Mycologia, 96(5), 2004, pp. 1015–1029.

Phylogenetic relationships of *Sparassis* inferred from nuclear and mitochondrial ribosomal DNA and RNA polymerase sequences

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Abstract: *Sparassis* species show extensive morphological variation, especially when materials from eastern Asia and Australia are compared with collections from North America and Europe. We have been studying the taxonomy of *Sparassis* from eastern Asia, North America, Australia and Europe, using both morphological and molecular data. DNA was extracted from 32 recent collections of *Sparassis* from Australia, Canada, China, Finland, France, Germany, Japan, Switzerland, Thailand, the United Kingdom and the United States. The report of a *Sparassis* taxon from Australia is the first report of this genus from the Southern Hemisphere. Sequences of nuclear and mitochondrial rDNA and the gene encoding RNA polymerase subunit II (RPB2) were used to examine relationships both within the genus *Sparassis* and between *Sparassis* species and other members of the polyporoid clade. Equally weighted parsimony analyses and Bayesian analyses were performed using independent datasets and combined datasets of sequences from different regions. Our results suggest that: (i) Polyporoid fungi producing a brown rot may form a clade; (ii) as suggested in a previous study, *Sparassis* and *Phaeolus* form a monophyletic group, which is united by the production of a brown rot, the presence of a bipolar mating system and the frequent habit of growing as a root and butt rot on living trees; (iii) at least seven lineages are within *Sparassis,* represented by *S. spathulata, S. brevipes, S. crispa, S. radicata* and three taxa that have not been described, which can be distinguished on the basis of fruiting body structure, presence or absence of clamp connections, presence or absence of cystidia and spore size.

Key words: polyporoid clade, multigene phylogeny, MrBayes, biogeography

Accepted for publication March 1, 2004.
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INTRODUCTION

Sparassis Fr. species frequently are reported from northern temperate forests. Members of this genus have conspicuous cream white or yellow, large and cauliflower-like basidiocarps, which are found arising from tree roots. *Sparassis* species are variable in basidiocarp shape and color and are associated with different plant host taxa in different ecosystems. Taxonomy of this genus has been based mainly on basidiocarp macromorphology, basidiospore size and host plant type (Burdsall and Miller 1988a, b). Twelve species with three varieties have been recorded in this genus, according to the CABI Index of Fungi (http://www.indexfungorum.org/). Three species, *Sparassis brevipes* Krombh., *S. crispa* Wulf. : Fr., and *S. spathulata.* Schw. : Fr., have been accepted in recent studies (Martin and Gilbertson 1976, Kreisel 1983, Burdsall and Miller 1988a, b, van Zanen 1988). *Sparassis radicata* Weir was described on Douglas-fir from western North America, but Martin and Gilbertson (1976) suggested that it should be considered conspecific with *S. crispa* based on di-mon mating tests and culture studies.

Sparassiella longistipitata Schwarzman is grouped in the family Sparassidaceae Herter (Kirk et al 2001), but no collections of this species are available (Burdsall and Miller 1988a).

The stalked form of the basidiocarp, highly branched flabellae with hymenium on both sides in initial stages, and the monomitic hyphal system make it hard to position the genus *Sparassis.* Donk (1964) reviewed earlier studies and, apparently based on basidiocarp morphology, concluded that the Sparassidaceae could be a tribe in the Clavariaceae or a distinct family close to the Clavariaceae. Later, the Sparassidaceae was put in the Cantharellales without explanation by Jülich (1981), and this was accepted in the Dictionary of Fungi 8th edition by Hawksworth et al (1995). Hibbett et al (1997) included *Sparassis spathulata* in their molecular study on major groups of gilled mushrooms, polypores and puffballs, which

was based on phylogenetic analyses of nuc-ssu and mt-ssu rDNA. *Sparassis* was resolved as the sister group of a clade including two polypores *Laetiporus sulphureus* (Bull. : Fr.) Murr. and *Phaeolus schweinitzii* (Fr.) Pat. (Hibbett et al 1997). The position of *Sparassis* in the polyporoid clade was supported in other studies (Hibbett and Donoghue 2001), and that placement is accepted in the Dictionary of Fungi 9th edition (Kirk et al 2001).

Sparassis species are known as brown-rot producers with a bipolar mating system (Weir 1917, Martin and Gilbertson 1976). By analyzing character correlations among wood-decay types, mating systems and host ranges in homobasidiomycetes, Hibbett and Donoghue (2001) did not support Gilbertson's hypothesis of a correlation between production of a brown rot and possession of a bipolar mating system. They inferred that brown-rot fungi are not monophyletic in the polyporoid clade and that the pattern of transformations between different wood-decay types is complicated. Two strongly supported groups of genera—one of them is *Laetiporus-Phaeolus-Sparassis*—were united by the production of brown rot on the butt or root of living or dead trees and a bipolar mating system (Hibbett and Donoghue 2001). More genes, including partial nuclear gene sequences coding for the second largest subunit of RNA polymerase II (RPB2), were used in this study to test the relationships among *Sparassis* and other brown-rot polypores. Genes that encode the subunits of nuclear RNA polymerase, especially RPB1 and RPB2, are promising phylogenetic markers in fungal systematics (Liu et al 1999, Matheny et al 2002).

Sparassis crispa has been reported in Asia from Japan to the Tibet highland in western China (Imazeki et al 1988, Teng 1995, Mao et al 1993). In many aspects, Asian "*S. crispa*" resembles *S. radicata* Weir from western North America more than "*S. crispa*" from eastern North America and Europe. The relationship between Asian and North American floras has been discussed for many years among botanists and mycologists (Mueller et al 2001). Early fungal biogeographic studies were based on specimens and descriptions in the literature. These studies were limited by the high degree of morphological variation of macrofungi, the existence of huge unexplored areas in eastern Asia and inconsistent applications of morphological species concepts. Several distribution patterns, such as eastern Asia-eastern North America disjuncts, eastern North America-western North America disjuncts or eastern Asia-western North America disjuncts, had been suggested by studies of vascular plants, bryophytes and lichens (Redhead 1989, Zang 1992, Wu and Mueller 1997, Wen 1999, Wu et al 2000, Mueller et al 2001). Molecular phylogenetic hypotheses coupled with information from morphological studies are becoming key to assessing species concepts and biogeographic relationships in fungi (Hibbett et al 1998, Mueller et al 2001). For example, Wu et al (2000) and Mueller et al (2001) tested the distribution pattern of species of *Armillaria, Suillus* and *Xerula* from eastern Asia and North America based on ITS sequences. Given that the ITS sequences can be too variable to be aligned across distantly related taxa, combined data from lsu-rDNA, ITS and partial RPB2 genes were used in this study to resolve the distribution pattern of *Sparassis* species.

MATERIALS AND METHODS

Specimens and morphological studies.—Specimens used in this study and their GenBank accession numbers are given in TABLE I and TABLE II. Morphological descriptions are based on observations of fresh, dried or rehydrated specimens. Microscopic studies used squashed tissues and sections cut with a freezing microtome to a thickness of 15–20 μm. Measurements were made under 1% Congo red in ammonium hydroxide using bright field (Olympus CH-2).

Molecular techniques.—DNA was isolated from dried fruiting bodies. Approximately 20–30 mg of tissue was ground in liquid nitrogen and extracted in 600 μl of extraction buffer (1% SDS, 0.15 M NaCl, 50 mM EDTA) at 75 C for 1 h and purified with phenol-chloroform-isoamyl alcohol (25:24:1) and precipitated with 95% ethanol and 3M NaCl overnight. Crude DNA extracts were purified with GeneClean (Bio 101, La Jolla, California). Cleaned DNA samples were diluted with distilled water up to 500-fold for use as PCR templates.

Sequence data of *Sparassis* species were generated from six regions (TABLE I): (1) partial mitochondrial small subunit (mt-ssu) rDNA bounded by primers MS1and MS2 (White et al 1990), representing four species of *Sparassis* in seven isolates; (2) partial mitochondrial large subunit (mt-lsu) bounded by primers ML5 and ML6 (White et al 1990), representing four species of *Sparassis* in five isolates; (3) nearly complete nuclear small subunit (nuc-ssu) rDNA bounded by primers PNS1 and NS8 (White et al 1990, Hibbett 1996), representing four species of *Sparassis* in 10 isolates; (4) partial nuclear large subunit (nuc-lsu) rDNA bounded by primers LR0R and LR5 (Vilgalys and Hester 1990), representing seven species of *Sparassis* in 29 isolates; (5) complete internal transcribed spacers 1 and 2 and the 5.8S rDNA (nuc-its rDNA) bounded by primers ITS4 and ITS5 (White et al 1990), representing seven species of *Sparassis* in 29 isolates; (6) partial gene encoding RNA polymerase subunit II (RPB2) bounded by primers RPB2-6F, 5′-TGG GGK WTG GTY TGY CCT GC-3′, and RPB2-7R, 5′-CC CAT WGC YTG CTT MCC CAT-3′ (http://faculty.washington.edu/benhall/), representing five species of *Sparassis* in 16 isolates. Additional data of other fungal materials were generated from these six regions or download-

