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Phylogenetic and taxonomic evaluation of *Chalara*, *Chalaropsis*, and *Thielaviopsis* anamorphs associated with *Ceratocystis*

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Abstract: Parsimony analysis of sequences of the internal transcribed spacer region of the nuclear rDNA and partial sequences of the large subunit (LSU) place four anamorphic *Chalara* species as a monophyletic grouping within the teleomorph genus *Ceratocystis*. *Chalara ovoidea*, *Ch. thielavioides*, *Ch. populi*, and *Ch. elegans* (synanamorph: *Thielaviopsis basicola*) form aleurioconidia typical of the anamorph genus *Thielaviopsis*, to which the species are transferred. Three of these species (*T. ovoidea*, *T. thielavioides*, and *T. populi*) are morphologically similar to each other but are shown to be distinct by rDNA sequences. The anamorphic genera *Chalaropsis* and *Hughesiella* are considered synonyms of *Thielaviopsis*. *Thielaviopsis punctulata*, which forms aleurioconidia singly, is shown to be the anamorph of *Ce. radicicola*. The respective anamorphs for *Ce. coerulescens*, *Ce. fagacearum*, and *Ce. eucalypti*, which lack aleurioconidia, are also transferred to the amended genus *Thielaviopsis as T. ungeri*, *T. quercina*, and *T. eucalypti*. Although *Ch. australis* and *Ch. neocaledoniae* do not form aleurioconidia, they are placed in *Thielaviopsis* based on their endoconidial state and clear affinities to *Ceratocystis eucalypti*. Three apparently asexual *Ambrosiella* species belong in the *Ce. moniliformis* clade based on LSU rDNA sequences, but the cultures available are not suitable for detailed morphological study, and these species are not transferred to *Thielaviopsis*.

Key Words: Ascomycetes, *Ceratocystis paradoxa*, rDNA, *Thielaviopsis paradoxa*

INTRODUCTION

Four apparently asexual species of *Chalara* (*Ch. ovoidea* Nag Raj et Kend., *Ch. thielavioides* (Peyr.) Nag Raj et Kend., *Ch. populi* Veldeman ex Kiffer et Delon, and *Ch. elegans* Nag Raj et Kend.) were confirmed as being anamorphs of *Ceratocystis* Ellis et Halstead based on phylogenetic analysis of rDNA sequences (Paulin and Harrington 2000). These four plant pathogenic species produce thick, dark-walled aleurioconidia (FIGS. 1–6) in addition to endoconidia from phialidic conidiophores. *Ceratocystis sensu stricto* is a monophyletic genus of insect dispersed, plant pathogenic fungi, and all *Ceratocystis* species have *Chalara* anamorphs (de Hoog and Scheffer 1984, Harrington 1981, 1987, Paulin and Harrington 2000, Witthuhn et al 1999). Aside from members of the *Ce. coerulescens* (Münch) Bakshi complex, *Ce. moniliformis* (Hedgecock) Moreau, and *Ce. fagacearum* (Bretz) Hunt, most species of *Ceratocystis* also produce thick-walled aleurioconidia from the tips of specialized hyphae. These aleurioconidia are produced in chains (synanamorph = *Thielaviopsis* Went) or singly (synanamorph = *Chalaropsis* Peyr.). Three apparently asexual symbionts of ambrosia beetles (*Ambrosiella xylebori* Brader ex v. Arx et Hennebert, *A. hartigii* Batra, and *A. ferruginea* (Math.-Käärik) Batra) also have affinities to *Ceratocystis* (Cassar and Blackwell 1996), but they apparently do not produce aleurioconidia (Batra 1967).

The synonymization of *Thielaviopsis* and *Chalaropsis* with *Chalara* (Nag Raj and Kendrick 1975) has gone uncontested until recently, when *Chalara* was found to be polyphyletic using partial small subunit (SSU, 18S) and large subunit (LSU, 28S) nuclear rDNA sequences (Paulin and Harrington 2000). The genus *Chalara* is typified by *Chalara fusidioides* (Cda.) Rabenh., a saprobe with deep-seated phialides that produces enteroblastic conidia by ring-wall building (Minter et al 1982, 1983, Nag Raj and Kendrick 1975, 1993). Most of the studied *Chalara* species without known teleomorphs were shown to have Leotialian affinities using rDNA sequences (Paulin and Harrington 2000). The description and biology of *Ch. fusidioides*, especially its slow growth rate on agar media (Nag Raj and Kendrick 1975), suggest that it may also belong within the Leotiales. Thus, *Chalara* does not appear to be an appropriate name for anamorphic species with *Ceratocystis* affinities.

The genus *Thielaviopsis* is based on *Thielaviopsis ethacetia* Went (Nag Raj and Kendrick 1975, Went 1893), which was later recognized as a synonym of *Thielaviopsis paradoxa* (de Seynes) Höhn, the ana-

