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Phylogeny and a new species of *Sparassis* (Polyporales, Basidiomycota): evidence from mitochondrial *atp*6, nuclear rDNA and *rpb*2 genes

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Abstract: Three nuclear genes, lsu-rDNA (encoding nuclear large subunit rDNA), ITS (encoding the rDNA internal transcribed spacers and 5.8 S rDNA) and *rpb*2 (encoding the second largest subunit of RNA polymerase II), and the mitochondrial gene *atp*6 (encoding the sixth subunit of ATP synthase), were sequenced from all recognized *Sparassis* lineages. *Sparassis latifolia* sp. nov. from boreal coniferous forests in China is described based on morphological, ecological, geographical and molecular data. The nuclear gene phylogeny strongly supported groups corresponding to morphological differences, geographic distribution and host shifts among species that produce clamp connections, such as *S. crispa* from Europe, *S. radicata* from western North America and *S. latifolia* from Asia. The *atp*6 phylogeny however showed no divergence among these three species. For clampless *Sparassis* species, such as *S. spathulata* from eastern North America, *S. brevipes* and a new species from Europe, the *atp*6 phylogeny was congruent with the nuclear gene phylogeny. *Sparassis cystidiosa* is basal in the nuclear tree but sister to *S. brevipes*-*S. spathulata* clade in the ATP6 tree. The differences between the phylogenetic inferences from the *atp*6 gene and those from nuclear genes within *Sparassis* species are discussed.

Key words: clamp connections, mating tests, mitochondrial inheritance, sexual compatibility

INTRODUCTION

The taxonomy and systematics of cauliflower fungi, species of *Sparassis* Fr., recently have received much attention (Blanco-Dios et al unpublished, Desjardin et al 2004, Wang et al 2004). *Sparassis* species have a bipolar mating system and produce a brown rot on conifers and Fagales. This is a derived wood decay mode in the Polyporales, which otherwise is dominated by white rot fungi (Hibbett and Donoghue 2001). Seven clades representing widely recognized species in *Sparassis* were reported based on molecular and morphological data (Desjardin et al 2004, Wang et al 2004). Presence of clamp connections is variable among *Sparassis* species, as well as among tissue in the basidiocarps of some species. In *S. brevipes* Krombh. and *S. spathulata* (Schwein.) Fr. no clamp connections are produced in the context, although clamp connections may be found in subhymenium and at the base of basidia. Species in four other clades produce clamp connections, including *S. cystidiosa* Desjardin and Zheng Wang from Thailand, which appears to be the sister group to all other *Sparassis* lineages, *S. radicata* Weir from western North America, *S. crispa* (Wulfen) Fr. from Europe and *S.* cf. *crispa* from Asia. Two collections from Spain with few clamp connections in the subhymenium represent a putatively new species, provisionally named *S.* ''*miniensis*'' and might be closely related to *S. brevipes* from northern Europe based on a rDNA phylogeny (Blanco-Dios et al pers. comm.).

Asian collections identified as *S. crispa* are morphologically different from *S. crispa* in Europe, and the Asian *S.* cf. *crispa* clade is strongly supported by molecular data. Wang et al (2004) did not describe these Asian collections as new, in part because appropriate materials for designating a type specimen were not available.

Mating studies (Martin and Gilbertson 1976) have cast doubts on the biological boundaries between *S. crispa* and *S. radicata*. Martin and Gilbertson (1976) crossed dikaryotic isolates of *S. crispa* from Europe and Japan with monokaryotic isolates of *S. radicata* from North America. The resulting Di-Mon mycelia produced clamp connections but did not produce fruiting bodies or basidia. Based on these results Martin and Gilbertson (1976) suggested that *S. crispa* and *S. radicata* were conspecific. They confirmed that *S. radicata* is heterothallic, that two *S. radicata* isolates exhibited a bipolar mating type and that matings between the two isolates were compatible. This was

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interpreted as evidence of multiple alleles for incompatibility in the *S. radicata* population (Martin and Gilbertson 1976). The eastern North American *S. spathulata* (*S. crispa* from southeastern north American in Martin and Gilbertson 1976), which produces no clamp connections in the context, produced basidiospores but no clamp connections in dikaryotic culture. However no mating studies with single spore isolates of *S. spathulata* were performed (Martin and Gilbertson 1976).

It is difficult to apply the biological species concept to fungi because little is known about the mating behavior of fungi in the field. Dikaryons produced by crossing monokaryotic isolates in the lab could fail to produce functional basidiospores for many reasons. Mating type genes regulate sexual compatibility and reproduction in fungi. Mating incompatibility inhibits crosses between closely related isolates of similar mating types. Some basidiomycetes possess complicated genetic systems that control the crossing from the very beginning of anastomosis between compatible hyphae to the production of fruiting bodies. For example more than 20 000 mating types can be formed in *Schizophyllum commune* Fr. because of the tremendous number of specificities generated by the subloci of two unlinked mating loci (Kronstad and Staben 1997). In the case of *Sparassis* no basidiospores were observed in Di-Mon matings within clamp producing species. This could indicate incompatibility among these fungi. However failure to produce fruiting bodies also could be a result of culture conditions or other factors not connected to genetic incompatibility.

