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ORIGINAL ARTICLE

Nimbya and *Embellisia* revisited, with *nov. comb* for *Alternaria celosiae* and *A. perpunctulata*

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Abstract Previous phylogenetic analyses revealed that species within the genera *Nimbya* and *Embellisia* reside within a large monophyletic clade that also includes the genera *Alternaria*, *Ulocladium*, *Undifilum*, *Sinomyces*, and *Crivellia* with *Stemphylium* as the sister taxon. This study expands upon previous work by including many contemporary species of each genus and utilizes molecular and morphological characters to further examine relationships. Maximum parsimony and Bayesian analysis reveals that *Nimbya* is not a monophyletic genus but is split into two phylogenetically distant clades, which have different and distinct conidial morphologies. One of these clades resides completely within *Alternaria*. Phylogenetic analyses also reveals that *Embellisia* does not form a monophyletic genus but is split into four monophyletic lineages. Moreover, several species of *Embellisia* cluster individually with clades that are predominantly *Alternaria*, *Ulocladium*, or *Stemphylium*, yet these *Embellisia* spp. possess morphological characters that are diagnostically *Embellisia*. Thus, these data reveal that both *Nimbya* and *Embellisia* are polyphyletic as currently defined and taxonomic restructuring is necessary in order to resolve conflict between historical morphological and contemporary molecular-based phylogenies.

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Introduction

Nimbya E. G. Simmons (Simmons 1989) and *Embellisia* E. G. Simmons (Simmons 1971) are closely related to the genera *Alternaria*, *Ulocladium*, *Undifilum*, *Sinomyces*, and *Crivellia*, and together these taxa comprise a large monophyletic assemblage of phaeodictyosporic Hyphomycetes. This assemblage includes plant pathogens that cause a variety of important crop plant diseases as well as numerous saprobic species that cause post-harvest rots of agricultural products and decay of organic matter in natural ecosystems. Many species of *Nimbya* and *Embellisia* were previously assigned to the genera *Sporidesmium*, *Helminthosporium*, or *Alternaria* because they share the principal morphological characteristics of being ovate to obclavate to elongate phaeodictyospores or phaeophragmospores. However, critical morphological studies also revealed diagnostic features that permitted differentiation.

The genus *Nimbya* was established in 1989 to accommodate the atypical *Sporidesmium scirpicola* Fuckel, a pathogen of river bulrush (Simmons 1989). Historically, the taxonomy of *Sporidesmium* has been problematic as the type of the genus, *Sporidesmium scirpicola*, has been previously described as *Clasterosporium scirpicola* (Fuckel) Sacc., *Cercospora scirpicola* (Fuckel) v. Zinderen Bakker, or *Alternaria scirpicola* (Fuckel) Sivanesan (Fuckel 1863; Saccardo 1886; Zinderen Bakker Van 1940; Sivanesan 1984). *Nimbya scirpicola* displays apically tapering multicelled conidia and short conidial chains, distoseptate conidia which become partially or completely euseptate at maturity, and the excessive rarity of longisepta, which collectively

differentiate the conidia from those of *Sporidesmium*, *Alternaria*, and *Drechslera* (Simmons 1989). Since Simmons erected *Nimbya* as a new genus, 17 additional species have been described or transferred from other genera (Chen et al. 1997; Johnson et al. 2002; Simmons 1989, 1995, 1997, 2000). Importantly, the teleomorph of *N. scirpicola* has long been recognized as *Macrospora scirpicola* Fuckel, which is morphologically similar to the teleomorph of several *Alternaria* species, *Lewia* Barr & Simmons, and the teleomorph of *Stemphylium* species, *Pleospora* Rabenh. ex Ces. & De Not. (Crivelli 1983).

The genus *Embellisia* was established to accommodate the atypical *Helminthosporium* species, *H. allii*, which was originally isolated from a garlic bulb. *Helminthosporium allii* displays a low percentage of dictyoconidia among a dominant population of phragmoconidia. In addition, the conidia possess distinctly thick and dark transsepta and are variously swollen, smoothly curved or sigmoid as an exception to the usually straight-elliptical or oblong-elliptical population. Sites of conidium production at conidiophore geniculations are umbilicate and intra-hyphal proliferating chlamydospores and hyphal coils occur in culture (Simmons 1971, 1983). On the basis of a combination of these characters, the genus *Embellisia* was circumscribed with *Embellisia allii* designated as the type. Since then, 25 additional species have been newly described or transferred from other genera (David et al. 2000; de Hoog and Muller 1973; de Hoog et al. 1985; Hoes et al. 1965; Muntanola-Cvetkovic and Ristanovic 1976; Simmons 1971, 1983, 1990). In addition, the teleomorphs of *E. proteae* and *E. eureka* have been circumscribed by the genus *Allewia* Simmons (1990), which is similar, yet distinct from the *Alternaria* teleomorph *Lewia* although some studies have synonymized the two genera (Eriksson and Hawksworth 1991).

Molecular delimitation among genera closely related to *Alternaria* and *Stemphylium*, including *Nimbya* and *Embellisia*, has been accomplished by the use of rDNA sequences, including the internal transcribed spacer (ITS) region, the mitochondrial small subunit (mtSSU), and the protein coding genes *gpd* (glyceraldehyde-3-phosphate dehydrogenase) and *Alt a1*, the primary *Alternaria* allergen (Pryor and Bigelow 2003; Zhang et al. 2009; Wang et al. 2010; Tóth et al. 2011; Wang et al. 2011). Analyses of these sequences revealed that phylogenetic groups generally corresponded with previously described groups based on morphological characterization and that *Nimbya* and *Embellisia* were more closely related to *Alternaria* than to *Stemphylium*. Analysis of a combined ITS, mtSSU, and *gpd* dataset provided perhaps the most comprehensive review of *Nimbya* and *Embellisia* systematics to date (Pryor and Bigelow 2003). That study revealed a strongly supported *Embellisia* clade that included *E. hyacinthi*, *E. novae-zelandiae*, *E. proteae*, and *E. leptinellae*.However, *E. allii*, the type of the genus, clustered with *Nimbya scirpicola* and *N. caricis* indicating the current circumscription was polyphyletic. Moreover, *Embellisia indefessa* grouped with *A. cheiranthi* and *Ulocladium* species in a strongly supported *Ulocladium* group, further supporting the polyphyly. A subsequent study including the *Alt a1* locus and additional taxa revealed that *E. allii* and *E. tellustris* were strongly supported as a monophyletic group and that *Nimbya scirpicola* and *N. caricis* were strongly supported as a monophyletic group. That study was the first evidence that the morphologically circumscribed *Nimbya* and *Embellisia* had molecular support for their designation. However, a more comprehensive study including most contemporary species and most contemporary phylogenetic loci has yet to be accomplished and is required to resolve these phylogenetic relationships as well as those within the larger *Alternaria-Embellisia-Nimbya-Undifilum-Ulocladium-Sinomyces-Crivellia-Stemphylium* clade.

The objective of this study was to build upon previous studies and re-evaluate the phylogenetic relationship of *Nimbya* and *Embellisia* based upon examination of a larger number of species and a more comprehensive phylogenetic analyses. The multi-locus sequence analysis of ITS, *Alt a1*, and *gpd* was used in an effort to further clarify the relationship between these two genera as well as their relationship within the Pleosporaceae and to establish new species designations.

