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Freshwater ascomycetes: two new species of *Lindgomyces* (Lindgomycetaceae, Pleosporales, Dothideomycetes) from Japan and USA

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Abstract: During independent surveys of freshwater ascomycetes in Japan and USA two new species of *Lindgomyces* were collected from submerged wood in freshwater. These species are described and illustrated based on morphological data and phylogenetic relationships based on analyses of nuclear ribosomal sequence data (partial SSU and LSU, and ITS). *Lindgomyces apiculatus*, collected in Japan, is characterized by immersed to erumpent, globose to subglobose ascomata; fissitunicate, cylindrical to clavate asci; and fusiform, one-septate ascospores with acute ends and short terminal appendages. *Lindgomyces lemonweirensis*, collected in Wisconsin, USA, differs from *L. apiculatus* in having clavate to cymbiform asci and oblong to fusiform ascospores that are distinctively multiguttulate and surrounded by an oval, ephemeral gelatinous sheath. The new species formed a strongly supported clade within the family Lindgomycetaceae (Pleosporales, Dothideomycetes) based on analyses of combined SSU and LSU sequence data. In addition phylogenetic analyses with ITS sequence data support the establishment of the new taxa as separate species within *Lindgomyces* because they were separated from each other and other *Lindgomyces* species based on maximum likelihood bootstrap and Bayesian analyses.

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Key words: aquatic fungi, *Massarina*, *Massariosphaeria*, saprobe, systematics

INTRODUCTION

The family Lindgomycetaceae was introduced recently based on morphological characteristics and phylogenetic analyses of nrDNA sequence data for bitunicate freshwater ascomycetes from temperate and tropical freshwater habitats (Hirayama et al. 2010). The family currently includes one genus, *Lindgomyces* K. Hiray., Kaz. Tanaka & Shearer, with four species (viz. *L. ingoldianus*, the type species, *L. breviappendiculatus*, *L. cinctosporae* and *L. rotundatus*). *Lindgomyces* is characterized by globose to subglobose ascomata, fissitunicate, clavate to cylindrical asci that are rounded at the apex, and one-septate, hyaline ascospores with a gelatinous sheath, which sometimes extends to form bipolar mucilaginous appendages (Hirayama et al. 2010).

During our continuing studies of freshwater ascomycetes in Japan and USA, (Tanaka and Harada 2003, Tanaka et al. 2004, 2005a, b; Raja et al. 2009, 2010a, b, c; Hirayama et al. 2010) we found two undescribed taxa with affinities to species in *Lindgomyces* based on morphological data. We sequenced the partial 18S small subunit (SSU) and 28S large subunit (LSU) of nuclear ribosomal DNA for the newly found taxa to confirm their taxonomic affinities to the Lindgomycetaceae. In addition we also sequenced the internal transcribed spacer (ITS) for the newly found species along with strains of previously described *Lindgomyces* species to reveal their phylogenetic relationships at the species rank.

MATERIALS AND METHODS

Sample collection and morphological examination.—Collection methodology and morphological examination of samples followed Hirayama et al. (2010). Specimens cited in this paper are maintained at the Herbarium of Hirosaki University (HHUF), the University of Illinois Herbarium (ILL) or the Illinois Natural History Survey Herbarium (ILLS). The fungal cultures obtained in this study were deposited at the Japan Collection of Microorganisms (JCM), and the National Institute of Agrobiological Sciences, Japan (MAFF), and the University of Illinois, Plant Biology fungal collection.

DNA extraction and PCR amplification.—DNA extraction and PCR amplification of SSU and LSU were performed

