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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 their esculent properties, they are collected commonly for culinary purposes. Species delimitations in *Sparassis* historically have been based on a combination of macromorphology, basidiospore size and host plant associations. Eleven epithets have been proposed in the genus, but either two species (viz., *S. crispa* Wulfen : 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 gasteromycetes, 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; $\bar{Q}$, the mean of Q-values (± SD). The

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TABLE I. Specimens used in molecular studies and GenBank accession numbers

Isolates	LSU	ITS	RPB2	Location
Sparassis crispa FIN3	AY218387	AY218425	AY218532	Finland
S. crispa FRA5	AY218389	AY218427	AY218534	France
S. crispa GER25	AY218404	AY218442	AY218544	Germany
S. crispa AME9	AY218393	AY218430	AY218537	USA
S. cf. *crispa* CHN19M	AY218398	AY218436	AY218540	China (middle)
S. cf. *crispa* CNH21S	AY218400	AY218438	AY218542	China (south)
S. cf. *crispa* CNH20M	AY218399	AY218437	AY218541	China (middle)
S. cf. *crispa* CHN17N	AY218397	AY218435	AY218539	China (north)
S. cf. *crispa* CHN1N	AY218385	AY218423	AY218530	China (north)
S. cystidiosa DED7410	AY256890	AY256891	AY256892	Thailand
S. spathulata AME11	AY218395	AY218432	AY218538	USA (MA)
S. spathulata AME7	AY218391	AY218428	AY218535	USA (MA)
S. spathulata AME8	AY218392	AY218429	AY218536	USA (NH)
S. brevipes GER24	AY218403	AY218441	AY218543	Germany
Grifola frondosa	AY218413	AY218415	AY218521	
Oligoporus rennyi	AY287876	AY218416	AY218499	

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 I). *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 rpb2-6f/7r = 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 Desjardin et Zheng Wang, sp. nov. FIGS. 2–4

Carpophora magna, 200–250 mm diam., rami erecti flabelliformes usque ad 120 mm lati × 1–2 mm crassi, ad marginem intacti, azonati, pallide luteobrunnei vel aurantiobrunnei, obscurior in siccitate. Hymenium griseobrunneum, rugosum basin versus. Odor fortis gratusque. Basidiosporae 7–9 × 6–7 μm, subglobosae vel late ellipsoideae, leves, hyalinae, inamyloideae. Basidia 4-spora. Cystidia 100–144 × 7–11 μm, plasma refractiva impleta, hyalina. Caro flabellarum 6-stratis composita. Hab. solitarius ad Quercum

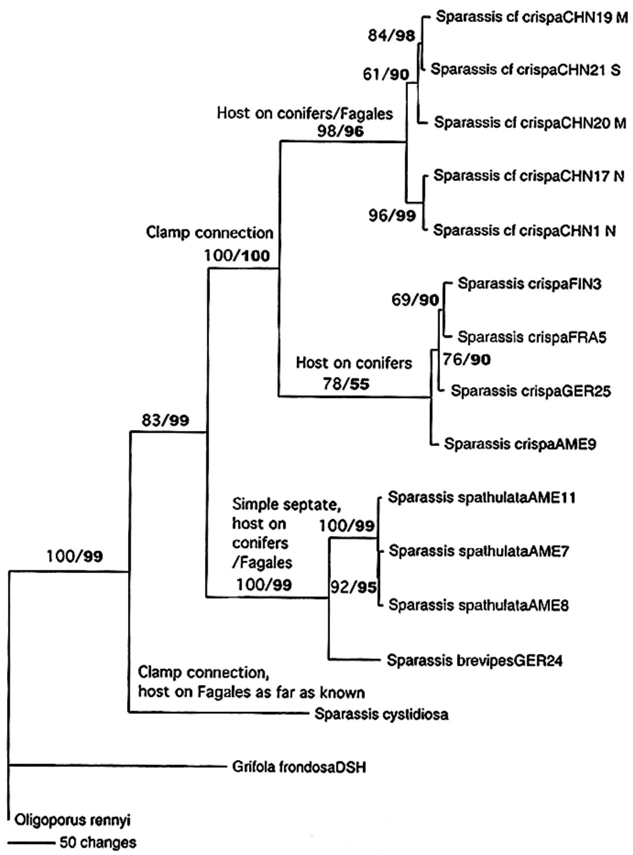
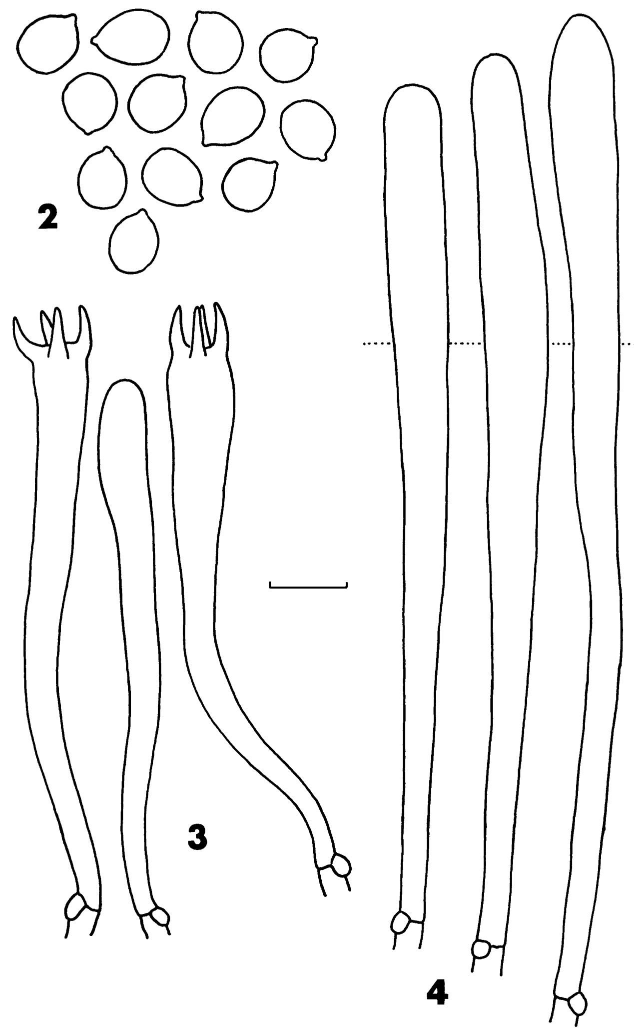


