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RESEARCH ARTICLE

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Novel *Sarcoscypha* Species from National Parks in Korea: *Sarcoscypha humida* sp. nov.

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ABSTRACT

Sarcoscypha (Sarcoscyphaceae, Pezizales) is a saprobic fungus characterized by the cup or disc-shaped blight red apothecium and oblong to ellipsoid ascospores. The 18 species of *Sarcoscypha* were known to occur in Europe, North America, and tropical Asia. However, up to date, only two *Sarcoscypha* species have been reported in Korea. In this study, novel *Sarcoscypha* specimens were collected from Juwangsan, Odaesan, and Taebaeksan National Parks from September to October in Korea. This species is well distinguished from other *Sarcoscypha* species according to the molecular and phylogenetic analysis based on internal transcribed spacer (ITS) region. Here, we provided detailed descriptions with illustrations and a phylogenetic tree to report our specimens as novel *Sarcoscypha* species.

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1. Introduction

The genus *Sarcoscypha* (Fries.) Boud., which belongs to the Sarcoscyphaceae, Pezizales, Pezizomycetes, and Ascomycota, was established more than 130 years ago [1]. This genus is a general decomposer on beech, elm, hazel, willow, oak, and rose family, which forms blight red apothecium surface; cup or disc or saucer-shape apothecium; and oblong to ellipsoid ascospores [2,3]. According to the Index Fungorum database, about 82 species were listed in the genus *Sarcoscypha* (Index Fungorum: http://www.indexfungorum.org, accessed August 2023). However, many species remain unknown or recognized as other genera of the Sarcoscyphaceae, or other families of the Pezizales, even the Helotiales [2]. Baral [2] recognized that 18 species are provisionally accepted as *Sarcoscypha* species which are distributed in North America, Europe, and tropical Asia. However, 20 taxa can be tentatively assigned into the genus to date because *Sarcoscypha chudei* was re-classified as *Kompsoscypha chudei* [4], and three novel *Sarcoscypha* species were additionally reported from Africa and Taiwan after 2011 [3,5]. Although 10 species are known to be distributed in Asia, only two species have been recorded in Korea (*Sarcoscypha coccinea* and *S. hosoyae*) [6].

Traditionally, the genus *Sarcoscypha* has been classified through morphological analysis and taxonomic identification and requires fresh specimens due to the plasticity of the specimens [7]. The fresh materials provide reliable taxonomical keys (e.g., spore guttulation) to distinguish the taxa from different environments [7,8]. However, it is not easy to obtain fresh materials, so additional classification methods are needed. In the previous study, the genus *Sarcoscypha* was identified using molecular DNA-based analysis with the internal transcribed spacer (ITS) region, and it was confirmed that they form the core clade of *Sarcoscypha* and have a phylogenetic relationship [3,9,10]. These results support successfully to report novel *Sarcoscypha* species [3].

The National Institute of Biological Resources (NIBR) has conducted a project to survey them in the national parks, in Korea. During the study, novel *Sarcoscypha* species candidates were collected from three different national parks. We performed

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morphological and molecular analysis for the accurate identification of *Sarcoscypha* specimens. Here, we present them as a novel species and provide a detailed description of novel *Sarcoscypha* species based on the morphological characteristics.

2. Materials and methods

2.1. Sampling

Sampling was conducted in Juwangsan National Park on October 11 2019 and October 30 2021. The specimens NIBRFG0000509969 and NIBRFG0000510138 were found on dead hardwood branches near the gorge. We desiccated the sample in a drying oven at 60 °C for 24 h. Subsequently, the samples were stored in zipper bags with silica gel. The additional dried specimens were obtained from the NIBR. One was found near the Danggol Valley in Taebaeksan National Park on October 30 2019 and the other was collected from Needle fir (*Abies holophylla* MAX.) forest beside of valley in Odaesan National Park on September 8 2017. The specimens are deposited in the herbarium of the NIBR.

