

Sulzbacheromyces sinensis, an Unexpected Basidiolichen, was Newly Discovered from Korean Peninsula and Philippines, with a Phylogenetic Reconstruction of Genus *Sulzbacheromyces*

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ABSTRACT

Most of lichens are formed by Ascomycota, less than 1% are lichenized Basidiomycota. The flora investigation of lichenized Ascomycota of South Korea has been well studied in the past three decades; however, prior to this study, none of basidiolichens was discovered. During the recent excursion, an unexpected clavarioid basidiolichen, *Sulzbacheromyces sinensis* was collected. Morphology and ecology has been recorded in detail. DNA was extracted, and ITS, 18S, 28S nuclear rDNA were generated. In order to further confirm the systematic position of the Korean specimens, maximum likelihood and Bayesian inference analysis including all the species of the order Lepidostromatales were conducted based on the ITS. As a result, the phylogenetic tree of the order Lepidostromatales was reconstructed, which differed from the previous studies. The inferred phylogenetic tree showed that species of *Sulzbacheromyces* in three different continents (Asia, South Africa and South America) were separated into three clades with support. In this study, the species worldwide distribution map of Lepidostromatales was illustrated, and *S. sinensis* had a widest distribution range (paleotropical extend to the Sino-Japanese) than other species (paleotropical or neotropical). Prior to this study, the range of distribution, southernmost and northernmost points and the fruiting time of *S. sinensis* were recorded, and the genus *Sulzbacheromyces* was firstly reported from Korean peninsula and Philippines.

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1. Introduction

Lichens are the typical symbionts in which lichenized fungi is associated with photobiont and with the basidiomycete yeasts occurring in the cortex of some species [1]. Over 99% lichen are formed by Ascomycota, and less than 1% were lichenized Basidiomycota, which comprised ca. 172 species belonging to 15 genera and five family, and most of them are a member of family Hygrophoraceae (i.e., *Dictyonema* and *Lichenomphalia*), Lepidostromataceae [2–5]. Recently, Galapagos Islands were confirmed to have a higher diversity of *Dictyonema* groups [6], and some species of *Lichenomphalia* were also reported from the Southwest of China [7].

The family Lepidostromataceae consists of three major genera according to the different thallus type, squamulose thallus with medulla for *Lepidostroma* Mägd. & S. Winkl. (4 spp.), microsquamulose thallus without medulla for *Ertzia* B.P. Hodk. & Lücking (1 spp.) and crustose and membranaceous

thallus for *Sulzbacheromyces* B.P. Hodk. & Lücking (8 spp.). Species of Lepidostromataceae is characterized by the clavarioid basidiome associated with green algae and is most similar with species of genus *Multiclavula* (Cantharellales). Actually, some species of Lepidostromataceae were primarily treated as members of *Multiclavula* for a long time [8,9], until application of molecular phylogeny in last decade, the relationship between *Multiclavula* and Lepidostromataceae has been well solved following with several new combinations and new species [3,10–12].

The genus *Sulzbacheromyces* belonging to the family Lepidostromataceae, was erected by Hodkinson and Lücking based on multi-gene analysis and the thallus morphology [10], and characterized by clavarioid white to orange basidiome and crustose thallus. Prior to this study, eight species are now included in this genus: *S. caatingae* from Brazil and Columbia [13,14], *S. bicolor* and *S. yunnanensis* from China, *S. fossicolus* from China and Thailand,

S. miomboensis from Congo, *S. sinensis* from China and Japan [3,15,16], *S. chocoensis* and *S. tutunendo* from Columbia [17].

The lichen flora investigation has been well conducted in last three decades, [18] listed a checklist including 113 genera and 510 taxa, following many new species and record were discovered from the mainland and island (i.e., Jeju Island) [19,20], up to

now, over 180 genera, 788 taxa were reported from South Korea [21,22]; however, all of lichens in these publications were Ascolichens, and no Basidiolichens had been recorded. Here, we have collected some basidiolichen specimens during the lichen investigation excursion and then morphological and molecular analysis have been done for the identification of them.

Table 1. GenBank accessions of ITS used in the phylogenetic analysis of the order Lepidostromatales, together with detailed collection information.