Materials and methods

Fungal strains

Nineteen species of *Embellisia*, seven species of *Nimbya*, 26 species of *Alternaria*, seven species of *Ulocladium*, two species of *Undifilum*, three species of *Stemphylium*, and one species of *Sinomyces alternariae*, *Brachycladium papaveris*, *Crivellia papaveracea*, *Pleospora herbarum*, and *Exserohilum pedicellatum* were used in this study (Table 1). Most isolates were acquired from international culture collections and all taxon identification was confirmed based upon morphological characters produced under standard culture conditions and reference to published descriptions (Pryor and Michailides 2002; Simmons 1992).

DNA extraction and PCR amplification

DNA extraction and purification were conducted according to previously described protocols (Pryor and Gilbertson 2000). Amplification of portions of the 18S and 28S rDNA including the ITS region was conducted according to

Table 1 Species used for phylogenetic analyses in this study, their sources, and GenBank accession numbers

Species	Source	GenBank accession		
		ITS	*gpd*	*Alt a1*
Alternaria alternata	E.G.S. 34-016	AF347031	AY278808	AY563301
A. arborescens	E.G.S. 39-128	AF347033	AY278810	AY563303
A. brassicicola	E.E.B. 2232	AF229462	AY278813	AY563311
A. carotiincultae	E.G.S. 26-010	AF229465	AY278798	AY563287
A. cetera	E.G.S. 41-072	**JN383482**	AY562398	AY563278
A. cheiranthi	E.G.S. 41-188	AF229457	AY278802	AY563290
A. cinerariae	E.G.S. 33-169	AY154700	AY562413	AY563308
A. conjuncta	E.G.S. 37-139	FJ266475	AY562401	AY563281
A. dauci	ATCC 36613	AF229466	AY278803	AY563292
A. ethzedia	E.G.S. 37-143	AY278833	AY278795	AY563284
A. infectoria	E.G.S. 27-193	AF347034	AY278793	FJ266502
A. limoniasperae	E.G.S. 45-100	FJ266476	AY562411	AY563306
A. longipes	E.G.S. 30-033	AY278835	AY278811	AY563304
A. macrospora	D.G.G. Ams1	AF229469	AY278805	AY563294
A. mimicula	E.G.S. 01-056	FJ266477	AY562415	AY563310
A. oregonensis	E.G.S. 29-194	FJ266478	FJ266491	FJ266503
A petroselini	E.G.S. 09-159	AF229454	AY278799	AY563288
A. porri	ATCC 58175	AF229470	AY278806	AY563296
A. pseudorostrata	E.G.S. 42-060	**JN383483**	AY562406	AY563295
A. radicina	ATCC 96831	AF229472	AY278797	AY563286
A. selini	E.G.S. 25-198	AF229455	AY278800	FJ266504
A. smyrnii	E.G.S. 37-093	AF229456	AY278801	AY563289
A. solani	ATCC 58177	AF229475	AY278807	AY563299
A. sonchi	E.G.S. 46-051	**JN383484**	AY562412	AY563307
A. tagetica	E.G.S. 44-044	FJ266479	AY562407	AY563297
A. tenuissima	E.G.S. 34-015	AF347032	AY278809	AY563202
Brachycladium papaveris	P.351	FJ357310	FJ357298	**JN383501**
Crivellia papaveracea	P.354.8	FJ357311	FJ357299	**JN383502**
Embellisia abundans	CBS 534.83	**JN383485**	FJ214852	**JN383503**
E. allii	E.G.S. 38-073	AY278840	AY278827	AY563322
E. annulata	CBS 302.84	**JN383486**	**JN383467**	
E. chlamydospora	E.G.S. 33-022	**JN383487**	**JN383468**	**JN383504**
E. conoidea	CBS 132.89	FJ348226	FJ348227	FJ348228
E. dennisii	CBS 476.90	**JN383488**	**JN383469**	**JN383505**
E. didymospora	CBS 766.79	**JN383489**	**JN383470**	**JN383506**
E. eureka	E.G.S. 36-103	**JN383490**	**JN383471**	**JN383507**
E. indefessa	E.G.S. 30-195	AY278841	AY278828	AY563323
E. hyacinthi	E.G.S. 49-062	AY278843	AY278830	FJ266506
E. leptinellae	E.G.S. 40-187	**JN383491**	**JN383472**	
E. lolii	E.G.S. 43-054	**JN383492**	**JN383473**	**JN383508**
E. novae-zelandiae	E.G.S. 39-099	AY278844	AY278831	AY563324
E. phragmospora	E.G.S. 27-098	**JN383493**	**JN383474**	**JN383509**
E. planifunda	CBS 537.83	FJ266480	FJ266492	FJ266507
E. proteae	E.G.S. 39-031	AY278842	AY278829	FJ266505
E. tellustris	E.G.S. 33-026	**JN383494**	**JN383475**	AY563325
E. thlaspis	E.G.S. 45-069	**JN383495**	**JN383476**	**JN383510**
E. tumida	CBS 589.83	FJ266481	FJ266493	FJ266508

Table 1 (continued)

Species	Source	GenBank accession		
		ITS	*gpd*	*Alt a1*
Exserohilum pedicellatum	B.M.P. 0384	AF229478	AY278824	
Nimbya alternantherae	E.G.S. 52-039	**JN383496**	**JN383477**	**JN383511**
N. caricis	E.G.S. 13-094	AY278839	AY278826	AY278856
N. celosiae	E.G.S. 42-013	**JN383497**	**JN383478**	**JN383512**
N. perpunctulata	E.G.S. 51-130	**JN383498**	**JN383479**	**JN383513**
N. scirpicola	E.G.S. 19-016	AY278838	AY278825	AY278855
N. scirpinfestans	E.G.S. 49-185	**JN383499**	**JN383480**	**JN383514**
N. scirpivora	E.G.S. 50-021	**JN383500**	**JN383481**	**JN383515**
Pleospora herbarum	ATCC 11681	AF229479	AY278823	AY563277
Stemphylium botryosum	ATCC 42170	AF229481	AY278820	AY563274
S. callistephi	E.E.B. 1055	AF229482	AY278822	AY563276
S. vesicarium	ATCC 18521	AF229484	AY278821	AY563275
Sinomyces alternariae	B.M.P. 0352	AF229485	AY278815	AY563316
Ulocladium atrum	ATCC 18040	AF229486	AY278818	AY563318
U. botrytis	ATCC 18043	AF229487	AY278817	AY563317
U. chartarum	ATCC 18044	AF229488	AY278819	AY563319
U. consortiale	CBS 201-67	AY278837	AY278816	FJ266509
U. obovoideum	CBS 101229	FJ266487	FJ266498	FJ266513
U. oudemansii	CBS 114-07	FJ266488	FJ266499	FJ266514
U. septosporum	CBS 109.38	FJ266489	FJ266500	FJ266515
Undifilum bornmuelleri	DAOM 231361	FJ357317	FJ357305	**JN383516**
U. oxytropis	R.C. OlB9	FJ357320	FJ357308	**JN383517**

