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Short communication

First Report of Two *Plectus* Species (Nematoda: Plectida) from Korea

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ABSTRACT

The genus *Plectus* Bastian, 1865 represents a group of free-living freshwater nematodes belonging to the family Plectidae Örley, 1880. However, only one species has been reported thus far from Korea. *Plectus aquatilis* Andrássy, 1985 and *Plectus cirratus* Bastian, 1865 are reported for the first time from Korea, from sediments collected from the Nakdong River. *Plectus aquatilis* is distinguished from other *Plectus* species by its three longitudinal alae in the lateral field, thin and directed cephalic setae, continuous lip region (head not set-off), and rectangular shaped tail. *Plectus cirratus* is distinguished from other *Plectus* species by its large body, two longitudinal alae in the lateral field, larger stoma, and longer tail. Morphological characters and measurements of the specimens generally agree with the original descriptions of *Plectus* species. Here, the morphological characters (detailed morphometrics) of two species in the genus *Plectus* are fully redescribed and illustrated using optical microscopy. DNA barcode sequence information from the 18S rDNA gene is also provided for molecular species identification and compared with sequences from other *Plectus* species available on GenBank.

Keywords: nematode, Plectida, *Plectus*, new record, freshwater, Korea

INTRODUCTION

The genus *Plectus* Bastian, 1865 belongs to the family Plectidae Örley, 1880. This group is one of the most widely distributed and common nematode taxa in freshwater and terrestrial habitats around the world, but only one species has previously been reported from Korea: *P. parietinus* Bastian, 1865 (Eun et al., 2016). In this study, we report *P. aquatilis* Andrássy, 1985 and *P. cirratus* Bastian, 1865 for the first time from Korea and provide descriptions of their morphological characters, morphometrics, and the molecular sequences of the 18S rDNA gene, useable as DNA barcodes.

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

For DNA barcoding, total genomic DNA was extracted from single individual specimens using a nematode lysis buffer (Holterman et al., 2006) according to the manufacturer's instructions. Each nematode was placed in 25 μL sterile water in a 0.2 mL tube, 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. The tube was then heated at 65°C for 2 h, and incubated at 100°C for 5 min. Lysate was stored at −20°C if not used immediately. The 18S rDNA gene was amplified using PCR with universal primer sets (988-F [5′-CTCAAAGATTAAGCCATGC-3′]/1096-R [5′-GGTAATTCTGGAGCTAATAC-3′]/1912R [5′-TTTACGGTCAGAACTAGGG-3′], 1813-F [5′-CTGCGTGAGAGGTGAAAT-3′]/2646-R [5′-GCTACCTTGTTACGACTTTT-3′]) (Holterman et al.,

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Table 1. Morphometrics of *Plectus aquatilis* and *Plectus cirratus*

Character	*Plectus aquatilis*	*Plectus cirratus*
	♀, n=4	♀, n=1
L	950.3±101.2 (886.2–1,100.5)	1,309.5
Body width	33.6±9.0 (28.4–47.0)	51.9
Pharynx length	223.8±16.2 (206.1–245.3)	263.2
Tail length	118.6±4.5 (113.6–123.5)	168.9
Anal region body width	22.6±3.2 (20.7–27.4)	32.1
a	29.1±4.0 (23.4–32.4)	25.3
b	4.2±0.2 (4.0–4.5)	5.0
c	8.0±0.6 (7.6–8.9)	7.8
c'	5.3±0.6 (4.5–5.8)	5.3
Lip region height	2.7±0.5 (2.0–3.2)	2.1
Lip region width	7.2±0.4 (6.8–7.8)	9.0
Longer cephalic seta	2.7±0.3 (2.3–3.1)	3.1
Amphid position from anterior end	10.5±1.0 (9.18–11.5)	11.8
Amphid aperture	3.3±0.5 (3.0–4.0)	3.6
Stoma length	20.2±1.1 (18.5–20.8)	21.6
Stoma width	4.3±0.4 (4–4.9)	4.7
Nerve ring from anterior end	111.8±7.2 (105.5–121.7)	134.5
Excretory pore from anterior end	128.4±7.3 (119.5–137.2)	155.2
Nerve ring (% pharynx)	50.0±1.8 (47.7–52.1)	51.1
Excretory pore (% pharynx)	57.4±1.2 (55.9–58.7)	59.0
V (%)	52.3±8.4 (47.4–64.9)	46.5
Anterior reproductive	169.2±62.5 (123.1–261.5)	326.9
Posterior reproductive	160.4±53.7 (130.0–240.6)	309.4
G1 (%)	17.5±4.3 (13.9–23.8)	25.0
G2 (%)	16.6±3.6 (14.3–21.9)	23.6
Rectum	27.2±3.5 (24.4–32.2)	37.5
Rectum/anal region body width	1.2±0.0 (1.2–1.2)	1.2
Vulva-anus distance	363.2±39.5 (336.3–421.1)	535.4

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/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.