TABLE I. Isolates of *Sparassis* species used in this study

			GenBank accessions (consult for isolate data)					
			r-DNA		mt-rDNA			
Isolates	Coll. No.	Locality	ssu	lsu	its	ssu	lsu	RPB2
Sparassis brevipes								
GER22	RB8/78	GERMANY		AY218401	AY218439			
GER24	ILKKA-96-1044	GERMANY	AY218381	AY218403	AY218441		AY218464	AY218543
S. cf. *cripa*								
CHN1	YCDA12145	CHINA	AY218373	AY218385	AY218423	AY218451	AY218458	AY218530
CHN2	YCDA12470	CHINA	AY218374	AY218386	AY218424	AY218452	AY218459	AY218531
CHN13	HMAS30269	CHINA			AY218433			
CHN14	HMAS30270	CHINA			AY218434			
CHN17	HMAS60590	CHINA		AY218397	AY218435		AY218463	AY218539
CHN19	HKAS15728	CHINA		AY218398	AY218436			AY218540
CHN20	HKAS32363	CHINA		AY218399	AY218437			AY218541
CHN21	HKAS17477	CHINA		AY218400	AY218438			AY218542
JAP34	FFPRI(?)	JAPAN		AY218412				
S. crispa								
FIN3	YCDA12637	FINLAND		AY218387	AY218425			AY218532
FIN4	SAVOLAINEN	FINLAND	AY218375	AY218388	AY218426			AY218533
FRA5	ILKKA94-1587	FRANCE	AY218376	AY218389	AY218427			AY218534
SWE6	ILKKA88-2036	SWEDEN		AY218390				
AME9	ZW-Claru003	USA/MA	AY218379	AY218393	AY218430	AY218455	AY218462	AY218537
ENG10	BMS2857	ENGLAND		AY218394	AY218431			
GER23	RB9/6/87	GERMANY		AY218402	AY218440			
GER25	DORISLABER	GERMANY		AY218404	AY218442			AY218544
AME27	TENN45811	USA/WA		AY218406	AY218444	AY218457		
AME28	TENN44575	USA/GA		AY218407	AY218445			
AME32	TENN50232	USA/TN		AY218410	AY218449			
AME33	TENN52558	USA/WA		AY218411	AY218450			
AME29	TENN56253	USA/CA		AY218408	AY218446			
S. radicata								
CAN26	UBC-F12464	CANADA		AY218405	AY218443	AY218456		
S. spathulata								
AME7	ZW-Claru001	USA/MA	AY218377	AY218391	AY218428	AY218453	AY218460	AY218535
AME8	ZW-Claru002	USA/NH	AY218378	AY218392	AY218429	AY218454	AY218461	AY218536
AME11	ZW-Claru004	USA/MA	AY218380	AY218395	AY218432			AY218538
JAP12	CUP-JA1385	JAP		AY218396				
AME30	TENN51767	USA/SC			AY218447			
S. sp. THAI	DEDesjardin7410	THAILAND		AY256890	AY256891			AY256892
S. sp. AUS31	TENN50289	AUSTRALIA	AY218382	AY218409	AY218448			

TABLE II. Isolates of other fungi used in this study

Species	GenBank accessions (consult for isolate data)					
	18s-rDNA	LR0R-LR5	ITS1-ITS4	MS1-MS2	ML5-ML6	RPB2/6.3-7
Agaricus bisporus	AJ012396	U11910				AF107785
Albatrellus fletti	AF518569	AF518596			AD001540	AY218465
Albatrellus skamanius	AF287829	AF393044		AF287817	AD001542	AY218466
Albatrellus syringae	AF026632	AF393045		AF026674	AD001543	AY218467
Amanita muscaria	AF026631	AF042643		AF026673	AD001549	AY218468
Amylostereum laevigatum	AF334901	AF287843		AF334871	AF393088	AY218469
Antrodia carbonica	AF026570	AF287844		U27023	AF393089	AY218470
Auriporia aurea	AF334903	AF287846		AF334873	AF393091	AY218471
Auriscalpium vulgare	AF026581	AF287847		U27024	AF393092	AY218472
Boletus satanas	M94337	AF071528		M91009	AD001566	AY218473
Botryobasidium isabellinum	AF026610	AF393047		AF393083	AF393097	AY218475
Ceriporia purpurea	AF026594	AF287852		U27029	AF393100	AY218476
Chondrostereum purpureum	AF082851	AF518607		AF518672	AF518704	AY218477
Coltricia perennis	AF026583	AF287854		U27028	AF393103	AY218526
Coniophora arida	AY293123	AF098375		AF518674		AY218478
Dacrymyces chrysospermus	L22257	AF287855		AF026642	AF393106	AY218480
Dentocorticium sulphurellum	AF026604	AF393055		AF026647	AF393111	AY218481
Echinodontium tinctorium	AF026578	AF393056		U27035	AF393112	AY218482
Entoloma strictius	AF287832	AF393057		AF287820	AF393113	AY218483
Fomitopsis pinicola	AF026599	AF287858		U27038	AF393115	AY218484
Ganoderma australe	AF026629	X78780		AF026672	AF393116	AY218485
Gautieria otthii	AF393043	AF336249		AF393085	AF393117	AY218486
Grifola frondosa	AY218383	AY218413	AY218415			AY218521
Heliocybe sulcata	AF334915	AF518619		AF334881		AY218487
Heterobasidion annosum	AF026576	AF139949		U27042	AD001593	AY218488
Hydnum repandum	AF026641	AJ279576		AF026683	AF393124	AY218489
Hyphodontia alutaria	AF026615	AF393061		AF026660	AF393125	AY218490
Laeticorticium roseocarneum	AF334910	AF393053		AF334875	AF393109	AY218491
Laetiporus sulphureus	AY218384	AY218414	AY218417			AY218522
Lentinula lateritia	AF026596	AF287872		U27047		AY218492
Lentinus tigrinus	U59080	AF518627	AY218419	U27050		AY218493
Lycoperdon sp.	AF026619	AF287873		AF026663	AF393131	AY218495
Meripilus giganteus	U59082	AF287874		U27053		AY218496
Neolentiporus maculatissimus	AF334921	AF518632		AF334884		AY218497
Nia vibrissa	AF334754	AF334750		AF334753	AF334748	AY218498
Oligoporus lacteus	AY293152	AY293205		AY293241	AY293267	AY218510
Oligoporus rennyi	AF334922	AF287876	AY218416	AF334885	AF393132	AY218499
Peniophora nuda	AF026586	AF287880		U27063	AF393134	AY218500
Phaeolus schweinitzii	AF026598	AF287882	AY218422	U27066		AY218501
Phlebia radiata	AF026606	AF287885		AF026649	AF393136	AY218502
Pleurotus ostreatus	U23544	U04160		U27064	AF393137	AY218504
Plicaturopsis crispa	AY293148	AY293203	AY218421	AY293239	AY293266	AY218505
Polyporus squamosus	AF026573	AF393069	AY218418	U27068	AF393142	AY218508
Pycnoporellus fulgens	AF518587	AF518643		AF518690		AY218527
Russula compacta	AF026582	AF287888		U27074	AF393148	AY218514
Sarcodon imbricatus	AY293157	AF518646		AF518693		AY218528
Schizophyllum commune	X54865	AF334751		AF069639	AF334747	AY218515
Scytinostroma alutum	AF026607	AF393075		AF026650	AF393150	AY218516
Serpula himantioides	AF518589	AF518648		AF518694		AY218517
Sistotrema eximum	AF334935	AF393076		AF334891	AF393151	AY218518
Sistotrema muscicola	AF334936	AF518649		AF334892		AY218519
Stereum hirsutum	AF026588	AF393078		U27076	AF393153	AY218520
Suillus cavipes	M90828	AF071535		M91016	AD001641	AY218523
Thelephora sp.	AF026627	AF287890		AF026670	AD001646	AY218529
Typhula phacorrhiza	AF026630	AF393079		AF026687	AF393154	AY218525

ed from GenBank (TABLE II). Strain numbers of some isolates were provided in Binder and Hibbett (2002).

PCR reaction mixes (Promega Corp., Madison, Wisconsin) contained 2.5 μL 10× PCR buffer, 5 μM dNTP, 12.5 pM of each PCR primer and 5 μL DNA in 25 μL. The amplification program included 40 cycles of 94 C for 30s, 45 C for 30s, and 72 C for 1 min. PCR products were purified using GeneClean (Bio 101, Carlsbad, California) and sequenced using the ABI Prism BigDye-terminator cycle sequencing kit (Applied Biosystems, Foster City, California) according to the manufacturer's protocols. Primers used for sequencing were PNS1, NS19bc, NS19b, NS41, NS51, NS6, NS8, MS1, MS2, ML5, ML6, RPB2-6F, RPB2-6.3F, RPB2-7R, RPB2-7.1R, LR0R, LR3, LR3R, LR5, ITS4, and ITS5. Sequencing reactions were purified using Pellet Paint (Novagen, Madison, Wisconsin) and were run on an Applied Biosystems 377XL automated DNA sequencer. Sequences were edited with Sequencher version 3.1 (GeneCodes Corp., Ann Arbor, Michigan). Sequences generated in this study were submitted to GenBank (accession numbers AY218373–AY218547).