Sequences that were determined in the course of this study appear in bold. Sequences that were determined in the course of this study appear in bold. Abbreviations for source are as follows: ATCC, American Type Culture Collection, Manassas, VA 20108; B.M.P., B. M. Pryor, Division of Plant Pathology, Department of Plant Sciences, The University of Arizona, Tucson, AZ 85721; D.G.G., D. G. Gilchrist, Department of Plant Pathology, University of California, Davis, CA 95616; E.E.B., E. E. Butler, Department of Plant Pathology, University of California, Davis, CA 95616; E.G.S., E. G. Simmons, Mycological Services, Crawfordsville, IN 47933; CBS, Centraalbureau voor Schimmelcultures, Royal Netherlands Academy of Arts and Sciences, Uppsalalaan 8, 3584 CT Utrecht, The Netherlands; DAOM, Department of Agriculture, Ottawa, Mycological Collection, Ottawa, Ontario K1A 0C6; P, P. Inderbitzin, Department of Plant Pathology, Cornell University, Ithaca, NY 14850;R.C., Rebecca Creamer, Department of Entomology, Plant Pathology, and Weed Science,New Mexico State University, Las Cruses, NM 88003

previously described protocols using primer pairs ITS5/ITS4 (White et al. 1990). Amplification of protein coding genes *Alt a1* and *gpd* was accomplished using primers pairs Alt-for/Alt-rev (Hong et al. 2005) and gpd1/gpd2 (Berbee et al. 1999), respectively. When PCR failed using Alt-for and Alt-rev, amplification was conducted using modified primers (Alt-4for; 5'-ATGCAGTTCACCACCATCGCYTC-3' and Alt-4rev; 5'-ACGAGGGTGAYGTAGGCGTCRG-3'). Each PCR mixture contained 10 μM of each primer, 200 μM dNTP, 1X *Taq* reaction buffer, 2 units of Ampli*Taq*-DNA polymerase, 2.5 mM $MgCl_2$ and 10 ng of template DNA in a final reaction volume of 25 μl. PCR conditions for *gpd* were an initial denaturation step at 94°C for five minutes followed by 35 cycles of 94°C for 40 s denaturing, 55°C for 40 s annealing, and 72°C for 1 min followed by a 5 min final extension at 72°C. For *Alt al*, PCR conditions were an initial denaturation step at 95°C for 5 min followed by 35 cycles of 95°C for 40 s denaturing, 57°C for 40 s annealing, and 72°C for 1 min followed by a 10 min final extension at 72°C.

Sequencing, alignment, and phylogenetic analyses

The nucleotide sequence of PCR products was determined with FS DyeTerminator reactions (Applied Biosystems, Foster City, CA) and ABI automated DNA sequencer. Sequences were determined for both forward and reverse DNA strands of PCR products for sequence confirmation. Some sequences of ITS, *Alt a1*, and *gpd* used in this study were determined from previous studies (Pryor and Bigelow 2003; Hong et al. 2005; Pryor et al. 2009). The sequences were proofread, edited, and aligned in MacVector version 6. Sequence alignments were adjusted manually where necessary using MacClade (Maddison and Maddison 2003).

Phylogenetic analyses were performed in PAUP* 4.0b10 (Swofford Swofford 2002). Ambiguously aligned regions were excluded from analyses. Sequence gaps were treated as missing data. Maximum parsimony (MP) analyses were estimated by heuristic searches consisting of 1000 stepwise random addition replicates and branch swapping by the tree-bisection-reconnection (TBR) algorithm. Branch stability was assessed by 1000 bootstrap replications using a heuristic search with simple sequence addition. Bayesian analyses were performed using the best-fit model (GTR+I+G for ITS and HKY+I+G for *gpd* and *Alt a1*), which was deduced as the best fit for these data by the likelihood ratio test using MODELTEST version 3.06 (Posada and Crandall 1998). Each Bayesian analysis was performed in MrBayes version 3.1.1 (Huelsenbeck and Ronquist 2001) and consisted of two independent runs with four chains each for five million generations sampling every 1,000th generation. Convergence was estimated based on the standard deviation of split frequencies <0.01 and plots of the –lnL values which stabilized after approximately 3 million generations. The first 3 million generations were removed and served as the burn-in; majority-rule consensus trees with Bayesian posterior probabilities (BPP) were produced in PAUP* 4.0b10 (Swofford 2002). Sequences of *Stemphylium* or *Exserohilum* were used as the outgroup based on results from previous studies (Hong et al. 2005; Pryor and Bigelow 2003; Pryor and Gilbertson 2000).

Tests of hypotheses

In addition to examining resulting topologies for placement that violated the null hypothesis of congruence with the monophyly of *Nimbya* and *Embellisia*, respectively, we used a constraint analysis to explicitly test this hypothesis. In this analysis, *Embellisia* and *Nimbya* constraint trees were produced in MacClade (Maddison and Maddison 2003) for a small subset of taxa in each analysis. These trees, which depict the phylogenetic relationships of a few representative *Alternaria*, *Embellisia*, and *Nimbya*, were loaded as backbone constraints for MP analyses in PAUP* 4.0 b10 (Swofford 2002) and subjected to heuristic search as mentioned above. This analysis permits all unconstrained taxa to assort across the tree with regard to the placement of exemplar (constrained) taxa. Tree quality scores, including tree length, consistency- and retention indices were compared for each dataset using results of constrained and unconstrained parsimony analyses. Results from each dataset were subjected to Kishino-Hasegawa (KH), Templeton Test (TT), and Winning-Sites (WS) tests to test for topological congruence with unconstrained trees (Hasegawa and Kishino 1989; Goldman et al. 2000; Rokas et al. 2003). Significant discordance between constrained and unconstrained trees, coupled with significantly lower tree-value scores (Table 2), provides strong evidence against the null hypothesis of congruence of these genes with organismal relationships.

Conidial morphological characterization of Embellisia and Nimbya species

Selected species of *Embellisia* and *Nimbya* were cultured on weak potato dextrose agar (WPDA, Pryor and Michailides 2002), potato carrot agar, and V8 agar (PCA and V8A, Simmons 1992) for seven days under light/dark photoperiods (10 h/14 h). Slide mounts in lactophenol were prepared for each species. Pictures were taken at 40x, 20x, and 10x using an Olympus DP71 digital camera (Olympus America, Inc., Center Valley, PA) attached to an Olympus BX51 compound microscope (Olympus America). Examination of sporulation patterns and conidial morphology was used to confirm the identity of each taxon and to compare morphological characters.

Results

Phylogenetic analyses: ITS

PCR amplification of the ITS region for all species generated 590–630 bp fragments. PCR products from the infectoria species-group, *E. abundans*, *A. cetera*, *E. thlaspis*, *E. conoidea*, *E. allii*, *E. tellustris*, *E. chlamydospora*, *E. phragmospora*, *E. didymospora*, *E. dennisii*, *N. caricis*, *N. scirpivora*, *N. scirpinfestans*, and *N. scirpicola* were longer by approximately 30 bp (data not shown). Alignment of the ITS region sequences resulted in a 624 character dataset (445 characters were constant, 46 characters were parsimony-uninformative, and 133 characters were parsimony informative). All sequences generated in this study have been accessioned in GenBank (JN383467-JN383517), and all alignments have been submitted to TreeBASE (S11665).