Taxon	Accession (ITS)	Collection information
<i>Ertzia akagerae</i>	KU999883	Rwanda, Ertz 7673 et al. (BR), basidiome
<i>Lepidostroma calocerum</i> 1	KT354923	Colombia, Lücking 35836a (F), basidiome
<i>L. calocerum</i> 2	KT354924	Colombia, Lücking 35836b (F), basidiome
<i>L. calocerum</i> 3	KT354925	Colombia, Lücking 35836b (F), thallus
<i>L. rugaramae</i>	KU999884	Rwanda, Ertz 8544 et al. (BR), basidiome
<i>L. vilgalysii</i>	KU999885	Mexico, Vilgalys-MX16 (DUKE), basidiome
<i>L. winklerianum</i>	KX431118	Mexico, Egan 18705 (OMA), basidiome
<i>Sulzbacheromyces bicolor</i> 1	KU999886	China, Wang & Wang 13-38188 (KUN-L), basidiome
<i>S. bicolor</i> 2	KU999887	China, Wang & Wang 13-38187 (KUN-L), basidiome
<i>S. caatingae</i> 1	KT354934	Brazil, Sulzbacher 235 (UFRN-Fungos 1478), thallus
<i>S. caatingae</i> 2	KT354935	Brazil, Sulzbacher 237 (UFRN-Fungos 1479), basidiome
<i>S. caatingae</i> 3	KT354936	Brazil, Sulzbacher 237 (UFRN-Fungos 1479), thallus
<i>S. caatingae</i> 4	NR120240	Brazil, Sulzbacher 235 (UFRN-Fungos 1478, type), basidiome
<i>S. caatingae</i> 5	KT354933	Brazil, Sulzbacher 235 (UFRN-Fungos 1478, isotype), basidiome
<i>S. chocoensis</i> 1	MG434495	Colombia, Coca 5821, LFC129 (FAUC), basidiome
<i>S. chocoensis</i> 2	MG434496	Colombia, Coca 5824, MON3373 (FAUC), basidiome
<i>S. chocoensis</i> 3	MG434497	Colombia, Coca 5821, MON3375 (FAUC), basidiome
<i>S. fossicolus</i> 1	KX431121	Thailand, Stubbe D. & Walley R. DS 07-379 (GENT), basidiome
<i>S. fossicolus</i> 2	KX431120	Thailand, Verbeken A. & Walley R. 2004-056 (GENT), basidiome
<i>S. fossicolus</i> 3	KU999888	China, Wang et al. 14-44144 (KUN-L), basidiome
<i>S. miomboensis</i> 1	KX431122	Democratic Republic of the Congo, A. De Kesel 5403 (BR), basidiome
<i>S. miomboensis</i> 2	KX431123	Democratic Republic of the Congo, A. De Kesel 6004 (BR), basidiome
<i>S. sinensis</i> 1	KU999889	China, Wang & Liu 11-32799 (KUN-L), basidiome
<i>S. sinensis</i> 2	KU999890	China, Wang & Liu 11-32785 (KUN-L), basidiome
<i>S. sinensis</i> 3	KU999891	China, Wang & Liu 11-32786 (KUN-L), basidiome
<i>S. sinensis</i> 4	KU999892	China, Ma 12-4313 (KUN-L), basidiome
<i>S. sinensis</i> 5	KU999893	China, Jia FJ1034 (KUN-L), basidiome
<i>S. sinensis</i> 6	KU999894	China, Wang & Liu 11-32822 (KUN-L), basidiome
<i>S. sinensis</i> 7	KU999895	China, Wang et al. 12-38189 (KUN-L), basidiome
<i>S. sinensis</i> 8	KU999896	China, Wang et al. 14-44135 (KUN-L), basidiome
<i>S. sinensis</i> 9	KU999897	China, Wang et al.14-44136 (KUN-L), basidiome
<i>S. sinensis</i> 10	KU999898	China, Wang et al. 14-44138 (KUN-L), basidiome
<i>S. sinensis</i> 11	KU999899	China, Wang et al. 14-44146 (KUN-L), basidiome
<i>S. sinensis</i> 12	KU999900	China, Wang et al. 14-44147 (KUN-L), basidiome
<i>S. sinensis</i> 13	KU999901	China, Wang et al. 12-34600 (KUN-L), basidiome
<i>S. sinensis</i> 14	AB819619	Japan, TUMH 50299, basidiome
<i>S. sinensis</i> 15	AB819620	Japan, TUF 100100, basidiome
<i>S. sinensis</i> 16	AB819621	Japan, TUF 100116, basidiome
<i>S. sinensis</i> 17	AB819622	Japan, TUF 14267, basidiome
<i>S. sinensis</i> 18	MK091291	South Korea, D. Liu 180204 (KoLRI), basidiome
<i>S. sinensis</i> 19	MK091292	South Korea, D. Liu 180206 (KoLRI), basidiome
<i>S. tutunendo</i> 1	MG434489	Colombia, Coca 5825, LFC126 (FAUC), basidiome
<i>S. tutunendo</i> 2	MG434490	Colombia, Coca 5822, LFC128 (FAUC), basidiome
<i>S. tutunendo</i> 3	MG434491	Colombia, Coca 5823, LFC127 (FAUC), basidiome
<i>S. tutunendo</i> 4	MG434492	Colombia, Coca 5823, MON3372 (FAUC), basidiome
<i>S. tutunendo</i> 5	MG434493	Colombia, Coca 5825, MON3385 (FAUC), basidiome
<i>S. tutunendo</i> 6	MG434494	Colombia, Coca 5822, MON3388 (FAUC), basidiome
<i>S. yunnanensis</i> 1	KU999902	China, Wang et al. 13-38191 (KUN-L), basidiome
<i>S. yunnanensis</i> 2	KU999903	China, Wang & Liu 11-32797 (KUN-L), basidiome
<i>S. yunnanensis</i> 3	KU999904	China, Wang & Liu 14-44141 (KUN-L), basidiome
<i>S. yunnanensis</i> 4	KU999905	China, Wang et al. 12-34448 (KUN-L), basidiome
<i>S. yunnanensis</i> 5	KU999906	China, Wang et al. 12-34445 (KUN-L), basidiome
<i>S. yunnanensis</i> 6	KU999907	China, Wang et al. 12-34614 (KUN-L), basidiome
<i>S. yunnanensis</i> 7	KU999908	China, Wang et al. 12-34446 (KUN-L), basidiome
<i>S. yunnanensis</i> 8	KU999909	China, Wang & Liu 14-44133 (KUN-L), basidiome
<i>S. yunnanensis</i> 9	KU999910	China, Wang et al. 14-44123 (KUN-L), basidiome
<i>S. yunnanensis</i> 10	KU999911	China, Wang et al. 12-34447 (KUN-L), basidiome
<i>S. yunnanensis</i> 11	KU999912	China, Wang et al. 12-34444 (KUN-L), basidiome
<i>S. yunnanensis</i> 12	KU999913	China, Wang et al. 13-38192 (KUN-L), basidiome
<i>S. yunnanensis</i> 13	KU999914	China, Wang et al. 13-38190 (KUN-L), basidiome