Phylogenetic analyses.—Two datasets were prepared, one for higher-level analyses (HLA) and one for lower-level analyses (LLA). The datasets for the HLA included sequences from five genes: mt-ssu rDNA (68 isolates), mt-lsu rDNA (59 isolates), nuc-ssu rDNA (72 isolates), nuc-lsu rDNA (72 isolates) and RPB2 (71 isolates). HLA were intended to resolve the placement of *Sparassis* among 61 homobasidiomycete genera (TABLE I and TABLE II). The datasets for the LLA included sequences from three genes nuc-lsu (36 isolates), ITS (37 isolates) and RPB2 (24 isolates). LLA were intended to resolve the relationships among *Sparassis* species (TABLE I and TABLE II). Sequences of nuc-rDNA and mt-rDNA were aligned by eye in the data editor of PAUP* 4.0b (Swofford 1999). Sequences of RPB2 were translated into amino acid sequences and aligned by eye in Sequencher and then converted back to nucleotides for the analyses. Both datasets were analyzed in PAUP* 4.0b (Swofford 1999) and MrBayes 2.01 (Huelsenbeck and Ronquist 2001), with gaps treated as missing data and ambiguous or unalignable positions excluded. Ambiguous positions were excluded from the datasets before performing the analyses.

The HLA dataset was rooted with *Dacrymyces chrysospermus* Berk. & M.A. Curtis (Hibbett and Donoghue 2001). Ssu-rDNA sequences of *Bondarzewia montana* (Quél.) Singer, *Piptoporus betulinus* (Bull. : Fr.) P. Karst., *Pycnoporus cinnabarinus* (Jacq. : Fr.) P. Karst., *Ramaria formosa* (Pers. : Fr.) Quél, *Trametes versicolor* (L. : Fr.) Pilát, *Polyporus varius* (Pers.) Fr., *Polyporus melanopus* (Pers.) Fr., *Polyporus arcularius* (Batsch) Fr., *Cryptoporus volvatus* (Peck) Shear and *Lenzites betulina* (Fr.) Fr. represented a shorter region than sequences of other taxa. Mt-ssu sequences of *Albatrellus fletti* (Morse) Pouzar, *Grifola frondosa* (Fr.) S.F. Gray, *Sparassis brevipes* GER24, and *S.* sp. AUS31, and mt-lsu sequences of *S. crispa* USA9, *S. brevipes* GER24, *Trametes versicolor, Heliocybe sulcata* (Berk.) Redhead & Ginns, *Lentinula lateritia* (Berk.) Pegler, *Lentinus tigrinus* (Fr.) Fr., *Neolentiporus maculatissimus* (Lloyd) Rajchenb., *Phaeolus schweinitzii* (Fr.) Pat., *Piptoporus betulinus, Ramaria formosa, Sparassis* sp. AUS31, *Pycnoporellus fulgens* (Fr.) Donk and one unidentified resupinate homobasidiomycete were not available, and those taxa were excluded from the analysis based on mtDNA. No sequence of RPB2 of *S.* sp. AUS31 was generated, and it was excluded in analyses using RPB2 data.

Parsimony analyses were performed using equal weighting of characters and transformations. Heuristic searches were performed with 1000 replicate searches, each with a random taxon addition sequence, MAXtrees set to autoincrease, and TBR branch swapping. A bootstrap analysis was performed with 1000 replicates, each with 10 random taxon addition sequences, MAXtrees set to 1000, and TBR branch swapping. Bootstrap consensus trees generated from analysis of each gene were compared to check whether there is an apparent conflict in tree topologies. Bootstrap values higher than 80% were used as the criterion for "strong" conflict. The combined analyses were performed using nuc-rDNA and RPB2 sequences, which were congruent according to this criterion.

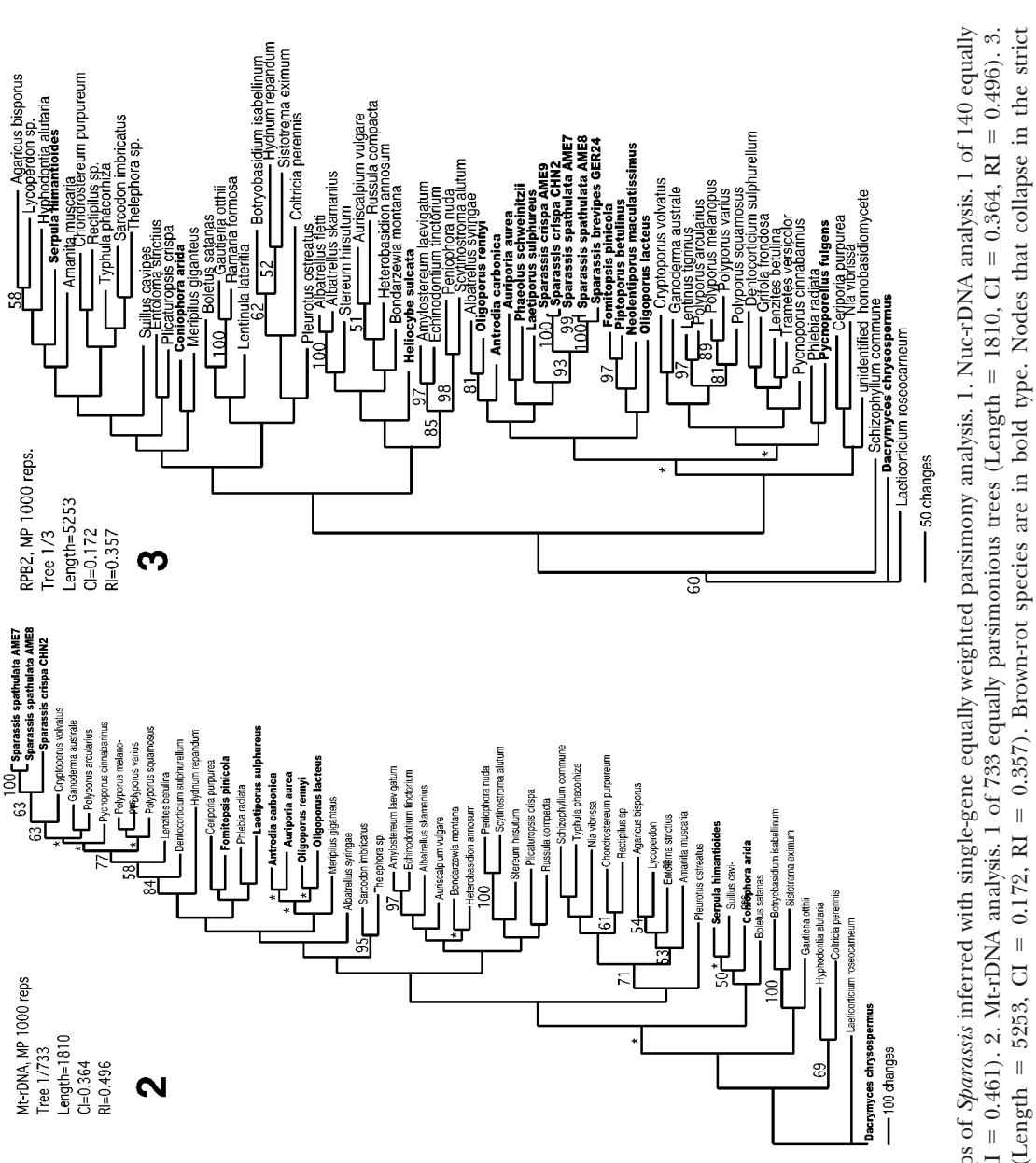
Bayesian posterior probabilities were computed using Metropolis-coupled Markov Chain Monte Carlo (MCMCMC) under the GTR+G model in MrBayes 2.01 (Huelsenbeck and Ronquist 2001) by running four chains with 200 000 generations using the program default priors on model parameters. Trees were sampled every 100 generations, and a total of 2001 trees were saved. Likelihoods converged to a stable value after 5000 generations, so the first 50 trees were discarded as "burn-in" before computing a majority rule consensus tree in PAUP*.

The LLA dataset was rooted using *Lentinus tigrinus* based on the results of the HLA. The same analytical settings as that for the HLA were applied to the LLA for separate and combined data of nuc-lsu rDNA, ITS and RPB2. Isolates without RPB2, ITS and nuc-lsu rDNA sequences were excluded from different analyses. Alignments are available at TreeBASE (accession number M1814-M1815).

RESULTS

Phylogenetic relationships.—HLA. The higher-level placement of *Sparassis* was estimated using five different datasets: nuc-rDNA; mt-rDNA; RPB2; and, nuc-rDNA + RPB2 (FIGS. 1–5).