Most *Alternaria* species-groups and *Ulocladium* groups described in previous studies are supported by this analysis (Fig. 1). However, *Nimbya* and *Embellisia* are separated into multiple clades with some species placed individually into clades dominated by other genera. The alternata, sonchi, and porri species-groups are well-defined with strong bootstrap and Bayesian posterior probability (BPP) support (≥95%/1.0), respectively. The radicina species-group is supported by moderate to strong support (69%/0.96). *Ulocladium* is also supported by moderate to high support (82%/1.0) as a monophyletic group which includes *U. atrum*, *U. consortiale*, *U. botrytis*, *U. obovoideum*, *U. chartarum*, *U. septosporum*, *A. cheiranthi*, and *E. inde-*

Table 2 Tests of topology for constrained monophyly of *Nimbya* and *Embellisia* using four molecular datasets

Dataset	Steps		Consistency Index		Retention Index		*P values*		
	Constrained	Unconstrained	Constrained	Unconstrained	Constrained	Unconstrained	KH[a]	TT[b]	WS[c]
ITS	611	498	0.44	0.53	0.72	0.81	<0.001*	<0.001*	<0.001*
gpd	947	906	0.44	0.456	0.78	0.76	<0.001*	<0.001*	<0.001*
Alt a1	1553	1333	0.36	0.41	0.65	0.730	<0.001*	<0.001*	<0.001*
Combined	3447	2866	0.36	0.429	0.65	0.74	<0.001*	<0.001*	<0.001*

[a] Kishino-Hasegawa
[b] Templeton Test
[c] Winning-Sites

fessa. *Ulocladium oudemansii* and *Sinomyces alternariae*, recently re-described as *nov. comb*. in the newly erected genus *Sinomyces* (Wang et al. 2011), cluster in a poorly supported monophyletic group (72%/0.70) and is circumscribed as *Sinomyces*. The brassicicola species-group which includes *A. brassicicola*, *A. mimicula*, and *E. conoidea* forms a strongly supported monophyletic group (96%/.98) basal to clades containing all taxa of *Alternaria*, except for the infectoria species-group. The infectoria species-group is well-defined with strong support (98%/1.0), whereas *E. abundans* and *A. cetera* form a sister monophyletic group with moderate to high support (70%/0.99). *Crivellia papaveracea* and *Brachycladium papaveris* cluster with weak bootstrap support (65%) and high BPP support (0.91) as a sister clade to *Sinomyces*. The two species of *Undifilum* form a monophyletic group with strong support (100%/1.0) and is basal to clades containing *Alternaria*, *Ulocladium*, *Sinomyces*, *Nimbya*, and *Crivellia*. *Embellisia dennisii* and *E. thalaspis* form distinct lineages and do not cluster with other *Embellisia* species. Surprisingly, *Embellisia annulata* is sister to the *Stemphylium* and *Pleospora* clade and its position is strongly supported (98%/0.98).

The remaining *Embellisia* species and all *Nimbya* species fall into five defined clades. *Embellisia tellustris*, *E. chlamydospora*, and *E. allii* cluster into a strongly supported monophyletic group (100%/1.0) and are circumscribed as *Embellisia* group I. *Embellisia didymospora* and *E. phragmospora* form a weakly supported monophyletic clade (55%) and are circumscribed as *Embellisia* group II. Eight species of *Embellisia* including *E. planifunda*, *E. tumida*, *E. lolii*, *E. hyacinthi*, *E. proteae*, *E. novaezelandiae*, *E. eureka*, and *E. leptinellae* form a weakly supported (50%/0.81) monophyletic group and are circumscribed as *Embellisia* group III. *Nimbya scirpivora*, *N. scirpinfestans*, and *N. scirpicola* form a well-supported monophyletic group (97%/1.0) and are circumscribed as *Nimbya* group I. *Nimbya caricis* resolved as a distinct lineage. Unexpectedly, *Nimbya alternantherae*, *N. perpunctulata*, and *N. celosiae* form a strongly supported monophyletic group (100%/1.0) sister to the sonchi and alternata species-groups and are circumscribed as *Nimbya* group II.

Phylogenetic analyses: gpd

PCR amplification of the *gpd* gene for most species generated 543–628 bp fragments. PCR products from *A. brassicicola*, *A. mimicula*, and *E. conoidea* were smaller by approximately 30 bp, the infectoria species-group was 50 bp smaller, and *E. abundans* and *A. cetera* were 80 bp smaller (data not shown). Alignment of the *gpd* gene sequences resulted in a 606 character dataset (350 characters were constant, 39 characters were parsimony-uninformative, and 217 characters were parsimony infor-