It has been demonstrated that mitochondria do not migrate along with nuclei in sexual crosses in some basidiomycetes, although recombination between different mtDNAs still may occur in some cases (Baptista-Ferreita et al 1983, Hintz et al 1988, May and Taylor 1988). In *Armillaria* and *Neurospora* species both parental mitochondrial types are present in mycelia soon after mating but only one mitochondrial type becomes dominant during subsequent vegetative growth (Smith et al 1990, Lee and Taylor 1993). Homogenization of mitochondrial populations after mating also has been observed in Di-Mon pairings of *Pleurotus ostreatus* (Jacq. ex Fr.) Kummer, with mitochondria from the monokaryotic donor taking over the whole mycelium (Fischer and Wolfrath 1997). Thus the evidence to date suggests that mitochondria are uniparentally inherited in fungi.

Sequences of fungal mitochondrial rDNA (mt-lsu and mt-ssu) have been used for population studies and higher-level phylogenetic studies (e.g. Binder and Hibbett 2002, Lumbsch et al 2005). However the utility of mitochondrial rDNA is limited by high substitution rates in variable regions, the variable size of introns and the relatively small number of conserved regions that can be aligned across distantly related taxa (Hibbett and Donoghue 1995, Kretzer and Bruns 1999, Wang et al 2004, Lutzoni et al 2004). Few mt-lsu rDNA sequences have been generated from *Sparassis* species because of difficulties in primer design. A more promising candidate to trace mitochondrial inheritance is the mitochondrial gene *atp*6. The *atp*6 gene codes subunit 6 in a $F_0 \cdot F_1$-H^+-ATP synthase, which is the main enzyme responsible for producing ATP in aerobic cells (Vinogradov 1999). Primers are available from the study of Kretzer and Bruns (1999), which suggested that phylogenetic inferences from mt-lsu and *atp*6 sequences are congruent and the combination of both genes increases support for the key clades in Boletales. A phylogeny of the genus *Agaricus* Fr. based on *atp*6 sequences suggested that variation within the *atp*6 genes was sufficient for studying species level relationships but was inadequate for lower level relationships (Robison et al 2001). In this study we discuss relationships among *Sparassis* species based on a combined nuclear gene phylogeny (nuclear large subunit rDNA, ITS, *rpb*2) with data drawn from Wang et al (2004) and comparative analyses using *atp*6.

MATERIALS AND METHODS

Morphological studies.—Materials are deposited at the Herbarium of the Institute of Applied Ecology, Chinese Academy of Sciences (IFP). For comparison seven specimens from the Botanical Museum of the University of Helsinki (H) were studied. The microscopic procedures followed those described by Dai (1999). These abbreviations are used: L = mean spore length (arithmetical mean of all spores), W = mean spore width (arithmetical mean of all spores), Q = mean L/W ratios (quotient of the mean spore length and the mean spore width), n = number of spores measured from given number of specimens. In presenting the variation in the size of spores 5% of the measurements were excluded from each end of the range and are given in parentheses. The abbreviation IKI stands for Melzer's reagent (IKI– means inamyloid), KOH for 5% potassium hydroxide and CB for cotton blue (CB+ means cyanophilous).

Molecular techniques.—DNA was isolated from dried herbarium material following standard protocols as described in Wang et al (2004). Sequence data of lsu-rDNA, *rpb*2, and ITS generated in Wang et al (2004) and Blanco-Dios et al (generated by ZW at Clark University unpublished) were used in this study. Twenty isolates representing all seven *Sparassis* lineages in Wang et al (2004) and eight polypores producing either a brown rot or a white rot were included.

The *atp*6 region bounded by primers ATP6-3 and ATP6-4 was amplified from 14 *Sparassis* and 14 polypore isolates with four primers, ATP6-1, ATP6-2, ATP6-3 and ATP6-4

