MYCOBIOLOGY
2022, VOL. 50, NO. 4, 213–218
https://doi.org/10.1080/12298093.2022.2097770

RESEARCH ARTICLE

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Mitrula aurea sp. nov., A New Aero-Aquatic Species from the Republic of Korea

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ABSTRACT

The genus *Mitrula* (Mitrulaceae, Helotiales), as also known as swamp beacons, inhabits submerged, decaying vegetation in standing or decaying needles, twigs, leaves, and shallow water. They play an important role in carbon cycling in some freshwater ecosystems. In the herbarium of the Korea National Arboretum (KH), seven *Mitrula* specimens were collected during mushroom forays in the period from 2019 to 2021. The Korean collections were found to be macromorphologically closely related to *M. paludosa* and *M. elegans*, but micromorphologically they could be distinguished by characteristics of slightly narrower asci and aseptate ascospores. Our molecular phylogenetic analyses of the internal transcribed spacer (ITS) and 28S rDNA regions also revealed that our specimens were related to *M. paludosa* and *M. elegans*, but formed a distinct clade. Based on these results, we reported our specimens as new to science and discussed the phylogeny and diversity of *Mitrula* species.

ARTICLE HISTORY
Received 21 March 2022
Revised 30 June 2022
Accepted 30 June 2022

KEYWORDS
Aero-aquatic; *Helotiales*; *Mitrula*; new species; phylogeny

1. Introduction

Aero-aquatic fungi play an important role in carbon cycling, specifically, during the decomposition of wood debris and leaf litter in woodland streams and rivers [1,2]. Most aero-aquatic fungi inhabiting freshwater habitats are known to form only asexual morphs (e.g., aquatic hyphomycetes) [3–5]. However, some species belonging to the families *Mitrulaceae* and *Vibrissaceae* are characterized by the formation of only the sexual morph in nature [5].

The family *Mitrulaceae* includes only one genus, namely *Mitrula* [6]. Approximately 80 *Mitrula* species have been recorded to date (ref. Index Fungorum; www.indexfungorum.org, accessed on March 18 2022), but after revisions, 12 species remained (namely *M. lunulatospora*, *M. luteola*, *M. microspore*, *M. multiformis*, *M. norvegica*, *M. omphalostoma*, *M. paludosa*, *M. pistillaris*, *M. roseola*, *M. serpentine*, *M. sphaerocephala*, and *M. ushuaiae*) [2,5,7]. Most *Mitrula* species have yellow to orange and club-shaped apothecia and occur on submerged, decaying vegetation in shallow standing waters [2]. However, some *Mitrula* species have white to flesh-pinkish apothecia and occur on sandy soil associated with dead plant material [1,6].

Only one species, *M. paludosa*, has been recorded in South Korea [8,9]. It was reported that the species closely resembles *M. paludosa*, although its microscopic features differ slightly from those of European *M. paludosa*. However, they did not perform molecular phylogenetic analysis or detailed morphological comparisons with related taxa.

In the present study, we reevaluated the Korean *Mitrula* species based on morphological observations and molecular analysis. We described the Korean *Mitrula* species and proposed a species new to science.

2. Materials and methods

2.1. Sample collection

In 2019, 100 *Mitrula* ascocarps were collected in woodland streams in South Korea. Since then, several samples of *Mitrula* species have been collected during mycological surveys in Korea. These samples were collected in the period from April to May, and most of the ascocarps emerged above the needles of *Pinus densiflora* or leaf litter in streams and rivers (Figure 1). Both yellow and whitish apothecia (KA21-0148) were collected from the same location. The detailed collection dates and localities of the

