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Sistotrema subconfluens sp. nov. (Cantharellales, Basidiomycota) from Changbaishan Nature Reserve, northeastern China

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ABSTRACT

Sistotrema, typified by *S. confluens*, is characterized mainly by its urniform basidia mostly with 6–8 sterigmata as well as by a monomitic hyphal system, oil rich hyphae and smooth basidiospores. The species of this genus have various hymenophore configurations and basidiospore shapes. During a field trip in 2011, two specimens with urniform basidia were collected from Changbaishan Nature Reserve, northeastern China. After careful morphological and molecular studies, they are described and illustrated here as a new species, *S. subconfluens*. The new species shares a terrestrial habit, stipitate basidiocarps and poroid hymenophores with *S. confluens*, besides urniform basidia. These characters make the two species different from all other species of *Sistotrema* with resupinate basidiocarps on wood. *Sistotrema subconfluens* differs from the type by having larger basidiocarps, shorter basidiospores and consistent poroid hymenophores. In phylogeny inferred from nuclear large subunit rDNA, the two species were sister taxa but clearly separated. The difference of internal transcribed spacer sequences between the two species was 3.6%.

1. Introduction

Sistotrema Fr. is characterized by urniform basidia mostly with 6–8 sterigmata and often oil rich hyphae. In other respects, such as hymenophore configuration and basidiospore shape, the genus is highly heterogenous (Eriksson et al. 1984). Its type species, *S. confluens* Pers., has a terrestrial habit with stipitate basidiocarps, while all other species of this genus form resupinate basidiocarps on wood (Eriksson et al. 1984). In phylogeny, *Sistotrema* appears as a polyphyletic genus that clusteres together with *Cantharellus* Adans. ex Fr., *Clavulina* J. Schröt., *Craterellus* Pers., *Hydnum* L. and *Multiclavula* R.H. Petersen within the order Cantharellales (Moncalvo et al. 2006).

Changbaishan Nature Reserve, situating in northeastern China, has a high diversity of polypores, and many new species are described from here (Dai 1995; Dai and Niemelä 1997, 2002; Langer and Dai 1998; Dai et al. 2003; Wei and Dai 2007). During a field trip in 2011, two specimens forming stipitate basidiocarps on the ground were collected. After careful morphological examination and molecular sequencing, they were identified as a new species of *Sistotrema* and an illustrated description is provided here. Although eight species of *Sistotrema* have previously been documented in

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multiple provinces of China (Dai et al. 2004a, b; Dai et al. 2007; Li et al. 2008; Yuan and Dai 2008), this is the first species originally described from the country (Dai 2011, 2012).

2. Materials and methods

2.1. Morphological study

The studied specimens are deposited in the herbarium of the Institute of Applied Ecology, Chinese Academy of Sciences (IFP). The microscopic procedure follows Dai (2010). Sections were examined using a microscope (Eclipse 80i, Nikon, Japan) at magnification up to ×1000. In presenting the size of basidiospores, 5% of measurements from each end of the range were excluded and given in parentheses. Drawings were made with the aid of a drawing tube. Special color terms follow Petersen (1996). The following abbreviations are used in the text: L = mean basidiospore length (arithmetic average of all basidiospores), W = mean basidiospore width (arithmetic average of all basidiospores), Q = variation in the L/W ratios between the specimens studied, and *n* = number of basidiospores measured from the given number of specimens.

2.2. Molecular phylogeny

The products of polymerase chain reaction (PCR) were directly generated from herbarium specimens (Dai 12577 and Dai 12578) using Phire® Plant Direct PCR Kit (Finnzymes Oy, Finland) according to the manufacturer's instructions. Nuclear large subunit rDNA (LSU-rDNA) region was amplified with primer pair LR0R and LR7 (Bunyard et al. 1994), while primers ITS1 (White et al. 1990) and ITS4-B (Gardes and Bruns 1993) were used to amplify the internal transcribed spacer (ITS) sequences. The PCR procedure was as follows: initial denaturation at 98 °C for 5 min, followed by 39 cycles at 98 °C for 5 s, 48 °C for 5 s for LSU-rDNA region/59 °C for 5 s for ITS region and 72 °C for 5 s, and a final extension of 72 °C for 10 min. The products were purified, and then directly sequenced in Beijing Genomics Institute, China. The sequencing primers were LR0R, LR7, LR3R and LR3 (Bunyard et al. 1994) for LSU-rDNA region, while ITS1, ITS4-B, ITS2 and ITS3 (White et al. 1990) for ITS region. For both specimens, LSU-rDNA region was successfully sequenced, while ITS sequence (including ITS1 + 5.8S + ITS2 regions) was obtained only from specimen Dai 12577. The three sequences were deposited in GenBank (accession numbers JX076810–JX076812).