(Kretzer and Bruns 1999), in a modified nested PCR reaction, which performed better in amplifying *atp*6 products than using one pair of primers in regular PCR setting. PCR reaction mixes (Promega Corp., Madison, Wisconsin) contained 2.5 µL 10× PCR buffer, 5µ M dNTP, 10 pM of primers ATP6-3 and ATP6-4 and 2.5 pM of primers ATP6-1 and ATP6-2, and 5 µL DNA in 25 µL. *Taq* polymerase was added to the PCR reaction mixes after they were heated to 95 C. The touchdown amplification program included 10 cycles of 94 C for 30 s, 43 C for 1 min, reducing by 0.5 C on every cycle, and 72 C for 1 min, followed by 30 cycles of 94 C for 30 s, 38 C for 1 min and 72 C for 1 min. PCR products were purified with GeneClean (Bio 101, Carlsbad, California) and sequenced with the ABI Prism BigDye-terminator cycle sequencing kit (Applied Biosystems, Foster City, California) according to the manufacturer's protocols. Primers used for sequencing were ATP6-3 and ATP6-4. Sequencing reactions were purified with Pellet Paint (Novagen, Madison, Wisconsin) and were run on an Applied Biosystems 377XL automated DNA sequencer. Sequences were edited with Sequencer version 3.1 (GeneCodes Corporation, Ann Arbor, Michigan) and submitted to GenBank (TABLE I).

Phylogenetic analyses.—Two datasets were analyzed, one was composed of mitochondrial *atp*6 data (ATP6) and the other was composed of nuclear gene data (NUC). The NUC dataset included sequences from nuc-lsu rDNA, *rpb*2, and ITS. Sequences were aligned by eye in the data editor of PAUP* 4.0b (Swofford 1999). Both datasets were analyzed in PAUP* 4.0b, with gaps treated as missing data and ambiguous or unalignable positions excluded.

The ATP6 dataset was rooted with *Climacodon septentrionalis* (Binder et al 2005). Parsimony analyses were performed with equal weighting of characters and transformations. Heuristic searches were performed with 1000 replicate searches, each with a random taxon addition sequence. MAXTREES was set to auto increase, and TBR branch swapping was employed. A bootstrap analysis was performed with 1000 replicates, each with 10 random taxon addition sequences, saving ten trees per replicate. MAXTREES was set to 1000, and TBR branch swapping was employed. The NUC dataset was rooted with *Lentinus tigrinus*, *Polyporus squamosus* and *P. tuberaster* (Wang et al 2004). Parsimony analyses were performed the same as the ATP6 dataset. A bootstrap analysis was performed with 1000 replicates, each with 10 random taxon addition sequences. MAXTREES was set to 1000, and TBR branch swapping was employed. Alignments are available at TreeBase (accession number SN2453)

TAXONOMY

Sparassis latifolia Y.C. Dai & Zheng Wang *sp. nov.* FIG. 1a–d

Carpophorum annuum, solitarium, stiptatum, flabellatum, albidum vel cremeum Systema hypharum monomiticum, hyphae fibulatae vel septatae, hyphae contexti 4.5–9.5 µm diam.

Sporae hyalinae, IKI–, CB–, 4.5–5.5×3.5–4 µm.

Type. China: Jilin Prov., Antu County, Changbaishan Nat. Res., on the ground in conifer forest, 14-VIII-1997 *Dai 2441* (HOLOTYPE in IFP).

Etymology. Sparassis latifolia, *Sparassis* species with broad leaf flabellae.

Basidiocarps. Annual, solitary, stipitate, up to 30 cm high, 25 cm diam, composed of numerous loosely arranged flabellae. Flabellae mostly extend from a common central mass, broad, dissected and slightly contorted, white and soft when fresh, becoming cream colored and leathery with age, pale ochraceous and corky when dry, azonate, up to 1 cm broad, 1 mm thick, margin wavy, sometimes tooth-like. Stipe up to 15 cm long, 1.5 cm thick at base, thinning out.

Hyphal structure. Hyphal system monomitic; generative hyphae with both clamp connections and simple septa (FIG. 1c, d); tissues unchanged in KOH.

Context. Contextual hyphae hyaline, thin- to slightly thick-walled, frequently branched, interwoven, (4.5–)5.4–10.5(–11.7) µm diam (n = 30/1). Gloeoplerous hyphae present, refractive, thin-walled, flexuous, frequently branched, 8–14(–16) µm diam (n = 30/1).

Flabellae. Composed of a hymenial layer, a subhymenium, and trama layer. Tramal hyphae hyaline, thin-walled, frequently branched, interwoven, (3–)4.5–9.5(–9.7) µm diam (n = 30/1). Gloeoplerous hyphae present, refractive, thin-walled, flexuous, occasionally branched, 7–12.5 (–13) µm diam (n = 30/1). Hymenia dominated by basidia and basidioles; basidia clavate, with four sterigmata and a basal clamp connection (FIG. 1b), 25–29–2.6–8 µm; basidioles similar in shape to basidia, 20–27–5.4–5.2 µm. Subhymenium distinct and thick, made up of delicate, hyaline, thin-walled, tortuous, densely interwoven hyphae.

Spores. Basidiospores ellipsoid (FIG. 1a), hyaline, thick-walled, smooth, IKI–, CB– (4–)4.5–5.5(–5.9) × (3.2–)3.5–4(–4.1) µm, L = 5.03 µm, W = 3.85 µm, Q = 1.23–1.35 (n = 90/3).