TABLE I. Species used in this study

Species	Voucher info[a]	GenBank accession numbers[b]		
		nucSSU rDNA	nuc ITS	nucLSU rDNA
Amniculicola immersa	CBS 123083	GU456295	—	FJ795498
Amniculicola lignicola	CBS 123094	EF493861	—	EF493863
Amniculicola parva	CBS 123092	GU296134	—	FJ795497
Anguillospora longissima	CS869-1D	GU266222	—	GU266240
Byssothecium circinans	CBS 675.92	AY016339	—	AY016357
Bimuria nova-zelandiea	CBS 107.79	AY016338	—	AY016356
Cochliobolus heterostrophus	CBS 134.39	AY544727	—	AY544645
Cucurbita elongata	CBS 171.55	U42482	—	DQ678061
Delitschia didyma	UME 31411	AF242264	—	DQ384090
Delitschia winteri	CBS 225.62	DQ678026	—	DQ678077
Didymella cucurbitacearum	IMI 373225	AY293779	—	AY293792
Didymella exigua	CBS 183.55	EU754056	—	EU754155
Dothidea insculpta	CBS 189.58	DQ247810	—	DQ247802
Dothidea sambuci	DAOM 231303	AY544722	—	NG_027611.1
Herpotrichia juniperi	CBS 468.64	U42483	—	DQ384093
Lentithecium aquaticum	CBS 123099	FJ795477	—	FJ795434
Lentithecium fluviatile	CBS 123090	FJ795492	—	FJ795450
Lepidosphaeria nicotiae	CBS 559.71	DQ384068	—	DQ384068
Leptosphaeria biglobosa	CBS 532.66	EU754090	—	EU754189
Letendraea helminthicola	CBS 884.85	AY016345	—	AY016362
Lindgomyces apiculatus	JCM 13091/MAFF 239601 Type	**JF419886**	**JF419892**	**JF419884**
Lindgomyces apiculatus	JCM 13092/MAFF 239602	**JF419887**	**JF419893**	**JF419885**
Lindgomyces breviappendiculatus	JCM 12702/MAFF 239291	AB521734	**JF419896**	AB4332749
Lindgomyces breviappendiculatus	JCM 12701/MAFF 239292 Type	AB521733	**JF419897**	AB521748
Lindgomyces cinctosporae	R56-1 Type	AB522430	**JF419905**	AB522431
Lindgomyces cinctosporae	R56-3	GU266238	—	GU266245
Lindgomyces ingoldianus	ATCC 200398 Type	AB521719	**JF419898**	AB521736
Lindgomyces ingoldianus	JCM 16479/NBRC 106126	AB521720	**JF419899**	AB521737
Lindgomyces sp.	JCM 16480/NBRC 106130	AB521721	**JF419900**	AB521738
Lindgomyces lemonweirensis	A632-1a Type	**JF419890**	**JF419892**	**JF419888**
Lindgomyces lemonweirensis	A632-1b	**JF419891**	**JF419895**	**JF419889**
Lindgomyces rotundatus	JCM 16481/MAFF 239473 Type	AB521722	**JF419901**	AB521739
Lindgomyces rotundatus	JCM 16482/NBRC106127	AB521723	**JF419902**	AB521740
Lindgomyces rotundatus	JCM 16483/NBRC 106128	AB521724	**JF419903**	AB521741
Lindgomyces rotundatus	JCM 16484/NBRC 106129	AB521725	**JF419904**	AB521742
Lophiostoma heterosporum	—	AY016345	—	AY016369
Lophiostoma macrostomum	JCM 13545	AB521731	—	AB433273
Lophiostoma macrostomum	JCM 13546/MAFF 239447	AB521732	—	AB433274
Massaria platani	CBS 221.37	DQ678013	—	DQ678065
Massarina eburnea	JCM 14422	AB521718	—	AB521735
Massariosphaeria typhicola	MAFF 239218	AB521729	—	AB521746
Massariosphaeria typhicola	MAFF 239219	AB521730	**JF419906**	AB521747
Montagnula opulenta	—	AF164370	—	DQ678086
Neotestudina rosatii	CBS 690.82	DQ384069	—	DQ384107
Neottiosporina paspali	CBS 331.37	EU754073	—	EU754172
Ophiosphaerella herpotricha	CBS 620.86	DQ678010	—	DQ678062
Phaeosphaeria avenaria	AFTOL-ID 280	AY544725	—	AY544684
Phaeodothis winteri	CBS 182.58	DQ678021	—	DQ678073
Phoma herbarum	CBS 615.75	EU754087	—	EU754155
Pleospora herbarum	CBS 714.68	DQ767648	—	DQ678049
Pleomassaria siparia	CBS 279.74	DQ678027	—	AY004341
Preussia terricola	DAOM 230091	AY544726	—	AY544686
Setomelanomma holmii	CBS 110217	AF525677	—	AF525678
Setosphaeria monoceras	CBS 154.26	AY016352	—	AY016368

TABLE I. Continued

Species	Voucher info[a]	GenBank accession numbers[b]		
		nucSSU rDNA	nuc ITS	nucLSU rDNA
Sporormia lignicola	CBS 264.69	U42478	—	DQ34098
Tingoldiago graminicola	JCM 16485/NBRC 106131 Type	AB521726	—	AB521743
Tingoldiago graminicola	MAFF 239472	AB521727	—	AB521744
Tingoldiago graminicola	JCM 16486/NBRC 106132	AB521728	—	AB521745
Trematosphaeria pertusa	CBS 400. 97	DQ678020	—	DQ678072
Ulospora bilgramii	CBS 110020	DQ384071	—	DQ384108
Verrculina enalia	CBS 304.66	DQ678028	—	AY016363
Zopfia rhizophila	CBS 270.26	L76622	—	DQ384104

[a] CBS, Centraalbureau voor Schimmelcultures; CS, Carol Shearer; IMI, International Mycological Institute; UME, Umeå University, Sweden; DAOM, Agriculture and Agri-Food Canada National Mycological Herbarium; JCM, Japan Collection of Microorganisms; MAFF, National Institute of Agrobiological Sciences, Japan; NBRC, National Biological Resource Center, Japan; R, Raja Freshwater Ascomycetes; A, Carol Shearer, Ascomycetes; AFTOL, Assembling the Fungal Tree of Life; ATCC, American Type Culture Collection.

[b] Numbers in boldface indicate newly obtained sequences in this study.

following the procedures of Hirayama et al. (2010). For the amplification of ITS we used a combination of ITS1/ITS1F and ITS4 primers (White et al. 1990, Gardes and Bruns 1993) with the thermo-cycler parameters outlined in Promputtha and Miller (2010).

Taxon sampling and phylogenetic analyses.—We assembled three datasets for phylogenetic analyses: (i) the SSU dataset that consisted of 62 taxa, 59 of these represented 16 of the 28 families currently included in the order Pleosporales (Schoch et al. 2009, Lumbsch and Huhndorf 2010); (ii) the LSU dataset that consisted of the same taxa as the SSU dataset; and (iii) the ITS dataset that consisted of 15 taxa, including two strains of *L. apiculatus*, two strains of *L. breviappendiculatus*, one strain of *L. cinctosporae*, two strains of *L. ingoldianus*, one strain of *Lindgomyces* sp., two strains of *L. lemonweirensis* and four strains of *L. rotundatus*. *Massariosphaeria typhicola*, which is a sister species to *Lindgomyces* (Hirayama et al. 2010), was used as an outgroup.

Sequences of a number of taxa used in this study were obtained from studies on Lindgomycetaceae (Shearer et al. 2009, Hirayama et al. 2010). In addition we also included taxa from recently introduced freshwater fungal families in the Pleosporales, such as Amniculicolaceae and Lentitheciaceae (Schoch et al. 2009, Shearer et al. 2009, Zhang et al. 2009). All taxa used in this study along with their GenBank numbers is provided (TABLE I). Each of the three datasets was aligned initially with the multiple alignment program MUSCLE® (Edgar 2004) with default parameters in operation. MUSCLE® was implemented with the program Seaview 4.1 (Gouy et al. 2010). Alignments were optimized by visual examination and manually corrected with MacClade 4.08 (Maddison and Maddison 2000). After the datasets were aligned ambiguous regions, gaps and introns were excluded from the final alignment with the default parameters in the program Gblocks (Castresana 2000, Talavera and Castresana 2007). Nucleotides from the 5′ and 3′ end also were removed in all the three datasets due to missing characters in most taxa.

