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First Report of *Aphelenchoides bicaudatus* (Nematoda: Aphelenchoididae) from South Korea

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ABSTRACT

The genus *Aphelenchoides* (Fischer, 1894) includes a diverse group of species, some of which are of economic importance. *A. bicaudatus* (Imamura, 1931) Filipjev and Schuurmans Stekhoven, 1941 is reported for the first time from South Korea, with a detailed redescription of the species. Specimens were collected from chrysanthemum (*Chrysanthemum morifolium*) leaves and shoot tips in South Korea. The species was identified by morphological traits and molecular sequencing. A bifurcated tail distinguishes *A. bicaudatus* from its congeneric species. To confirm species identification, we determined the partial 18S ribosomal DNA sequence of the specimens and compared with those obtained from other *Aphelenchoides* species available on GenBank.

Keywords: Nematoda, Aphelenchoididae, *Aphelenchoides bicaudatus*, 18S ribosomal DNA, South Korea

INTRODUCTION

Aphelenchoides Fischer, 1894 is large and abundant genus with a worldwide distribution. They are found in a wide range of habitats; free-living forms in soil (including many mycetophagous species that feed on mushroom mycelium), plant parasitic forms, and some others associated with insects (Nickle, 1970). To date, eight *Aphelenchoides* species have been recorded from South Korea: *A. besseyi* (Christie, 1942), *A. fragariae* (Ritzema Bos, 1891) Christie, 1923, *A. parasaprophilus* (Sanwal, 1965), *A. parietinus* (Bastian, 1865) Steiner, 1932, *A. ritzemabosi* (Schwartz, 1911) Steiner and Buhrer, 1932, *A. subtenius* (Cobb, 1926) Steiner and Buhrer, 1932, *A. paradalianensis* (RuQiang, 2011), and *A. rotundicaudatus* (Fang, 2014).

A. bicaudatus (Imamura, 1931) Filipjev and Schuurmans Stekhoven, 1941 has previously been considered a primarily mycophagous species and is known to feed on mushroom mycelium (Siddiqui and Taylor, 1967); however, it has also been found living on buds and leaves of crops and plants (Jen et al., 2012; this study). Here we provide a detailed redescription of *A. bicaudatus*, collected from chrysanthemum (*Chrysanthemum morifolium*) leaves and shoot tips in South Korea, together with a molecular information of 18S ribosomal DNA sequence.

MATERIALS AND METHODS

Nematode isolation and culture

Live specimens were collected from the roots and shoot tips of *C. morifolium* from a greenhouse in Chungcheongnam-do, Taean-gun, Sowon-myeon, Yeongjeon-ri, South Korea (GPS information: 36°46′62.9″N, 126°13′38.1″E). Nematodes were isolated using sieving and the Baermann funnel method (Baermann, 1917). Individual adult female specimens were cultured at room temperature (18−20°C) on potato dextrose agar plates containing *Botrytis cinerea*.

Fixation and morphological observation

To prepare specimens for morphological observations, nematodes were transferred to a 15 mL tube containing 2 mL water and followed by fixation step with adding 4 mL of 80°C TAF (2% triethanolamine and 7% formaldehyde) solu-

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tion. The fixed nematodes were processed to glycerin (Seinhorst, 1959) and mounted in pure glycerin on permanent HS-slides (Shirayama et al., 1993). Morphological characters were examined with an optical microscope (BX-51; Olympus, Tokyo, Japan) equipped with differential interference contrast. Morphometric characters were measured in the program QCapture Pro 5, from images taken with a Cool-Snap Photometrics color CCD digital camera. Voucher specimens have been deposited in the National Institute of Biological Resources (slide Nos. KOSPIV0000226303 and KO SPIV0000226304) and the Animal Phylogenomics Laboratory at Ewha Womans University (slide Nos. 07010101001-07010101004), South Korea.