Additional specimens (paratypes) examined. China. Jilin Prov., Antu County, Changbaishan Nat. Res., on ground in conifer forest, 14-VIII-1997 *Dai 2470 & 2472*; 26-VII-2005 *Wei 2549a*, *2472*; 26.VII.2005 *Wei 2576*.

Other specimens examined. Sparassis brevipes: Germany: Baden-Württemberg, Karlsruhe, Freudenstadt, Alpirsbach, Baierhof, conifer forest, 24-IX-1995 *Kytövuori 96-1044*; Freiburg, near Hornberg, Langenschiltach, conifer forest, 27.IX.1996 *Laber*. *S. crispa*: Germany: Baden-Württemberg, Freiburg, west of Freiburg, Haslach Rod, Biederbach, Heid-

TABLE I. Isolates of *Sparassis* and other polypores in this study

Isolates	Coll. no.	Locality	GenBank accessions (consult for isolate data)			
			rDNA			
			Lsu	ITS	*rpb2*	*atp6*
Sparassis brevipes Krombh.	ILKKA-96-1044	GERMANY	AY218403	AY218441	AY218543	**DQ250709**
S. crispa (Wulfen) Fr.						
FIN3	YCDAI2637	FINLAND	AY218387	AY218425	AY218532	**DQ250700**
FIN4	SAVOLAINEN	FINLAND	AY218388	AY218426	AY218533	
FRA5	ILKKA94-1587	FRANCE	AY218389	AY218427	AY218534	**DQ250701**
AME9	ZW-Clarku003	USA/MA	AY218393	AY218430	AY218537	**DQ250703**
GER25	DORISLABER	GERMANY	AY218404	AY218442	AY218544	**DQ250702**
S. cystidiosa Desjardin & Zheng Wang	DEDesjardin7410	THAILAND	AY256890	AY256891	AY256892	**DQ250704**
S. latifolia Y.C. Dai & Zheng Wang						
CHN1	YCDAI2145	CHINA-north	AY218385	AY218423	AY218530	
CHN2	YCDAI2470	CHINA-north	AY218386	AY218424	AY218531	
CHN17	HMAS60590	CHINA-north	AY218397	AY218435	AY218539	**DQ250698**
CHN19	HKAS15728	CHINA-middle	AY218398	AY218436	AY218540	**DQ250699**
CHN20	HKAS32363	CHINA-middle	AY218399	AY218437	AY218541	
CHN21	HKAS17477	CHINA-south	AY218400	AY218438	AY218542	**DQ250697**
S. miniensis nom. prov	Lou-Fungi 18390	SPAIN	UNPUBL	UNPUBL.	UNPUBL.	**DQ250710**
S. radicata Weir						
AME32	TENN50232	USA/TN	AY218410	AY218449	AY218546	**DQ250706**
AME33	TENN52558	USA/WA	AY218411	AY218450		
AME29	TENN56253	USA/CA	AY218408	AY218446	**DQ270673**	**DQ250707**
CAN26	UBC-F12464	CANADA	AY218405	AY218443	**DQ270672**	**DQ250705**
S. spathulata (Schwein.) Fr.						
AME7	ZW-Clarku001	USA/MA	AY218391	AY218428		
AME8	ZW-Clarku002	USA/NH	AY218392	AY218429		**DQ250708**
AME11	ZW-Clarku004	USA/MA	AY218395	AY218432	AY218535	
Climacodon septentrionalis (Fr.) P. Karst.					AY218536	**DQ250684**
Fomitopsis pinicola (Sw. : Fr.) P. Karst.					AY218538	**DQ250685**
Grifola frondosa (Dicks. : Fr.) S. F. Gray			AY218413	AY218415		**DQ250686**
L. sulphureus (Bull. : Fr.) Murrill			AY218414	AY218417		**DQ250687**
Lentinus tigrinus (Fr.) Fr.			AF518627	AY218419	AY218521	**DQ250688**
Oligoporus rennyi (Berk. & Broome) Donk			AF287876	AY218416	AY218522	**DQ250689**
Phaeolus schweinitzii (Fr.) Pat.			AF287882	AY218422	AY218493	**DQ250690**
Phlebia radiata Fr.					AY218499	**DQ250691**
Piptoporus betulinus (Bull. : Fr.) P. Karst.					AY218501	**DQ250694**
Polyporus arcularius Batsch : Fr.						**DQ250695**
P. squamosus Huds. : Fr.			AF393069	AY218421		**DQ250692**
P. tuberaster (Jacq.) Fr.			AJ488116	AF516597		
Postia lacteal (Fr.) P. Karst					AY218508	**DQ250693**
Pycnoporellus fulgens (Fr.) Donk			AF518643	AY218418	UNPUBL. AY218527	**DQ250696**

Sequences generated in this study are in boldface.

burg, mixed forest, 29-IX-1996 *Kytövuori 96-1281*. Sweden, Vätergötland, Hökensåa, Madengsholm, east of the road 48, pine forest, 18-IX-1984 *Kytövuori 84640*. Finland: Etelä-Häme, Lammi, Evo, Kotinen virgin forest, conifer forest, 7-IX-1982 *Niemelä 2769*, 19-IX-1985 *Niemelä 3290*, 12-IX-1997 *Dai 2637*.