Accessions in bold are sequences newly generated in this study.

2. Materials and methods

2.1. Morphological observation

Specimens in this study were collected from South Korea and deposited in Suncheon National University, Korean Lichen Research Institute (KoLRI). Tracing observations of field growth status on the population of *S. sinensis* in Yeosu were conducted biweekly. Morphology examination and anatomical characters were recorded under a dissecting microscope (Nikon SMZ 745 T; Nikon, Tokyo, Japan) and Olympus BX 50 microscope (Olympus,

Tokyo, Japan), and photographs were taken under HD-Measure LTHS-300 (Leetech Co., Seoul, Korea) microscope and Carl Zeiss MicroImaging with Axio Cam ERc 5s imaging system (Carl Zeiss MicroImaging, Gottingen, Germany).

2.2. DNA extraction and molecular phylogenetic analysis

Total genomic DNA was extracted using the NucleoSpin Plant II Kit (Clontech Laboratories, Mountain View, CA) from newly collected

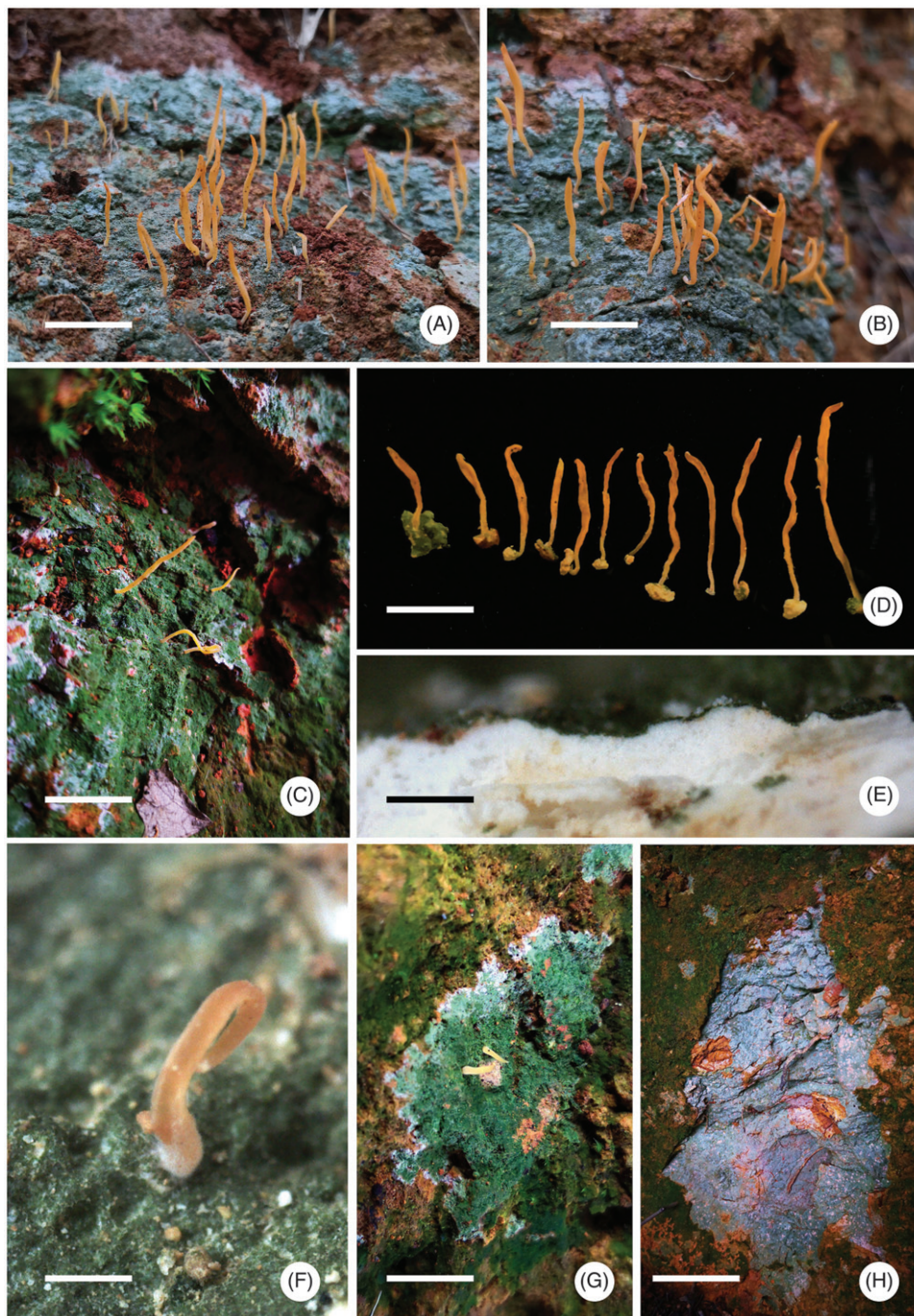


Figure 1. *Sulzbacheromyces sinensis* (D. Liu 180204). (A, B), population in natural open habit; (C), population in natural shade habit; (D), basidiome; (E), thallus; (F), specimen in the herbarium; (G), thallus with silvery margin (prothallus); (H), thallus after basidiome disappeared (Scale bars: A–C = 1.5 cm, D = 1 cm, E = 1 mm, F = 0.5 mm, G = 2 cm, H = 8 cm).

specimens following the manufacturer's instructions. The internal transcribed spacer (ITS) region, the large subunit (28S) and small subunit (18S) of the ribosomal RNA were targeted via PCR using the primers pairs ITS4/ITS1F [23], LR0R/LR5 [24,25] and NS24/NSSU97A [26,27], respectively. Protocols of PCR amplification were detail in [3]. Sequencing was accomplished by the genomic companies GenoTech (Daejeon, Korea) and Macrogen (Daejeon, Korea). Newly obtained sequences for the

ITS region of *Sulzbacheromyces sinensis* (bold in Table 1) were assembled and edited using SeqMan and Mega 7.0 [28], then complemented with publicly available sequences of order Lepidostromatales (Table 1) into a matrix and aligned with Mafft v7.273 [29]. Species of *Lepidostroma* were used as outgroup.

Maximum likelihood (ML) and Bayesian inference (BI) were used to methods were used to reconstruct phylogenetic trees of Lepidostromatales. ML

