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 laciniate, azonate, and arise from an orange to rose-purple 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, 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) (Burdasall & 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 macro morphology of basidiomata are critical in recognizing Sparassis species. Biogeographic relationships among Sparassis species still remain unclear and data from collections in Europe and
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. spatulata 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. spatulata (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: $x$, 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; Q, the mean of Q-values ($\pm$ s.d.). The specimens are deposited in LOU-Fungi herbarium (Centro de Investigación Forestal e Ambiental de Lourizán, 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. spatulata, 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. spatulata (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 latissimis × 2 mm crasis, fragilibus, dispersis, lanciati (usque ad 38 mm) et pilatis, azonatis, ab albidos ad, denique, ochres, basi debili, ab aurantiaca ad rosea et purpurea. Caro carpe sapore ocreis, basi debili, ab aurantiaca ad rosea et purpurea. Carpophora 110–180 mm, rami flabelliformes, usque ad 60 mm latissimis × 1 mm crassis, fragilibus, dispersis, lanciati (usque ad 38 mm) et pilati, azonati, ab albidos ad, denique, ochres, basi debili, ab aurantiaca ad rosea et purpurea. Caro carpe sapore ocreis, basi debili, ab aurantiaca ad rosea et purpurea.

Basidiospore 4.5–5 (± 0.5) μm, ellipsoidae, raro latae ellipsoidae vel oblongae, inamyloideae. Basidia bi- vel tetrasporigera, afibulata. Hyphae subhyphalii rari fibulae.

Typus: Spain: Pontevedra Province: Salceda de Caselas, Entenza, 29NTG3658, 90 m, forest of Pinus pinaster, 4 Nov. 2004, J. B. Blanco-Dios et al. (LOU-Fungi 18390 — holotypos).

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, ellipsoidae, raro latae ellipsoidae vel oblongae, inamyloideae.

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

Distribution: South of Pontevedra province, Galicia, Spain.

Discussion

Sparassis miniensis is morphologically distinct from other known species in Sparassis because of the following combination of characters: flabellae are scattered and strongly laciniated; 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 laciniated, 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
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 (4.4–5.3 × 3.4–4.4 μm) deviate considerably from those of others, for example, 5–6.5 × 4–5 μm in Burdsall & Miller (1988) or 4.9–6 × 4–4.9 μm in Wang et al. (2004). Similar discrepancies concern the spore size of S. brevipes, for which Kreisel (1983) reports 4.4–5.8 × 3.4–4.4 μm, while Wang et al. (2004) report 6.8–7.9 × 4.4–5.2 μm. 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 (5–9 × 3.5–5 μm). 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.

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

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

REFERENCES


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