Mycologia, 87(5), 1995, pp. 618–638.

Phylogenetic diversity in shiitake inferred from nuclear ribosomal DNA sequences[1]

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Abstract: Phylogenetic relationships of the edible shiitake mushroom (*Lentinula*, Tricholomataceae) were studied using DNA sequences from the internal transcribed spacers (ITS) of nuclear ribosomal DNA. The ingroup consisted of seven isolates of *L. edodes* from Japan and Thailand, nine isolates of *L. lateritia* from Borneo, Papua New Guinea, and Tasmania, and five isolates of *L. novaezelandieae* from New Zealand. These species designations are based on morphological species concepts in *Lentinula*. However, because *Lentinula* isolates from throughout Asia-Australasia are mating compatible, some authors treat all of these as belonging to *L. edodes*. The outgroup included two isolates of *L. boryana* from Mexico. Parsimony, distance, and maximum likelihood analyses were performed, with various combinations of taxa, characters, and character codings, and bootstrap and decay index measures of robustness. Alternate topologies were evaluated in terms of tree lengths, maximum likelihood ratios, and Templeton's nonparametric test of parsimony. Results suggest that there are four independent lineages of shiitake in Asia-Australasia, which provides partial support for the morphologically based species concepts. *Lentinula novaezelandieae* and *L. edodes sensu stricto* were supported as monophyletic, but *L. lateritia* appears to be paraphyletic. A corollary of this is that the morphology of *L. lateritia* should be plesiomorphic for shiitake, which seems plausible based on outgroup comparison. In general, there is a strong correlation between the geographic origins of the isolates and the lineages supported. Biogeographic interpretation of ITS trees suggests that the ancestral area for shiitake in Asia-Australasia is in the South Pacific, which was the most phylogenetically diverse area examined. Phylogenetic analyses including previously published ITS2 sequences of three shiitake isolates of unknown origin placed the unknown isolates in a group of *L. edodes sensu stricto* isolates from Japan and Thailand, which suggests that the unknown isolates are from northeast or continental Asia. ITS-based cladograms have points of agreement as well as disagreement with previously published mitochondrial DNA-based dendrograms from a subset of the isolates used in this study. Discrepancies between the ITS and mtDNA trees could mean that the nuclear ITS and the mtDNA have different evolutionary histories. However, comparison of mtDNA and ITS data are complicated by the fact that the mtDNA data are based on pairwise distances, whereas the ITS data are discrete. Practical implications of the results for shiitake breeding and conservation are discussed.

Key Words: biogeography, conservation, edible fungi, ITS, *Lentinula*, phylogeny, shiitake

INTRODUCTION

Shiitake, also known as xianggu or black forest mushroom, is an edible, wood-decaying basidiomycete that has been cultivated in east Asia for approximately one thousand years (Chang and Miles, 1987). Today, the annual volume of commercial shiitake production is exceeded only by that of the button mushroom, *Agaricus bisporus* (Lange) Imbach and the oyster mushroom, *Pleurotus ostreatus* (Jacq.: Fr.) Kummer *sensu lato* (Chang, 1994). Shiitake is abundant in the wild, and natural isolates have provided the raw material for developing cultivars for the highly competitive shiitake industry (Ellingboe, 1993). In recent years, mushroom breeders have started to explore novel techniques for improving strains, including protoplast fusion and genetic transformation (Anderson, 1993; Peberdy and Fox, 1993). In addition to applied agricultural research, shiitake has been the subject of basic investigations of cytology, genetics, wood decay, etc. (e.g., Arima and Morinaga, 1993; Murakami and Takemaru, 1985; Nakai, 1986; Tsuneda et al., 1991).

Considering the attention that shittake has received,

Accepted for publication May 25, 1995.

[1] This is publication number 302 of the Tottori Mycological Institute.

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it is rather surprising that its classification remains unsettled (for reviews, see Hibbett, 1992, Ito and Imai, 1925, and Pegler, 1983). Much of the taxonomic literature on shiitake has been based on collections from Japan, which are presently recognized as *Lentinula edodes*. Nevertheless, the range of the genus *Lentinula* in Asia-Australasia extends far beyond Japan, to New Zealand and Tasmania in the south, and at least to the Himalayan regions of Bhutan, Nepal, and India in the west (Pegler, 1983). The actual western limit of the range is questionable. *Lentinula edodes* has been reported from Kazakhstan (Samgina, 1981), which lies northwest of Pakistan and Afghanistan. The report from Kazakhstan, however, states that the mushrooms were growing on conifer wood, whereas shiitake (literally, "oak mushroom" in Japanese) is usually found on Fagaceae, such as *Quercus, Castanopsis,* and *Lithocarpus* (Pegler, 1983). Thus, it is possible that the mushrooms from Kazakhstan were misidentified. As used here, the informal term "shiitake" encompasses all Asian-Australasian populations of *Lentinula.*

Shiitake has been classified in no less than 12 genera (Hibbett, 1992; Pegler, 1983). In the latter half of the 20th century, most mycologists have followed Singer (1936, 1941, 1986), who placed shiitake in *Lentinus.* However, Pegler (1975, 1983) transferred shiitake into *Lentinula* (Tricholomataceae, Collybieae), primarily on the grounds that shiitake is monomitic, whereas *Lentinus s. str.* is dimitic. Pegler's generic classification for shiitake is supported by molecular phylogenetic studies which suggest that shiitake is closely related to *Collybia,* but not to *Lentinus s. str.* (Hibbett and Vilgalys, 1993; Hibbett and Donoghue, 1995; Molina et al., 1992). In spite of these arguments, shiitake is still frequently referred to as *Lentinus edodes* (Berk.) Singer (e.g., Chang, 1994).

Both morphological and biological species concepts have been applied in *Lentinula.* Not surprisingly, this has resulted in disagreements over species limits (Hibbett, 1992). Pegler's (1983) monograph of *Lentinula* includes five morphologically defined species: *L. boryana* (the type species), *L. guarapiensis* (Speg.) Pegler, *L. lateritia, L. novaezelandieae,* and *L. edodes. Lentinula boryana* occurs in Central America, northern South America, and the Gulf Coast states of North America. *Lentinula guarapiensis* is known from only a handful of collections from Paraguay (Pegler, 1983). Neither is commercially important, although *L. boryana* is reported to be eaten (Pegler, 1983). These American taxa have been placed in various genera at one time or another (Pegler, 1983), but there seems to have been little argument regarding their validity as distinct species. In contrast, the limits of the Asian-Australasian species have been the subject of much debate. The characters that Pegler used to delimit species of shiitake are derived primarily from macromorphology and are corroborated by geographic distribution. In Pegler's key (1983), *L. edodes* is distinguished by a thick-fleshed pileus with fibrillose squamules or areolate cracks, and is limited to eastern Asia; *L. lateritia* is distinguished by a thin-fleshed, smooth pileus and slender stipe, and is limited to southeast Asia and Australasia; *L. novaezelandieae* is distinguished by a covering of fibrillose squamules on the stipe and narrow spores, and is limited to New Zealand. Hongo (in Kobayashi et al., 1973) also employed a morphological species concept, but considered collections of *Lentinula* from Japan and Papua New Guinea (PNG) to be conspecific because of their similarity in anatomical features. Nevertheless, he noted their macroscopic differences (Hongo, 1973). Other workers have suggested that the range of morphological variation among Japanese shiitake populations encompasses that among all species of shiitake *sensu* Pegler (Shimomura et al.,1992; A. Tsuneda, unpubl. results). If only one species of shiitake is to be recognized, nomenclatural priority dictates that it be called *L. edodes* (Berkeley, 1878).

The biological species concept has had a strong impact on *Lentinula* taxonomy (e.g., Komatsu and Kimura, 1968; Mori et al., 1974; Petersen, 1994; Tokimoto et al., 1973). The most comprehensive mating study in *Lentinula* to date is that of Shimomura et al. (1992), who found interstrain compatibility among isolates from Japan, Thailand, Borneo, Nepal, PNG, and New Zealand. The isolates that Shimomura et al. tested represent all three of Pegler's morphological species of Asian-Australasian *Lentinula.* Thus, based on mating criteria, some mycologists now regard all *Lentinula* populations in Asia-Australasia as *L. edodes* (e.g., Fukuda et al., 1994). Attempts to cross *L. boryana* from Mexico and *L. edodes* have been unsuccessful (Mata and Guzman, 1989). As far as we are aware, *L. guarapiensis* has never been cultured and therefore has never been used in mating studies.

Application of a phylogenetic species concept (Donoghue, 1985) in *Lentinula* was advocated by Hibbett (1992). However, evolutionary relationships within *Lentinula* are poorly understood and it has not yet been possible to delimit phylogenetic species. The morphological characters used by Pegler (1983) to define species in *Lentinula* might be useful in constructing a phylogenetic hypothesis for the genus. However, other than Pegler's monograph (1983), there has been no rigorous assessment of the variation of morphological characters within and among putative morphological species. Genetic and physiological characters may also help resolve phylogenetic species in *Lentinula.* A number of studies have assessed the distribution of genetic variation in *Lentinula* (Hibbett, 1992; Royse and Nicholson, 1993), but many of these have

focused on cultivars derived from northeast Asian stocks, often with an eye to strain typing, and have not been able to address species limits. Such studies have analyzed isozymes (e.g., Ohmasa and Furukawa, 1986; Royse and May, 1987), restriction fragment length polymorphisms (RFLPs, Kulkarni, 1991; Molina et al., 1992), randomly amplified polymorphic DNA (e.g., Kwan et al., 1992), and mating type alleles (Tokimoto et al., 1973).

Fukuda and Tokimoto (1991) and Fukuda et al. (1994) also studied the distribution of genetic variation in *Lentinula*, but their studies used large samples of broadly distributed, wild-collected isolates and are therefore especially relevant to the species controversy in shiitake. Using isozyme data, Fukuda and Tokimoto (1991) produced a dendogram that grouped 93 isolates into three clusters: 1) China and Japan (81 isolates), 2) PNG (seven isolates), and 3) New Zealand (five isolates). These groupings are perfectly correlated with the distribution of Pegler's (1983) morphologically defined species. Fukuda et al. (1994) examined RFLPs in mitochondrial DNA (mtDNA) in 51 isolates. The dendrograms derived from mtDNA RFLPs grouped the isolates into five major clusters: 1) Thailand (one isolate), 2) Japan (36 isolates), 3) New Zealand (four isolates), 4) PNG (three isolates), and 5) PNG (four isolates), Japan (two isolates), and Borneo (one isolate). The higher-order relationships of these clusters to each other were poorly resolved. The major conflict between the mtDNA trees and Pegler's classification is that the mtDNA topologies split the PNG isolates into two distinct clusters, one of which contained a mixture of PNG and Japanese isolates.

The goal of the present study was to use DNA sequences to infer phylogenetic relationships in shiitake. An understanding of phylogeny in *Lentinula* would have implications for species-level classification and biogeographic hypotheses, and could also help guide breeding programs and conservation efforts. At present, the only published DNA sequences for *Lentinula* are those of Kwan et al. (1992), who presented internal transcribed spacer 2 (ITS2) sequences of nuclear ribosomal DNA (rDNA) from three isolates of *L. edodes*. The low level of variation that Kwan et al. found suggested that ITS2 sequences are too conservative for strain typing in *L. edodes*. However, a gene that has appropriate variation for differentiating individual cultivars would probably be too variable for the species and population level analyses intended here. Therefore, for this study we also chose to examine the ITS region (both ITS1 and ITS2), which has previously been informative for phylogenetic problems at low taxonomic levels in fungi (e.g., Gardes et al., 1991; Baura et al., 1992; O'Donnell, 1993). This region was also selected because a nuclear gene phylogeny would complement the previous analyses of the mitochondrial genome (Fukuda et al., 1994).

MATERIALS AND METHODS

Material examined.—The ingroup consisted of 22 wild isolates of *Lentinula* from Japan, Borneo, Thailand, PNG, Tasmania, and New Zealand (TABLE I). Based on their geographic distribution, these isolates represent all three of Pegler's (1983) Asian-Australasian *Lentinula* species (TABLE I). Fourteen of the isolates that we used are from the Tottori Mycological Institute (TMI) culture collection and are a subset of the isolates used by Fukuda et al. (1994) in their mtDNA RFLP study. Ten of the TMI isolates that we used were also included in Fukuda and Tokimoto's (1991) isozyme study. The three sequences of ITS2 that were previously published by Kwan et al. (1992) were included in some analyses. The isolates from which these sequences were derived were identified as *L. edodes* strain nos. L74, L76, and L88, and were said to have been received from a source in Hong Kong (Kwan et al., 1992, p. 166), but no location data or morphological descriptions were published.