Maximum likelihood (ML) was performed on all three datasets. We used Modeltest 3.7 (Posada and Crandel 1998) as well as jModeltest (Posada 2008) (with 88 possible evolutionary models) to obtain the best-fit model of nucleotide evolution. We initially ran separate ML analyses on the individual SSU and LSU datasets with a general time reversible model (GTR) (Rodríguez et al. 1990), including invariable sites and a discrete gamma shape distribution with 1000 ML bootstrap (BS) replicates with a combined nearest neighbor interchange (NNI) and subtree pruning and regrafting (SPR) tree search option in effect with PHYML (Guindon and Gascuel 2003). After the bootstrap values (BV) were obtained individual SSU and LSU phylogenies were examined for conflict by comparing clades with BV $\geq$ 70% (Wiens 1998). Because we did not detect any significant clade conflict between the SSU and LSU ML trees we concatenated the two datasets and performed a ML analysis using PHYML with the same parameters as above with 1000 ML BS replicates to assess clade support (Felsenstein 1985). For the ITS dataset ML analysis was performed separately as above. Additional ML analyses were performed with RAxML 7.0.4 (Stamatakis et al. 2008) on both the combined SSU and LSU dataset as well as separately on the ITS dataset; these analyses were run on the CIPRES Portal 2.0 (Miller et al. 2010) with the default rapid hill-climbing algorithm and GTR model employing 1000 fast bootstrap searches. We considered clades with a BV $\geq$ 70% as significant and strongly supported (Hills and Bull 1993).

Bayesian analyses were performed on the combined SSU and LSU dataset as well as separately on the ITS dataset with MrBayes 3.12 (Huelsenbeck and Ronquist 2001, 2005) using CIPRES Portal 2.0 (Miller et al. 2010) to assess clade support. The programs Mr. Modeltest 2.2 (Nylander 2004) and PAUP 4.0b10 (Swofford 2003) were used with the implementation of Akaike information criterion (AIC) (Posada and Buckley 2004) to select the best-fit model of evolution. As above, the GTR + I + G was selected for the combined SSU and LSU dataset and GTR + G was selected

for the ITS dataset. The above models of evolution were implemented in a Markov chain Monte Carlo method (MCMC) performed with MrBayes 3.1.2. Constant characters were included. We ran 10 000 000 or 100 000 000 generations with trees sampled every 1000th generation, resulting in 10 000 or 100 000 total trees for the combined SSU + LSU and ITS datasets respectively. The first 1000 or 10 000 trees that extended beyond the burn-in in each analysis were discarded, and the remaining 9000 or 90 000 trees were used to calculate the posterior probability (PP). The consensus of the trees were viewed in PAUP 4.0b10 (Swofford 2003). The Bayesian analysis was run twice, starting from a different random tree each time to ensure that trees from the same tree space were being sampled.

RESULTS

The original SSU alignment consisted of 2864 nucleotides. Three intron regions, 996–1340 in *Delitschia didyma* AF242264, 1511–1889 in *Delitschia didyma* AF242264 and *Phaeosphaeria avenaria* AY544725, and 2153–2513 *Trematosphaeria pertusa* DQ678020, were excluded from all further analyses. The final SSU dataset consisted of 1043 nucleotides. The original LSU dataset consisted of 1471 nucleotides. After ambiguous regions and introns were delimited and excluded with G blocks the final LSU dataset consisted of 1304 nucleotides. Because no significant conflict was found between the separate SSU and LSU tree topologies based on PHYML BP (data not shown) we concatenated the two genes. The combined SSU and LSU alignment consisted of 2347 nucleotides. PHYML analyses of the combined dataset produced a single most likely tree (FIG. 1). All species of *Lindgomyces* occurred in a highly supported clade within the family Lindgomycetaceae (Hirayama et al. 2010) with ≥ 95% PP, 81% PHYML BP, 80% RAxML BP. Isolates of the two new species, *L. apiculatus* and *L. lemonweirensis*, formed well supported clades with ≥ 95% PP, 98% PHYML BV, 98% RAxML BV for *L. apiculatus*, and ≥ 95% PP, 98% PHYML BV, 98% RAxML BV for *L. lemonweirensis* respectively (FIG. 1).

The ITS dataset consisted of 15 taxa and the alignment consisted of 1639 nucleotides including introns and ambiguous regions. After the exclusion of introns and ambiguous regions the final ITS sequence alignment consisted of 1127 nucleotides. Among ITS sequences the average intraspecific variation between different strains of *Lindgomyces* was 2.5% whereas the interspecific difference between species of *Lindgomyces* was 13.25% (data not shown). PHYML analyses of the ITS dataset generated a single most likely tree (FIG. 2). *Lindgomyces apiculatus* (≥ 95% PP, 100% PHYML BV, 100% RAxML BV) and *L. lemonweirensis* (≥ 95% PP, 100% PHYML BV, 100% RAxML BV) formed distinct monophyletic groups separated from the previously described species of *Lindgomyces* (viz. *L. breviappendiculatus*, *L. cinctosporae*, *L. ingoldianus*, *L. rotundatus*). Although *Lindgomyces* sp. KH241 (JCM16479) groups with the type of *L. ingoldianus* in the ITS phylogeny (FIG. 2), we currently retain it as a *Lindgomyces* sp. based on differences in ascospore features and habitat of *Lindgomyces* sp. KH241 until additional specimens become available for further investigation (see Hirayama et al. 2010). The molecular phylogenetic analyses of both the combined SSU and LSU (FIG. 1) as well as the ITS phylogeny (FIG. 2) clearly support the establishment of *L. apiculatus* and *L. lemonweirenesis* as new and separate taxa within *Lindgomyces*. This placement also is corroborated by morphological data. These taxa therefore are described and illustrated herein as new species of *Lindgomyces*.

