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FULL PAPER

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Phylogenetic placement of *Marasmiellus juniperinus*

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Abstract Recent collections and the type specimen of *Marasmiellus juniperinus*, the type species of the genus, were examined. Phylogenetic placement, based on ribosomal large subunit (LSU) and internally transcribed spacer (ITS) sequences, is within the lentinuloid clade, nested among *Gymnopus* taxa. This placement dictates genus name usage and phylogenetic position of other putative species of *Marasmiellus*. The mating system is tetrapolar.

Key words *Collybia* · *Gymnopus fusipes* · Lentinuloid clade · rDNA

Introduction

When Murrill (1915) introduced the genus name *Marasmiellus*, it represented three species [*M. inconspicuous* Murrill (Cuba), *M. purpureus* (Berk. & Curt.) Murrill (Cuba), and *M. juniperinus* Murrill (Jamaica), with the latter designated as *typus generis*]. *Marasmiellus juniperinus* was represented by a single collection of small basidiomata on *Juniperus barbadensis* (Linn.) Gordon. From this inauspicious beginning, *Marasmiellus* has been interpreted as inclusive of many taxa (viz. Singer 1973), chiefly distributed in the tropics, but also found in temperate regions. From marasmielloid basidiomata, several genera have been segregated (e.g., *Chaetocalathus*, *Tetrapyrgos*, *Campanella*) based on unique micromorphological structures, but the taxonomic limits and phylogenetic placement of *Marasmiellus* sensu stricto have remained elusive (Moncalvo et al. 2002).

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Gilbertson and Blackwell (1985, 1987) described numerous basidiomycetous fungi fruiting on *Juniperus* taxa through the Gulf Coastal region of the United States from central Texas to central Florida. Among these fungi was *M. juniperinus*, reported from *J. virginiana* Linn. in Louisiana and Florida and from *J. phoenicea* Linn. in Louisiana. No less than nine collections were reported, indicating that the fungus was more common than previously thought (although collections of agarics from bark of living junipers had not been emphasized by previous workers). Desjardin (1997) summarized *Marasmiellus* from the southern Appalachian Mountains, but did not report *M. juniperinus*, which presumably fruited only farther south.

Singer (1973) interspersed a detailed neotropical floristic monograph of *Marasmiellus* among the editions of his *Agaricales in Modern Taxonomy* (Singer 1962, 1975). In accepting ten sections, including 134 neotropical species and their infraspecific taxa, the genus was greatly expanded from its humble origin. Characters of basidiomata were combined with micromorphological considerations (i.e., presence or absence of clamp connections, shapes and sizes of basidiospores, structures of the pileipelli).

Singer apparently dismissed Murrill's note on substratum and reported *M. juniperinus* (var. *juniperinus*) as fruiting on "woody sticks of coniferous, dicotyledonous and monocotyledonous trees and shrubs." *Juniperus*, *Nothofagus*, and *Podocarpus* were noted particularly. Seasonality and geographic range: ". . . fruiting in the rainy season, not common but widely distributed in temperate, subtropical and tropical zones, from Jamaica south to Chile and Patagonia." Singer's (1973) detailed description may be interpreted as including several forms of the species, or perhaps several species, so we have chosen to return to a few collections of less disputed identity.

This article is intended to supplement micromorphological data furnished by Gilbertson and Blackwell (1985, 1987), to which collections of putative *Marasmiellus* taxa can be compared, and to report the phylogenetic position of the species as a guide to the use of the genus name and possible disposition of "*Marasmiellus*" names phylogenetically differently placed from the type species.

Materials and methods

Collections examined

Jamaica, Cinchona, 4500–5200 ft, Dec. 25, 1908, W.A. Murrill, no. 484 (holotypus, NY). United States, Louisiana, Baton Rouge Parish, Louisiana State University, T.J. Baroni and M. Blackwell, Aug. 31, 1996, TJB 8128 (CORT); same location, M. Blackwell, Oct. 4, 2002, TFB 9889 (TENN 59540).

Terminology

Colors in alphanumeric codes in parentheses are from Kornerup and Wanscher (1978). Basidiospore measurements and statistics are as follows: spore length × spore width is given for all measured basidiospores in terms of their range of variation; n = total number of spores measured; x = arithmetic mean of spore length × spore width for all spores measured; Q = spore length divided by spore width in any spore, indicated as a range of variation in n spores measured; Qx = arithmetic mean of Q values. Herbarium abbreviations are according to Holmgren et al. (1990). In regard to mating types, "*" = tester strain deposited at ATCC.

Microscopy

Sections of dried specimens were rehydrated in aqueous 95% alcohol, then placed in aqueous 3% KOH, observed under phase-contrast microscopy, or with Congo red and phloxine with bright-field microscopy. Melzer's reagent was used to conduct amyloidy tests.

