AY552974.1; 100%). These 18S rDNA gene sequences are useful as DNA barcoding information. Further studies should focus on obtaining a large amount of DNA data for *Nygolaimus* species to be used for molecular identification.

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CONFLICTS OF INTEREST

No potential conflicts of interest relevant to this article are reported.

ACKNOWLEDGMENTS

This work was supported by a grant from the Nakdonggang National Institute of Biological Resources (NNIBR) funded by the Ministry of Environment (NNIBR202001107) and Korea Research Fellowship Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science and ICT (2020R1A2C2005393) of the Republic of Korea.

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Received January 14, 2021
Revised March 12, 2021
Accepted March 15, 2021