Sparassis crispa was reported from China and Japan (Tai 1979, Imazeki et al 1988) but Wang et al (2004) and Desjardin et al (2004) found that Asian collections referred to *S. crispa* are different from European *S. crispa* both in morphology and molecular characters (TABLE II). Here we describe *S. latifolia* based on a collection from northern China. This species

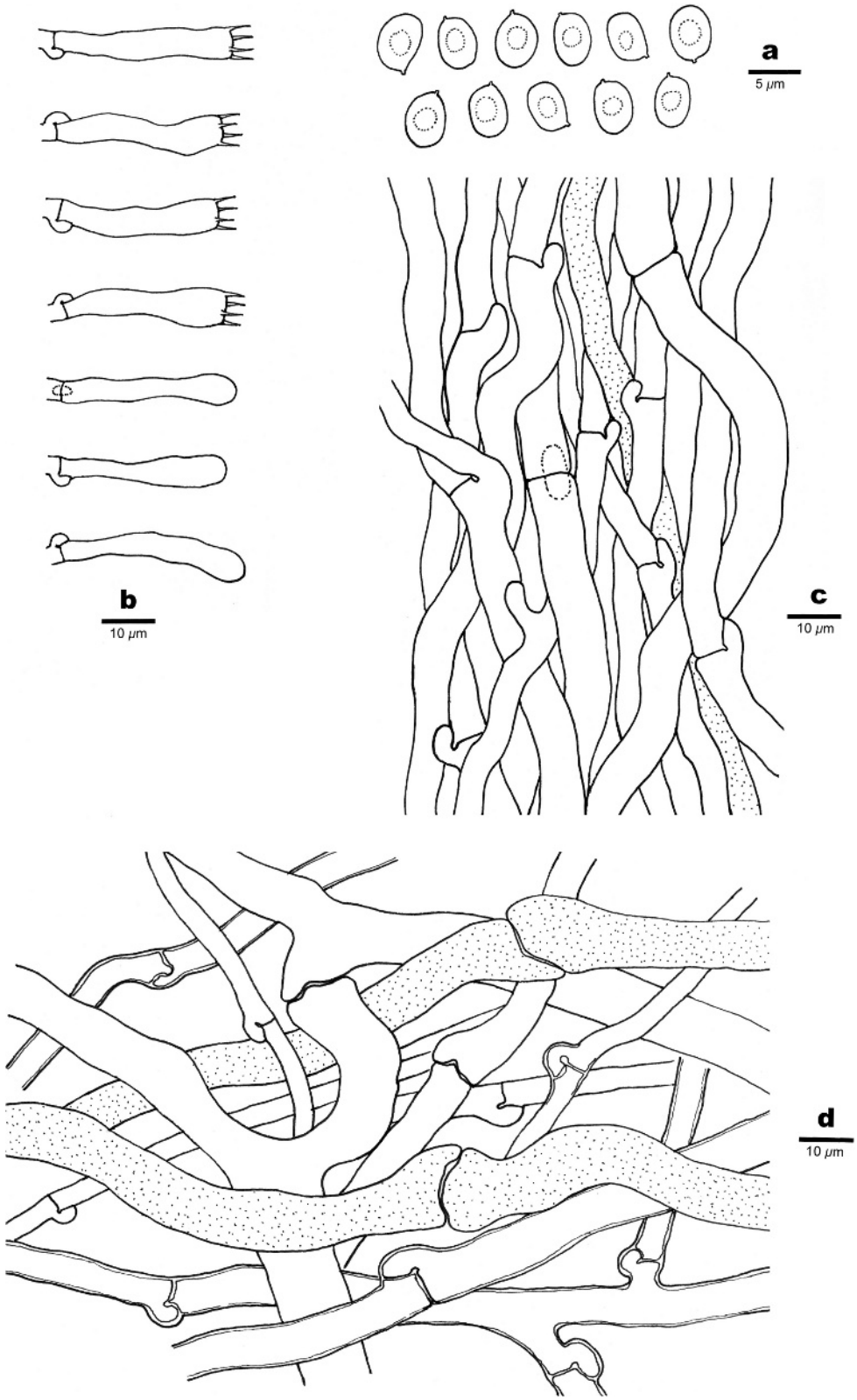

FIG. 1. Microscopic structures of *Sparassis latifolia* Y.C. Dai & Zheng Wang (drawn from holotype). a. Basidiospores. b. Basidia and basidioles. c. Trama hyphae. d. Context hyphae. Carpophorum annuum, solitarium, stipitatum, flabellatum, albidum vel cremeum Systema hypharum monomiticum, hyphae fibulatae vel septatae, hyphae contexti 4.5–9.5 μm diam. *Sporae hyalinae*, IKI–, CB–, 4.5–5.5 × 3.5–4 μm.