The outgroup consisted of two isolates of *L. boryana* from Mexico (TABLE I). Morphological characters that support monophyly of *Lentinula* were discussed by Pegler (1975, 1983). A close relationship between *L. boryana* and *L. lateritia* was also supported by an analysis of mitochondrial small subunit rDNA sequences (Hibbett and Donoghue, 1995). Nevertheless, there have been no critical analyses of the monophyly of *Lentinula* relative to other collybioid fungi, or of relationships between populations of *Lentinula* in the Americas and those in Asia-Australasia. Until such studies have been performed our outgroup choice must be regarded as provisional.

Culturing and molecular techniques.—Cultures were maintained on 1.5% malt-extract agar or potato dextrose agar. Cultures for DNA isolation were grown on liquid MYG media (1% malt extract, 1% yeast extract, 0.4% glucose) for 2–3 wk, harvested, and freeze-dried. DNAs were isolated with a SDS miniprep based on the method of Raeder and Broda (1985). Crude DNA preparations were usually digested with RNAase, followed by chloroform extraction and ethanol precipitation.

The polymerase chain reaction (PCR) was used with primer pair ITS4-ITS5 (White et al., 1990) to amplify the region containing ITS1 and 2 and the 5.8S rDNA. PCR products were purified using GeneClean II (Bio 101) and sequenced with DyeTerminator cycle sequencing kits (Applied Biosystems). Sequencing reactions were carried out using primers ITS3, ITS4,

TABLE I. Material examined

Species[a]	Culture number[b]	Origin	Isolate code	Genbank accession
Lentinula boryana (Berk. & Mont.) Pegler	R39	Mexico	MEX1	U33077
	R52	Mexico	MEX2	U33078
L. edodes (Berk.) Pegler	TMI-571	Japan	JPN1	U33088
	TMI-646	Japan	JPN2	U33089
	TMI-818	Japan	JPN3	U33091
	TMI-941	Japan	JPN4	U33092
	TMI-951	Japan	JPN5	U33093
	TMI-1148	Japan	JPN6	U33080
	TMI-1633	Thailand	THL	U33087
L. lateritia (Berk.) Pegler	TMI-689	Borneo	BOR	U33090
	TMI-1476	PNG	PNG1	U33083
	TMI-1485	PNG	PNG2	U33084
	TMI-1499	PNG	PNG3	U33085
	TMI-1502	PNG	PNG4	U33086
	D92-143	PNG	PNG5	U33070
	D92-145	PNG	PNG6	U33071
	D92-147	PNG	PNG7	U33072
	D92-149	PNG	PNG8	U33073
	RHP3577	Tasmania	TAS	U33076
L. novaezelandieae (Stev.) Pegler	TMI-1172	New Zealand	NZL1	U33081
	TMI-1449	New Zealand	NZL2	U33082
	N156	New Zealand	NZL3	U33074
	N210	New Zealand	NZL4	U33075
	RHP7563	New Zealand	NZL5	U33079

[a] Morphological species *sensu* Pegler (1983). Designations based on geographic distribution.

[b] R isolates from Pennsylvania State University Mushroom Culture Collection. T isolates from Tottori Mycological Institute culture collection. D isolates from personal culture collection of DSH. RHP isolates donated by Dr. Ronald H. Petersen. N isolates from New Zealand Forest Research Institute.

ITS5 (White et al., 1990), and 5.8S (5′-CGC TGC GTT CTT CAT CG-3′, R. Vilgalys, pers. comm.). We found that the commonly used primer ITS2 (White et al., 1990) is not effective for sequencing in *Lentinula* because of a single base mismatch at its 3′ end [however, it is reported to have been used successfully for PCR by Kwan et al., (1992)]. Sequencing reactions were purified using centrisep columns (Princeton Separations) and analyzed with Applied Biosystems 370A or 373A automated DNA sequencers.

Sequences were aligned initially using CLUSTAL V (Higgins et al., 1992), followed by manual adjustment. Approximate end points of the 18S, 5.8S, and 25S rDNAs were located by alignment to homologous rDNA sequences and secondary structures from *Saccharomyces* (Georgiev et al., 1981; Rubstov et al., 1980; Thweatt and Lee, 1990; Yeh and Lee, 1990).

Parsimony analyses.—Phylogenetically informative positions were determined from aligned sequences (FIG. 1). Alignment gaps, representing putative insertion-deletion (indel) sites, were coded either as "missing data" (gap = missing coding) or as character states, using a coding scheme described below (indel coding). For examples of coding under indel coding and gap = missing coding, see FIG. 2. Gap = missing coding is commonly employed (e.g., Hibbett and Vilgalys, 1993) but has been criticized on the grounds that it ignores

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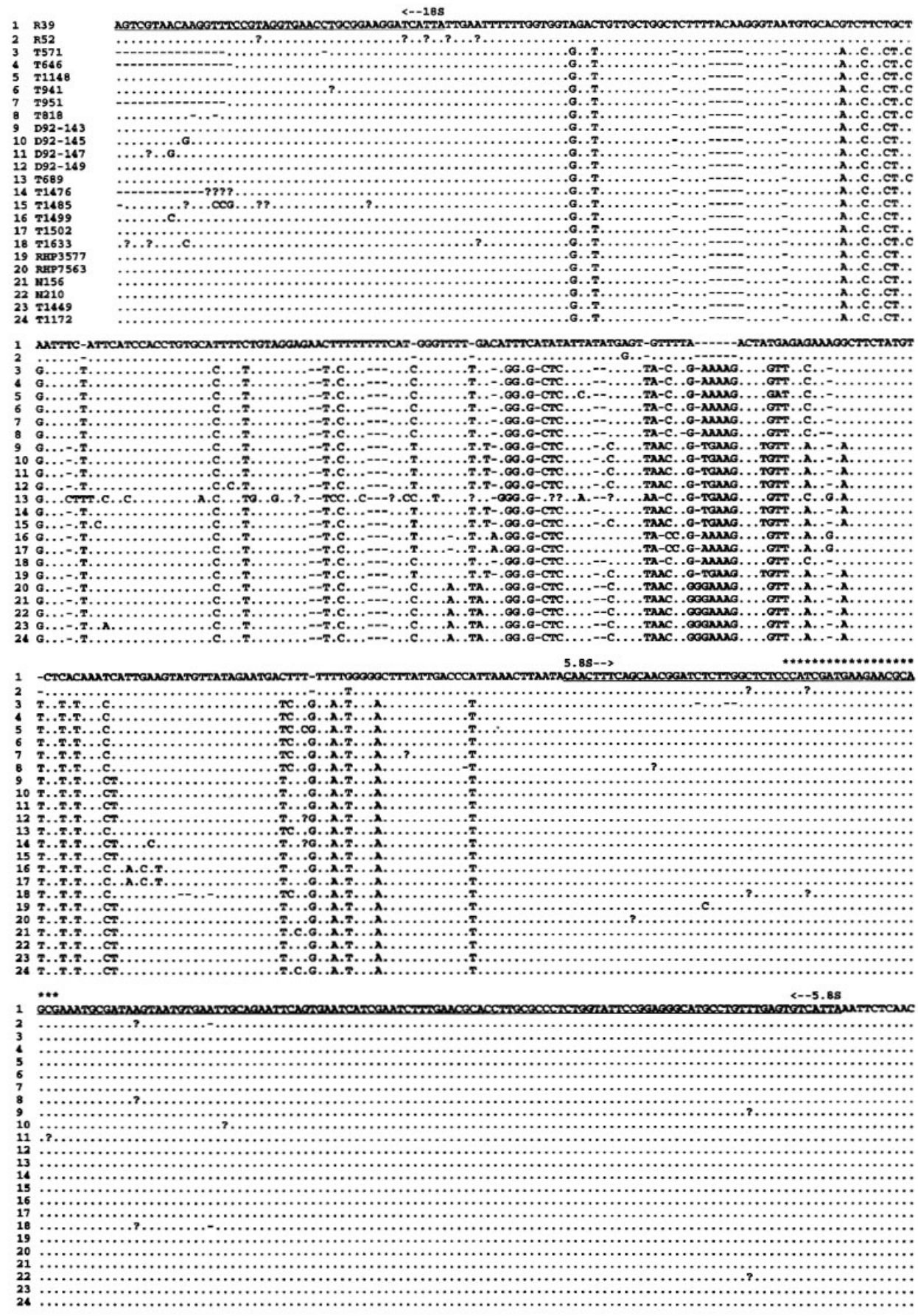
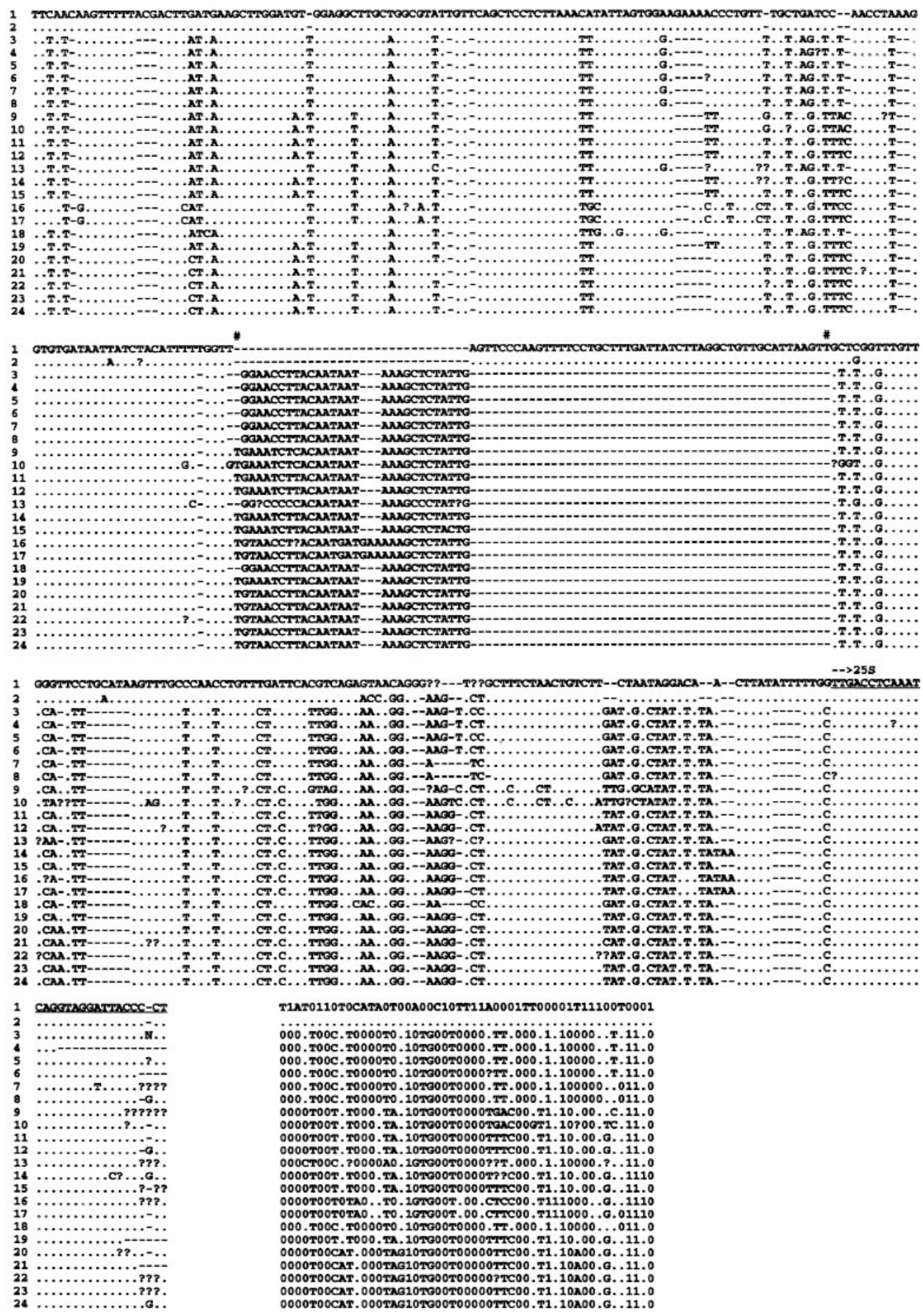
FIG. 1. Aligned sequences of ITS1, ITS2 and 5.8S rDNA of *Lentinula spp.* Isolate identification numbers given in first block of sequences. Ribosomal RNA coding sequences are underlined and labeled at their end points. Overlapping sites for primers ITS3 and 5.8S indicated by asterisks. End points of the region in ITS2 that could not be aligned between the ingroup and outgroup (offset) are indicated by # symbols. Positions identical to reference sequence (*L. boryana* R39) indicated by dots. Gaps, representing putative insertion-deletion sites, indicated by dashes. Ambiguous sites indicated by question marks. Final block of characters, after aligned sequences, are recoded indel characters.