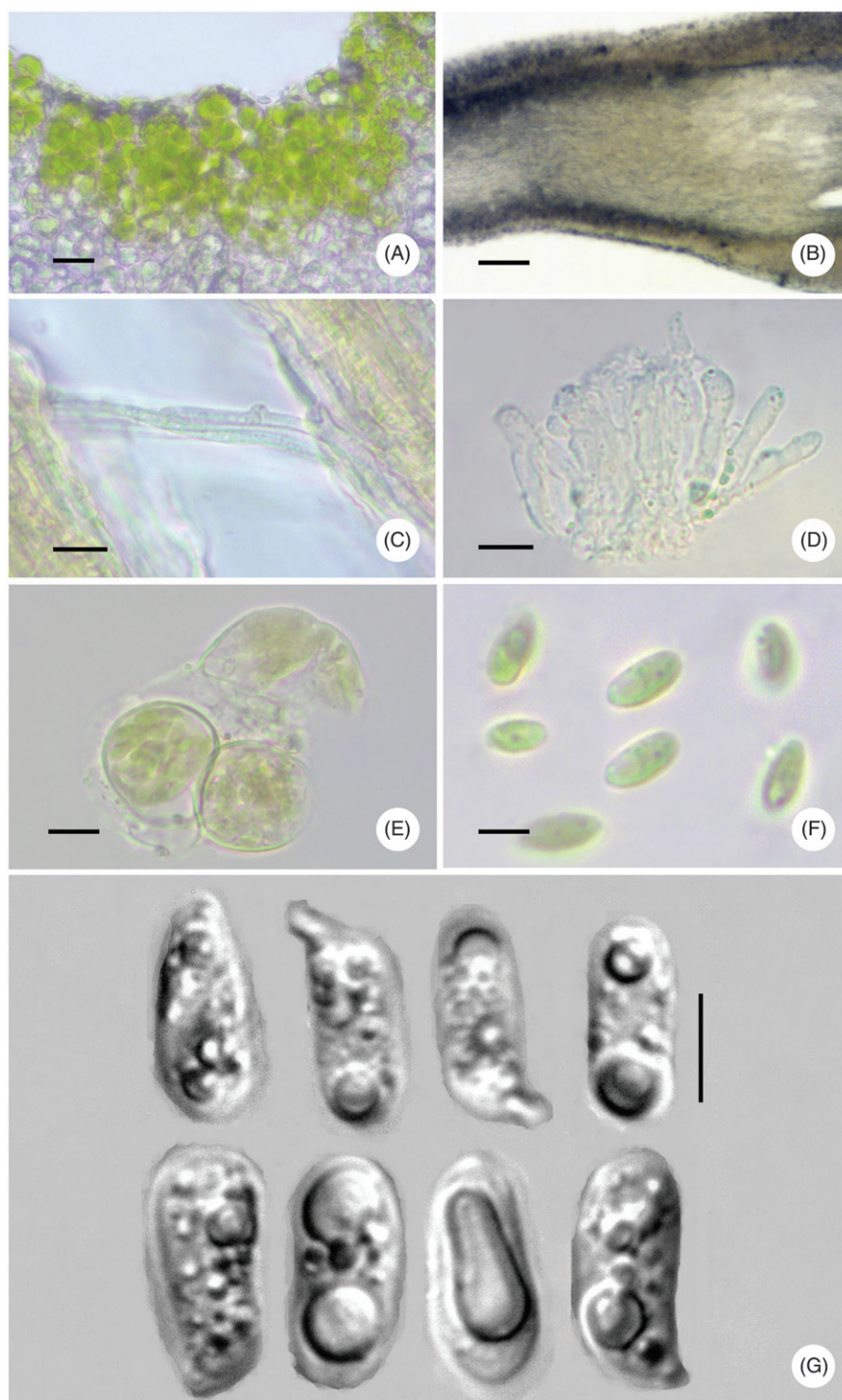


Figure 2. Microscopic characters of *Sulzbacheromyces sinensis* (D. Liu 180204). (A), cross section of thallus; (B), longitudinal section of basidiome; (C), hyphae with clamps; (D), basidia; (E, F), algae in the basidiome; (G), spores (Scale bars: A, C–E = 10 μ m, B = 100 μ m, G = 5 μ m).

was analyzed using RAxML v7.2.6 [30] with the GTR + I + G model. Bootstrap values were estimated from the consensus trees obtaining from 1000 non-parametric bootstrapping pseudoreplicates, clade value $\geq 70\%$ infers strong supported. Bayesian inference (BI) was performed with MrBayes v3.1.2 [31], applying the best-fitted substitution models (TVM + I + G) based on the Akaike information criterion (AIC) by jModelTest 3.7 [32]. Bayesian inferences were conducted using four chains and run for 2 million generations. Trees were sampled every 1000 generations with the first 25% of tree discarded. The remaining trees were used to generate a majority-rule consensus tree with posterior probabilities (PP), clades of PP value ≥ 0.95 were considered as significantly supported.

3. Result and discussion

3.1. Taxonomy

Sulzbacheromyces sinensis (R. H. Petersen & M. Zang) D. Liu & Li S. Wang, [Figures 1 and 2](#)

\equiv *Multiclavula sinensis* R. H. Petersen & M. Zang, *Acta Bot. Yunnanica* 8: 284. 1986.

= *Lepidostroma asianum* Yanaga & N. Maek, *Mycoscience* 56: 3. 2015.

