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Phylogenetic Relationships of *Lentinus* (Basidiomycotina) Inferred from Molecular and Morphological Characters

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ABSTRACT. Phylogenetic relationships of the basidiomycete *Lentinus* were investigated using 20 morphological and 133 nucleic acid sequence characters from three regions in the 5′ half of the nuclear-encoded large subunit rRNA. Molecular data were obtained from 34 individuals that represent 11 species in *Lentinus*, nine in the Polyporaceae, eight in the Tricholomataceae, and one in the Corticiaceae. *Thanetephorus cucumeris* (Tulasnellales) was used as an outgroup for rooting purposes. Most of the sequence variation was in regions that correspond to eukaryote-specific divergent domains D1 and D2. Molecular data alone yielded a well-resolved cladogram but morphological data alone were insufficient to resolve phylogenetic relationships. The most resolved cladograms were obtained with a combined analysis of molecular and morphological characters. Bootstrap and decay index measures of branch robustness had a significant positive correlation, but some branches with high bootstrap values were contradicted by near-minimal trees. Monophyly of *Lentinus* sensu Pegler was not supported. Rather, three monophyletic groups of *Lentinus* species were resolved. These largely correspond to *Neolentinus*, *Panus*, and *Lentinus* s. str. The latter appears to be derived from the Polyporaceae, suggesting that lamellae are products of convergent evolution.

Fungi are among the most challenging organisms for morphological systematics. At low taxonomic levels many important characters are subtle and preserve poorly in herbarium materials, whereas at higher levels morphological simplicity and a poor fossil record hinder phylogenetic inference. Understandably, increasing numbers of mycological systematists are turning to molecular characters (see reviews by Bruns et al. 1991; Hibbett 1992; Kohn 1992). Here we employ cladistic analyses of ribosomal DNA (rDNA) sequence data and morphological characters to elucidate phylogenetic relationships of *Lentinus* Fr.

Lentinus has been classified in the Tricholomataceae, Agaricales, because it has lamellae and a white spore print (Miller 1972; Miller and Manning 1976). However, it also has anatomical similarities to certain poroid taxa in the Aphyllophorales, and in most modern treatments it is placed in or near the Polyporaceae (Kühner 1980; Moser 1978; Pegler 1983; Singer 1986). The primary character that supports this placement is the fact that *Lentinus* species are dimitic [the sporocarp is composed of both thin-walled generative hyphae and thick-walled skeletal or ligative hyphae (Corner 1981; Pegler 1983; Pegler and Young 1983)]. Dimiticity is common in the Aphyllophorales, whereas sporocarps of the Agaricales sensu Singer are usually monomitic [composed only of generative hyphae (Corner 1966, 1981; Pegler 1975, 1983; Singer 1986)]. In addition, hyphal pegs, fascicles of sterile hyphae that emerge from the hymenium, are found in certain *Lentinus* species and approximately 10 genera of polypores (Corner 1981; Gilbertson and Ryvarden 1986, 1987; Pegler 1983).

Lentinus was monographed by Pegler (1983). Pegler restricted *Lentinus* to dimitic species, and therefore, transferred the monomitic shiitake fungus, traditionally known as *Lentinus edodes* (Berk.) Singer, into *Lentinula* Earle [Collybieae, Tricholomataceae (Pegler 1975)]. Pegler combined *Lentinus* and *Panus* Fr. as subgenera. Subgenus *Lentinus* was restricted to species with ligative hyphae and subg. *Panus* was restricted to species with skeletal hyphae.

Despite Pegler's comprehensive monograph, the delimitation and evolutionary relationships of *Lentinus* remain controversial. Alternatives to Pegler's treatment have emphasized hyphal anatomy (Corner 1981), the hymenophoral trama (Kühner 1980; Singer 1986), and wood decay (Redhead and Ginns 1985). Many of the disagreements concern the limits of *Lentinus*, *Panus*, and *Pleurotus* Fr. Redhead and Ginns (1985) created the segregate genera *Neolentinus* Redhead and Ginns and *Heliocybe* Redhead and Ginns for nine species of *Lentinus* subg. *Panus*.

Pegler hypothesized that "*Lentinus* represents

TABLE 1. Species and isolates of *Lentinus*, Polyporaceae, Tricholomataceae, Corticiaceae, and Tulasnellales included in phylogenetic analyses of rDNA and morphological characters. Nomenclature follows Pegler (1983) for *Lentinus*. All "D" cultures are maintained at Duke. "VT" cultures were obtained from Dr. Orson K. Miller Jr., Virginia Polytechnic Institute, Blacksburg, Virginia. "FPL" cultures were obtained from Dr. Harold H. Burdsall Jr., U.S.D.A. Forest Products Laboratory, Madison, Wisconsin. The *Lentinula edodes* isolate was obtained from Kunkel Mushroom Farms. DUKE, VT, and FPL collections housed at Duke, Virginia Polytechnic Institute, and U.S.D.A. Forest Products Laboratory respectively. Cloned rDNA fragment codes with positions relative to *Saccharomyces cerevisiae* rDNA: A = *Eco* RI/*Bgl* II fragment from position 78 in 5.8S rRNA to position 1436 in 25S rRNA. B = *Eco* RI/*Eco* RI fragment from position 78 in 5.8S rRNA to 310 in 25S rRNA. C = *Eco* RI/*Bgl* II fragment from position 310 to 1436 in 25S rRNA. D = *Eco* RI/*Eco* RI fragment from 78 in 5.8S rRNA to 1181 in 25S rRNA. E = complete rDNA repeat, cloned from genomic DNA, gift of Dolores Gonzelez. Numbers in parentheses indicate the number of individual clones of each fragment.

Species/Isolate	Voucher culture	Voucher collection	DNA source	Clones
Lentinus subg. *Panus* (Fr.) Pegler				
sect. *Squamosi* Fr.				
Lentinus lepideus (Fr.: Fr.) Fr. 13	D484	DUKE EK 88-1	culture	A (12)
Lentinus lepideus 17	D612/VT306	VPI OKM 2304	culture	A (12)
Lentinus lepideus 47	D622/VT1254/ FPL534-R	no information	culture	A (5)
Lentinus ponderosus O. K. Miller 14	D585/VT302	VPI OKM 2361	culture	A (8)
Lentinus ponderosus 15	D606/VT303	VPI OKM 7233	culture	A (8)
Lentinus ponderosus 16	D592/VT304	VPI 105765	culture	A (6)
Lentinus dactyloides Clel.	none	VPI OKM 23622	herbarium	D (1)
sect. *Pulverulenti* Fr.				
Lentinus kauffmannii A. H. Smith	D619/VT1033.7	VPI OKM 19226	culture	A (5)
Lentinus sulcatus Berk.	FPL4655/D797	FPL OKM 8302	culture	A (4)
sect. *Panus* (Fr.) Pegler				
Lentinus strigosus (Schwein.) Fr. 22	D743	DUKE DSH 89-1	culture	B (3), C (8)
Lentinus strigosus 23	D635/VT340	VPI CHD 30684	culture	B (1), C (8)
Lentinus strigosus 48	D631/VT343	VPI OKM 6666	culture	B (3), C (5)
Lentinus torulosus (Pers.: Fr.) Lloyd	D613/VT1502	no information	culture	B (2), C (8)
sect. *Velutini* Pegler				
Lentinus velutinus Fr.	D795/FPL4147	FPL LCF 573	culture	B (5), C (5)
Lentinus subg. *Lentinus*				
sect. *Tigrini* Pegler				
Lentinus tigrinus (Bull.: Fr.) Fr.	D608/VT296	VPI ERT 226	culture	B (2), C (9)
sect. *Lentinus*				
Lentinus crinitus (Linn.: Fr.) Fr.	D796/FPL4647	FPL HHB 9765	culture	B (4), C (6)
Polyporaceae s.l.				
Polyporus arcularius Batsch: Fr.	D603/VT959	VPI OKM 9875	culture	B (7), C (8)
Polyporus squamosus Huds.: Fr.	none	DUKE SAR 89-468	field coll.	A (8)
Polyporus alveolaris (D.C.: Fr.) Bond. et Sing.	D785	DUKE DSH 90-36	culture	A (5)
Grifola frondosa (Dicks.: Fr.) S. F. Gray	none	DUKE SAR 89-478	field coll.	A (8)
Laetiporus sulphureus (Bull.: Fr.) Murr.	none	DUKE SAR 89-466	field coll.	A (10)
Pycnoporus cinnabarinus (Jacq.: Fr.) Karst.	D614/VT875	VPI FP 103633-s	culture	A (9)
Lenzites betulina (Fr.) Fr.	D781	DUKE JSH 155	culture	B (4)
Trametes versicolor (L.: Fr.) Pilat	D775	DUKE E. Kay	culture	B (3), C (5)
Ganoderma lucidum (W. Curt.: Fr.) Karst.	D780	DUKE JSH 0093	culture	B (1)
Corticiaceae				
Stereum complicatum (Fr.) Fr.	D783	DUKE P. Schultz	culture	B (1), C (1)

TABLE 1. Continued.

Species/Isolate	Voucher culture	Voucher collection	DNA source	Clones
Tricholomataceae s.l.				
Pleurotus eryngii (D.C. ex Fr.) Quel.	D625/VT1477	DUKE DSH 91-42	culture	D (8)
Ossicaulis lignatilis (Pers.: Fr.) Redhead	D483/VT1122	VPI OKM 17605	culture	B (2), C (1)
Collybia earleae (Murr.) Murr.	D50	VPI OKM 18761	culture	D (6)
Panellus stipticus (Bull.: Fr.) Karst.	D611	DUKE DSH 89-28	culture	A (1)
Lentinula edodes (Berk.) Pegler	D607/VT1484	no information	culture	D (1)
Lentinellus omphalodes (Fr.) Karst.	none	DUKE DSH 89-9	culture	B (2), C (4)
Lentinellus montanus O. K. Miller	D595/VT242	OKM 6414	culture	B (5), C (6)
Mycena galericulata (Fr.) S. F. Gray	D198	DUKE RV 87-14	culture	D (12)
Tulasnellales				
Thanetephorus cucumeris (Frank) Donk	D9RS	none	culture	E (1)

the most agaricoid development derived from a polyporoid ancestry" (1983, p. 11), and therefore, that the gills of *Lentinus* and the Agaricales are the result of convergent evolution. However, Corner proposed that *Lentinus*, *Panus*, and *Pleurotus* "are rather primitive agarics" (1981, p. 25), and therefore, that gills in *Lentinus* and Agaricales s. str. are homologous. *Lentinus* is morphologically intermediate between the Agaricales and the Aphyllophorales. Therefore, an understanding of its evolutionary relationships is important to a phylogenetic classification for the Hymenomycetes.

The main questions addressed by this study are: is *Lentinus* sensu Pegler monophyletic; and, what are the relationships of *Lentinus* to the Tricholomataceae and Polyporaceae?

MATERIALS AND METHODS

For simplicity, ingroup taxa were classified as members of *Lentinus*, the Tricholomataceae, Polyporaceae, or Corticiaceae (Table 1). No family placement for *Lentinus* was endorsed *a priori*, and so it was treated separately from the Tricholomataceae and Polyporaceae (Table 1). The delimitation of Tricholomataceae employed here follows Miller (1972), except that *Lentinus* is not included. The delimitation of the Polyporaceae employed here follows Donk (1964), except that Donk placed *Ganoderma* P. A. Karsten in the Ganodermataceae. Classification of *Stereum* Persoon ex S. F. Gray in the Corticiaceae follows Eriksson et al. (1984). Certain aspects of the family-level classification employed here are controversial. For example, some authors now classify *Lentinellus* P. A. Karsten in the Auriscalpiaceae (Donk 1964; Maas Geesteranus 1963; Singer 1986), *Pleurotus* in the Polyporaceae (Singer 1986), and *Stereum* in the Stereaceae (Donk 1964).

