Sparassis cystidiosa sp. nov. from Thailand is described using morphological and molecular data

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Abstract: Sparassis cystidiosa, collected recently from a primary montane cloud forest in northern Thailand is described as new. It is distinct from all others species in the genus because of the presence of hymenial cystidia, relatively large basidiospores and flabellae composed of six distinct layers of tissue. Analyses of a combined dataset of DNA sequences from three genes support its distinction and suggest that the S. cystidiosa lineage is the sister group of all other Sparassis.

Key words: Bayesian phylogenetics, brown rot, Homobasidiomycetes, polyporoid clade, rDNA, rpb2

INTRODUCTION

Sparassis Fr. species are reported commonly from north temperate forests of the Northern Hemisphere because of their large, conspicuous, cauliflower-like basidiomes. In addition, because of the esculent properties, they are collected commonly for culinary purposes. Species delimitations in Sparassis historically have been based on a combination of macro-morphology, basidiospore size and host plant associations. Eleven epithets have been proposed in the genus, but either two species (viz., S. crispa Wallenf., Fr. and S. spathulata Schwein., Fr.; sensu Burdsall and Miller [1988a, b], Martin and Gilbertson [1976], van Zanen [1988]), or three species (including S. brevipes Krombh.: sensu Kreisel [1983]) currently are accepted in Sparassis. In their type studies, Burdsall and Miller (1988a) reduced most published epithets to synonymy with S. crispa or S. spathulata and they accepted S. brevipes (without an extant type specimen) as a nomen dubium. Molecular data, in part presented here, support the recognition of S. brevipes sensu Kreisel, S. radicata Weir, an Asian S. cf. crispa, an unnamed Australian taxon, and S. cystidiosa, newly delimited here, as additional distinct species. A more detailed discussion of the nomenclature, taxonomy and phylogeny of the worldwide members of Sparassis is presented elsewhere (Wang et al. 2004).

In a molecular phylogenetic study of the relationships among selected agarics, polypores and gastromycetes, Hibbett et al. (1997) suggested that S. spathulata was the sister taxon of the polypores Laetiporus sulphureus (Bull.: Fr.) Murrill and Phaeolus schweinitzii (Fr.) Pat. (bootstrap support 87%). All three species have bipolar mating systems and are brown-rot fungi that cause root and heart rot of living trees (Gilbertson and Ryvarden 1986, 1987, Hibbett and Donoghue 2001). Support for the genus Sparassis being included in the polyporoid clade is presented by Wang et al. (2004) who include sequences of many additional specimens that were not studied by Hibbett et al. (1997). In Wang et al. (2004), two previously unknown taxa of Sparassis are included. One of these, Sparassis spAUS31, was collected from Australia and will be described as a new species elsewhere. A second taxon, collected recently from northern Thailand, is described as a new species in this work, based on morphological and molecular data. Before this report of a new Asian Sparassis species, only S. crispa had been reported from Japan westward to Tibet (Imazeki et al. 1988, Teng 1996, Mao et al. 1993). A Sparassis species, reported as S. laminosa, is displayed on a 2002 issue DPR Korea postage stamp. The latter image probably represents S. cf. crispa as we report here from China.

MATERIALS AND METHODS

Morphological studies.—In the morphological description, color terms and notations in parentheses are from Kornerup and Wanscher (1978). In the micromorphological analyses, data were obtained from the dried specimen after sectioning and mounting in water, 3% KOH, Phloxine or Melzer’s reagent. Spore statistics include: $\bar{x}$, the arithmetic mean of the spore length by spore width (± SD) for n spores measured; $Q$, the quotient of spore length and spore width in any one spore, indicated as a range in variation in n spores measured; $Q$, the mean of Q-values (± SD). The

\[ \text{Accepted for publication March 5, 2004.} \]

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specimen is deposited in SFSU and BBH (herbarium acronyms from Holmgren et al 1990).

Molecular phylogenetics.—A subset of the sequence data reported by Wang et al (2004) was analyzed, including sequences of partial nuclear large subunit (nuc-lsu) rDNA, complete internal transcribed spacers 1 and 2 and the 5.8S rDNA (nuc-ITS rDNA), and part of the gene encoding the second largest RNA polymerase, subunit 2 (rpb2). Sequences were aligned by eye in the data editor of PAUP* 4.0b (Swofford 1999), and the matrix was submitted to TreeBase (No. M1815). The taxa included one specimen of S. cystidiosa from Thailand; nine specimens of S. crispa sensu lato from Europe, North America and East Asia; one specimen of S. brevipes from Germany; and three specimens of S.spathulata from North America (Table 1). Oligoporus rennyi and Grifola frondosa were included for rooting purposes.