Culture establishment

With the collection of basidiomata from Louisiana (TFB 9889) came a light spore deposit on the surface of the Petri dish. These spores were suspended in sterile water and a dilution series was plated on malt extract (Difco, 15 g/l) agar (Difco, Bacto-agar, 20 g/l; malt extract agar, MEA). Germinated spores were excised using a sharpened dentist's probe and transferred to individual MEA agar Petri dishes. Once grown, single-basidiospore isolates (SBIs) were screened for presence of clamp connections (indicative of dikaryon), and clampless SBIs were used in a self-cross experiment.

In the self-cross, clamp connections were small and scattered, so portions of contact zones of all pairings were excised, squashed, and observed at 1500× under phase-contrast oil immersion microscopy.

Molecular procedures

Monokaryon and dikaryon cultures were grown in 30 ml PD broth (24 g/l Difco potato dextrose broth) until the mycelial cultures were ~2 cm in diameter. Cultures were filtered through a fine mesh cloth and blotted to remove excess medium. Tissue (0.25 g) was ground in 750 µl Carlson lysis buffer with a mortar and pestle, then incubated at 74°C for 30 min (Carlson et al. 1991). DNA was recovered as described in Jin et al. (2001). For a few taxa, including *M. juniperinus*, the ribosomal internal transcribed spacer (ITS) region and 5′-end of the ribosomal large subunit gene (LSU) were amplified using the forward primer ITS1F (Gardes and Bruns 1993) and reverse primer LR7 (Moncalvo et al. 2000). Cycle parameters were 94°C for 3 min followed by 30 cycles of 94°C for 30 s, 60°C for 60 s, and 72°C for 90 s with a final extension at 72°C for 3 min (Jin et al. 2001). The polymerase chain reaction (PCR) product was visualized by gel electrophoresis in a 1.5% Tris-borate + EDTA (ethylenediaminetetraacetic acid) (TBE) agarose gel and was sequenced with an automated ABI 3100 DNA sequencer (ABI Prism Dye Terminator cycle sequencing; Perkin-Elmer, Norwalk, CT, USA) with primers ITS5 and ITS3 (Bruns et al. 1991), and LR5 and LR12 (www.biology.duke.edu/fungi/mycolab). Sequences were spliced, manually corrected, manually aligned using the SEQLAB program in the Genetics Computer Group package (GCG 2000), and deposited in GenBank (Tables 1, 2). Related lentinuloid LSU sequences were identified by a Blast search of GenBank (Table 1) and were aligned with the LSU portion of *M. juniperinus*. Related *Gymnopus* ITS sequences were from those by Mata (2002) and Hughes (unpublished data) using protocols given by Mata (2002).

Analysis of sequence data

Phylogenetic relationships were estimated by maximum parsimony (MP) implemented in PAUP* 4.0 (Swofford 2001) and by Bayesian analysis implemented in Mr. Bayes (Huelsenbeck and Ronquist 2000). In the LSU data set, gaps were usually small (1–3 bp). For the ITS data set, gaps were many and of varying size, ranging from a few bases to more than 100 bp. No attempt was made to code gaps as characters due to the complexity of the ITS sequence data. Two regions, a 40-bp region in ITS1 and a 140-bp region in ITS2, were of uncertain alignment and were eliminated from the aligned data set before analysis.

For MP analyses, gaps were treated as missing or as a fifth base in separate analyses. Characters were unordered and unweighted. Heuristic searches were conducted under the following conditions: the starting tree was obtained via stepwise addition with the addition sequence set as furthest; the maxtrees setting was 100; the branch-swapping algorithm was tree-bisection-reconnection (TBR). Branch robustness was evaluated by 100 bootstrap replicates (Felsenstein 1985) using the same conditions as above. For all analyses, *Omphalotus* was used as the outgroup.

Bayesian analysis was performed using rates set to a gamma distribution approximated using four categories of nuclear substitution. The number of generations was 100 000, sampled every 100th generation. The burn-in value was 100. The strict consensus tree was calculated based on the last 901 trees generated.