Nomenclature follows Pegler (1983) for *Lentinus*. Nine species from *Lentinus* were examined, two species from subg. *Lentinus*, including *L. tigrinus*, which has been conserved as the type species of *Lentinus* (David Hawksworth, pers. comm.), and seven species from subg. *Panus* (Table 1). Species from subg. *Panus* include the type species and other representatives of *Neolentinus*, *Heliocybe*, and *Panus*. The remaining species represent the Polyporaceae (nine species), Tricholomataceae (eight species), and Corticiaceae (one species). Most species were represented by single isolates, but *Lentinus lepideus*, *L. ponderosus*, and *L. strigosus* were each represented by three isolates.

Thanetephorus cucumeris (Tulasnellales) was used as an outgroup. A previous cladistic analysis of morphology and 5S ribosomal RNA (rRNA) sequences suggests that the Tulasnellales may be the sister group to the hymenomycetes (Hibbett et al., unpubl. data).

Cultures were maintained on 1.5% malt-extract 2% agar at 4°C. Mycelium for DNA isolation was grown for 1–3 wk at room temperature in 50 ml MYG liquid media (1% malt-extract,

0.4% yeast-extract, 1% glucose). DNA was isolated essentially as described by Raeder and Broda (1985) from lyophilized cultured mycelia, field-collected sporocarps, or herbarium materials (Table 1). Genomic DNA's were gel-purified in low melting-point agarose (0.6% Sea Plaque, FMC Bioproducts) and ribosomal DNA was amplified as described in Vilgalys and Hester (1990). The Polymerase Chain Reaction (PCR; Saiki et al. 1988) was used to amplify an approximately 1.7 kilobase sequence that is homologous to a region in *Saccharomyces cerevisiae* Meyers and Hansen rDNA from base position 34 in the 5.8S coding sequence to position 1448 in the 25S rRNA coding sequence (see Vilgalys and Hester 1990, for primer sequences). Control reactions in which the genomic DNA template was replaced with water were performed to check for contamination by exogenous DNA.

PCR products were digested with the restriction enzymes *Eco*RI and *Bgl*II and cloned into the plasmid pUC 119 (Table 1). Transformed *E. coli* was grown in TB broth with 25–50 μg/ml ampicillin and plasmids were harvested by alkaline lysis followed by polyethylene glycol precipitation (Sambrook et al. 1989). For most isolates, up to 12 individual clones were pooled but some isolates are represented by single clones (Table 1). Plasmids were sequenced using Sequenase (U.S. Biochemicals). Partial sequences from the 25S coding region (Appendices 1–3) were obtained using oligonucleotide primers LR0R, LR3, and LR6 which align to positions 26–42, 654–638, and 1141–1125 in *S. cerevisiae* 25S rRNA, respectively. Hereafter these will be referred to as the LR0R, LR3, and LR6 sequences. Partial internal transcribed spacer 2 (ITS2) sequences (Appendix 4) for a subset of the taxa were obtained using primer LR1 which aligns to positions 73–56 in *S. cerevisiae* 25S rRNA (Vilgalys and Hester 1990). LR3 and LR6 sequences were not obtained from *Lenzites betulina* and *Ganoderma lucidum*.

Partial 25S rDNA coding sequences for all species except *Mycena galericulata* and *Thanetephorus cucumeris* have been deposited in GenBank (Intelligenetics Inc., Mountain View, California; accession numbers listed in Appendices 1–3). The *Mycena* and *Thanetephorus* sequence data will be deposited in GenBank as complete 25S rDNA sequences (available on request).

Sequences were recorded using the MICROGENIE computer package (Beckman), and aligned with the aid of the ALIGN computer package (vers. 1.0, Scientific and Educational Software, State Line, Pennsylvania) which implements the algorithm of Myers and Miller (1988). ALIGN parameters were: mismatch penalty = 2, open gap penalty = 4, and extended gap penalty = 1. Nucleotide substitutions from regions of unambiguous alignment were used as characters (Appendices 1–3), with gaps coded as missing. Autapomorphies were not used in construction of the data matrix. Consensus character distributions were constructed for *Lentinus lepideus*, *L. ponderosus*, and *L. strigosus*. Positions that varied within these species were coded as polymorphic ("uncertain").

Morphological characters previously used to delimit genera and formulate phylogenetic hypotheses were chosen for cladistic analysis and were scored from published descriptions. Character descriptions and references are listed in Appendix 5.

Phylogenetic analyses were performed with PAUP version 3.0 (Swofford 1990) configured for the Macintosh. Because of the size of the data matrix (available on request), heuristic methods had to be used. Effectiveness of the heuristic tree-building methods is sensitive to the addition sequence used, so in each analysis a variety of addition sequences were employed, following Swofford's recommendation (1990). Branch swapping was performed with the tree bisection-reconnection option. Strict and 50% majority-rule consensus trees were constructed from the most parsimonious trees.

Trees up to five steps longer than the most parsimonious trees were examined. Strict consensus trees were constructed from the six nested sets of successively longer trees and these were compared to the most parsimonious trees. The decay index, here abbreviated d.i., is the number of extra steps required to lose resolution of a particular branch in the strict consensus tree (Mishler et al. 1991). All of the trees up to three steps longer than the most parsimonious tree could be stored in memory and used to calculate the first three decay indices. For calculation of the fourth and fifth decay indices, up to approximately 15,000 trees could be stored.

Bootstrapping was performed with up to 861 replicates (Felsenstein 1985). To run the bootstrap within 24 hr, MAXTREES had to be set at ten for each replicate.

Three data sets were analyzed: morphological

data alone, rDNA data alone, and a combined data set. In all analyses, all characters were weighted equally. We chose not to weight the molecular sequence characters for transition-transversion bias (Kimura 1980) because we wanted to combine morphological and molecular characters in analyses. We are unaware of a method for rationally assigning weights to morphological characters in a weighting scheme whose parameters are based on transition-transversion bias. For rDNA sequences, weighting on the basis of secondary structure has also been advocated (Hixson and Brown 1986; Steele et al. 1988; Wheeler and Honeycutt 1988; Wolters and Erdman 1986). We chose not to perform this kind of molecular character weighting for two additional reasons: first, this type of weighting requires that an accurate secondary structure model be constructed for each sequence. We have constructed secondary structure models for a portion of our sequences using the method of Zuker implemented under the UWGC computer package (Zuker and Stiegler 1981; Hibbett and Vilgalys, unpubl. data). The thermodynamically optimal secondary structures obtained from homologous sequences were often very different from each other so "phylogenetic" or comparative methods had to be used to infer the most likely structure (Guttell and Fox 1988). The secondary structure models constructed with this method are supported by the occurrence of some apparent compensatory base changes in putative stem regions. However, we feel that using these models as sources of information for character weighting would add untested assumptions to the phylogenetic analysis. Second, our secondary structure models showed that non-canonical base pairing is common which complicates this kind of weighting.

The morphological data set was analyzed as an unrooted network. Analyses that included rDNA characters used *Thanetephorus cucumeris* as an outgroup. Outgroup and Lundberg rooting were performed (Lundberg 1972). For Lundberg rooting the *Thanetephorus* character states were used as ancestral states.

An outgroup-rooted analysis of the combined data set was performed under a user-defined topological constraint that forced *Lentinus* to be monophyletic. Tree lengths and consistency indices of the constrained and unconstrained trees were compared.

Results

Morphology. The distribution of morphological characters is summarized in Table 2. The unrooted analysis of morphological characters resulted in 385 equally parsimonious networks of 77 steps, with a consistency index of 0.597. *Lentinus* was monophyletic in a majority of the equally parsimonious networks, but was monophyletic in only 8% of the bootstrap replicates (Fig. 1). Three monophyletic groups of *Lentinus* species were resolved (Fig. 1): Group 1) *L. tigrinus* and *L. crinitus* (65%, d.i. = 2); Group 2) *L. lepideus*, *L. ponderosus*, *L. kauffmannii*, and *L. sulcatus* (50%, d.i. = 1); Group 3) *L. velutinus*, *L. torulosus*, and *L. strigosus* (44%, d.i. = 0). These groups form an unresolved polychotomy that includes *L. dactyloides* (Fig. 1). Support for the overall topology is weak as measured by bootstrapping and decay indices (Fig. 1).

Variation in ITS2. Aligned ITS2 partial sequences from 10 isolates are shown in Appendix 4. Between the isolates of *Lentinus lepideus* and *L. ponderosus* there was over 95% sequence similarity. *Lentinus lepideus* and *L. ponderosus* are morphologically similar and are presumably closely related (Pegler 1983; Miller 1965). Other than for this pair of species, the sequences are very divergent and the alignments are ambiguous because of numerous small length mutations and point substitutions. ITS2 was judged to be too variable for this study and was not examined further.

Variation in the 25S rRNA Coding Sequence. Aligned partial sequences from the 25S rRNA coding sequences are shown in Appendices 1–3. The LR0R, LR3, and LR6 sequences align to positions 70–306, 371–597, and 845–1093 in *Saccharomyces cerevisiae* 25S rRNA, respectively. A total of 704 bases were aligned of which 240 (34%) were variable positions. 133 of the variable positions were phylogenetically informative; the remainder were autapomorphies. Differences were observed between the levels of conservation of the three sequences. The LR0R, LR3, and LR6 sequences were composed of 32%, 62%, and 10% variable positions with 48, 77, and 8 informative sites, respectively.

Within-species sequence variability for *Lentinus lepideus*, *L. ponderosus*, and *L. strigosus* was low: there were 17, 15, and 5 variable positions, respectively. The average within-species se-