<--18S
5.8S-->
<--5.8S



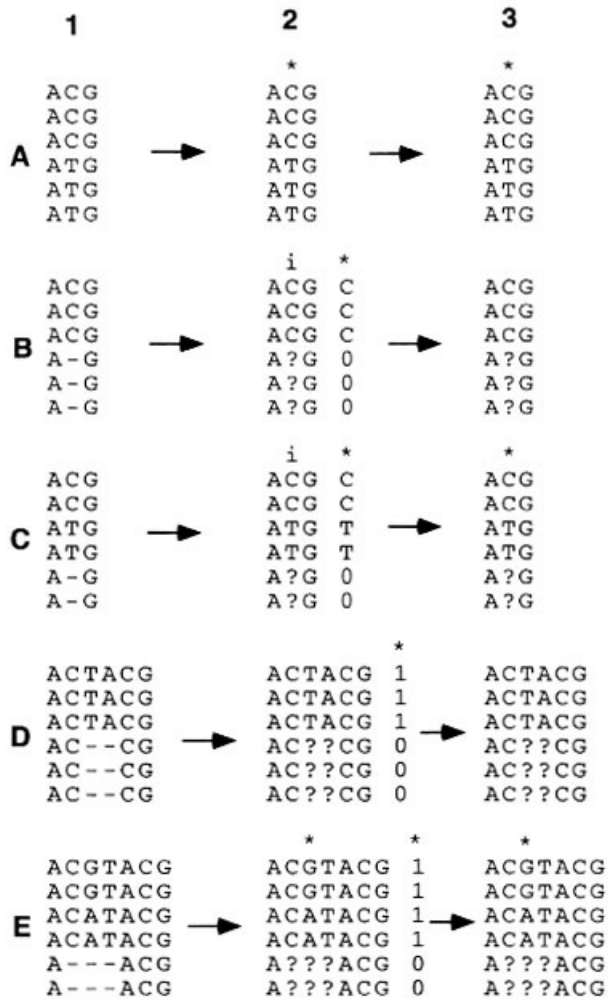


FIG. 2. Comparison of character coding from hypothetical DNA sequences under indel coding and gap = missing coding. Column 1: aligned DNA sequences, with gaps indicated by dashes. Column 2: character coding under indel coding. Column 3: character coding under gap = missing coding. Potentially informative characters recognized under indel coding and gap = missing coding are indicated by asterisks. Positions that are omitted from analyses under indel coding are indicated by "i." Recoded indel characters under indel coding are appended to sequences in column 2. Rows A–F depict different classes of sequence variation. Row A: single base position with informative nucleotide variation and no gaps. Row B: single base position with gaps but without informative nucleotide variation. Row C: single base position with informative nucleotide variation *and* informative gaps. Row D: multiple base indel with no informative nucleotide variation. Row E: multiple base indel with informative nucleotide variation at one position. From the set of sequences in column 1, six characters are recognized under indel coding, whereas three characters are recognized under gap = missing coding.

potentially informative indels (e.g., Baum et al., 1994), or can lead to errors in phylogentic estimates because of the way parsimony analyses (e.g., PAUP) may assign values where character states are unknown (Maddison, 1994). One alternative to treating gaps as missing data is to code all gaps as a fifth state (Swofford, 1993). However, under this coding, a single insertion-deletion event that results in a multiple-base indel will be overweighted because adjacent nucleotide positions (with gaps) are coded as independent characters. Indels may also be coded as separate characters, with gaps coded as missing data in the body of the alignment. The indel coding that we used is a hybrid of these approaches (FIG. 2). Steps in coding for indels, using MacClade 3.0 (Maddison and Maddison, 1993) and PAUP 3.1s (Swofford, 1993), were as follows: 1) Indels were identified from the alignments and divided into two categories: single-base indels and multiple-base indels. 2) For each single-base indel, a separate character was created that reproduced the position with the indel, with nucleotides intact, except that the gap symbol (-) was replaced by 0 (e.g., a single column in an alignment with AA--CC would be reproduced as AA00CC). These new characters were appended to the matrix at the end of the aligned sequences (FIG. 2, row B, C). 3) A character set was defined that included all the positions in the body of the aligned sequences with single-base indels (the "single indel set"). 4) For each multiple-base indel, a separate binary character (states = 0 or 1) was created and these were appended to the aligned sequences (FIG. 2, row D, E). 5) A character set (the "all indels set") was designated which combined the set of single- and multiple-base indel characters. The "all indels set" was set off at the end of the block of aligned sequences (as in FIG. 1). To run an analysis under gap = missing coding, the "all indels" character set was simply excluded (with the format command gapmode = missing data). To run an analysis under indel coding, the "all indels" character set was included, and the "single indels" character set was excluded (gapmode = missing data). In other words, under indel coding, nucleotide positions with single base indels would be scored as characters, with gaps as a fifth state; multiple-base indels would be scored as binary characters; and single nucleotide positions aligned to multiple-base indel sites would be scored as additional characters with gaps as missing data. The goals of this coding scheme were to include potentially informative indels in the analyses, avoid overweighting single insertion-deletion events, and preserve phylogenetically informative nucleotide variation in sequences that aligned to gaps. The configuration of the data matrix (which is available from DSH on request) facilitated exploration of the sensitivity of phylogenetic hypotheses to indels, which are

commonly observed in fungal ITS sequences (e.g., Baura et al., 1992; Carbone and Kohn, 1993; Curran et al., 1994; Gardes et al., 1991; O'Donnell, 1992). For examples of other indel coding methods, see Baldwin (1995), Bruns et al. (1992), and Wojciechowski et al. (1993).

Parsimony analyses were performed using PAUP 3.1s (Swofford, 1993) running on Macintosh computers (mostly on a Quadra 800). The main analyses were performed with indel coding and gap = missing coding. Because of the number of taxa, we were limited to heuristic searches (10 random taxon addition sequences, TBR branch swapping, MAXTREES unrestricted, MULPARS on). Relative robustness of individual clades was assessed by the bootstrap (Felsenstein, 1985a) (100 or 1000 replicates, simple addition sequence, TBR branch swapping, MAXTREES = 100) and the decay index (Bremer, 1978; Donoghue et al., 1992). The decay index was only calculated under indel coding. For the first and second decay index, all trees up to two steps longer than the most parsimonious trees were saved and semistrict consensus trees were generated. For clades with a decay index greater than 2, the decay index was calculated using inverse monophyly constraint trees (Swofford, 1993) and strict consensus trees. In most analyses, all transformations were weighted equally. To explore the effects of incorporating a transition:transversion bias, we also ran a series of analyses with gap = missing coding and stepmatrices specifying transition:transversion biases of 1:5, 1:10, or 1:30 (no bootstrap or decay index, other settings as in main analyses). Transition:transversion bias was not incorporated into analyses under indel coding because of the difficulty of assigning relative weights to transitions, transversions, and length mutations.

A second set of analyses was performed using Lundberg (1972) rooting with indel coding. In this method, an unrooted topology is constructed using ingroup taxa only, and is then rooted by attaching the outgroup to an internode such that parsimony is maximized. Lundberg rooting in PAUP requires designation of ancestral states for all characters, for which we used the sequence of *L. boryana* isolate R39 (using the "vector" option in the ANCSTATES command). The goal of these analyses was to determine whether inclusion or exclusion of the outgroups had an effect on ingroup topology or robustness, as measured by the bootstrap (100 replicates, all other settings as in main analyses).

A final set of parsimony analyses was performed using the three ITS2 sequences published by Kwan et al. (1992), which were manually aligned to the sequences of the TMI isolates and the outgroups. ITS1 and 5.8S rDNA sequences were omitted from these analyses. By comparing the rDNA sequences of the Kwan et al. isolates to those of the TMI isolates (for which location data are known), we hoped to estimate the geographic origin of the Kwan et al. isolates. Separate analyses were performed with the Kwan et al. sequences included or excluded, under indel coding. Because these analyses had only 16–19 taxa, we were able to use branch and bound which assures that all most parsimonious trees are found (Swofford, 1993). Robustness was assessed by bootstrapping (100 replicates).

Distance and maximum likelihood (ML) analyses.—Distance analyses were performed using PHYLIP 3.5p (Felsenstein, 1993), running on a Macintosh Quadra 800. Aligned sequences in Nexus format were transformed into PHYLIP format using MacClade 3.0 (Maddison and Maddison, 1992). Pairwise distance matrices were generated from the aligned sequences using the DNADIST program of PHYLIP with Kimura (1980) and ML (Felsenstein, 1993) models of nucleotide substitution. Under both models a 2:1 transition: transversion bias was assumed. The Kimura model assumes equal frequencies of all nucleotides, but the ML model allows for varying frequencies, which in this case were determined empirically from the observed base composition of the sequences. Pairwise distances estimated with the Kimura and ML models are based on nucleotide substitutions at all positions, and do not take indels into account. Phylogenies were estimated from the distance matrices using Fitch-Margoliash (FM, Fitch and Margoliash, 1967) and Neighbor-Joining (NJ, Saitou and Nei, 1987) analyses using the FITCH and NEIGHBOR programs, respectively. FITCH analyses used global rearrangements and 25 random taxon addition sequences. Trees generated by both FITCH and NEIGHBOR can have unequal branch lengths, which allows for differences among evolutionary rates across lineages. Bootstrapping was performed with ML distances and FM and NJ analyses (100 replicates, using the SEQBOOT, DNADIST, FITCH, NEIGHBOR, and CONSENSE programs).

Maximum likelihood (ML) analysis was performed using fastDNAml 1.0 (Olsen et al., 1992) on a PowerMacintosh 7100 (empirical base frequencies, global rearrangements, transition:transversion ratio = 2). FastDNAml implements a modification of the ML algorithm used in the DNAML program of PHYLIP (Felsenstein, 1993).

Evaluation of alternate ingroup rootings.—PAUP was used with topological constraints to generate trees that forced alternate rootings of the ingroup topology. Constraint trees were constructed that forced the root to be attached to a particular internode, thus implying monophyly of two groups of ingroup taxa, but no other topology was specified. Five such constraint trees were used, each of which placed the root along a branch

TABLE II. Distribution of different classes of characters between ITS1 and ITS2

Classes of characters	Number of characters ITS1	ITS2	ITS1 + ITS2
Single nucleotide positions			
—without gaps[a]	44	60	104
—with gaps and informative nucleotide variation[a]	7	8	15
—with gaps, without informative nucleotide variation[b]	12	10	22
Multiple-base indels[b]	4	15	19
Total under indel coding	67	93	160
Total under gap = missing coding	51	68	119
% informative sites—all taxa[c]	27.4	27.8	27.6
% informative sites—ingroup only[c]	11.3	16.7	41.2

[a] Scored under both indel coding and gap = missing coding.

[b] Not scored under gap = missing coding.

[c] (All characters except multiple base indels)/(average length of sequences). Excludes 18S, 5.8S, and 25S rDNA.

that was strongly supported by bootstrapping and decay index. Indel coding was used, with other settings as in the main parsimony analyses.

Constrained and unconstrained topologies were compared by three criteria: tree length (given by PAUP), log-likelihood ratios (Felsenstein, 1993; Kishino and Hasegawa, 1989), and Templeton's (1983) nonparametric test for parsimony. Log-likelihood ratios were calculated using the DNAML program in PHYLIP (Felsenstein, 1993). This method compares the difference in log-likelihood for individual sites (aligned nucleotide positions) between the most likely tree and a suboptimal tree. If the mean of the log-likelihood differences is 1.96 standard deviations or greater, then the tree with the lower likelihood is considered a significantly worse hypothesis than the most likely tree. MacClade was used to transform tree topologies generated by PAUP into PHYLIP format, which necessitated arbitrary resolution of polytomies in trees generated by PAUP.

Templeton's (1983) test makes pairwise comparisons of tree topologies and asks if, given a single dataset, a suboptimal tree suggests a significantly less parsimonious hypothesis of character evolution than the shortest tree. The protocol we followed was outlined by Larson (1994) for comparison of trees derived from molecular vs. morphological characters, but it is equally applicable to comparison of optimal and constrained topologies: 1) Constrained topologies were compared to the most parsimonious tree using the "compare trees" function in MacClade, and characters that changed a different number of times on the two trees were identified. 2) Characters with different numbers of changes on the two trees were ranked by the absolute value of the difference in the number of steps on the two trees, with tied ranks receiving the midpoint rank if rankings were consecutive. 3) Ranked characters were segregated based on whether the number of changes on the most parsimonious tree minus the number of changes on the constrained tree was positive or negative. 4) Positive and negative ranks were summed separately and the sum with the smaller absolute value was taken as the test statistic T_s. 5) The test was deemed significant if the values of T_s were less than the critical values for the Wilcoxon rank sum (Rohlf and Sokal, 1981, Table 30), with n equal to the number of characters with different numbers of changes on the two trees under comparison. Following Felsenstein (1985b) and Larson (1994), the probabilities used here are for a two-tailed test (TABLE III).