Besides two LSU-rDNA sequences generated in this study, 36 other LSU-rDNA sequences from related species as ingroup and two from *Dacrymyces* Nees (Dacrymycetales) as outgroup (Nilsson et al. 2006; Münzenberger et al. 2012) were included in our dataset. The dataset was aligned in ClustalX 2.0 (Larkin et al. 2007) with default parameters. The resulting alignment was deposited in TreeBase (http://www.treebase.org; accession number S12749), and its best-fit evolutionary model was estimated using jModelTest (Guindon and Gascuel 2003; Posada 2008) according to corrected Akaike information criterion. Under the best model, MrBayes 3.2 (Ronquist and Huelsenbeck 2003) was used to infer Bayesian inference (BI). Two independent runs were performed, each starting from random trees and employing a four-chain Metropolis-coupled Markov chain Monte Carlo analysis. Each chain sampled trees every 100th generation from total 10,000,000. The first quarter of trees was discarded as burn-in. All other trees were accounted for when computing a 50% consensus tree and calculating Bayesian posterior probability (BPP). Maximum parsimony (MP) tree was constructed using PAUP* 4.0b10 (Swofford 2001) with 1000 bootstrap (BS) replicates. Heuristic searches were conducted using the following conditions: starting tree obtained via stepwise addition, tree-bisection-reconnection branch swapping, steepest descent option not in effect, and 'multrees' option in effect. All characters were equally weighted and gaps were set as missing data.

Only one full-length ITS sequence (including ITS1 + 5.8S + ITS2 regions) of *S. confluens*, sequenced from a strain FCUG 298, was available from GenBank (accession numbers DQ267125). It was downloaded to calculate genetic distance between *S. confluens* and specimen Dai 12577 using MEGA 5 (Tamura et al. 2011) under substitution model of maximum composite likelihood, uniform rates among sites, same pattern among lineages and complete deletion of gaps/missing data.

3. Results

3.1. Molecular phylogeny

Our dataset of 40 LSU-rDNA sequences resulted in an alignment with 1418 characters, of which 839 were constant, 256 parsimony-uninformative and 323 parsimony-informative. The best-fit evolutionary model of this alignment was GTR + G with unequal base frequencies of A = 0.2768, C = 0.2006, G = 0.2808 and T = 0.2418, substitution rates of AC = 0.8819, AG = 2.5647, AT = 0.8379, CG = 0.6825, CT = 4.9427 and GT = 1.0000, and a gamma distribution parameter of 0.3980. MP analysis generated 84 equally best trees of 1167 steps (CI = 0.648 and RI = 0.716).

The phylogenetic trees from BI and MP analyses were highly similar in topology. Therefore, only the topological structure from BI was shown, and the statistical values of both BPP > 0.95 and BS > 70 were labeled at the nodes (Fig. 1). Our phylogeny generated similar results as previous studies (Nilsson et al. 2006; Münzenberger et al. 2012). In addition, the two newly generated sequences from specimens Dai 12577 and Dai 12578 formed a strongly supported terminal clade, which clustered together with four sequences of *S. confluens*. Noteworthily, of the four sequences, three (accession numbers AY586712, AY647214 and DQ898711) formed a separated clade with strong supports, while the fourth one (accession number AM259216) appeared on a branch separated from others as well as the sequences from Dai 12577 and Dai 12578.

The ITS sequences (including ITS1 + 5.8S + ITS2 regions) from Dai 12577 and FCUG 298 (also the source of LSU-rDNA sequence with accession number DQ898711) differed by 3.6%.