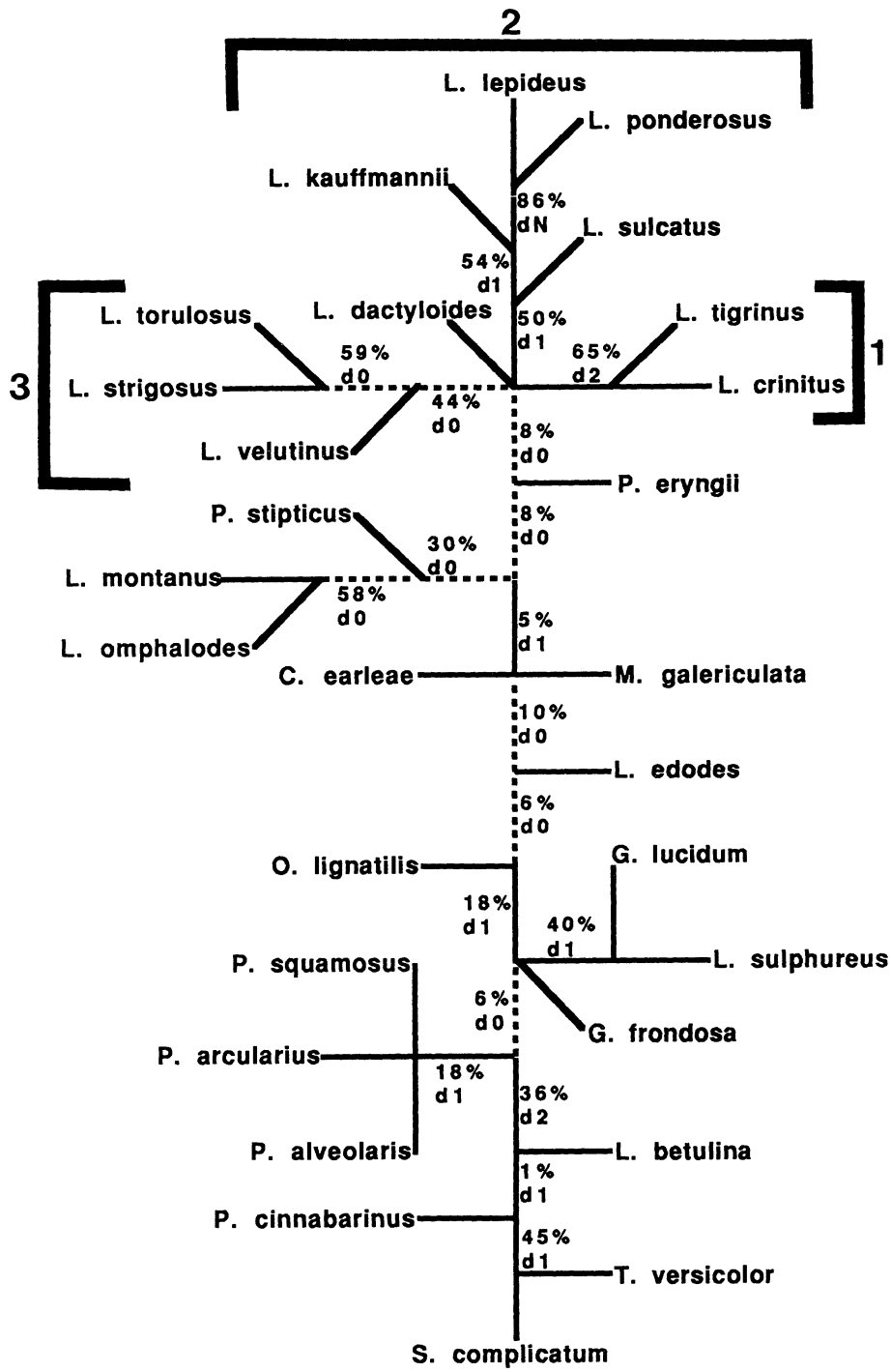

FIG. 1. Fifty percent majority-rule consensus unrooted network based on morphological characters of representatives of *Lentinus*, Tricholomataceae, Polyporaceae, and Corticiaceae (see Table 1 for genus abbreviations). 385 input trees, length 77 steps, consistency index = 0.597. Dashed lines indicate branches that were present in the majority-rule tree, but not in the strict consensus tree. Bootstrap intervals from 116

quence similarity was 97.6%, 97.9%, and 99.3%, respectively, or 98.2% overall.

Phylogenetic Analyses of Molecular Characters Alone. Cladograms based on rDNA data alone are shown in Figures 2 and 3. Under outgroup rooting, there were 22 equally parsimonious rDNA-only trees with 479 steps and consistency index of 0.497 (Fig. 3). The majority-rule consensus tree supports the monophyly of the same three groups of *Lentinus* species as the morphology-only tree, but *Lentinus* as a whole is shown as being polyphyletic (Fig. 3). Group 1 is in the polypores and has *Polyporus arcularius* as its sister group. Group 2 is the sister group to the rest of the ingroup taxa. Group 3 is one node removed from Group 2. *Lentinus dactyloides* is the sister group to *Pleurotus eryngii* (Fig. 3).

Under Lundberg rooting, there were 26 equally parsimonious trees with 453 steps and consistency index of 0.510 (Fig. 2). In the majority-rule consensus tree *L. sulcatus* is not monophyletic with Group 2. Otherwise, the Lundberg and outgroup-rooted trees support the same monophyletic groups of *Lentinus* species. The major difference between the outgroup and Lundberg-rooted rDNA-only trees is that under Lundberg-rooting, the polypores plus Group 1 are paraphyletic. In all of the equally parsimonious Lundberg-rooted trees *Lenzites betulina* is the sister group to the clade that contains the Tricholomataceae and *Stereum*. The remaining polypore species are in a single lineage that also includes Group 1 and Group 3 (Fig. 2).

Bootstrapping and decay indices provided similar levels of support for comparable branches under both Lundberg and outgroup-rooting (Figs. 2 and 3). For most branches there was general agreement between the bootstrap and the decay index: for the branches in Figure 2 the Pearson product-moment correlation of the decay index and bootstrap interval is 0.741 ($P < 0.001$). For the sample of all branches in Figures 2–4 the correlation was 0.744 ($P < 0.001$). Both the decay index and the bootstrap support certain terminal groups of species. Branches that did not decay after five extra steps were designated as d.i. = N. Strongly supported groups include: Group 3 (98%, d.i. = N, outgroup; 97%, d.i. = N, Lundberg); *L. lepideus* and *L. ponderosus* (99%, d.i. = 4, outgroup; 100%, d.i. = 3, Lundberg); *L. dactyloides* and *Pleurotus eryngii* (100%, d.i. = N, outgroup and Lundberg); *Lentinula edodes* and *Collybia earleae* (100%, d.i. = N, outgroup and Lundberg); and *Lentinellus omphalodes* and *L. montanus* (100%, d.i. = N, outgroup and Lundberg). There was a discrepancy between the bootstrap and decay index values regarding the monophyly of Group 1 plus *Polyporus arcularius* (95%, d.i. = 0, outgroup; 91%, d.i. = 0, Lundberg). Thus, even though this branch is strongly supported by bootstrapping, there are equally parsimonious topologies that contradict it.

Support for many of the internal branches was weak as measured by both the bootstrap and decay index. Lack of robustness is also reflected by sensitivity of the results to choice of rooting method (compare Figs. 2 and 3).

Phylogenetic Analyses of the Combined Data Set. Outgroup and Lundberg-rooted trees for the combined data set are shown in Figures 3 and 4. Under outgroup-rooting, there were 14 equally parsimonious trees with 578 steps and consistency index of 0.491. The outgroup-rooted majority-rule consensus tree based on the combined data set is topologically identical to the outgroup-rooted majority-rule consensus tree based on the rDNA characters alone (Fig. 3).

The outgroup-rooted combined analysis in which *Lentinus* was topologically constrained to be monophyletic resulted in 18 equally parsimonious trees that were 50 steps longer than the minimal outgroup-rooted tree and that had a consistency index of 0.452.

The Lundberg-rooted analysis of the combined data set yielded a single tree with 552 steps and a consistency index of 0.502.

Under both rooting methods, there were fewer trees with the combined data set than with rDNA characters alone. The greatest topological difference between the combined and rDNA-

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replicates indicated with "%." Decay indices preceded by "d." Decay indices calculated up to 79 steps. Branches remaining at last decay level indicated by "dN." Bracketed groups designated 1, 2, and 3 correspond to species groups 1–3 discussed in text.

TABLE 2. Morphological characters used in cladistic analyses of *Lentinus*, Polyporaceae, Tricholomataceae, and Corticiaceae. See Appendix 5 for character codes. Polymorphic characters enclosed in parentheses.

Species	Characters																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
L. lepideus	0	0	1	0	0	1	0	0	0	0	1	1	0	0	1	1	2	0	0	1
L. ponderosus	0	0	1	0	0	1	0	0	0	0	1	1	0	0	1	0	2	0	0	1
L. kauffmannii	0	1	1	0	0	1	0	0	0	0	1	9	0	0	1	0	0	0	0	1
L. sulcatus	0	1	0	0	1	1	0	0	0	0	1	9	0	0	1	0	(02)	0	0	0
L. dactyloides	0	1	0	0	0	1	0	0	0	0	9	9	0	0	0	0	(02)	0	1	0
L. strigosus	0	1	0	1	0	1	1	1	0	0	0	9	0	1	0	0	1	0	0	0
L. torulosus	0	1	0	1	0	1	1	1	0	0	0	9	0	1	0	0	0	0	0	(01)
L. velutinus	0	1	0	1	0	1	1	0	0	0	9	9	0	0	0	0	1	0	1	9
L. tigrinus	1	0	0	0	0	2	0	0	0	0	0	0	0	0	0	1	2	0	0	0
L. crinitus	1	0	0	0	0	2	1	0	0	0	0	9	0	0	0	0	0	0	0	9
Polyporus arcularius	0	0	0	0	0	2	0	0	0	0	0	0	1	0	9	0	2	0	0	0
P. squamosus	0	0	0	0	0	2	0	0	0	0	0	0	1	1	9	0	2	0	0	0
P. alveolaris	1	0	0	0	0	2	0	0	0	0	0	0	1	(12)	9	0	2	0	0	0
Grifola frondosa	0	0	0	0	0	2	0	1	0	0	0	9	1	1	9	0	0	0	0	(01)
Laetiporus sulphureus	0	0	0	0	1	2	0	1	0	0	1	9	1	(12)	9	0	0	1	0	(01)
Pycnoporus cinnabarinus	1	0	0	0	0	3	0	0	0	0	0	0	1	3	9	0	0	0	0	(01)
Lenzites betulina	0	0	0	0	0	3	0	0	0	0	0	0	1	3	9	0	0	1	0	(01)
Trametes versicolor	0	0	0	0	0	3	0	0	0	0	0	0	1	3	9	0	1	1	0	0
Stereum complicatum	0	0	0	0	2	0	9	0	0	1	0	1	2	3	9	0	1	1	0	(01)
Ganoderma lucidum	0	0	0	0	0	2	0	1	1	0	0	0	1	(12)	9	0	0	1	0	0
Pleurotus eryngii	0	9	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	2
Ossicaulis lignatilis	0	0	0	0	0	0	0	1	0	0	1	0	0	1	2	0	0	0	0	(01)
Collybia earleae	0	1	0	0	0	0	0	1	0	0	0	0	0	0	2	0	0	0	0	3
Panellus stipticus	0	1	0	0	0	0	1	0	0	1	0	9	0	1	2	0	0	0	0	0
Lentinellus omphalodes	0	1	0	0	0	0	1	2	2	1	0	9	0	0	2	0	0	0	0	0
L. montanus	0	1	0	0	0	0	1	2	2	1	0	9	0	1	0	0	0	0	0	(013)
Mycena galericulata	0	1	0	0	0	0	0	1	0	1	9	9	0	0	2	0	0	0	0	0
Lentinula edodes	0	0	0	0	0	0	0	1	0	0	0	9	0	0	2	1	2	0	0	0

