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

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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 lacinated, azonate, and arise from an orange to rose-purplish base. The sporadic presence of clamp connections is restricted to subhyphal 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.

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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*) (Burdson & 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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adjacent areas are critical for resolving this biogeographic puzzle (Wang *et al.* 2004). Based on rDNA and *rpb2* 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* (Burdshall & 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 (\pm 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 (\pm s.d.). The specimens are deposited in LOU-Fungi herbarium (Centro de Investigaci3n Forestais e Ambientais de Louriz3n, Pontevedra, Spain).

Molecular techniques and phylogenetic analyses

Sequence data of three regions, nuLSU rDNA, ITS and *rpb2* 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 *rpb2* 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 *rpb2*,

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 Mi3no, 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) μ m, 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 lacinate 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) μ m, (\bar{x} = 7 ± 0.5 × 4.5 ± 0.5 μ m, *Q* = (1.15) 1.34–1.58 (1.80), *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 μ m, 2–4 spored, with sterigmata 3.5–4.5 μ m, 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 μ m diam, densely compacted and interwoven, with hyphae subclavate, subisodiametric, thin-walled, simple septate, clamp connections scarce. *Contextual hyphae* (Fig 3D) 4–7.5 μ m in diam., irregularly cylindrical, mostly thin-walled, with simple septa, no clamp connections observed; oleiferous hyphae, 5.5–12.5 μ m 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 Mi3no.

Distribution: South of Pontevedra province, Galicia, Spain.

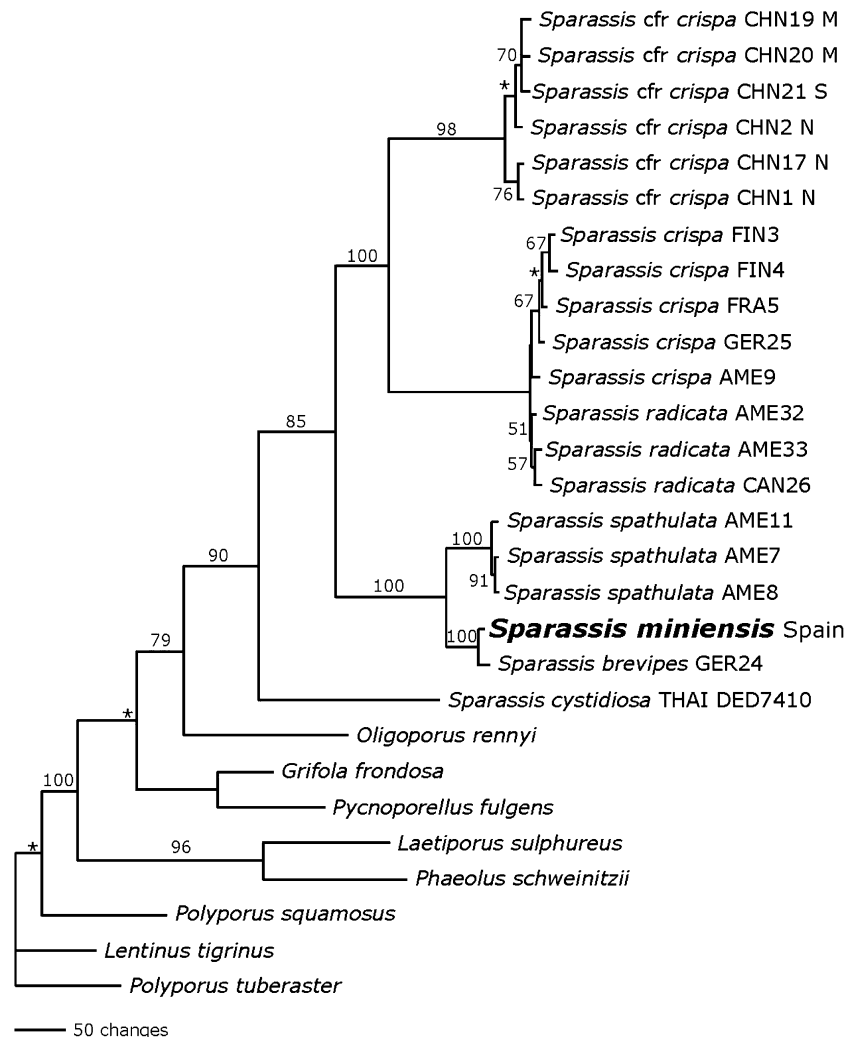


Fig 1 – Phylogenetic relationships of *Sparassis* based on parsimony analysis of the combined dataset of LSU-rDNA, ITS and *rpb2* sequences. One of the 16 equally parsimonious trees. BS values greater than 50 % are indicated along branches. Nodes marked with asterisks collapse in the strict consensus tree. Species names of *Sparassis* are followed by the geographical location and strain number (AME, North America; CAN, Canada; CHN, China [N, north, M, middle; S, south]; FIN, Finland; FRA, France; GER, Germany; THAI, Thailand).