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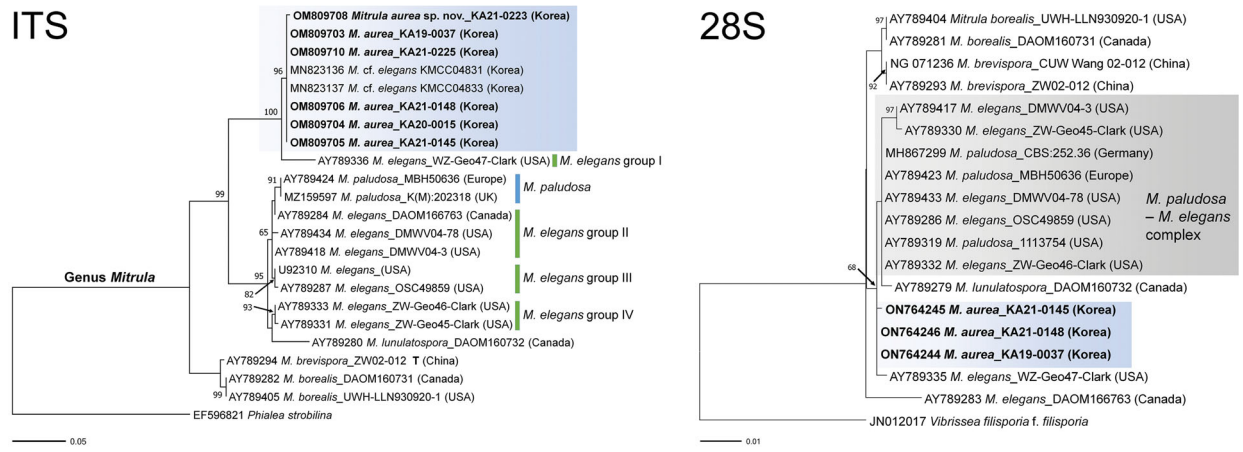

Figure 1. Phylogenetic tree of *Mitrula* species based on the RAxML analysis of their ITS and 28S regions. Specimens identified during this study are indicated in bold. Bootstrap values higher than 60% are shown in the branches. The scale bar equals the number of nucleotide substitutions per site.

Korean samples are listed in Table 1. Type specimens have been deposited in the herbarium of the Korea National Arboretum (KH), and MycoBank numbers have been provided for the new species.

2.2. Morphological observations

Macro-morphological characteristics were examined using a dissecting microscope. For micro-morphological characterization, the asci, paraphyses, and ascospores were examined using a compound microscope (Zeiss Plan-Apochromat, Oberkochen, Germany) and photographed with an Axiocam 506 color camera (Zeiss). Microscopic parameters were measured and calculated using the ZEN 3.1 blue edition software (Zeiss). Whenever possible, 30 representative images were acquired for each microscopic characteristic. Morphological characteristics of *M. elegans*, *M. lunulatospora*, and *M. paludosa* were from Redhead [1].

2.3. DNA isolation, PCR, sequencing, and phylogenetic analysis

Genomic DNAs was extracted from six specimens using a DNeasy Plant Mini DNA Extraction Kit (Qiagen Inc., Valencia, CA) according to the manufacturer's instructions. The genomic DNA was used for PCR amplification. The internal transcribed spacer (ITS) and 28S regions of rDNA were amplified. The obtained PCR amplicons were purified using a QIAquick purification Kit (Qiagen Inc., Germantown, MD, USA) and sequenced at the Macrogen sequencing facility (Macrogen Inc., Seoul, South Korea). A Blastn search against the NCBI database (http://www.ncbi.nlm.nih.gov) was performed, and new sequences were deposited in GenBank (www.ncbi.nlm.nih.gov/genbank/). For phylogenetic analyses, the dataset was aligned using MAFFT v. 7.475 [13], selecting the auto option. The DNA sequences were assembled and manually edited in BioEdit v. 7.2.5 [14] and MEGA v. 7.0.26 [15]. A phylogenetic tree was constructed using RAxML on the CIPRES web server (https://www.phylo.org). The relative robustness of individual branches was estimated by bootstrapping with 1000 replicates. *Phialea strobilina* (EF596821) and *Vibrissea filisporia* f. *filisporia* (JN012017) were used as the basal taxa.