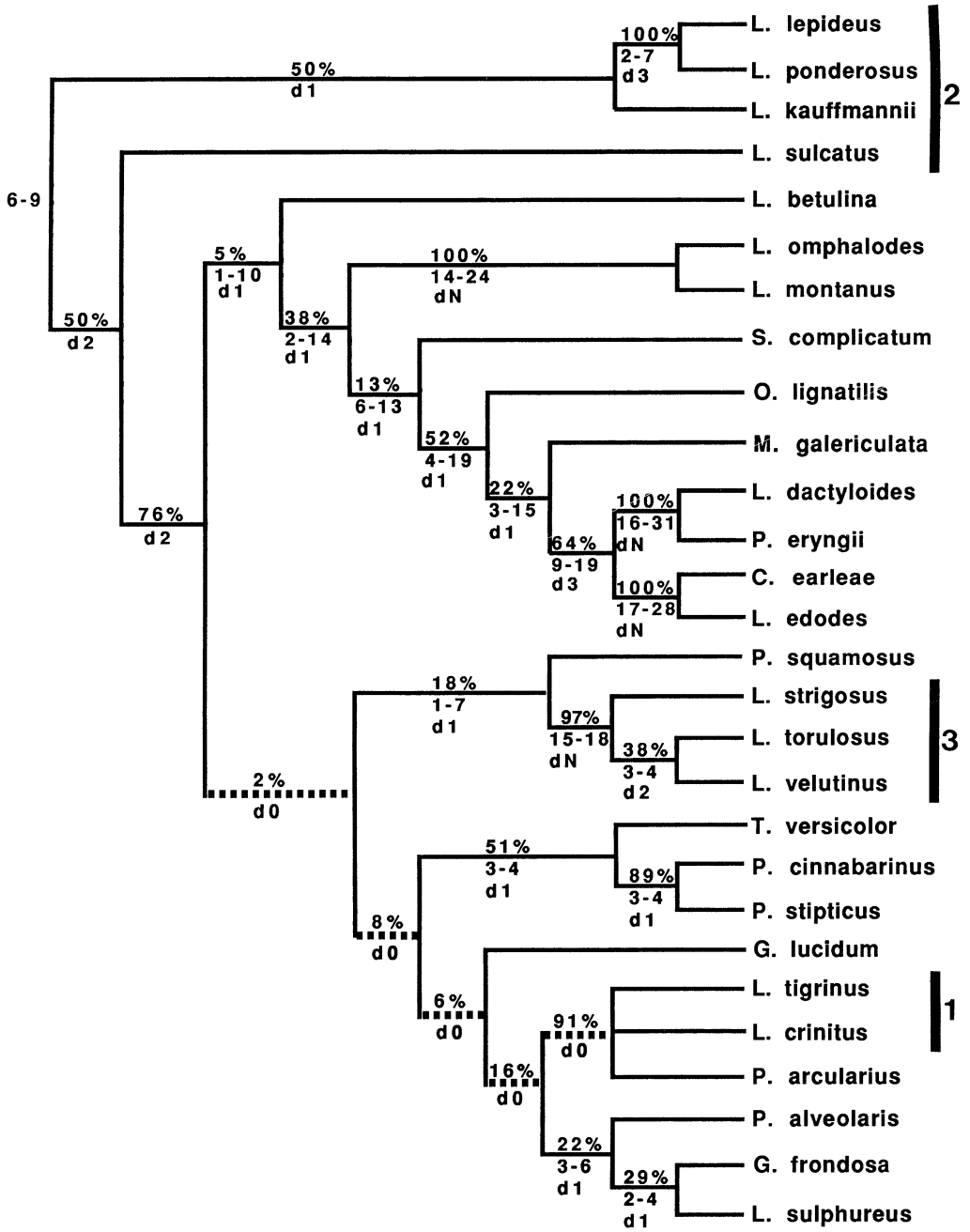

FIG. 2. Fifty percent majority-rule consensus tree based on Lundberg-rooted analysis of rDNA sequence characters of representatives of *Lentinus*, Tricholomataceae, Polyporaceae, and Corticiaceae using *Thanetephorus cucumeris* as an outgroup (see Table 1 for genus abbreviations). Twenty-six input trees, length 453 steps, consistency index = 0.510. Bootstrap intervals based on 861 replicates. Length ranges of branches present in strict consensus tree indicated by hyphenated numbers. Decay index calculated up to 458 steps. See caption to Figure 1 for other symbols.

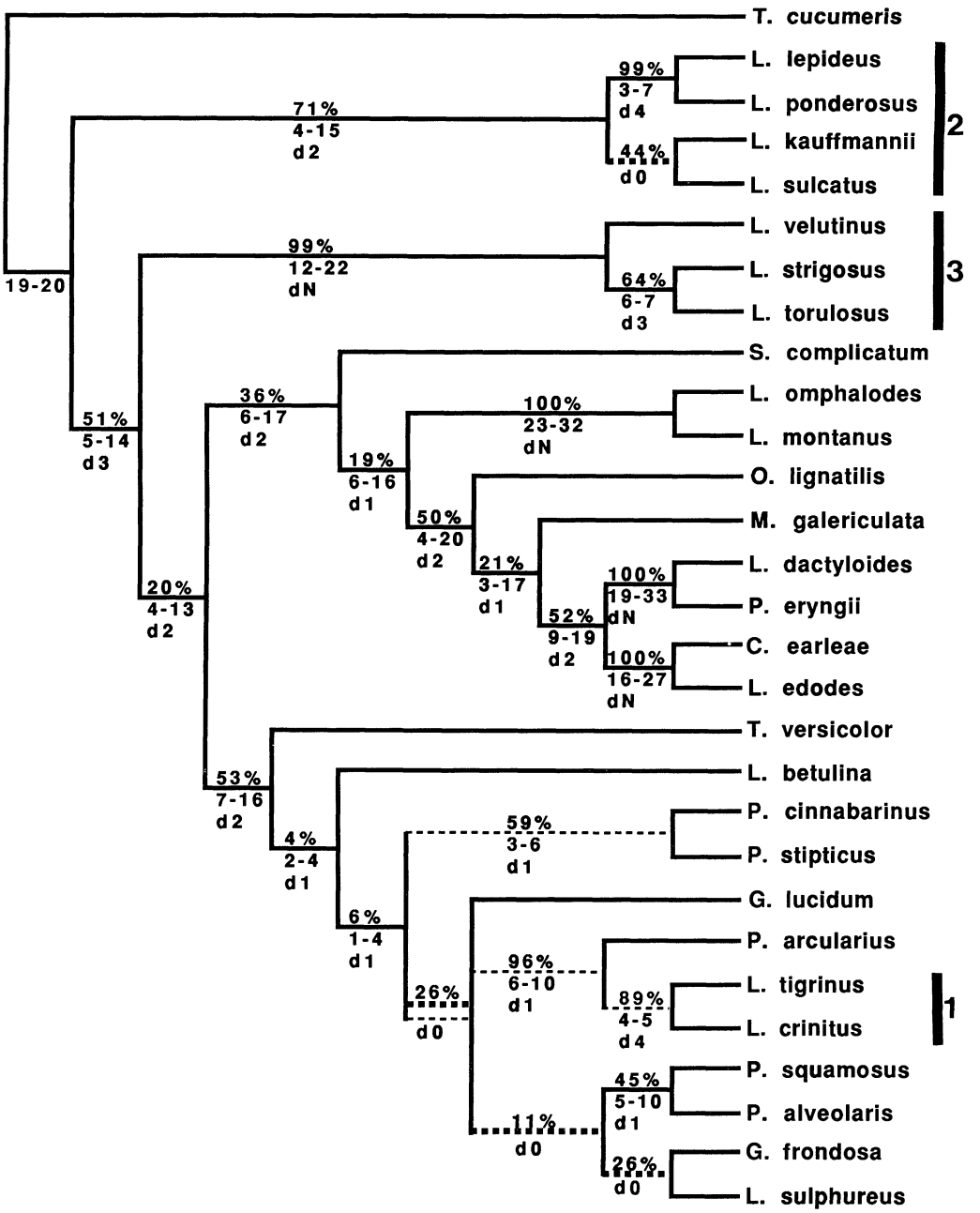

FIG. 3. Fifty percent majority-rule consensus tree based on outgroup-rooted analyses of rDNA sequence characters alone or combined data set for *Lentinus*, Tricholomataceae, Polyporaceae, and Corticiaceae with *Thanetephorus cucumeris* as an outgroup (see Table 1 for genus abbreviations). Twenty-two input rDNA-only trees, length 479 steps, consistency index = 0.497. Fourteen input combined trees, length 578 steps, consistency index = 0.491. Topology of fifty-percent majority-rule consensus tree is the same with either data set. Bootstrap intervals based on 653 replicates, decay indices up to 583 steps and branch length ranges are for the combined data set. Heavy dashed lines indicate branches not present in strict consensus of combined data set. Light dashed lines indicate branches not present in strict consensus of molecular-only trees. See captions to Figures 1 and 2 for other symbols.

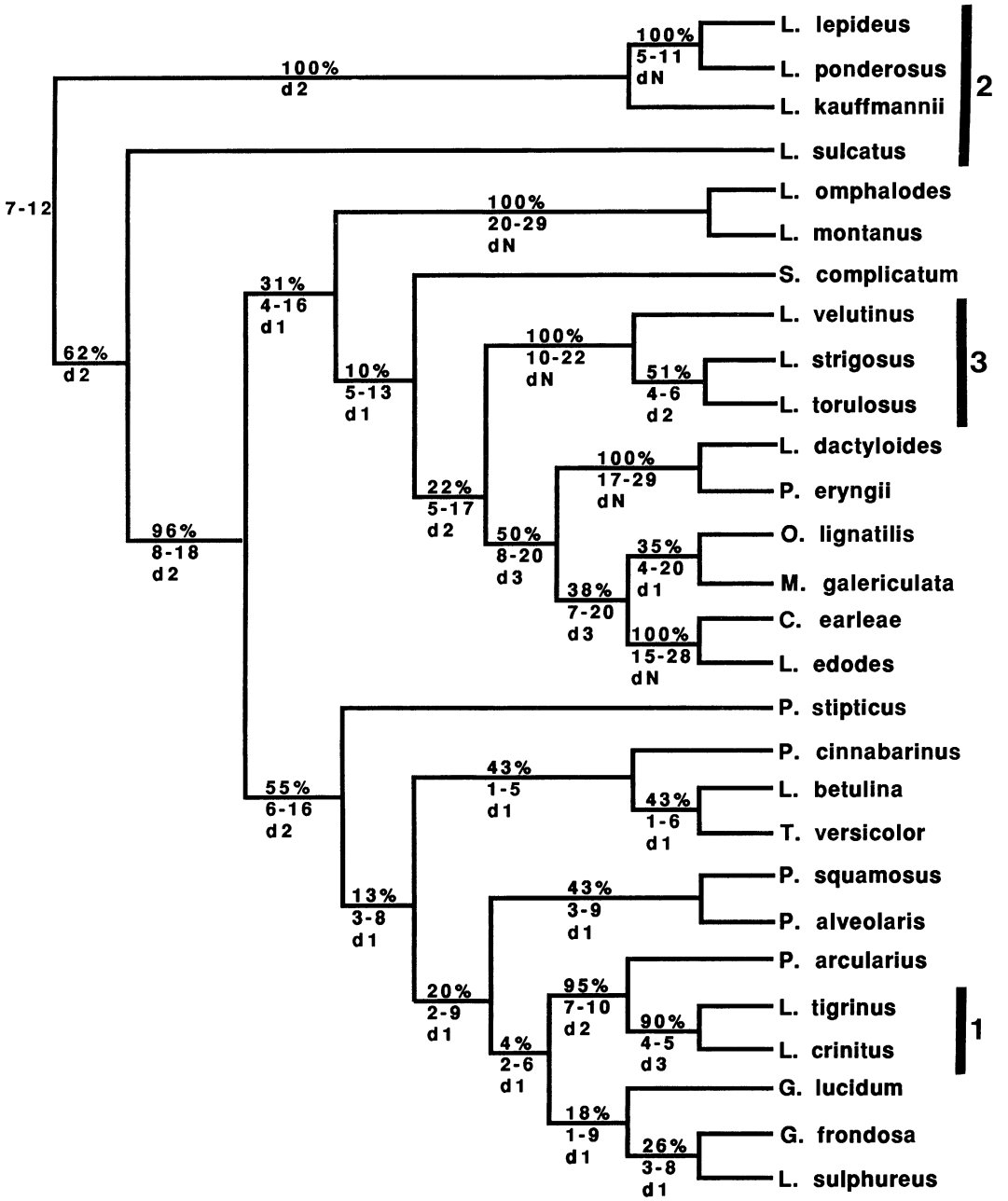

FIG. 4. Single most parsimonious tree from Lundberg-rooted analysis of combined data set for *Lentinus*, Tricholomataceae, Polyporaceae, and Corticiaceae using *Thanetephorus cucumeris* as an outgroup (see Table 1 for genus abbreviations). Length = 552 steps, consistency index = 0.502. 697 bootstrap replicates. Decay indices calculated up to 557 steps. See captions to Figures 1 and 2 for symbols.

only trees was observed under Lundberg rooting. In the combined Lundberg-rooted tree the polypores plus Group 1 form a completely resolved monophyletic group that has *Panellus stipticus* as its sister group and that does not contain Group 3 (Fig. 4).

