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A new *Sparassis* species from Spain described using morphological and molecular data

Jaime B. BLANCO-DIOS[a,*], *Zheng WANG*[b], *Manfred BINDER*[b], *David S. HIBBETT*[b]

[a]*Centro de Capacitación e Experimentación Agraria de Lourizán, P.O. Box 127. Lourizán, Pontevedra, 36080, Spain*
[b]*Department of Biology, Clark University, 950 Main Street, Worcester, Massachusetts, 01610, USA*

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ABSTRACT

Sparassis miniensis, collected in *Pinus pinaster* forests in Galicia (northwest Iberian Peninsula) is described as a new species, based on morphological and molecular data. *Sparassis miniensis* is morphologically distinct from all other species in the genus *Sparassis* based on scattered flabellae, which are strongly laciniated, azonate, and arise from an orange to rose-purplish base. The sporadic presence of clamp connections is restricted to subhymenial hyphae. Molecular data from LSU-rDNA, ITS and partial gene coding RNA polymerase subunit II (*rpb2*) suggest a close relationship between the new species *S. miniensis* and *S. brevipes*, another European species producing large fruiting bodies but with entire flabellae and no clamp connections.

Introduction

Sparassis species are distributed worldwide, and they have been collected and studied morphologically in Europe for many years (e.g. Hennig 1952, Kreisel 1983, Reid 1958). The phylogenetics of the genus *Sparassis* were recently studied using materials from eastern Asia, North America, Australia, and to a lesser extent from Europe. A close relationship between *Sparassis* species and two polypores, *Laetiporus sulphureus* and *Phaeolus schweinitzii*, which also produce brown rot, was suggested by sequence data from rDNA (including the ITS region) and the partial gene coding RNA polymerase subunit II (*rpb2*) (Wang *et al.* 2004). Using both morphological and molecular data, seven potential *Sparassis* species have been recognized: *S. crispa*, *S. radicata, S. brevipes* (syn. *S. laminose*, *S. nemecii*) (Kreisel 1983), *S. spathulata* (syn. *S. herbstii*) (Burdsall & Miller 1988), *S. cystidiosa*, and two undescribed taxa, one from Australia and one from Asia (Desjardin *et al.* 2004; Wang *et al.* 2004).

The objectives of the present study are the morphological characterization of a new *Sparassis* species, *S. miniensis*, and estimation of its phylogenetic position in the genus *Sparassis*, extending the data of Desjardin *et al.* (2004) and Wang *et al.* (2004). Their studies suggested that the presence of clamp connections, geographic distribution and macromorphology of basidiomata are critical in recognizing *Sparassis* species. Biogeographic relationships among *Sparassis* species still remain unclear and data from collections in Europe and

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[*] Corresponding author.
E-mail address: jblanco@infobrion.com.

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adjacent areas are critical for resolving this biogeographic puzzle (Wang *et al.* 2004). Based on rDNA and *rpb*2 data, European species of *Sparassis* are closely related to those from North America, and a sister relationship between North American *S. spathulata* and European *S. brevipes* received strong support from the molecular data. However, European materials of *Sparassis* species were incompletely sampled. For example, material of the European *S. simplex*, which is potentially synonymous with *S. spathulata* (Burdsall & Miller 1988), was not available for this study and the study of Wang *et al.* (2004). In this study, several recent collections from Galicia in the northwest of Spain are described as a new species, *S. miniensis*.

Materials and methods

Morphological studies

Morphological descriptions are based on observations of fresh and dried specimens. In the microscopic studies, data were obtained from the dried specimens after sectioning and mounting in water, 5 % potassium hydroxide, 1 % Congo red in water, and/or Melzer's reagent, using brightfield microscopy. Spore statistics include: $\bar{x}$, the arithmetic mean of the spore length by spore breadth (±s.d.) for *n* spores measured; Q, the quotient of spore length and spore breadth in any one spore, indicated as a range in variation in *n* spores measured; $\bar{Q}$, the mean of Q-values (±s.d.). The specimens are deposited in LOU-Fungi herbarium (Centro de Investigacións Forestais e Ambientais de Lourizán, Pontevedra, Spain).

Molecular techniques and phylogenetic analyses

Sequence data of three regions, nuLSU rDNA, ITS and *rpb*2 were generated following Wang *et al.* (2004), using the same primer pairs and procedures, and sequence data generated in this study were submitted to GenBank (accession numbers DQ270672–DQ270676). A dataset of combined sequences from nuLSU rDNA, ITS, and *rpb*2 was prepared (28 isolates representing 15 taxa) using ClustalX (Thompson *et al.* 1997) with default settings, which was manually adjusted in the editor of PAUP 4.0b10 (Swofford 2002). In addition to new sequences of *Sparassis miniensis*, published data from *S. cystidiosa*, *S. brevipes*, *S. spathulata*, *S. radicata*, and *S. crispa* from Europe, North America and Asia were included. The dataset was rooted with *Lentinus tigrinus*, and MP analyses basically followed Wang *et al.* (2004). In addition to a branch-and-bound analysis, a BS analysis was performed in PAUP with 1K heuristic search replicates, tree bisection–reconnection (TBR) branch swapping, with MAXTREES set to autoincrease. The dataset is available at TreeBASE (accession number SN2542).