3. Results

3.1. Phylogenetic analysis

Six sequences from the Korean collections were newly generated and deposited in GenBank. GenBank accession numbers for the ITS and 28S regions are listed in Table 1. In the phylogenetic tree of *Mitrulaceae* obtained based on the ML analysis, the Korean collections formed a supported monophyletic group which was distant from the rest of *Mitrula* species, with 100% bootstrap support (Figure 1). In the ITS tree, *M. paludosa* and *M. elegans* groups II, III, and IV were closely related, except for *M. elegans* group I. However, *M. elegans* group I was closely related to the Korean collections (Figure 1). In the 28S tree, the Korean collections formed a monophyletic group, but *M. paludosa* and *M. elegans* formed a complex group.

3.2. Taxonomy

Mitrula aurea sp. nov.: S-E. Cho, H.S. Kim, Y-N Kwag & C.S. Kim [#MB843007] (Figures 1 and 2).

Holotype: Found on the soil or dead foliage of *Pinus densiflora* in Goesan-gun, Chungcheongbuk-do, Korea, on May 16 2019; collected by H.S. Kim and Y-

Table 1. A list of species, specimens, and GenBank accession numbers of sequences used for phylogenetic trees in this study.

Species	Specimen no.	Locality	GenBank accession no.		References
			ITS	28S	
Mitrula aurea	KA19-0037	Korea	**OM809703**	**ON764244**	In this study
M. aurea	KA20-0015	Korea	**OM809704**	–	In this study
M. aurea	KA21-0145	Korea	**OM809705**	**ON764245**	In this study
M. aurea	KA21-0148	Korea	**OM809706**	**ON764246**	In this study
M. aurea	KA21-0188	Korea	**OM809707**	–	In this study
M. aurea	KA21-0223	Korea	**OM809708**	–	In this study
M. aurea	KA21-0225	Korea	**OM809710**	–	In this study
M. cf. *elegans*	KMCC04831	Korea	MN823136	–	Derived from NCBI
M. cf. *elegans*	KMCC04833	Korea	MN823137	–	Derived from NCBI
M. borealis	DAOM160731	Canada	AY789282	AY789281	Wang et al. [2]
M. borealis	UWH-LLN930920-1	USA	AY789405	AY789404	Wang et al. [2]
M. brevispora	ZW02-012	China	AY789294	AY789293	Wang et al. [2]
M. brevispora	CUW Wang 02-012	China	–	NG071236	Wang et al. [2]
M. elegans	WZ-Geo47-Clark	USA	AY789336	AY789335	Wang et al. [2]
M. elegans	DAOM166763	Canada	AY789284	AY789283	Wang et al. [2]
M. elegans	DMWV04-78	USA	AY789434	AY789433	Wang et al. [2]
M. elegans	DMWV04-3	USA	AY789418	AY789417	Wang et al. [2]
M. elegans	OSC49859	USA	AY789287	AY789286	Wang et al. [2]
M. elegans	ZW-Geo46-Clark	USA	AY789333	AY789332	Wang et al. [2]
M. elegans	ZW-Geo45-Clark	USA	AY789331	AY789330	Wang et al. [2]
M. elegans	–	USA	U92310	–	Gernandt et al. [10]
M. lunulatospora	DAOM160732	Canada	AY789280	AY789279	Wang et al. [2]
M. paludosa	MBH50636	Europe	AY789424	AY789423	Wang et al. [2]
M. paludosa	K(M):202318	UK	MZ159597	–	Royal Botanic Garden Kew Fungarium
M. paludosa	CBS:252.36	Germany	–	MH867299	Vu et al. [11]
M. paludosa	1113754	USA	–	AY789319	Wang et al. [2]
Phialea strobilina	CBS 643.85	Norway	EF596821	–	Derived from NCBI
Vibrissea filisporia f. *filisporia*	ILLS60499	USA	–	JN012017	Hustad and Miller [12]

The GenBank accession numbers of sequences generated in this study are in bold.
NCBI: National Center for Biotechnology Information.