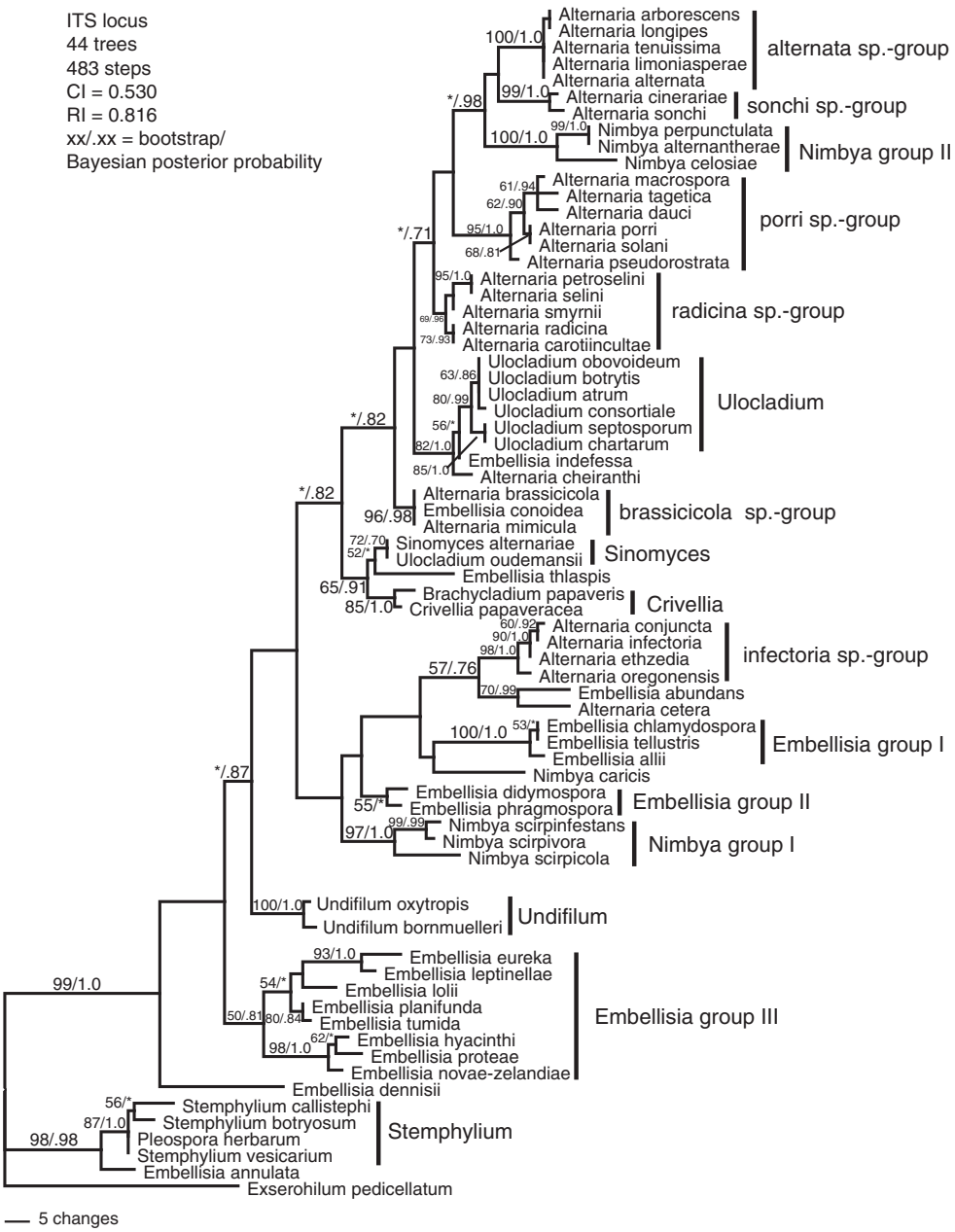


Fig. 1 One of 44 most parsimonious trees generated from maximum parsimony analysis of ITS sequences. Number in front of "/" represents parsimony bootstrap values from 1,000 replicates and number after "/" represents Bayesian posterior probabilities. Values represented by an "*" were less than 50% for bootstrap or less than 0.70 for Bayesian posterior probability respectively. The scale bar indicates the number of nucleotide substitutions

mative). The three distinct *Embellisia* clades suggested in the ITS phylogeny are very similar in the *gpd* phylogeny (Fig. 2). In addition, most *Alternaria* species-groups described in previous studies are also supported by *gpd* analysis (Pryor and Bigelow 2003; Hong et al. 2005; Runa et al. 2009). The alternata, porri, sonchi, brassicicola, and infectoria species-groups and *Nimbya* group II form strongly supported clades (≥99%/1.0), whereas the radicina species-group is supported by moderate to strong support (70%/0.98). *Ulocladium* is maintained, but weakly supported (50%/0.70). *Sinomyces* is maintained with strong support (100%/1.0), but it is positioned basal to clades containing all taxa of *Alternaria*, *Nimbya*, *Ulocladium*, and *Embellisia* groups I and II. *Embellisia dennisii* is placed as an independent lineage within the large *Alternaria-Ulocladium-Embellisia-Nimbya* group in the *gpd* phylogeny directly basal to the brassicicola species-group. The infectoria species-group is well-defined and strongly supported (100%/1.0) with *E. abundans* and *A. cetera* as a strongly supported sister clade (100%/1.0). The *Crivellia* clade is positioned more basal in the *gpd* phylogeny as compared to the ITS phylogeny. Additionally, the *Undifilum* clade is positioned basal to *Ulocladium* and all *Alternaria* species-groups excluding the infectoria species-group. *Embellisia dennisii* and *E. thalaspis* remain as distinct lineages unclustered to other *Embellisia* species,

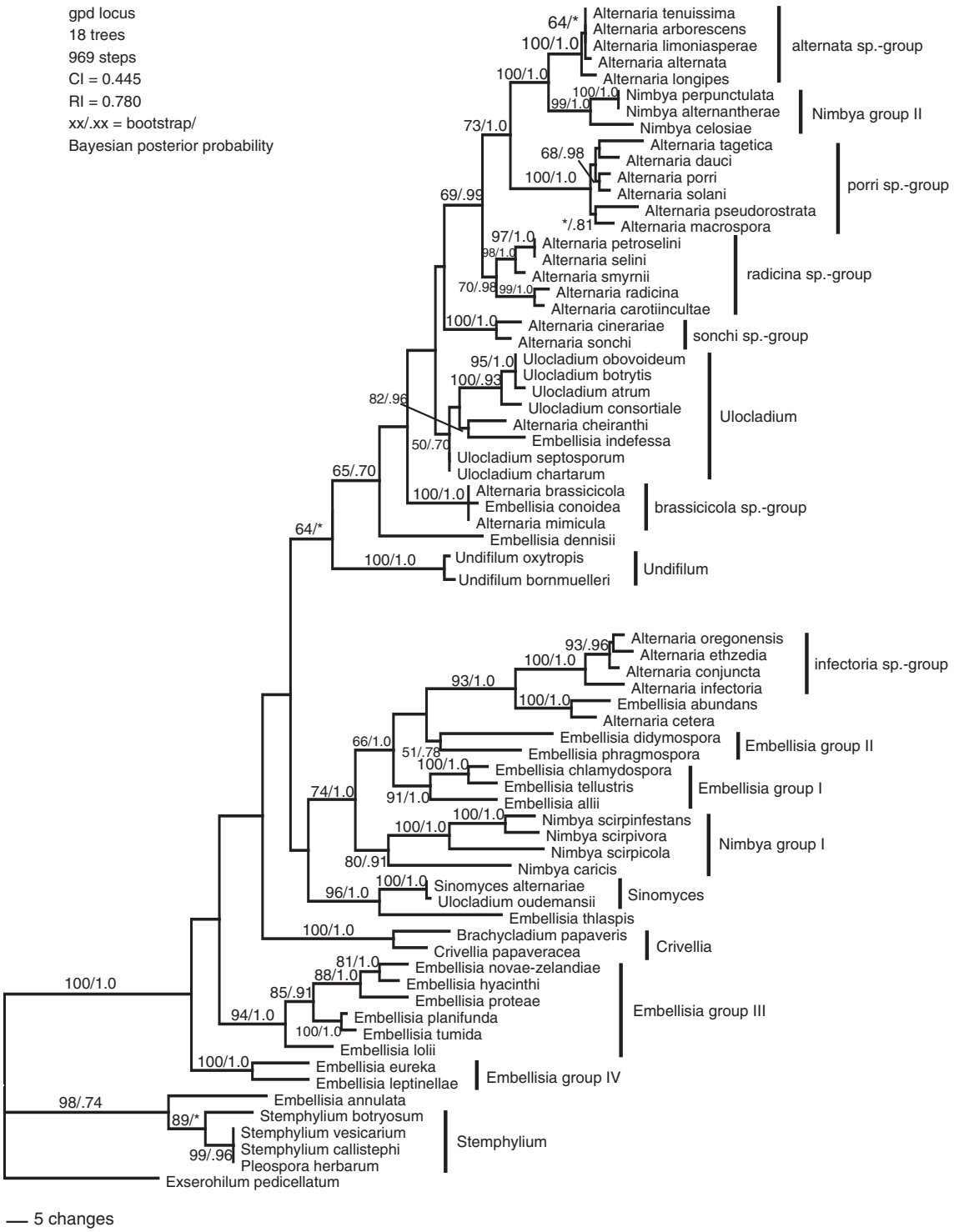


Fig. 2 One of 18 most parsimonious trees generated from maximum parsimony analysis of *gpd* sequences. Number in front of "/" represents parsimony bootstrap values from 1,000 replicates and number after "/" represents Bayesian posterior probabilities. Values represented by an "*" were less than 50% for bootstrap or less than 0.70 for Bayesain posterior probability respectively. The scale bar indicates the number of nucleotide substitutions

as in the ITS analysis, as did *E. annulata* as a sister lineage to the *Stemphylium* clade.

