Arthrobotrys koreensis, a new nematode-trapping species from Korea

H. Y. Wu[1], D. G. Kim[2]*, Y. H. Ryu[2] & X. B. Zhou[3]*

[1] College of Plant Protection, Shandong Agricultural University, Tai'an 271018, People's Republic of China
[2] Institute for Natural Products Research, Gyeongbuk Agricultural Technology Administration, Sangri-ri 133-6, Euiseong, Korea
[3] State Key Laboratory of Crop Biology, Shandong Agricultural University, Tai'an 271018, People's Republic of China

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Arthrobotrys koreensis, a nematode-capturing fungus with adhesive 3-D networks was isolated from soil around the roots of *Dystaenia takeshimana* (Apiaceae) on Ulleung Island, Korea. It has monoseptate, obovoid conidia with a wide distal cell, measuring 18–30 × 9.2–16.7 µm, not or slightly constricted at the septum. Conidiophores are often unbranched and 150–400 µm in height, bearing 1–5 conidia on short denticles at the apex.

Keywords: sp. nov., adhesive network, taxonomy, molecular phylogeny.

The predacious behavior of *Arthrobotrys* species had been impressively recorded by Zopf (1888). Then many nematologists identified predatory and endoparasitic nematode-destroying fungi (e.g. Barron 1977). Some potential biological control agents for nematodes have been extensively reviewed and applied to control nematodes in soil (Sayre & Walter 1991, Sikora 1992, Zhang *et al*. 2008). During the surveys of nematode-trapping fungi in soil from plant rhizosphere of Ulleung Island, Korea, a new *Arthrobotrys* species was isolated. It was morphologically distinct from the known *Arthrobotrys* species, and our molecular analysis using the nuclear ITS region sequence supports its separation. The new species is described and illustrated.

Materials and methods

Fungal isolates and identification

Soil was collected from around the roots of *Dystaenia takeshimana* (Nakai) Kitagawa (Apiaceae) on Ulleung Island, Korea. This plant species is endemic to Korea. Chemical constituents from its root have anti-inflammatory activity (Kim J. S. *et al*. 2006). A modified sprinkling–baiting technique was used for isolating nematode-trapping fungi (Barron 1977). About 1.0 g soil was sprinkled onto plates of 1.7 % corn meal agar (CMA, Difco) and 2 %

* Corresponding Authors e-mails: kimdgkr@naver.com; whyzxb@yahoo.com.cn

water agar (WA), and about 200 nematodes (*Rhabditis* spp.) were added to the surface of the petri dish plate (D = 10 cm) as bait for the nematode-trapping fungi. Two CMA plates and two WA plates were used for each soil sample. Plates were incubated for 2–4 weeks at 25 °C and were examined every other day under a dissecting microscope (Olympus SZ-12) to detect the appearance of nematode-trapping fungi. When a nematode-trapping fungus was detected, its image was captured with an attached digital camera (Nikon DXM1200F) and transferred to CMA for pure culture.

Extraction of DNA, PCR amplification and DNA sequencing

DNA of the fungal isolate was extracted by a previously described procedure (Kim D. G. *et al.* 2006). The extracted DNA was suspended in TE buffer (10 mM Tris-HCl and 10 mM EDTA, pH 8.0). The primers ITS1 (5′-TCCG-TAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) were used to amplify ITS region I and 5.8S rDNAs, ITS region II, and a portion of the 28S rDNA (White *et al.* 1990). The PCR reaction was conducted by Mygenie Thermal Block (BioNeer) and 0.5 pM ITS 1 and ITS 4 primers were used (White *et al.* 1990). The thermal cycling parameters were initial denaturation at 94 °C for 4 min followed by 30 cycles of denaturation at 94 °C for 60 s, annealing at 56 °C for 40 s, and extension at 72 °C for 70 s; a final extension at 72 °C for 10 min was performed at the end of the amplification. PCR products were electrophoresed in a 2 % agarose gel, and expected products were excised and purified with a Gel Purification Kit (Bioneer, Korea). Purified PCR products were sequenced directly.

Phylogenetic analysis

The nucleotide sequences were compared visually and aligned using Clustal X (ver. 1.8, Thompson *et al.* 1997), and a phylogenetic tree was constructed from genetic distance values calculated using the neighbor joining method (Saitou & Nei 1987). The phylogenetic tree was visualized and edited by TreeView software version 1.6.6 (Page 1996). The sequences extracted from GenBank are listed in Tab. 1.

Conidia measurements

Conidia from fresh culture were measured in water using a Nikon DXM1200F interference contrast microscope. Measurements are given as follows: (21.4)24.7 ± 1.6(26.9) × (11.6)13.9 ± 1.1(15.6) µm, Q = (1.5)1.8 ± 0.2(2.1) (n = 50).