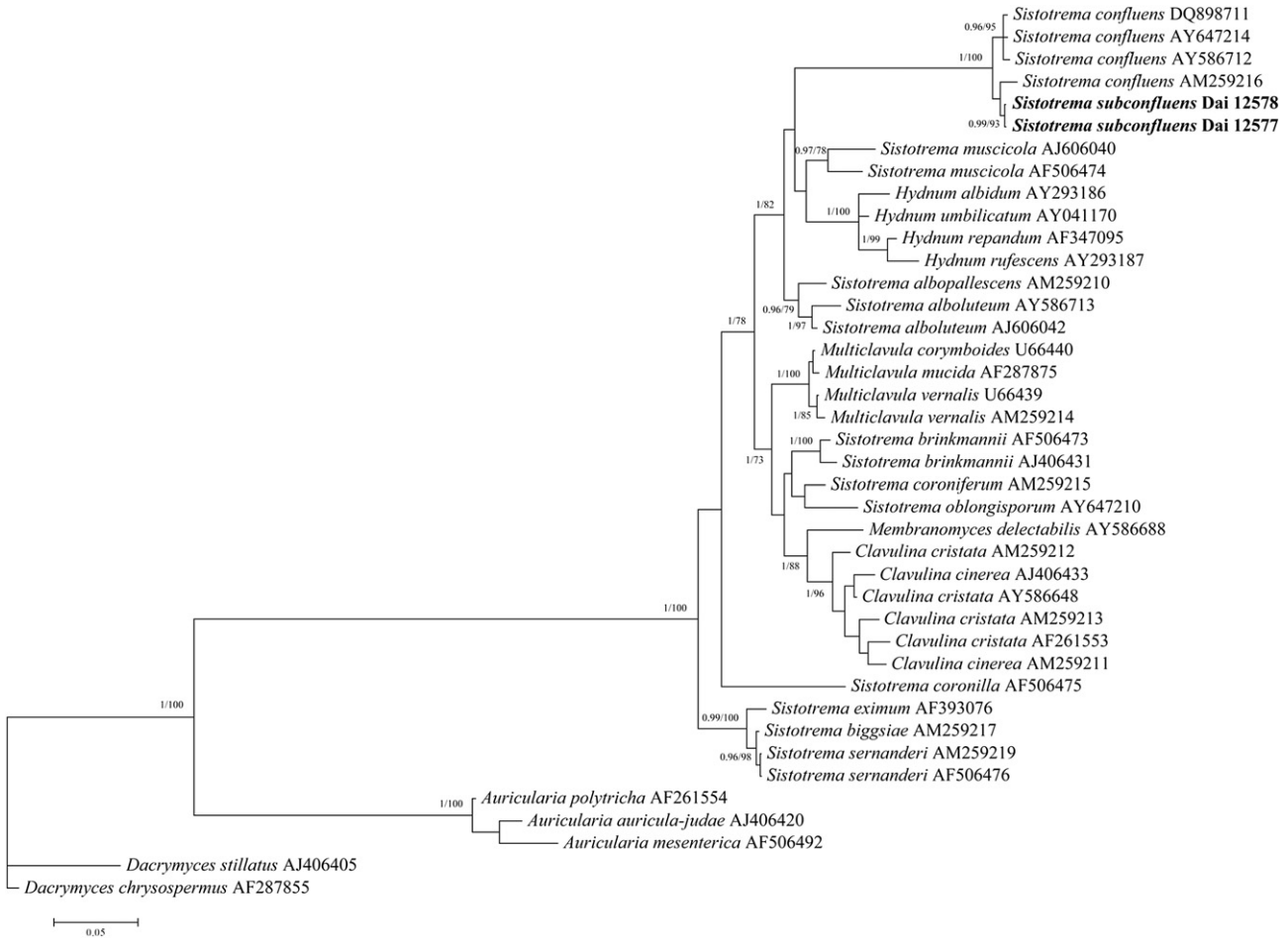

Fig. 1 – Phylogenetic position of *Sistotrema subconfluens* inferred from nuclear LSU-rDNA regions. Topology from BI tree and statistical values from both BI (>0.95) and MP (>70%) analyses.

3.2. *Taxonomy*

Sistotrema subconfluens L.W. Zhou, sp. nov. Fig. 2.
MycoBank no.: MB 800429.