The remaining *Embellisia* species and all *Nimbya* species fall into six defined clades. In the *gpd* phylogeny,

Embellisia group I (*Embellisia chlamydospora*, *E. tellustris*, and *E. allii)* is strongly supported (100%/1.0), as in the ITS analysis, but *Embellisia* group II (*E. didymospora* and *E. phragmospora*) is weakly supported (51%/0.78). *Embellisia* group III from the ITS analysis is divided into two strongly supported lineages in the *gpd* analysis as *Embellisia* group III (94%/1.0) consisting of *E. tumida*, *E. planifunda*, *E. proteae*, *E. hyacinthi*, *E. novae-zelandiae*, and *E. lolii*, and *Embellisia* group IV consisting of *E. eureka* and *E. leptinellae* (100%/1.0). *Nimbya caricis* clusters with *N. scirpinfestans*, *N. scirpicola*, and *N. scirpivora* with moderate support (80%/0.91) and is circumscribed as *Nimbya* group I in the *gpd* phylogeny. *Nimbya* group II as defined in the ITS phylogeny has strong support in the *gpd* analysis (99%/1.0),but clusters with the alternata species-group as opposed to the alternata and sonchi species-groups in the ITS phylogeny.

Phylogenetic analyses: Alt a1

PCR amplification of the *Alt a1* gene for all species generated 432–499 bp fragments. PCR products from *Stemphylium vesicarium* and *E. eureka* are smaller by approximately 60 bp (data not shown). No amplification could be obtained with DNA from *Exserohilium pedicellatum*, *Embellisia annulata*, or *E. leptinellae* despite repeated attempts and repeated primer redesign. Alignment of the *Alt a1* sequences resulted in a 493 character dataset (127 characters were constant, 57 characters were parsimony-uninformative, and 309 parsimony informative characters).

Three distinct *Embellisia* clades as suggested in the ITS and *gpd* gene phylogenies are maintained in the *Alt a1* gene phylogeny with slight changes in taxon placement and most species-groups described in previous studies (Pryor and Bigelow 2003; Hong et al. 2005) are maintained (Fig. 3). The alternata, porri, radicina, and sonchi species-groups are strongly supported (≥97%/1.0). In the ITS and *gpd* phylogenies *Ulocladium* forms one distinct group; however, the *Alt a1* analysis further resolves *Ulocladium* into two distinct clades designated as *Ulocladium* group I and *Ulocladium* group II with high support (99%/1.0 and 94%/1.0, respectively). *Sinomyces* is positioned as sister to the clade that contains *Nimbya* group I, *Embellisia* group I, and the infectoria species-group. *Embellisia eureka*, *E. didymospora*, and *E. dennisii* all remain as distinct lineages without well-supported clustering with other *Embellisia* species and as in the ITS and *gpd* analyses, *E. annulata* is sister to the *Stemphylium* clade.

The remaining *Embellisia* species and all *Nimbya* species fall into four defined clades. *Embellisia* group I is strongly supported (100%/1.0) and consists of a monophyletic group that includes *E. chlamydospora*, *E. allii*, and *E. tellustris*. *Embellisia* group II consists of two species, *E. phragmospora* and *E. thlaspis* that is strongly supported (100%/1.0). *Embellisia* group III has strong support (86%/1.0) and contains a monophyletic group that consists of *E. novae-zelandiae*, *E. protea*, *E. hyacinthi*, *E. planifunda*, *E. tumida*, and *E. lolii*. As in the *gpd* analyses, *Nimbya* group I and *Nimbya* group II are both strongly supported (95%/1.0 and 100%/1.0, respectively) and circumscribed as the same taxa.

Phylogenetic analyses: combined

The combined datasets of *gpd*, ITS, and *Alt* a*1* produced an alignment with 1723 total characters (921 characters were constant, 143 characters were parsimony-uninformative, and 659 characters were parsimony informative). Figure 4 shows that the alternata, porri, sonchi, radicina, and brassicicola species-groups are strongly supported (100%/1.0). The infectoria species-group is strongly supported (100%/1.0) with *E. abundans* and *A. cetera* as a well-supported sister group (100%/1.0). *Ulocladium* is divided into two distinct clades as in the *Alt a1* analysis, I and II, and both are strongly supported (100%/1.0 and 99%/1.0, respectively). *Sinomyces* has strong support (100%/1.0) and is sister to the *Crivellia* clade (100%/1.0), which are both sister the *Undifilum* clade (100%/1.0). The *Embellisia* species, *E. didymospora*, *E. dennisii*, and *E. annulata* form independent lineages, with *E. annulata* sister to the *Stemphylium* clade (100%/1.0).

The remaining *Embellisia* species and all *Nimbya* species fall into five well-defined clades. *Embellisia* group I forms a monophyletic clade (100%/1.0) and contains *E. chlamydospora*, *E. tellustris*, and *E. allii*. *Embellisia* group II contains *E. phragmospora* and *E. thlaspis* and is well-supported (94%/1.0). *Embellisia* group III consists of a large monophyletic group (100%/1.0) that includes *E. novae-zelandiae*, *E. hyacinthi*, *E. proteae*, *E. planifunda*, *E. tumida*, and *E. lolii*. *Embellisia eureka* and *E. leptinellae* comprise the early diverging lineage *Embellisa* group IV (100%/1.0). As in most other analyses, *Nimbya* groups I and II are also maintained with strong support (99%/1.0 and 100%/1.0, respectively).

Tests of hypotheses

Hypotheses of topological congruence were tested with the parsimony criterion. The hypothesis stated that there is no statistical difference between the topology of the genealogy represented in the constrained and unconstrained parsimony analyses as described in the Materials and Methods. Maximum parsimony heuristic searches with ITS, *gpd*, *Alt a1*, and combined datasets under no constraints produced phylograms with signifi-