The combined nuc-ssu rDNA and nuc-lsu rDNA genes had an aligned length of 2694 base pairs (bp) with 374 uninformative variable positions and 628 parsimony-informative positions. Parsimony analysis based on nuc rDNA generated 140 equally parsimonious trees of 4416 steps and consistency index (CI) = 0.336 (FIG. 1). A clade including *S. spathulata, S. brevipes, S. crispa, S.* sp. AUS31, several other brown-rot species and *Grifola frondosa* was weakly supported (bootstrap = 53%) within the polyporoid clade. A clade (bootstrap = 90%) containing *S. spathulata* and *S. brevipes* and a clade (bootstrap = 100%) containing two isolates of *S. crispa* were grouped together with the *Phaeolus-Laetiporus* clade (bootstrap = 50%) as the sister group (bootstrap = 72%). *Spa-*



FIGS. 1–3. Higher-level phylogenetic relationships of *Sparassis* inferred with single-gene equally weighted parsimony analysis. 1. Nuc-rDNA analysis. 1 of 140 equally parsimonious trees (Length = 4416, CI = 0.336, RI = 0.461). 2. Mt-rDNA analysis. 1 of 733 equally parsimonious trees (Length = 1810, CI = 0.364, RI = 0.496). 3. RPB2 analysis. 1 of 3 equally parsimonious trees (Length = 5253, CI = 0.172, RI = 0.357). Brown-rot species are in bold type. Nodes that collapse in the strict consensus tree are marked with an asterisk above the branch. Bootstrap values greater than 50% are indicated along nodes.

rassis sp. AUS31 was grouped with *Grifola frondosa* and *Pycnoporellus fulgens* (bootstrap = 100%). The brown-rot polypores *Fomitopsis, Piptoporus* and *Neolentiporus* formed a clade (bootstrap = 67%). Relationships between white-rot and brown-rot species in the polyporoid clade were not well resolved.

The mt-rDNA had an aligned length of 684 bp with 117 uninformative variable positions and 290 parsimony-informative positions. Parsimony analysis based on mt-rDNA (FIG. 2) generated 733 equally parsimonious trees of 1810 steps with CI = 0.364. A clade containing *Sparassis spathulata* and *S. crispa* was weakly supported (bootstrap = 63%) and was grouped with white-rot species including *Ganoderma, Pycnoporus* and *Polyporus* (bootstrap = 84%).

The RPB2 sequences had an aligned length of 510 bp with 79 uninformative variable positions and 333 parsimony-informative positions. Parsimony analysis based on RPB2 (FIG. 3) generated three equally parsimonious trees of 5253 steps with CI = 0.172. Brown-rot species of *Oligoporus, Antrodia, Auriporia, Phaeolus, Laetiporus, Sparassis, Fomitopsis, Piptoporus, Neolentiporus* and the white-rot species *Albatrellus syringae* (Parm.) Pouz. formed a weakly supported clade, within which there was a clade containing *S. crispa, S. spathulata* and *S. brevipes* (bootstrap = 93%).

The combined nuc-rDNA and RPB2 genes had an aligned length of 3149 bp with 449 uninformative variable positions and 954 parsimony-informative positions. Parsimony analysis based on this dataset generated 19 equally parsimonious trees of 9866 steps with CI = 0.240 (FIG. 4). *Sparassis* species, including *S. spathulata, S. brevipes* and *S. crispa,* were monophyletic (bootstrap = 87%) with the *Phaeolus-Laetiporus* clade (bootstrap = 64%) as its sister group (bootstrap = 68%). A brown-rot polypore clade, the *Fomitopsis-Piptoporus-Neolentiporus* clade, also was supported (bootstrap = 87%), but the relationships among brown-rot and white-rot species in the polyporoid clade still were not well resolved.

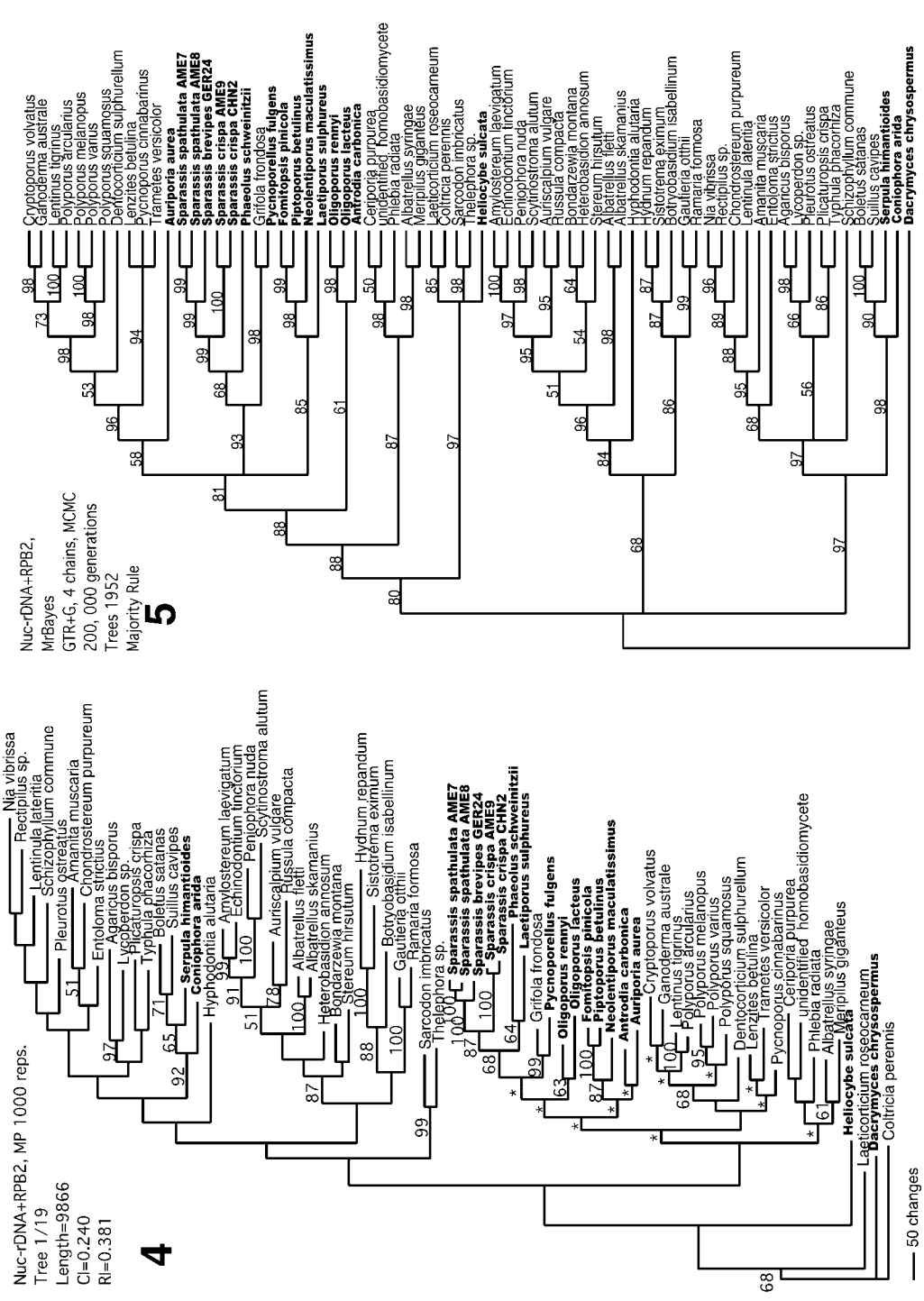
Bayesian analysis based on combined nuc-rDNA and RPB2 sequences (FIG. 5) strongly supported (posterior probability = 93%) a clade containing *Sparassis, Phaeolus, Pycnoporellus,* and *Grifola,* in which *Sparassis* species are monophyletic (posterior probability = 99%). Two more clades of brown rot polypores were supported: the *Fomitopsis-Piptoporus-Neolentiporus-Laetiporus* clade (posterior probability = 85%) and the *Oligoporus-Antrodia* clade (posterior probability = 61%). In addition, the clade including white rot species of *Cryptoporus, Ganoderma, Lentinus, Polyporus, Dentocorticium, Lenzites, Pycnoporus* and *Trametes* is strongly supported (posterior probability = 96%).

LLA. The lower-level relationships among *Sparassis* species were estimated using five different datasets: nuc-lsu rDNA; ITS; RPB2; nuc-lsu rDNA+ITS; and nuc-lsu rDNA+ITS+RPB2. Trees inferred from single regions showed similar topologies to trees inferred from the combined sequences but with weaker branch support (FIGS. 6–9).