Description: Thallus crustose, distinct, membranaceous, green to dark green, turning grey or silvery following long term drying, forming a thin layer on the substrate, covering areas 2–60 cm diam.; thallus 20–101 μm thick, containing clusters of single-celled green, chlorococcoid algae; prothallus obvious, white, gray or silvery, shiny; photobiont spherical, round or slightly angular, rough, contiguous, surrounded by hyaline hyphae, 5–14 μm .

Basidiome solid, clavarioid, or cylindrical, sometimes fusiform, simple, occasionally 2–3 branched

near the tip, up to 4 cm tall, 0.5–1.5 mm wide, yellow, orange to red orange, turning ochraceous upon drying. Apex obtuse and yellow or orange when mature or when growing in shade, and acute ochraceous when young or in direct sunshine. Basidiomes always with white tomentose stipe at base, 0.5–3 mm tall. Tasteless and with a mushroom-like odor. Trama orange, composed of parallel, clamped, thin-walled, hyaline hyphae, 1–2 μm diam., densely agglutinated, oily contents always present, round, 3–6.5 μm diam. Hymenium sometimes containing a mass of algal cells, elliptical, 5.5–8.5 \times 3.5–4.4 μm . Basidia 26–38 \times 3.5–8 μm , oblong, subclavate to clavate, thin-walled, hyaline, guttulate, apex inflated, with 4 sterigmata, 2–6 μm long. Basidiospores elliptical, slightly reniform or obpyriform, thin-walled, hyaline, smooth, with hilar appendix, usually guttulate, non-amyloid, 8.6–12.5 \times 3.9–5.3 μm .

Habitat: Grow on the slopes with red clay and rocks.

Distribution: *S. sinensis* occurs in paleotropical region of Asia and extends to subtropical parts of China, Japan, Korean Peninsula, new record to South Korea and Philippines, [Figure 3](#).