TABLE II. Comparison of *Sparassis crispa*, *S. latifolia*, *S. radicata* and *S. spathulata* (Data are based on Burdsall and Miller 1988a, b; Martin and Gilbertson 1976; Wang et al 2004; and personal observations)

		S. crispa	*S. latifolia*	*S. radicata*	*S. spathulata*[3]
Geographic distribution		Europe, eastern North America	Eastern Asia	Western North America	Eastern North America
Flabellae		Small, broad but short, dissected and strongly contorted, margin entire	Large, broad, dissected and slightly contorted, margin sometimes tooth-like	Large, broad, dissected and slightly contorted, margin entire	Large, broad, distinctively zonate, upright standing, margin entire
Size of basidiospores (μm)		4.0–4.9 × 4.9–6.0 (–6.9)	3.5–4 × 4.5–5.5[1]	3.9–5.0 × 6.3–7.0	4.7–5.8 × (5.9) 6.9–8.0
Clamp connections in basidiocarps		Present	Present	Present	Absent[4]
Culture characters		Clamps and chlamydospores observed	Clamps and chlamydospores observed	Clamps and chlamydospores observed	Clampless, basidiospores and basidiocarps observed
Mating compatibility (Di-Mon) X *S. radicata* (mon).		Clamps were found within a *S. radicata* clone in a zone < 15 mm from the area of contact with the dikaryotic mycelium	Clamps were found within a *S. radicata* clone in a zone < 15 mm from the area of contact with the dikaryotic mycelium[2]		NA
Host		Conifers	Conifers and Fagales	Conifers	Conifers and Fagales
Bootstrap support (%).	LSU+ITS	70	94	67	100
	LSU+ITS+*rpb2*	67	98	51	100

[1] Based on dried specimens, the measurements were recorded as 4.0–5.0 × 5.0–6.0 in Wang et al. (2004).
[2] *S. crispa* from Japan in Marin and Gilbertson (1976) probably is *S. latifolia*, but no material from Japan had been examined.
[3] *S. crispa* from southeastern North America in Martin and Gilbertson (1976) based on their descriptions.
[4] Clamps were reported in Martin and Gilbertson (1976) but not in Burdsall and Miller (1988), and we did not detect any clamp connections in recent collections of *S. spathulata* from northeastern North America.

represents the Asian *S.* cf. *crispa* clade in Wang et al (2004). *S. latifolia* is characterized morphologically by its large, broad, dissected and slightly contorted flabellae and by the production of clamp connections. The species is distributed broadly in east Asia and grows in association with conifers and Fagales. *S. crispa* and *S. radicata* also produce clamp connections but they mainly are found associated with conifers. *S. crispa* might be strictly distributed in Europe and eastern North America while, *S. radicata* has been found only in western North America. *S. cystidiosa* also produces clamp connections and, in addition, cystidia. *S. crispa* was reported from the Russian Far East (Lyubarskii and Vasilyeva 1975), but it probably represents *S. latifolia*.

PHYLOGENETIC ANALYSES

Molecular inference from the ATP6 dataset.—The relationships within *Sparassis* were investigated with the mitochondrial gene *atp*6 (FIG. 2a). The data had an aligned length of 642 base pairs with 72 uninformative variable positions and 302 parsimony informative positions.

For the most part the higher-level topology within *Sparassis* based on *atp*6 sequences was congruent with the topology based on the NUC dataset (FIG. 2b). *Sparassis* species formed a weakly supported clade (BP = 62%) with a clade (BP = 95%) of two brown rot fungi *Oligoporus rennyi* and *Postia lactea* as the sister group. Three clampless species formed a clade (BP = 100%) with *S. cystidiosa* as the sister branch (96%). Two European species *S. brevipes* and *S. miniensis* nom. prov. formed a clade (BP = 100%). *Sparassis latifolia*, *S. crispa* and *S. radicata* formed a strongly supported clade (BP = 100%).

Molecular inference from the NUC dataset.—The NUC dataset (LSU+ITS+*rpb*2) had an aligned length of 2326 base pairs (246 ambiguous positions were excluded from the analyses) with 276 uninformative variable positions and 518 parsimony informative positions.