TAXONOMY

Lindgomyces apiculatus K. Hiray. & Kaz. Tanaka, sp. nov. FIGS. 3–14

MycoBank MB561162

Ascomata 300–330 μm alta, 320–340 μm diam, gregaria, immersa vel erumpentia, subglobosa vel globosa, nigra, cum ostiolo rotundato. Rostrum leviter papillata vel none, centrale. Paries ascomatis 25–30 μm crassus. Pseudoparaphyses copiosae, 2–4 μm latae, septatae. Asci 85–125 × 17–25(–27) μm, fissitunicati, cylindrici vel clavati, apice rotundati, camera vadosae apicali formantes vel none, octosporis. Ascosporae (31–)33–43 × 8–11 μm, fusiformes, ad apicum acutum, 1-septatae, ad septum primo medio constrictae, hyalinae, appendiculatae.

Anamorphe ignota.

Ascomata 300–330 μm high, 320–340 μm diam, gregarious, immersed to erumpent, subglobose to globose, black, ostiolate. Ostiole central, rounded. Beak absent or short papillate. Ascomal wall 25–30 μm thick, composed of 5–7 layers of rectangular to polygonal thin-walled cells, 6–10 × 4–5 μm. Pseudoparaphyses numerous, 2–4 μm wide, septate, hyaline, branched, associated with gelatinous material. Asci 85–125 × 17–25(–27) μm (av. 103.2 × 21.5 μm, n = 21), fissitunicate, cylindrical to clavate, rounded at the apex, with or without an apical chamber, with eight overlapping biseriate to triseriate ascospores. Ascospores (31–)33–43 × 8–11 μm (av. 36.4 × 9.5 μm, n = 50), L/W 3.1–4.2(–4.5) (av. 3.8, n = 50), fusiform to clavate with acute ends, slightly curved, one-septate, with the primary septum almost median (0.45–0.55; av. 0.51, n = 50), constricted at primary septum, with broad upper cell, hyaline, smooth, guttulate, with short terminal appendages 2–5 μm long.

Anamorph: Unknown.

Habitat: On submerged twigs of woody plant.

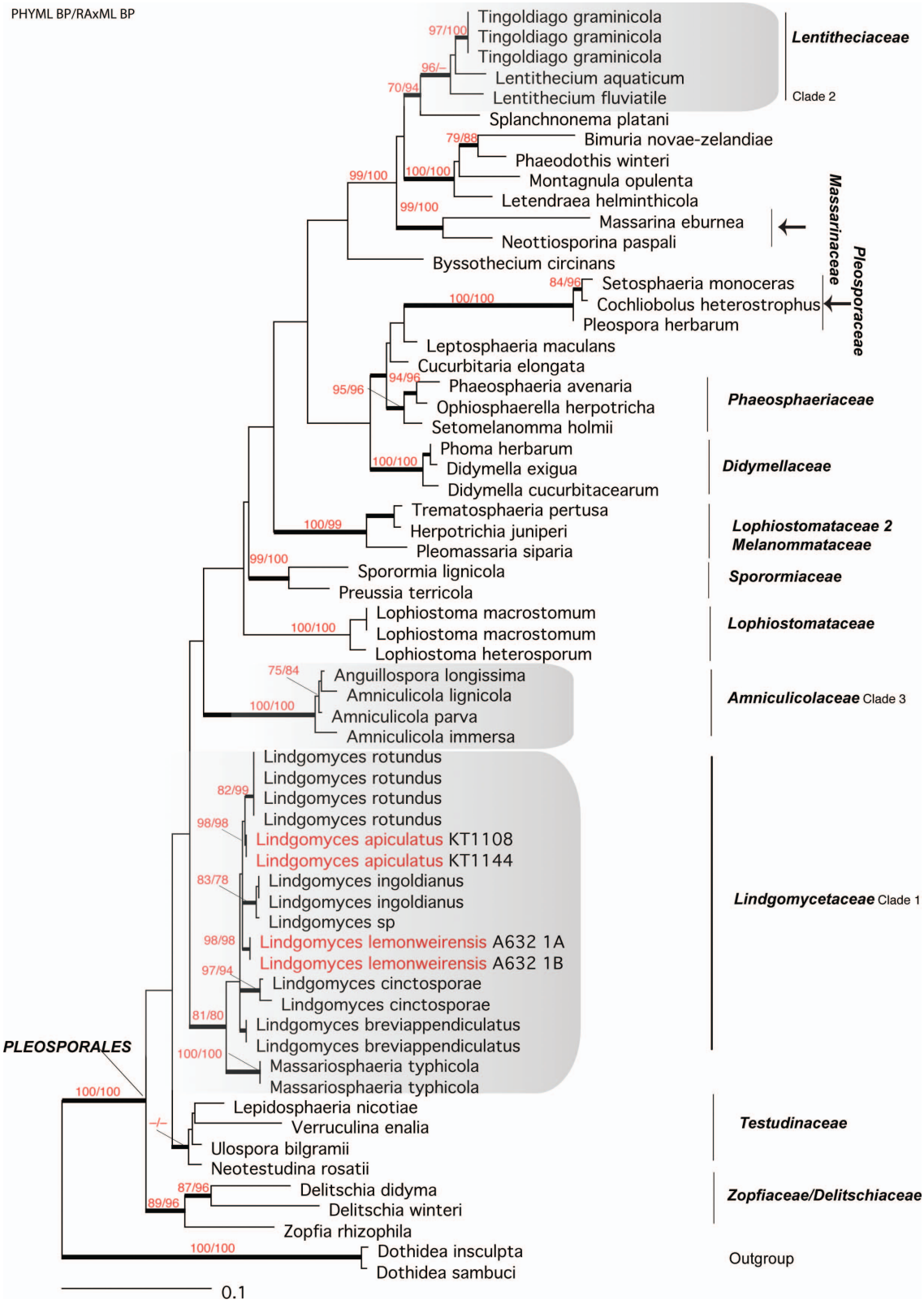


FIG. 1. Phylogram of the most likely tree (−lnL = 9855.08) from a PHYML analysis of 62 taxa based on combined SSU and LSU nrDNA (2346 bp). Thickened branches indicate significant Bayesian posterior probabilities ≥ 95%; numbers refer to PhyML/RAxML bootstrap support values ≥ 70% based on 1000 replicates. Members of the Dothideales were used as outgroup taxa. The two new species are in boldface. Families with taxa from freshwater habitats are shaded. Classification following Lumbsch and Huhndorf 2010 is on the right.