Specimens examined: South Korea. Jeollanam-do, Yeosu-si, Mandeok-dong, the base of Malaesan Mt., 34°45'24"N, 127°44'46"E, 140 m, on soil and rock, 08 Sept. 2018, D. Liu 180204 (18S = MK091295, ITS = MK091291, 28S = MK091294), 180205, 180206 (18S = MK091296, ITS = MK091292, 28S = MK091293), 180207; *ibidem*, 22 Sept. 2018, D. Liu 180208, 180209, 180210; *ibidem*, 15 Oct. 2018, D. Liu 180213, 180214; Suncheon-si, Jorye-dong, Jorye Lake Park, 34°57'55"N, 127°31'26"E, 78 m, soil and rock, 09 Oct. 2018, D. Liu 180215. **Philippines.** Benguet, Municipality of Bokod, Daclan, Daclan Sulfur Spring, 16°31'11"N, 120°49'03"E, 1326 m, on

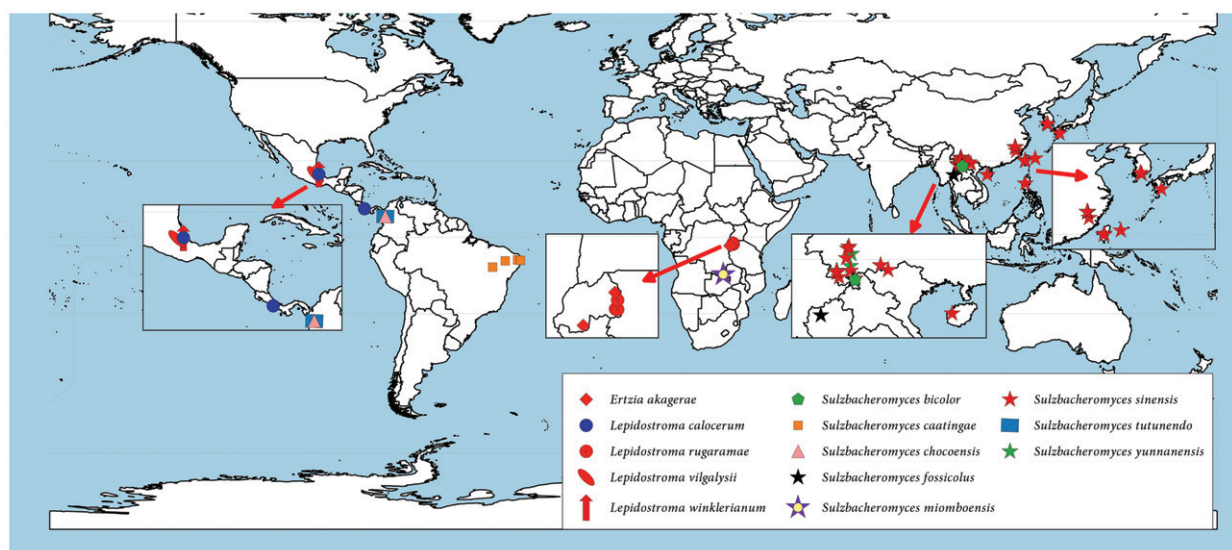


Figure 3. The world distribution of the species in the order Lepidostromatales. The overlap areas of different species were enlarged in the map.