2006). Total 50 μL PCR reactions were performed using 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). 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. PCR products were purified with a QIAquick Gel Extraction Kit (Qiagen, Hilden, Germany) following the manufacturer's protocol, and a sequenced using Big Dye Terminator Cycle-Sequencing (Applied Biosystems, Waltham, MA, USA). The resulting 18S rDNA sequences are deposited in GenBank. Sequence analyses were done with 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). Based on 18S rDNA sequences, we reconstructed a phylogenetic tree in MEGA 5.2.2 using the maximum likelihood (ML) method with 1,000 bootstrap replications (Tamura et al., 2011). Genetic distances were calculated using the Kimura-2-parameter model (Kimura, 1980).

SYSTEMATIC ACCOUNTS

Order Plectida Malakhov, 1982
Superfamily Plectoidea Örley, 1880
Family Plectidae Örley, 1880
Genus *Plectus* Bastian, 1865

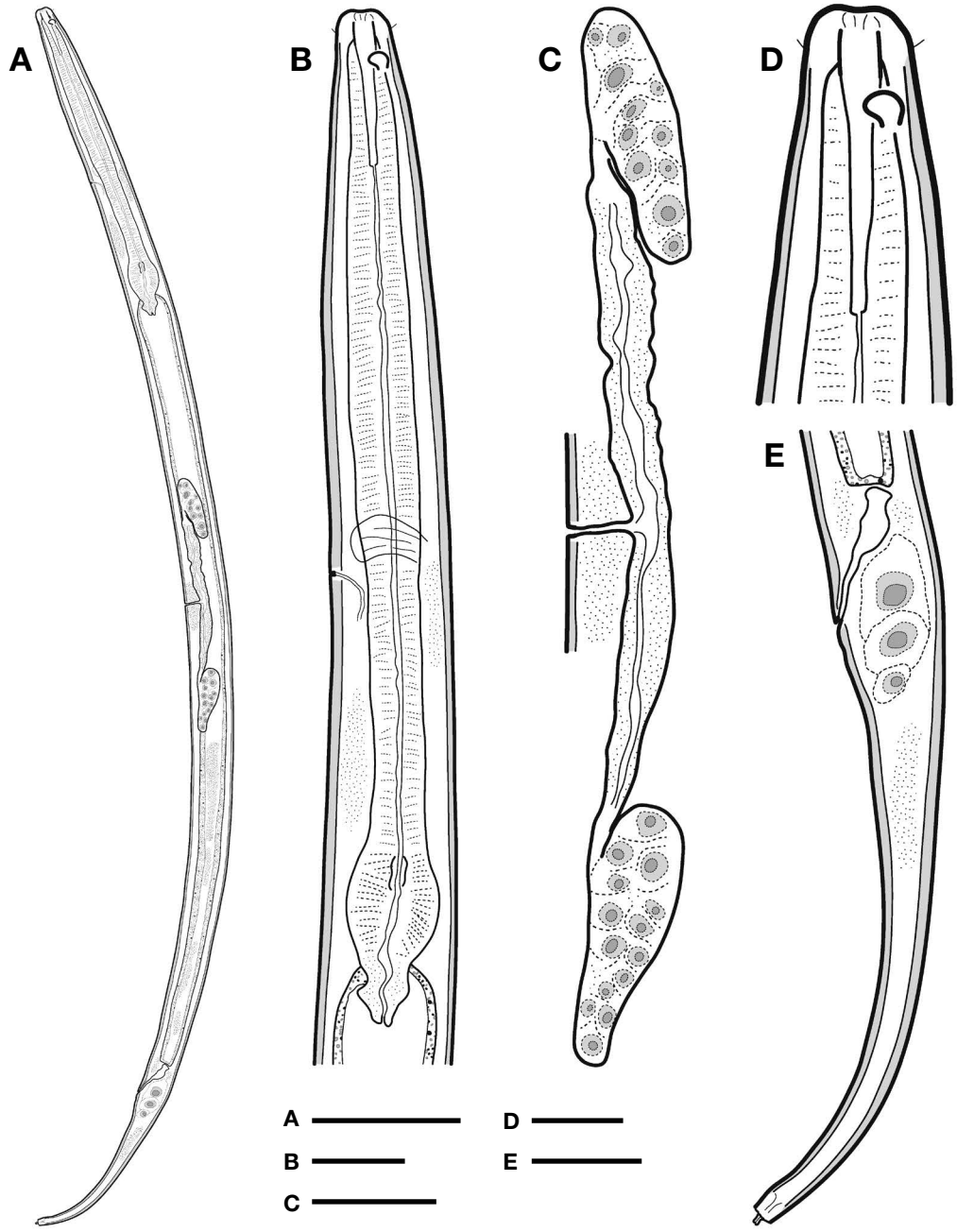

Fig. 1. *Plectus aquatilis* Andrássy, 1985. A, Entire female; B, Pharyngeal region; C, Reproductive system; D, Head region; E, Posterior region. Scale bars: A = 100 μm, B, C, E = 20 μm, D = 10 μm.

[1]****Plectus aquatilis* Andrássy, 1985 (Table 1, Fig. 1)**

Plectus aquatilis Andrássy, 1985: 9, fig. 3.

Material examined. 4♀♀, Korea: Gyeongsangbuk-do, Chilgok-gun, Yangmok-myeon, Gwanho-ri, 851-16, 36°0′5.61″N, 128°23′30.93″E, 3 Mar 2017. Voucher specimens are deposited in the Nakdonggang National Institute of Biological Resources (NNIBR), Korea.

Measurements. See Table 1.

Description. Female: Body cylindrical, length 886.2−1,100.5 μm, width 28.4−47.0 μm (maximum value at a level of vulva), ventrally curved after fixation, more curved in posterior

Korean name: [1]*직각꼬리감공선충 (신칭)