Sparassis species formed a monophyletic group (BP = 90%) with the brown rot fungus *Oligoporus rennyi*

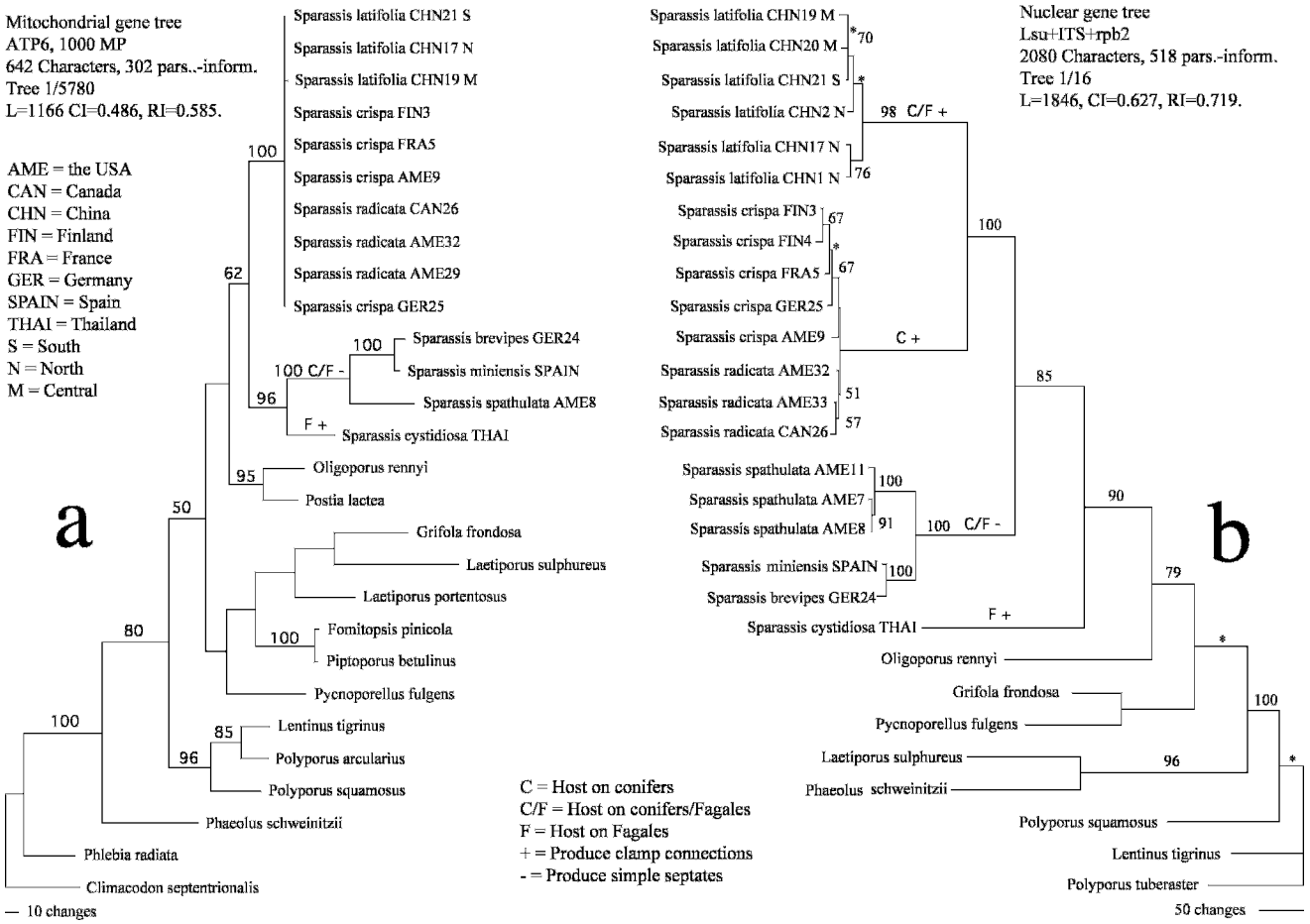

FIG. 2. Phylogenetic relationships of *Sparassis*. a. Parsimony analysis based on *atp*6 sequences. One of 5780 equally parsimonious trees (length = 1166, CI = 0.486, RI = 0.585). Bootstrap values greater than 50% are indicated along nodes. b. Parsimony analysis based on the combined lsu-rDNA, *rpb*2 and ITS sequences. One of 16 equally parsimonious trees (length = 1864, CI = 0.627, RI = 0.719). 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.

as the sister group (BP = 79%). *Sparassis cystidiosa* from Thailand was the sister group of all other lineages of *Sparassis*. Three clampless species, *S. spathulata* from eastern North America, *S. brevipes* from Germany and *S. miniensis* nom. prov. from Spain, formed a clade (BP = 100%), within which three collections of *S. spathulata* formed a lineage (BP = 100%), and *S. brevipes* and *S. miniensis* nom. prov. formed a clade (BP=100%). Three clamp connection producing species, *S. latifolia* from China, *S. crispa* from Europe and eastern North America and *S. radicata* from western North America, formed a clade (BP = 100%), within which there were three groups, including *S. latifolia* (BP = 98%), European *S. crispa* (BP = 67%) and *S. radicata* (BP = 51%). *Sparassis crispa* from eastern North America was placed as the sister group of *S. crispa* from Europe without bootstrap support. Within *S. latifolia* two collections from northern China formed a clade (BP = 76%) while three collections from middle and southern parts of China formed the sister clade (BP = 70%) to the northern collections.