2.2. Molecular approach

DNA extraction was conducted using the AccuPrep Genomic DNA Extraction Kit (Bioneer, Daejeon, South Korea) from the dried specimens according to the manufacturer's protocol. PCR for the ITS region was carried out according to the previously described method with primer pairs ITS1F/ITS5 and ITS4/LR3 [11–13]. Sequencing of the PCR amplicons was performed by Macrogen Inc. (Seoul, South Korea). The large subunit rRNA (LSU), RNA polymerase II (*RPB2*), and the translation elongation factor-1 alpha (*TEF-1α*) sequence regions were additionally amplified to provide sequence information of type material following the previously described method with primer pairs: LR0R/L5 for *LSU*, fRPB2-5f/fRPB2-7cR for *RPB2*, and 983F/2218R for *TEF-1α* region, respectively [12,14–16].

For phylogenetic analysis, the closest relative sequences of *Sarcoscypha* and two outgroup sequences were obtained from the NCBI GenBank database (www.ncbi.nlm.nih.gov/genbank/). The obtained ITS sequences were assembled, proofread, and edited using MEGA v. 7 and were aligned using MAFFT 7.130 [17,18]. The alignment was checked by eye and was manually adjusted. The phylogenetic analyses were performed by MrBayes 3.2.3 on XSEDE with a total of 20,000,000 generations with sampling every 1000 generations for Bayesian inference (BI) [19]. jModeltest 2.1.10 with the Bayesian information criterion (BIC) with default options was used to test the best-fitting DNA substitution models [20]. The model for ITS was K80 + G. The phylogenetic analysis was also conducted by RAxML v. 7.03 with GTR + G model of the evolution and 1000 bootstrap replicates for maximum-likelihood (ML) analysis [21]. The first 25% of trees were eliminated as burn-in and constructed a 50% majority rule consensus tree. All phylogenetic analyses were conducted on the CIPRES web portal [22]. Phylogenetic tree editing was conducted using FigTree-version 1.4.3 [23] and Adobe Illustrator CS6 (Adobe Systems, Inc., San Jose, CA). The sequences used for phylogenetic analysis were listed with GenBank accession number in Table 1.

2.3. Morphological analysis

The macro-morphological characteristics of the species were observed simultaneously with sampling. The color of the macroscopic structures in descriptions follows Ridgway [24]. The microscopic feature observation was performed from slide preparations of dried specimens mounted in distilled water using an Olympus BX51 light microscope (Tokyo, Japan). The 5% potassium hydroxide (KOH) was treated to examine the guttulation of dried ascospores. At least 30 ascospores and asci structures were measured. In this paper, the following abbreviations are used to indicate the size of spores: L = mean of spore length, W = mean of spore width, n = number of spores from a given specimen, and Q = variation in the L/W ratios.

3. Results

3.1. Phylogenetic analyses

The genomic DNA sequences of the ITS of the four specimens were obtained from Macrogen Inc. (Seoul, South Korea). The ITS phylogeny contained 32 sequences, including *Pithya vulgaris* and *P. cupressina* as outgroup taxa. The ML analysis and the Bayesian analysis showed the same tree topology, and the ML tree is represented (Figure 1). In the phylogeny, most of the *Sarcoscypha* species were well divided at the species level, except *S. austriaca* and *S. humberiana*. The closely related species of novel candidate *S. humida* sp. nov. are *S. hosoyae* (97.83% identity in the ITS sequence matrix), *S. dudleyi* (96.39% identity in the ITS sequence matrix), and *S. emarginata* (95.83% identity in the ITS sequence matrix). *Sarcoscypha humida* sp. nov. was clearly separated from other taxa with high support value (PP: 0.91/BP: 91%) (Figure 1).

Table 1. The list of *Sarcoscypha* species in this study for phylogenetic analysis.