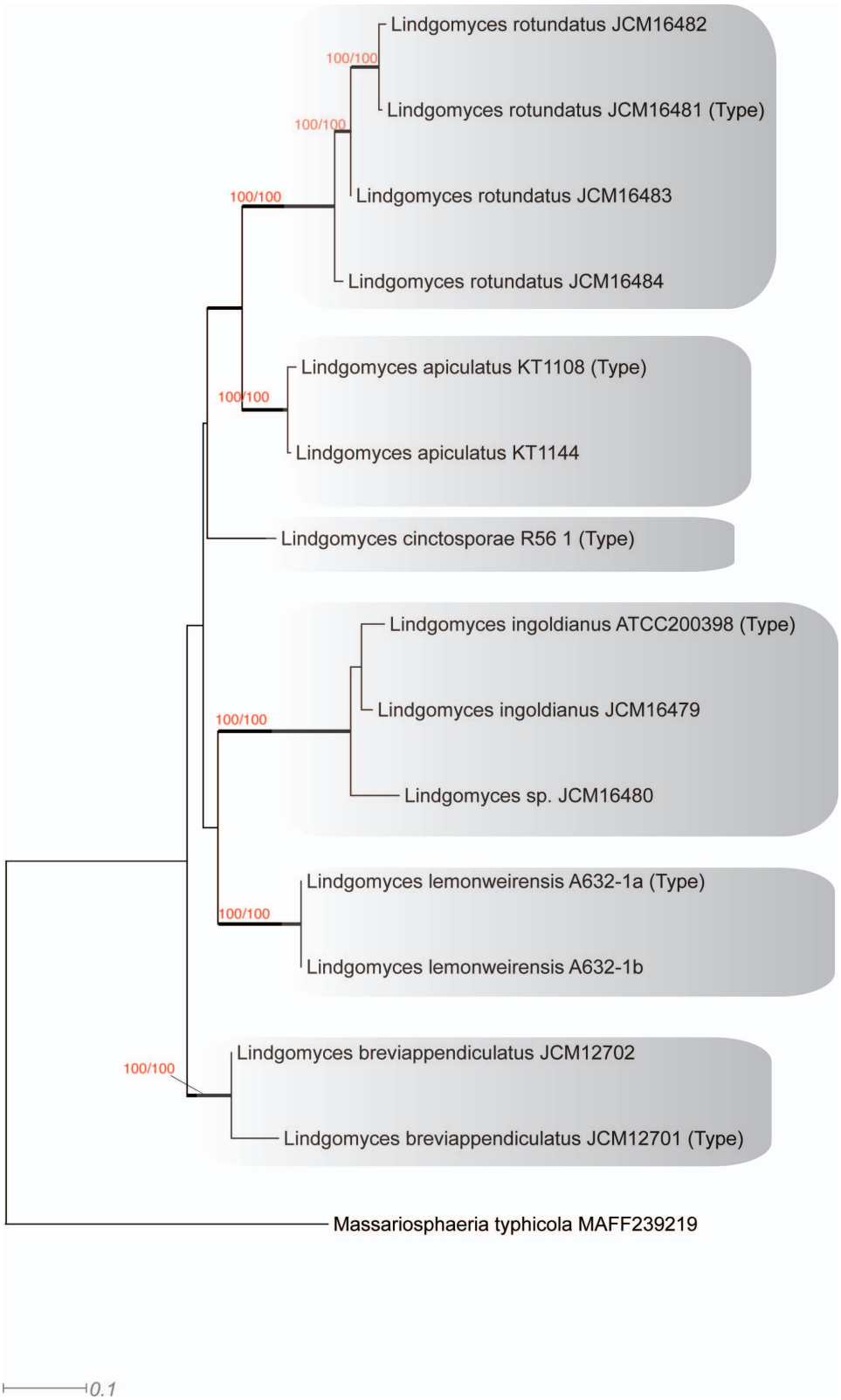
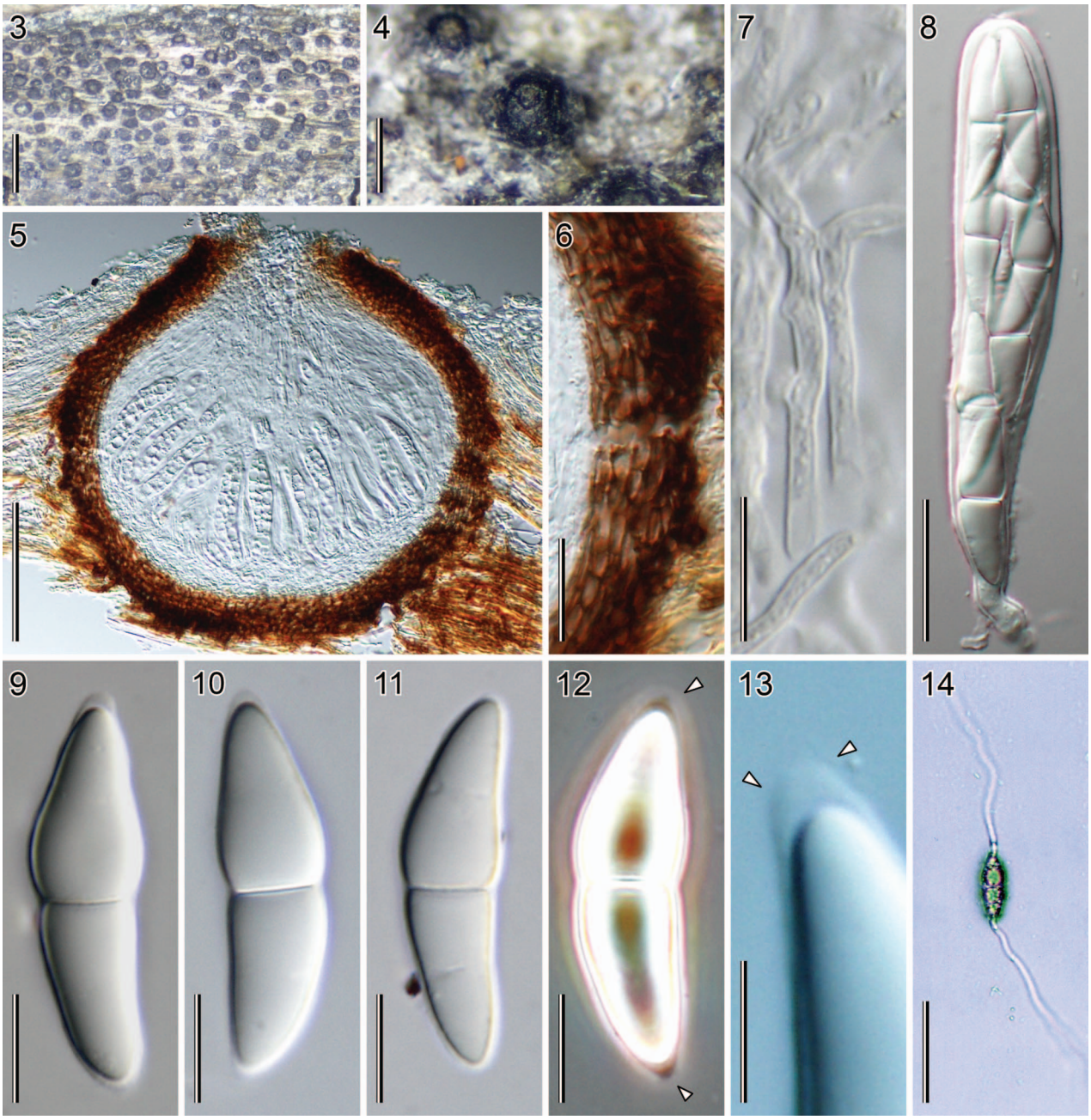