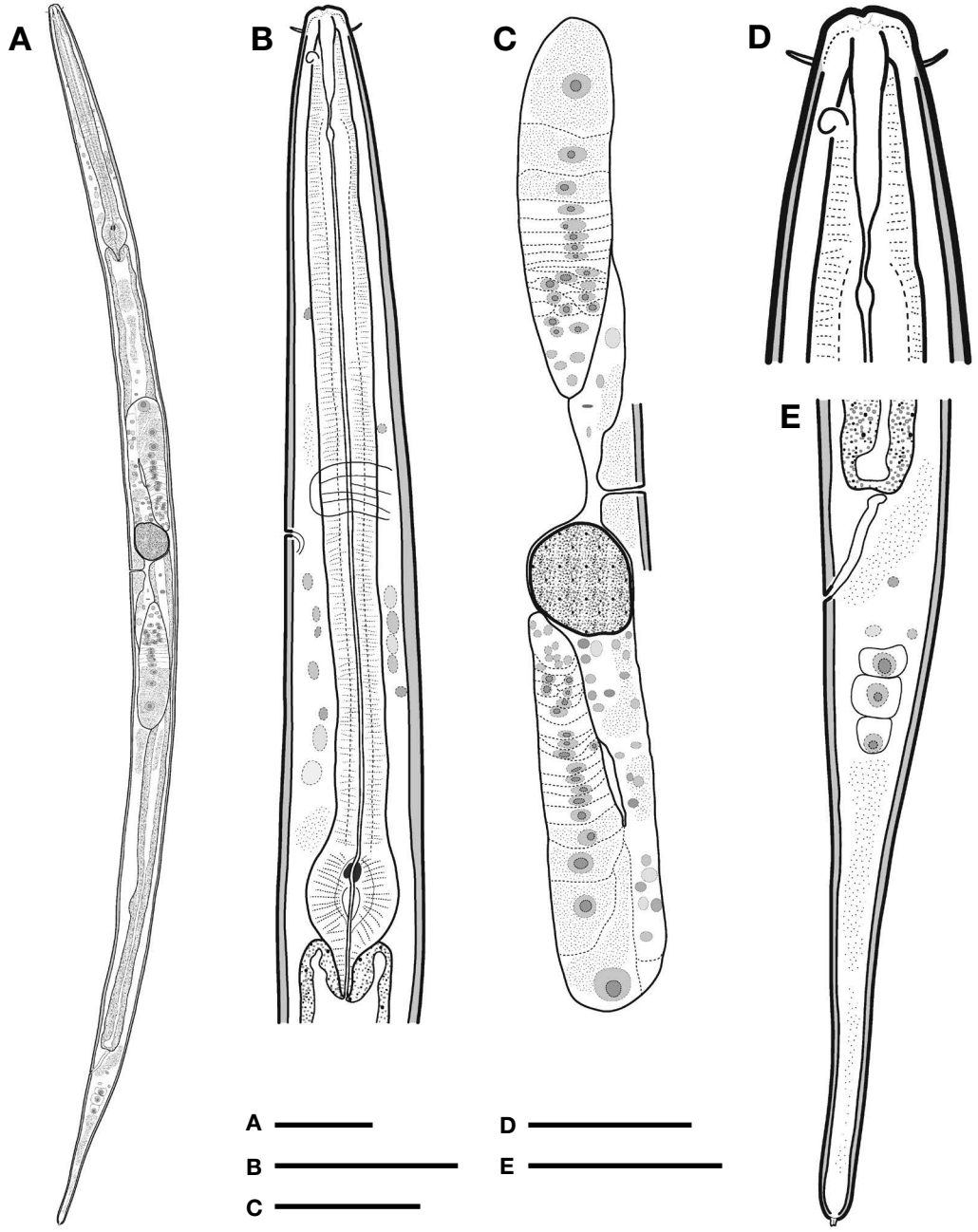

Fig. 2. *Plectus cirratus* Bastian, 1865. A, Entire female; B, Pharyngeal region; C, Reproductive system; D, Head region; E, Posterior region. Scale bars: A, D = 100 µm, B, C, E = 50 µm.

end. Cuticle annulated; 1.0–2.0 µm thick at mid-body. Lateral fields with three closely-placed longitudinal alae. Lip region continuous with body contour, 6.8–7.8 µm wide. Four cephalic setae, about 2.3–3.1 µm long, directed forward. Stoma cylindrical; cheilostom arcuate, cuticularized, gymnostom thick, cuticularized; stegostom slightly narrower. Pharynx 206.1–245.3 µm long, with cylindrical corpus and continuous isthmus, then ovoid basal bulb with a grinder having 8–10 pairs of denticulate ridges. Cardia covered by post-bulbar extension. Amphids circular, its aperture 3.0–4.0 µm, located middle of stoma. Nerve ring located 105.5–121.7 µm from anterior end of body. Excretory pore slightly posterior to nerve ring, located at 56.0–58.7% of pharynx length. Excretory duct forming one and a half loops, then connecting with renette