Table 1. Collections used for ribosomal large subunit comparisons

GenBank number	Name	Voucher number(s)	Reference
AF042595	*Gymnopus dryophilus* as *Collybia dryophila*	RV83/180	Moncalvo et al. 2000
AF042596	*Gymnopus polyphyllus* as *Collybia polyphylla*	RV182.01	Moncalvo et al. 2000
AF223172	*Gymnopus acervatus*	CBS174.48	Moncalvo et al. 2002
AF261336	*Gymnopus biformis*	RV98/32	Moncalvo et al. 2002
AJ406564	*Gymnopus dryophilus* as *Collybia dryophila*	GEL4613	Langer E, unpublished data
AF135795	*Gymnopus fusipes*	TENN55904	Krueger et al., unpublished data and this paper
AY256710	*Gymnopus fusipes*	11333, TENN59217[a]	This paper
AY256711	*Gymnopus fusipes*	11439, TENN59300[a]	This paper
AY256709	*Gymnopus luxurians*	10350, TENN57910[a]	This paper
AF223173	*Gymnopus peronatus*	CBS 426.79	Moncalvo et al. 2002
AF042579	*Lentinula edodes*	ATCC 42962	Moncalvo et al. 2000
AF261562	*Lentinula lateritia*	TMI1485	Moncalvo et al. 2002
AF356162	*Lentinula lateritia*	TMI1172	Hibbett et al. 1995
AY256708	*Marasmiellus juniperinus*	9889, TENN59540[a]	This paper
AF261329	*Marasmiellus opacus*	JEJ.574	Moncalvo et al. 2002
AF261330	*Marasmiellus opacus*	HN2270	Moncalvo et al. 2002
AF042626	*Marasmiellus ramealis*	DED3973	Moncalvo et al. 2000
AF261331	*Marasmius scorodonius*	JEJ.586	Moncalvo et al. 2002
AF261332	*Marasmius scorodonius*	DAOM175382	Moncalvo et al. 2002
AF261328	*Micromphale foetidum*	JEJ.VA.567	Moncalvo et al. 2002
AF042008	*Omphalotus japonicus*	456	Binder et al. 1997
AF135172	*Omphalotus japonicus* as *Lampteromyces japonicus*	JMlegMURAKAMI	Thorn et al. 2000
AF042621	*Omphalotus nidiformis*	T1946.8	Moncalvo et al. 2000
AF042010	*Omphalotus olearius*	CBS 141.34	Binder et al. 1997
AF261325	*Omphalotus olivascens*	VT645.7	Moncalvo et al. 2002
AF042597	*Rhodocollybia maculata*	RV94/175	Moncalvo et al. 2000
AF261585	*Setulipes androsaceus* as *Marasmiellus androsaceus*	HN4730	Moncalvo et al. 2002

[a] Culture number followed by herbarium number

Results

Morphology

Pileus 5–17 mm diameter, dimidiate, convex, often pleated and remaining so; surface almost smooth, with a suede-like appearance, dry, opaque, sulcate-striate, reddish-brown (6D4) becoming paler (5C4), when dry cream to tan (4–5B3); margin lobed, when dry somewhat inrolled. Context less than 1 mm thick, concolorous to pileus surface; consistency rather coriaceous to chewy, very tough. Lamellae adnate to adnexed, some free, about 1 mm broad, distant, 6–8 per basidiome, ± concolorous to pileus surface; margin smooth; intervenose ridges several. Stipe 1–3 × 1–2 mm, eccentric to nearly lateral, terete, equal, tough; surface mealy, appressed fibrillose, dark brown to pale brown. Odor mild; taste ± sweet. Habitat gregarious on cortex of living *Juniperus* spp.

Pileus epicutis a loosely arranged cutis; hyphae 2–5 μm diameter, cylindrical, occasionally diverticulate, some ± radially oriented, pale brown in mass, lightly banded pigment-encrusted, hyaline singly; wall thin. Terminal cells often coralloid, knobbed, or apically lobed, prostrate to erect, single or in fascicles. Pileus trama loosely interwoven; hyphae 4–6 μm diameter, cylindrical, hyaline; wall thin. Gleoplerous hyphae occasional. Lamellar trama irregular to interwoven; hyphae 2–6 μm diameter, hyaline; wall thin. Subhymenium pseudoparenchymatous. Basidia 24–30 × 6–7 μm, clavate, four-sterigmate; basidioles (16–)24–29 × 4–7 μm, clavate, often submucronate to subfusoid, some ± flexuous. Pleurocystidia absent. Lamellar margin fertile. Cheilocystidia (19–)26–36 × 4–7 μm, narrowly clavate to clavate, occasionally flexuous, typically knobbed and apically diverticulate, often embedded within hymenium and prostrate, arising from horizontal hyphae. Stipe epicutis parallel; hyphae 3–9 μm diameter, hyaline; wall up to 1.6 μm thick. Caulocystidia absent. All tissues inamyloid; clamp connections present.

Basidiospores (6.8–)7.2–9.6(–10.0) × 4.0–5.2(–5.6) μm (n = 50, x = 8.4 × 4.6 μm, Q = 1.43–2.18, Qx = 1.84), lacrymoid, ellipsoid to broadly ellipsoid in side view, ellipsoid in profile, hyaline, inamyloid, acyanophilous; wall thin; contents sometimes uniguttulate or "oily."

Mating system

When 12 SBIs were paired in all combinations, a tetrapolar mating system was revealed. Isolates 3*, 13 = A_1B_1; 2*, 12 = A_2B_2; 6*, 7, 11 = A_1B_2; 1, 5, 9*, 14 = A_2B_1. Tester strains of mating types have been deposited at ATCC.