Evaluation of congruence between mtDNA RFLPs and rDNA sequences.—Assessing phylogenetic congruence of the mtDNA RFLP data of Fukuda et al. (1994) and the rDNA sequence data was complicated by the fact that the mtDNA data are represented as a matrix of pairwise distances, whereas the rDNA data are discrete. If the mtDNA data had been in discrete form (e.g., restriction site presence/absence, sequence data), then we would have been able to explore combined vs. independent parsimony analyses of the mtDNA and ITS data (cf. Barrett et al., 1991, Bull et al., 1993, and de Queiroz, 1993). This would have also permitted us to perform reciprocal evaluations of mtDNA and ITS trees and data (e.g., Rodrigo et al., 1993). Given the constraints of the data formats, we followed two approaches to assess congruence of the ITS and mtDNA:

1) The pairwise distances based on mtDNA RFLPs published by Fukuda et al. (1994) were reanalyzed using FM and NJ analyses in PHYLIP, including only the 14 isolates common to both the mtDNA and rDNA datasets. The topology derived from the mtDNA RFLPs was used to guide construction of a constraint tree that forced monophyly of the major clusters suggested by the mtDNA analyses, with no other topology specified. Constrained and unconstrained parsimony analyses of the rDNA sequences were performed, using a reduced datset that included the 14 isolates common to the mtDNA study and the present study, plus the outgroups (indel coding, other settings as in analyses of the entire rDNA dataset). Results of constrained and unconstrained analyses were evaluated by the same criteria as the analyses of alternate ingroup rootings (tree length, log-likelihood ratios, and Templeton's nonparameteric test of parsimony).

2) Dendrograms from the FM and NJ analyses of mtDNA RFLPs were used to code a single mtDNA character with five unordered character states, corresponding to the major clusters discussed above. Parsimony analyses of the reduced rDNA dataset were performed with the mtDNA character included or excluded, and the resulting topologies were compared. It has previously been shown that in certain cases the addition of a small number of characters can change topologies derived from large datasets, particularly in areas of the cladogram where character support is weak (Donoghue and Sanderson, 1992). Therefore, we thought it was worthwhile to see whether inclusion of the single mtDNA character in the analysis would affect the topology, even though it is far outnumbered by the rDNA sequence characters.

Biogeography.—Geographic origins of the isolates were mapped onto the ITS topologies in MacClade using parsimony. Geographic distribution was coded as a single character, with 6 unordered states: South Pacific (Borneo and PNG), Thailand, Japan, New Zealand, Tasmania, and Mexico.

RESULTS

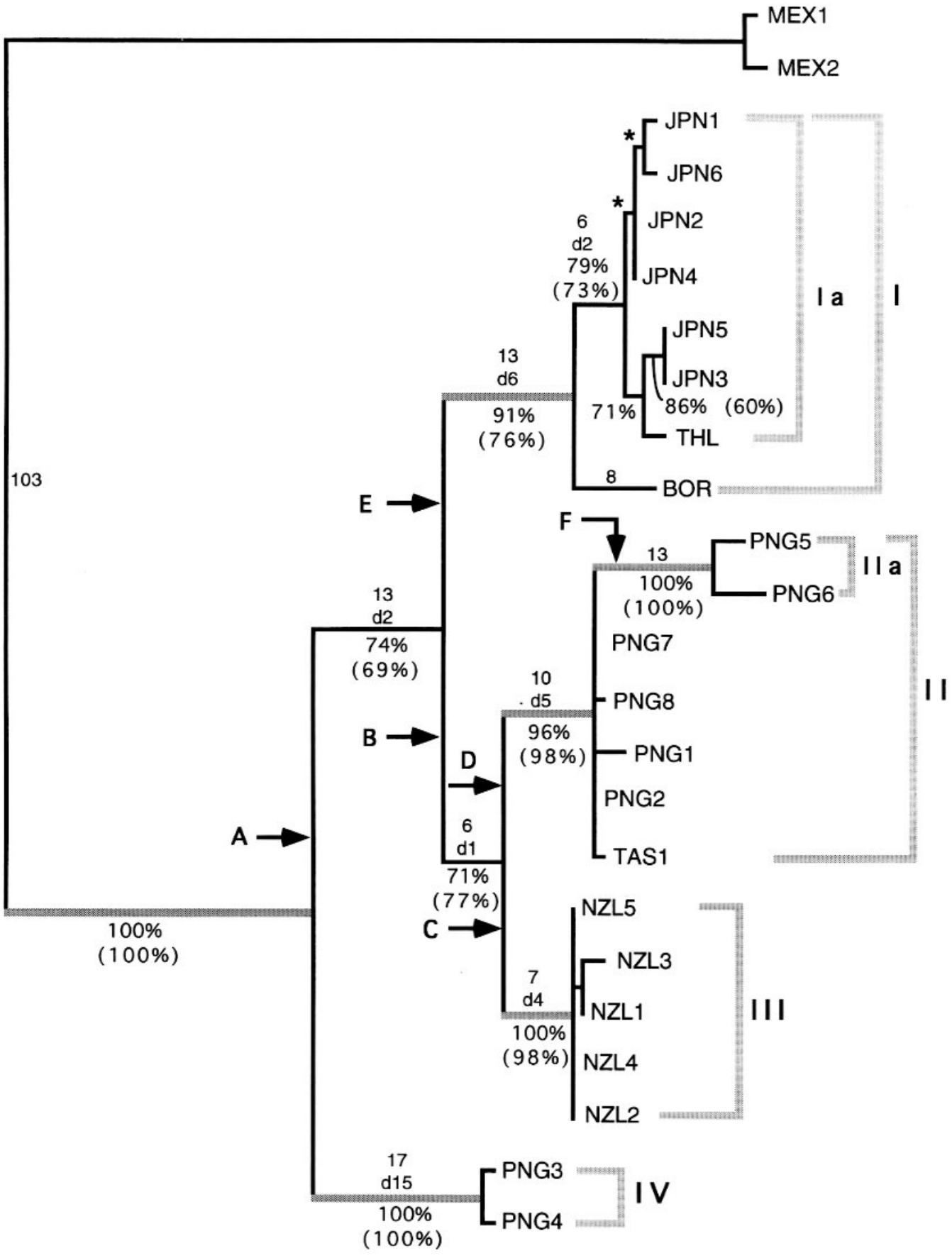
Alignment.—Aligned sequences (excluding sequences of Kwan et al., 1992) are shown in FIG. 1. Virtually all positions were readily alignable across both the outgroup and the ingroup, except for one region in ITS2 that was unambiguously alignable only within the ingroup, or within the two *L. boryana* isolates. This region consisted of 49 base pairs (bp) in *L. boryana* and 29–33 bp in the ingroup (FIG. 1, see region bracketed by # symbols). The approximate length of ITS1 was 234 bp in *L. boryana* and 229 bp in the ingroup; ITS2 was 314 bp in *L. boryana* and 276 bp in the ingroup. The 5.8S rDNA was 157 bp. Base composition of the entire region, from DNAML output, was approximately A = 25%, C = 17%, G = 21%, T = 37%.

Parsimony analyses.—Under gap = missing coding, there were 119 characters, whereas under indel coding there were 160 characters (TABLE II). The difference is due to two classes of characters that are ignored under gap = missing coding: 1) positions that have a potentially informative distribution of gaps, but no informative nucleotide variation (22 characters; e.g., FIG. 2, row B), and, 2) multiple-base indels (19 characters; e.g., FIG. 2, row D). Distribution of the different classes of characters between ITS1 and ITS2 is summarized in TABLE II. There was no informative variation in the 5.8S rDNA or the flanking sequences of 18S rDNA (about 30 bp) and 25S rDNA (about 25 bp).

Parsimony analysis of the entire dataset under indel coding yielded 30 equally parsimonious trees (223 steps, CI = 0.871, FIG. 3), whereas analysis under gap = missing with no transition:transversion bias coding yielded 541 trees (156 steps, CI = 0.897). Despite the large difference in the number of equally parsimonious trees produced under the different coding schemes, there is no positive conflict between the strict consensus trees of the two sets of analyses (in each consensus tree there is one node that resolves part of a polychotomy in the other consensus tree). Analyses with gap = missing coding and transition:transversion bias weighted at 1:5, 1:10, or 1:30 each yielded 150 trees, the strict consensus of which was identical to the strict consensus of the trees produced under gap = missing coding with no transition:transversion bias.

Under indel coding, there were four well-supported groups, as measured by bootstrapping and decay index, within which there was generally little resolution. These clades have been designated groups I–IV (FIG. 3). Group I (bootstrap 91%, decay = 6) includes six isolates from Japan, one isolate from Thailand, and one isolate from Borneo. The isolate from Borneo is the sister group of the others, which form a moderately well-supported clade (bootstrap = 79%, decay = 2), designated group Ia. Group II (bootstrap 96%, decay = 5) consists of six isolates from PNG, and one isolate from Tasmania. A clade consisting of two of the PNG isolates, designated group IIa, is strongly supported as monophyletic, but there is no other structure in group II. Group III (bootstrap 100%, decay = 4) includes five isolates from New Zealand. Group IV (bootstrap 100%, decay = 15) includes two isolates from PNG. Higher-order relationships among groups I–IV are resolved, but not very strongly supported: group I + II + III (bootstrap = 74%, decay = 2), group II + III (bootstrap = 71%, decay = 1). Under gap = missing coding, bootstrap support was almost unchanged for groups Ia, II, IIa, III, IV, I + II + III, and II + III (FIG. 3). However, support for group I fell to 76% (FIG. 3).

In the analysis of the entire dataset under indel coding, 103 of the 160 characters changed along the branch leading from the outgroup to the ingroup, which was by far the longest branch on the tree (FIG. 3). In the Lundberg-rooted analysis, there were only 74 informative characters, which is 86 fewer than in analyses with outgroups included. In other words, 51.875% of the characters included in the outgroup-rooted, global parsimony analysis of the entire dataset under indel coding are noninformative for the ingroup topology. The Lundberg-rooted analysis produced 30 equally parsimonious trees (109 steps, CI = 0.862). The strict consensus tree (not shown) was identical to that from the comparable analysis of the entire dataset (FIG. 3). Bootstrap values for groups I, II, III,


MEX1
MEX2
JPN1
JPN6
JPN2
JPN4
JPN5
JPN3
THL
BOR
PNG5
PNG6
PNG7
PNG8
PNG1
PNG2
TAS1
NZL5
NZL3
NZL1
NZL4
NZL2
PNG3
PNG4
I a
I
II a
II
III
IV
A
B
C
D
E
F
103
100%
(100%)
6
d2
79%
(73%)
13
d6
91%
(76%)
71%
86%
(60%)
8
13
d2
74%
(69%)
13
100%
(100%)
10
d5
96%
(98%)
6
d1
71%
(77%)
7
d4
100%
(98%)
17
d15
100%
(100%)

and IV were 93%, 93%, 100%, and 100%, respectively, which are similar to those from the global parsimony analysis (FIG. 3). However, support for group Ia rose to 100% in the Lundberg-rooted analysis (from 79%).

Analyses of ITS2 data from the TMI isolates plus the outgroups yielded 10 trees (145 steps, CI = 0.876) whose strict consensus is topologically compatible with that derived from analyses of the total dataset (FIGS. 3, 4). Levels of bootstrap support for the monophyly of exemplars of groups I–IV were comparable to those from analyses of the entire dataset (FIG. 4). However, strict consensus trees derived from the reduced dataset show groups I, II, and III forming a trichotomy, and thus do not positively support monophyly of group II + III. Inclusion of the three Kwan et al. (1992) sequences did not change overall relationships among the TMI isolates (12 trees, 154 steps, CI = 0.851). The three sequences from Kwan et al. (1992) were included in the group I clade, which was supported at 84% by bootstrapping (FIG. 4).

Distance and ML analyses.—Distance analyses using ML or Kimura models of nucleotide substitution and FM or NJ analyses all yielded virtually identical topologies. Groups I–IV, as defined above, were all supported as monophyletic in distance-based topologies. The major difference between results of the distance analyses and those of parsimony analyses was that in all the distance trees group I was the sister group of the rest of the ingroup, with groups II, III, and IV forming a clade (FIG. 5). Groups II and III were still supported as monophyletic in the distance trees. Thus, the ingroup topology from the distance analyses can be obtained merely by rerooting the ingroup topology from the parsimony analyses (FIGS. 3, 5). Bootstrap levels of support for groups II, IIa, III, and IV in both FM and NJ analyses of ML distances were comparable to those from parsimony analyses. However, support for group I fell to 62%, and support for group Ia rose to 100%. FastDNAml yielded a topology (not shown) that is completely compatible with the strict consensus of the maximum parsimony topologies.