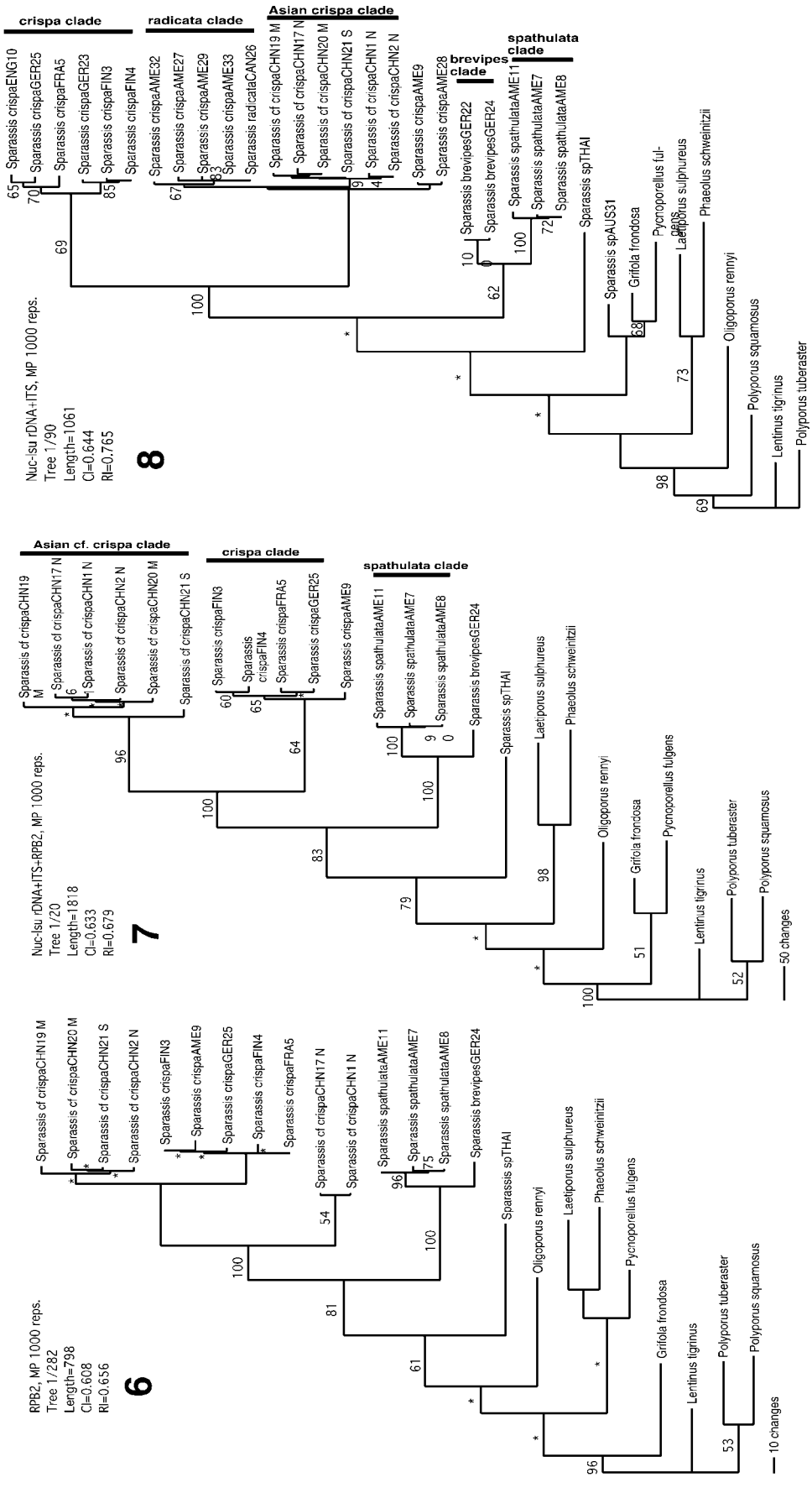
The RPB2 sequences had an aligned length of 642 bp with 87 uninformative variable positions and 225 parsimony-informative positions. Parsimony analysis based on the RPB2 gene generated 282 equally parsimonious trees of 789 steps with CI = 0.608 (FIG. 6). A clade containing *Sparassis crispa, S.* cf. *crispa, S. spathulata, S. brevipes* and *S.* sp. THAI was supported weakly (bootstrap = 61%) with *S.* sp. THAI as a basal branch. One North American isolate and four European isolates of *S. crispa* and six Chinese isolates of *S.* cf. *crispa* formed a clade (bootstrap = 100%). Isolates of *S. spathulata* formed a clade (bootstrap = 96%) with *S. brevipes* as its sister group (bootstrap = 100%). The relationship among the *Sparassis* clade and other brown-rot polypores were not resolved. Unfortunately, no RPB2 sequences were obtained for *S. radicata, S.* sp. AUS31 and isolates of *S. crispa* collected from Georgia (AME28), Tennessee (AME32) and western North America (TABLE I).

The combined sequences of nuc-lsu rDNA, ITS and RPB2 had an aligned length of 2080 bp with 286 uninformative variable positions and 505 parsimony-informative positions. Parsimony analysis based on this dataset generated 20 equally parsimonious trees of 1818 steps with CI = 0.633 (FIG. 7). *Sparassis* species formed a monophyletic group (bootstrap = 79%) with *S.* sp. THAI as a basal branch. Five clades were found within the *Sparassis* clade: Asian *S.* cf. *crispa* clade (bootstrap = 96%), European-North American *S. crispa* clade (bootstrap = 64%), *S. spathulata* clade (bootstrap = 100%), *S. brevipes* and *S.* sp. THAI. *Laetiporus* and *Phaeolus* formed a clade (bootstrap = 98%), which is the sister group of the *Sparassis* clade.

The combined sequences of nuc-lsu rDNA and ITS had an aligned length of 1432 bp with 193 uninformative variable positions and 295 parsimony-informative positions. Parsimony analysis based on this dataset generated 90 equally parsimonious trees of 1061 steps with CI = 0.644 (FIG. 8). Eight clades were recognized among *Sparassis* species: *crispa* clade (European isolates of *S. crispa,* bootstrap = 69%), *radicata* clade (western North American isolates of *S. crispa* and *S. radicata,* bootstrap = 67%), Asian *crispa* clade (Chinese isolates of *S.* cf. *crispa,* bootstrap = 94%), *brevipes* clade (European isolates of *S. brevipes,* bootstrap = 100%), *spathulata* clade (eastern North American isolates of *S. spathulata,* bootstrap = 100%), and two unidentified *Sparassis*



FIGS. 4–5. Higher level phylogenetic relationships of *Sparassis* inferred with multigene parsimony and Bayesian analysis. 4. Parsimony analysis based on the combined nuc-rDNA and RPB2 sequences. 1 of 19 equally parsimonious trees (Length = 9866, CI = 0.240, RI = 0.381). Brown-rot species are in bold type. Nodes that collapse in the strict consensus tree are marked with an asterisk above the branch. Bootstrap values greater than 50% are indicated along nodes. 5. Bayesian analysis based on the combined nuc-rDNA and RPB2 sequences. The majority rule consensus of 1952 MCMCMC-sampled trees. Brown-rot species are in bold type. Group frequencies greater than 50% are indicated as posterior probability (%) along nodes.



FIGS. 6–8. Lower-level phylogenetic relationships of *Sparassis* inferred with single-gene and multi-gene analysis. 6. Relationships among *Sparassis* species inferred from the RPB2 sequences. 1 of 282 equally parsimonious trees (Length = 798, CI = 0.608, RI = 0.656). 7. Relationships among *Sparassis* species inferred from the combined nuc-lsu-rDNA, ITS, and RPB2 sequences. One of 20 equally parsimonious trees (Length = 1818, CI = 0.633, RI = 0.679). 8. Relationships among *Sparassis* species inferred from the combined nuc-lsu-rDNA and ITS sequences. One of 90 equally parsimonious trees (Length = 1061, CI = 0.644, RI = 0.765). Clades are named and indicated by black bars. Nodes that collapse in the strict consensus tree are marked with an asterisk above the branch. Bootstrap values greater than 50% are indicated along nodes.

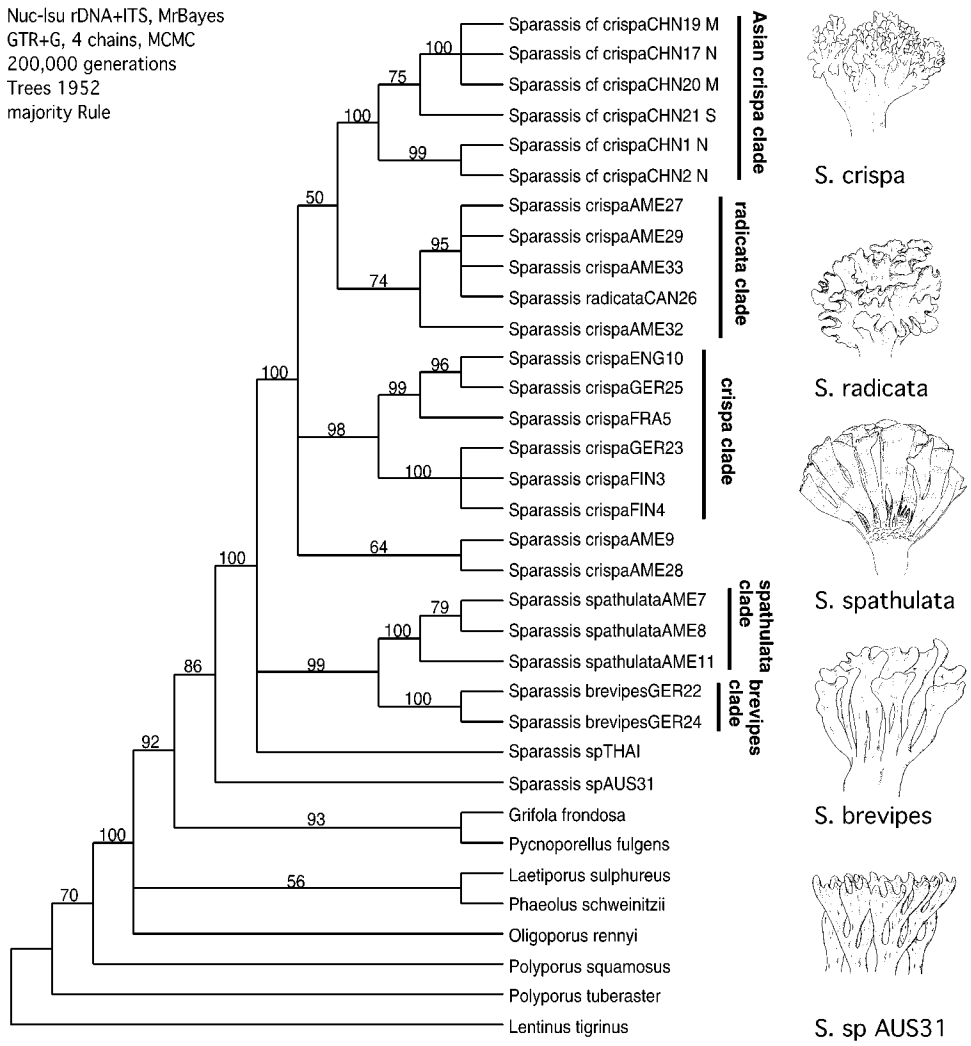


FIG. 9. Lower-level phylogenetic relationships of *Sparassis* inferred with multigene Bayesian analysis and representative fruiting body morphologies. The majority rule consensus of 1952 MCMCMC-sampled trees. Group frequencies greater than 50% are indicated as posterior probability (%) along nodes.