Although poorly defined "flat" and "barrage" contact zone morphologies were noted, (1) they were only roughly patterned, and (2) some compatible pairings also showed flat or barrage contact zones. Subordinate mating types, therefore, were assigned arbitrarily.

Table 2. Collections used for ribosomal internal transcribed spacer (ITS) comparisons

GenBank number	Name	Voucher numbers		Reference
		Culture number	TENN number	
AY256696	*Gymnopus cylindricus*	10084	58024	This paper
AY256693	*Gymnopus* sp.[a]	11584	59532	This paper
AY256691	*Gymnopus aquosus*	10310	57958	This paper
AY256699	*Gymnopus biformis*	9127-11	55753	This paper
AY256698	*Gymnopus confluens*	9875	59500	This paper
AY256697	*Gymnopus confluens*	10653	58242	This paper
AY256702	*Gymnopus dichrous*	10014	56726	This paper
AY256690	*Gymnopus dryophilus*	9952	57012	This paper
AY256694	*Gymnopus earleae*	11043	59457	This paper
AY256710	*Gymnopus fusipes*	11333	59217	This paper
AF505777	*Gymnopus fusipes*	11439	59300	Mata et al. 2002
AY256705	*Gymnopus lodgae*	11030-10	58638	This paper
AF505765	*Gymnopus luxurians*	10350-1	57910	Mata et al. 2002
AY256700	*Gymnopus omphalodes*	10022	56734	This paper
AY256706	*Gymnopus peronatus*	6983	55902	This paper
AY256701	*Gymnopus polygrammus*	9631-10	56592	This paper
AY256695	*Gymnopus polyphyllus*	11041	59455	This paper
AY256707	*Gymnopus subnudus*	6928	Culture only: specimen held by S.C. McCleneghan	This paper
AY256692	*Gymnopus subsulphureus*	10006	56718	This paper
AY016443	*Lentinula aciculospora*	9447	56421	Mata et al. 2001
AY016440	*Lentinula boryana*	10827	58368	This paper
AY256687	*Lentinula raphanica*	9921	56663	This paper
AY256708	*Marasmiellus juniperinus*	9889	59540	This paper
AY256703	*Marasmiellus opacus*	11499	Culture only	This paper
AY256704	*Marasmiellus stenophyllus*	11558	59444	This paper
AY313271	*Omphalotus illudens*	6951	54507	This paper
AY313286	*Omphalotus japonicus*	2305	Culture only: specimen held by Izawa	This paper
AY313274	*Omphalotus mexicanus*	4866	51283	This paper
AY313277	*Omphalotus oleareus*	9061b	Culture only	This paper
AY313282	*Omphalotus subilludens*	8258	54323	This paper
AY256689	*Rhodocollybia* cf. *butyracea*	9000	55660	This paper
AY256688	*Rhodocollybia maculata*	11045	59459	This paper

[a] In Table 2 and Fig. 2, *Gymnopus* sp. is a new species of sect. *Levipedes* to be described elsewhere

Basidiospore germination

Germination on MEA occurred within 4 h. By 12 h, germination tubes were 25–50 μm long, single, and sinuous. At 24 h, germination tubes were 50–250 μm long, single (unbranched), and generally submerged. For this reason, the connection between spore and visible germination tube was difficult to discern. SBIs were harvested at this time, with additional SBIs taken over the subsequent 48 h (total = 54 h). Subsequent survival of SBIs was low (15 of original 54).

Although basidiospore germination was rapid and vigorous, colony growth was slow, attaining a radius on MEA of 2–5 mm in 6 weeks. At about 8 weeks, growth rate increased dramatically, increasing in radius by 10 mm in 3 weeks. Three SBIs never increased growth rate or modified early colony morphology.

Colony morphology

Hyphal differentiation was minimal, as follows: (1) aerial hyphae were abundant, almost always involved in slime and usually associated in loose ropes of 2–5 individuals; on such hyphae clamps were difficult to see; (2) at 250×, hyphae were (a) thin walled and easily crumpled; (b) smooth, thin walled, and often vacuolated; (c) somewhat thick walled and flagelliform; and (d) often inflated at branch points and sometimes intercalary and then vacuolated.

Results of LSU analyses

A phylogenetic reconstruction based on ribosomal LSU placed *M. juniperinus* within the lentinuloid clade as delineated by Moncalvo et al. (2002). Parsimony analysis of ribosomal LSU sequences treating gaps as missing produced 24 trees of 271 steps (832 total characters, 92 parsimony informative characters). When gaps were treated as a fifth base, 24 trees were also recovered with 279 steps (832 total characters, 94 parsimony informative characters). The consensus tree produced by Bayesian analysis was 266 steps long. Clade 1 (*Lentinula*), clade 2 (*Setulipes androsaceus*, *Micromphale foetidum*, *Gymnopus fusipes*, *G. polyphyllus*, *G. dryophilus*, and *G. acervatus*), clade 3 (*G. luxurians*, *G. biformis*, *G. dryophilus*, *G. peronatus*, *Marasmiellus*