N. Kwag, KA19-0037. GenBank Nos.: OM809703 (ITS sequence) and ON764244 (28S sequence).

Etymology: From the Latin word "*aureus*", meaning "gold-colored", because of the goldish or golden yellow cap-color of the ascocarps.

Description: Ascocarps gregarious, solitary to caespitose, fleshy, 1.5–4.0 cm high. Cap ovoid to irregularly pyriform, cylindrical or clavate, smooth to rugose, slightly tremellose, mostly luteous to yellow-luteous, but rarely whitish to pale pinkish, 5–8 mm wide. Stipes unbranched, glabrous, and slightly lubricous above, 1–3 mm side above, hyaline; basal part occasionally enlarged, matted hyphal hairs below moderately, developing reddish-brown stains when damaged. Asci eight-spored, elongate to clavate, 95–130 × 5.5–7.0 µm. Paraphyses filiform, slightly enlarged above, 100–150 × 2.5–3 µm. Ascospores elliptical to ovoid, broadly cylindrical, 12.0–17.0 × 2.3–3.0 µm, not septate.

Habitat: Gregarious in shallow water, on decaying needles, twigs, and leaves of *Pinus densiflora*.

Sporulating period: April–May (spring).

Specimens examined: KOREA: Chungcheongbuk-do, Goesan-gun, May 16 2019 (KA19-0037); April 30 2021 (KA21-0145); May 3 2021 (KA21-0148), Gangwon-do, Yangyang-gun; May 7 2020 (KA20-0015), Gangwon-do, Inje-gun; May 15 2021 (KA21-0188), Gangwon-do, Pyeongchang-gun; May 29 2021 (KA21-0223); May 30 2021 (KA21-0225).

Notes: The asci of the Korean collections were slightly narrower (95–130 × 5.5–7.0 µm) than those of *M. paludosa* (asci: 119–133 × 6.5–7.5 µm [1]). Microscopic features of the samples from the Korean collections were similar to those of *M. elegans*. However, samples from the Korean collections had a shorter stipe and no septate of ascospores compared with those of *M. elegans*. In addition, the Korean collections were clearly distinct from *M. paludosa* and *M. elegans* in the ITS and 28S trees (Figure 1), and geographical distribution and sporulating period (Table 2).

4. Discussion

The taxonomic position of the genus *Mitrula* among the Helotiales families is controversial [2,16]. Until 1994, the genus *Mitrula* was identified as a member of the family Geoglossaceae based on its clavate apothecia [1]. However, based on the observation of ascus ultrastructures, Verkley [17] concluded that the genus *Mitrula* (specifically, *M. paludosa*) is closely related to members of the family Sclerotiniaceae. This placement was accepted until 2004 [18]. In the early 1990s, with the introduction of molecular phylogeny into fungal taxonomy, Wang et al. [2] placed the genus *Mitrula* within Leotiomycetes based on ITS rDNA sequences. Currently, based on the comprehensive classification