Results and discussion

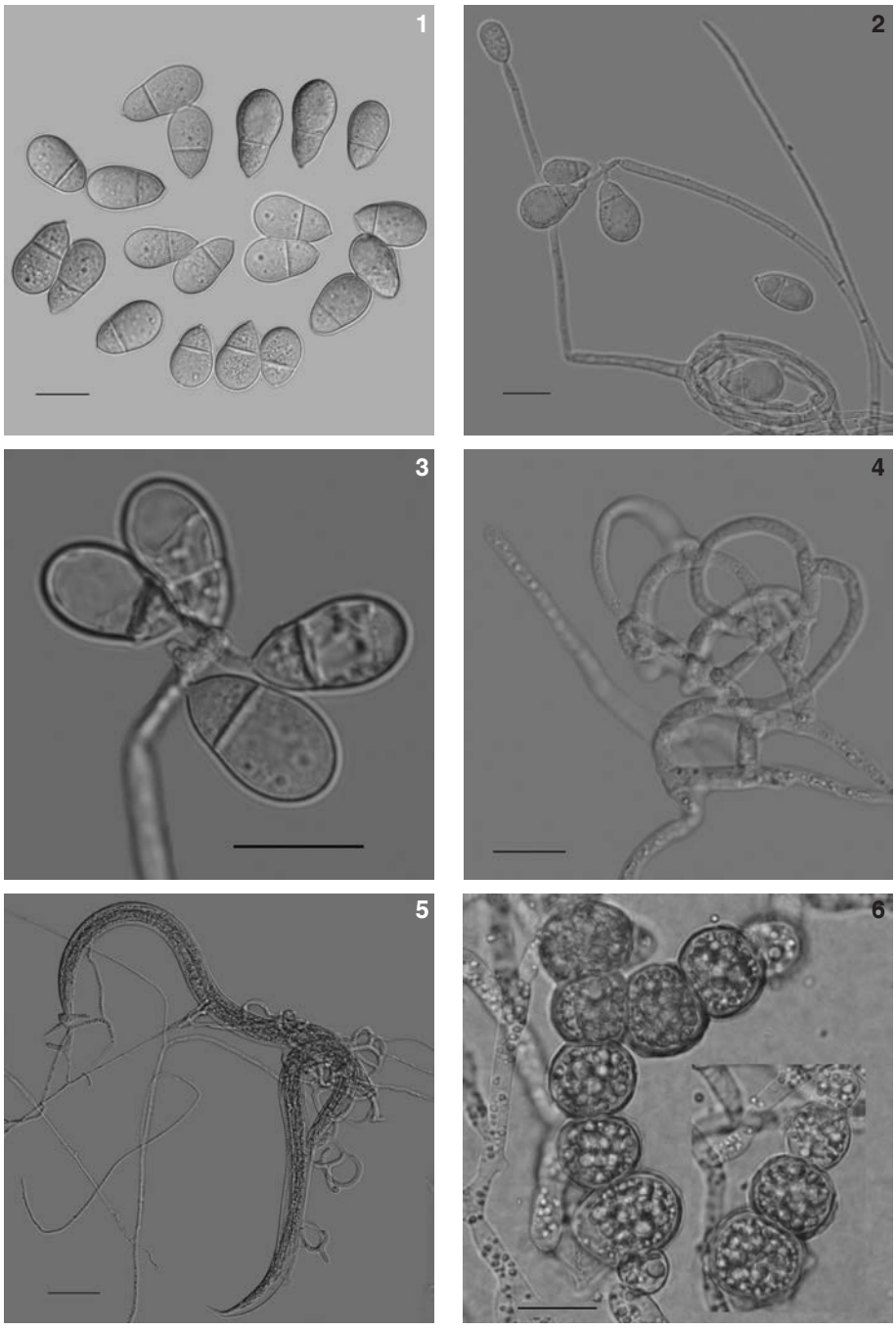
Taxonomy

Arthrobotrys koreensis H.Y. Wu & D. G. Kim, **sp. nov.** – Figs. 1–6
MycoBank no: MB560797

Coloniae in CMA effusae, post 10 dies 25 ºC 6 cm diam., hyphis aeriis sparsis. Hyphis hyalinis, septatis, 2,4–7,9 µm crassis. Laqueis vermiculos nematodeos illaqueantibus,

Tab. 1. – List of fungal species and GenBank accession numbers used in phylogenetic analysis.