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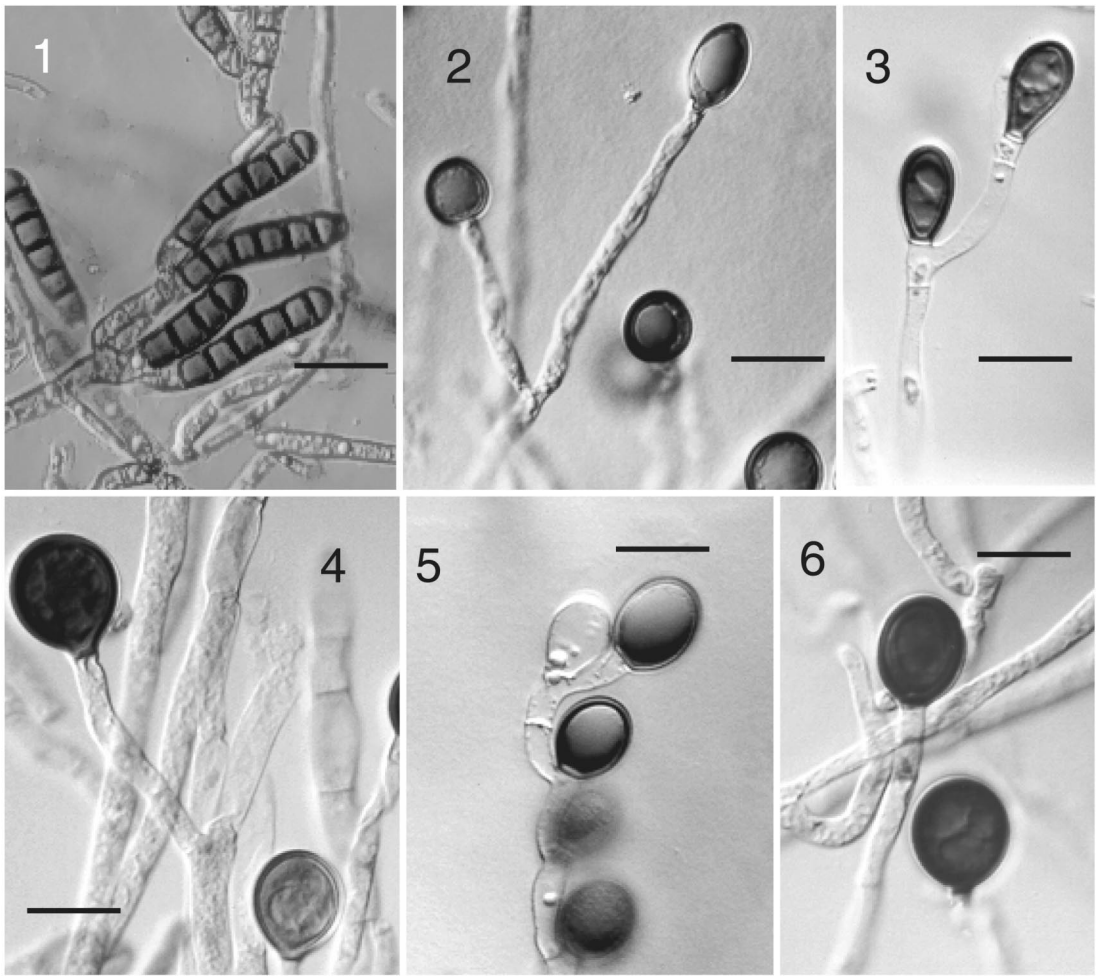

FIGS. 1–6. Aleurioconidia. 1. *Chalara elegans* (C1373). 2. *Ch. populi* (C1368). 3. *Ch. ovoidea* (C1375). 4. *Ch. thielavioides* (C1377). 5. *Ch. thielavioides* (C1630). 6. *Ch. thielavioides* (C1379). Scale bars: 1 = 25 μm; 2–6 = 10 μm.

morph of *Ceratocystis paradoxa* (Dade) Moreau. Went (1893) gave the generic diagnosis for *Thielaviopsis* as "*Hyphae steriles repentes, subhyalinae, fertiles simplices, septatae. Conidia dimorpha, majora catenulata, ovata, fusca; minora cylindracea, hyalina, ex interiore hypharum catenulatim generata et ex apice exsilientia.*" Thus, his concept of the genus included conidia of two types, endoconidia from phialides and larger, pigmented aleurioconidia in chains at the tips of specialized hyphae. Peyronel (1916) erected the genus *Chalaropsis*, using the type species *Chalaropsis thielavioides*, for species with aleurioconidia produced singly. *Thielaviopsis basicola* (Berk. et Br.) Ferr (synanamorph = *Chalara elegans*) produces aleurioconidia from a basal sporogenous cell, and this conidium undergoes division into an aleurioconidial chain that fragments into individual barrel-shaped spores (Riggs and Mims 2000).

We used parsimony analysis of sequences from the large subunit and internal transcribed spacer regions (ITS) of the nuclear ribosomal DNA operon to examine the phylogenetic placement of anamorphic species with *Ceratocystis* affinities. We tested the hypothesis that four of these *Chalara* species form an asexual lineage within *Ceratocystis*. We also used morphological comparisons to determine if three of these are distinct species or if they are a single species. The genus *Thielaviopsis* is amended, and *Chalara* species with *Ceratocystis* affinities are transferred to *Thielaviopsis*.

MATERIALS AND METHODS

Morphological analyses.—Isolates (TABLES I and II) were grown on 3.9% Difco potato dextrose agar or malt yeast extract agar (MYEA, 0.2% Difco yeast extract, 2% Difco malt extract, 2% agar) for microscopic examination. Incubation was at room temperature (21–24 C) and lighting for 7–21 d.

DNA isolation.—Template DNA for PCR was obtained by extracting from mycelium grown in broth medium or from mycelium scraped from 1–2 wk-old cultures on MYEA. For DNA extraction in liquid culture, strains were grown at room temperature (approximately 21 C) in 30 mL of broth medium (MYEA without agar) for 2 to 6 wk. DNA extrac-