The dataset was analyzed in PAUP* 4.0b (Swofford 1999) and MrBayes 2.01 (Huelsenbeck and Ronquist 2001), with gaps treated as missing data and ambiguous positions excluded. An equally weighted parsimony analysis was performed in PAUP* using branch and bound. A bootstrapped parsimony analysis was performed in PAUP*, with 1000 replicates, each with a heuristic search with 10 random taxon addition sequences, MAXtrees set to 1000, and TBR branch swapping. A Bayesian analysis was performed in MrBayes, under the GTR+I+G model (Gu et al 1995), with 50,000 generations and four chains (one cold, three incrementally heated), as per the default program settings (Huelsenbeck 2000). Trees were sampled every 100 generations, and a total of 501 trees were saved for each analysis. Likelihoods converged to a stable value after the first 1000 generations, so the first 10 trees sampled were discarded before computing a majority rule consensus of 491 trees in PAUP*.

RESULTS

Molecular phylogenetics.—The combined nuc-lsu, ITS and rpb2 dataset contained 2267 aligned positions with 343 parsimony-informative positions, distributed as follows: nuc-lsu = 880 aligned positions, ITS = 746 aligned positions, and rpb2 = 64/76 = 691 aligned positions. Parsimony analysis resulted in one most parsimonious tree of 928 steps (Fig. 1). Isolates of Sparassis crispa sensu lato (European S. crispa and Asian S. cf. crispa) formed a strongly supported clade (bootstrap = 100%). Isolates of S. spathulata formed a monophyletic group (bootstrap = 100%) with S. brevipes as its sister group (bootstrap = 100%). Sparassis cystidiosa was placed as the sister group of all other Sparassis isolates. The monophyly of Sparassis was supported strongly (bootstrap = 100%), and the monophyly of all Sparassis isolates except S. cystidiosa was moderately supported (bootstrap = 83%). The majority-rule consensus tree produced in the Bayesian analysis (Fig. 1) supported the same tree topology as the parsimony analysis, with higher confidence along some branches. Sparassis cystidiosa again was placed as the sister group of all other Sparassis isolates, with strong support (posterior probability = 99%).

TAXONOMY

Sparassis cystidiosa Desjardins et Zheng Wang, sp. nov.

Fig. 1. Phylogenetic relationships of Sparassis spp inferred from a combined dataset of nuc-lsr-rDNA, ITS, and rpb2 sequences. The single most parsimonious tree (928 steps, CI = 0.837, RI = 0.863) is shown, with branch lengths proportional to the number of mutations inferred with parsimony. Bootstrap values greater than 50% are indicated in plain type. Posterior probabilities generated from the Bayesian analysis are indicated in bold type.


Basidiomes 200–250 mm diam, composed of a rosette of loosely arranged flabellae arising from a poorly developed central core: flabellae up to 120 mm broad, 1–2 mm thick; margin entire or dissected, wavy; sterile upper surface rugulose and radially wrinkled, glabrous, azonate, yellowish brown (5D6–8) to brown (6D5E5–8), darkening with age, becoming brownish orange (6C5E5–8) to light brown (6D6E8) in exsicciata; fertile hymenium (lower surface) radially wrinkled, glabrous to minutely pruinose, azonate, concolorous with the sterile upper surface when fresh, becoming dark greyish brown (6E7E3) in exsiccati; basidiomes distinctly bicolorous (upper versus lower surfaces) when dried. Context tough, pliant, concolorous with surface. Odor strong, cheddar cheese-like, pleasant. Taste not recorded.

Flabellae composed of six distinct layers of tissue, itemized from the lower/outer layer toward the upper/inner layer as follows: (i) Hymenium layer 65–80 μm thick, hyaline, inamylloid, composed of basidia and cystidia; dark greyish brown in exsicciata. Basidiospores (Fig. 2) 7–9 × 6–7 μm (t = 7.8 ± 0.8 × 6.6 ± 0.5 μm, Q = 1–1.3, Q = 1.24 ± 0.07, n = 25 spores), subglobose (rarely globose) or broadly ellipsoid-ovoid, smooth, hyaline, inamylloid, thin-walled. Basidia (Fig. 3) 65–74 × 8–9.5 μm, narrowly elongate-subclavate, 4-spored, hyaline, clamped. Basidioles (Fig. 3) narrowly elongate-subclavate. Hymenial cystidia (Fig. 4) scattered, 100–144 × (6.4–7)–11 μm (w = 8.7 μm, n = 20), narrowly clavate, arising from deep in the subhymenium (possibly as terminal cells of the gloeplerosus hyphae) and projecting 28–52 μm beyond the basidia, refractive (gloeocystidia-like), hyaline to pale yellow, inamylloid, thin-walled. (ii) Sub-