Basidiocarps terrestrial, centrally or laterally stipitate. Pilei up to 3.8 cm in diam. Stipe up to 1.9 cm long, 4 mm thick, swollen near the base (8 mm in diam). Basidia urniform, mostly with six sterigmata. Basidiospores 3.9–4.2 × 2–2.3 μm, L = 4.01 μm, W = 2.08 μm, Q = 1.90–1.94 (n = 60/2).

Type: China, Jilin Province, Changbaishan Nature Reserve, in a natural secondary poplar-birch forest, on the ground, 24 August 2011, Dai 12577 (holotype in IFP 016008), Dai 12578 (paratype in IFP 015999).

rDNA sequences ex holotype: JX076810 (LSU-rDNA); JX076812 (ITS).

rDNA sequence ex paratype: JX076811 (LSU-rDNA).

Etymology: *subconfluens* (Lat.): similar to *S. confluens*.

Fruitbody: Basidiocarps annual, terrestrial, centrally or laterally stipitate. Pilei circular when fresh, infundibuliform, up to 3.8 cm in diam, up to 3 mm thick, sometimes confluent with a few adjacent ones. Pileal surface buff to cinnamon-buff, glabrous, wrinkled when dry, concentrically zonate and sulcate; margin acute, cream, curved down when dry. Pore surface buff to curry-yellow; sterile margin distinct, buff, up to 1.5 mm; pores angular to irregular, 2–4 per mm; dissepiments thick, lacerate. Context buff, soft corky when dry, up to 1 mm thick. Tubes buff, not decurrent on stipe, fragile when dry, up to 2 mm long. Stipe cream to cinnamon-buff, corky when dry, up to 1.9 cm long, 4 mm thick, swollen near the base (8 mm in diam).

Hyphal structure: Hyphal system monomitic; generative hyphae bearing clamp connections, acyanophilous, inamyloid and indextrinoid; tissue unchanged in 5% KOH. Context: Contextual hyphae hyaline, thin-walled, occasionally branched, with frequent clamp connections, sometimes with oily contents, more or less straight, regularly arranged, 3–5 μm in diam, covered by numerous small crystals; hyphae in stipe similar to contextual hyphae. Tubes: Tramal hyphae hyaline, thin-walled, rarely branched, with abundant clamp connections, more or less straight, subparallel along the tubes, 2–4 μm in diam. Cystidia and cystidioles absent; basidia urniform, hyaline, thin-walled, bearing a basal clamp connection, mostly with six sterigmata, occasionally with two or four sterigmata, 10–18 × 4–7 μm; basidioles in shape similar to basidia, but slightly smaller. Spores: Basidiospores

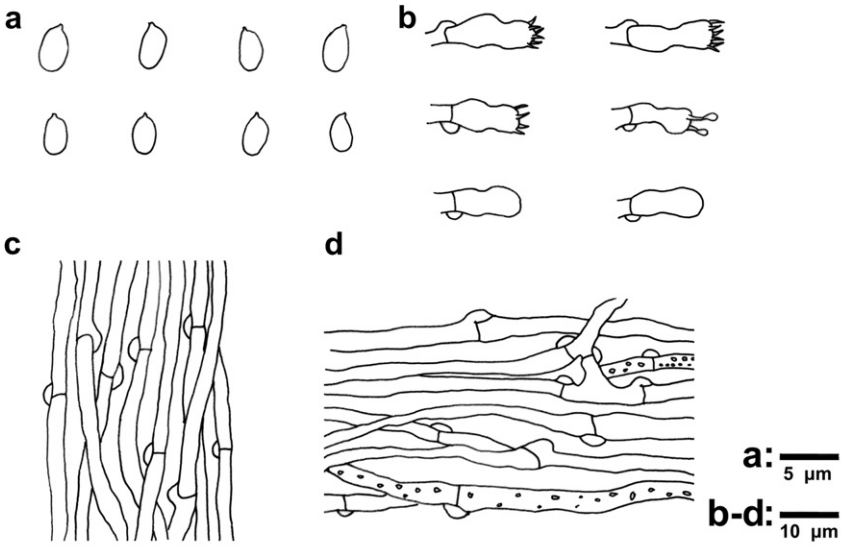

Fig. 2 – Microscopic structures of *Sistotrema subconfluens* (holotype). a Basidiospores. b Basidia and basidioles. c Hyphae from trama. d Hyphae from context.

short-cylindrical to oblong ellipsoid, hyaline, thin-walled, smooth, inamyloid and indextrinoid, acyanophilous, (3.7–) 3.9–4.2(–4.5) × 2–2.3(–2.4) μm, L = 4.01 μm, W = 2.08 μm, Q = 1.9–1.94 (n = 60/2).