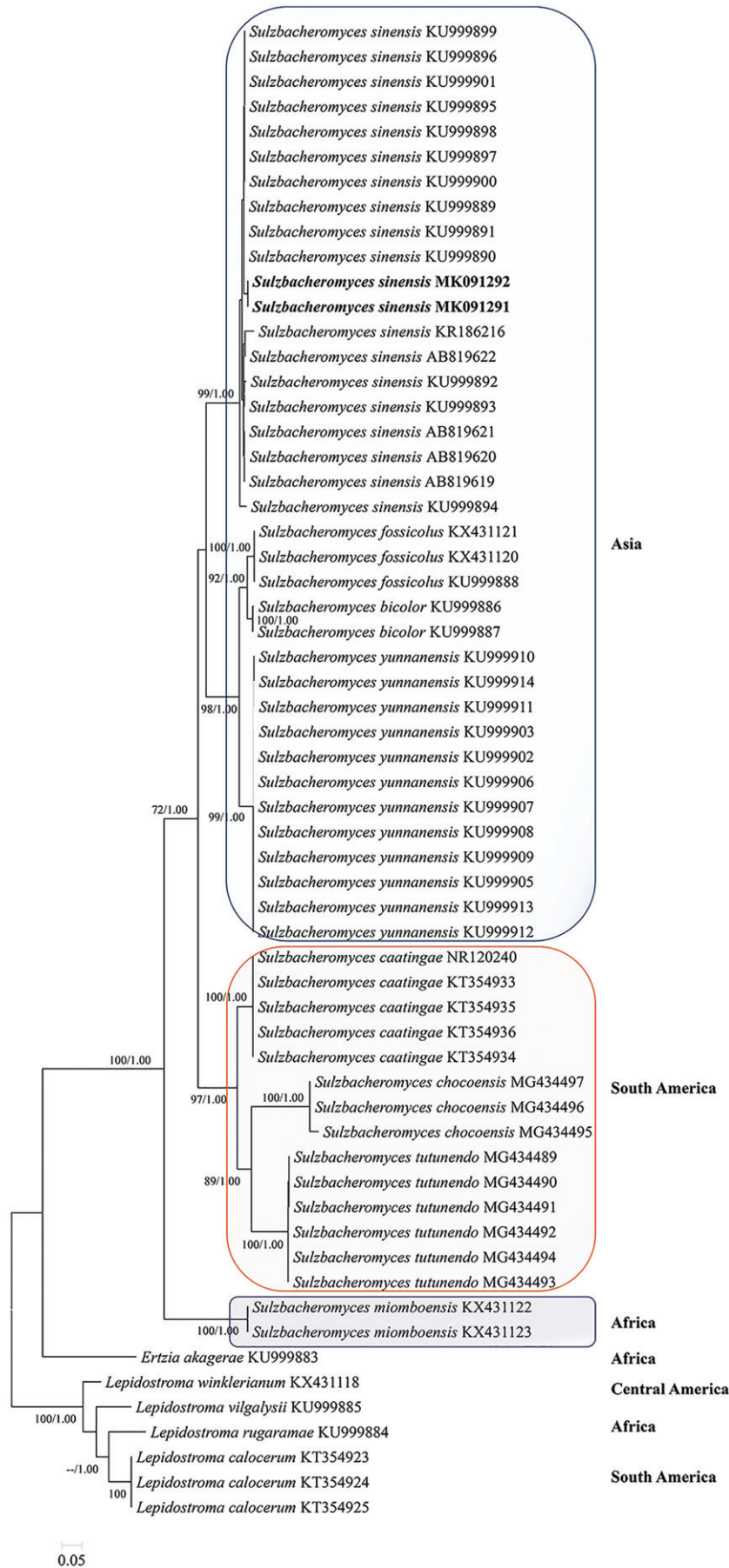


Figure 4. Most likely phylogenetic relationships among all species of Lepidostromatales inferred from ITS sequences. Nodes supported by ML bootstrap values $\geq 70\%$ and Bayesian posterior probabilities ≥ 0.95 are noted on the branches, and bootstrap values are erased on lower support clades.

soil, 16 May 2013, R.G. U. Jayalal & J. S. Hur PH130094.

3.2. Phylogenetic results

Topologies were constructed based on the 60 sequences of 675 bp including 13 species of the order Lepidostromatales (Figure 4). The specimens from South Korea clusters into the *S. sinensis* clade with strong support, and the *S. sinensis* clade forms a sister group with other Asia species, *S. bicolor*, *S. fossicolus* and *S. yunnanensis*. The genera *Lepidostroma*, *Ertzia* and *Sulzbacheromyces* were separated into three clades with support. Groups with three different thallus (squamulose, micro-squamulose and crustose) had been reported from the neotropical region of American Continent and African paleotropical region, while only crustose species were recorded from Asia.

Differing from the previous studies [3,17], we combined all the species of Lepidostromatales including newly discovered species from South America. As a result, *S. sinensis* clustered a single clade out of the Asian group; however, in our reconstructed phylogenetic tree, species in the genus *Sulzbacheromyces* formed into three clades by different continents with support, the Africa cluster with *S. miomboensis*, the South America cluster with *S. caatingae*, *S. chocoensis* and *S. tutunendo*, and the Asia cluster with *S. bicolor*, *S. fossicolus*, and *S.*

sinensis, *S. yunnanensis*. The backbone of Asian clade had lower support, and *S. sinensis* covered broader distribution than other Asian species of *Sulzbacheromyces*, which were paleotropical.

3.3. Distribution and field observation

S. sinensis was characterized by the crustose thallus with shiny silvery margin and prothallus, and chronically treated as *Multiclavula sinensis* since it was firstly described from Yunnan Province, China [8]. Later, Yanaga et al. [16] discovered a new species *L. asianum* from Japan, following the record of this species from China [15]. Recently, Liu et al. [3] recombined *M. sinensis* and *L. asianum* as *S. sinensis* based on the morphological and phylogenetic revision on the clavarioid basidiolichen of China. All these records seemed to gain a summary of the latitude and altitude range for *S. sinensis*, 19°15'31"N (Changjiang Co., China) ~31°16'N (Miyazaki City, Japan), 17 m ~ 1782 m; nevertheless, our collection extended distribution of the genus *Sulzbacheromyces* and species *S. sinensis* from Philippines (16°31'11"N, Bokod) to the Korean peninsula (34°57'55"N, Suncheon-si), where were the southernmost northernmost point prior to this study (Figure 3). Meanwhile, it is the first time that the basidiolichen was found in South Korean and *S. sinensis* was the firstly discovered from Korean peninsula and Philippines.