TABLE I. Fungal isolates and sequence accession numbers

Species	Host	Strain number[a]	GenBank	
			ITS rDNA	LSU rDNA
Ambrosiella ferruginea	unknown	C1571, CBS 408.68	—	AF275505
Ambrosiella hartigii	*Acer*	C1574, CBS 403.82	—	AF275506
Ambrosiella xylebori	*Coffea*	C1650, CBS 110.61	—	AF275508
Ceratocystis adiposa	*Prunus*	C998, CBS 600.74	AF275545	AF222481
Ceratocystis albofundus	*Acacia*	C1042	—	AF275500
Ceratocystis coerulescens	*Pinus*	C301, CBS 100.98	—	AF275510
Ceratocystis eucalypti	*Eucalyptus*	C457	—	AF222482
Ceratocystis fagacearum	*Quercus*	C1305	—	AF222483
Ceratocystis fimbriata	*Populus*	C685	—	AF275512
Ceratocystis moniliformis	*Theobroma*	C1582	AF275476	—
	Theobroma	C1008, CBS 155.62	—	AF275499
Ceratocystis paradoxa	*Ananus*	C1001, CBS 601.70	—	AF275498
	unknown	C1091	AF275477	—
Ceratocystis pinicola	*Pinus*	C448	—	AF275511
Ceratocystis radicicola	*Phoenix*	C869, CBS 114.47	AF275495	AF275513
Ceratocystis virescens	*Quercus*	C74	—	AF222489
Chalara australis	*Nothofagus*	C448	—	AF222479
Chalara elegans	*Betula*	C1373, CBS 430.74	AF275482	—
	Daucus	C853	AF275494	AF222480
	Daucus	C1602	AF275490	—
	Pelarogonium	C185	AF275493	AF275509
	Primula	C1372, CBS 414.52	AF275481	AF222459
Chalara neocaledoniae	*Coffea*	C694, CBS 149.83	—	AF222471
Chalara ovoidea	*Quercus*	C1376, CBS 136.88	AF275484	AF222472
	Fire wood	C1375, CBS 354.76	AF275483	AF275502
Chalara populi	*Populus*	C1368, CBS 484.71	AF275479	AF222475
	Populus	C1369, CBS 486.71	AF275480	AF275501
Chalara punctulata	*Lawsonia*	C1631, CBS 167.67	AF275492	—
Chalara thielavioides	*Carya*	C1362	AF275478	AF222479
	Lupinus	C1377, CBS 148.37	AF275485	AF275503
	Populus	C1379, CBS 180.75	AF275487	—
	Plant remains	C1630, CBS 543.69	AF275491	AF275507
	Ulmus	C1378, CBS 130.39	AF275486	AF222480
	Ulmus	C1486, ATCC 11234	AF275488	—
	unknown	C1509, ICMP 11355	AF275489	AF275504
Petriella sordida	*Pyrus*	A104, CBS 258.31	—	AF275497
Glomerella phacidiomorpha	unknown	A100, CBS 198.35	—	AF275496

[a] CBS = Centraalbureau voor Schimmelcultures, Netherlands; A and C = Culture collection of T. C. Harrington; ICMP = Landcare Research New Zealand Culture Collection; ATCC = American Type Culture Collection, USA.

tions were performed using the protocol of DeScenzo and Harrington (1994).

PCR, DNA sequencing, and RFLP analysis.—A portion of the LSU and the internal transcribed spacer regions (ITS) of the nuclear rDNA were amplified and sequenced for *Chalara*, *Ambrosiella*, and *Ceratocystis* species. The primers (ITS1-F, ITS-4, LR3, LR5, and LROR) and protocols for amplification and sequencing were as described by Paulin and Harrington (2000).

Partial sequences of the LSU gene were obtained from 10 species within the genus *Ceratocystis* and six species within *Chalara* (TABLE I). The outgroup taxa *Glomerella phacidiomorpha* (Cesati) Petrak and *Petriella sordida* Barron et Gilman are closely related to *Ceratocystis* (Paulin and Harrington 2000). The sequences were manually aligned, but 40 of the 590 LSU characters, including gaps, were ambiguously aligned and eliminated before parsimony analysis. After excluding the ambiguously aligned characters, the largest insertion/deletion (indel) within the LSU data set was one base pair.

Sequences of the ITS and the 5.8S rDNA from four species of *Ceratocystis* were compared to those of five species of *Chalara* (*Ch. ovoidea*, *Ch. elegans*, *Ch. thielavioides*, *Ch. populi*, and *Ch. punctulata* Hennebert). Among the *Ceratocystis* species, only the ITS sequences of *Ce. moniliformis*, *Ce. adiposa*, *Ce. radicicola* and *Ce. paradoxa* could be rea-

TABLE II. Measurements (in microns) of *Chalara thielavioides* and *Chalara ovoidea* spores[a]

Species	Isolate	Host	Aleurioconidia			Endoconidia		
			Ratio (L/W)	Range	Mean	Ratio (L/W)	Range	Mean
Ch. thielavioides	C1379	Poplar	1.15/1	12 × 10–11	12 × 10.4	4.5/1	8–12 × 2–4	10 × 2.2
	C1486	Elm root	1.25/1	10–16 × 8–12	12.8 × 10.2	none	none	none
	C1362	*Carya*	1.10/1	12–18 × 12–16	14.4 × 13.7	2.8/1	9–20 × 2–10	12.6 × 4.5
	C1377	*Lupinus*	1.10/1	12–16 × 9–11	13.5 × 10	none	none	none
	C1509	unknown	1.20/1	12–20 × 12–16	15.4 × 12.8	6.56/1	12–24 × 2–4	16.4 × 2.5
	C1630	plant	1.3/1	14–17 × 11–12	15 × 11.5	7.0/1	15–20 × 2.5	18.5 × 2.5
Ch. ovoidea	C1376	*Quercus*	1.76/1	10–16 × 6–10	13.0 × 7.4	4.8/1	14–21 × 2–4	16.8 × 3.5
	C1375	firewood	1.62/1	8–14 × 4–8	11.4 × 7	5.26/1	10–16 × 2–4	12.1 × 2.3

[a] All isolates were grown on potato dextrose agar except for isolate C1377, which was grown on malt yeast extract agar.

sonably aligned with those of the five *Chalara* species. *Ceratocystis moniliformis* was used as the outgroup taxon because it is distinct molecularly (LSU sequences and *MAT-2* sequences, data not shown) and morphologically (hat-shaped ascospores and no aleurioconidia) from the *Chalara* species. The sequences were manually aligned, but 69 of the 483 ITS characters, including gaps, were ambiguously aligned and, therefore, eliminated before parsimony analysis. After excluding ambiguously aligned characters, the largest indel within the ITS data set was two base pairs.

In both data sets, gaps were treated as a fifth character. Only parsimony informative sites were used in the phylogenetic analyses (PAUP 4.04b, Swofford 1998). Maximum parsimony heuristic searches were performed with all characters having equal weight, starting trees were obtained via stepwise addition, and tree-bisection-reconnection was used. Robustness of the internal branches of the tree was evaluated by 1000 bootstrap replications using heuristic searches (Felsenstein 1985). Decay indices (Bremer 1988) were calculated using AutoDecay version 4.0 (Eriksson 1998). Trees were rooted at the internal node with basal polytomy.