Discussion

Variability in rDNA Regions. Studies of rRNA secondary structure indicate that eukaryotic large-subunit rRNA's are composed of a conserved "core" that has a similar secondary structure to prokaryotic rRNA's, and interspersed "divergent domains" that appear to have no prokaryotic homologue and that account for much of the size difference between eukaryotic and prokaryotic rRNA's (Guttell and Fox 1988; Hillis and Davis 1987; Michot et al. 1984; Michot and Bachellerie 1987). In this study, most of the coding sequence variation was in the LR0R and LR3 sequences whereas the LR6 sequences were highly conserved (Appendices 1–3). The LR0R and LR3 sequences align to the divergent domains D1 and D2, respectively, whereas the LR6 sequence aligns to a part of the conserved core regions (Michot et al. 1984). This pattern of variation in large subunit rRNA is similar to that noted by Guadet et al. (1989) in studies on *Fusarium* Link ex E. M. Fries. In the present study, D1 and D2 provided a more useful level of variation than the LR6 or ITS2 sequences. However, the high level of homoplasy and the low bootstrap and decay index values, particularly at the internal nodes of the trees, suggest that some sites have been saturated by multiple substitutions and are therefore no longer informative for the most ancient divergences (Mishler et al. 1988; Smith 1989).

Independent Vs. Combined Analyses. Morphological characters alone were insufficient to resolve overall relationships. Still, three groups of *Lentinus* species were weakly supported as monophyletic (Fig. 1). The monophyly of these groups is also supported by the rDNA characters alone (Fig. 3). This congruence suggests that each data set does contain some accurate phylogenetic information (Cracraft and Mindell 1989). The trees based on rDNA characters alone are more resolved than the morphology-only tree and provide strong support for the monophyly of certain groups, as measured by bootstrapping and the decay index (Figs. 2 and 3). This suggests that rDNA sequence characters are superior to morphological characters for phylogenetic inference in these fungi, even when the morphological characters are analyzed cladistically.

The rationale for combining molecular and morphological evidence has been discussed by Donoghue and Sanderson (1991), Doyle (1992), Hillis (1987), Kluge (1989), and Miyamoto (1985). As noted by these authors, combined analyses maximize parsimony for all putative homologies. Therefore, results of the combined analyses are preferred to those of the independent analyses of rDNA or morphological characters.

Combined analyses resulted in more resolved cladograms than the independent analyses (Figs. 1–4). For example, in the Lundberg-rooted analysis of rDNA data alone, the monophyly of Group 1 and *Polyporus arcularius* is supported by a majority of the minimal-length trees and by bootstrap intervals (91%) but alternate equally parsimonious topologies exist (d.i. = 0; Fig. 2). With the combined data set, under the same rooting method, these three species form a fully resolved monophyletic group that is strongly supported by bootstrapping (95%; Fig. 4). However, the low decay index (d.i. = 2; Fig. 4) indicates that there is character conflict in the data and that alternate topologies are nearly as parsimonious. Even though the morphological characters did not resolve the overall relationships on their own, they did add resolution in parts of the combined analysis, especially where molecular characters do not strongly support the topology (Donoghue and Sanderson 1991). Far from being overwhelmed, morphological characters had a significant impact on the results, despite the fact that they were not given greater weight than the more numerous rDNA characters (Figs. 2–4).

Rooting. The choice of rooting method had an effect on both the combined and rDNA-only topologies (Figs. 2–4). In outgroup rooting, a global parsimony analysis is performed for a set of taxa that includes the designated outgroup. Lundberg rooting is designed to minimize ingroup homoplasy that can result from use of a distantly related outgroup (Lundberg 1972). Under Lundberg-rooting, an unrooted ingroup network is constructed and this is rooted by attaching the outgroup at the internode where it minimizes tree length. Thus, the Lundberg-rooted topology preserves the cladistic rela-

tionships of the most parsimonious unrooted ingroup network. At present, higher-order evolutionary relationships of the hymenomycetes are poorly understood and so it was not possible to choose an outgroup from among the hymenomycetes. *Thanetephorus cucumeris*, representing the Tulasnellales, was a conservative outgroup choice and was scored for only molecular characters. Our results suggest that some variable sites may already be saturated by multiple substitutions at the level of the ingroup. If this is true, then it is probably misleading to use a distantly related outgroup taxon to polarize rDNA sequence character states (Miyamoto and Boyle 1989; Wheeler 1990). The Lundberg-rooted topologies are, therefore, preferred to the outgroup-rooted topologies.

Bootstrapping and Decay Index. Although decay indices and bootstrap values were positively correlated, the branch uniting *Polyporus arcularius* and Group 1 shows that a high bootstrap value on a branch does not necessarily mean that there is not an equally parsimonious, or near-minimal tree that contradicts the branch (Figs. 2–4). On the other hand, branches with high decay indices always had high bootstrap intervals (Figs. 2–4). These results suggest that the decay index is a more conservative estimate of robustness than the bootstrap (Mishler et al. 1991).

The decay index may lack the sensitivity necessary for ranking robustness of branches within a tree. For example, in Figure 2 the 11 branches with a decay index of one have bootstrap values of 4–43% and occur in 22–98% of the trees one step longer than the minimal tree. This suggests that some branches are more robust than others, even though they all have the same decay index. For within-tree ranking, it might be preferable to base the decay index of a branch on the frequency of its occurrence in the near-minimal trees, rather than on strict consensus. The situation is complicated, however, by the fact that "families" of topologically similar trees may exist among the set of trees of a given length (Hendy et al. 1988). The number of individual trees that contain a branch may be less significant than the number of families of trees that contain the branch. Families of trees can be identified by phenetic analysis of a tree comparison metric, such as the partition metric (Penny and Hendy 1985). The relationship between families of trees, the decay index, and the bootstrap remains to be explored.

Another drawback of the decay index, one that is shared by the bootstrap, is that its calculation is computer-intensive. With large data sets, it may not be possible to examine all the trees that are more than a few steps longer than the minimum length tree. Large data sets also require the use of heuristic methods which are sensitive to local optima and may fail to find all the possible families of trees (Swofford 1990). Both of these problems could lead to overestimates of the decay index.

Taxonomic Conclusions. Our strongest conclusions are about the monophyly of certain terminal groups of species, which may therefore deserve recognition as genera. The sensitivity of the results to the choice of rooting method, and the low bootstrap and decay index values of many internal nodes indicate that certain aspects of the topologies are weakly supported. Nevertheless, for reasons given above, we propose that the Lundberg-rooted analysis of the combined data set is the best estimate of the overall phylogeny that is possible with the data at hand (Fig. 4). This topology is consistent with previously published results from a Fitch-Margoliash analysis of rDNA restriction fragment length polymorphism data from a subset of taxa in this analysis (Hibbett and Vilgalys 1991). The tree suggests that the Agaricales are a paraphyletic group that has given rise to the Corticiaceae and the Polyporaceae. This is inconsistent with the Friesian classification of the Agaricales and Aphyllophorales, which has long been regarded as an artificial taxonomic system (Fayod 1889; Patouilliard 1900, and later authors).

The placement of *Panellus stipticus* as the sister group to the polypore clade (Fig. 4), or as derived from the polypores (Figs. 2 and 3), was unexpected because *P. stipticus* is monomitic and lamellate. Still, *P. stipticus* does have a number of polypore-like characters, including the ability to revive from the dry state upon rewetting and the growth habit of imbricate clusters on wood. These similarities are not proposed as unique synapomorphies of *P. stipticus* and polypores, however. The most parsimonious distribution of hymenophore characters in Figure 4 is for *P. stipticus* to be plesiomorphically lamellate. However, *Panellus* has been combined with the poroid genus *Dictyopanus* Pat., based on an-

atomical similarities and bioluminescence (Burdsall and Miller 1975, 1978; Corner 1950). If *Dictyopanus* and *Panellus* are placed in a single genus, then it becomes equivocal whether the lamellate hymenophore of *Panellus* is homologous to the lamellae of other agarics.

The transfer of shiitake out of *Lentinus* as *Lentinula edodes* was supported (Pegler 1975). The sister-group relationship of *Lentinula edodes* and *Collybia earleae* is consistent with Pegler's (1975) placement of *Lentinula* in the Collybieae (Figs. 2–4).

Monophyly of *Lentinus* was not supported, suggesting that dimiticity is polyphyletic (Figs. 2–4). In the Lundberg-rooted analysis of the combined data set (Fig. 4) *Lentinus* is distributed among the following groups:

GROUP 1: *LENTINUS* S. STR. Group 1 is shown as being derived from the polypores with *Polyporus arcularius* as its sister group. This is consistent with Pegler's (1983) hypothesis on the origin of *Lentinus*, which suggested that the lamellae of these species (and those of *Lenzites betulina*) are the result of an evolutionary reversal (Fig. 4). Group 1 represents *Lentinus* subg. *Lentinus* which includes 27 species characterized by ligative hyphae and hyphal pegs [except sect. *Lentodiellum* Murrill which lacks hyphal pegs (Pegler 1983)]. Among the taxa included in this analysis, hyphal pegs are also found in *Pycnoporus cinnabarinus* and *Polyporus alveolaris*, which occur on two separate lineages (Figs. 2–4). The distribution of hyphal pegs is most parsimoniously attributed to three parallel gains, suggesting that they are not homologous. However, with two extra steps, it is possible to infer that hyphal pegs are plesiomorphic for the polypores and that they have been independently lost repeatedly. There are approximately seven genera of polypores that have members with hyphal pegs that were not included in this study, and therefore it is premature to infer the evolutionary history of this character.

GROUP 2: *NEOLENTINUS* AND *HELIOCYBE*. Representatives of *Neolentinus* in this study include *Lentinus dactyloides*, *L. kauffmannii* (which is the type species), *L. lepideus*, and *L. ponderosus* (Redhead and Ginns 1985). *Lentinus kauffmannii*, *L. lepideus*, and *L. ponderosus* were supported as being monophyletic (Fig. 4) but *L. dactyloides* was not supported as part of this group. *Lentinus kauffmannii*, *L. lepideus*, and *L. ponderosus* all produce a brown rot and have a bipolar mating system, which are important characters in the concept of *Neolentinus* (Redhead and Ginns 1985). We are unaware of published descriptions of the type of rot or mating system for *L. dactyloides*.

Pegler placed *L. dactyloides*, *L. lepideus*, *L. ponderosus*, and three other species in sect. *Squamosi* and noted that these species all have a "reduced dimitic hyphal construction" (Pegler 1983, p. 11) and large spores that he hypothesized were indicative of a close relationship to *Pleurotus*. Although our results do not support the monophyly of sect. *Squamosi*, they do suggest that *L. dactyloides* is closely related to *Pleurotus eryngii* (Figs. 2–4). Our results do not support a close relationship between *Pleurotus* and the Polyporaceae (Figs. 2–4).

Lentinus sulcatus was transferred to the monotypic genus *Heliocybe* by Redhead and Ginns (1985) as *H. sulcata* (Berk.). The results of this study indicate that *L. sulcatus* is closely related to *Neolentinus*. Both have a brown rot and bipolar mating system (Redhead and Ginns 1985). In the outgroup-rooted analysis of the combined data *L. sulcatus* is the sister group to *L. kauffmannii*, but in the Lundberg-rooted analysis it is one node removed from the *Neolentinus* species (Figs. 3 and 4).

Laetiporus sulphureus and *Ossicaulis lignatilis* are also brown rot species. Under Lundberg-rooting, the most parsimonious character distribution is to have brown rot and bipolar mating system plesiomorphic for the ingroup as a whole, but under outgroup-rooting it is equivocal (Maddison et al. 1984). Under either rooting option, our results are consistent with previous hypotheses that brown rot has evolved repeatedly (Gilbertson 1980; Figs. 2–4).