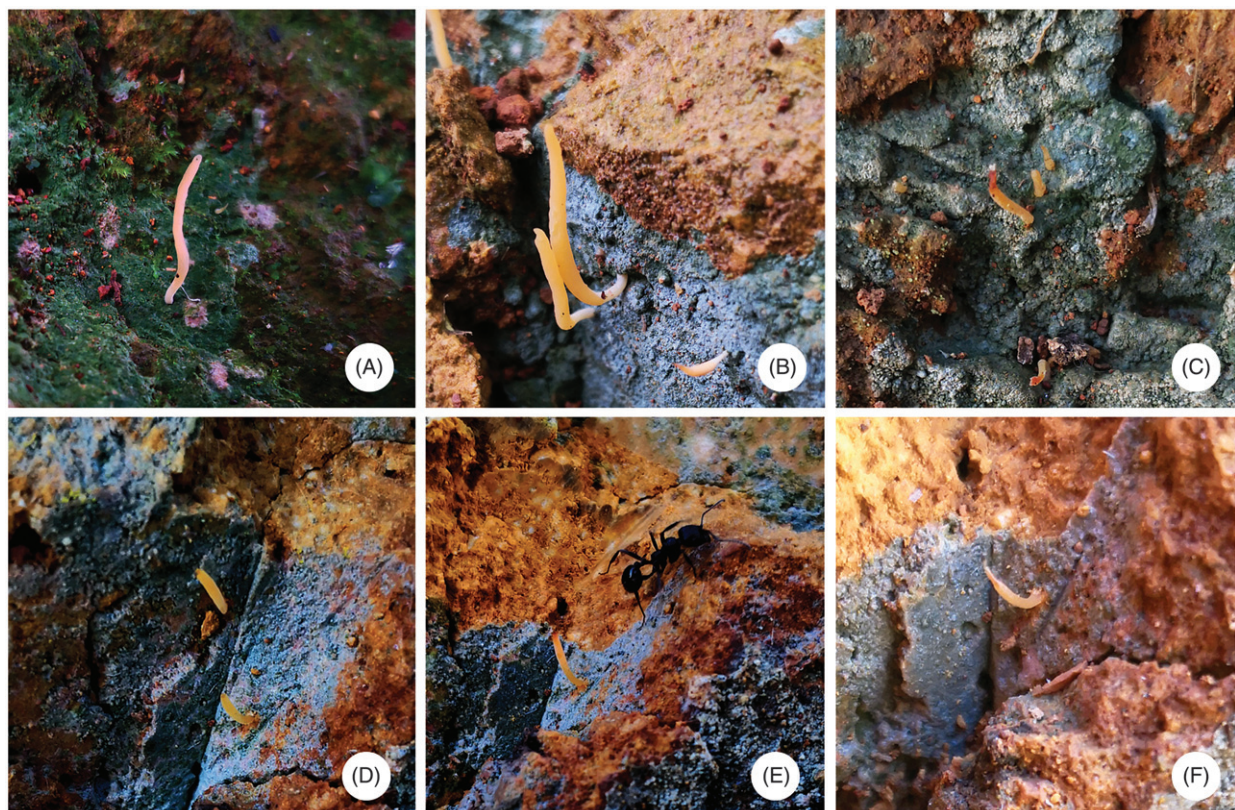


Figure 5. Field observation time on the fruiting in Korea. (A), 2018.09.08; (B), 2018.09.22; (C), 2018.10.15; (D), 2018.10.27; (E), 2018.11.14; (F), 2018.12.24.

It's difficult to monitor the fruiting time of fungi in the field, which was effected by many different factors, including temperature, moisture, geography, hosts, and the fungal physiology. Most mushrooms fruiting during the autumn in temperate and boreal ecosystems, statistical data [33] showed that the spores size was significantly related to the fruiting time of autumn-fruiting mushroom, and species fruiting earlier in general producing larger spores. Unlike Ascolichens, whose individual sexual reproductive organ can exist or keep growth for a rather long time, the fruit body of *S. sinensis* usually existed for a very short duration (4–9 days). Even so, it's surprising that the fruiting time of *S. sinensis* lasted for a long time. Based the collection date of specimens with fruit body and our field observation, species of *S. sinensis* fruiting time was from March to the October (March~August in Japan [16], May to October in China [3]) in the Northern Hemisphere. During our field observation for Korean specimens, even near the end of December (24th), there were still sparsely fruiting, and it's also the latest record on the fruiting of *S. sinensis*, period filed observation in Figure 5.

The difference of the macro-morphology on the basidiome is tiny among some species of *Sulzbacheromyces*; however, the thallus variation in different stages may give some new clues. The thallus of *S. sinensis* spreading from small to large size, and the morphology vary greatly before and after fruiting. The portions before fruiting is usually greenish to dark greenish, while become silvery after fruiting due to forming a thin mycelium on the surface. Normally, a individual population will not finish fruiting all at once, some parts early, the other late. After the portions fully fruiting, the thallus will become shiny silvery with dark green, then silvery with greenish spot, later whitish without algae cell, eventually disappeared. This processing completed in three or four month in South Korea. However, the physiology reason of *S. sinensis* conducting the annual growth in short time need to futher study and observation.

Disclosure statement

No potential conflict of interest was reported by the authors.

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