RESULTS

Phylogenetic analyses.—Analysis of the LSU sequences placed six *Chalara* species into two groups: two species (*Ch. australis* Kile and *Ch. neocaledoniae* Dadant ex Kiffer et Delon) are in the *Ceratocystis coerulescens* complex (Witthuhn et al 2000), and the other four species are in a distinct clade within *Ceratocystis* (FIG. 7). Eighteen equally most parsimonious trees of 185 steps were derived from analysis of the 63 parsimony informative positions of the LSU data set. The consistency (CI), homoplasy (HI), retention (RI), and rescaled consistency (RC) indices were 0.8000, 0.2000, 0.8490, and 0.6792, respectively. Based on LSU data, the clade of four *Chalara* species (*Ch. ovoidea*, *Ch. thielavioides*, *Ch. populi*, and *Ch. elegans*) was not supported by bootstrap analysis but had a decay value of d1 and was found in each of the 18 most parsimonious trees. *Ambrosiella* species were placed within the strongly supported *Ce. moniliformis* clade with *Ce. adiposa* (Butler) Moreau and *Ce. fagacearum*, confirming the SSU rDNA findings of Cassar and Blackwell (1996). When gaps were treated as missing data, 18 trees of 174 steps were found, and these trees had the topology of the trees in which gaps were treated as a fifth character.

A neighbor joining analysis (Swofford 1998) of the LSU data set was also performed, and a tree with a topology only slightly different from FIG. 7 was produced (FIG. 8). As in parsimony analysis, the four *Chalara* species (*Ch. ovoidea*, *Ch. thielavioides*, *Ch. populi*, and *Ch. elegans*) grouped together in the neighbor joining analysis, and there was support for the *Ce. fimbriata*, *Ce. coerulescens*, *Ce. paradoxa* and *Ce.*

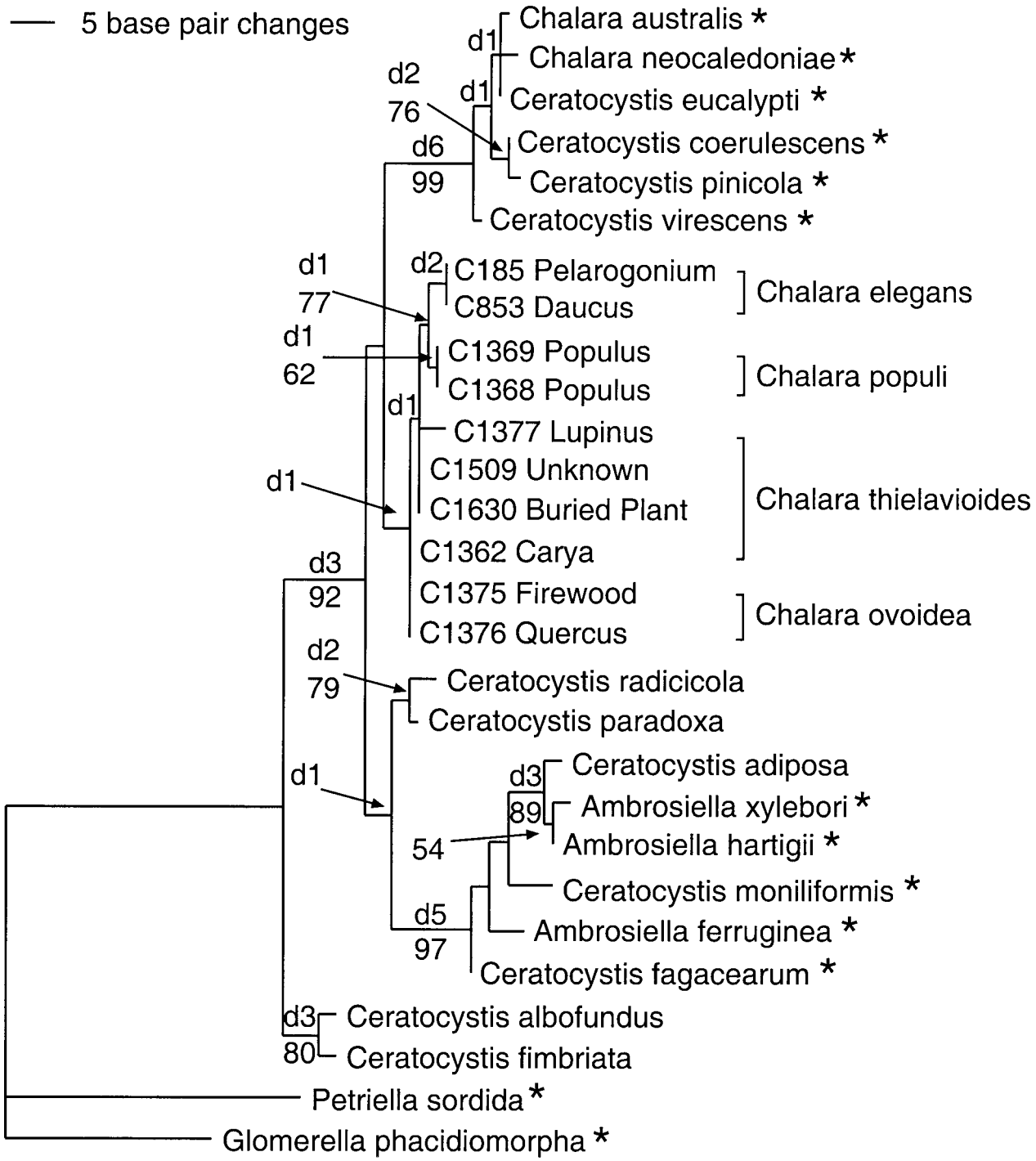

FIG. 7. One of 18 most parsimonious trees based on 550 characters, including gaps, of a partial sequence of the large subunit rDNA gene. The tree is rooted to *Petriella sordida* and *Glomerella phacidiomorpha*. Branches with decay indices are indicated with a "d" above the branch, and branches with bootstrap values >50% are indicated below the branch. Those species not known to produce aleurioconidia are indicated with an asterisk.

moniliformis clades. The *Ce. paradoxa* clade was sister to the *Ce. coerulescens* clade in the neighbor joining tree, as opposed to being sister to the *Ce. moniliformis* clade in parsimony analysis (FIG. 7), but there was little resolution of the relationships among the five major clades of *Ceratocystis*, except that the *Ce. fimbriata* complex was basal to the genus in both parsimony and neighbor joining analyses.