GROUP 3: *PANUS*. The monophyly of Group 3 is strongly supported, but its placement is ambiguous (compare Figs. 2–4). The species in Group 3 have all been previously classified in *Panus*, and *Lentinus torulosus* is the type species of *Panus*, as *P. conchatus* Fr. (Corner 1981; Pegler 1983; Singer 1986). These species have similarities in rDNA sequences and anatomical features (strongly developed skeletal hyphae, pleurocystidia, and radiate hymenophoral trama) but contrast sharply in stature: *L. strigosus* and *L. torulosus* have short lateral to excentric stipes whereas *L. velutinus* has an elongate central stipe. Furthermore, *L. velutinus* sporocarps develop from a pseudosclerotium that is lacking

in *L. strigosus* and *L. torulosus* (Corner 1981; Pegler 1983). This suggests that sporocarp gross morphology and life history strategy have evolved rapidly relative to rDNA and anatomical characters.

This study supports the view that *Lentinus, Panus, Neolentinus,* and *Heliocybe* should be recognized as distinct genera. However, because of limited taxon sampling, the limits of these genera cannot be addressed. Outgroups to *Panus, Neolentinus,* and *Heliocybe* remain unclear. The results of this study are consistent with Pegler's (1983) hypothesis that *Lentinus* is derived from polypores.

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APPENDIX 1. Aligned rDNA sequences from primer LR0R. See Table 1 for genus abbreviations. Gaps or unsequenced end regions indicated by dashes. Unreadable bases indicated by "N." Phylogenetically informative positions used in data matrix indicated by asterisks. Homologous position in *Saccharomyces cerevisiae* rDNA and polarity indicated by arrows and numbers above alignment. GenBank accession numbers, beginning with "M," follow sequences.

```
                ↓70(5')                                                    135(3')↓
                                                    *           **   *   **   * **
L. lepideus13   ----GATTCCCCTAGTAACTGCGAGTGAAGCGGGAAGAGCTCAAATTTAAAATCTGGCGGTCCTTTG
L. lepideus17   ------------------------------------------------------........T....
L. lepideus47   ---------------------------.......................................N
L. ponderosus14 -----..............................................................
L. ponderosus15 --------..............T............................................
L. ponderosus16 --------...........................................................
L. kauffmannii  ----.G.-------------------------------------------------------.....
L. sulcatus     ---------......G...G................A........................-...C.
L. dactyloides  -----...........................-...A.....................T..-.....
L. strigosus22  -----...............................A.................G...A..-.....
L. strigosus23  -----------------------.............A....C................A..-.....
L. strigosus48  -AAG................................A.....................A..-.....
L. torulosus    -------.............................A.....................A..-.....
L. velutinus    -AAG................................A.....................A..-.....
L. tigrinus     ----................................A.....................N..-.....
L. crinitus     ---G................................A........................-.....
P. arcularius   -------.............................A.................G......-.....
P. squamosus    ----................................A....................T..-......
P. alveolaris   ----------------------------------..A.......................---....
G. frondosa     -------......................................................-..CA.
L. sulphureus   --------........................N...A.............N..........-.....
L. betulina     ------------......NN............-...A........................-.....
T. versicolor   ------------------------------------------------.............-.....
S. complicatum  ---------...........N...........-...A.........................T.C..
G. lucidum      ----T.-------G...GN............-....A........................-.....
P. eryngii      ------..............................A.....................T..-.....
O. lignatilis   --------.....................G......A................-...TA..-.....
C. earleae      --------.......................A....A............N.N......A..-.....
P. stipticus    ---------------------------------------------------------....-.....
L. edodes       AAG-................................A.....................A..-..C..
L. omphalodes   AAG-................................A...........GT..........-....C.
L. montanus     AAG-................................A.......N...GT........N.-....C.
M. galericulata CAAG................................A............................C.
T. cucumeris    -------------------------------------------------------------------
                ↓136(5')                                                   203(3')↓
                ****     *     * * *      * ****  ** *           *    ** *     ** *
L. lepideus13   GCCGTCCGAGTTGTATTCTGGAGAAGTGCTTTCCGCGCTGGACCGTGTATAAGTCTCTTGGCCCAGA
L. lepideus17   ..T..........................................................AA....
L. lepideus47   N...............................G............................AA....
L. ponderosus14 .............................................................AA....
L. ponderosus15 ..T.....T....................................................AA....
L. ponderosus16 .............................................................AA....
L. kauffmannii  .............................................................AA....
L. sulcatus     ................................-NNNN........................AA....
L. dactyloides  ...A...........A-..A........T.A.....................-..C.C...AATG.G
L. strigosus22  ..T......A................C.T.....N.N........................AAT...
L. strigosus23  ..T......A................C.T................................AAT...
L. strigosus48  ..T......A....-...........C.T................................AAT...
L. torulosus    ..T.......................C.T....................C...........AAT...
L. velutinus    ..T......A................C.TC...................C...........AAT...
L. tigrinus     .....N...C.....G..................N.N........................AA....
L. crinitus     ...............G.............-...............................AA....
P. arcularius   ...............G.............................................AA....
P. squamosus    .T.A...........G.....................T.......................AAT...
P. alveolaris   ...............G.............................................AA....
G. frondosa     ...............G.................................C...........AA....
L. sulphureus   ...............G..........C.TC...TA.N.C..........C.....C.....AA.G.G
L. betulina     ...............G.............-...................C...........AA....
T. versicolor   ...............G..........C.TC.......T...........C...........AA...G
S. complicatum  ...............G..........C.T........T...........C....T..C...AA.G..
G. lucidum      ...............G...............C.GA..........................AA....
P. eryngii      ...A...........A...A........T.A..................C.....C.C...AATG.G
O. lignatilis   ..T.C..........A...A......-CA..AT................C.....------------
C. earleae      ATT............A.T.A........T.GC...T.T...........C....T..C...AATG..
P. stipticus    .....N.........G................NNN.N........................AA....
L. edodes       ATT............A.T.A........T.AC...T.T...........C.......C...AATG..
L. omphalodes   .T.............A...A.............................C.......C...AA.G..
L. montanus     .T.............A...A.............................C.......C...AA.G..
M. galericulata ...............A.T.A........T.A..................C.......C...AATG..
T. cucumeris    ---------------------.....CATCA....T........A....C...........AA....
```

APPENDIX 1. Continued.

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                 ↓204(5')                                                   267(3')↓
                      *                     *          **   *** **    * * *     *
L. lepideus13    GCGTCATAGAGGGTGAGAATCCCGTCTTTGACACGGACTACCAGTGCTTTGTGATGCGCTCTCAAAG
L. lepideus17    ...................................................................
L. lepideus47    ............-------------------------------------------------------
L. ponderosus14  .....G.............................................................
L. ponderosus15  ..........................AAA..............................G.G.....
L. ponderosus16  ..........................AAA......................................
L. kauffmannii   ...................................................................
L. sulcatus      ......C............................................................
L. dactyloides   ............................................G..-.........A.......C.
L. strigosus22   ....................N.......................N......................
L. strigosus23   ...................................................................
L. strigosus48   ...................................................................
L. torulosus     .....G.............................................................
L. velutinus     ...................................................................
L. tigrinus      ..................NNN........N....................N.N..N.N.........
L. crinitus      ...................................................................
P. arcularius    ...................................................................
P. squamosus     ...................................................................
P. alveolaris    ............................................NN.....................
G. frondosa      ...............................................................G...
L. sulphureus    ..........................CA..........CG..G.C.G................G...
L. betulina      .....................................................TA............
T. versicolor    ...........................................A.......................
S. complicatum   ......................................CC.A.TGCT....................
G. lucidum       ....................-..............................................
P. eryngii       ............................................G............A.........
O. lignatilis    -------------------------------------------------------------------
C. earleae       A....................................--T...A...A.....G.A.A..T..G...
P. stipticus     ..-................................................................
L. edodes        ...............................T.....-.C...A.A.A.....G.A.A.....G...
L. omphalodes    .....G..........................................C..............G...
L. montanus      .....G..............-...........................C..............G...
M. galericulata  .......................................G....G..A...............G...
T. cucumeris     ..........................C......T.....C.....A.......T..T........C.

                 ↓268(5')                       306(3')↓
                                         *         *   *
L. lepideus13    AGTCGAGTTGTTTGGGAATGCAGCTCAAAATGGGTGGTA  M98644
L. lepideus17    .......................................  M98647
L. lepideus47    ---------------------------------------  M98663
L. ponderosus14  .......................................  M98647
L. ponderosus15  .......................................  M98645
L. ponderosus16  .......................................  M98646
L. kauffmannii   ..........................-...........-  M98656
L. sulcatus      .......................................  M98655
L. dactyloides   .......................................  M98666
L. strigosus22   ......................................G  M98651
L. strigosus23   ..........................-..........--  M98652
L. strigosus48   ..........................-------------  M98664
L. torulosus     ......................................G  M98650
L. velutinus     ......................................G  M98658
L. tigrinus      N..................N......-...........G  M98653
L. crinitus      ......................................G  M98659
P. arcularius    ......................................G  M98642
P. squamosus     .......................................  M98643
P. alveolaris    .......................................  M98665
G. frondosa      .......................................  M98641
L. sulphureus    ..........................-...C........  M98662
L. betulina      ......................................G  M98668
T. versicolor    .....C..................G.........A...G  M98657
S. complicatum   ........................-.............G  M98669
G. lucidum       ......................................G  M98667
P. eryngii       ..................................GT.GT  M98649
O. lignatilis    ---------------------------------------  M98660
C. earleae       ..........................-............  M98661
P. stipticus     .....-C.................G...-----------  M98654
L. edodes        ..........................T..........--  M98640
L. omphalodes    -N................................A...G  M98638
L. montanus      -N................................A...G  M98639
M. galericulata  .......................................
T. cucumeris     .......................................
```

APPENDIX 2. Aligned sequences from primer LR3. See Table 1 for genus abbreviations. See Appendix 1 caption for explanation of symbols and GenBank accession numbers.

```
                  ↓597(3')                                                        530(5')↓
                     * *     **    ** **** *  *** ***** *      *     *** **      *  *
L. lepideus13     ----------GCTAGGGCTGGTGACATATGCTGGTCTCCGATATTGTGTGGGCTTCCACGGTGTAAG
L. lepideus17     ---------------......................................C.............
L. lepideus47     ----------------------------------------------.....................
L. ponderosus14   --------------.....................G...............................
L. ponderosus15   -----------------.................C................................
L. ponderosus16   ----------------...................................................
L. kauffmannii    GACGCCAGGNN......T..A.......C.......G..............................
L. sulcatus        ACGCCAGGA.T.....T..AC.G..............................C............
L. dactyloides   CGACTCAAGGA.TC....TC..........-A.A.....T..............C............A
L. strigosus22    GACGCCAGGA.T.....T...C.......A.....AC..............TT..............
L. strigosus23     ACGCCAGGA.T.....T...C.......A.....AC..............TT..............
L. strigosus48    -ACGCCAGGA.T.....T...C.......A.....AC..............TT..............
L. torulosus      GACGCCAGGA.TC....T...C.......A.....A.GG...............C............
L. velutinus      --CGCCAGGA.TC....T...C.......A.....G...............................
L. tigrinus       -ACGCAAGGA.......T...C.G....C....ACTC.T................-..N.......A
L. crinitus       ----CAAGGA...........C.G....C....ACTC.T...........................A
P. arcularius     --CGCTAGGA.......T...CTG....C....ACTC.T.............T.............A
P. squamosus      ---GCCAGGA.T.....T...CA.....CA...T.......................-.........
P. alveolaris     -ACGCAAGGA.......T...CAG....C...AACT...................-..........-
G. frondosa       --CTCAAGGA.......T...CAG....CA...AC................................
P. cinnabarinus   --CGNAAGGA.......T...C.G....C....ACT...............................
L. sulphureus     ------AGGA.......T...CAG....C.....CT...........................A...
T. versicolor     --CGCAAGGA.......T...C......C....ACT...............................
S. complicatum    -----AAGGA.TC....T...C.CTG..GA...C.TC........T..A.....CT.T........-
P. eryngii        -ACTCAAGGA.TC....TC.........GA..A..T..T...........................A
O. lignatilis    -GACTCAAGGA.T.....T...CT.....C.T...TC.............A......T..........
C. earleae       CGACTCAAGGA.TC.-..T...CT.......T..T...........-..........T..........
P. stipticus     CGACGCAAGGA.......T...C.G....C....ACT...............................
L. edodes        CGACTCAAGGA.T.....T.A..T......ATC.T.....A................T........G.
L. omphalodes     ----------.AGTTA.GGCTG.CGGC..A.GCTG....CCG..AT.TGT..GC.T.......G...
L. montanus       --------GA.T.....T...C.G....C....C..C..........................G...
M. galericulata  ---CTCAAGGA.T.....TC..CT.....C.....GTCT..................T..........
T. cucumeris    CGTGATTCTGGA.T.....T....T.....CA...T..C..................-...........