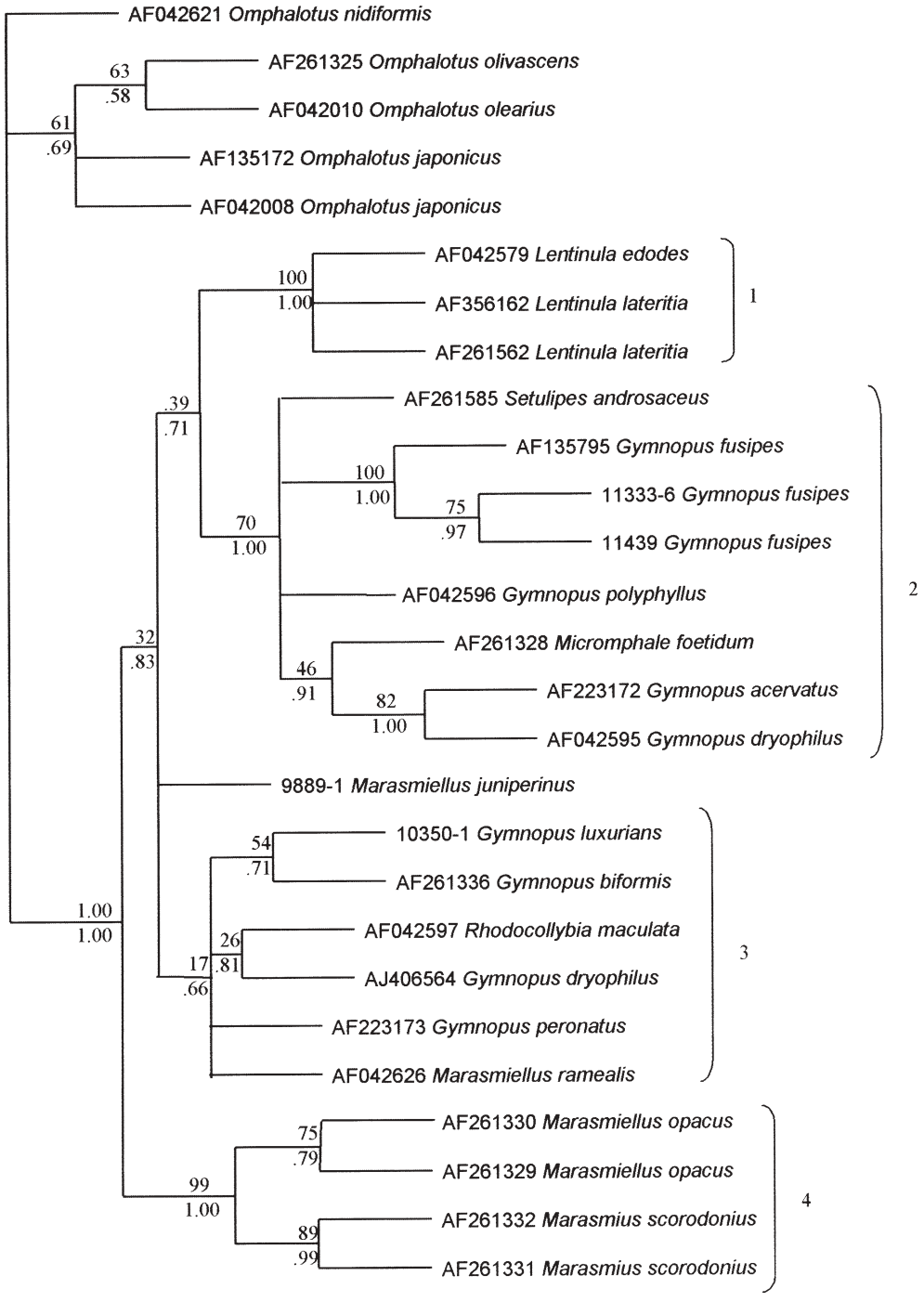

Fig. 1. Phylogeny based on the ribosomal large subunit (*LSU*) region. This tree represents a bootstrap 50% majority rule tree plus other groups compatible with this tree obtained from a maximum-parsimony analysis of ribosomal LSU sequences with gaps treated as a fifth base. Branches not supported by either maximum parsimony or Bayesian analysis have been collapsed. Bootstrap values (*top number*) and Bayesian posterior probabilities (*bottom number*) are given to the left of the supported node. Total characters = 831; parsimony informative characters = 94; tree length = 283 steps; CI = 0.58, HI = 0.41, RI = 0.75

ramealis, and *Rhodocollybia maculata*), and clade 4 (*Marasmiellus opacus* and *M. scorodonius*) were recovered in both parsimony analyses and Bayesian analysis. Bootstrap 50% majority-rule consensus trees for MP treating gaps as a fifth base or as missing data had identical topologies. Figure 1 gives the results of a maximum-parsimony analysis using gaps as a fifth base. Bayesian analysis produced a consensus tree from 901 trees with a topology identical to that recovered by MP (Fig. 1) with the exception of *M. juniperinus*, which appeared basal to clades 1 and 2 in Bayesian analysis (posterior probability = 1.00).