Additional specimens examined: Spain: Pontevedra Province: Tomiño, O Seixo, 29TNG2249, 20 m, forest of *Pinus pinaster*, 30 Oct. 2004, J. B. Blanco-Dios et al. (LOU-Fungi 18389); Tui, Caldeas de Tui, 29TNG3557, 70 m, forest of *Pinus pinaster*, 25 Nov. 2002, M. A. Rodríguez-Martínez (LOU-Fungi 18388).

Discussion

Sparassis miniensis is morphologically distinct from other known species in *Sparassis* because of the following combination of characters: flabellae are scattered and strongly lacinate; sporocarps have an orange to rose purplish central core; context with a fungoid or bitter taste; and the sporadic presence of clamp connections in the subhymenial hyphae. *Sparassis miniensis* forms a clade with *S. brevipes*, and the sister group to this European clade is the North American species *S. spathulata*. Efforts to amplify *rpb2* from additional collections were not successful, however, rDNA genes were

obtained from at least one additional isolate of *S. miniensis* and *S. brevipes* that confirm the phylogeny to be presented in Fig 1. This result is consistent with an *atp6* phylogeny presented elsewhere (Dai et al. unpubl.).

Morphologically, *S. miniensis* is easily distinguished from both *S. brevipes* and *S. spathulata* by its conspicuous habit including strongly lacinated, azonate flabellae, and by the possession of clamp connections. It has previously been thought that clamp connections were lost in the *S. spathulata* – *S. brevipes* clade (Wang et al. 2004). The presence of clamp connections in *Sparassis* species is presumably a plesiomorphic character that is unequally distributed over several clades, but it consistently occurs in the following European species: clamp connections are present, though rarely, in the subhymenial hyphae of *S. miniensis*. *S. crispa* produces clamp connections at the base of basidia and in subhymenial hyphae, while *S. simplex* produces clamp connections at the base of basidia (Jülich 1989; Reid 1958). *S. simplex* collections need to

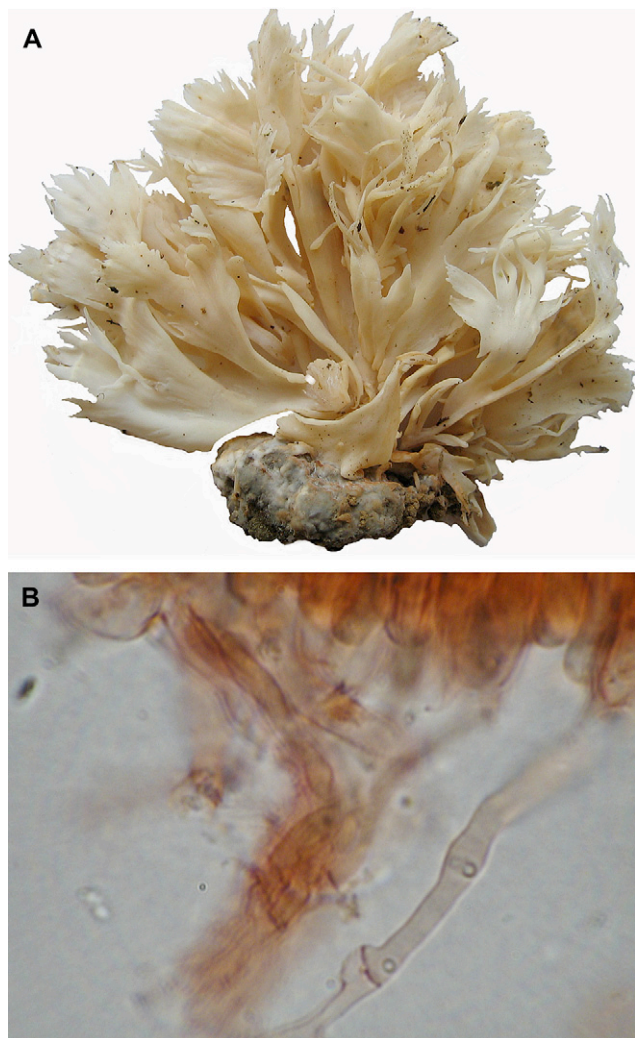


Fig 2 – *Sparassis miniensis* (LOU-Fungi 18390—holotypus).
A. Basidiome. B. Clamp connection in subhymenial hyphae.

be included in future phylogenetic studies to understand the evolution of gains and losses of clamp connections in *Sparassis* species.

In addition, host shift appears to be associated with geographic patterns among *Sparassis* species (Wang et al. 2004). European species are strictly associated with either conifers or *Fagales*, or sometimes with eucalypts (*Eucalyptus globulus*), for example *S. brevipes* in Galicia (López-Prada & Castro 2002), whereas Asian and North American species show a wider range of hosts (e.g. *Tsuga*, *Larix*). The European species *S. crispa*, *S. simplex*, and *S. miniensis* have so far only been associated with conifers, while *S. brevipes* is associated with members of *Fagales* and conifers (Kreisel 1983).

