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Short note

Schizophyllum amplum (Agaricales, Schizophyllaceae): a rare Basidiomycete from Malta and Estonia

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Abstract
Schizophyllum amplum (Agaricales, Schizophyllaceae) is reported for the first time in Malta and Estonia. The collections from Malta were examined morphologically and compared with previously published accounts of this species. The specimens from Estonia and Malta were sequenced after PCR amplification with ITS1F and ITS4 for ITS region. The sequences were then compared to other sequences deposited in public databases using BLASTn. Genetically the ITS rDNA data obtained from the Maltese and Estonian specimens are 99% similar but show some differences from other sequences of *S. amplum* (L43381 and L43382) deposited in GenBank. A multigenetic approach with a larger number of specimens should be employed to define the currently accepted genetic limits for *Schizophyllum* species.

Key words: *Auriculariopsis ampla*, ITS rDNA, mycobiota, Mediterranean, morphology, phylogeny

Introduction

Schizophyllum Fr. is a fungal genus with lamellar hymenophore classified within family Schizophyllaceae, order Agaricales, along with the sister genera *Fistulina* Bull. and *Porodisculus* Murrill (Singer, 1949; Binder et al., 2002; Moncalvo et al., 2002; Bodensteiner et al., 2004; Matheny et al., 2006; Binder et al., 2010). *Schizophyllum* was erected by Fries (1815) for *Schizophyllum commune* Fr., one of the most widespread basidiomycete species, and a model taxon for research whose complete genome has been already sequenced (Ohm et al., 2010). Most sequences of *Schizophyllum* in public databases come from *S. commune* (99.4% in July 2019), and very few other species of this genus are represented. *Schizophyllum radiatum* Fr., *S. umbrinum* Berk., *S. fasciatum* Pat., *S. leprieurii* Linder, and *S. amplum* (Lév.) Nakasone are the only other species present in GenBank. *Schizophyllum radiatum* was sometimes considered a synonym of *S. commune* because of the morphological and ITS rDNA genetic similarity of both species (Cooke, 1961), but recent multigenic analyses concluded that both represent closely related but independent taxa (Siqueira et al., 2016). *Schizophyllum umbrinum* has a brownish surface and striking orange-brown lamellae (Robledo et al., 2014). *Schizophyllum leprieurii* has been rarely collected, and was always reported to resemble *S. umbrinum* (Linder, 1933; Martin, 1941; Cooke, 1961; Robledo et al., 2014), both species displaying brownish basidiomata with a whitish attachment disc. *Schizophyllum leprieurii* and *S. umbrinum* have a similar structure of the context and basidia but differ by the slightly larger and yellowish-brown colored basidiospores (*S. leprieurii*). Robledo et al. (2014) found also some genetic differences between two sequences of *S. umbrinum* and one of *S. leprieurii* which support their recognition as distinct taxa. *Schizophyllum fasciatum* was proposed by Patouillard (1887) to accommodate specimens collected in Mexico and displaying a zonate pileus and fuliginous lamellae. All sequences available in public databases come from a single strain (CBS267.60) obtained from a Mexican specimen.

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More recently, Nakasone (1996) combined *Cyphella ampla* Lév., a species with a smooth hymenophore, into *Schizophyllum* as *S. amplum* (Lév.) Nakasone, because of its dimitic hyphal structure, ellipsoid basidiospores, and also genetic data, in agreement with previous observations of similarities in the development of the hymenophore by Stalpers (1988). *Schizophyllum amplum* is generally found on dead twigs or branches of *Populus* and *Salix*, mainly in Europe, but also in North America, central Asia, Australia and New Zealand. The purpose of the present work is to cite *S. amplum* from Malta and Estonia providing a brief morphological and genetic study to support the identification.

Materials and Methods

Specimens

Two collections of *S. amplum* from Malta and one from Estonia were examined. The Maltese specimens are preserved in the herbarium of one of the authors (CS) and the Estonian specimen is preserved in the fungarium of the University of Tartu (TU).

Morphological studies

Fresh samples were examined with a light microscope. Thin sections and squashed tissue were stained using Congo Red/Phloxine (1% each in 5% ammonia), Melzer reagent and/or Toluidine blue (1%). All measurements were taken on fresh material mounted in water with the aid of Piximètre (downloadable from http://ach.log.free.fr/Piximetre/).