Molecular techniques and phylogenetic analysis

Total genomic DNA was extracted using an Epicentre MasterPure DNA Purification Kit (Epicentre, Madison, WI, USA), following the manufacturer's instructions. Partial 18S ribosomal DNA was amplified by polymerase chain reaction (PCR) using nematode-specific primer sets (988F [5′-CTCA AAGATTAAGCCATGC-3′]/1912R [5′-TTTACGGTCAGA ACTAGGG-3′] and 1813F [5′-CTGCGTGAGAGGTGAA AT-3′]/2646R [5′-GCTACCTTGTTACGACTTTT-3′]; Holterman et al., 2006). PCR reactions were performed in a total volume of 50 μL, containing 32.5 μL distilled water, 5 μL 10× Ex Taq buffer including $MgCl_2$, 4 μL dNTP mixture, 10 pmole of each primer, 0.5 μL TaKaRa Ex Taq (Takara, Otsu, Shiga, Japan) and 4 μL template DNA. The PCR amplification procedure entailed an initial denaturing step at 94°C for 1 min, 39 cycles of denaturation at 95°C for 30 s, annealing at 45°C for 30 s, and extension at 72°C for 1 min, and a final extension at 72°C for 10 min. The amplified PCR products were purified using a QIAquick Gel Extraction Kit (Qiagen, Hilden, Germany) following the manufacturer's protocols. Negative controls with no template were run in all PCR amplifications in order to check for potential contamination. The amplified fragments were sequenced with a Big Dye Terminator Cycle-Sequencing (Applied Biosystems, Foster City, CA, USA). A partial 18S ribosomal DNA sequence of *A. bicaudatus* was deposited in GenBank (accession No. KX345119), combined with the homologous sequences of other *Aphelenchoides* species and two outgroups (*Bursaphelenchus mucronatus* and *B. xylophilus*). Sequences were multiple-sequence aligned using CLUSTAL W (Thompson et al., 1994). Molecular phylogenetic tree was reconstructed 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

Phylum Nematoda Diesing, 1861
Class Secernentea Shitwood, 1958
Order Aphelenchida Siddiqi, 1960
Familly Aphelenchoididae Skarbilovich, 1947
Genus *Aphelenchoides* Fischer, 1894

[1]**Aphelenchoides bicaudatus* (Imamura, 1931) Filipjev and Schuurmans Stekhoven, 1941 (Fig. 1)

Aphelenchus bicaudatus Imamura, 1931: 217, text-fig. 30.
Aphelenchoides bicaudatus: Filipjev and Schuurmans Stekhoven, 1941: 456, fig. 274.

Material examined. 7♀♀, South Korea: Chungcheongnam-do, Taean-gun, Sowon-myeon, Yeongjeon-ri, 36°46′62.9″N, 126°13′38.1″E, 27 Mar 2015.

Measurements. Female (n = 7): L = 517.9 ± 3.8 (513.6–522.6) μm; a = 28.3 ± 0.5 (27.7–28.8); b = 7.3 ± 0.0 (7.3–7.4); c = 11.3 ± 0.5 (10.7–11.9); c′ = 4.6 ± 0.1 (4.4–4.8); tail = 45.9 ± 2.5 (43.1–48.8) μm; V = 66.0 ± 0.2 (65.7–66.4)%; stylet = 11.2 ± 0.5 (10.4–11.7) μm.

Male: Extremely rare.

Description. Female: Body slender, tapering slightly anteriorly, and more prominently toward posterior end (Fig. 1A). Body straight and tail region only slightly curved when relaxed by gentle heat. Cuticle annulated; annuli 0.47–0.58 μm wide and 0.39–0.51 μm thick. Lateral field with two incisures at mid-body region. Head distinctly set off from body (Fig. 1B). Lip region rounded, offset, 5.05–5.23 μm wide and 2.52–2.88 μm high; no annules. Stylet weak, with small basal swellings. Procorpus wider anteriorly, gradually narrowing posteriorly, then widening at metacorpus. Metacorpus rounded, occupying approximately 73% of body width, and measuring 8.63–8.92 μm wide and 12.80–12.97 μm long. Pharyngeal-intestinal valve conoid and surrounded by intestinal tissue. Nerve ring about 1/2 body width behind metacorpus. Excretory pore opposite anterior margin of nerve ring. Intestine filled with tiny globules throughout its length. Vulva a transverse slit and slightly protruding, about 66% of body length from anterior end. Ovary usually extending to region of esophageal gland lobe, sometimes even beyond median bulb (Fig. 1C). Post-vulvar uterine sac extending for one-fifth of distance from vulva to end of tail. Rectum prominent, straight, near ventral body wall, and in length approximately three-fourths of anal body width. Tail gradually tapering to terminus, which is unevenly bifurcateda with one prong longer than the other (Fig. 1D).