species, *S.* sp. THAI and *S.* sp. AUS31. Two eastern North American isolates of *S. crispa* (*S. crispa* AME9 and AME28) formed a branch close to the *radicata* clade and the Asian *crispa* clade without bootstrap support. Relationships among those clades and lineages were not completely resolved. The *crispa* clade, the *radicata* clade, the Asian *crispa* clade and two eastern North American *S. crispa* were supported as monophyletic (bootstrap = 100%), whereas the *brevipes* clade and the *spathulata* clade were grouped together (bootstrap = 62%). *S.* sp. AUS31 was grouped with *Grifola* and *Pycnoporellus* (bootstrap value less than 50%).

Bayesian analysis based on the combined sequences of nuc-lsu rDNA and ITS provided higher confidence for all clades and lineages of *Sparassis* observed in the parsimony analysis (FIG. 9). The *crispa* clade (posterior probability = 98%), the *radicata* clade (posterior probability = 74%), the Asian *crispa* clade (posterior probability = 100%), the *brevipes* clade (posterior probability = 100%), and the *spathulata* clade (posterior probability = 100%) were confirmed. Two eastern North American isolates of *S. crispa* formed a weakly supported clade (posterior probability = 64%). *Sparassis* species were strongly supported as monophyletic (posterior probability = 86%) with *Grifola* and *Pycnoporellus* as the sister group. All species from the Northern Hemisphere formed a clade (posterior probability = 100%). *S. brevipes* and *S. spathulata* were grouped together (posterior probability = 99%), and the *crispa* clade, the *radicata* clade, the Asian *crispa* clade and eastern North American *S. crispa* formed a monophyletic group (posterior probability = 100%).

MORPHOLOGY AND TAXONOMY

The lack of type specimens hampers the taxonomic and nomenclatural studies of the genus *Sparassis*. Martin and Gilbertson (1976), Kreisel (1983), and Burdsall and Miller (1988a, b), have produced authoritative taxonomies of *Sparassis*. This study used recent collections because they provide high quality DNA for molecular studies. For nomenclatural purposes, however, it would be useful to attempt to extract DNA from the older specimens studied by Gilbertson and others. All the collections were studied morphologically following widely accepted species concepts, which are based on Weir (1917), Kreisel (1983) and Burdsall and Miller (1988a, b). Simplified taxonomic descriptions and habit illustrations (FIG. 9) based on our own observations are provided in addition to the major clades found in phylogenetic analyses (as in FIG. 8). Commentary is provided regarding morphological incongruence or conflict among the members of each clade.

brevipes clade (represented by *Sparassis brevipes* Krombh.)

Base of basidiocarps composed of a round mass. Flabellae spathulate, all originating directly from a common base, split into pieces and then fused with others several times on the way to the top, azonate to not distinctively zonate, margin entire. Hyphal system monomitic, simple septate, no clamp connection observed. Basidiospores hyaline, thin-walled, smooth, broadly elliptic to subglobose, (3.9–)4.4–5.2(–5.5) × (6.2–)6.8–7.8(–8.7) μm. Distribution: Europe.

Specimens examined. GERMANY. BADEN-WÜRTTEMBERG: In mixed forest of *Abies, Picea, Pinus* and *Fagus* on limestone, 24-IX-1995, MBUH (ILKKA96-1044); VIII-1978, RB8/78.

Commentary. *Sparassis brevipes* has been documented in Europe without precise typification (Burdsall and Miller 1988a). Morphological studies showed that there are at least two distinct *Sparassis* species in European samples. We followed Kreisel's (1983) concept and accepted the widely used name *S. brevipes* to represent the European species mostly growing on *Abies, Fagus* and *Quercus*. *S. brevipes* is similar to the eastern North American *S. spathulata* in spore size and possession of simple septate hyphae, but the flabellae of *S. brevipes* are azonate.

crispa clade (represented by *Sparassis crispa* Wulf. : Fr.)

Base of basidiocarps branched and elongated to form several flattened branches. Flabellae extend from the branches, broad but short, dissected and contorted, azonate, margin entire. Hyphal system monomitic, clamp connections present, gelatinous hyphae rarely present. Basidiospores hyaline, thin-walled, smooth, subglobose, 4.0–4.9 × 4.9–6.0(–6.9) μm. Distribution: Europe, eastern North America.

Specimens examined. USA. MASSACHUSETTS: Purgatory Chasm State Park, 15-IX-2001, *FH* (*ZW-Clarku003*); GEORGIA: Fort Top Mountains, 13-IX-1983, *TENN44575*. FINLAND. North Kotinen Forest: on *Pinus,* 12-IX-1997, *MBUH* (*YCD2637*); PAINO TUOREENAN: 25-IX-1983, *MBUH* (*Savolaninen*). FRANCE. LOZERE: in mixed forest of *Fagus, Picea* and *Quercus,* 21-X-1994, *MBUH* (*PIRJO&ILKKA94-1587*). SWEDEN. NARKE: on *Pinus,* 26-X-1988, *MBUH* (*ILKKA88-2036*). UK. ESHER COMMON, SURREY: collected by P.M. Kirk, on *Pinus sylvestris,* 29-X-2000, *BMS2857*. GERMANY. 6-IX-1987, *RB9/6/87*; collected by Doris Laber, 27-IX-1996, *MBUH*.

Commentary. Neotypification of *Sparassis crispa* was based on a European collection (Burdsall and Miller 1988b). *S. crispa* has been reported from Europe, eastern Asia and North America (Breitenbach and Kränzlin 1986; Burdsall and Miller 1988a, 1988b; Gilbertson 1980; Imazeki et al 1988; Mao et al 1993; Martin and Gilbertson 1976). Asian collections under the name of *S. crispa* are morphologically different from European *S. crispa*. European *S. crispa* collections are characterized by branched basidiocarps, strongly dissected and contorted flabellae and are strictly growing on conifers. Two North American isolates, FH-ZW-Clarku003 (*S. crispa* AME9) and TENN44575 (*S. crispa* AME28), were not nested in the *crispa* clade in two analyses (FIGS. 8–9) and need further study. FH-ZW-Clarku003 collected from Massachusetts is similar to the European *S. crispa* except for possessing gelatinous hyphae. TENN44575 collected from Georgia is similar to the western North American *S. radicata,* which has bigger, slightly dissected and contorted flabellae.

Asian crispa clade (represented by *Sparassis* cf. *crispa* Wulf. : Fr.)

Base of basidiocarps branched or not. Flabellae mostly extend from a common central mass, broad, dissected and slightly contorted, azonate, margins sometimes tooth-like. Hyphal system monomitic, clamp connections present. Basidiospores hyaline, thin-walled, smooth, subglobose, 4.0–5.0 × 5.0–6.0 (–6.6) μm. Distribution: eastern Asia (Japan and China).

Specimens examined. CHINA. JILIN, ANTU: Changbaishan Mountains, on *Larix* sp., 16-IX-1995, *MBUH (YCD2145)*; on *Larix* sp., 14-VIII-1997, *MBUH(YCD2470)*; on conifers, 25-VII-1960, *HMAS30269*; on conifers, 1-VIII-1960, *HMAS30270*; NEIMENGU: Arxan Mountains, 14-VIII-1991, *HMAS60590*; SICHUAN, DAOCHENG: on *Picea,* 11-VIII-1984, *HKAS15728*; SICHUAN, XIANGCHENG: on *Quercus* sp., 17-VII-1998, *HKAS 32363*; YUNNAN, ZHONG-

DIAN: on *Larix,* 25-VII-1986, *HKAS17477*. JAPAN. NAGANO PREF, USUDA: on *Larix,* VII-1988, *FFPRI(Tsengoku)*; 5-IX-1989, *FFPRI(F-15706)*.