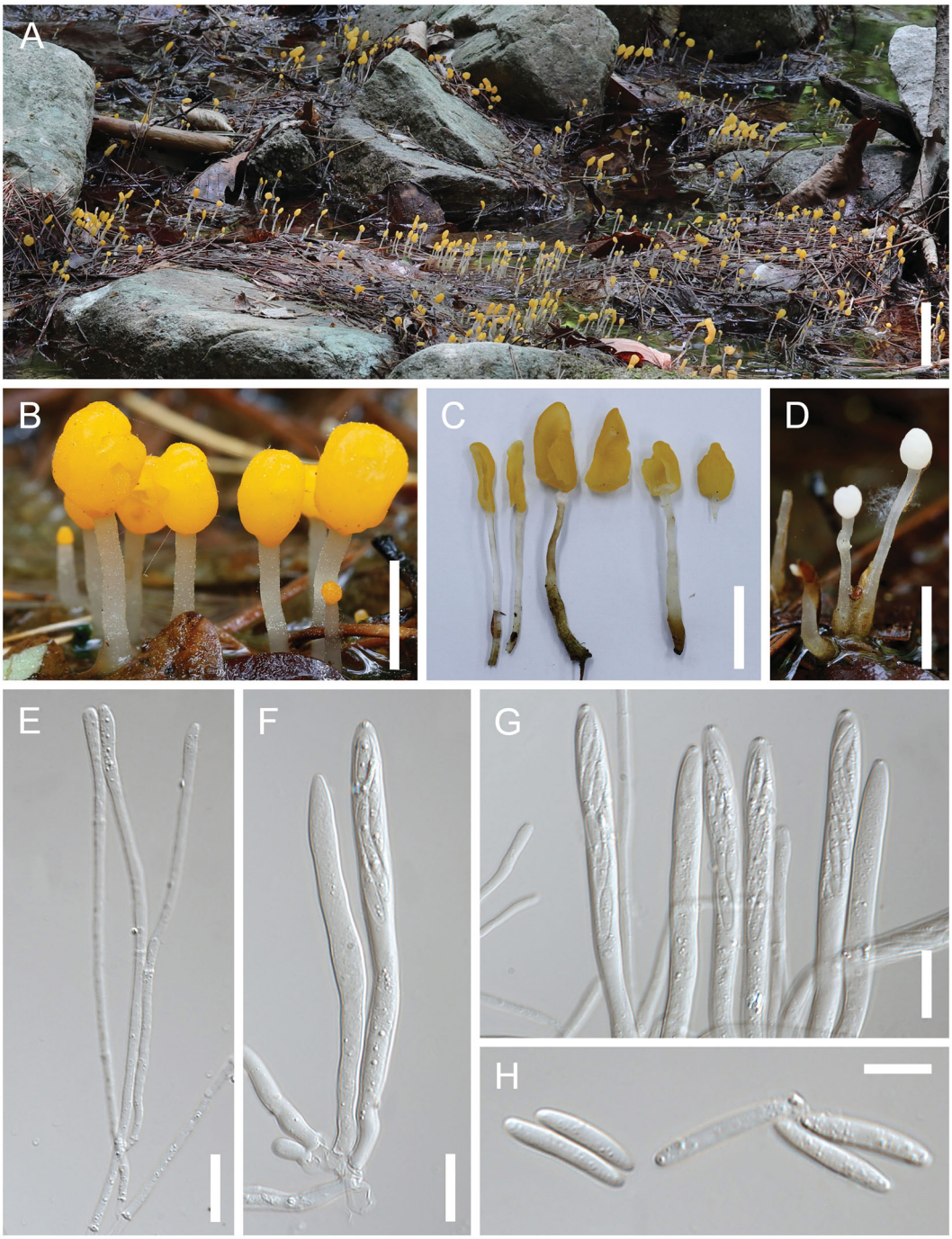

Figure 2. Morphological characteristics of *Mitrula aurea* sp. nov. (A–C) Most of our specimens have yellow apothecia, but one collection (KA21-0148) produced white apothecia (D); (E) Paraphyses; (F, G) Asci; (H) Ascospores. Scale bars: A = 5 cm; B–D = 1 cm; E–H = 10 µm.

of Leotiomycetes by Ekanayaka et al. [6], the genus *Mitrula* is placed within the family Mitrulaceae and characterized as saprobic, with white to flesh-pinkish or yellow-orange hymenium, clavate to stipitate apothecia, eight-spored asci, and no recorded asexual morphs in nature.

Mitrula paludosa is a type species of the genus *Mitrula*. It was first described by Elias Magnus Fries in 1816. However, this species never had a type specimen designated until Redhead provided a neotype based on European specimens [1]. In South Korea, Park et al. [9] reported the Korean *Mitrula* species as *M. paludosa*. However, the present study revealed that the Korean *Mitrula* species are phylogenetically distinct from *M. paludosa* and other related *Mitrula* species (see Figure 1). According to Redhead [1], *M. paludosa* and related North American *Mitrula* species (*M. borealis*, *M. elegans*, and *M. lunulatospora*) are clearly distinctive based on their geographic distribution and sporulation period. Similarly, in our ITS and 28S trees, *M. aurea*, including two Korean *Mitrula* samples (KMCC04831 and KMCC04833 in ITS tree only, collected from the National Institute of Horticultural and Herbal Science, Rural Development Administration, Eumseong, South Korea), was geographically very far from other reported *Mitrula*

Table 2. Morphological comparison between *Mitrula aurea* sp. nov. and morphologically related *Mitrula* species.