Korean name: [1]*두갈래꼬리잎선충(신칭)

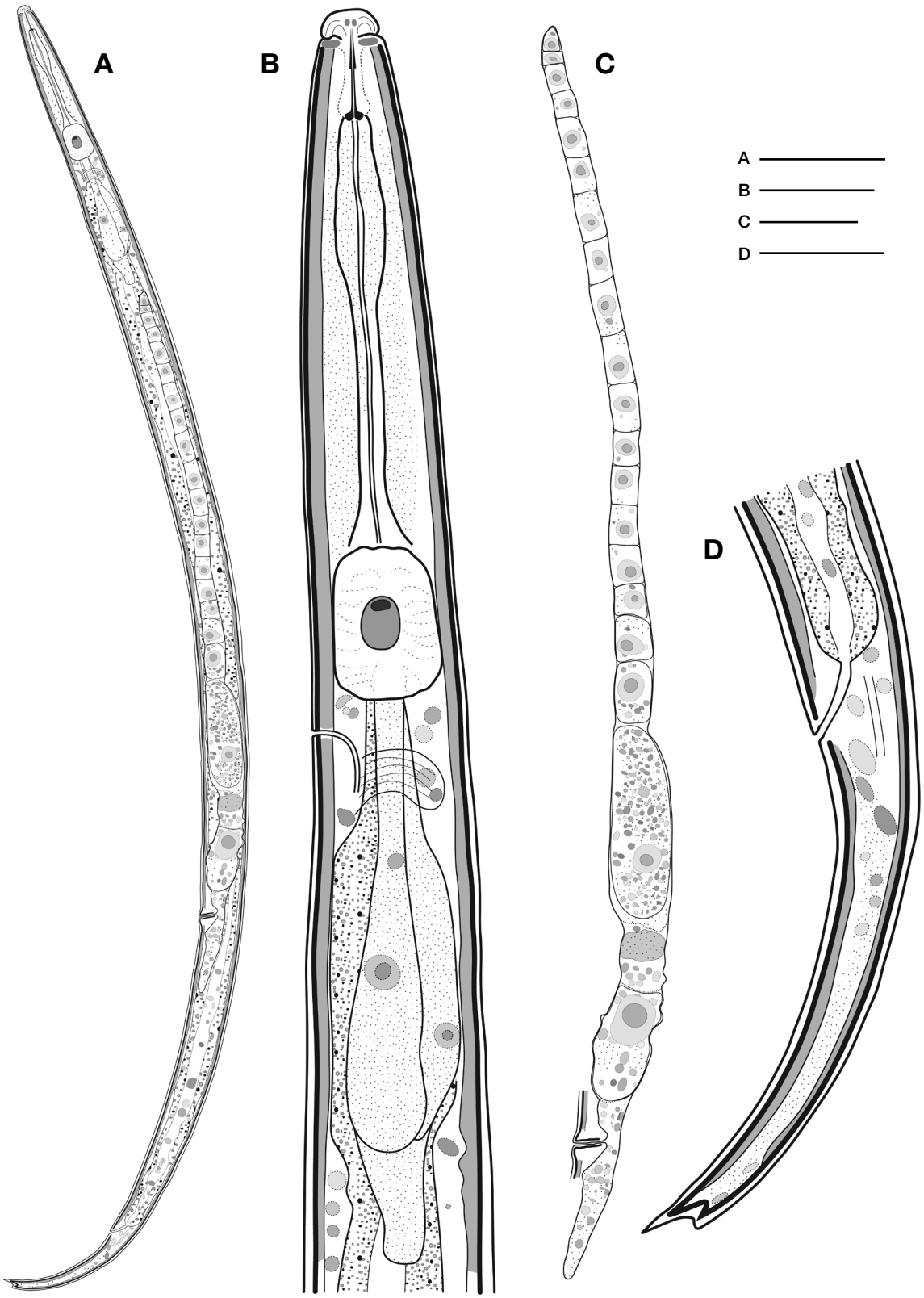

Fig. 1. *Aphelenchoides bicaudatus* (Imamura, 1931) Filipjev and Schuurmans Stekhoven, 1941. A, Entire female; B, Neck region; C, Female reproductive system; D, Female posterior region. Scale bars: A = 50 µm, B, D = 10 µm, C = 20 µm.

Table 1. Morphometrics of *Aphelenchoides bicaudatus* in previous publications and this study

	This study	Imamura (1931)	Filipjev and Schuurmans Stekhoven (1941)	Siddiqui and Taylor (1967)
Population	South Korea	Japan	Japan	USA
L (μm)	517.9±3.8 (513.6–522.6)	430 (380–470)	430 (380–470)	460 (410–550)
a	28.3±0.5 (27.7–28.8)	31.5 (31.3–31.7)	31.5 (31.3–31.7)	28.0 (25–31)
b	7.3±0.0 (7.3–7.4)	7.4 (6.8–8.4)	7.4 (6.8–8.4)	8.2 (7.3–9.6)
c	11.3±0.5 (10.7–11.9)	10.6 (9.4–12.6)	10.6 (9.4–12.6)	11.4 (9.8–13.7)
c′	4.6±0.1 (4.4–4.8)	–	–	–
Tail (μm)	45.9±2.5 (43.1–48.8)	–	–	–
V (%)	66.0±0.2 (65.7–66.4)	70.4 (61.7–90.2)	70.4 (61.7–90.2)	67.5 (65–70)
Stylet (μm)	11.2±0.5 (10.4–11.7)	–	–	11.2 (10–12)