Results

Phylogenetic relationships

The systematic position of *Sparassis miniensis* was estimated using combined sequences of nuLSU rDNA, ITS and *rbp*2, which had an aligned length of 2320 base pairs with 276 uninformative variable positions and 518 parsimony-informative positions. Equally weighted parsimony analysis generated 16 equally parsimonious trees of 1846 steps with a CI of 0.627 (Fig 1).

Species of *Sparassis* formed a monophyletic group with *S. cystidiosa* as the basal branch (BS = 90 %), and five additional clades that were recognized. The Asian *S.* cfr *crispa* formed a clade (BS = 98 %) in the monophyletic *S. crispa s. lat.* (BS = 100 %). A clade including European and eastern North American isolates of *S. crispa*, and western North American *S. radicata* was resolved without high BS values (BS < 70 %). A clade including *S. miniensis* and *S. brevipes* was strongly supported (BS = 100 %), and this group is the sister group to *S. spathulata* (BS = 100 %).

Taxonomy

Sparassis miniensis Blanco-Dios & Zheng Wang, **sp. nov.**

Etym.: *miniensis*, from the river Miño, Galicia (Spain).

Carpophora 110–180 × 140–210 mm, rami flabelliformibus, usque ad 100 mm latis × 2 mm crassis, fragilibus, dispersis, laciniatis (usque ad 38 mm) et plicatis, azonatis, ab albidis ad, denique, ocreis, basi debili, ab aurantiaca ad rosea et purpurea. Caro sapore fungico vel amaro et odore dulci. Sporae (6–) 6.5–7.5 (–8) × (4–) 4.5–5 (–5.5) µm, ellipsoideae, raro latae ellipsoideae vel oblongae, inamyloideae. Basidia bi- vel tetrasporigera, afibulata. Hyphae subhymenii raro fibulatae.

Typus: **Spain:** *Pontevedra Province*: Salceda de Caselas, Entenza, 29TNG3658, 90 m, forest of *Pinus pinaster*, 4 Nov. 2004, *J. B. Blanco-Dios et al.* (LOU-Fungi 18390— holotypus).

Basidiomata 110–180 mm wide × 140–210 mm tall, composed of a scattered group of flabellae arising from a poorly developed central base (up to 80 × 35 mm), orange to rose purplish (Fig 2A). *Flabellae* up to 100 mm broad, 2 mm thick, with fragile habit, scattered, margin strongly laciniate and folding, with laciniae up to 38 mm. *Surface* rugulose, minutely pruinose to glabrous, azonate, whitish, creamy ochre with age, with hymenium whitish greyish. *Context* soft, slightly pliant, concolorous with surface. *Odour* pleasant, slightly-sweet. *Taste* fungoid or bitter. *Basidiospores* (Fig 3A) (6–) 6.5–7.5 (–8) × (4–) 4.5–5 (–5.5) µm, ($\bar{x}$ = 7 ± 0.5 × 4.5 ± 0.5 µm, Q = (1.15) 1.34–1.58 (1.80), $\bar{Q}$ = 1.45 ± 0.1, *n* = 30), mainly ellipsoid, rarely broadly ellipsoid or oblong, smooth, thin-walled, hyaline, inamyloid. *Basidia* (Fig 3B) 30.5–56 × 4.5–9.5 µm, 2–4 spored, with sterigmata 3.5–4.5 µm, hyaline, narrowly elongate-subclavate, no clamp connections observed, closely packed. *Basidioles* (Fig 3B) narrowly elongate-subclavate. *Cystidia* or other sterile hymenial elements were not observed. Hyphal system monomitic. *Subhymenial hyphae* (Figs 2B, 3C) 2.5–7 µm diam, densely compacted and interwoven, with hyphae subclavate, subisodiametric, thin-walled, simple septate, clamp connections scarce. *Contextual hyphae* (Fig 3D) 4–7.5 µm in diam., irregularly cylindrical, mostly thin-walled, with simple septa, no clamp connections observed; oleiferous hyphae, 5.5–12.5 µm diam, interspersed, irregularly cylindrical, sinuous, thick-walled, aseptate, greyish brownish in Congo red.

Habitat: solitary at the roots of afflicted, living Pinus trees (*Pinus pinaster*) in ancient fluvial terraces from the river Miño.

Distribution: South of Pontevedra province, Galicia, Spain.