Results of ITS analyses

A maximum-parsimony bootstrap consensus tree (together with branches consistent with the tree) is given in Fig. 2. Results of maximum-parsimony analysis of the ITS region with 180 characters eliminated and gaps treated as a fifth base produced nine trees, each 1978 steps long (857 total characters, 286 constant, 428 parsimony informative). When gaps were treated as missing (857 total characters, 298 parsimony informative), eight trees were recovered, each 1173 steps long. The bootstrap consensus tree from

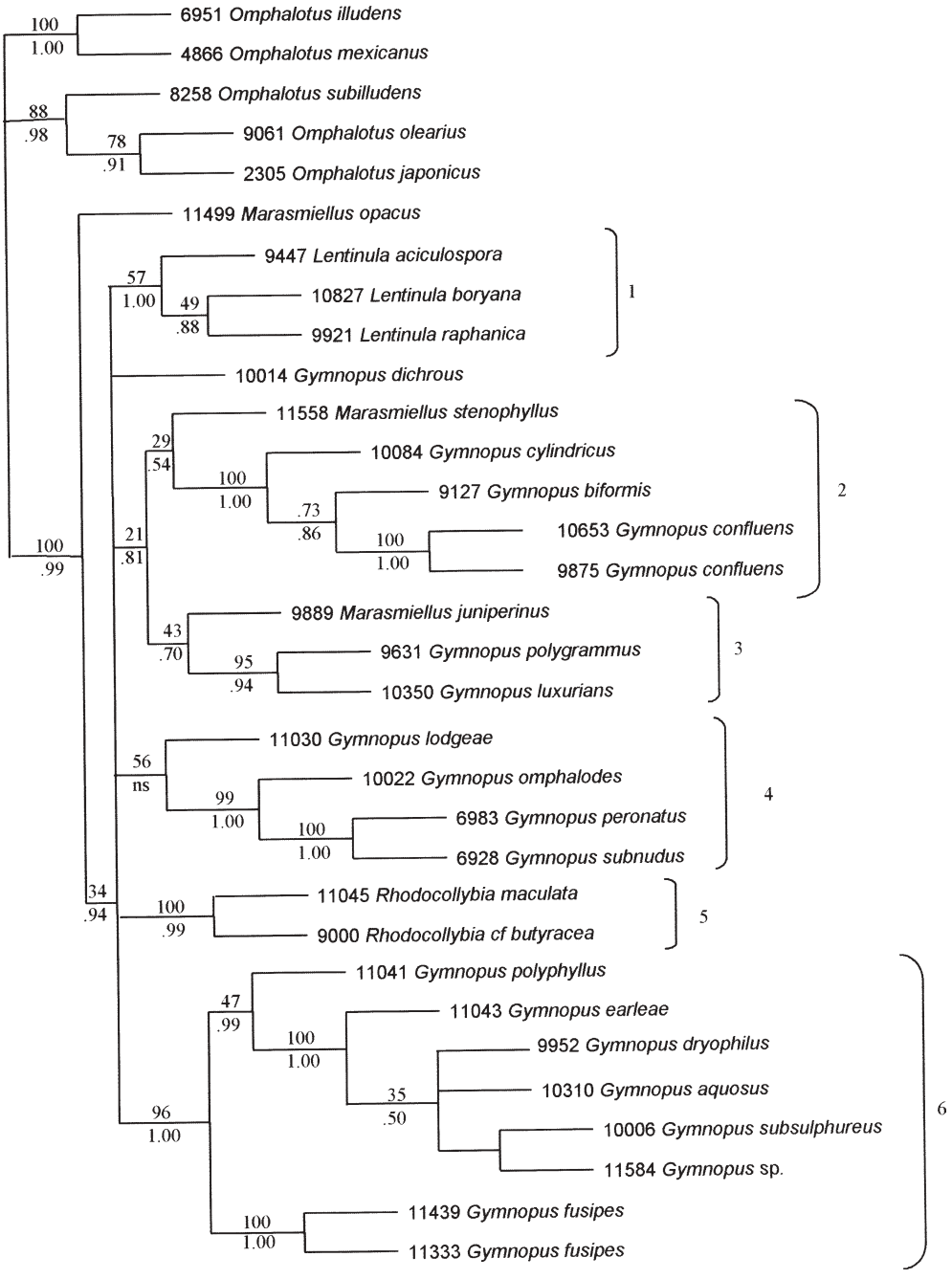

Fig. 2. Phylogeny based on the ribosomal internal transcribed spacer (*ITS*) region. The tree represents a bootstrap 50% majority rule tree plus other groups compatible with this tree obtained from a maximum-parsimony analysis of ribosomal ITS sequences with gaps treated as a fifth base and areas of ambiguous alignment (a total of 182 characters) deleted. Branches not supported by either maximum parsimony or Bayesian analysis have been collapsed. Bootstrap values (*top number*) and Bayesian posterior probabilities (*bottom number*) are given to the *left* of the supported node. Total characters = 857; parsimony informative characters = 428; tree length = 1989 steps; CI = 0.52, HI = 0.47, RI = 0.65