Identity	Strain no.	Country	ITS	References
Sarcoscypha austriaca	CUP 63162	USA	U66011	[10]
	CUP 62771	Norway	U66010	[10]
	mh 193	Slovakia	U66012	[10]
Sarcoscypha coccinea	CUP 63157	USA	U66014	[10]
	CUP 62113	USA	U66013	[10]
	CUP 63160	USA	U66015	[10]
Sarcoscypha dudleyi	CUP 62775	USA	U66018	[10]
	mh 192	USA	U66019	[10]
Sarcoscypha emarginata	CUP 62723	Luxembourg	U66020	[10]
	HB2861	Switzerland	U66021	[10]
Sarcoscypha hosoyae	TRL 456	Japan	U66031	[10]
Sarcoscypha humberiana	TNM F28630	China	KT716833	[3]
	CUP 63489	China	U66028	[10]
Sarcoscypha javensis	HMAS 61198	China	U66026	[10]
Sarcoscypha humida	**NIBRFG0000509969**	**Korea**	**MW116458**	**This study**
	NIBRFG0000509970	**Korea**	**MW116459**	**This study**
	NIBRFG0000504788	**Korea**	**MW116460**	**This study**
	NIBRFG0000510138	**Korea**	**OL439727**	**This study**
Sarcoscypha korfiana	mh 705	–	AF026308	[28]
	HMAS 61202	China	U66027	[10]
Sarcoscypha macaronesica	CUP MM 2628	Canary Islands	U66022	[10]
	TFC MIC 6460	Canary Islands	U66023	[10]
Sarcoscypha mesocyatha	TNM F3688	China	KT936558	[3]
	TNM F5134	China	KT936559	[3]
	CUP 62699	USA	U66029	[10]
Sarcoscypha minuta	TNM F28831	China	KT716834	[3]
Sarcoscypha occidentalis	CUP 62777	USA	U66024	[10]
	CUP 63484	USA	U66025	[10]
Sarcoscypha tatakensis	TNM F0754	China	KT716835	[3]
	TNM F0993	China	KT716836	[3]
Pithya cupressina	mh 208	USA	AF006316	[10]
Pithya vulgaris	RK 90001	–	U66008	[10]

The strains in this study are shown in bold.

3.2. Taxonomy

Sarcoscypha humida M. Cho, S.L. Kwon & J.J. Kim, sp. nov. (Figure 2).

MycoBank: MB 840449.

Type: KOREA, Gyeongsangbuk-do, Cheongsong-gun, Juwangsan National Park, Mount Juwang, Jeolgol gorge, 36°24′10.33″N, 129°10′25.33″E, elev. 387 m, October 11 2019, Sun Lul Kwon, on dead wood branch (*Holotype:* NIBRFG0000509969; *GenBank:* ITS, MW116458; LSU, OR882785; *RPB2*, OR871740; *TEF-1α*, OR871739).

Etymology: Name refers to the habitat characteristic where this species is commonly found, typically in humid sites such as valleys or gorges.

Diagnosis: This species is diagnosed by irregularly narrow cup-shaped and sessile to subsessile apothecia with light salmon-orange surface in macroscopic characters, and slightly longer paraphyses than asci and ellipsoidal ascospores in microscopic characters.

Apothecia solitary to scattered, first cyathiform, then irregularly narrow cup-shaped at the mature stage, 5–40 mm diam, sessile to subsessile. *Disc* concave, flame scarlet inner surface, and light salmon-orange surface. *Margin* crenulate, first curved to center, then irregularly wavy in old specimens, inrolled when dry. *External hairs* abundant, distributed on receptacle in young stage, covering the external surface of apothecia surface in mature stage, smooth, straight to flexuous, 3.3–5.1 µm wide. *Ectal excipulum* thin, 97–138 µm thick, textura porrecta, hyphae 2–3.5 µm wide, parallel to outer surface. *Medullary excipulum* 200–250 µm thick, hyphae tightly arranged, 2.5–3.5 µm wide. *Asci* 8 – spored, cylindrical, 230–320 × 10.3–15.7 µm with rounded end. *Ascospores* smooth, hyaline, inamyloid, (11–)13.5–17(–21.5) × (5–)6.0–7.5(–8.5) µm, L = 15.5 µm, W = 6.9 µm, Q = (1.5–)1.9–2.5(–3.7) (n = 79/2), ellipsoid to broadly ellipsoid. *Paraphyses* filiform, septate, slightly longer than asci, 2–3 µm wide, slightly capitate tips, filled with red pigment.