DNA extraction, amplification and sequencing

Total DNA was extracted from dry specimens employing a modified protocol based on Murray & Thompson (1980). A portion of each sample was blended with the aid of a micropestle in 600 µL CTAB buffer (CTAB 2%, NaCl 1.4 M, EDTA pH 8.0 20 mM, Tris-HCl pH 8.0 100 mM). The resulting mixture was incubated for 15 min at 65 °C. A similar volume of chloroform: isoamyl alcohol (24:1) was added and carefully mixed with the samples until their emulsion. It was then centrifuged for 10 min at 13,000 g, and the DNA in the supernatant was precipitated with a volume of isopropanol. After a new centrifugation of 15 min at the same speed, the pellet was washed in cold ethanol 70%, centrifuged again for 2 min and dried. It was finally resuspended in 200 µL ddH_2O. PCR amplification was performed with the primers ITS1F and ITS4 (White et al., 1990; Gardes and Bruns, 1993) for ITS region. PCR reactions were performed under a program consisting of a hot start at 95 °C for 5 min, followed by 35 cycles at 94 °C, 54 °C and 72 °C (45, 30 and 45 s respectively) and a final 72 °C step 10 min. PCR products were checked in 1% agarose gels, and positive reactions were sequenced with one or both PCR primers. Chromatograms were checked searching for putative reading errors, and these were corrected.

Phylogenetic analyses

BLASTn (Altschul et al., 1990) was used to select the most closely related sequences from INSD public databases. Sequences came mainly from Nakasone (1996), Robledo et al. (2014), and Siqueira et al. (2016). Sequences first were aligned in MEGA 5.0 (Tamura et al., 2011) software with its Clustal W application and then corrected manually. The final alignment included 233/560 variable and 59/560 parsimony-informative sites. The alignment was loaded in PAUP* 4.0b10 (Swofford, 2001) and subjected to MrModeltest 2.3 (Nylander, 2004) in PAUP* 4.0b10. Model GTR+G was selected and implemented in MrBayes 3.1 (Ronquist and Huelsenbeck, 2003), where a Bayesian analysis was performed (data partitioned, two simultaneous runs, six chains, temperature set to 0.2, sampling every 100th generation) until convergence parameters were met after about 0.51 M generations, standard deviation having fell below 0.01. Finally, a full search for the best-scoring maximum likelihood tree was performed in RAxML (Stamatakis, 2006) using the standard search

algorithm (data partitioned, GTRMIX model, 2000 bootstrap replications). Significance threshold was set above 0.95 for posterior probability (PP) and 70% bootstrap proportions (BP).

Results and Discussion

ITS rDNA data obtained from the specimen found at Wied il-Qlejgha (MH013190) was found to be 100% similar to two *S. amplum* records in GenBank (DQ097353 and AF141873), and 99% close to one in UNITE (UDB031960; Kõljalg et al., 2013). Other sequences of *S. amplum* in GenBank (L43381 and L43382) showed a 50 bp deletion and 8 differences at other positions, resulting in a 87.5% similarity with the lineage of the samples analyzed in the present work. However, phylogenetic inferences grouped them all together in a significantly supported clade different from that of *S. commune*, *S. umbrinum* and *S. fasciatum* (Fig. 1).