Bayesian analysis was 1709 steps long. Maximum-parsimony analyses using gaps first as a fifth base then as missing data produced strict consensus trees that were identical with the exception of rearrangement of terminal taxa in the *G. polyphyllus* clade (Fig. 2, clade 6) and the position of *G. lodgeae*. Major clades were consistent in both MP and Bayesian analyses with *G. fusipes* appearing as sister to *G. polyphyllus* and *Gymnopus* sect. *Levipedes* (Fig. 2, clade 6). Clade 3 appeared as sister to clade 2, which included species of *Gymnopus* sects. *Vestipedes* and *Subfumosae*. *Rhodocollybia* was associated with *Lentinula* sp. in Bayesian analysis (posterior probability 0.83) and in MP with gaps treated as missing data (37% bootstrap support). In MP analyses, *Lentinula* species were placed within a mixed clade together with *Marasmiellus*, *Gymnopus*, and *Rhodocollybia* (clades 1–6), and in Bayesian analysis, *Lentinula* and *Rhodocollybia* were in the same clade (posterior probability = 0.83).

In the ITS phylogenetic reconstruction, all *Marasmiellus* taxa appeared basal to clades dominated by *Gymnopus* taxa. *Marasmiellus juniperinus* was associated with *G. luxurians* and *G. polygrammus* (Fig. 2, clade 3) in all analyses but with less than 50% bootstrap support. The posterior probability from Bayesian analysis, however, was 0.70. *M.*

stenophyllus was basal to clade 2. *Marasmiellus opacus* was basal to *Gymnopus*/*Rhodocollybia*/*Lentinula* clades in both MP and Bayesian analyses (Fig. 2, clades 1–6).

Discussion

Overall macro- and micromorphology of the specimens studied for this article were consistent with reports by Horak (1968), Gilbertson and Blackwell (1985, 1987), and Singer (1973) for *M. juniperinus*. The pileus epicutis is reminiscent of a weak "rameales structure" where prostrate, diverticulate hyphae give rise to ± upright elements. No clear general orientation of the hyphae was noticed, although outer hyphae sometimes tend to be aggregated in fascicles and seem to be radially oriented.

Similar to Horak (1968), we could not observe any cheilocystidia in the type specimen, although they were present in more recently collected material (Gilbertson and Blackwell 1985, 1987). Cheilocystidia are embedded in the hymenium, frequently prostrate, and arise from horizontal hyphae; they seem to be similar in shape and origin to those of certain species placed in *Gymnopus* (i.e., *G. confluens* and *G. biformis* species complexes). Gilbertson and Blackwell (1985: 339) reported fusoid sterile elements in the hymenium, which in our concept represent basidioles. Spore measurements by Horak (1968) (7–9.5 × 3.5–4.5 μm), Singer (1973) [(6.8–)7.8–12 × (3–)3.8–5.7 μm, most frequently 8–9 × ±4 μm], and Gilbertson and Blackwell (1985) (8–9 × 4.5–5.5 μm) appear to be consistent with those reported here.

The type specimen of *M. juniperinus* is now broken into three very small pieces; the largest fragment includes three lamellae that are partially destroyed. To date, collection of material from the type locality and substrate has not been possible (D.J. Lodge, personal communication).

Basidiospore germination as described here, although unusually rapid, is typical of at least three agaric groups in our experience. Several species of *Marasmius*, some species of *Marasmiellus* (*s. l.*), and all observed taxa of *Xerula/Oudemansiella* form long, single germination tubes. In *Xerula/Oudemansiella*, germination is slow (usually 1 week or longer) and spores are quite different (i.e., usually large, uniguttulate). Germination in *Marasmius* and *Marasmiellus* is indistinguishable, although basidiome micromorphology separates the two genera.

Germination in *Gymnopus*, conversely, is often slow (2–10 days) and often with low germination percentage. Typically, a short germination tube, is formed, following by a quiescent time period of variable length (2–20 days). Subsequent growth is usually through densely branched germination tubes, often forming a stellate configuration. Moreover, basidiospores tend to clump together in spore prints (and spore dilutions), whereas spores of *Marasmiellus* (*s. l.*) appear to disperse in dilution platings. Thus, *M. juniperinus* spore germination is inconsistent with such behavior of surrounding *Gymnopus* taxa, although morphologically similar *Marasmiellus* taxa act in the same way.