                  ↓529(3')                                                       465(5')↓
                   ********  *   ** *** *   **    *  *   *  ***   ***  * *    *** ***
L. lepideus13     GGGATCGGGAATAGGTCGCCAGCTTTAGCTACGACCGGGCAGTTGG--CCTTTTATGTGGCTCGCCTT
L. lepideus17     ....CT................T..C....................--............T....T..
L. lepideus47     ......................T.......................--....................
L. ponderosus14   ......................T.......................--............T.......
L. ponderosus15   ......................T.......................--............T.......
L. ponderosus16   ......................N..........T............--......--------------
L. kauffmannii    .C.........C......T.G.T.......NNAC............--............T.......
L. sulcatus       .A.G.......C......T...T....A....A.........-CC.--............T.......
L. dactyloides    .A.G.T..A.........T.G.T...GA.......T...T......--TTC..C.C....AGT.-T..
L. strigosus22    ..T.....A..A..........T....A..................--TG..........T...-T..
L. strigosus23    ..T.....A..A..........T....A..................--T.........----------
L. strigosus48    ..T.....A..A..........T....A..................--T...........T...-T..
L. torulosus      .AT.-.C....A..........T...GA..................--T...........-..CGT..
L. velutinus      ..T.C...A..A..........T...GA..................--T....C......TC..T...
L. tigrinus       ...G...........CT.....T...T....N..........A...--.....C.C....T.G.TT..
L. crinitus       ...G.......A...CT.....T............T......A...--.....C.C....T.G.TTC.
P. arcularius     ...G.......A...CT.........................A...--.....C.C....T.G.TT..
P. squamosus      ...GA...A..A....T.....T....A..........A....AA.--T....C.C....T...-T..
P. alveolaris     ....GTT........CT.....T...................CAT.--T....C.C....TG..-T..
G. frondosa       ...G....A......CT.T.......................C...--.......C....T...-...
P. cinnabarinus   .A.G.......A.NN.......T...-----............A..--..C..C.C....T...G...
L. sulphureus     .A..C......G...CT.....T.......G...........C...--..C..C..T.......TT.G
T. versicolor     .A.G.......-.N........T..........-............--.....C.C...AT...G...
S. complicatum    ....GGC.......AG......T...GA..............C...--.....C......T......-
P. eryngii        .A..CT..A.........TTG.T...GA.......T...T......--T.C..C...C..AGT.-T..
O. lignatilis     ....-.T.........G.....T....A.......T......C...TT..C..C......T...----
C. earleae        .A..GT.A........G.....T...GA.......T..........--T.C..C.....--.T.GT..
P. stipticus      .A.G.......A..........T....................A..--..C..C.C....T...G...
L. edodes         .A.T.T.AA.......AA.T..T...GA....A..T.....AC.A.--T.C....C....T.G.TA..
L. omphalodes     ...GG......C...C......T....A..............G...--.TC..C......T...GTC.
L. montanus       ...GG......C...C..-...T...GA..............G...--.TC..C......T...GTCC
M. galericulata   A.AC....A.......G..TG.T....A.......T......C.T.--T....C......T...--..
T. cucumeris      ...T....A..A....GA.T..T....AG............TC..AT.C....C....T...-T..T.
```

APPENDIX 2. Continued.

```
                  ↓464(3')                                                    398(5')↓
                  * **   *   **********  *                            *
L. lepideus13     CCGTTCCGACTCAAGGCCGATTGCGCTGACTGAAGTTCGCAAAGGGAAANNNNGTTAAAGTGCATGAC
L. lepideus17     .............G.........................T.........-GTT...........GT..
L. lepideus47     .................................................-GTT...............
L. ponderosus14   ------------NN.N.N..C.----------------------------------------------
L. ponderosus15   .................................................-GTT...............
L. ponderosus16   --------------------------------------------------------------------
L. kauffmannii    .................................................-GTT...............
L. sulcatus       T............G.....G....................----------------------------
L. dactyloides    T.A....A...AGGAA....C...........................--GTT...............
L. strigosus22    T..G...........A.TT.C............................-GTC...............
L. strigosus23    --------------------------------------------------------------------
L. strigosus48    T..G...........A.TT.C............................-GTC...............
L. torulosus      T..G...........A.TT.C............................-GTC...............
L. velutinus      .--G...........A..T.C...........................--GTC...............
L. tigrinus       ..-................TC..N...............N.........-GTC...............
L. crinitus       ..-................TC............................-GTC...............
P. arcularius     ..-................TC............................-GTC...............
P. squamosus      T...............TTTG...T..............A......--..AGTC...............
P. alveolaris     T..C............TATG.............................-GTC...............
G. frondosa       ...C..............TGC............................-GTC...............
P. cinnabarinus   -...........-.....TG.............................-GTC...............
L. sulphureus     .TTACGTTC.GACTC.GGCCGGCT..GCTGACTGAAGTT.GC.AA.G..AGTC...............
T. versicolor     -....T............TGC............................-ATC...............
S. complicatum    -A...........G.....GC.............-..............-GTT....NN.......N.
P. eryngii        T.A....A...AGG.A...TC............................-GTT...............
O. lignatilis     --------------------------------------------------------------------
C. earleae        T.C........AGG....TG...---------------------------------------------
P. stipticus      -............G....TG......................--------------------------
L. edodes         ..-........AGG...T.G.............................-GTC...............
L. omphalodes     T.N..........GA....CC..-N.......................--GTT...............
L. montanus       T.N..........GA....TC...........................--GTT...............
M. galericulata   T...........-..A.TTGC............................-GTC...............
T. cucumeris      .........T.G..T.TC.........................T...-GTT.................

                  ↓397(3')           371(5')↓
L. lepideus13     AAATTGAGAGAAAGGTTTCACGAAA-A   M98583
L. lepideus17     .........................-.   M98587
L. lepideus47     .........................AG   M98603
L. ponderosus14   --------------------------T   M98584
L. ponderosus15   .........................-.   M98585
L. ponderosus16   --------------------------.   M98586
L. kauffmannii    ..............-------------   M98596
L. sulcatus       ---------------------------   M98595
L. dactyloides    ....................A..----   M98606
L. strigosus22    .........................AG   M98590
L. strigosus23    ---------------------------   M98591
L. strigosus48    ............N......--------   M98604
L. torulosus      .........................AG   M98589
L. velutinus      ......................-----   M98598
L. tigrinus       .........A...............AG   M98592
L. crinitus       ............N............AG   M98599
P. arcularius     ............N............AG   M98581
P. squamosus      -----------------------..--   M98582
P. alveolaris     .........................AG   M98605
G. frondosa       ..........-------------..--   M98580
P. cinnabarinus   ---------------------------   M98594
L. sulphureus     .................----------   M98602
T. versicolor     ............N......--.....A   M98597
S. complicatum    ............G......--.....N   M98607
P. eryngii        ...................--.A....   M98588
O. lignatilis     ---------------------------   M98600
C. earleae        ---------------------------   M98601
P. stipticus      ---------------------------   M98593
L. edodes         ............---------------   M98579
L. omphalodes     ..........-----------------   M98577
L. montanus       ..........---------------..   M98578
M. galericulata   .............--------------
T. cucumeris      ...----------------.......T
```

APPENDIX 3. Aligned sequences from primer LR6. See Table 1 for genus abbreviations. See Appendix 1 caption for explanation of symbols and GenBank accession numbers.

```
                ↓1093(3')                                        1036(5')↓
                                     *   *   *     *
L. lepideus17   -GGCT--C-G-C-A--GGTTAGTTCACTGCCG--AGCAA-GAATGTATAAATTTCAAACTCTTATCC
L. lepideus13   ---TC--G-N-N-N--N.........NNN...--....N-N..........................
L. lepideus47   -------------------------------------------------------------------
L. ponderosus14 -----------------.........NNN...--....N-N..........................
L. ponderosus15 C....--.-.-.C.--.........N......GA.....-...........................
L. ponderosus16 -----------.-.--.........N......--....N-N..........................
L. kauffmannii  -....--.-.-.C.--................--.....-...........................
L. sulcatus     -....--.-.-N-NATT...............--.....T...........................
L. dactyloides  -..T.--.-.T.-.--.....A..N....T..--.A...-...........................
L. strigosus22  ----------------.....A...TNNN...--....N-N..........................
L. strigosus23  -....--.-.-.C.--.....A...T......--.....-...........................
L. strigosus48  -....--.-.-.C.--.....A...T......--.....-...........................
L. torulosus    ---------N-.-.--.....A...TNN....--....N-N..........................
L. velutinus    C....--.-.-.C.--.....A...T......--.....-...........................
L. tigrinus     -....--.-.N.-.--.........GN.....--.....-...........................
L. crinitus     -....--.-.-.C.--.........G......--.....-...........................
P. arcularius   ----------------.........GN.....--.....-...........................
P. squamosus    ---------N-N-.--.........GN.....--.....-...........................
P. alveolaris   -------------------.TA...G......--.....-...........................
G. frondosa     C....--.-.-.C.--.........G......--.....-...........................
P. cinnabarinus -....--.G.-.-.--.........GG.....--.....-...........................
T. versicolor   -....--.-.-.C.--.........G......--.....-........-..................
S. complicatum  ----------------.........T......--.....-...........................
P. eryngii      -----------------------------...--.A...-...........................
O. lignatilis   -...C--.-.-.C.--.........T......--G....-...........................
C. earleae      -CT.ATCN-T-.-N--.....A...T...T..--.....-...........................
P. stipticus    -....--.-.-.C.--N........GN.....--.....-...........................
L. edodes       -C.------N-N-N--.....A..NTN.....--..N.N-...........................
L. omphalodes   C..T.--.-.-.C.--.........G......--.A...-...........................
L. montanus     C..T.--.-.-.C.--.........G......--.A...-...........................
M. galericulata C....--.-.-.C.--.........T......--.....-...........................
T. cucumeris    --------------------------------------------------...............T.