Additional specimens examined: KOREA, Odaesan-ro, Jinbumyeon, Pyeongchang-gun, Odaesan National Park, Mount Odae, 37°43′37.59″N, 128°35′44.1″E, elev. 650 m, September 8 2017, Changmu Kim, on dead wood (NIBRFG0000504788); KOREA, Sodo-dong, Taebaek-si, Taebaeksan National Park, Mount Taebaek, October 30 2019, Changmu Kim, on dead wood branch (NIBRFG0000509970); KOREA, Gyeongsangbuk-do, Cheongsong-gun, Juwangsan National Park, Mount Juwang, Jeolgol gorge, 36°24′12″N, 129°10′19.4″E, October 30 2021, Sun Lul Kwon, on dead wood branch (NIBRFG0000510138).

Notes. The spore guttulation of *S. humida* sp. nov. was not observed. *Sarcoscypha humida* is closely related to *S. hosoyae* and *S. dudleyi*. in the phylogenetic analysis (Figure 1). However, both *S. hosoyae*

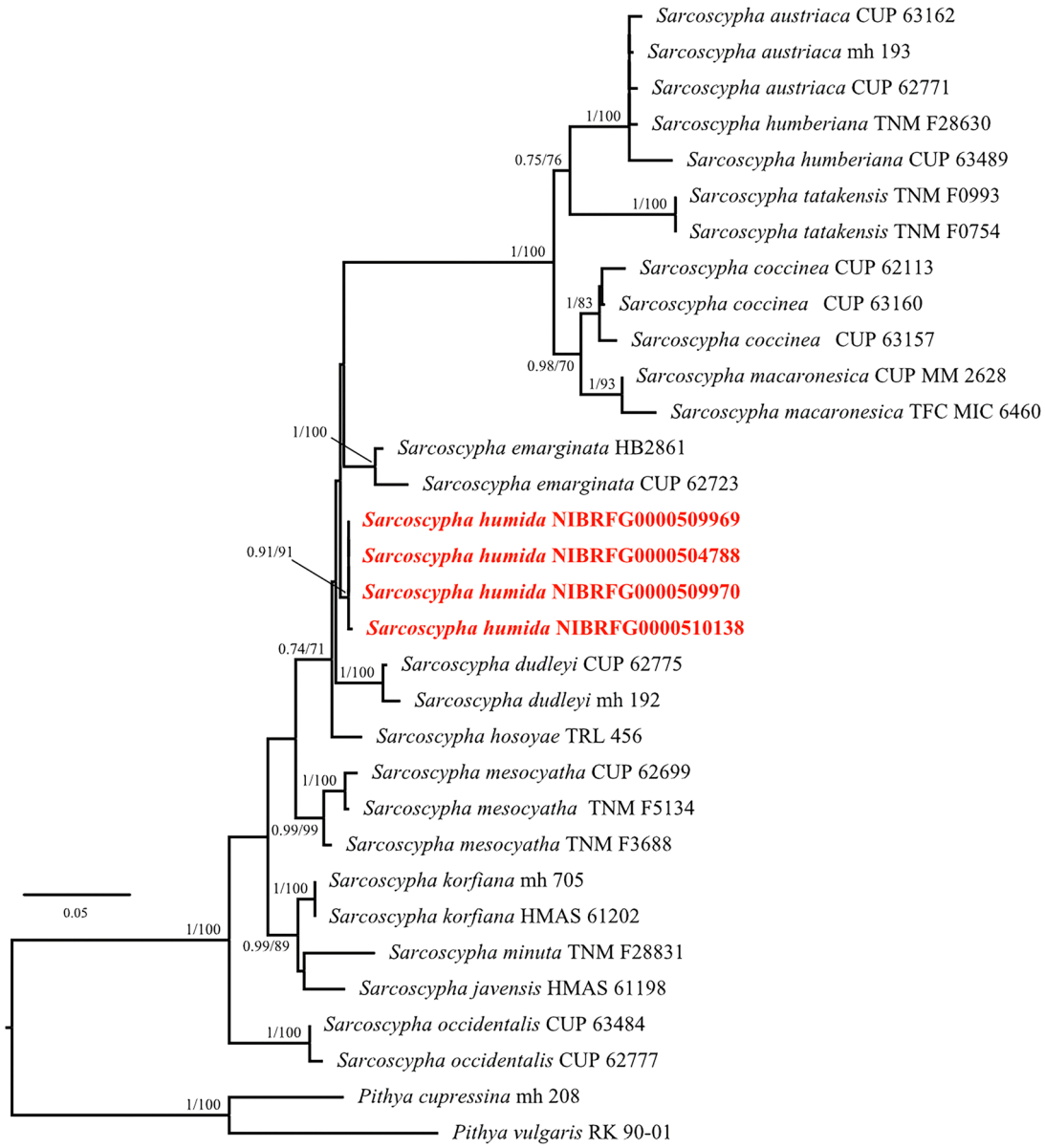

Figure 1. Maximum-likelihood (ML) tree based on the ITS rDNA region. The numbers at the nodes indicate the Bayesian posterior probabilities (PP) >0.70 and ML bootstrap proportion (BP) >70% as PP/BP. The *Pithya* species are used as outgroup. The specimens examined in this study are shown in red colored bold. The scale bar indicates the nucleotide substitutions per position.

(22–38(–45) × 9–12 µm) and *S. dudleyi* (25–33 × 12–14 µm) can be distinguished from *S. humida* by the length of ascospore [10,25]. The apothecia shape of *S. humida* is similar to *S. knixoniana* F.A. Harr. However, *S. knixoniana* is distinguished by the presence of stipitate and short paraphyses, and wider ascospore [10].

4. Discussion

To our best knowledge, a total of 20 species have been tentatively recognized as *Sarcoscypha* species. *Sarcoscypha javensis* Höhn. and *S. macaronesica* Baral & Korf can be distinguished from *S. humida* sp. nov. by phylogenetic analysis. They were in the core clade of *Sarcoscypha* and were delimited from *S. humida* with high support value (Figure 1). *Sarcoscypha macaronesica* Baral & Korf has ellipsoidal ascospores (21)22–23–29–33(40) × (8.5)9–11(13) µm [8]. Thus, it can be distinguished from *S. humida* by larger ascospore. *Sarcoscypha vassiljevae* has been studied with phylogenetic analysis using ITS in previous research [9,10]. The results show that *S. vassiljevae* is located outside of the core clade. This species can be delimited by morphological features, such as longer ascospores and asci, white color apothecia, and stipitate structure [26]. *Sarcoscypha serrata* (Le Gal) Eckblad can be delimited by the yellow color of the hymenium, strongly serrated-crenate margin, and short stipe [27].

The specimens of *S. humida* were collected from three different national parks in Korea during the fall season (September to October). They were found on dead hardwood branches near the wet areas (e.g., Jeolgol gorge). Its closely related species, *S. hosoyae*