Species	GenBank acc. No.	Strain number	Trapping devices
Arthrobotrys amerospora S. Schenck, W. B. Kendr. & Pramer	AF106533	SBUG M1257	Networks
A. botryospora G. L. Barron	U51955	CBS 321.83	Adhesive hyphae
A. cladodes	U51945	isolate CCRC 32697	Networks
A. conoides Drechsler	AF106534	Strain SBU GM12	Networks
A. cylindrospora (R. C. Cooke) S. Schenck, W. B. Kendr. & Pramer	DQ494364	CBS325.70	Networks
A. dactyloides Drechsler	AY965753	CBS109.37	Constricting rings
A. entomopaga Drechsler	AY965758	CBS642.80	Adhesive knobs
A. javanica (Rifai & R. C. Cooke) Jarow.	U51947	CBS 534.63	Networks
A. musiformis Drechsler	U51948	CCRC 32665	Networks
A. oligospora	AM412778		Networks
A. oligospora	EF445989	strain ATCC 96709	Networks
A. oligospora Fresen.	FJ557237	YMF1.01837	Networks
A. pyriformis (Juniper) Schenk, W. B. Kendr. & Pramer	EU977541	123	Networks
A. scaphoides (Peach) S. Schenck, W. B. Kendr. & Pramer	GU171370.1	Strain CBS 226.52	Networks
A. superba Corda	U51949	CBS 109.52	Networks
A. thaumasia	AF106526	strain CBS 322.94	Networks
A. thaumasia (Drechsler) S. Schenck, W. B. Kendr. & Pramer	U51972	isolate 124	Networks
A. vermicola	AY773454	629	Networks
A. vermicola (R. C. Cooke & Satchuth.) Rifai	U51944		Networks
A. cladodes Drechsler	FJ557236	YMF1.01839	Networks
Arthrobotrys sp.	JF304780	C45	Networks
Dactylaria sp.	AY773457		None
Monacrosporium megalosporum (Drechsler) Subram.	AB114475		Networks
M. eudermatum (Drechsler) Subram.	U51975		Networks
M. janus S. D. Li & Xing Z. Liu	AY773459	85-1	Networks
Vermispora sp.	AY773447		None



Figs. 1–6. *Arthrobotrys koreensis*, holotype. **1.** Conidia. **2–3.** Conidiophores and conidia. **4.** Adhesive 3-D network. **5.** Nematode captured by adhesive 3-D network and partially digested. **6.** Chlamydospores (Bars 1-4, 6: 20 µm, 5: 50 µm).

deinde tum integumentum perforantibus, tuber mortiferum intrudentibus, hyphas intus evolventibus quae carnem exhauriunt. Conidiophora hyalina, erecta, solitaria, septata, simplicia, non ramosa, plerumque 150–400 µm alta, basi 4,0–7,3 µm lata, ad apicem angustata 2,4–3,6 µm lata. Conidiis hyalinis, obovatis, apice rotundatis, basi truncatis, interdum aliquid constricta in septum 21,4–26,9 µm longis, 11,6–15,6 µm crassis, 1-septatis. Chlamydosporis globosis vel ellipsoideis.

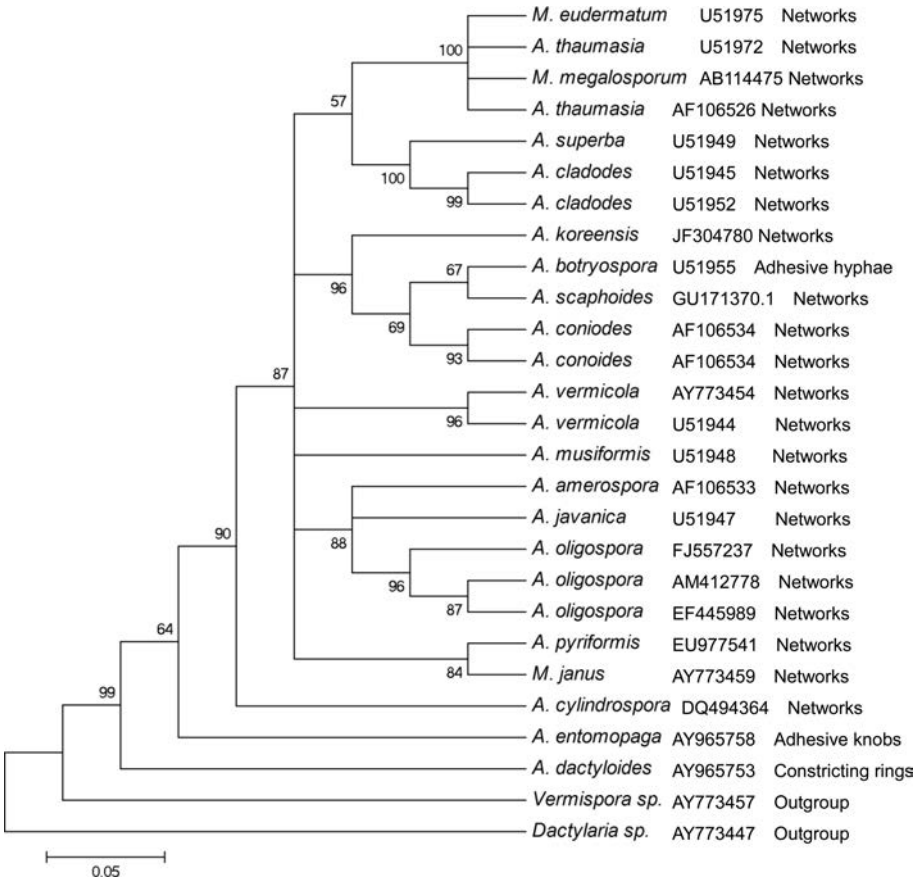
Holotypus. – KOREA, Ulleung Island, Sep 2009, leg. Young Hyun Ryu. The holotype and its living culture (C-4-5) were deposited in the laboratory of the Institute for Natural Products Research, Gyeongbuk Agricultural Technology Administration.