Ingroup rerooting costs.—The position of the ingroup root in the parsimony trees was designated root A (FIG. 3). Five alternate placements for the root along strongly supported branches were investigated, which were designated roots B–F (FIG. 3). Parsimony analyses using topological constraints to force these rootings produced from 10 to 75 trees each, under indel coding. The rerooting costs (difference in length between most parsimonious trees and constrained trees) were +6, +8, +8, +2, and +12 steps for roots B–F, respectively (TABLE III). Single trees from each constrained parsimony analysis were input into DNAML, along with one of the most parsimonious unconstrained trees, for comparison of log-likelihood differences. The most likely input topology, estimated by DNAML, was the tree with root E. Roots A, B and D were not considered significantly worse than the most likely tree, but roots C and F were rejected (TABLE III).

The trees used in the log-likelihood test were also used in Templeton's nonparametric test, except that polytomies were not resolved. The number of characters that changed a different number of times on the most parsimonious trees vs the constrained trees ranged from 8 (root B) to 20 (root F). Roots C, D, and F were rejected ($p < 0.01$–0.05), but roots B and E were not significantly less parsimonious than the shortest tree (TABLE III).

Congruence of mtDNA RFLPs and rDNA sequences.—Reanalysis of the mtDNA RFLP distance data (Fukuda et al., 1994) with NJ or FM analysis resulted in virtually identical topologies that supported the same five major clusters as the UPGMA and NJ dendrograms of Fukuda et al. (1994). We designated these clusters mt-I–V (FIG. 6). Mt-I contained four isolates from Japan. Mt-II contained two isolates from Japan, two from PNG, and one from Borneo. Mt-III contained two isolates from New Zealand. Mt-IV contained a single isolate from Thailand. Finally, mt-V contained two isolates from PNG. Mt-III and mt-IV correspond perfectly to group III and group IV, respectively. The major discrepancy between the mtDNA and rDNA groups is that the ITS group I isolates are spread among mt-I, mt-II, and mt-V (FIGS. 3, 6).

Unconstrained parsimony analysis of rDNA sequences from the TMI isolates plus outgroups under indel coding yielded five equally parsimonious trees (198 steps, CI = 0.871). Inclusion of a single unordered character with states corresponding to the mtDNA groups had no effect on this topology. When

←

FIG. 3. Phylogram depicting one of 30 equally parsimonious trees for ITS sequences, under indel coding (223 steps, CI = 0.871). Branches marked with an asterisk collapse in strict consensus tree. Numbers without prefixes or suffixes above branches are number of character state changes along branch under ACCTRAN optimization. Numbers preceded by "d" are decay index values. Numbers with % signs are bootstrap frequencies (1000 replicates) from analyses under indel coding or gap = missing coding (in parentheses). Rooting options discussed in text are indicated by arrows with letters. Bracketed groups Ia–IV are discussed in text.

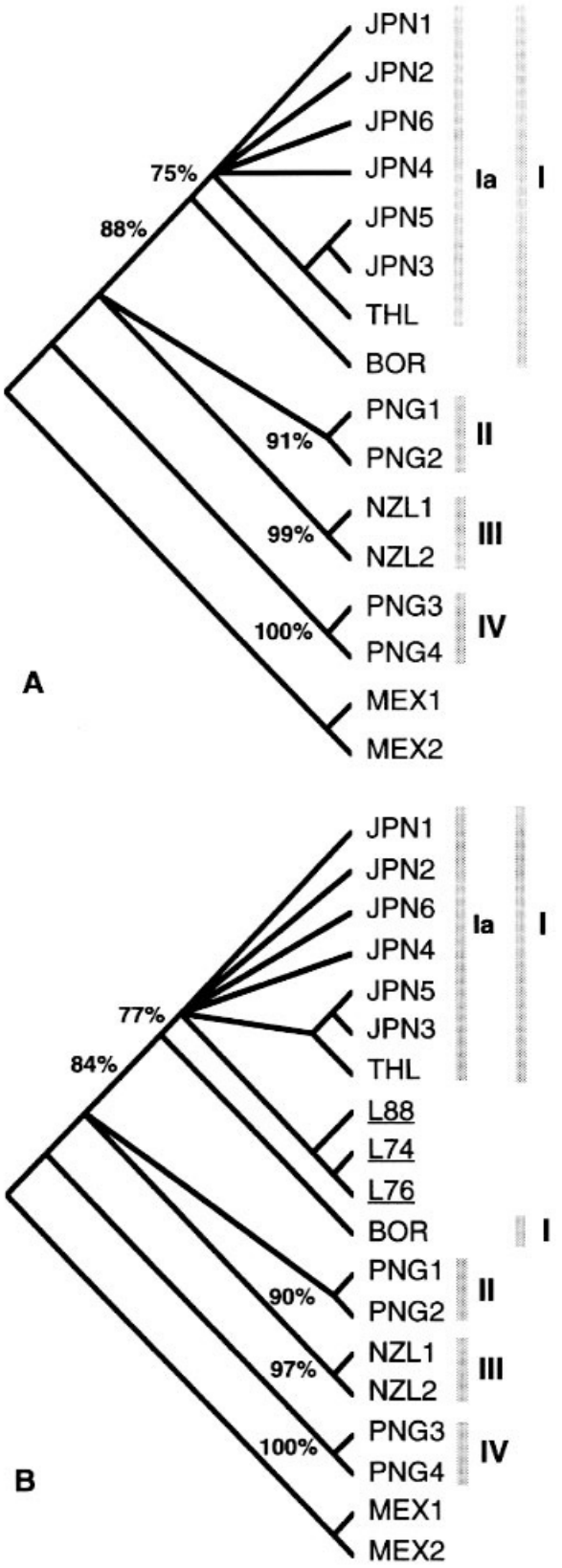


FIG. 4. Phylogenetic relationships of shiitake isolates inferred from ITS2 sequences only. Numbers by branches with % signs are bootstrap frequencies (100 replicates). Groups identified with Roman numerals Ia–IV correspond to groups Ia–IV in FIG. 3. A: TMI isolates plus outgroups only; strict consensus of 10 trees, 145 steps, CI = 0.876. B: TMI isolates plus outgroups, and sequences from Kwan et al. (1992); strict consensus of 12 trees, 154 steps, CI = 0.851. The Kwan et al. sequences (underlined) are nested in group I.

the mtDNA groups were constrained to be monophyletic (with no other topology specified) in analyses of rDNA sequences, a single tree was generated which required 17 additional steps (215 steps, CI = 0.814). The mtDNA topology is rejected by both log-likelihood ratios and Templeton's test (TABLE III).

DISCUSSION

ITS phylogeny.—The various analytical methods that we used for the rDNA sequence data each have unique tree-building algorithms and optimization criteria. In parsimony analyses, we varied the sample of taxa and characters and explored the effects of indels and transition:transversion biases on phylogenetic estimates. Throughout these analyses, certain aspects of the topologies remained constant, and we take these as our strongest phylogenetic hypotheses (FIGS. 3–5). The most robust clades were groups II, IIa, III, and IV, which were present in all analyses, and were consistently supported by over 95% of the bootstrap replicates (decay = 4–15). Groups I and Ia were also monophyletic in all analyses, but levels of support varied considerably among different analyses (bootstrap values = 62%–100%). Although groups I–IV were generally well-supported as monophyletic, there was little resolution within them (except for groups Ia and IIa). To understand relationships within groups I–IV, more rapidly evolving sequences may be useful, such as the intergenic nontranscribed spacers of rDNA (Anderson and Stasovski, 1992).

Support for group I in parsimony analyses was sensitive to the choice of character coding scheme. Group I received only moderate support under gap = missing coding (76% with outgroup rooting), whereas under indel coding it received strong support (91% with outgroup rooting, FIG. 3). This indicates that the indel characters provide considerable additional support for group I. The weakest support for group I was obtained with distance analyses (bootstrap = 62%). Both the Kimura and ML models of nucleotide substitution effectively ignore indels, which may account in part for the low support for group I in distance analyses.

Because of the extent of sequence divergence between the ingroup and outgroup, we were concerned that inclusion of the *L. boryana* isolates in global parsimony analyses could result in spurious resolution of clades in the ingroup. However, the ingroup topology

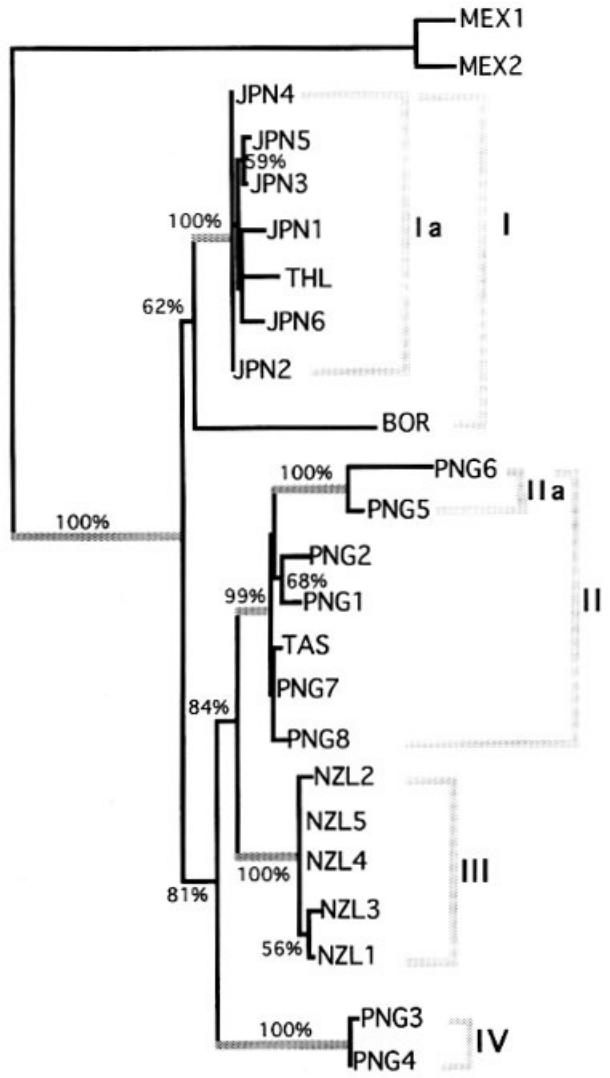


FIG. 5. Phylogenetic relationships of shiitake inferred from distance analyses of ITS sequences. Tree derived from FM analysis using ML distances. Numbers with % signs are bootstrap frequencies (100 replicates). Bracketed groups are same as in FIG. 3.

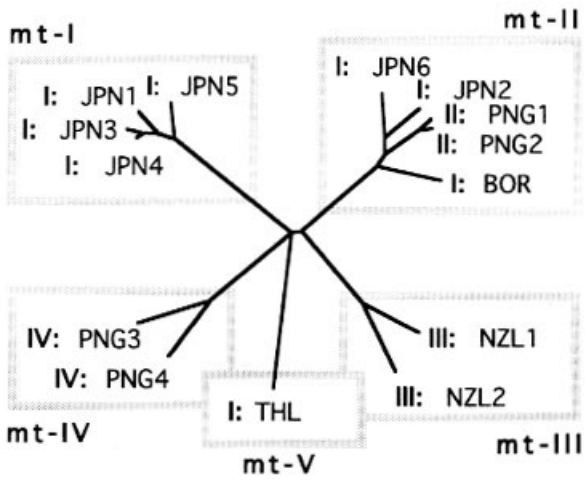


FIG. 6. Relationships of mitochondrial genome in TMI shiitake isolates based on FM analysis of distances published by Fukuda et al. (1994). Roman numerals preceding isolate numbers correspond to ITS groups identified in FIG. 3. Shaded boxes delimit putatively monophyletic mtDNA groups used to construct topological constraint tree and define mtDNA character states.

of the Lundberg-rooted analysis was identical to that of the global parsimony analysis. Thus, even though the ingroup and outgroup sequences are highly divergent, there is apparently not enough homoplasy introduced by inclusion of the outgroups to change the ingroup topology. Robustness of groups I–IV, as measured by bootstrapping, was also not strongly affected by exclusion of the outgroups (except for group Ia, which was strengthened), despite the fact that over half of the characters that are included in the global parsimony analyses are noninformative for the ingroup topology. These observations are consistent with the findings of Harshman (1994), who noted that inclusion or exclusion of large numbers of characters in a data matrix that are "irrelevant" to a particular node generally has little effect on the bootstrap support for that node.