FIG. 2. Phylogram of the most likely tree (−lnL = 4904.86) from a PHYML analysis of 15 taxa based on ITS nrDNA (1127 bp). (Support values as in FIG. 1.)



FIGS. 3–14. *Lindgomyces apiculatus* (From HOLOTYPE. HHUF 28988). 3, 4. Ascomata on host surface. 5, 6. Ascoma in longitudinal section. 7. Pseudoparaphyses. 8. Asci. 9–14. Ascospores (arrowheads indicate sheath of ascospore). Bars: 3 = 1000 µm; 4 = 200 µm; 5 = 100 µm; 6 = 25 µm; 7, 9–13 = 10 µm; 8 = 30 µm; 14 = 50 µm.

Known distribution: JAPAN.

Etymology: From the L. *apiculatus*, in reference to the position and shape of ascospore appendages.

Specimens examined: JAPAN. Aomori, Hirosaki, Aoki, Mohei-pond, 140°26.3′E, 40°34.1′N, on submerged twigs of woody plant, 3 May 2003, KT 1108 (HHUF 28988, HOLOTYPUS designated here; single ascospore isolate JCM 13091 = MAFF 239601); 17 May 2003, *KT 1144* (HHUF 28991; single ascospore isolate JCM 13092 = MAFF 239602); 28 Jun 2003, *KT 1263* (HHUF 28992); 19 Jul 2003, *KT 1307A* (HHUF 28993); 27 Sep 2003, KT 1495 (HHUF 28995); 25 Oct 2003, *KT 1531* (HHUF 28996).

Notes: The morphological features of *Lindgomyces apiculatus* agree with the generic characters of *Lindgomyces*: globose ascomata, cylindrical to clavate asci, and hyaline, one-septate ascospores.

Lindgomyces apiculatus has fusiform ascospores with acute ends, with a short terminal appendage at each apex. These ascospore features are similar to

those of *L. breviappendiculatus*. However the ascospore dimensions of *L. apiculatus* are smaller, (31–)33–43 × 8–11 µm vs.(40–)44–60(–63.5) × (9.5–)11–17.5 µm), than those of *L. breviappendiculatus* (Tanaka et al. 2005b).

In addition molecular data (SSU + LSU nrDNA, FIG. 1) as well as nuclear ribosomal ITS data (FIG. 2) clearly indicate that *L. apiculatus* is a phylogenetically distinct species.

Lindgomyces lemonweirensis Raja, A.N. Mill. & Shearer, sp. nov. FIGS. 15–25

MycoBank MB561163

Ascomata 218–250 µm alta, 295–310 µm diam, globosa vel subglobosa, papillata, ostiolata, nigra. Rostrum 40–50 × 60–100 µm, centrale, compositum brunneum cellularum. Peridium 30–40 crassum, ex 2 stratum. Pseudoparaphyses ca. 4 µm latae, 100–150 µm alta., numerosa, in materia glutinosa sitae. Asci 127–170 × 28–42 µm fissitunicati, clavati vel cymbiformi, rotundati ad apicem, octospori, longi stipitati. Ascosporae 30–44 × 10–15 µm, 1–2-seriatae, 1-septatae, oblongus vel fusiformis, ad apicem rotundatum, ad septum et apices depositis guttulatis; e vagina ephemera gelatinosa circumcincta.

Anamorphe ignota.