                ↓1035(3')                                         973(5')↓
                           *                                             * *
L. lepideus17   AATTCCAACAAAG-TTGGGGTTCCGGAGATTAGTAAGCGAAATGGTGTATTTAGACTATG-C--TCA
L. lepideus13   .............-.......................N.........AT...........-.GT...
L. lepideus47   ----------...-..............................................-.--...
L. ponderosus14 .............-....................-.NNN.....................-N--...
L. ponderosus15 .............-..............................................-.--...
L. ponderosus16 .............-.......................N......................-.--...
L. kauffmannii  .............-.N............................................-.--...
L. sulcatus     .............-..............................................-.--.--
L. dactyloides  .............-.............................................TA.--...
L. strigosus22  .............-.......................N...................G.A-N--...
L. strigosus23  .............-...........................................G.A-.--...
L. strigosus48  .............-...........................................G.A-.--...
L. torulosus    .............-.......................N...................G.A-.--...
L. velutinus    .............-...........................................G.A-.--...
L. tigrinus     .............-.............................................A-.--...
L. crinitus     .............-.............................................A-.--...
P. arcularius   .............-.............................................A-.--...
P. squamosus    .............-.............................................A-.--...
P. alveolaris   .............-.............................................A-.--...
G. frondosa     .............-............-................................A-.--...
P. cinnabarinus .............-.NN..........................................A-.--...
T. versicolor   .............-.............................................A-.--...
S. complicatum  ...........N.-.............................................A-.--...
P. eryngii      .............-.............................................A-.--...
O. lignatilis   ...........C.-...........................................T.A-.--...
C. earleae      ...........T.C...........................................G.TA.--...
P. stipticus    .............-.............................................A-.--...
L. edodes       ...........T.-.......................N...................G.TA.--...
L. omphalodes   .............-...........................................G..-.--...
L. montanus     .............-.................-.........................G..-.--...
M. galericulata .............-.............................................A-.--...
T. cucumeris    .............-...........A..........T...............T.......-.--...
```

APPENDIX 3. Continued.

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                    ↓972(3')                                                  911(5')↓

L. lepideus17       AAGACGATAGGACTCCCTTTGAAGCCGTCC--TTGGTCGATGATCT-ACCAAG-CTAATCAGAAAGC
L. lepideus13       .........................N.N..--..............-......-..........NNN
L. lepideus47       ..............................--..............-......-............N
L. ponderosus14     .NN.N...................NN.N..--..............-......-......N...NNN
L. ponderosus15     ..............................--..............-......-.............
L. ponderosus16     ..............................--..............-......-...........NN
L. kauffmannii      ..............................--..............-......-.............
L. sulcatus         -------------------------------------------------------------......
L. dactyloides      G........-....................--..............-......-.............
L. strigosus22      .NNNN...................NN....--..............-.N....-............N
L. strigosus23      .........-.......-............CT..............-......-......-------
L. strigosus48      ..............................--..............-......-.............
L. torulosus        .NN.....................NN....--..........-...-.N....-......N...NNN
L. velutinus        .........-....................--..............-......-.............
L. tigrinus         ..............................--..............-......G.............
L. crinitus         .........-....................--..............-......-.............
P. arcularius       ..............................--..............-......-.............
P. squamosus        ..............................--..............-......-.............
P. alveolaris       ..............................--..............-......-.............
G. frondosa         ..............................--..............-......-.............
P. cinnabarinus     .........-....................--..............-......-............N
T. versicolor       ......-..-....................--..............-......-.............
S. complicatum      .........-....................--..............-......-.............
P. eryngii          .........-....................--..............-......-.............
O. lignatilis       .........-....................--..............-......-......-......
C. earleae          .........-....................--..............-......-.............
P. stipticus        .........-....................--..............-......-.............
L. edodes           .NN...........................--..............-.-....-......N..NNNN
L. omphalodes       .........-....................-------------------------------------
L. montanus         .........-....................-------------------------------------
M. galericulata     .........-C...................--..........---------------...G-.C.T.
T. cucumeris        G........-....................--..............-......-.............


                    ↓910(3')                                                  845(5')↓
                                     *
L. lepideus17       GGGGATATGGGTTTAAACTGCTAGCTAAACGTGCAGTCT-TAGCGATGCTCGGAGGTG-GTCTCAA   M98618
L. lepideus13       N.............--------------------------------------------------..   M98614
L. lepideus47       .......................................-..................-.......   M98633
L. ponderosus14     N.............--------------------------------------------------..   M98615
L. ponderosus15     ..................................--------------------------------   M98616
L. ponderosus16     N................NN.N............---------------------------------   M98617
L. kauffmannii      .......................................-..................G.....--   M98627
L. sulcatus         .......................................-..................-.......   M98626
L. dactyloides      ................G......................-..................-.......   M98636
L. strigosus22      N................N..N....---------------------------------------..   M98621
L. strigosus23      ------------------------------------------------------------------   M98622
L. strigosus48      .......................................-........N.........-.......   M98634
L. torulosus        N................NN.NN...---------------------------------------..   M98620
L. velutinus        .......................................-..................-.......   M98629
L. tigrinus         ......................-................-...-------....T.-----...GG   M98623
L. crinitus         .......................................T..................-.......   M98630
P. arcularius       .......................................-...........---------------   M98612
P. squamosus        .......................................-..................-.......   M98613
P. alveolaris       .......................................-..................-.......   M98635
G. frondosa         ........-..............................-..................-..-----   M98611
P. cinnabarinus     .......................................-..................-..-----   M98625
T. versicolor       .......................................-..................-.......   M98628
S. complicatum      .........N.............................-..................-.......   M98637
P. eryngii          ................G......................-..................-.......   M98619
O. lignatilis       ........-.......G......................-.......-------------------   M98631
C. earleae          ................G......................-..................-.......   M98632
P. stipticus        .......................................-..................-.......   M98624
L. edodes           N.............----------------------------------------------------   M98610
L. omphalodes       ---------------------------------------------------------------.TT   M98608
L. montanus         -------------------------------------..-.-------------------------   M98609
M. galericulata     ....T.C.T.A..--G.T...G.T.A.-----T..A..G-G..-.G.CG...AGCCCC-AGT...C
T. cucumeris        -.......-...G..........................-..................-.......
```

APPENDIX 4. Aligned sequences from ITS2 from a subset of ten isolates. See Table 1 for genus abbreviations. Asterisks indicate invariant positions. Other symbols as in Appendix 1.

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                     ***      *                            *        *          *  ***    *     *
1  L. lepideus13     TTTAGAAGCCGATCAA-CCA----AA-GAC-GCTTCC-CAGAG-----ACG-GCGTAGA---CA
2  L. lepideus17     -----------------------------------..-.....-----...-.......---..
3  L. ponderosus14   ................A...----..-...-......-.....-----...-.......---..
4  L. ponderosus15   -------------------.----..-...A......-.....-----...-.......---..
5  L. ponderosus16   -------------------.----..-...-......-.....-----...-.......---..
6  L. strigosus22    G......-----...-GA.----C.-T.T-AT..TG-.T...CAACA..A-...C...---T.
7  L. tigrinus       G.....GAG..TAGTG-...TTAA..-CN.-.T.CA.-.G.TC-----N..T...GT..GAG..
8  P. arcularius     ---------.C..T..------------..-N....AA.G.TC-----N.N-N..C....---T.
9  P. squamosus      --...G...----TTG-..C----..-AT.-.....AA...CT-----G..-.....A.---..
10 G. frondosa       A...TN...---..GC-..G----.CT..G-.....A-AC..CC----...-.......---..

      *********    * *                **                 ** ** **       **    **** ***** *
1  A-TTATCACACC--GAGC-----CAC-TGTTCCGCAACGGGTTC--AGC-TA-ATA-CATTTA-GAGAG-GAGCCGACTT
2  .A..........--...-----...A..................--....-..-..G-......-.....-.........C.
3  .-..........--...-----...-..................--....-..T...T......T.....A..........
4  .-..........--...-----...-..................--....-..-...-......-.....-..........
5  .-..........--...-----...A..................--....-..-...-......-.....-..........
6  .-.........T--...-------GT-..GCA..GC.ACAT.C.--T..-..-..T-T....--C....-......----
7  .-...........--.T.GAGGCG..G-GAG.....G.AA.AGA.CA...G..-..G-.GA..T-A....-.---------
8  .-...........--.T.------AG.-C.A.......G.AACCA--...-..-..G-......--.....-.........-.
9  T-..........AG...-----..----A.......G.AACCA--...-..-..G-......--.......-......A.AGG
10 .-...........--..------AG.-C.A.......G..A.GG--...-..-..G-..-..C-C....-.........CC

                *           ***       *
1  GACAG----CCAGCA---AAGCCCCCAA-ATCCA-AGCC--CA
2  T..G.----......---.....T....-.....-..------
3  .....----......---..........-.....-....--.-
4  .....----......---..........-.....-....--.-
5  .....----......---..........-G....-....--.-
6  ------------GA..---C..AT....G-.AGG.T..T.--GC
7  ..GC.-----.G...C---.T..-----G-.AA..-GAGN--.G
8  .C..A----G....CGAC.....T....-G....-..-.--.T
9  A.TTT----...T..----------------G..-....TC..
10 ..AT.GGGG..G...G--..A..T....T.....-....--.-
```

APPENDIX 5. Morphological characters and references. References for morphological characters include: Corner (1981), Miller (1965), Miller and Manning (1976), Pegler (1975, 1983), Pegler and Young (1983), Redhead and Ginns (1985) for *Lentinus*; Gilbertson and Ryvarden (1986, 1987) for *Polyporus* spp., *Grifola frondosa*, *Laetiporus sulphureus*, *Pycnoporus cinnabarinus*, *Lenzites betulina*, *Trametes versicolor*, and *Ganoderma lucidum*; Chamuris (1988), Coates et al. (1981), and Eriksson et al. (1984) for *Stereum complicatum*; Bresinsky et al. (1977) and Hilber (1984) for *Pleurotus eryngii*; Redhead and Ginns (1985) for *Ossicaulis lignatilis*; Halling (1983), Vilgalys (1986), and Vilgalys and Miller (1983) for *Collybia earleae*; Burdsall and Miller (1975, 1978) and Miller (1970) for *Panellus stipticus*; Miller and Stewart (1971) for *Lentinellus omphalodes* and *L. montanus*; Miller (1972) and Singer (1986) for *Mycena galericulata*; and Pegler (1975) for *Lentinula edodes*. Character distributions are summarized in Table 2. All characters are unordered.

1) Hyphal pegs. 0 = absent; 1 = present.
2) Cheilocystidia. 0 = absent; 1 = present.
3) Cystidiiform hairs on lamella edge. 0 = absent; 1 = present.
4) Metuloidal pleurocystidia. 0 = absent; 1 = present.
5) Hyphal septation. 0 = clamped; 1 = simple; 2 = verticillate clamps.
6) Mitic system. 0 = monomitic; 1 = dimitic with skeletal hyphae; 2 = dimitic with ligative hyphae; 3 = trimitic.
7) Development of hymenophoral trama. 0 = descending; 1 = radiate. In descending growth the hyphae that form the gill trama initially grow downwards at a roughly 90° angle to the pileus. In radiate growth the hyphae initially grow out from the stipe more or less parallel to the surface of the pileus. Descending growth leads to a regular gill trama in tangential section whereas radiate growth leads to an irregular trama. Also, descending growth is thought to result in a serrate gill edge because not all of the descending hyphae cease growth at the same time (Corner 1981; Pegler 1983). For these reasons, the adult structure of the trama and the gill edge were not treated as independent characters even though they have been important characters in the classification of *Lentinus*.
8) Spore shape. 0 = cylindric; 1 = ovoid to ellipsoid; 2 = subglobose.
9) Spore ornamentation. 0 = smooth; 1 = with minute depressions in spore wall; 2 = with minute spines.
10) Amyloidity (reaction of spores to iodine). 0 = spores not amyloid; 1 = spores amyloid.
11) Wood decay type. 0 = white rot; 1 = brown rot.
12) Mating system. 0 = tetrapolar, bifactorial; 1 = bipolar, unifactorial.
13) Hymenophore configuration. 0 = lamellate; 1 = poroid; 2 = smooth.
14) Habit. 0 = centrally stipitate; 1 = laterally to excentrically stipitate; 2 = sessile; 3 = effused-reflexed.
15) Lamellar attachment. 0 = decurrent; 1 = decurrent by a tooth; 2 = adnexed to free.
16) Partial veil. 0 = absent; 1 = present.
17) Pileus surface texture. 0 = glabrous; 1 = strigose, hispid, tomentose; 2 = squamulose.
18) Pileus zonation. 0 = not zonate; 1 = zonate.
19) Pseudosclerotia. 0 = absent; 1 = present.
20) Substrate. 0 = hardwoods; 1 = conifers; 2 = live Umbelliferae; 3 = soil.