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Short communication

Prionchulus oleksandri (Nematoda: Mononchida) from Korea

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ABSTRACT

The genus *Prionchulus* Cobb, 1916 represents a group of predaceous nematodes belonging to the family Mononchidae Chitwood, 1937, and is found worldwide. However, only five species have been reported thus far from Korea. *Prionchulus oleksandri* Winiszewska and Susulovsky, 2003 is reported for the first time from Korea, from sediments collected from the Nakdong River. This species is distinguished from other *Prionchulus* species by its truncated lip region with small cephalic papillae and refringens vaginae. In this study, morphological characters (detailed morphometrics) of *P. oleksandri* are described and illustrated using optical microscopy. DNA barcode sequence information (the D2−D3 region of 28S rDNA, 18S rDNA, and internal transcribed spacer rDNA) is also provided for the molecular identification of the species.

Keywords: nematode, Mononchidae, *Prionchulus oleksandri*, Korea

INTRODUCTION

Member of the genus *Prionchulus* Cobb, 1916, which belongs to Mononchidae Filipjev, 1934, are known as predaceous nematodes and are found in soil and freshwater habitats. About 30 species have been described in the genus *Prionchulus* Cobb, 1916 worldwide, but only five species were previously listed as occurring in Korea: *P. koriensis* Khan, Choi, Lee and Choi, 2000; *P. mordax* Andrássy, 1993; *P. muscorum* (Dujardin, 1845) Cobb, 1916; *P. pachydermis* Khan, Choi, Lee and Choi, 2000; and *P. punctatus* Cobb, 1917 (see Cobb, 1916, 1917; Andrássy, 1993; Khan et al., 2000). In this study, we report *P. oleksandri* Winiszewska and Susulovsky, 2003 for the first time from Korea and describe its morphological characters and morphometrics. In addition, molecular sequences of the D2−D3 region of the 28S rDNA, 18S rDNA, and internal transcribed spacer (ITS) rDNA of this species are provided as DNA barcode sequence information.

Live specimens were collected from freshwater and sediment samples of the Nakdong River and were isolated by sieving and the Baermann funnel method (Baermann, 1917). Specimens were placed in a 15 mL tube with 2 mL water, and were fixed by adding 4 mL of 80°C TAF (2% triethanolamine and 7% formaldehyde) solution. After the fixed nematodes were processed into glycerin (Seinhorst, 1959), they were mounted in glycerin on HS-slides (Shirayama et al., 1993). An optical microscope (Olympus BX-51, Tokyo, Japan) with differential interference contrast was used for morphological observations, and morphometrics were measured with the program QCapture Pro 5 using digital photographs taken on a CoolSnap Photometrics color CCD camera.

Total genomic DNA was extracted from a single individual of *P. oleksandri* using a nematode lysis buffer (Holterman et al., 2006) according to the manufacturer's instructions. For PCR, a single nematode was placed in a 0.2 mL tube with 25 μL of sterile water, to which was added an equal volume of lysis buffer consisting of 0.2 M NaCl, 0.2 M Tris-HCl (pH 8.0), 1% (v/v) β-mercaptoethanol, and 800 μg/mL proteinase-K. Lysis was performed by placing the PCR tube in a 65°C heating block for 2 h, followed by 5 min of incubation at 100°C. The resulting lysate was either used promptly or stored at −20°C. The D2−D3 regions of 28S rDNA, 18S rDNA, and ITS rDNA were PCR-amplified using universal primer sets (D2A [5′-ACAAGTACCGTGAGGGAAAGTTG-3′]/D3B

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Table 1. Morphometrics of *Prionchulus oleksandri* Winiszewska and Susulovsky, 2003