Considering that other species of *Marasmiellus* have been reported as tetrapolar (Desjardin et al. 1993; Lamoure 1989; Petersen 1997; Petersen and Gordon 1994), discovery of tetrapolarity in *M. juniperinus* was expected. Nonetheless, no bipolar or amphithallic taxa of *Marasmiellus* have yet been reported, even though such mating behaviors might be expected in tropical agarics, for which rapid colonization and reproductive escape are advantages.

By placing *M. juniperinus* within the clade of taxa usually accepted as *Gymnopus*, the strictest use of the genus name *Marasmiellus* is dictated. A classic cladistic argument is revealed. Either *Gymnopus* is monophyletic, with *Marasmiellus* (*s. s.*) as a synonym, or the limited clade to which *M. juniperinus* belongs can be segregated as a genus (perhaps using *Marasmiellus*, as indicated by Wilson and Desjardin 2003), or *Collybiopsis* Earle, with *Gymnopus* regarded as polyphyletic. In the latter scenario, several sections usually accepted as belonging in *Gymnopus* (*Levipedes*, *Vestipedes*, *Subfumosae*, etc.) are left to seek new or alternative genus names. We prefer to treat *M. juniperinus* as part of *Gymnopus*, and propose a new combination accordingly (see following).

In any case, within *Gymnopus*, *Marasmiellus* is polyphyletic, but addition of other heretofore accepted *Marasmiellus* taxa may show even wider polyphyletic distribution outside the lentinuloid clade (i.e., taxa now placed in *Tetrapyrgos*, *Crinipellis*, etc.) (Moncalvo et al. 2002).

The phylogenetic reconstruction shown in Fig. 2, based on ITS sequences, places *Rhodocollybia* within a clade otherwise dominated by *Gymnopus* taxa, including *G. fusipes*, typus generis. From this illustration, it would be difficult to justify *Rhodocollybia* at genus rank, but when additional *Rhodocollybia* taxa or fewer *Gymnopus* taxa are included (Mata 2002; Mata et al. 2002), a discrete *Rhodocollybia* clade is resolved. The same is true of *Lentinula*.

This is the first published report of the phylogenetic position of *G. fusipes* (but see Mata 2002; Mata et al. 2002). Both in this analysis and previous phylogenetic reconstructions (Mata 2002; Mata et al. 2002), *G. fusipes* forms its own discrete clade, but always among other clades dominated by *Gymnopus* taxa. *Gymnopus*, as a genus name, must follow the position of *G. fusipes*, regardless of the nomenclatural ranks assigned to various nodes.

Although Moncalvo et al. (2002) showed *Setulipes androsaceus* as nested within the lentinuloid clade with *Gymnopus* and *Rhodocollybia*, Fig. 1 more particularly shows *S. androsaceus* in a clade with *G. fusipes*. Although LSU resolution is coarse, *Setulipes* also must be considered as part of *Gymnopus*. Likewise, both our analyses and that by Moncalvo et al. (2002) show *Micromphale foetidum* as within *Gymnopus*. As a result of such previous reports (Moncalvo et al. 2002), our phylogenetic reconstructions (see Figs. 1, 2), and Arts. 11.4 and 49.1 of the ICBN, the following new nomenclatural combinations are necessary:

Gymnopus androsaceus (Linn.: Fries) J.L. Mata & R.H. Petersen, comb. nov. (basionym: *Agaricus androsaceus* Linn.: Fries 1821. Syst. Mycol. 1: 137).

Gymnopus foetidus (Sowerby: Fries) J.L. Mata & R.H. Petersen, comb. nov. (basionym: *Agaricus foetidus* Sowerby: Fries 1821. Syst. Mycol.1: 138).

Gymnopus juniperinus (Murrill) J.L. Mata & R.H. Petersen, comb. nov. (basionym: *Marasmiellus juniperinus* Murrill. 1915. North Am. Flora 9(4): 243).

Gymnopus opacus (Berk. & Curtis) J.L. Mata & R.H. Petersen, comb. nov. (basionym: *Marasmius opacus* Berkeley & Curtis 1849. Hooker J. Bot. 1: 99).

Gymnopus ramealis (Bull.: Fries) J.L. Mata & R.H. Petersen, comb. nov. (basionym: *Agaricus ramealis* Bulliard: Fries 1821. Syst. Mycol. 1: 135).

Gymnopus scorodonius (Fries: Fries) J.L. Mata & R.H. Petersen, comb. nov. (basionym: *Agaricus scorodonius* Fries: Fries 1821. Syst. Mycol. 1: 130).

Gymnopus stenophyllus (Mont.) J.L. Mata & R.H. Petersen, comb. nov. (basionym: *Marasmius stenophyllus* Montagne 1854. Ann. Sci. Nat. Bot. 4(1): 116).

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