Ascomata 218–250 × 295–310 µm, globose to subglobose, papillate, black to brown, scattered or gregarious, immersed to superficial, ostiolate. Papillae short, 40–50 × 60–100 µm, central, composed of dark brown cells. Peridium 30–40 µm thick, composed of two layers in longitudinal view; inner layer of pseudoparenchyma cells of textura angularis, elongate, compressed or not; outer layer of dark brown cells occluded with brown amorphous material. Pseudoparaphyses ca. 100–150 × 4 µm, numerous, septate, hyaline, anastomosing, immersed in gel. Asci 127–170 × 28–42 µm (av. = 150 × 32 µm, n = 30), fissitunicate, clavate to cymbiform, rounded at the apex, tapering to a 2–3 µm long stipe, with or without an apical chamber. Ascospores 30–44 × 10–15 µm (av. = 40 × 13 µm, n = 50), overlapping uni- or biseriate, oblong-fusiform, somewhat rounded at the apices, hyaline, one-septate, slightly constricted at the septum, septum close to median (0.46–0.60; av. = 0.50, n = 50); upper cell slightly broader than lower cell, multiguttulate, with a band of small guttules at the septum and at the ascospore apices and a large guttule in each cell; guttules disappear in glycerin and lactic acid; surrounded by an oval gelatinous sheath; sheath extends ca. 4–8 µm from the wall of the spore at the septum and ca. 2–3 µm at the ascospore apices, ephemeral, not seen when ascospores are mounted in glycerin or lactic acid.

Anamorph: Unknown.

Habitat: On submerged, decorticated wood.

Known distribution: USA (Wisconsin).

Etymology: Named after the Lemonweir River in Wisconsin where the type species was collected.

Specimens examined: USA, Wisconsin, Lemonweir River, on submerged decorticated wood, 5 Oct, 2010, 43°46′16″N, 89°53′10″W, *Huzefa A. Raja* and *Andrew N. Miller*, *A632-1*. (ILL 40793, HOLOTYPUS designated here).

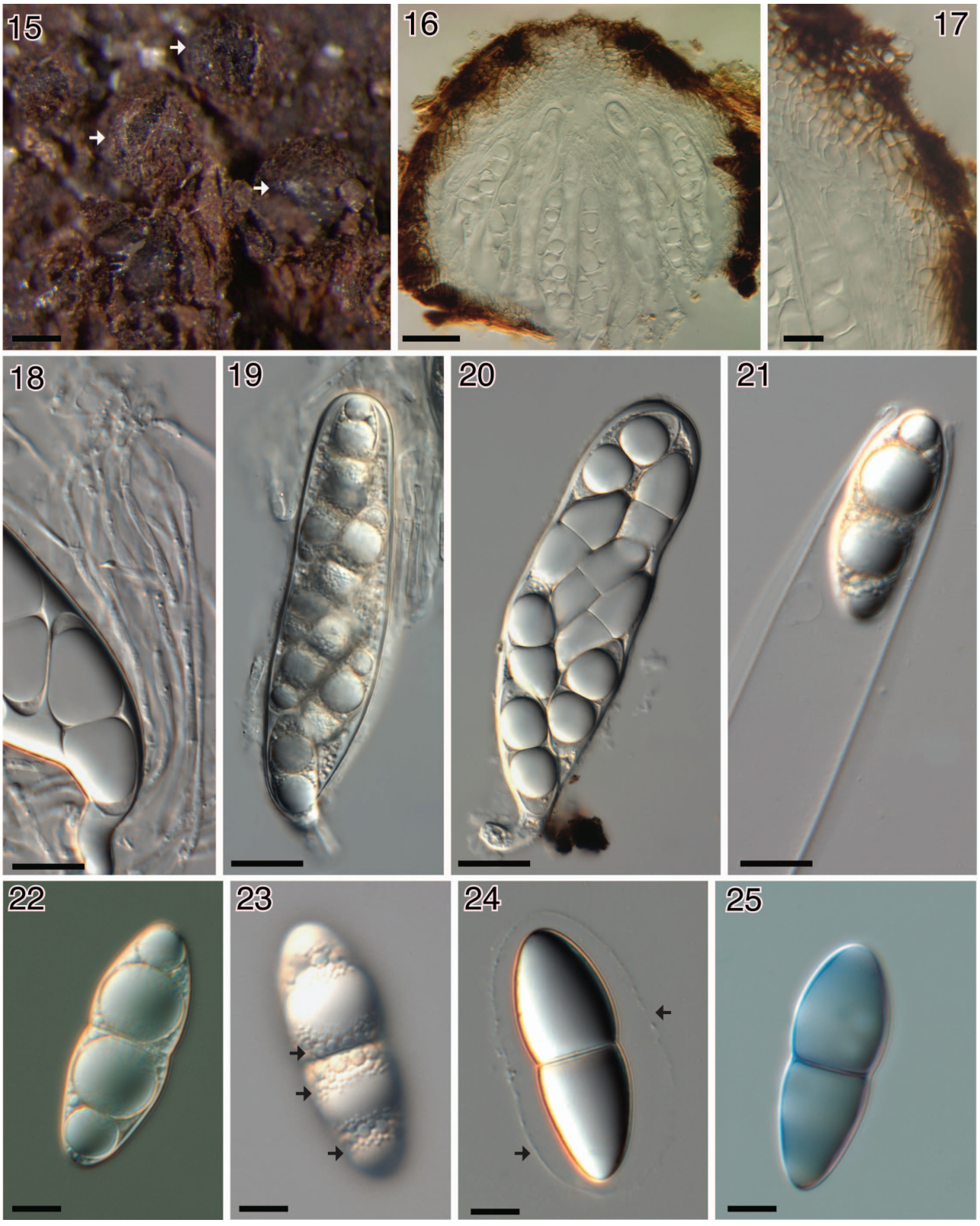
Notes: Lindgomyces lemonweirensis resembles *L. cinctosporae* in having morphologically similar ascomata, and ascospores. However it differs in having smaller ascomata (218–250 × 295–310 µm vs. 285–330 × 374–426 µm in *L. cinctosporae*). In addition the ascospores of *L. lemonweirensis* are smaller (30–44 × 10–15 µm) and surrounded by an oval gelatinous sheath that has an even margin. The ascospores of *L. cinctosporae* are larger (40–58 × 10–18 µm) and surrounded by an amorphous gelatinous sheath that expands in water and remains visible when fixed in glycerin and lactic acid (Hirayama et al. 2010). In addition molecular data (SSU + LSU nrDNA, FIG. 1) as well as nuclear ribosomal ITS data (FIG. 2) clearly indicate that *L. lemonweirensis* is a phylogenetically distinct species.