Character	This study (♀, n = 32)	Winiszewska and Susulovsky (2003)
L	2,718.6 ± 187.1 (2,586.3–2,850.9)	2,452 ± 104 (2,265–2,603)
Body width	117.6 ± 2.1 (116.1–119.0)	82.2 ± 6.9 (69.4–94.8)
Pharynx length	605.3 ± 49.4 (570.4–640.2)	558 ± 15.3 (536–593)
Tail length	160.1 ± 8.0 (154.4–165.7)	160 ± 7.5 (146–174)
Anal region body width	53.4 ± 0.8 (52.8–54.0)	49.2 ± 2.8 (44.8–57.8)
a	23.1 ± 2.0 (21.7–24.6)	30.0 ± 2.1 (27.1–34.5)
b	4.5 ± 0.1 (4.5–4.5)	4.4 ± 0.7 (4.1–4.7)
c	17.0 ± 0.3 (16.8–17.2)	15.4 ± 0.8 (14.0–17.2)
c′	3.0 ± 0.2 (2.9–3.1)	3.3 ± 0.3 (2.6–3.8)
Lip region height	10.8 ± 0.8 (10.2–11.4)	10.3 ± 1.1 (8.1–12.1)
Lip region width	51.4 ± 1.2 (50.5–52.2)	46.8 ± 1.7 (43.6–49.8)
Amphid aperture	5.2 ± 0.1 (5.1–5.2)	5.2 ± 0.8 (4.6–6.1)
Buccal cavity length	47.9 ± 3.5 (45.4–50.3)	48.3 ± 0.9 (47.4–50.0)
Buccal cavity width	30.5 ± 0.1 (30.4–30.6)	27.8 ± 1.1 (26.1–29.4)
Position of tooth apex (%)	26.2 ± 4.1 (23.3–29.1)	23.3 ± 1.3 (20.7–26.0)
Tooth length	3.9 ± 0.8 (3.3–4.5)	–
Subventral denticles number	12	11–12
Subventral denticles length	29.9 ± 4.0 (27.1–32.7)	–
Length of ventral wall	41.0 ± 1.8 (39.7–42.2)	36.2 ± 1.2 (34.4–38.2)
Subventral denticles length (%) (than length of ventral wall)	73.3 ± 12.8 (64.2–82.4)	59.0 ± 5.6 (49.4–70.3)
Subventral denticles length from first to last	22.4 ± 3.5 (19.9–24.8)	21.3 ± 1.9 (18.2–25.4)
Nerve ring from anterior end	165.8 ± 37.3 (139.4–192.2)	–
Excretory pore from anterior end	188.9 ± 29.8 (167.8–210.0)	187 ± 5.9 (175–196)
Nerve ring (% pharynx)	27.2 ± 3.9 (24.4–30.0)	–
Excretory pore (% pharynx)	31.1 ± 2.4 (29.4–32.8)	–
V (%)	62.7 ± 1.6 (61.5–63.9)	63.0 ± 1.4 (59.8–65.8)
Anterior reproductive	416.0 ± 25.4 (398–433.9)	301 ± 37.0 (222–370)
Posterior reproductive	424.0 ± 28.6 (403.8–444.2)	296 ± 34.5 (230–379)
G1 (%)	15.3 ± 0.1 (15.2–15.4)	12.3 ± 1.4 (9.0–14.5)
G2 (%)	15.6 ± 0.0 (15.6–15.6)	12.1 ± 1.4 (9.5–14.6)
Rectum	43.8 ± 1.8 (42.5–45.1)	–
Rectum/anal region body width	0.8 ± 0.0 (0.8–0.9)	0.8 ± 0.05 (0.7–0.9)
Hyaline part of tail length	5.8 ± 0.6 (5.3–6.2)	6.1 ± 0.9 (4.7–7.6)
Hyaline width	5.4 ± 0.4 (5.1–5.6)	–
Hyaline length/width	1.1 ± 0.0 (1.0–1.1)	1.4 ± 0.2 (1.1–1.9)

All measurements are in μm and in the form mean ± 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/tail 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.

[5′-TCGGAAGGAACCAGCTACTA-3′]; De Ley et al., 1999 for the D2–D3 region of 28S, 328-F [5′-TACCTGGTTGAT CCTGCCAG-3′]/329-R [5′-TAATGATCCTTCCGCAGGTT-3′]; Adl et al., 2014 for 18S, and TW81 [5′-GTTTCCGTAG GTGAACCTGC-3′]/AB28 [5′-ATATGCTTAAGTTCAGC GGGT-3′]; Joyce et al., 1994 for ITS). PCR took place in 50 μL reactions, which included 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). PCR amplification conditions consisted of 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 (extended to 2 min for the 328-F/329-R primer), 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 following the manufacturer's protocol. After purification, Big Dye Terminator Cycle-Sequencing (Applied Biosystems, Waltham, MA, USA) was used for sequencing the fragments. The resulting sequences from the specimens were aligned using ClustalX with default options (Thompson et al., 1997) with sequences of other representatives of *Prionchulus* downloaded from GenBank.