The ITS1 and ITS2 sequences of species from the *Ce. coerulescens* complex (Witthuhn et al 2000), *Ce.*

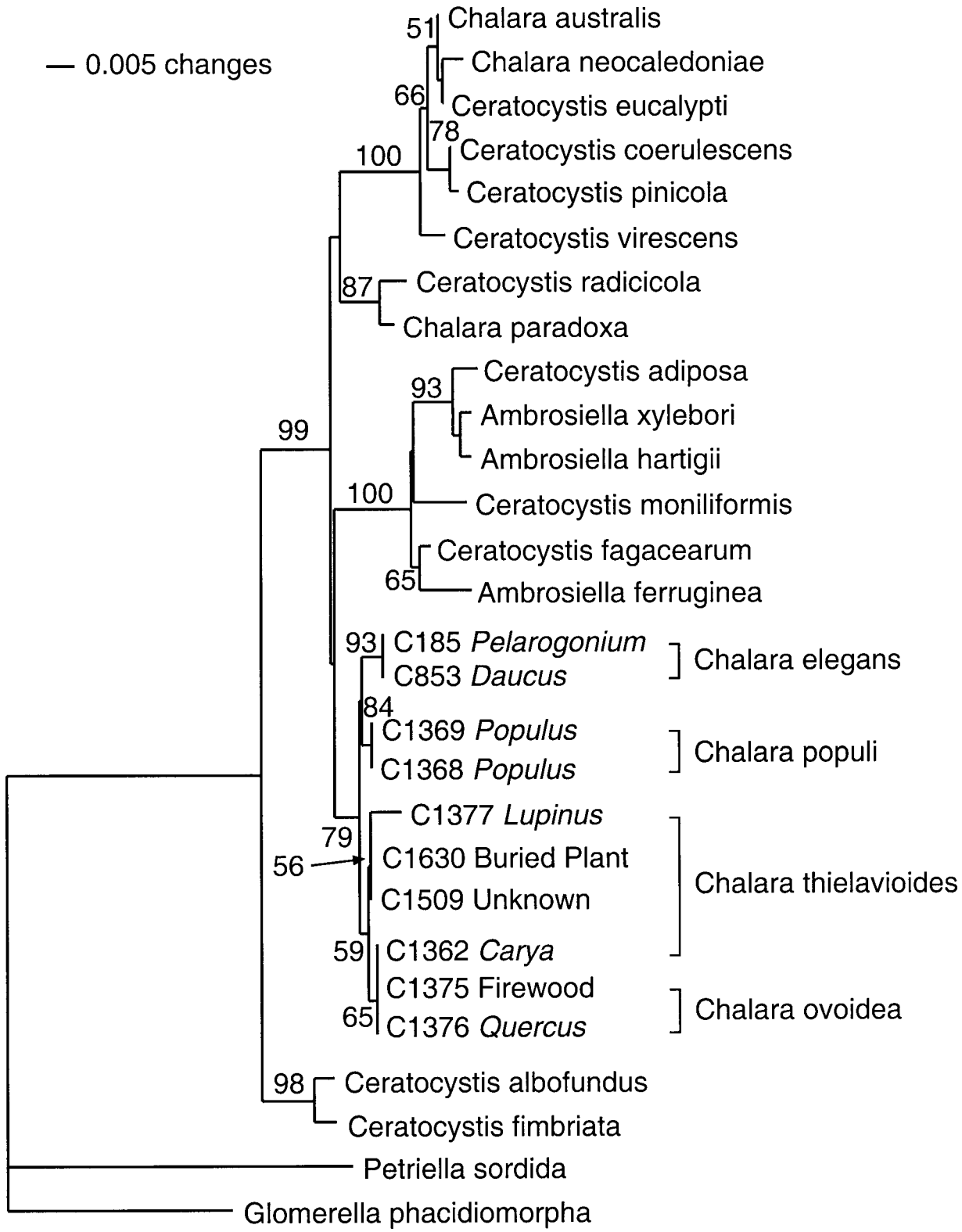

FIG. 8. Neighbor joining tree using large subunit rDNA sequence data rooted to *Petriella sordida* and *Glomerella phacidiomorpha*. Branches with bootstrap values >50% are indicated above the branch.