hymenium 90–105 μm thick, composed of tightly packed, pseudoparenchymatous to sinuous cells 4–10 mm diam, tawny brown in water and 3% KOH, dark brownish orange to pale reddish orange (weakly dextrinoid) in Melzer’s reagent, thin-walled or with walls up to 1 μm thick. (iii) Pseudoparenchymatous layer 140–220 μm thick, composed of irregularly cylindrical, vesiculose and irregularly ovoid to puzzle-shaped cells up to 30 μm diam, tightly adherent to each other, with hyaline, inamylloid, nongelatinized walls 1–5 μm thick. (iv) Medullary layer composed of loosely interwoven, cylindrical hyphae 3–10 μm diam, sometimes swollen up to 16 μm diam, hyaline, inamylloid, nongelatinous, thin-walled or with walls up to 1.5 mm thick, clamped. Gloeoplerous hyphae interspersed, 2–11 μm diam, refractive, irregularly cylindrical to sinuous or strangulate, thin-walled. (v) “Hypodermium” layer pseudoparenchymatous, 120–160 mm thick, similar to layer 3, composed of tightly packed hyphae 4–32 μm diam, irregularly cylindrical to vesiculose or puzzle-shaped, hyaline, inamylloid, nongelatinous, with walls 1–2.5 mm thick. (vi) On young flabellae, a hymenium layer as in layer 1; on mature flabellae the hymenium collapses and forms a sterile cuticle layer 12–24 μm thick, composed of irregularly cylindrical to vesiculose, collapsed hymenial elements 3–8(–10) μm diam, smaller-celled and more tightly packed than cells in layer 5, subhyaline to pale yellowish brown, inamylloid, nongelatinous, thin-walled or with walls up to 1 μm thick; layer 6 brownish orange to light brown in exsiccata.

Habit and distribution. Solitary at the base of a living oak tree (Quercus eumorpha Kurz). Thailand.

Habitat. Primary, montane, temperate, evergreen cloud forest with a closed canopy dominated by tree species of Acer, Symingtonia and Quercus, and an understory of Cornus oblonga Wall., Rhododendron dela-vayi Franch., and other shrubs.


DISCUSSION

S. cystidiosa is distinct morphologically from other known species of Sparassis because of these combination of features: (i) loosely arranged, very broad flabellae with nondissected and only slightly wavy margins; (ii) distinctly bicolorous flabellae when dried, with orange-toned sterile surface and dark greyish brown hymenium; (iii) basidiospores with mean width 6.6 μm; (iv) the presence of conspicuous, refractive, projecting hymenial cystidia; and (v) flabellae formed of six distinct layers of tissue. In all other species of Sparassis, the flabellae are arranged more tightly and have dissected and/or strongly wavy margins, dried specimens are not distinctly bicolorous, the basidiospores have a mean width of 4–5 μm, hymenial cystidia are lacking, and tranal tissues are not as distinctly layered. Although the hymenium is amphigenous in all Sparassis species, in S. cystidiosa the hymenium layer on the upper/inner surface of young flabellae is fertile but collapses early in development resulting in a primarily sterile surface. On mature flabellae, the lower/outer surface becomes geotropically and is functionally the surface contributing most significantly to sporulation.

Phylogenetic analyses of the combined nuc-its rDNA, ITS and rpb2 dataset suggest that S. cystidiosa is a unique species that is the sister group of all the other Sparassis species included in this study (Fig. 1). The only Sparassis taxa that were not included here are S. radicata, from western North America, and Sparassis spAUS31, from Australia. Both were excluded because rpb2 sequences were not available. Analyses by Wang et al (2004) suggest that S. radicata is nested within the clade that includes S. crispa sensu lato. The placement of Sparassis spAUS31 is more problematic. Parsimony analysis of nuc-its rDNA and ITS sequences suggests that Sparassis spAUS31 is the sister group of a clade containing Grifola frondosa and Pycnoporellus fulgens, suggesting that Sparassis is possibly not monophyletic. Bayesian analysis of the same data suggests, however, that Sparassis spAUS31 is the sister group of all Sparassis species, including S. cystidiosa (see Wang et al 2004). Until additional data (and collections) are available for Sparassis spAUS31, its placement, and the monophyly of Sparassis, will remain uncertain. Sparassis spAUS31 is the only collection from the South Hemisphere and is morphologically distinct from all known Sparassis species. The taxonomic status of the Australian taxon will be discussed elsewhere (Wang et al 2004).

It is noteworthy that S. crispa forms clamp connections and European and North American S. crispa is associated strictly with conifers whereas S. brevipes and S. spathulata both lack clamp connections and are associated both with conifers and Fagales (Fig. 1). In comparison, S. cystidiosa forms clamp connections and, as far as is known, is associated only with Fagales. If the itss/rpb2 tree reflects the true phylogeny of Sparassis, then this would suggest that the ancestor of Sparassis had clamp connections and was associated with Fagales hosts.

ACKNOWLEDGMENTS

This work is supported in part by National Sciences Foundation Grants DEB-0118776 to Desjardin and DEB-9903835
LITERATURE CITED