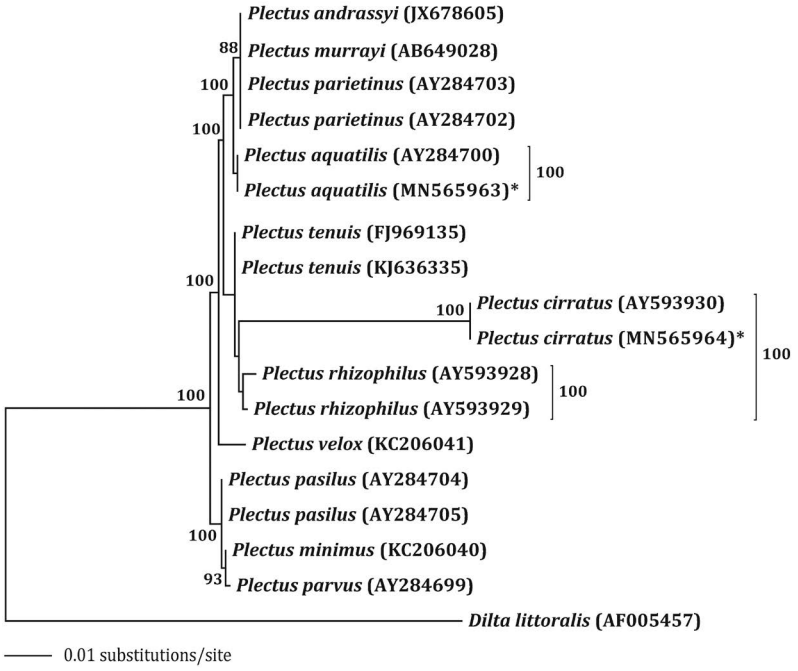

Fig. 3. Maximum likelihood phylogenetic tree of the partial 18S rDNA sequences of the eleven *Plectus* species, including *Plectus aquatilis* and *Plectus cirratus* (*determined in this study) and outgroup (*Dilta littoralis*). Bootstrap values ≥70% are shown above the branches. GenBank accession numbers are in parentheses after the species name.

cell. Female reproductive system didelphic, amphidelphic, ovary branched symmetrical, reflexed. Anterior ovary on right and posterior ovary on left side of intestine. Female genital branch symmetrical, anterior genital branch 123.1–261.5 μm, posterior 130.0–240.6 μm long. Sperm and spermatheca absent. Vulva a transverse slit, about 47.4–64.9% of body length from anterior end. Rectum straight, length 1.2 times anal body width. Three caudal glands present in tandem. Tail ventrally arcuate, narrowing gradually towards spinneret tip, with five caudal setae. Male: Not found.