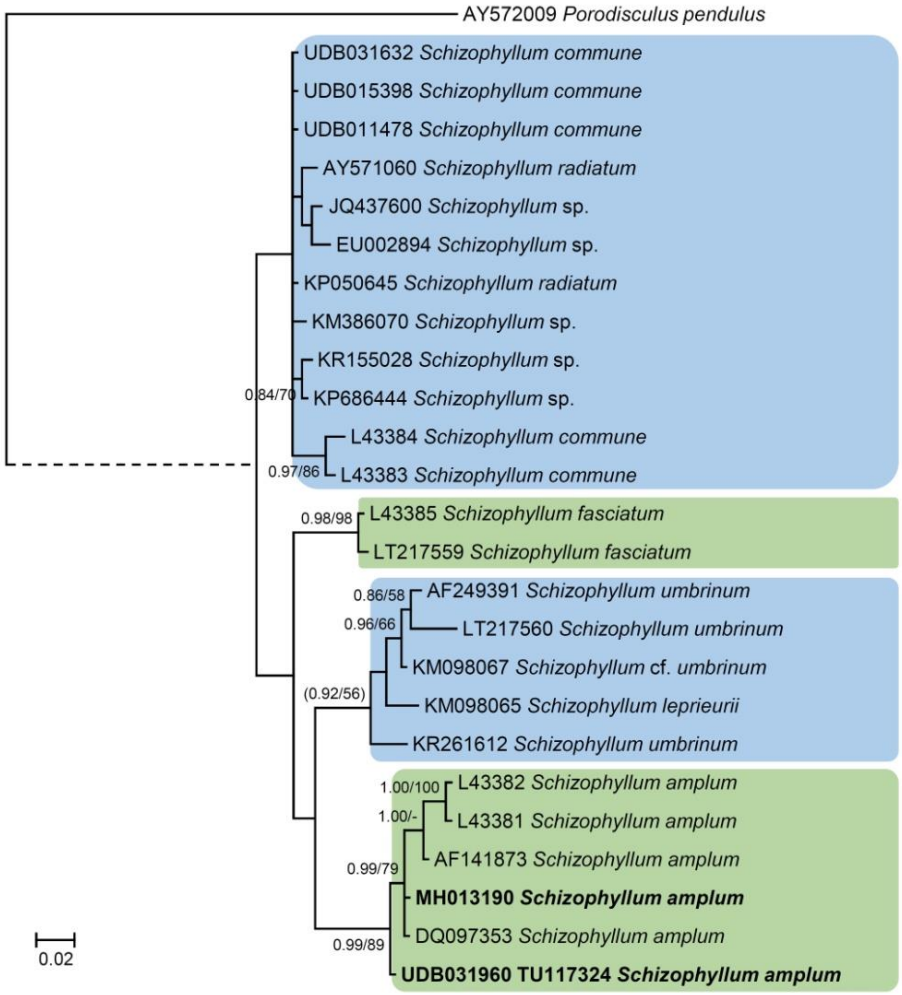

Fig 1 - Fifty percent majority rule consensus ITS rDNA phylogram of the genus *Schizophyllum* obtained in MrBayes from 3,825 sampled trees. Nodes supported by > 0.95 Bayesian PP or > 70% ML BP are shown annotated

Schizophyllum amplum (Lév.) Nakasone Figs. 2-3

MB 415948

= *Cyphella ampla* Lév., Ann. Sci. Nat., Bot., III 9: 126 (1848); = *Chaetocypha ampla* (Lév.) Kuntze, Revis. gen. pl. (Leipzig) 2: 847 (1891); = *Auriculariopsis ampla* (Lév.) Maire, Bull. Soc. mycol. Fr. 18(suppl.): 102 (1902); = *Merulius amplus* (Lév.) Spirin & Zmitr., Nov. sist. Niz. Rast. 37: 181 (2004);

= *Cantharellus coemansii* Rabenh., Fungi eur. exs., No 209: no. 209 (1867);

= *Auricularia syringae* Fuckel, in Jb. nassau. Ver. Naturk. 27-28: 9, Fung. Rhen. no 2508 (1874);

= *Auricularia leveillei* Cooke & Quél., Clav. Hym. Europ. (London): 213 (1878);

= *Cyphella cyclas* Cooke & W. Phillips, Grevillea 9(no. 51): 94 (1881); = *Chaetocypha cyclas* (Cooke & W. Phillips) Kuntze, Revis. gen. pl. (Leipzig) 2: 847 (1891);

= *Auricularia bresadolae* Schulzer, Hedwigia 24(4): 148 (1885);

= *Cytidia simulans* Lloyd, Mycol. Notes (Cincinnati) 6(Letter 64): 991 (1920);

= *Stereum pubescens* Burt, Ann. Missouri. bot. Gdn 7(2-3): 178 (1920).

Basidiomata: 4-19 mm in diameter, disc-shaped to cupulate, pileate, sessile, lignicolous, scattered. Abhymenial surface tomentose and white in colour; internally (hymenium) coloured a light brown to ochre, smooth to veined, gelatinous in appearance.