FIG. 1. Phylogenetic relationships of *Sparassis* spp inferred from a combined dataset of nuc-lsu-rDNA, ITS, and rpb2 sequences. The single most parsimonious tree (928 steps, CI = 0.837, RI = 0.863) is shown, with branch lengths proportional to the number of mutations inferred with parsimony. Bootstrap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



FIGS. 2–4. Micromorphological features of *Sparassis cystidiosa* (DED 7410—HOLOTYPE). 2. Basidiospores. 3. Basidia and basidiole. 4. Hymenial cystidia; dotted horizontal line represents surface of hymenium. Scale bar = 10 μm.

eumorpham in silvis montanis virgineis. Thailand. Holotypus D. E. Desjardin 7410 (BBH).

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 (6D E5–8), darkening with age, becoming brownish orange (6C5–8) to light brown (6D6–8) in exsiccata; fertile hymenium (lower surface) radially wrinkled, glabrous to minutely pruinose, azonate, concolorous with the sterile upper surface when fresh, becoming dark greyish brown (6–7E3) 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, inamyloid, composed of basidia and cystidia; dark greyish brown in exsiccati. *Basidiospores* (FIG. 2) 7–9 × 6–7 μm ($\bar{x}$ = 7.8 ± 0.8 × 6.6 ± 0.5 μm, Q = 1–1.3, $\bar{Q}$ = 1.24 ± 0.07, n = 25 spores), subglobose (rarely globose) or broadly ellipsoid-ovoid, smooth, hyaline, inamyloid, 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 ($\bar{w}$ = 8.7 μm, n = 20), narrowly clavate, arising from deep in the subhymenium (possibly as terminal cells of the gloeplerous hyphae) and projecting 28–52 μm beyond the basidia, refractive (gloeocystidia-like), hyaline to pale yellow, inamyloid, 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, inamyloid, 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, inamyloid, 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* pseudoparenthymatous, 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, inamyloid, 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, inamyloid, nongelatinous, thin-walled or with walls up to 1 μm thick; layer 6 brownish orange to light brown in exsiccati.

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 delavayi* Franch., and other shrubs.

Specimen examined. THAILAND. CHIANG MAI PROVINCE: Doi Inthanon National Park, summit area of Doi Inthanon at ca. 2500 m, Ang Ka Nature Trail, 27 Jun 2002, collected by D.E. Desjardin and E. Horak, *D.E. Desjardin 7410* (HOLOTYPE, BBH; ISOTYPE, SFSU).

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 tramal 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 geotropic and is functionally the surface contributing most significantly to sporulation.

Phylogenetic analyses of the combined nuc-lsu 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 problematical. Parsimony analysis of nuc-lsu 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 lsu/ITS/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.

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