KEY TO SPECIES OF *LINDGOMYCES*

1a. Ascospores surrounded by a large, expanding gelatinous sheath 2
1b. Ascospores not as in 1a 3
2a. Ascospores fusiform with acute apices *L. ingoldianus*
2b. Ascospores cylindrical with rounded apices . . . . . *L. rotundatus*
3a. Ascospores with apiculate gelatinous appendages . . . 4
3b. Ascospores lacking apiculate gelatinous appendages 5
4a. Ascospores 44–60 × 11–17.5 . . . *L. breviappendiculatus*
4b. Ascospores 33–43 × 8–11 . . . . . *L. apiculatus*
5a. Ascospores 40–58 × 10–18 µm, surrounded by a scalloped gelatinous sheath that stains in aqueous nigrosin . . . . . *L. cinctosporae*
5b. Ascospores 30–44 × 10–15 µm, surrounded by a regular, ephemeral sheath . . . . . *L. lemonweirensis*.

DISCUSSION

Four families of freshwater ascomycetes are currently included in the Dothideomycetes based on molecular sequence data, namely Aliquandostipitaceae, Amniculicolaceae, Lentitheciaceae and Lindgomycetaceae (Inderbitzin et al. 2001, Schoch et al. 2009, Shearer et al. 2009, Zhang et al. 2009). In addition freshwater ascomycetes also occur in some marine and mangrove fungal lineages within the Dothideomycetes and vice versa (Suetrong et al. 2009). For example *Manglicola guatemalensis* Kohlm. & E. Kohlm., which was isolated from Thailand on submerged fronds of *Nypa fruticans* Wurmb in mangrove habitats, is placed currently in



FIGS. 15–25. *Lindgomyces lemonweirensis* (From HOLOTYPE. A632-1). 15. Ascomata on wood (arrows). 16. Longitudinal section through ascomata. 17. Section through peridial wall. 18. Pseudoparaphyses. 19, 20. Ascus. 21. Extending endosascus with ascospore at the apex. 22. Ascospore in water. 23. Ascospore in water, note arrows showing guttulation. 24. Ascospore with oval gelatinous sheath in water. 25. Ascospore in glycerin. Bars: 15 = 200 µm; 16 = 50 µm; 16–25 = 20 µm.

the Aliquandostipitaceae based on nuclear ribosomal SSU and LSU sequence data (Suetrong et al. 2010). *Kirschsteiniothelia elaterascus* Shearer has been reported frequently from submerged wood in temperate and tropical freshwater habitats (Shearer 2001, Shearer and Raja 2010), however it currently is placed in the newly erected family Morosphaeriaceae, which consists of taxa mostly occurring in marine and mangrove habitats (Suetrong et al. 2009). Furthermore *Lentithecium arundinaceum* (Sowerby) K.D. Hyde et al. (= *Massarina phragmaticola* Poon & K.D. Hyde) is described from intertidal estuarine habitats (Poon and Hyde 1998) and placed in the Lentitheciaceae with other freshwater taxa (Suetrong et al. 2009). Thus far only Lindgomycetaceae (Clade 1) and Amniculicolaceae (Clade 2) consist of taxa described and reported exclusively from freshwater. In this regard it is interesting to note however that the ascospores of *L. lemonweirensis* contain lipid droplets (FIG. 23). Although multiguttulate ascospores occur commonly in freshwater taxa in both the Sordariomycetes and Dothideomycetes (Shearer and Raja 2010), the guttulation pattern observed in *L. lemonweirensis* is akin to that observed for marine Halosphaeriaceae such as *Nais inornata* Kohlm., which occurs in both marine (Kohlmeyer 1962) and freshwater habitats (Shearer and Crane 1978) and *Saagaromyces glitra* (J.L. Crane & Shearer) K.L. Pang & E.B.G. Jones (≡ *Nais glitra*), which has been reported previously on submerged wood of *Rhizophora mangle* L. (Crane and Shearer 1986). It will be interesting to note whether *L. lemonweirensis* will be reported from brackish water habitats in future studies.

In addition to the *Lindgomyces* species the Lindgomycetaceae also currently includes *Massariosphaeria typhicola* (P. Karst.) Leuchtm. (strain KT 667, KT 797) (Hirayama et al. 2010). The genus *Massariosphaeria* is currently polyphyletic within the order Pleosporales (Wang et al. 2007). Tanaka et al. (2010) reported remarkable morphological differences among various worldwide records of *M. typhicola* reported in the literature. The authors concluded that a reassessment of species monophyly is warranted based on molecular sequence data. We therefore continue to retain *M. typhicola* in the Lindgomycetaceae until further molecular work can be carried out to understand the phylogenetic relationship within the *Massariosphaeria* species complex. In a study, an isolate of a freshwater hyphomycete *Taniolella typhoides* Gulis & Marvanová (strain CCMF-10198) also showed phylogenetic affinities to the Lindgomycetaecae (Shearer et al. 2009). More recently a new freshwater coelomycete, *Lolia aquatica* Abdel-Aziz & Abdel-Wahab (Abdel-Aziz and Abdel-Wahab 2010), was described and reported from decayed submerged *Phragmites australis* (Cav.) Steud. stems in Egypt. Phylogenetic analysis of partial LSU sequences showed that it had affinities to Lindgomycetaceae (Abdel-Aziz and Abdel-Wahab 2010).

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