The application of spore size and shape is probably of limited value for separating European species of *Sparassis*, as ranges of spore size are clearly overlapping among the species and are sometimes subject to inconsistent reports within single species. Kreisel's (1983) spore measurements of *S. crispa* collections ($4.4\text{--}5.3 \times 3.4\text{--}4.4 \mu\text{m}$) deviate considerably from those of others, for example, $5\text{--}6.5 \times 4\text{--}5 \mu\text{m}$ in Burdsall & Miller (1988) or $4.9\text{--}6 \times 4\text{--}4.9 \mu\text{m}$ in Wang et al. (2004). Similar

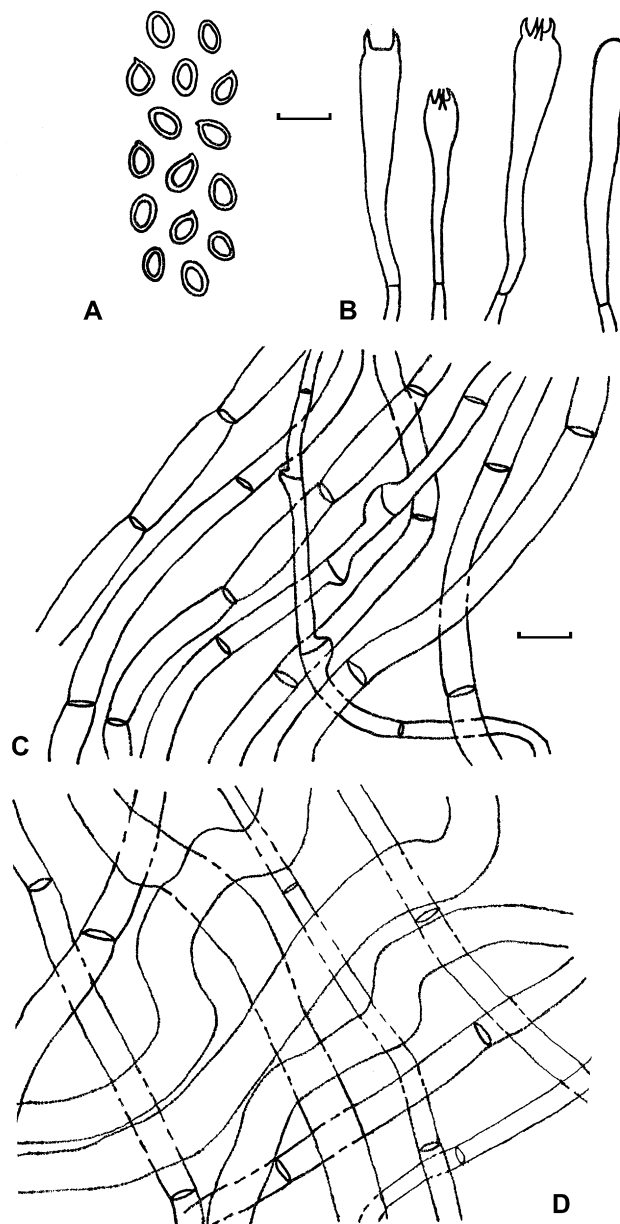


Fig 3 – Micromorphological features of *Sparassis miniensis* (LOU-Fungi 18390—holotypus).
A. Basidiospores. B. Basidia and basidiole. C. Subhymenial hyphae with clamp connections. D. Contextual hyphae with oleiferous hyphae.
Bar = 10 μm .

discrepancies concern the spore size of *S. brevipes*, for which Kreisel (1983) reports $4.4\text{--}5.8 \times 3.4\text{--}4.4 \mu\text{m}$, while Wang et al. (2004) report $6.8\text{--}7.9 \times 4.4\text{--}5.2 \mu\text{m}$. The latter measurement approximates to the size of *S. miniensis* spores. *S. simplex*, which is only known from the type collection (Reid 1958), is an exception as it produces larger and distinctly ellipsoid spores ($5\text{--}9 \times 3.5\text{--}5 \mu\text{m}$). Taken together, spore size is a variable character in European species that needs closer examination to verify morphological species concepts or to detect cryptic species complexes.

With the description of *S. miniensis*, we present a key to the species of *Sparassis* known worldwide.

Key to the described species of *Sparassis*

- 1 Hymenial cystidia present, flabellae not dissected **cystidiosa**
 Hymenial cystidia absent, flabellae dissected 2
- 2(1) Clamp connections absent, flabellae distinctly zonate 3
 Clamp connections present, flabellae azonate 4
- 3(2) Eastern North America **spathulata**
 Europe **brevipes**
- 4(2) Clamp connections scarce 5
 Clamp connections abundant in subhymenium and at the base of basidia 6
- 5(4) Clamp connections only in subhymenial hyphae, flabellae laciniate **miniensis**
 Clamp connections only at the base of basidia **simplex**
- 6(4) Flabellae arising from a branched or corticioid base 7
 Flabellae arising from a common centre **radicata**
- 7(6) Flabellae arising from a branched base [Asian material may represent a different species] **crispa**
 Flabellae arise from a corticioid base **simplex**

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