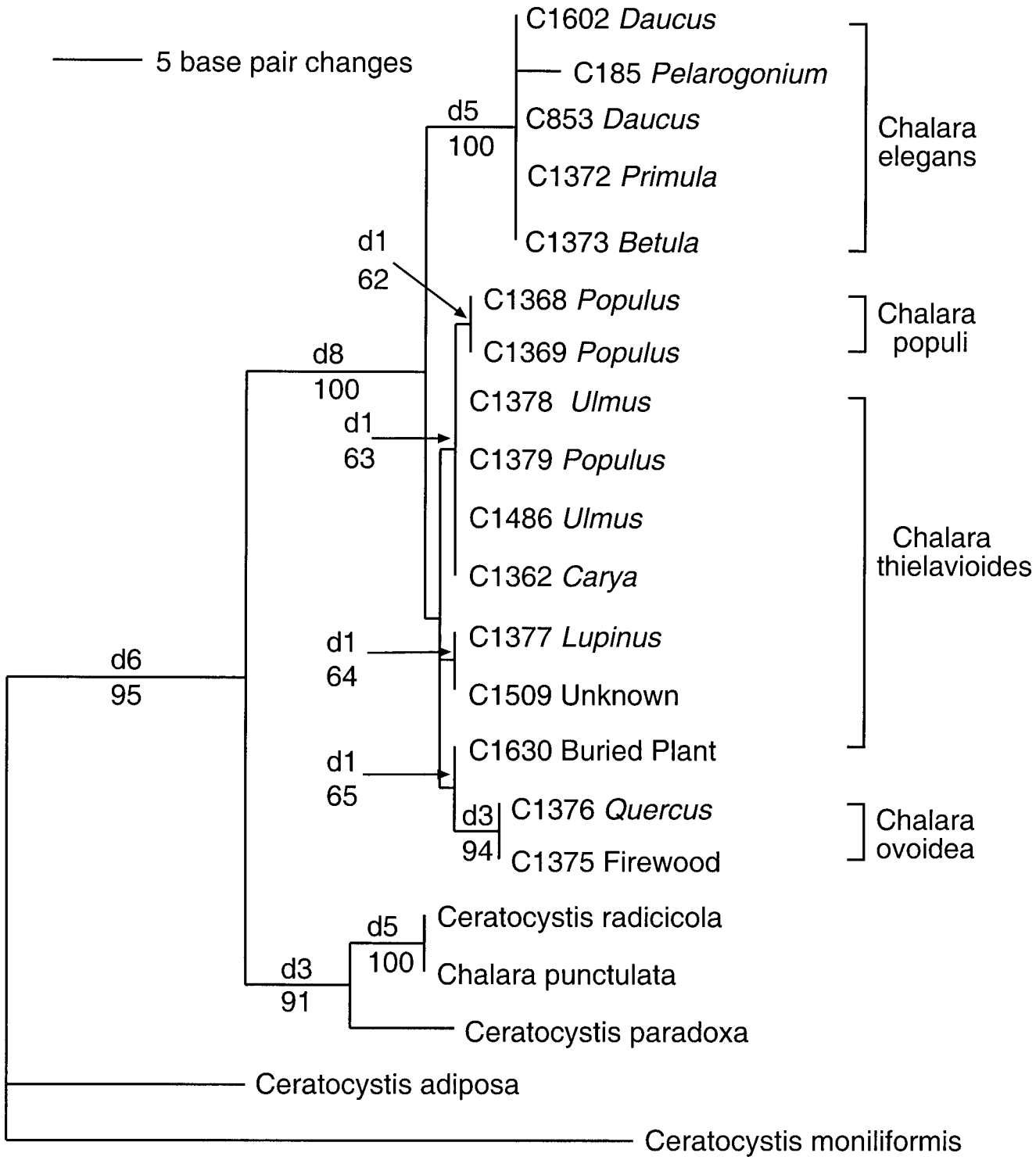

FIG. 9. One of two most parsimonious trees based on 414 characters, including gaps, of the ITS-1, 5.8S, and ITS-2 regions of the rDNA operon. The tree is rooted to *Ceratocystis moniliformis*. Branches with decay indices are indicated above the branch, and branches with bootstrap values >50% are indicated below the branch.

fimbriata complex and *Ce. fagacearum* (Witthuhn et al 1999) could not be unambiguously aligned with the ITS sequences of the four *Chalara* species and, therefore, were not used in parsimony analysis. Two equally most parsimonious trees of 122 steps were derived from analysis of the 47 parsimony informative positions of the ITS data set. The CI, HI, RI and RC indices were 0.8934, 0.1066, 0.8960, and 0.8005,

respectively. Strong bootstrap support was found for the grouping of the ''asexual *Chalara* clade'' (FIG. 9), and this branch had a decay index of d8. *Ceratocystis radicicola* (Bliss) Moreau and *Ce. paradoxa* had ITS sequences that were similar to those of the *Chalara* clade. *Chalara punctulata* was morphologically similar to the anamorph of *Ce. radicicola*, and these species had identical ITS sequences (FIG. 9). When gaps were treated as missing data, two trees of 97 steps were found, and the trees were identical to those found when gaps were treated as a fifth character. A neighbor joining analysis was performed on the ITS data, and a tree was produced that was identical to that shown in FIG. 9.

Morphological analyses.—Three species of *Chalara* (*Ch. ovoidea*, *Ch. thielavioides*, and *Ch. populi*) were morphologically very similar to each other, and both LSU and ITS sequences failed to clearly separate them. Ten measurements of conidia and aleurioconidia were made of isolates grown on PDA. Isolate C1377 of *Chalara thielavioides* did not produce aleurioconidia or endoconidia on PDA, so measurements were taken from MYEA. The aleurioconidia of isolates of *Ch. thielavioides* were 10–20 × 8–16 μm, with length to width ratios of 1.10–1.3/1.0, and endoconidia were 8–24 × 2–10 μm, with length to width ratios of 2.8–7.0/1.0 (TABLE 2). Our dimensions are similar to the ranges reported by Nag Raj and Kendrick (1975): 9–19 × 7.5–18 μm (ratio = 1.15/1) and 6.5–32 × 2.5–6.5 μm (ratio = 3.5/1), respectively. Peyronel (1916) reported that *Ch. thielavioides* has aleurioconidia 10–20 × 8–15 μm (ratio = 1.3/1) and endoconidia 8–55 × 3–4.5 μm (ratio = 7.8/1).

Chalara thielavioides is morphologically similar to *Ch. ovoidea*, but *Ch. ovoidea* has less globose and narrower aleurioconidia (Nag Raj and Kendrick 1975). We found aleurioconidia of *Ch. ovoidea* (isolates C1375 and C1376) to be 8–16 × 4–10 μm, with length to width ratios of 1.6–1.8/1, and endoconidia were 10–21 × 2–4 μm, with length to width ratios of 4.8–5.3/1, similar to measurements reported by Nag Raj and Kendrick (1975): 7.5–14 × 6–11 μm (1.25/1) and 5–22 × 2.5–5 μm (3.3/1), respectively.

Kiffer and Delon (1983) reported that *Ch. populi*, when compared to *Ch. ovoidea*, has longer phialides on branched conidiophores. They reported aleurioconidia to be 6.7–12 × 6–9 μm and endoconidia to be 6–18 × 2.2–3.8 μm, similar to those of *Ch. ovoidea*. We also found that aleurioconidia of *Ch. populi* are similar in width to those of *Ch. ovoidea* (FIGS. 2, 3).

TAXONOMY

The genus *Thielaviopsis* is amended to include all *Chalara*-like species with *Ceratocystis* affinities, with or without aleurioconidia, which when present may be produced in chains or singly (as in *Chalaropsis*). *Hughesiella* Bat. et Vital, distinguished from *Chalaropsis* by endoconidia that are dark walled (Batista and Vital 1956), is also considered a synonym.