SYSTEMATIC ACCOUNTS

Order Mononchida Jairajpuri, 1969
Suborder Mononchina Kirjanova and Krall, 1969

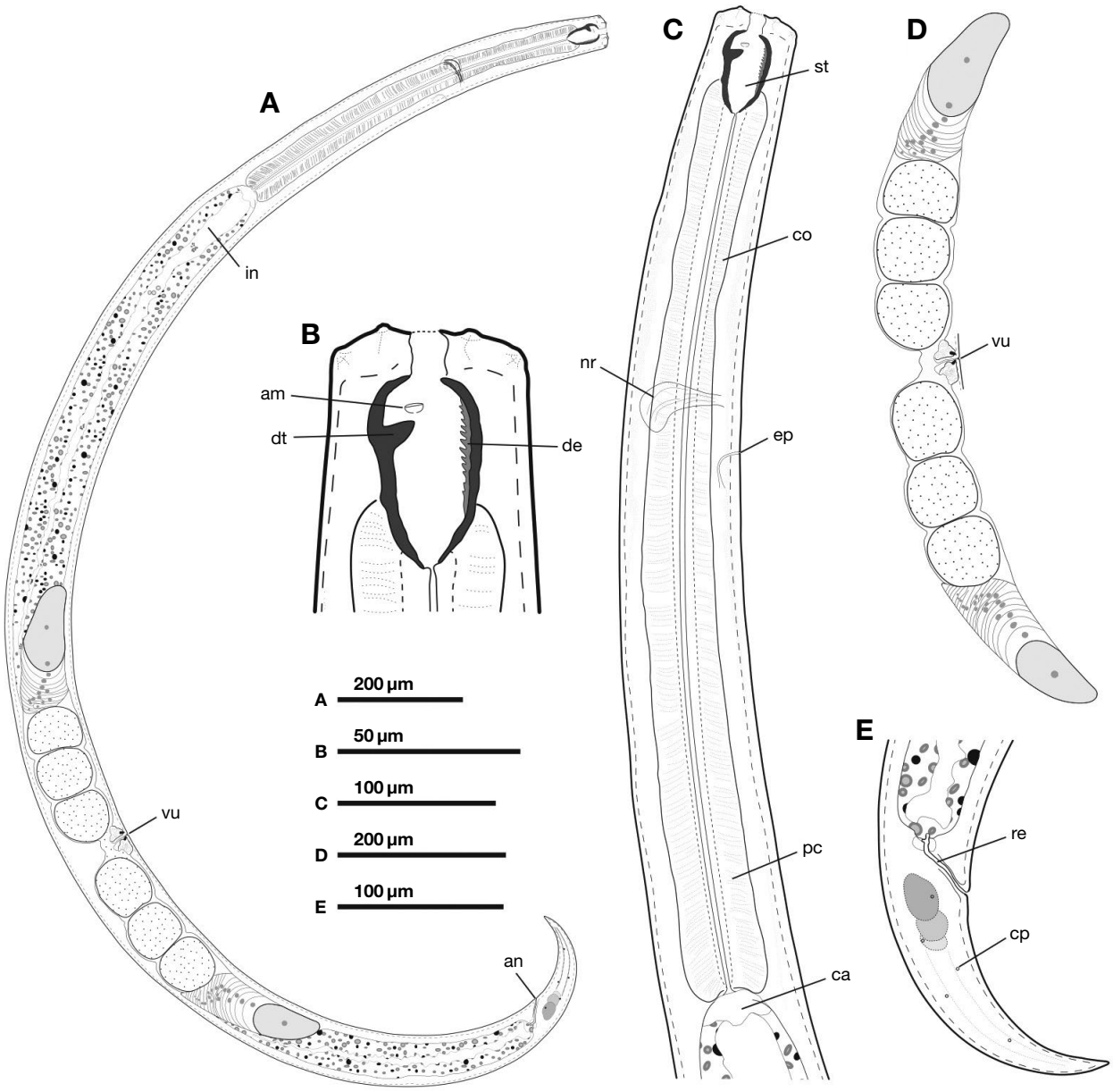

Fig. 1. *Prionchulus oleksandri* Winiszewska and Susulovsky, 2003. A, Entire female; B, Head region; C, Female neck region; D, Female reproductive system; E, Female posterior region. am, amphid; an, anus; ca, cardia; co, corpus; cp, caudal papillae; de, denticle; dt, dorsal tooth; ep, excretory pore; in, intestine; nr, nerve ring; pc, procorpus; re, rectum; st, stoma; vu, vulva.