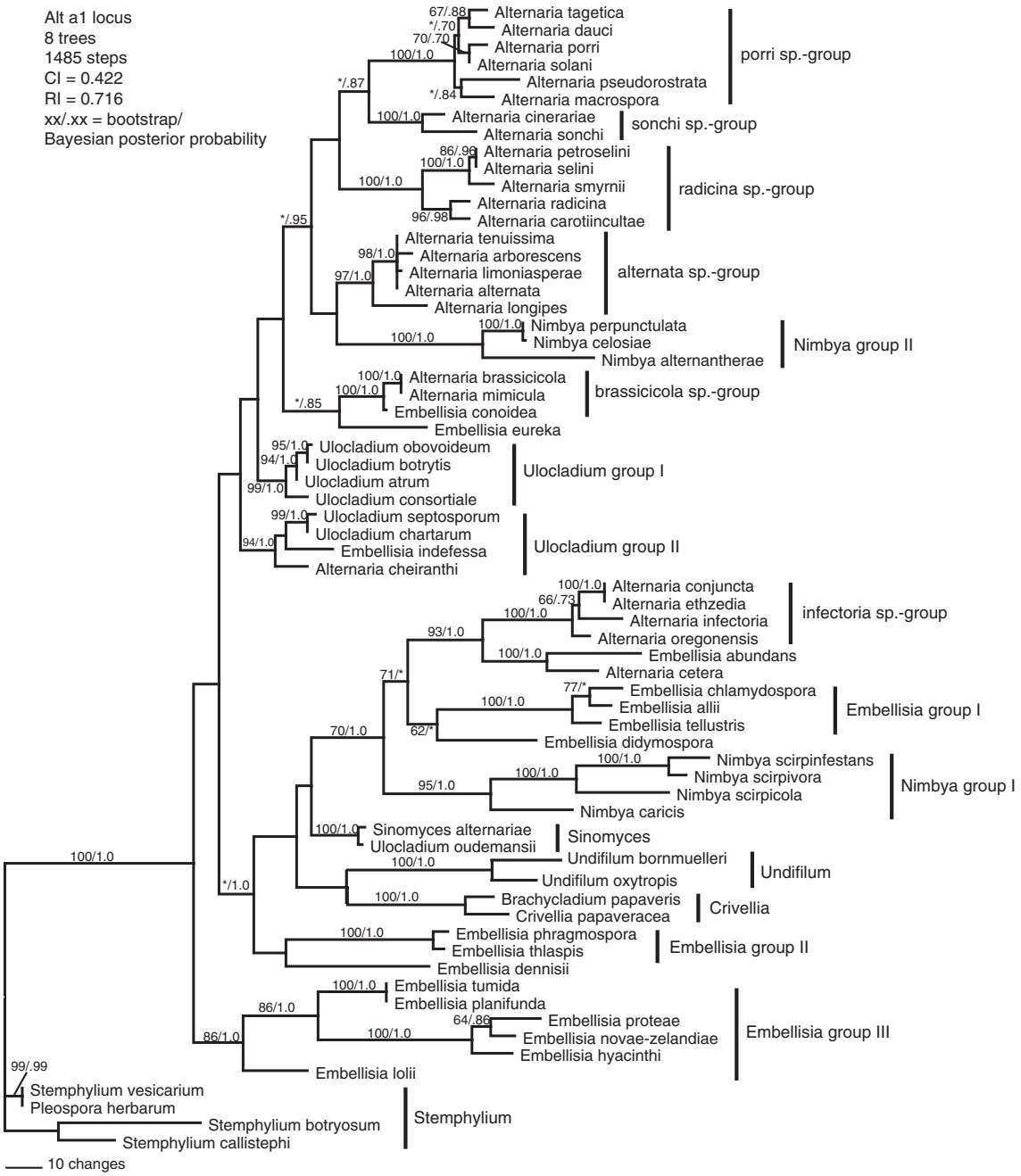


Fig. 3 One of eight most parsimonious trees generated from maximum parsimony analysis of *Alt a1* sequences. Number in front of "/" represents parsimony bootstrap values from 1,000 replicates and number after "/" represents Bayesian posterior probabilities. Values represented by an "*" were less than 50% for bootstrap or less than 0.70 for Bayesian posterior probability respectively. The scale bar indicates the number of nucleotide substitutions

cantly shorter tree lengths and higher consistency and retention indices as compared to constrained MP analyses of the same datasets, respectively (Table 2). Additionally, the null hypothesis of no difference between the constrained and unconstrained tree topologies of each respective dataset was rejected by all three tests of topology for each analysis ($P<0.001$; Table 2).

Conidial morphological characterization of Embellisia and Nimbya species

For most examined isolates, conidial morphological characters produced in cultures on PCA, WPDA, or V8A are consistent with previously published descriptions of these species (Simmons 1967, 1971, 1983, 1986, 1989, 1990,