Commentary. *Sparassis crispa* reported and collected from Japan and China are macro-morphologically distinct from European *S. crispa* and are referred to as *S.* cf. *crispa* in this study. Flabellae of *S.* cf. *crispa* are similar to those of *S. radicata,* but they are bigger and not as contorted as in European *S. crispa* collections. Chinese collections show a host range from conifers such as *Larix, Pinus* to hardwood plants (dactyls) such as *Quercus*. *S. radicata* so far has been reported only on Douglas-fir and Pines (Martin and Gilbertson 1976).

radicata clade (represented by *Sparassis radicata* Weir)

Basidiocarps expand from a round mass. Flabellae anastomosed, vertically subdivided, broad, dissected and slightly contorted, azonate. Hyphal system monomitic, clamp connections present. Basidiospores hyaline, thin-walled, smooth, broadly elliptic to subglobose, 3.9–5.0 × 6.3–7.0 μm. Distribution: western North America.

Specimens examined. CANADA. VANCOUVER: Stanley Park, on *Tsuga heterophylla,* collected by P. Koreger, 6-X-1985, *UBC(F12464)*. USA. WASHINGTON: on the base of *Tsuga* sp., 4-X-1984, *TENN45811*; on base of conifer, 3-X-1992, *TENN52558*; CALIFORNIA: 13-II-1998, *TENN56253*; TENNESSEE: Great Smoky Mountains National Park, on base of *Pinus* sp., 28-VII-1991, *TENN50232*.

Commentary. *S. radicata* is well studied and was thought to be conspecific with the European and Asian *S. crispa,* mainly based on culture studies and dikaryon-monokaryon mating tests (Martin and Gilbertson 1976). Morphologically, *S. radicata* is similar to Asian *S.* cf. *crispa* in having large but not strongly contorted flabellae. The flabellae arise radially from a common center rather than from a branched base as in the European *S. crispa*.

spathulata clade (represented by *Sparassis spathulata* Schw. : Fr.)

Base of basidiocarps composed of a round mass. Flabellae spathulate, all originating from a common base, anastomosing and vertically subdivided, distinctively zonate, margin entire. Hyphal system monomitic, simply septate, no clamp connections observed. Basidiospores hyaline, thin-walled, smooth, broadly elliptic to subglobose, 4.7–5.8 × (5.9–)6.9–8.0 μm. Distribution: eastern North America and Japan (?).

Specimens examined. USA, MASSACHUSETTS: on *Quercus* sp., 13-IX-2001, *FH (ZW-Clarku001)*; WORCESTER: on *Quercus* sp., 1-X-2000, *FH (ZW-Clarku004)*; NEW HAMPSPHERE: Walpole, 7-IX-2001, *FH (ZW-Clarku002)*; SOUTH CAROLINA: Oconee, 18-VIII-1992, *TENN51767*. JAPAN. HONSHU, TODAI-JI: collected by Kumiko and Korf, 22-VIII-1959, *CUP(JA-1385)*.

Commentary. The large, zonate, vertically oriented flabellae of *S. spathulata* distinguish this species from other *Sparassis* species. *S. spathulata* is similar to the European *S. brevipes* (synonym: *S. laminosa*) in some aspects, and therefore validity of *S. spathulata* as a separate species was doubted by Burdsall and Miller (1988a). Eastern North American *S. spathulata* have been found on both *Pinus* and *Quercus,* whereas *S. brevipes* has been found mostly on *Quercus*. One Japanese collection (CUP JA1385) was identified as *S. spathulata,* which might be introduced from North America and so far is the only report of this species from Asia. Basidiocarp development of *S. spathulata* begins with central daedaloid ridges on the surface. These ridges expand somewhat parallel to form flabellae with an amphigenous hymenium.

Sparassis sp. AUS

Base of basidiocarps complex, flabellae narrow and highly branched, azonate. Branch tips flattened, often daedalioid arranged, then crested. Lower branches light ochraceous buff, upper branches pinkish buff to light ochraceous salmon. Margin with 2–4 fork-like teeth when dried. Hyphal system monomitic, simply septate, no clamp connections observed. Basidiospores hyaline, thin-walled, smooth, broadly elliptic to subglobose, 4.8–5.9 × 5.8–7.5 μm. Distribution: Australia.

Specimens examined. Australia. TASMANIA, VIS. GEESTON: Tahune Forest Reserve, 5-VI-1991, by R. Peterson and A. Mills, *TENN50289, Alan Mills (3996)*.

Commentary. This fungus shows a unique macromorphology and was identified as *Sparassis* sp. by the collectors. The amphigenous hymenium and the arrangement of the flabellae make this fungus a *Sparassis* species. There is only one collection of this fungus, and the base was rotten away. More and better materials from the southern hemisphere are necessary for further studies.

Sparassis sp. THAI

Basidiocarps composed of a rosette of flabellae. Flabellae loosely arranged, up to 120 mm broad, margin not dissected, wavy, azonate. Hyphal system monomitic, clamp connections present. Hymenium layer composed of basidia and cystidia. Basidiospores hyaline, thin-walled, smooth, subglobose to broadly ellipsoid-ovoid, 6–7 × 7–9 μm. Distribution: Thailand.

Specimens examined. THAILAND, CHIANG MAI PROVINCE: Doi Inthanon National Park, at the base of a living oak tree (*Quercus eumorpha*), 27-VI-2002, *SFSU (D.E. Desjardin 7410)*.

Commentary. *Sparassis* sp THAI is distinct from other *Sparassis* species in possessing cystidia and larger basidiospores. Taxonomy of this fungus is discussed elsewhere (Desjardin et al 2004).

DISCUSSION

Sequences from five loci (nuc-ssu rDNA, nuc-lsu rDNA, mt-ssu rDNA, mt-lsu rDNA and RPB2) were used to infer the higher-level phylogenetic relationships of *Sparassis*. Not all the possible combinations of these loci have been analyzed, and nuc-rDNA (nuc-ssu rDNA + nuc-lsu rDNA), mt-rDNA (mt-ssu rDNA + mt-lsu rDNA) and RPB2 genes have been treated as three unlinked units. *Sparassis* species were nested within the polyporoid clade in all the analyses.

Parsimony analyses, except for the one based on the RPB2 gene alone, showed a similar tree topology for the major clades in homobasidiomycetes. The nucleotide sequences of the RPB2 gene, between the primer pairs bRPB2-6f and bRPB2-7r, are highly variable, which suggests that nucleotide sequences in this part of the gene are saturated among taxa sampled here. Analyses based on the amino acid sequences (data not shown) suggest that amino acid sequences in this part of the gene are too conserved to be informative for HLA, however. Combining RPB2 sequences with nuc rDNA sequences increased the number of resolved clades (FIGS. 1, 3, 4) and reduced the number of equally parsimonious trees.

Sparassis species were grouped with some white-rot polypores in the parsimony analysis based only on mitochondrial rDNA, whereas other analyses suggested a close relationship between *Sparassis* species and other brown-rot polypores. Much of the mt-rDNA has multiple deletions and insertions and was excluded from the dataset. The mt-lsu rDNA sequences of one-fifth of the taxa sampled, including *Phaeolus schweinitzii,* were not available, so inferences regarding the higher-level phylogenetic relationships of *Sparassis* species based mostly on the combined nuc-rDNA and RPB2 sequences.

Sparassis species form a monophyletic group within the polyporoid clade supported by multilocus molecular data (FIGS. 1–5). *Sparassis* is closely related to *Phaeolus, Laetiporus, Pycnoporellus, Auriporia, Oligoporus* and other brown-rot polypores. *Phaeolus* and *Laetiporus* were confirmed as the sister group of *Sparassis* using equally weighted parsimony (FIGS. 1, 3, 4) as in previous studies (Hibbett et al 1997). *Phaeolus, Laetiporus* and *Sparassis* are united by the production of a brown rot, bipolar mating system and the habit of growth as a root and butt rot on living trees (rarely on dead trees and logs). Bayesian analysis (FIG. 5) suggests a close relationship among *Sparassis* species, *Phaeolus, Grifola* and *Pycnoporellus*. *Pycnoporellus* was thought to be closely related to *Phaeolus* (Gilbertson and Ryvarden 1987), and the cystidia similar to those found in *Phaeolus* and *Pycnoporellus* were found in *Sparassis* sp. THAI as well (Desjardin et al 2004). Bayesian analysis supports a clade including *Fomitopsis, Piptoporus, Neolentiporus* and *Laetiporus,* which are all brown rot polypores with bipolar mating systems.

Sequences from three loci, nuc-lsu rDNA, ITS and RPB2, have been used to infer the lower-level phylogenetic relationships of *Sparassis*. ITS sequences of sampled taxa are divergent and difficult to align, and parsimony analysis based on ITS sequences alone generated more than 53 000 equally parsimonious trees with little resolution (data not shown). As expected, RPB2 were easier to align and provided more informative sites than ITS sequences. Combining RPB2 gene with nuc-lsu rDNA and ITS provided higher support to branches and better resolution (FIGS. 6–8). Unfortunately, RPB2 sequences were not obtained for most *Sparassis* collections from North America.