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

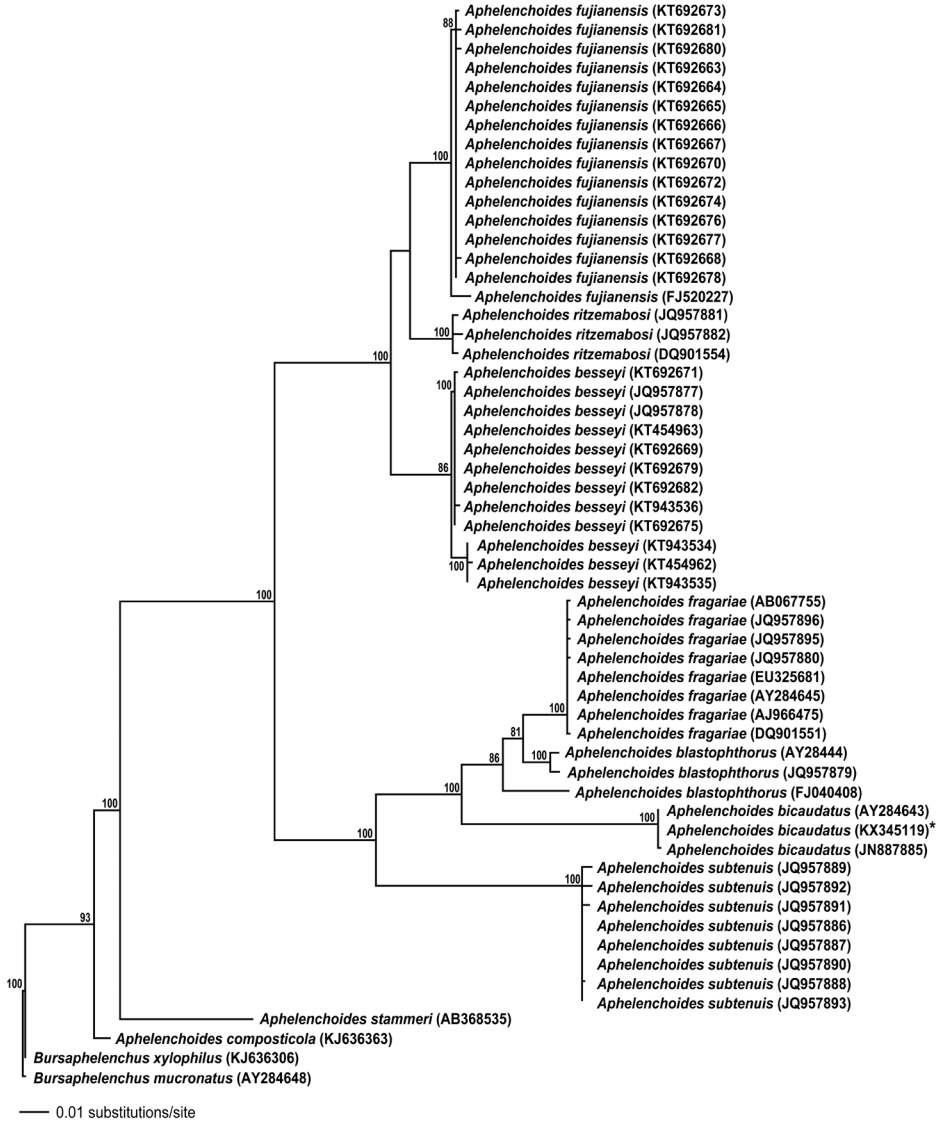

Fig. 2. Maximum likelihood phylogenetic tree of the partial 18S ribosomal DNA sequences of ten *Aphelenchoides* species, including *Aphelenchoides bicaudatus* (* determined in this study) and two outgroups (*Bursaphelenchus mucronatus* and *Bursaphelenchus xylophilus*). Bootstrap values ≥70% are shown above the branches. GenBank accession numbers are in parentheses after the name of the species.

DISCUSSION

The genus *Aphelenchoides* includes diverse species that are found abundantly in a wide range of habitats, but eight species have been recorded in South Korea thus far. *A. bicaudatus* is here reported for the first time in Korea. *A. bicaudatus* is distinguished from other members of the genus by having an unevenly bifurcated tail tip with prongs of different lengths (Sanwal, 1961). *A. hainanensis* (Rahm, 1938) T. Goodey, 1951 also has a bifurcated tail tip, but is 1.4−3.4 times longer than *A. bicaudatus* and has a relatively shorter tail and esophagus. In *A. bicaudatus*, only females have a bifurcated tail tip, whereas in *A. hainanensis* both males and females have a bifurcated tail. Males of *A. bicaudatus* were not found in the present study and appear to be extremely rare, consistent with observations by Siddiqui and Taylor (1967). The morphological characters of *A. bicaudatus* reported here are the same as previously described, and the morphometric parameters are also generally within the ranges given in other work (Siddiqui and Taylor, 1967) (Table 1). However, body length in the original species description (Imamura, 1931) varies from subsequent studies (Siddiqui and Taylor, 1967; this study). In addition to detailed redescription of its morphology, we reconstructed phylogenetic tree using ML method for the partial 18S rDNA sequences of some selected *Aphelenchoides* species available on GenBank. The resulting tree depicted two distinct species groups with 100% bootstrap supporting values (Fig. 2): One containing *A. fujianensis*, *A. ritzemabosi*, and *A. besseyi*, and the other containing *A. fragariae*, *A. blastophthorus*, *A. bicaudatus*, and *A. subtenuis*. Within the latter species group, *A. bicaudatus* sequences (including Korean isolates; KX345119) were clustered together and in turn sister to *A. fragariae* + *A. blastophthorus* with 100% bootstrap value. Intraspecific variation of 18S rDNA among three individuals of *A. bicaudatus* is lower ($\leq$0.6%) than interspecific variation among *Aphelenchoides* species ($\geq$20.8%). This result indicates that 18S rDNA sequence provides reliable molecular evidence for species identification in the genus *Aphelenchoides*.

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