The major difference between the ATP6 and NUC estimations is that there is no resolution in the *S. latifolia*-*S. crispa*-*S. radicata* clade in the *atp*6 tree, whereas the NUC tree divides these taxa into two highly divergent clades, one of which contains only *S. latifolia*. Another noticeable difference between the phylogenies is that *S. cystidiosa* is the sister taxon of the *S. brevipes*-*S.* ''*miniensis*''-*S. spathulata* clade in the ATP6 analysis, whereas *S. cystidiosa* is the sister taxon of all *Sparassis* lineages in the NUC analysis (FIG. 2).

DISCUSSION

Phylogenetic relationships within *Sparassis* species suggested in Wang et al (2004) with lsu-rDNA and ITS data were basically supported by NUC dataset in this study. Among the clamp connection producing

species, monophyly of western North American *S. radicata* was upheld (but with weak bootstrap support of 51%), and Asian *S. latifolia* and European *S. crispa* received respectively high (98%) and moderate (67%) bootstrap support. Although relationships among these closely related species were not supported by bootstrap values the *S. radicata* and *S. latifolia* clade (FIG. 8 in Wang et al 2004) and the *S. radicata* and *S. crispa* clade are consistently resolved (FIG. 2b). *Sparassis cystidiosa* was suggested to represent the earliest diverging *Sparassis* species based on an rDNA phylogeny (Desjardin et al 2004), and this is supported here with combined data from three nuclear genes (FIG. 2b). There is virtually no sequence divergence in the *atp*6 gene among clamp producing *Sparassis* species. The nuclear gene phylogeny in this study strongly supported *S. latifolia* as a separate species from *S. radicata* and *S. crispa*, but the *atp*6 phylogeny did not resolve the three species as expected. Nevertheless the *atp*6 phylogeny agreed with the nuclear gene phylogeny with regard to the relationships among clampless *Sparassis* species from different geographical regions and supported the divergence of the North American *S. spathulata* from European *S. brevipes* and *S. miniensis* nom. prov.

Because significant divergence of nuclear genes has been observed among *S. latifolia*, *S. crispa* and *S. radicata*, the highly conserved *atp*6 genes in these fungi could be evidence of clonal inheritance within partly overlapping populations. A decreased mating rate among the three related groups would enhance genetic drift of genes dominant in the population and diminish polymorphism, and a dominant copy of mitochondrial genes would be maintained. On the other hand quick changes in mitochondrial genes would be observed within small and separated populations, which have a less strict sexual incompatibility. Mating behavior may not affect the nuclear genes to this extent, because genetic changes from both partners can be fixed through sexual recombination. Multiple factors are involved in mating compatibility of *S. radicata*, and successful mating was shown to be more likely between distant isolates (Martin and Gilbertson 1976). However we cannot exclude the possibility that substitution rates of the *atp*6 gene are extremely low among clamp connections producing species in *Sparassis*. Data from other mitochondrial genes in *Sparassis* species are not available for comparison, and the uniformity of the *atp*6 gene among some *Sparassis* species is not necessarily evidence of homogenization of mitochondrial genomes of those fungi. Another explanation for the uniformity of the *atp*6 gene among clamp producing *Sparassis* species is that these fungi have been isolated geographically through recent radiation events and hybridization among them still exists but at a very low frequency.

Robinson et al (2001) studied the *atp*6 phylogeny of *Agaricus* species, and their results were similar to ours. Close relationships among *A. bisporus*, *A. subfloccosus* and *A. superonatus* were supported by rDNA data, and these taxa exhibited little distance in the *atp*6 phylogeny while several other *Agaricus* species had comparatively long internal and terminal branches (Robinson et al 2001). Unfortunately there is no rDNA phylogeny of all *Agaricus* species sampled by Robinson et al (2001), and neither is there information about the mating types of these fungi.

Phylogenetic analyses have been used widely in study of population to phylogenetic classification of higher-level taxa of fungi. Theoretically different substitution rates are expected in genes between populations of different mating behaviors and traits of mating behavior should be traceable in population level phylogenies. Our study demonstrates incongruence between nuclear gene phylogeny and mitochondrial *atp*6 gene phylogeny within three closely related *Sparassis* species, which is associated with the presence or absence of clamp connections. We are currently studying the mating behavior, distribution and colonization strategies of these fungi.

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