Except for two unidentified *Sparassis* species, *S.* sp. AUS and *S.* sp. THAI, five well-supported clades were found among *Sparassis* species. Asian isolates of *S.* cf. *crispa* show morphological differences compared with European *S. crispa*. Western North American collections of *Sparassis,* including three isolates identified as *S. crispa* and one as *S. radicata,* formed a clade, and they morphologically are similar to the Asian *S.* cf. *crispa*. *Sparassis spathulata, S. brevipes* and the European *S. crispa* are distinct species delimited by both molecular data and morphological characters. The recently collected cystidioid *Sparassis* sp. THAI was grouped with all *Sparassis* species and might provide clues to the plesiomorphic form of *Sparassis* (Desjardin pers comm). Recognizing the Australian collection (*S.* sp. AUS31) as a species of *Sparassis* denotes that this genus is distributed worldwide, but more material from the Southern Hemisphere is necessary to describe this species and to resolve the distribution pattern.

The relationships among members of *Sparassis crispa sensu lato,* which include European and eastern North American *S. crispa,* western North American *S. radicata* and Asian *S.* cf. *crispa,* were not well resolved in all the analyses. Asian *S.* cf. *crispa* and western North American *S. radicata* are morphologically similar, but they are not strongly supported to form a monophyletic group. The eastern North American *S. crispa* morphologically is identical to the European *S. crispa,* but molecular data do not suggest that the collections from both locations are conspecific. This eastern North America-Europe distribution pattern

was supported by the close relationship between eastern North American *S. spathulata* and European *S. brevipes*. North American *S. crispa* and *S. radicata* collections show a morphological transition between European *S. crispa* and Asian *S.* cf. *crispa* (i.e., eastern North American *S. crispa* is similar to European species whereas western North American *S. radicata* is similar to Asian *S.* cf. *crispa*). However, the lack of the RPB2 sequences from western North American collections make it premature to draw conclusions about biogeographic relationships among *S. crispa sensu lato*.

Sparassis is characterized morphologically by flabellae with an amphigenous hymenium and a central mass giving rise to the flabellae. The presence of clamp connections in *Sparassis crispa, S. radicata, S.* cf. *crispa* and *S.* sp. THAI suggests that clamp connections might have been lost in *S. spathulata* and *S. brevipes*. The spore size range in *Sparassis* species is overlapping and therefore not always reliable to use for recognizing species. Basidia with 2–4 sterigmata have been observed in all examined collections and basidiospores generally are larger when fewer spores are produced on single basidium.

Host shifts must have occurred in the evolution of *Sparassis* species. *S.* sp. THAI was thought to grow strickly on *Quercus,* and this trait has been kept in the lineages of *S. brevipes, S. spathulata* and Asian *S.* cf. *crispa,* but they all are known to have conifer hosts as well. European *S. crispa* and western North American *S. radicata* are found strictly on conifers. Additional ecological and phylogenetic studies could resolve patterns of host shifts in *Sparassis*.

ACKNOWLEDGMENTS

We thank R.H. Petersen, D.E. Desjardin, D.H. Pfister, R.J. Bandoni, T. Hattori, P.M. Kirk, G. Ringer, Z.L. Yang, Helmut Besl, and the curators of CUP, FH, HKAS, HMAS, MBUH, BMS, TENN and the herbaria in the University of Regensburg (REG) for providing specimens, and two reviewers for their helpful comments. This work was supported by National Sciences Foundation Grant DEB-9903835 to DSH and MB.

LITERATURE CITED

Binder M, Hibbett DS. 2002. Higher-level phylogenetic relationships of Homobasidiomycetes (mushroom-forming fungi) inferred from four rDNA regions. Mol Phylogenet Evol 22:76–90.

Breitenbach J, Kränzlin F. 1986. Fungi of Switzerland. Vol. 2: Nongilled fungi. 412 p.

Burdsall HH Jr, Miller OK Jr. 1988a. Type studies and nomenclatural considerations in the genus *Sparassis*. Mycotaxon 31:199–206.

———. 1988b. Neotypification of *Sparassis crispa*. Mycotaxon 31:591–593.

Desjardin DE, Wang Z, Binder M, Hibbett DS. 2004. *Sparassis cystidiosa* sp. nov. from Thailand is described using morphological and molecular data. Mycologia 96: 1008–1012.

Donk MA. 1964. A conspectus of the families of the Aphyllophorales. Persoonia 3:199–324.

Gilbertson RL. 1980. Wood-rotting fungi of North America. Mycologia 72:1–49.

———. 1981. North American wood-rotting fungi that cause brown rots. Mycotaxon 12:372–416.

———, Ryvarden L. 1987. North American polypores. Vol. 2. Fungiflora, Oslo.

Hawksworth DL, Kirk PM, Sutton BC, Pegler DN. 1995. Ainsworth & Bisby's dictionary of the fungi. 8th ed. Wallingford, UK: CAB International. 616 p.

Hibbett DS. 1996. Phylogenetic evidence for horizontal transmission of group I introns in the nuclear ribosomal DNA of mushroom-forming fungi. Mol Biol Evol 13: 903–917.

———, Donoghue MJ. 1994. Progress toward a phylogenetic classification of the Polyporaceae through parsimony analysis of mitochondrial ribosomal DNA sequences. Can J Bot 73 (Suppl. 1):S853–S861.

———, Pine EM, Langer E, Langer G, Donoghue MJ. 1997. Evolution of gilled mushroom and puffballs inferred from ribosome DNA sequences. Proc Nat Acad Sci USA 94:12002–12006.

———, Hansen K, Donoghue MJ. 1998. Phylogeny and biogeography of *Lentinula* inferred from an expanded rDNA data set. Myc Res 102:1041–1049.

———, Donoghue MJ. 2001. Analysis of character correlations among wood decay mechanisms, mating system, and substrate ranges in Homobasidiomycetes. Syst Biol 50:215–242.

———, Binder M. 2002. Evolution of complex fruiting-body morphologies in homobasidiomycetes. Proc R Soc Lond B269:1963–1969.

Hirt RP, Logsdn JM, Healy B, Dorey MW, Doolittle WF, Embley TM. 1999. Microsporidia are related to fungi: evidence from the largest subunit of RNA polymerase II and other proteins. Proc Natl Acad Sci, USA 96:580–585.

Huelsenbeck JP, Ronquist F. 2001. MrBayes: Bayesian inference of phylogenetic trees. Bioinformatics 17:754–755.

Imazeki R, Otani Y, Hongo T. 1988. Fungi of Japan. Tokyo, Japan: Yama-kei Publishers Co. Ltd. 623 p.

Jülich W. 1981. Higher taxa of basidiomycetes. J Cramer, Vaduz. 485 p.

Kirk PM, Cannon PF, David JC, Stalpers JA. 2001. Ainsworth & Bisby's dictionary of the fungi. 9th ed. Wallingford, UK: CAB International. 655 p.

Kreisel H. 1983. Zur Taxonomie von *Sparassis laminosa* Fr. s. l. Fed Report 94:675–682.

Liu YJ, Whelen S, Hall BD. 1999. Phylogenetic relationships among ascomycetes: evidence from a RNA polymerase II subunit. Mol Biol Evol 16:1799–1808.

Mao XL, Jiang CP, Ciwang OZ. 1993. Economic macrofungi

of Tibet. Beijing: Beijing Science & Technology Press. 651 p.

Martin KJ, Gilbertson RL. 1976. Cultural and other morphological studies of *Sparassis radicata* and related species. Mycologia 68:622–639.

Matheny PB, Liu YJ, Ammirati JF, Hall BD. 2002. Using RPB1 sequences to improve phylogenetic inference among mushroom (*Inocybe*, Agaricales). Am J Bot 89: 688–698.

Mueller GM, Wu QX, Huang YQ, Guo SY, Aldana-Gomez R, Vilgalys R. 2001. Accessing biogeographic relationships between North American and Chinese macrofungi. Journal of biogeography 28:271–281.

Redhead SA. 1989. A biogeographical overview of the Canadian mushroom flora. Can J Bot 67:3003–3062.

Swofford DL. 1999. PAUP*. Phylogenetic analysis using parsimony (*and other methods). Version 4. Sunderland, Massachusetts: Sinauer Associates.

Teng SC. 1995. Fungi of China. Mycotaxon, LTD.

van Zanen GCN. 1988. *Sparassis laminosa* versus *Sparassis crispa*. Coolia 31:93–95.

Vilgalys R, Hester M. 1990. Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several species of *Cryptococcus*. J Bacteriol 172: 4238–4246.

Weir JR. 1917. *Sparassis radicata*, an undescribed fungus on the roots of conifers. Phytopathology 7:166–177.

Wen J. 1999. Evolution of eastern Asia and eastern North American disjunct distributions in flowering plants. Ann Rev Ecol Syst 30:421–455.

White TJ, Bruns T, Lee S, Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ, eds. PCR protocols. San Diego, California: Academic Press. p 315–322.

Wu QX, Mueller GM. 1997. Biogeographic relationships between the macrofungi of temperate eastern Asia and eastern North America. Can J Bot 75:2108–2116.

———, ———, Lutzoni FM, Huang YQ, Guo SY. 2000. Phylogenetic and biogeographic relationships of eastern Asian and eastern North American disjunct Suillus species (fungi) as inferred from nuclear ribosomal RNA ITS sequences. Mol Phylogenet Evol 17:37–47.

Zang M. 1992. Endemic higher fungi with a note on China and adjacent areas. Acta Bot Yunnanica 14:385–400.