4. Discussion

The urniform basidia mostly with six sterigmata make *S. subconfluens* a typical representative for the concept of *Sistotrema* (Eriksson et al. 1984). The terrestrial habit, stipitate basidiocarps and poroid hymenophores of *S. subconfluens* make it similar to *S. confluens*, but different from all other species of *Sistotrema*. In our phylogeny inferred from LSU-rDNA sequences (Fig. 1), *S. subconfluens* also clustered together with *S. confluens*, confirming the close relationship indicated by morphology and habit. However, *S. confluens* has smaller basidiocarps (pilei up to 2 cm in diam and stipe up to 1 cm long; Eriksson et al. 1984; Ryvarden and Gilbertson 1994) and, more importantly, longer basidiospores (4.5–6 μm long in Eriksson et al. 1984; 4–5.5 μm long in Ryvarden and Gilbertson 1994) than *S. subconfluens*. Besides, the hymenophore configurations of *S. confluens* are poroid to irpicoid or lamelloid, while *S. subconfluens* produces poroid hymenophores only. Furthermore, the phylogenetic tree (Fig. 1) supported *S. confluens* and *S. subconfluens* were isolated from each other. Since only a single ITS sequence could be obtained for each species, the intraspecific differences of ITS variation could not be calculated. However, the 3.6% ITS sequence (including ITS1 + 5.8S + ITS2 regions) difference observed for the two species is higher than the 3% threshold value commonly used for species delimitation (Tedersoo et al. 2003; Izzo et al. 2005; Smith et al. 2007; Zhou and Kõljalg 2013), and was enough to accept *S. confluens* and *S. subconfluens* as separated species.

Traditionally, the type species of *Sistotrema*, *S. confluens*, as well as *Sistotrema alboluteum* (Bourdot & Galzin) Bondartsev & Singer, *Sistotrema dennisii* Malençon and *Sistotrema muscicola* (Pers.) S. Lundell were placed in the *S. confluens* group, which shared irpicoid, hydnoid to poroid hymenophores (Eriksson et al. 1984). In our phylogeny, this group and the new species *S. subconfluens* formed a strongly supported clade with several species of *Hydnum*. *Hydnum* is accepted as an ectomycorrhizal genus (Tedersoo et al. 2010) and the ectomycorrhizal status of *S. alboluteum* and *S. muscicola* has been confirmed by molecular and morphological studies (Nilsson et al. 2006; Di Marino et al. 2008; Münzenberger et al. 2012). As such, *S. confluens* was postulated as an ectomycorrhizal species (Nilsson et al. 2006). Whether *S. subconfluens* likewise has an ectomycorrhizal habit should be further examined.

The four *S. confluens* LSU-rDNA sequences available in GenBank did not form a single clade. The sequence originating from Canary Islands (accession number AM259216) sat on a single branch separated from the other three sequences. This indicates that some specimens, representing unknown species similar to *S. confluens*, might be misidentified and buried in herbaria under the labels of *S. confluens*. Future careful morphological examination and ITS sequencing might help to resolve this issue.

The current concept of *Sistotrema* is undoubtedly polyphyletic (Larsson et al. 2004; Moncalvo et al. 2006; Nilsson et al. 2006; Münzenberger et al. 2012). The newly described species *S. subconfluens* was closely related to the type species, *S. confluens*, both from a morphological and a phylogenetic perspective. For most other species of *Sistotrema*, especially those with smooth hymenophores, the taxonomic position should be further inferred from more gene loci.

Disclosure

The authors declare no conflict of interest. All the experiments in this study comply with the current laws of PR China.

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