The position of the root of the ingroup is the most problematic aspect of the rDNA topology. We examined the effect of placing the root along each of the six strongly supported internodes, designated roots A–F (FIG. 3, TABLE III). Roots C and F were rejected by both log-likelihood ratios and Templeton's test, and have rerooting costs of +8 and +12 steps, respectively. Root D was rejected only by Templeton's test, and has a rerooting cost of +8 steps. Roots A, B, and E were not significantly different by either log-likelihood ratios or Templeton's test. Root A was favored in all parsimony analyses, whereas root E was favored by distance analyses. Root B was not rejected by the Templeton or ML tests, but it is considerably less parsimonious than root A (+6 steps) and was not favored by any analyses (TABLE III). Trees with root E are only two steps longer than the most parsimonious trees. DNAML evaluation of alternate roots found the tree with root E to be the most likely. This was somewhat surprising because the tree inferred by fastDNAml had root A. Possible explanations for this result are that fastDNAml failed to find the optimal tree, or, perhaps more likely, that MacClade's arbitrary resolution of polytomies resulted in trees that are suboptimal by the likelihood criterion. We regard root A to be only marginally better supported than root E.

The phenomenon of "long branch attraction" (Felsenstein, 1978) could cause error in the placement of

TABLE III. Evaluation of alternate roots and mtDNA topology

Root	Favored by which analyses?	Tree length[a]	Templeton test results			Log likelihood (L) ratio test results			
			T_s	n	Rejected?	ln L	Difference[c]	S.D.	Rejected?
Evaluation of alternate rooting options									
A	Parsimony, fastDNAml	223	Not applicable (shortest tree)			−2328.55262	−4.06713	5.4867	No
B	None	229 (+6)	8.0	8	No	−2330.67062	−6.18513	4.8403	No
C	None	231 (+8)	12.0	12	Yes ($p < 0.02$)	−2340.41686	−15.93136	7.1903	Yes
D	None	231 (+8)	32.0	16	Yes ($p < 0.05$)	−2336.66832	−12.18283	7.3860	No
E	FM, NJ, DNAML	225 (+2)	42.0	14	No	−2324.48549	Not applicable (best tree)		—
F	None	235 (+12)	40.0	20	Yes ($p < 0.01$)	−2347.77386	−23.28836	8.7055	Yes
Evaluation of mtDNA topology[b]									
Unconstrained parsimony tree		198	Not applicable (shortest tree)			−2111.15107	Not applicable (best tree)		—
Constrained by mtDNA topology		215 (+17)	31.5	22	Yes ($p < 0.01$)	−2158.04134	−46.89027	16.1835	Yes

[a] Under indel coding. Difference in length between most parsimonious tree and constrained trees in parentheses.
[b] TMI isolates plus outgroups only.
[c] Difference in ln likelihood (L) between best tree and suboptimal tree.

the root in our parsimony analyses. Long branch attraction is most likely in situations where there is considerable inequality among lengths of branches of a (true) phylogeny. In such cases, it is possible that so many character state transformations will occur along two long branches that by chance the convergent characters will come to overwhelm the "true" phylogenetic signal. Thus, taxa that are not actually closely related can appear to be monophyletic due to convergence. In our parsimony trees, root A is placed along the longest internode in the ingroup (FIG. 3), which increases our suspicion that long branch attraction may be a factor. Measures that might help to resolve the position of the root with greater confidence, by counteracting the effects of long branch attraction, include addition of more isolates, which could increase the branch density of the ingroup topology, or examination of a region that evolves more slowly than the ITS, such as large subunit rDNA divergent domains (Bruns et al., 1991; Hibbett, 1992).

Congruence of ITS and mtDNA topologies.—In comparing the mtDNA and ITS topologies, we wish to focus only on aspects of both that are strongly supported (de Queiroz, 1993). In the case of the ITS sequence data, branch robustness within analyses and congruence between analyses provide measures of confidence and suggest that we should focus on groups I–IV. Unfortunately, the mtDNA dendrograms are based on distance measures rather than discrete characters, which makes it impossible to estimate topological robustness with the same bootstrap or decay index measures. In the absence of such robustness measures, we arbitrarily collapsed short internodes in the mtDNA dendrograms, which resulted in an unordered set of groups that are phenetically distinct (mt-I to V, FIG. 6). We think that this is a conservative interpretation of the mtDNA data, but acknowledge that there are sources of error that could bias the inferred distances. Most significant is the problem of length mutations. The pairwise distance measures published by Fukuda et al. (1994) were based on presence or absence of comigrating fragments in restriction endonuclease-digested mtDNAs, visualized by ethidium bromide staining. Gels were not blotted and probed to assess homology of fragments or to perform restriction mapping. Length mutations introduce error into this sort of analysis because single indels can affect multiple fragments (Bruns et al., 1991; Hibbett, 1992). From the sums of fragment sizes in individual mtDNA digests, Fukuda et al. (1994) estimated that the mitochondrial genome in shiitake ranges from approximately 70 to 90 kilobase pairs, which indicates that length mutations have indeed occurred. For these reasons, we view the mtDNA topology with caution, and we treat mtDNA

groups I–V as tentative hypotheses of monophyletic mitochondrial lineages.

The dendrograms based on mtDNA RFLPs and the cladograms based on rDNA ITS sequences have points of agreement as well as disagreement (FIGS. 3, 6). Both support groups III and IV, and both suggest that PNG isolates occur in two separate clades (ITS groups II and IV, or mtDNA groups II and IV). The major difference is that ITS group I contains exemplars of the mtDNA groups I, II, and V. From log-likelihood ratios, Templeton's (1983) test, and comparison of tree lengths, it is clear that the mtDNA topology is rejected by the ITS sequence data (TABLE III). Thus, the mtDNA and ITS topologies are incongruent in aspects that were deemed internally well-supported. How could this come about? Given our confidence in the ITS topology and our skepticism about the mtDNA topology, one interpretation would be that the mtDNA tree simply does not reflect the actual mtDNA phylogeny. Nevertheless, the degree of congruence between the mtDNA and ITS trees suggests that there is phylogenetic signal in the mtDNA distance matrix or, at the very least, that not all branches in the mtDNA dendrogram are artifacts. We therefore must entertain the possibility that both the ITS and mtDNA topologies correctly estimate their underlying gene phylogenies, and that their incongruence is due to the fact that the nuclear ITS and the mitochondrial genome have different evolutionary histories.

In recent years, a substantial body of literature has accumulated describing situations where phylogenies of different genes from the same organisms can be incongruent with each other, or with the phylogenies of species or populations (Doyle, 1992; Maddison, 1995; Pamilo and Nei, 1988; for an example in fungi, see Lutzoni and Vilgalys, 1995). Presumably common phenomena that could result in conflicts between gene phylogenies and species phylogenies include lineage sorting, contemporary gene flow, and hybridization (excluding exotic mechanisms such as virus-mediated horizontal gene transfer). Lineage sorting is most likely when times between population divergences are short and population sizes are large. Thus, lineage sorting may be an especially serious source of error for phylogenetic studies whose operational taxa are closely related and presumably recently diverged, as is the case here. Hybridization is certainly possible in shiitake because of the demonstrated ability of strains from throughout Asia-Australasia to interbreed (Shimomura et al., 1992). Long distance dispersal of shiitake isolates, which could facilitate hybridization, is possible through aerial spore dispersal and transportation of cultivars by humans. The latter may be an especially significant factor in northeast Asia where shiitake has been cultivated for many centuries (Chang and Miles, 1987). Thus, based on our understanding of the biology of shiitake and its recent human-influenced history, there are reasons to think that different loci in shiitake could have different evolutionary histories.

In spite of our suspicion that the ITS and mtDNA have incongruent phylogenies, our lack of confidence in the mtDNA topology precludes making strong statements about whether this is true. For the moment, we take the ITS topology to be our best-supported estimate of the organismal phylogeny of shiitake, with the caveat that we suspect that the mitochondrial phylogeny may be incongruent. To critically address this issue it will be necessary to have data that permit rigorous assessment of the mtDNA phylogeny as well as reciprocal comparisons of trees and data (e.g., Rodrigo et al., 1993) for mtDNA and ITS. The mitochondrial large-subunit rDNA might have appropriate levels of sequence variation for inferring the mtDNA phylogeny. Restriction mapping of the mitochondrial genome could also provide discrete characters for phylogenetic analysis. In shiitake it appears that there is no recombination of mtDNA in normal meiosis and mating (Matsumoto and Fukumasa-Nakai, 1993). Thus, for the purpose of estimating gene phylogenies, the entire mitochondrial genome can be treated as a single locus. Ideally, we would also like to have data for multiple, unlinked nuclear genes. By comparing and combining (where appropriate) evidence and trees from multiple loci we should achieve a better understanding of the evolutionary history of populations in *Lentinula,* including dispersal, establishment, and gene flow.

Taxonomic and biogeographic conclusions.—Because of the limited sampling in this study, both of genes and isolates, we are reluctant to propose taxonomic changes at this time. Nevertheless, some preliminary conclusions can be drawn. The ITS topology suggests that there are at least four major lineages of shiitake in Asia-Australasia (FIGS. 3–5). Because they are capable of mating, we anticipate that some mycologists will continue to call them all *L. edodes.* We think that this would be unfortunate, however, because a treatment that lumps all lineages of shiitake into the same species would convey no information about the apparent phylogenetic diversity in shiitake, information that could be important for understanding character evolution, biogeography, and distribution of genetic variation. Ultimately, we think that the most useful, predictive classification will be one that recognizes separate lineages in shiitake as distinct species, even though organisms in these lineages have retained the ability to interbreed.

Pegler (1983) recognized three species of shiitake, but our results provide only limited support for his

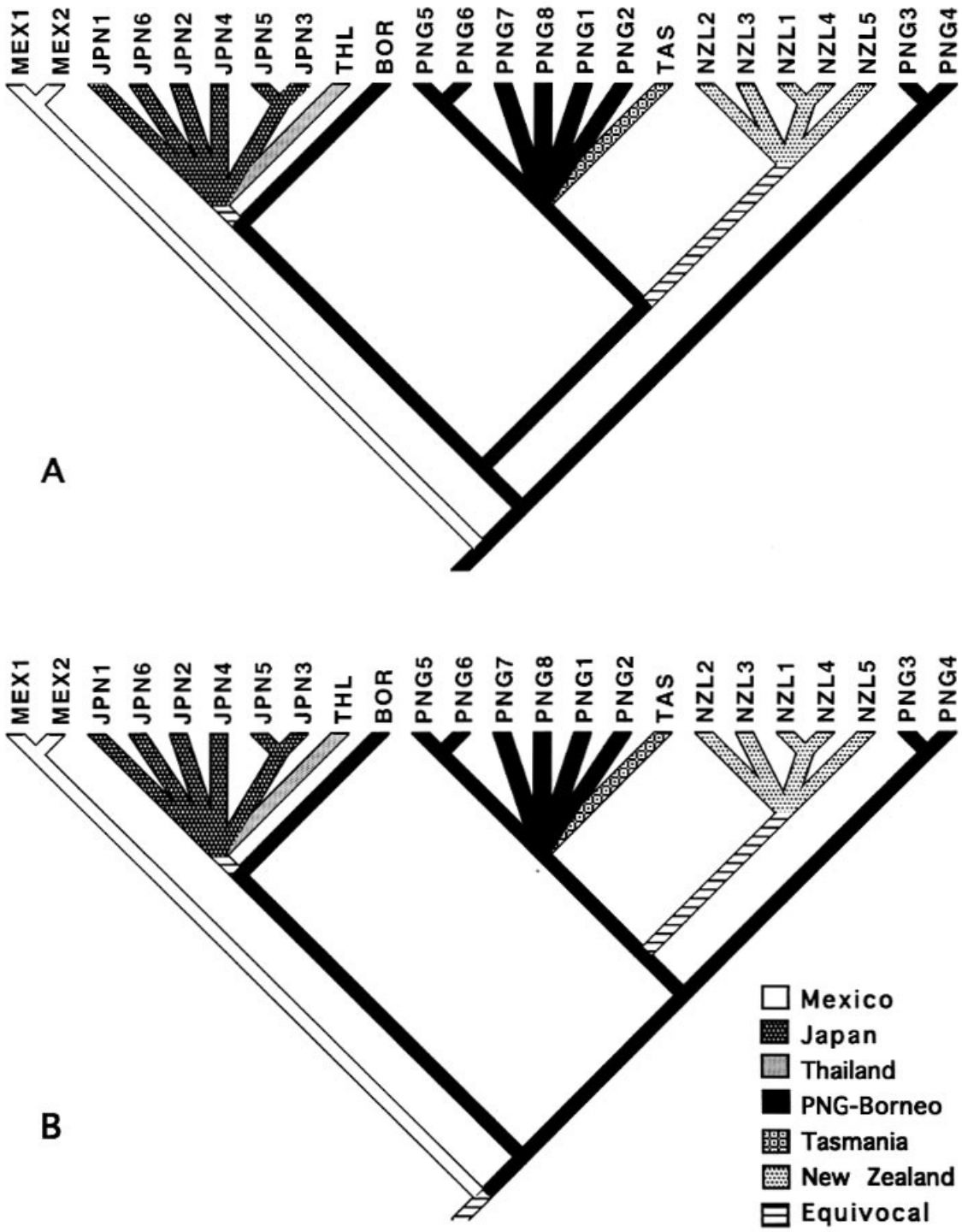


FIG. 7. Biogeographic hypotheses for shiitake. A. Hypothesis under ingroup root A, which is favored by parsimony analyses (FIG. 3). B. Hypothesis under root E, which is favored by distance analyses (FIG. 5). Under either rooting, the South Pacific region (black) appears to be the ancestral area for shiitake.

classification of *Lentinula*. The ITS data strongly support monophyly of the New Zealand isolates (group III, FIGS. 3–5), which correspond to *L. novaezelandieae sensu* Pegler. Monophyly of the New Zealand form of shiitake is also suported independently by mtDNA RFLPs (Fukuda et al., 1994, FIG. 6) and isozymes (Fukuda and Tokimoto, 1991).