Habitat. Freshwater and sediment.

Distribution. Czech, Germany, Hungary, Poland, Romania, Sweden, Ukraine, India, Korea.

[1]**Plectus cirratus* Bastian, 1865 (Table 1, Fig. 2)**

Plectus cirratus Bastian, 1865: 119, figs. 81–82.

Material examined. 1♀, Korea: Gyeongsangbuk-do, Sangju-si, Jungdong-myeon, Osang-ri, 968-1, 36°26′24.24″N, 128° 15′26.60″E, 19 Oct 2017. Voucher specimen is deposited in the Nakdonggang National Institute of Biological Resources (NNIBR), Korea.

Measurements. See Table 1.

Description. Female: Body cylindrical, length 1,309.5 μm, width 51.9 μm (maximum value at a level of vulva), ventrally curved after fixation, more curved at posterior end. Cuticle annulated; 1.3 μm thick at mid-body. Lateral fields with two closely-placed cuticular alae. Lip region continuous with body contour, 9.0 μm wide. Four cephalic setae, about 3.1 μm long, directed forward. Stoma cylindrical; cheilostom arcuate, cuticularised, gymnostom thick, cuticularised; stegostom slightly narrower. Pharynx 263.2 μm long, with cylindrical corpus and continuous isthmus, then ovoid basal bulb with a grinder having 8–10 pairs of denticulate ridges. Cardia covered by post-bulbar extension. Amphids circular, its aperture 3.6 μm, located in the middle of the stoma. Nerve ring located 134.5 μm from anterior end of body. Excretory pore slightly posterior to nerve ring, located at 59.0% of pharynx length. Excretory duct forming one and a half loops, then connecting with renette cell. Female reproductive system didelphic, amphidelphic, ovary branched symmetrical, reflexed. Anterior ovary on right and posterior ovary on left side of intestine. Female genital branch symmetrical, anterior genital branch 326.9 μm, posterior 309.4 μm long. Sperm and spermatheca absent. Vulva a transverse slit, about 46.5% of body length from anterior end. Rectum straight, length 1.2 times anal body width. Three caudal glands present in a tandem. Tail ventrally arcuate, narrow-

Korean name: [1]긴꼬리감공선충 (신칭)

ing gradually towards spinneret tip, with four caudal setae. Male: Not found.

Habitat. Freshwater and sediment.

Distribution. United States, England, Netherlands, Bulgaria, Turkey, Korea.

Molecular sequence information. Molecular sequences (partial 18S rDNA sequences) deposited in GenBank: *P. aquatilis* (GenBank accession No. MN565963) and *P. cirratus* (GenBank accession No. MN565964).

Diagnosis and molecular analysis. Morphological characters reported herein match those previously reported for these two *Plectus* species, and the morphometric characters in this study also generally fall within the ranges of earlier studies (Andrássy, 1985 for *P. aquatilis*; Bastian, 1865 for *P. cirratus*). The present two *Plectus* species are distinguishable from other *Plectus* species by specific characters (middle length body, head continuous [not set-off], cephalic setae thin and directed outward, amphids located at middle stoma, lateral field with three longitudinal alae, tail rectangular shape in the tip for *P. aquatilis*; long body, head slightly set off, amphids large, eggs more globular than in other species, tail arcuate and slender for *P. cirratus*).

In addition to redescribing the specimens' morphology, we obtained partial 18S rDNA sequences and used them to reconstruct a ML phylogenetic tree with other selected *Plectus* species available on GenBank (Fig. 3). We also assessed sequence similarity between our specimens 18S rDNA sequences and other *Plectus* species from GenBank. The specimens' sequences respectively matched those of *P. aquatilis* (AY284700; 100%) and *P. cirratus* (AY593930; 100%) from Genbank. In the phylogenetic tree, the *P. aquatilis* sequences (AY284700 and MN565963 [Korean isolates]) clustered together and were in turn sister to *P. andrassyi*+*P. parietinus*+*P. murrayi* with high statistical support (100% bootstrap value) (Fig. 3). The *P. cirratus* sequences (AY593930 and MN565964 [Korean isolates]) clustered together and were sister to *P. rhizophilus* with a bootstrap value of 100% (Fig. 3). Intraspecific variation in 18S rDNA among three individuals of *P. aquatilis* and *P. cirratus* was lower ($\leq$0.00%) than interspecific variation among *Plectus* species ($\geq$8.92%). This result indicates that the 18S rDNA sequence is a reliable molecular tool for species identification in the genus *Plectus*.

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CONFLICTS OF INTEREST

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

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