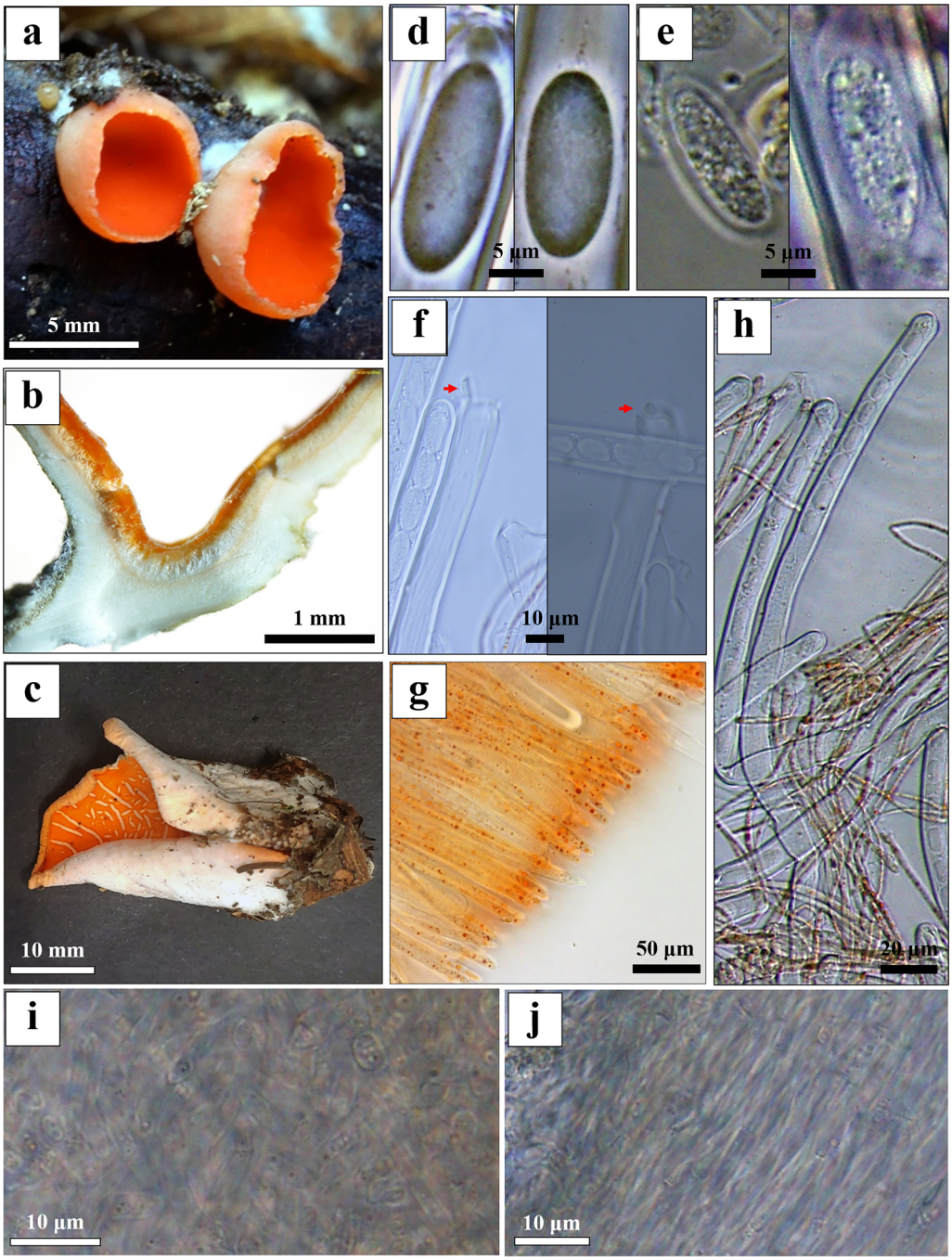

Figure 2. *Sarcoscypha humida*: (a) fresh apothecia of specimen; (b) longitudinal section of apothecia; (c) dried apothecia of mature specimen; (d) dried ascospores; (e) dead ascospores; (f) operculum; (g) paraphyses; (h) asci with eight spores; (i) medullary excipulum; (j) ectal excipulum.

F.A. Harr., is also found in cool and wet areas at the Kamataki waterfall, Japan, in February. *Sarcoscypha dudleyi* (Peck) Baral. is also found in the cool area of the Rocky Mountains in spring. These results indicated that *Sarcoscypha* species in the *S. humida* clade is likely to occur near the wet area and prefer cool weather. Meanwhile, the spore guttulation of *S. humida* was not observed after 24 h desiccation of *S. humida* specimens. According to the previous study, large polar guttules can be found even with dried materials [10]. It might indicate that the spores of *S. humida* have low drying tolerance.

5. Conclusions

In this study, we collected four *Sarcoscypha* specimens from three different national parks during the fall season in Korea. These four specimens are found near wet areas such as valleys and gorges. This species is taxonomically analyzed using morphological and phylogenetic methods. Phylogenetically, *Sarcoscypha humida* is closely related to *S. hosoyae* and *S. dudleyi*. They are found in wet and cool regions like *S. humida*. Morphologically, *S. humida* has a similar apothecia shape to *S. knixoniana*. Here, the

morphological characteristics of novel candidate *S. humida* with detailed illustrations were provided. This is the third report on *Sarcoscypha* species in Korea.

Disclosure statement

Y.M. Heo is employed by COSMAX BTI. The rest of the authors declare that the research was conducted in the absence of any commercial or financial relationship that could be construed as a potential conflict of interest.

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