Microscopy: External hyphae thick walled, straight hyphae; internal layer a filamentous mesh of hyphae 2.3-4.0 µm in width with distinct clamps and with several anastomoses, many hyphae with gelatinous sheaths. Basidia clavate-elongated, 4-spored, 26-33x4-4.8µm, with clamps. Basidiospores hyaline to pale yellowish, smooth, allantoid to cylindrical, (8.1) 8.4-11.1 (11.3) × (2.3) 2.5-3.6 (4.5) µm, Q = (2.4) 2.6-3.6 (4.5), N = 20, mean = 9.8×3.1 µm, Qe = 3.2.

Material examined: MALTA, Buskett (Wied il-Luq) on dead twigs under *Populus alba*, 35.85887 °N 14.40157 °E, 29.09.2010, (CS95); Wied il Qlejgha, on dead broken *P. alba* branch, 35.89395 °N 14.3922 °E, 22.03.2017, (CS1112, GenBank MH013190); ESTONIA, Lääne-Viru county, Vinni, on fallen branch of *Populus tremula*, 59.2941667 °N 26.4408333 °E, 30.09.2015, leg. Urmas Ojango (TU117324, UNITE UDB031960).

Fig 2 - *Schizophyllum amplum*. a-c. collection CS1112; a) *in habitus*; b) younger specimen; c) abhymenial surface. Scale Bar = 10mm

This is the first published record of *S. amplum* in the Maltese Islands and Estonia. *Schizophyllum amplum* is a very rare species in Malta, always associated with *Populus alba* in areas near small streams. The single Estonian collection was growing on *Populus tremula*. The species is also rare in other parts of Europe (Wojewoda, 2006; Robinson et al., 2011). The Maltese specimens are morphologically similar to other published records (Eriksson and Ryvarden, 1975; Nakasone, 1996).

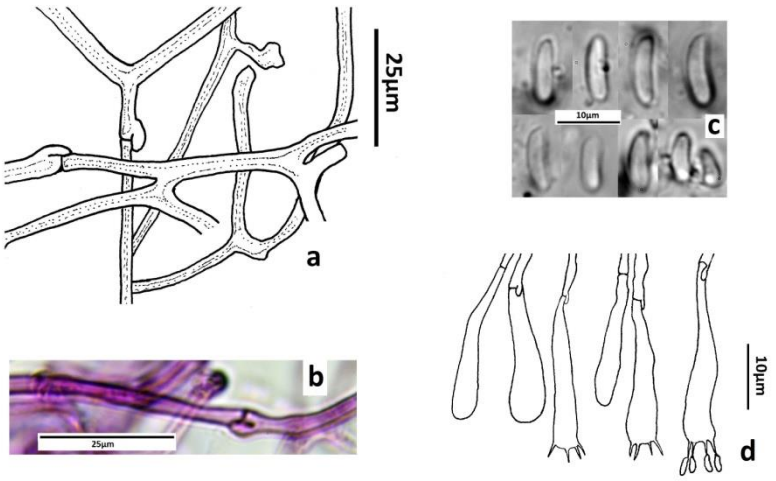

Fig 3 - *Schizophyllum amplum*. Microscopic characters of collection CS1112. a) Tramal hyphae; b) Tramal hyphae stained in toluidine blue; c) Basidiospores; d) Basidia, lateral view

Genetically, the clade of *S. amplum* shows variability in ITS rDNA (8/496 bp variable sites), but most remarkably, sequences L43381 and L43382 present important indels (up to 56 bp in 5 sites). This could mean that two closely related species are hidden within the concept of *S. amplum*. The limited phylogenetic signal provided by ITS rDNA alone does not support the existence of homogeneous and genetically isolated groups within any of the clades of *Schizophyllum*, and so all variability found here is considered intraspecific by now. However, multigenic analyses have already shown that different species could have been misidentified within these clades, e.g. *S. radiatum* within the *S. commune* complex (Siqueira et al., 2016). Therefore, a multigene approach including a high number of specimens analyzed from a broad geographical range could change the currently accepted genetic limits of *Schizophyllum* species.

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