Etymology. – The species epithet refers to Korea, the place where the fungus was first isolated.

Colonies growing on potato dextrose agar (PDA, Difco) denser than those growing on CMA, attaining a diameter of 5.5 cm after 10 days at 25 °C with aerial hyphae; colorless on CMA and yellow on PDA. Spreading of mycelium with vegetative hyphae hyaline, septate and branched, measuring mostly 2.4–7.9 (average 4.6 µm) µm in diameter; presence of nematodes resulting in more or less extensive networks (Fig. 4); nematodes captured by the networks through adhesion and entanglement (Fig. 5). Conidiophores hyaline, erect, 2–6-septate, usually unbranched (Figs. 2, 3), mostly 150–400 µm in height (on average 243 µm), 4.0–7.3 (av. 5.2) µm wide at the base, tapering upwards gradually to a width of 2.4–3.6 (av. 3.0) µm near the tip, bearing 1–4 conidia on 1.6–5.3 (av. 2.5) µm long denticles at the apex. Conidia hyaline, obovoid, widely rounded at the distal end, tapering noticeably toward the slightly protruding base, 1-septate, not or only slightly constricted at the septum, length/width ratio = 1.8, 21.4–26.9 (av. about 24.1) µm long and 11.6–15.6 (av. about 13.9) µm wide, with 14.3–18.0 (av. 16.1) µm and 8.1–10.2 (av. 9.1) µm long upper and lower cells respectively (Figs. 1, 2). Chlamydospores observed 50 days after inoculation; being spherical to ellipsoidal, terminal or intercalary by forming long chains, mostly 14–25 (av. 18.7) × 14.3–27.7 (av. 22) µm in size.

In conidial shape *Arthrobotrys koreensis* most closely resembles *A. oligospora* and *A. conoides* (Drechsler 1937, Van Oorschot 1985). The new species is distinguished from these morphologically similar species by the presence of short denticles at the uninflated tip instead of nodules on an inflated node, also by the small number of conidiogenous loci, and the frequency of conidiophores which did not elongate by repeated proliferation. Comparison between *A. koreensis* and closely related *Arthrobotrys* spp. was conducted by phylogenetic analysis (Fig. 7), which showed that it is most closely related to *A. conoides* and *A. cladodes*. BLAST result showed that it was closely related to *A. botryospora,* which differs in non-septate conidia.

BLAST results also showed that it is most closely related to *A. conoides*, *A. scaphoides*, *Orbilia fimicola*, *A. botryospora*, *A. cladodes*, *A. thaumasia,* and *O. auricolor*, with greater than 93 % identity (not shown). However, there is a notable difference between *A. koreensis* and other species with respect to the morphology of conidia and denticles, and these characteristics did not change when the strain was transferred or the culture conditions were changed.



Figs. 7. Parsimony analyses of ITS regions. Bootstrap values were obtained from 1000 replications, and only >50 % are shown. Evolutionary analyses were conducted in MEGA4, outgroups without traps.

In pure culture on maize meal agar, for *Arthrobotrys oligospora*, sporulation is much more profuse, 20–30 clusters. *Arthrobotrys conoides* has an irregularly expanded tip as many as 30 conidia in dense capitate arrangement; subsequently often, following repeated elongation, giving rise successively to additional clusters of conidia. The conidiophore of *A. cladodes* is branched. *Arthrobotrys koreensis* produces 1–5 conidia on short denticles at the tip of unbranched conidiophores.

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