If group Ia, containing isolates from Japan and Thailand, is accepted as monophyletic, then *L. edodes sensu* Pegler is also supported (FIGS. 3–5). However,

this clade was strongly supported only in distance analyses (FIG. 5), whereas it received moderate support in parsimony analyses (FIG. 3). Furthermore, it is not clear whether the isolate from Thailand in group Ia (THL, TMI-1633) would have been assigned to *L. edodes* or to *L lateritia* by Pegler. In Pegler's monograph, *L. edodes* was based on material from Japan, China, and Vietnam, whereas *L. lateritia* was based on material from Borneo, Australia, India, and Bhutan. Thailand is geographically intermediate between these poorly delimited ranges. Furthermore, group Ia is nested within group I which includes the isolate from Borneo (BOR, TMI-689), which is definitely in the range of *L. lateritia*. Pegler himself expressed reservations about the distinction of *L. edodes* and *L. lateritia*, saying that "*Lentinula lateritia* may be no more than a tropical form of *L. edodes*" (1983, p. 233).

The ITS data suggest that *L. lateritia sensu* Pegler is a paraphyletic group from which *L. edodes s. str.* and *L. novaezelandieae* have been independently derived. If this is true, then the morphology of *L. lateritia* should be plesiomorphic for shiitake. Comparison with the putative sister-group, *L. boryana*, supports this view. Based on Pegler's (1983) descriptions, *L. boryana* and *L. lateritia* share several features that may be primitive for *Lentinula*, including a smooth stipe (vs fibrillose-squamulose stipe in *L. novaezelandieae*) and a smooth, thin-fleshed pileus (vs thick-fleshed pileus with squamules or fissures in *L. edodes*). Thus, our results suggest that *L. lateritia sensu* Pegler is an evolutionary grade united only by symplesiomorphies (rather than a monophyletic group with uniquely derived characters).

We mapped the geographic origins of the isolates onto topologies with both root A and root E (FIG. 7). Under either rooting, the most parsimonious interpretation is that the South Pacific (Borneo and PNG) is the ancestral area for shiitake. These results suggest that populations of shiitake in continental and northeastern Asia, New Zealand, and Tasmania have been derived from South Pacific populations (FIG. 7). Admittedly, our interpretations are based on a limited geographic sample of isolates. Areas from which we do not have representatives include Australia, China, and the Himalayan nations of Bhutan, Nepal, India, etc. More isolates are also needed from throughout southeast Asia and the south Pacific, including Vietnam, Thailand, Taiwan, Phillipines, Indonesia, etc. Nevertheless, based on the sample of isolates at hand, it appears that the South Pacific area has the highest phylogenetic diversity of shiitake in Asia-Australasia. In particular, PNG stands out because ITS groups II and IV both occur on this island. Exemplars of group II (TMI-1485) and group IV (TMI-1499) have been shown to be mating compatible (Shimomura et al., 1992). The presence of ITS groups II and IV in PNG could reflect a) biogeographic barriers to mating between groups II and IV, or, b) a maintained polymorphism for ITS type in PNG shiitake populations.

Analyses of ITS2 sequences alone placed the three Kwan et al. (1992) isolates in group Ia, which includes all of the isolates from Japan and the one isolate from Thailand (FIG. 4). This suggests that the Kwan et al. isolates are derived from northeast or continental Asian material. This seems plausible because the majority of shiitake isolates in culture collections are probably derived from Chinese, Japanese, and Korean strains. Even though Kwan et al. found the ITS2 sequences to be too conservative for strain identification, our results suggest that ITS sequences permit coarse estimation of the geographic origin of isolates.

Practical implications.—The ITS, mtDNA, and isozyme data all agree that there are multiple, genetically divergent lineages of shiitake in Asia-Australasia, some of which may have restricted distributions, such as group III, which occurs only in New Zealand, or group IV, which is limited to PNG (FIGS. 3–7). Because all shiitake lineages tested to date have been mating compatible (Shimomura et al., 1992), the genetic diversity present in nature is readily accessible to mushroom breeders for use in strain development. However, we suspect that most (if not all) commercial cultivars of shiitake in use today are derived only from Japanese, Chinese, or Korean strains. If so, then the wild populations of shiitake outside of northeast Asia represent an untapped genetic resource that could prove valuable for future breeding programs. Thus, wild biodiversity of shiitake deserves protection for economic as well as ethical reasons.

Unfortunately, throughout their range, natural populations of shiitake face serious threats to their existence. Foremost among these is habitat loss through deforestation. A more insidious potential threat to indigenous shiitake populations is contamination by exogenous genotypes originating from mushroom farms. Shiitake cultivation, whether on logs or in bags, produces a high density of fruiting bodies of a single genotype. Escape of cultivars under such circumstances is quite likely, as has already been shown to have occurred in *A. bisporus* (Kerrigan and Ross, 1989). Many nations that have native shiitake populations are developing shiitake cultivation, such as PNG, Nepal, Vietnam, Bhutan, Thailand, etc. (International Symposium on Production and Products of Lentinus Mushroom, abstracts distributed in Qingyuan, China, Nov. 1, 1994), presumably using imported cultivars from northeast Asia. It would be ironic if development of local shiitake industries in these countries ultimately came to limit the growth of the global shiitake industry because of loss of wild genetic diversity.

We think that it is imperative that fieldwork be un-

dertaken to document the remaining biodiversity of shiitake, and to establish culture collections for conservation of germplasm. We also have two suggestions that might help protect local populations of shiitake by reducing the probability that cultivars will be released. The simplest solution would be for mushroom growers in sensitive areas to collect and cultivate only native strains of shiitake. Of course, this would probably require that many local isolates be screened in order to find those with desirable market properties. Alternately, it might be possible to develop methods of cultivation that limit the release of spores to the environment. Perhaps the most elegant solution (requiring no modification of cultivation practices) would be to develop cultivars of shiitake that do not release spores. Already, a sporeless mutant of shiitake that produces normal fruiting bodies, but has defective spore maturation has been described (Hasebe et al., 1991). With protoplast fusion techniques and di-mon matings, it may be possible to incorporate such strains in breeding programs aimed at producing environmentally friendly shiitake cultivars.

ACKNOWLEDGMENTS

We are indebted to the individuals who generously donated isolates for this study, including Mike Nicholson and Daniel J. Royse (Pennsylvania State University), Ronald H. Petersen (University of Tennessee), and Geoff Ridley (New Zealand Forest Research Institute). For their helpful comments and suggestions, we thank David Baum, Rick Kerrigan, Anne Yoder, Dennis Desjardin, and an anonymous reviewer. We also thank Svetlana Ordian for translating passages from Samgina (1981) from Russian into English, and our colleagues at the Tottori Mycological Institute for their assistance with this project. This research was supported in part by NSF Postdoctoral Fellowship DEB-9303268 to DSH.

LITERATURE CITED

Anderson, J. B. 1993. Molecular tools in breeding *Agaricus*. Pp. 207–223. *In: Genetics and breeding of edible mushrooms*. Eds., S. T. Chang, J. A. Buswell, and P. G. Miles. Gordon and Breach Science Publishers, Philadelphia, Pennsylvania.

———, and E. Stasovski. 1992. Molecular phylogeny of northern hemisphere species of *Armillaria*. *Mycologia* 84: 505–516.

Arima, T., and T. Morinaga. 1993. Electrophoretic karyotype of *Lentinus edodes*. *Trans. Mycol. Soc. Japan* 34: 481–485.

Baldwin, B. G., M. J. Sanderson, J. M. Porter, M. F. Wojciechowski, C. S. Campbell, and M. J. Donoghue. 1995. The ITS region of nuclear ribosomal DNA: a variable source of evidence on angiosperm phylogeny. *Ann. Missouri Bot. Gard.* (In press)

Barrett, M., M. J. Donoghue, and E. Sober. 1991. Against consensus. *Syst. Zool.* 40: 486–493.

Baum, D. A., K. J. Sytsma, and P. C. Hoch. 1994. A phylogenetic analysis of *Epilobium* (Onagraceae) based on nuclear ribosomal DNA sequences. *Syst. Bot.* 19: 363–388.

Baura, G., T. M. Szaro, and T. D. Bruns. 1992. *Gastrosuillus laricinus* is a recent derivative of *Suillus grevillei*: molecular evidence. *Mycologia*: 84: 592–597.

Berkeley, M. J. 1878. Contributions to the botany of H. M. S. 'Challenger.' XXXVIII. Enumeration of the Fungi collected during the expedition of H. M. S. 'Challenger,' 1874–75. *J. Linn. Soc., Bot.* 16: 38–54.

Bremer, K. 1988. The limits of amino acid sequence data in angiosperm phylogenetic reconstruction. *Evolution* 42: 795–803.

Bruns, T. D., T. J. White, and J. W. Taylor. 1991. Fungal molecular systematics. *Annual Rev. Ecol. Syst.* 22: 525–564.

———, R. Vilgalys, S. M. Barns, D. Gonzalez, D. S. Hibbett, D. J. Lane, L. Simon, S. Stickel, T. M. Szaro, W. G. Weisburg, and M. L. Sogin. 1992. Evolutionary relationships within the fungi: analyses of nuclear small subunit rRNA sequences. *Molec. Phylogenet. Evol.* 1: 231–241.

Bull, J. J., J. P. Hulsenbeck, C. W. Cunningham, D. L. Swofford, and P. J. Waddell. 1993. Partitioning and combining data in phylogenetic analysis. *Syst. Biol.* 42: 384–397.

Carbone, I., and L. M. Kohn. 1993. Ribosomal DNA sequence divergence within internal transcribed spacer 1 of the Sclerotiniaceae. *Mycologia* 85: 415–427.

Chang, S. T. 1994. Mushroom biology: the impact on mushroom production and mushroom products. Pp. 3–20. *In: Mushroom biology and mushroom products*. Eds., S. T. Chang, J. A. Buswell, and S. W. Chiu. The Chinese University Press, Hong Kong.

———, and P. G. Miles. 1987. Historical record of the early cultivation of *Lentinus* in China. *Mushroom J. Tropics* 7: 31–37.

Curran, J., F. Driver, J. W. O. Ballard, and R. J. Milner. 1994. Phylogeny of *Metarhizium*: analysis of ribosomal DNA sequence data. *Mycol. Res.* 98: 547–552.

de Queiroz, A. 1993. For consensus (sometimes). *Syst. Biol.* 42: 368–372.

Donoghue, M. J. 1985. A critique of the biological species concept and recommendations for a phylogenetic alternative. *Bryologist* 88: 172–181.

———, R. G. Olmstead, J. F. Smith, and J. D. Palmer. 1992. Phylogenetic relationships of Dipsacales based on rbcL sequences. *Ann. Missouri Bot. Gard.* 79: 333–345.

———, and M. J. Sanderson. 1992. The suitability of molecular and morphological evidence in reconstructing plant phylogeny. Pp. 340–368. *In: Molecular systematics in plants*. Eds., P. S. Soltis, D. E. Soltis, and J. J. Doyle. Chapman and Hall, New York.

Doyle, J. J. 1992. Gene trees and species trees: molecular systematics as one-character taxonomy. *Syst. Bot.* 17: 144–163.