Thielaviopsis Went, Arch. voor de Java Suekerr., p 4. 1893, emend. Paulin, Harrington, et McNew
= *Chalaropsis* Peyr., Staz. Sper. Agr. Ital. 49: 595. 1916.
= *Hughesiella* Bat. et Vital, Anais. Soc. Biol. Pernamb., 14: 41. 1956.

Thick, dark-walled aleurioconidia present or absent, produced either singly or in chains upon specialized hyphae. *Chalara*-like conidia are produced by ring wall building within phialides, extruded in chains, cylindrical, remaining hyaline or becoming thick-walled and dark. When known, teleomorphs are placed in *Ceratocystis*.

Type: *Thielaviopsis paradoxa* (de Seynes) Höhn., Hedwigia 43: 295. 1904.
≡ *Sporoschisma paradoxum* de Seynes, Recherches pour Servir à d'Histoire Naturelle des Végétaux Inférieurs, 3: 30. 1886.
≡ *Chalara paradoxa* (de Seyenes) Sacc, Sylloge Fung., 10: 595. 1892.
= *Thielaviopsis ethacetica* Went, Arch. voor de Java Suekerrind, p 4. 1893.
= *Endoconidium fragrans* Delacr., Bull. Soc. Mycol. Fr., 9: 184. 1893.
= *Stilbochalara dimorpha* Ferd. et Winge., Bot. Tidsskr., 30: 220. 1910.
Teleomorph: *Ceratocystis paradoxa* (Dade) C. Moreau, Rev. Mycol. (Paris) Suppl. Col. 17: 22. 1952.
≡ *Ceratostomella paradoxa* Dade, Trans. Br. Mycol. Soc., 13: 191. 1928.
≡ *Ophiostoma paradoxum* (Dade) Nannf., Svenska Skogsfor. Tidskr. 32: 408. 1934.
≡ *Endoconidiophora paradoxa* (Dade) Davidson, J. Agric. Res. 50: 802. 1935.

Thielaviopsis australis (Kile) Paulin, Harrington, et McNew, *comb. nov.*
≡ *Chalara australis* Kile, Austr. J. Bot. 35: 7. 1987.

Thielaviopsis basicola (Berk. et Br.) Ferr., Flora Italica Cryptogama. Pars. I: Fungi, Hyphales, Tubercolariaceae-Stilbaceae. Fasc. 6: 113. 1910.
≡ *Torula basicola* Berk. et Br., Ann. Mag. Nat. Hist., ser. 2, 13: 456. 1854.
= *Chalara elegans* Nag Raj et Kend., A Monograph of *Chalara* and Allied Genera. p. 111. 1975.

Thielaviopsis eucalypti (Z.Q. Yuan et Kile) Paulin, Harrington, et McNew, *comb. nov.*
≡ *Chalara eucalypti* Z.Q. Yuan et Kile, Mycol. Res. 100: 573. 1996.
Teleomorph: *Ceratocystis eucalypti* Z.Q. Yuan et Kile, Mycol. Res. 100: 573. 1996

Thielaviopsis euricoi (Bat. et Vital) Paulin, Harrington, et McNew, *comb. nov.*
≡ *Hughesiella euricoi* Bat. et Vital, Anais. Soc. Biol. Pernamb., 14: 42. 1956.

Thielaviopsis neocaledoniae (Dadant ex Kiffer et Delon) Paulin, Harrington, et McNew. *comb. nov.*
≡ *Chalara neocaledoniae* Dadant ex Kiffer et Delon, Mycotaxon 18: 166. 1983.
≡ *Thielaviopsis neocaledoniae* Dadant, *nom. inval.,* Art. 36, 37. Rev. Gén. Bot. 57: 176. 1950.

Thielaviopsis ovoidea (Nag Raj et Kend.) Paulin, Harrington, et McNew, *comb. nov.*
≡ *Chalara ovoidea* Nag Raj et Kend., A Monograph of *Chalara* and Allied Genera. p. 127. 1975.

Thielaviopsis populi (Veldeman ex Kiffer et Delon) Paulin, Harrington, et McNew, *comb. nov.*
≡ *Chalara populi* Veldeman ex Kiffer et Delon, Mycotaxon 18: 171. 1983.
≡ *Chalaropsis populi* Veldeman, *nom. inval.* Art. 36, 37. Meded. Fak. Land. Wet. Gent 36: 1001. 1971.

Thielaviopsis punctulata (Hennebert) Paulin, Harrington, et McNew, *comb. nov.*
≡ *Chalaropsis punctulata* Hennebert, Anton. Leeuw. 33: 334. 1967.
Teleomorph: *Ceratocystis radicicola* (Bliss) Moreau, Mycol (Paris) Suppl. Col. 17: 22. 1952.
≡ *Ceratostomella radicicola* Bliss, Mycologia 33: 468. 1941.

Thielaviopsis quercina (Henry) Paulin, Harrington, et McNew, *comb. nov.*
≡ *Chalara quercina* Henry, Phytopathology 34: 631. 1944.
Teleomorph: *Ceratocystis fagacearum* (Bretz) Hunt, Lloydia 19: 21. 1956.
≡ *Endoconidiophora fagacearum* Bretz, Phytopathology 42: 437. 1952.

Thielaviopsis thielavioides (Peyr.) Paulin, Harrington, et McNew, *comb. nov.*
≡ *Chalaropsis thielavioides* Peyr., Staz. Sper. Agr. It. 49: 596. 1916
≡ *Chalara thielavioides* (Peyr.) Nag Raj et Kend., A Monograph of *Chalara* and Allied Genera. p. 136. 1975.
= *Chalaropsis thielavioides* Peyr. var. *ramosissima* Sugiyama, J. Fac. Sci. Univ. Tokyo 10: 33. 1968.

Thielaviopsis ungeri (Sacc.) Paulin, Harrington, et McNew, *comb. nov.*
≡ *Chalara ungeri* Sacc., Sylloge Fung., 4: 336. 1886.
Teleomorph: *Ceratocystis coerulescens* (Münch) Bakshi, Trans. Br. Mycol. Soc. 33: 114. 1950.
≡ *Endoconidiophora coerulescens* Münch, Naturw. Z. Land. Forstw. 5: 54. 1907.
≡ *Ophiostoma coerulescens* (Münch) Nannf., Svensa Skogsfor. Tidskr. 32: 408. 1934.