Character	*M. aurea* sp. nov.	*M. elegans*	*M. lunulatospora*	*M. paludosa*[a]
Cap color	Golden-yellow, rarely whitish to pale pinkish	Bright orange to bright yellow, becoming ochraceous orange, rarely pinkish	Buff, yellow-orange	Orange to yellow-orange, becoming pinkish with age when submerged
Stipe color	Whitish	White to faintly pinkish	White or tinted pink	Whitish
Stipe size	1.5–4 cm	2–10 cm	2.4–5.2 cm	2–5 cm
Asci shape	Elongated-clavate	Elongated-clavate	Elongated-clavate	Elongated-clavate
Asci size	95–130 × 5.5–7 μm	115–123 × 5–7.5 μm	91–135 × 6.5–8.5 μm	119–133 × 6.5–7.5 μm
Ascospore color	Hyaline	Hyaline	Hyaline	Hyaline
Ascospore size	12–17 × 2.3–3 μm	11–17.5 × 2–2.5 μm	11–19 × 2.5–3.5 μm	11–17.5 × 2–3 μm
Ascospore shape	Elongated-clavate, aseptate	Narrowly cylindrical or clavate, one- or rarely two celled	Lunate to allantoid or cymbiform, one- or rarely two celled	Cylindrical, oblong-elliptical to elongated-clavate, one- or two celled
Habitat	On decaying needles, twigs, and leaves of *Pinus densiflora*	On the remains of conifer needles, cones, and aquatic plants in standing water	On sandy soil associated with *Pinus* spp.	On rotting wood and plant remains, conifer needles in flowing or standing water
Sporulating period	Spring only	Spring to autumn	Spring to summer	Spring to autumn
Distribution	South Korea	Canada, USA	Canada, USA	Europe

[a]Mainly derived from neotype-description of *M. paludosa* in Redhead [1].

species except for *M. elegans* group I (see Figure 1), and its sporulating period is in spring (Table 2).

In the present study, *M. elegans* was not monophyletic (Figure 1). It was divided into four groups in ITS tree, and all specimens were reported from North America. *M. elegans* is characterized by slender, cylindrical to clavate ascospores. Although *M. elegans* groups I, II, III, and IV are morphologically very similar, they can be divided into four species based on the phylogenetic concept of fungal taxonomy; this is especially true for *M. elegans* group I, which was distinct from the other *M. elegans* groups in the ITS tree [2]. Due to the lack of microscopic differences, further morphological, ecological, and molecular studies are required to clarify the relationship between *M. elegans* groups. The Korean *Mitrula* species was found to be morphologically similar to *M. elegans*; however, they were clearly distinct in the ITS and 28S trees (Figure 1; Table 2).

Interestingly, one specimen of Korean *Mitrula*, KA21-0148, had white apothecia (see Figure 2). Although this morphological characteristic is significantly different from that of the other Korean specimens, it was well-supported by a clade in phylogenetic trees (Figure 1). Similarly, *M. elegans* and *M. paludosa* have yellowish apothecia, but they become pinkish or whitish when aging or submerged (Table 2) [1]. Therefore, we concluded that KA21-0148 is the same Korean *Mitrula* species (*M. aurea*) because apothecial color may not be an important feature in *Mitrula* taxonomy.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This research was supported by a research fund from the Korea National Arboretum (Project No.: KNA1-1-25, 19-2) and the Research Program of Agricultural Science and Technology Development (PJ01437001) of the National Institute of Horticultural and Herbal Science.

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