Ellingboe, A. H. 1993. Breeding for mushroom production in *Lentinula edodes*. Pp. 111–122. *In: Genetics and breeding of edible mushrooms*. Eds., S. T. Chang, J. A.

Buswell, and P. G. Miles. Gordon and Breach Science Publishers, Philadelphia, Pennsylvania.

Felsenstein, J. 1978. Cases in which parsimony or compatibility methods will be positively misleading. *Syst. Zool.* 27: 401–440.

———. 1985a. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39: 783–791.

———. 1985b. Confidence limits on phylogenies with a molecular clock. *Syst. Zool.* 34: 152–161.

———. 1993. *PHYLIP (Phylogeny inference package)* version 3.5p. Department of Genetics SK-50, University of Washington, Seattle.

Fitch, W. M., and E. Margoliash. 1967. Construction of phylogenetic trees. *Science* 155: 279–284.

Fukuda, M., Y. F. Nakai, D. S. Hibbett, T. Matsumoto, and Y. Hayashi. 1994. Mitochondrial DNA restriction fragment length polymorphisms in natural populations of *Lentinula edodes*. *Mycol. Res.* 98: 169–175.

———, and K. Tokimoto. 1991. Variation of isozyme patterns in the natural population of *Lentinus edodes*. *Proc. Japan Acad.*, ser. B, 67: 43–47.

Gardes, M., T. J. White, J. A. Fortin, T. D. Bruns, and J. W. Taylor. 1991. Identification of indigenous and introduced symbiotic fungi in ectomycorrhizae by amplification of nuclear and mitochondrial ribosomal DNA. *Canad. J. Bot.* 69: 180–190.

Georgiev, O. I., N. Nikolaev, and A. A. Hadjiolov. 1981. The structure of the yeast ribosomal RNA genes. 4. Complete sequence of the 25 S rRNA gene from *Saccharomyces cerevisiae*. *Nucl. Acids Res.* 9: 6953–6958.

Harshman, J. 1994. The effect of irrelevant characters on bootstrap values. *Syst. Biol.* 43: 419–424.

Hasebe, K., S. Murakami, and A. Tsuneda. 1991. Cytology and genetics of a sporeless mutant of *Lentinus edodes*. *Mycologia* 83: 354–359.

Hibbett, D. S. 1992. Towards a phylogenetic classification for shiitake: taxonomic history and molecular perspectives. *Rept. Tottori Mycol. Inst.* 30: 30–42.

———. 1992. Ribosomal RNA and fungal systematics. *Trans. Mycol. Soc. Japan* 33: 533–556.

———, and M. J. Donoghue. Progress toward a phylogenetic classification of the Polyporaceae through parsimony analysis of mitochondrial ribosomal DNA sequences. *Canad. J. Bot.* (In press)

———, and R. Vilgalys. 1993. Phylogenetic relationships of *Lentinus* (Basidiomycotina) inferred from molecular and morphological characters. *Syst. Bot.* 18: 409–433.

Higgins, D. G., A. J. Bleasby, and R. Fuchs. 1992. CLUSTAL V: improved software for multiple sequence alignment. *Computer Applic. Biosci.* 8: 189–191.

Ito, S., and S. Imai. 1925. On the taxonomy of shii-take and matsu-take. *Bot. Mag. (Tokyo)* 39: 319–327.

Kerrigan, R. W., and I. K. Ross. 1989. Allozymes of a wild *Agaricus bisporus* population: new alleles, new genotypes. *Mycologia* 81: 433–443.

Kimura, M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J. Molec. Evol.* 16: 111–120.

Kishino, H., and M. Hasegawa. 1989. Evaluation of the maximum likelihood estimate of the evolutionary tree topologies from DNA sequence data, and the branching order in Hominoidea. *J. Molec. Evol.* 29: 170–179.

Kobayashi, Y., Y. Otani, and T. Hongo. 1973. Some higher fungi found in New Guinea. *Rept. Tottori Mycol. Inst.* 10: 341–356.

Komatsu, M., and K. Kimura. 1968. Sexuality of *Lentinus edodes* (Berk.) Sing. collected in Borneo. *Rept. Tottori Mycol. Inst.* 6: 1–8.

Kulkarni, R. K. 1991. DNA polymorphisms in *Lentinula edodes*, the shiitake mushroom. *Appl. Environm. Microbiol.* 57: 1735–1739.

Kwan, H. S., S. W. Chiu, K. M. Pang, and S. C. Cheng. 1992. Strain typing in *Lentinula edodes* by polymerase chain reaction. *Exp. Mycol.* 16: 163–166.

Larson, A. 1994. The comparison of morphological and molecular data in phylogenetic systematics. Pp. 371–390. *In: Molecular ecology and evolution: approaches and applications*. Eds., B. Schierwater, B. Streit, G. P. Wagner, and R. DeSalle. Birkhäuser Verlag, Basel, Switzerland.

Lundberg, J. 1972. Wagner networks and ancestors. *Syst. Zool.* 21: 398–413.

Lutzoni, F., and R. Vilgalys. 1995. Integration of morphological and molecular datasets in estimating fungal phylogenies. *Canad. J. Bot.* (In press)

Maddison, W. P. 1994. Missing data versus missing characters in phylogenetic analysis. *Syst. Biol.* 42: 576–581.

———. 1995. Phylogenetic histories within and among species. Pp. 273–287. *In: Experimental and molecular approaches to plant biosystematics*. Eds., P. C. Hoch, and A. G. Stephenson. Missouri Botanical Garden, St. Louis.

———, and D. R. Maddison. 1992. *Maclade* version 3. Sinauer Associates Inc., Sunderland, Massachusetts.

Mata, G., and G. Guzman. 1989. Hybridization between a Mexican strain of *Lentinus boryanus* and an Asiatic strain of *Lentinus edodes*. *Revista Mex. Micol.* 5: 77–80.

Matsumoto, T., and Y. Fukumasa-Nakai. 1993. Mitochondrial DNA polymorphism and inheritance in *Lentinula edodes* and *Pleurotus ostreatus*. *Rept. Tottori Mycol. Inst.* 31: 153–161.

Molina, F. I., P. Shen, and S. C. Jong. 1992. Molecular evidence supports the separation of *Lentinula edodes* from *Lentinus* and related genera. *Canad. J. Bot.* 70: 2446–2452.

Mori, K., S. Fukai, and A. Zennyozi. 1974. Hybridization of Shii-ta-ke (*Lentinus edodes*) between cultivated strains of Japan and wild strains grown in Taiwan and New Guinea. Pp. 391–403. *In: Mushroom science IX: proceedings of the IXth annual congress on the cultivation of edible fungi*. Ed., K. Mori. Japan Science Press, Tokyo, Japan.

Murakami, S., and T. Takemaru. 1985. Nuclear behavior during basidiospore formation in *Lentinus edodes*. *Trans. Mycol. Soc. Japan* 26: 253–260.

Nakai, Y. 1986. Cytological studies on shiitake, *Lentinus edodes* (Berk.) Singer. *Rept. Tottori Mycol. Inst.* 24: 1–202.

O'Donnell, K. 1992. Ribosomal DNA internal transcribed spcers are highly divergent in the phytopathogenic ascomycete *Fusarium sambucinum* (*Gibberella pulicaris*). *Curr. Genet.* 22: 213–320.

Ohmasa, M., and H. Furukawa. 1986. Analysis of esterase and malate dehydrogenase isozymes of *Lentinus edodes* by isoelectric focusing for the identification and discrimination of stocks. *Trans. Mycol. Soc. Japan* 27: 79–90.

Olsen, G. J., R. Overbeek, N. Larsen, T. L. Marsh, M. J. McCaughey, M. A. Maciukenas, W. M. Kuan, T. J. Macke, Y. Xing, and C. R. Woese. 1992. The ribosomal database project. *Nucl. Acids Res.* 20 (supplement): 2199–2200.

Pamilo, P., and M. Nei. 1988. Relationships between gene trees and species trees. *Molec. Biol. Evol.* 5: 568–583.

Peberdy, J. F., and H. M. Fox. 1993. Protoplast technology and edible mushrooms. Pp. 125–155. *In: Genetics and breeding of edible mushrooms.* Eds., S. T. Chang, J. A. Buswell, and P. G. Miles. Gordon and Breach Science Publishers, Philadelphia, Pennsylvania.

Pegler, D. N. 1975. The classification of the genus *Lentinus* Fr. (Basidiomycota). *Kavaka* 3: 11–20.

———. 1983. The genus *Lentinula* (Tricholomataceae tribe Collybieae). *Sydowia* 36: 227–239.

Petersen, R. H. 1994. Mating systems of antipodal agarics: unreported taxa and range extensions. *New Zealand J. Bot.* (In press)

Raeder, U., and P. Broda. 1985. Rapid preparation of DNA from filamentous fungi. *Lett. Appl. Microbiol.* 1: 17–20.

Rodrigo, A. G., M. Kelly-Borges, P. R. Bergquist, and P. L. Bergquist. 1993. A randomisation test of the null hypothesis that two cladograms are sample estimates of a parametric phylogenetic tree. *New Zealand J. Bot.* 31: 257–268.

Rohlf, F. J., and R. R. Sokal. 1981. *Statistical tables.* W. H. Freeman and Co., San Francisco, California.

Royse, D. J., and B. May. 1987. Identification of shiitake genotypes by multilocus enzyme electrophoresis: catalog of lines. *Biochem. Genet.* 25: 705–716.

———, and M. S. Nicholson. 1993. Allozymes, ribosomal DNA and breeding in *Lentinula. Rept. Tottori Mycol. Inst.* 31: 162–167.

Rubstov, P. M., M. M. Musakhanov, V. M. Zakharyev, A. S. Krayev, K. G. Skryabin, and A. A. Bayev. 1980. The structure of the yeast ribosomal RNA genes. I. The complete nucleotide sequence of the 18S ribosomal RNA gene from *Saccharomyces cerevisiae. Nucl. Acids Res.* 8: 5779–5794.

Saitou, N., and M. Nei. 1987. The neighbor-joining method: a new method for inferring evolutionary trees. *Molec. Biol. Evol.* 4: 406–425.

Samgina, D. I. 1981. Agarikovye Griby I. Agaricales. *Fl. Spor. rast. Kazahst.* 13: 1–268.

Shimomura, N., K. Hasebe, Y. Nakai-Fukumasa, and M. Komatsu. 1992. Intercompatibility between geographically distant strains of shiitake. *Rept. Tottori Mycol. Inst.* 30: 26–29.

Singer, R. 1936. Das System der Agaricales. *Ann. Mycol.* 34: 286–378.

———. 1941. Is shiitake a *Cortinellus? Mycologia* 33: 449–451.

———. 1986. The Agaricales in modern taxonomy. 4th ed. Koeltz Scientific Books, Koenigstein, Germany. 981 pp.

Swofford, D. L. 1993. *PAUP: Phylogenetic analysis using parsimony,* version 3.1. Illinois Natural History Survey, Champaign, Illinois.

Templeton, A. 1983. Phylogenetic inference from restriction endonuclease cleavage site maps with particular reference to the evolution of humans and the apes. *Evolution* 37: 221–224.

Thweatt, R., and J. C. Lee. 1990. Yeast precursor ribosomal RNA: molecular cloning and probing the higher-order structure of the internal transcribed spacer I by kethoxal and dimethylsulfate modification. *J. Molec. Biol.* 211: 305–320.

Tokimoto, K., M. Komatsu, and T. Takemaru. 1973. Incompatibility factors in the natural population of *Lentinus edodes* in Japan. *Rept. Tottori Mycol. Inst.* 10: 371–376.

Tsuneda, A., N. Maekawa, I. Ohira, and I. Furukawa. 1991. Incipient decay of *Quercus serrata* sapwood by *Lentinus edodes* and its inhibition by an antagonistic hyphomycete, *Leptodontidium elatus. Canad. J. Bot.* 69: 2797–2805.

White, T. J., T. Bruns, S. Lee, and J. W. Taylor. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Pp. 315–322. *In: PCR protocols.* Eds., M. A. Innis, D. H. Gelfand, J. J. Sninsky, and T. White. Academic Press, San Diego, California.

Wojciechowski, M. F., M. J. Sanderson, B. G. Baldwin, and M. J. Donoghue. 1993. Monophyly of aneuploid *Astragalus* (Fabaceae): evidence from nuclear ribosomal DNA internal transcribed spacer sequences. *Amer. J. Bot.* 80: 711–722.

Yeh, L. C., and J. C. Lee. 1990. Structural analysis of the internal transcribed spacer 2 of the precursor ribosomal RNA from *Saccharomyces cerevisiae. J. Molec. Biol.* 211: 699–712.