Superfamily Mononchoidea Filipjev, 1934
Family Mononchidae Filipjev, 1934
Genus *Prionchulus* Cobb, 1916

[1]**Prionchulus oleksandri* Winiszewska and Susulovsky, 2003 (Table 1, Fig. 1)

Prionchulus oleksandri: Winiszewska and Susulovsky, 2003: 576, figs. 70–78.

Material examined. 32♀♀, Korea: Gyeongsangbuk-do, Sangju-si, Ian-myeon, Ian 1-gil, 14-9, 36°32′37.3″N, 128° 09′27.6″E, 4 Apr 2017. Voucher specimens are deposited in the Nakdonggang National Institute of Biological Resources (NNIBR) and the Animal Phylogenomics Laboratory at Ewha Womans University (slide no. 08010101001), Korea.

Measurements. See Table 1.

Description. Female: Body cylindrical, length 2,586–2,850

Korean name: [1]*각진입술톱이선충(신칭)

μm, width 116.1–119.0 μm (maximum value at a level of vulva), ventrally curved after fixation. Cuticle annulated; 1.9–2.3 μm thick at mid-body. Cuticular pores small, numerous, covering the body surface. Truncated lip region 50.5–52.2 μm wide, with small cephalic papillae. Buccal cavity cylindrical, thick-walled, with length about 1.5–1.7 times the width, and with funnel-shaped base. Amphid cup-like, its aperture 5.1–5.2 μm, located 16.8–17.7 μm from anterior end. Dorsal tooth apex 3.3–4.5 μm in length, located 11.7–13.2 μm forward from buccal cavity. Subventral denticles 12 in number, 27.1–32.7 μm in length, covering 64.2–82.4% of ventral wall. Nerve ring located 139.4–192.2 μm from anterior end of body. Excretory pore weak, located at 29.4–32.8% of pharynx length. Female reproductive system amphidelphic. Female genital branch symmetrical, anterior genital branch 398–433.9 μm, posterior 403.8–444.2 μm long. Vulva a transverse slit, about 61.5–63.9% of body length from anterior end. Pars refringens vagina one-third of body width at vagina region. Egg in genital branches, 64.8–83.3 μm long and 74.4–92.0 μm wide. Rectum straight, length 0.8–0.9 times anal body width. Tail ventrally curved, elongated conoid. Hyaline part of tail 1.0–1.1 times longer than wide. Tail with five pores: three subdorsal, one subventral and one lateral. **Male:** Not found.

Habitat. Freshwater and sediment.

Distribution. Ukraine, Korea.

Diagnosis and relationships. Although habitats between the specimens and type species were different (freshwater river vs. mountain moss), the morphological characters described herein are the same as those previously reported for *P. oleksandri*, and the morphometric characters are also generally within the range of earlier study (Winiszewska and Susulovsky, 2003) except for some intraspecific variation among populations (body length : width [a = 21.7–24.6 vs. 27.1–34.5], lip region width [50.5–52.2 μm vs. 43.6–49.8 μm], length of ventral wall in buccal cavity [39.7–42.2 μm vs. 34.4–38.2 μm] and reproductive length [G1 = 15.2–15.4% vs. 9.0–14.5% and G2 = 15.6–15.6% vs. 9.5–14.6%]) (Table 1). *Prionchulus oleksandri* is distinguishable from other *Prionchulus* species by its truncate lip region with small cephalic papillae, funnel-shaped buccal cavity, lower position of dorsal tooth and amphid, distinctly sclerotized pars refringens vaginae, and conical and regularly tapering tail.

Molecular sequence information. Molecular sequences deposited on GenBank: D2–D3 region of 28S rDNA (GenBank accession No. MG969499); 18S rDNA (GenBank accession No. MG969498); ITS rDNA (GenBank accession No. MG 969500).

Molecular analysis. Sequences of the D2–D3 region of 28S rDNA, 18S rDNA, and ITS rDNA were successfully obtained from *P. oleksandri*. As the ITS rDNA sequences from other *Prionchulus* species are not yet available on GenBank, we compared the *P. oleksandri* 18S rDNA sequence with other *Prionchulus* species available on GenBank. *Prionchulus oleksandri* showed high genetic distances from *P. muscorum* (AJ 966500; 6.897% and AY284745; 7.291%) and *P. punctatus* (AY284746; 7.224% and AY284747; 7.247%). Since this analysis is based on only a few species for which sequence information is publically available, additional sequence information from other *Prionchulus* species is needed to develop molecular markers that can be used for species-level identification of *Prionchulus* species.

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