DISCUSSION

By amending Went's concept of *Thielaviopsis*, we are able to accommodate the anamorphs of all described *Ceratocystis* species into *Thielaviopsis* and distinguish *Chalara*-like species with Leotialian affinities from those related to *Ceratocystis*. Aleurioconidia are produced by species in four of the five major clades of *Ceratocystis*, including the apparently basal *Ce. fimbriata* clade, and thus aleurioconidia production appears to be an ancestral state in *Ceratocystis*. Aleurioconidia appear to be associated with species that are soilborne, including the species *T. thielavioides*, *T. populi*, *T. ovoidea*, and *T. basicola*. All eleven species within the *Ce. coerulescens* complex (Harrington and Wingfield 1998, Witthuhn et al 2000), *Ce. moniliformis*, and *Ce. fagacearum* have apparently lost the ability to produce aleurioconidia, and none of these species are known to be soilborne.

Four asexual species are the only known plant pathogenic *Chalara* species that produce aleurioconidia, either singly (*T. thielavioides*, *T. populi*, *T. ovoidea*) or in chains (*T. basicola*), and they group as an asexual, monophyletic lineage within *Ceratocystis* based on rDNA sequences. Analysis of DNA sequences from a portion of the *MAT-2* gene also groups these *Thielaviopsis* species as a monophyletic lineage (unpubl). These four *Thielaviopsis* species appear to be reproductively isolated, soilborne, root pathogens that are no longer dependent upon insects for dispersal, and it is possible that the four species are derived from a common asexual ancestor. The ITS sequences of *T. thielavioides*, *T. ovoidea* and *T. populi* isolates did not clearly delineate these species, but minor morphological differences suggest they are distinct species. *Thielaviopsis ovoidea* and *T. populi* may be recently derived species in the *T. thielavioides* complex.

Two other asexual species, *T. australis* and *T. neocaledoniae*, have no known teleomorph. However, only one isolate of *T. neocaledoniae* is available, and all available isolates of *T. australis* are of a single mating type (Harrington et al 1998). Both *T. australis* and *T. neocaledoniae* will form sterile perithecia and aborted ascospores when paired with strains of the opposite mating type of *Ce. eucalypti* or *Ce. virescens*, respectively (Harrington et al 1998). Thus, teleomorphs of *T. australis* and *T. neocaledoniae* may yet be found.

Three asexual *Ambrosiella* species (*A. ferruginea*, *A. hartigii*, and *A. xylebori*) form conidia at the tips of hyphal branches that are fed upon by ambrosia beetle symbionts (Batra 1967). Using partial sequences of the small subunit rDNA, Cassar and Blackwell (1996) placed these three *Ambrosiella* species within *Ceratocystis*, while six other *Ambrosiella* species were placed in the Ophiostomatales, indicating that the genus *Ambrosiella* is polyphyletic. Using partial sequences of the large subunit rDNA, we show that the

three *Ambrosiella* species, *A. ferruginea*, *A. hartigii*, and *A. xylebori*, are placed within the *Ce. moniliformis* group, with *A. ferruginea* near *Ce. fagacearum*, and *A. xylebori* (the type species for *Ambrosiella*) and *A. hartigii* closely related to *Ce. adiposa*. A close examination of the conidia of these three *Ambrosiella* species may indicate a similarity to the endoconidia of their *Ceratocystis* relatives.

Chalaropsis punctulata, found on the roots of *Lawsonia inermis* by Hennebert (1967), was determined by Nag Raj and Kendrick (1975) to be morphologically similar to the anamorph of *Ce. radicicola*. Comparison of ITS sequences showed no difference between *Chalaropsis punctulata* and *Ce. radicicola*, and isolate C1631 from the holotype of *Ch. punctulata* successfully mated with an isolate of *Ce. radicicola* in our tests (unpubl). *Ceratocystis paradoxa*, a close relative to *Ce. radicicola*, produces aleurioconidia in chains, so the distinction between aleurioconidia produced in chains (*T. paradoxa*) and aleurioconidia produced singly (*Chalaropsis punctulata*) appears trivial at the genus level, and we have synonymized *Chalaropsis* with *Thielaviopsis*.

There was some minor variation in LSU and ITS sequences among isolates of *T. basicola* and *T. thielavioides*. Punja and Sun (1999) saw substantial variation in RAPD markers among isolates of *T. basicola*, and they speculated that genetically distinct strains of *T. basicola* may be adapted to specific hosts. Hammond (1935) reported on a strain of *T. thielavioides* that appeared to have adapted to peach (*Prunus persica*). The morphological and genetic variation found in *T. thielavioides* suggest that *T. thielavioides* may also be comprised of host specialized forms, perhaps in the process of speciation.

Other described *Thielaviopsis* species warrant further study. *Thielaviopsis wallemiaeformis* Dom. et Ihn. (Dominik and Ihnatowicz 1975) was considered an invalid name by Kiffer and Delon (1983). *Thielaviopsis abuensis* Chouhan et Panwar (Chouhan and Panwar 1980) appears morphologically similar to *T. basicola*. Sugiyama (1968) described *Chalaropsis thielavioides* var. *ramosissima* from buried plant material and distinguished it from *T. thielavioides* var. *thielavioides* based on more elongated and larger aleurioconidia. Our examination of the isolate from the holotype of var. *ramosissima* (C1630) did not reveal a distinction in morphology between var. *ramosissima* and var. *thielavioides,* and we have synonymized these varieties.

Our attempts to produce perithecia through pairings of different isolates of *T. basicola* and *T. thielavioides* have consistently failed, though other *Ceratocystis* species readily form perithecia and ascospores on agar media. From the data presented here, it appears that loss of the sexual state has occurred at least once in the evolution of *Ceratocystis*, and speciation appears to have occurred in this asexual lineage. Although delineation of species can be difficult for asexual fungi (Harrington and Rizzo 1999), minor morphological characters separate *T. ovoidea* and *T. populi* from *T. thielavioides*, and further speciation may be taking place in *T. thielavioides* and *T. basicola*.

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