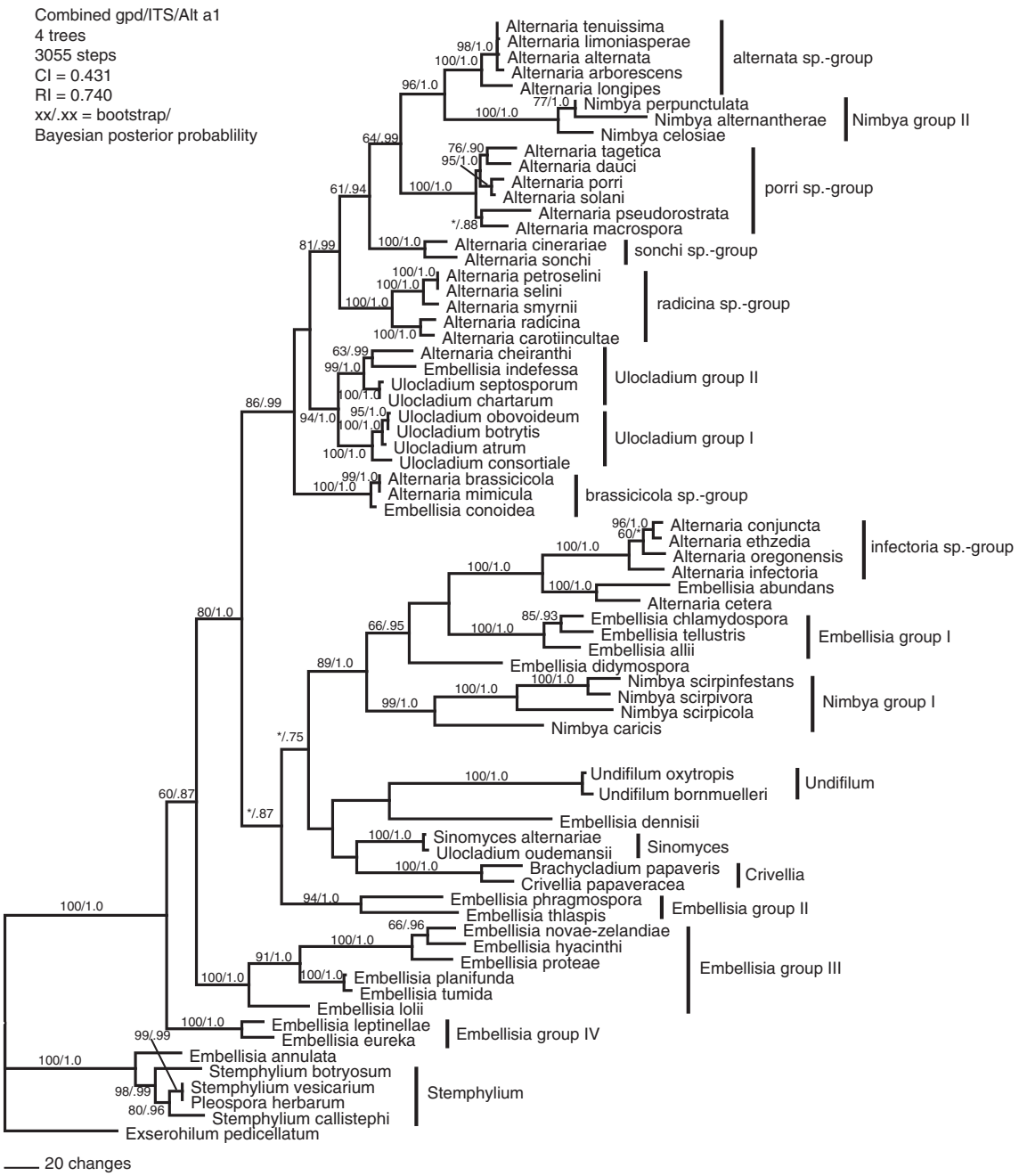
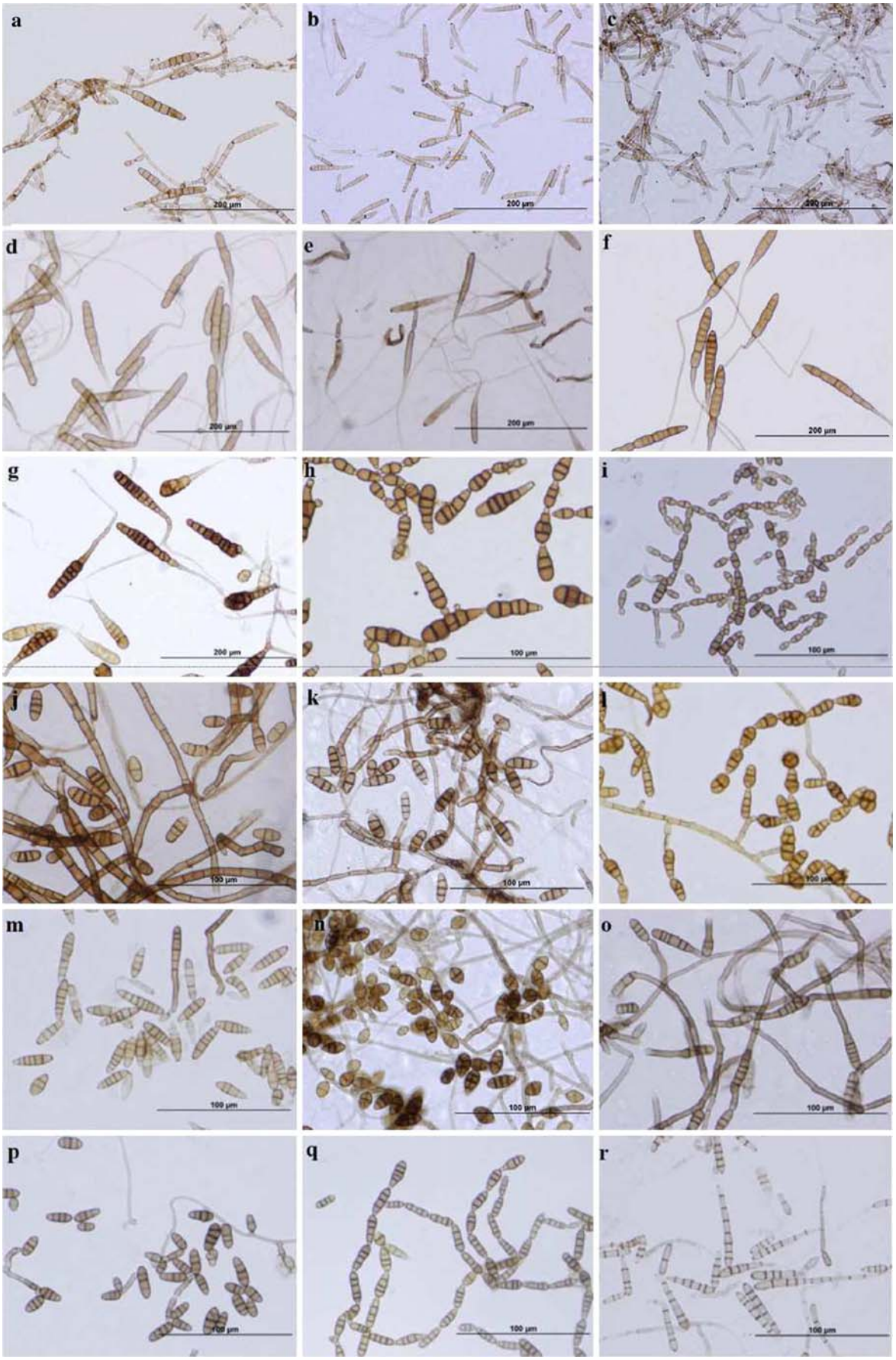


Fig. 4 One of four most-parsimonious trees generated from maximum parsimony analysis of combined sequences. Number in front of "/" represents parsimony bootstrap values from 1,000 replicates and number after "/" represents Bayesian posterior probabilities. Values represented by an "*" were less than 50% for bootstrap or less than 0.70 for Bayesian posterior probability respectively. The scale bar indicates the number of nucleotide substitutions

1995, 1997, 2000, 2004, 2007; David et al. 2000; de Hoog and Muller 1973; de Hoog et al. 1985; Muntanola-Cvetkovic and Ristanovic 1976; Chen et al. 1997; Johnson et al. 2002; Fig. 5). The only exception to this is the isolate of *E. conoidea*, CBS 132.89, which produces conidia in catenulate arrangement in contrast to the original description based upon isolate EGS 29–179 (Simmons 1983). However, interpretation of morphological characters for many species was novel in some respects when viewed comparatively with taxa associated based on molecular characters. For example, based upon growth on PCA, the conidia of *E. indefessa* more closely resembles certain *Alternaria* and *Ulocladium* species when the comparative taxa are observed side by side (Fig. 5), particularly in


a
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◀ **Fig 5** Conidia of (**a**) *N. scirpicola*, (**b**) *N. scirpivora*, (**c**) *N. scirpinfestans*, (**d**) *N. alternantherae*, (**e**) *N. perpunctulata*, (**f**) *N. celosiae*, (**g**) *A. dauci*, (**h**) *A. brassicicola*, (**i**) *E. conoidea*, (**j**) *E. allii*, (**k**) *E. tellustris*, (**l**) *E. indefessa*, (**m**) *E. leptinellae*, (**n**) *E. protea*, (**o**) *E. dennisii*, (**p**) *E. abundans*, (**q**) *E. thlaspis*, (**r**) *E. annulata*, on V8A (**a-g**), PCA (**h-q**) or WPDA (**r**) after one week

regard to the catenulate arrangement of conidia. Furthermore, when current imaging was coupled with original published descriptions, *E. annulata* clearly has features similar to those of *Stemphylium*, particularly in regard to the swollen conidiophore terminus. Phylogenetic placement of both species into respective clades is concordant in all analyses and is concordant with morphological characterization. In contrast, phylogenetic placement of *E. abundans* is strongly supported in all analyses, yet, *E. abundans* does not have obvious characteristics of its sister taxon *A. cetera* or the closely related taxa in the infectoria species-group. However, the phylogenetic placement of the specific clades that includes each of the three taxa in question is not strongly supported and concordant across all loci. Similarly, although the morphological characters of *E. dennisii*, *E. thalaspis*, and *E. didymospora* are within the description of *Embellisia*, the phylogenetic placement of these taxa is not strongly supported across the three loci examined.

Embellisia groups I-IV all have, in general, typical *Embellisia* characteristics, but there are some noted differences between some groups. *Embellisia* group I taxa have exceptionally rare longisepta while *Embellisia* groups II-IV taxa have longisepta that are quite common such as evident in *Embellisia protea* (Fig. 5, Table 3). *Embellisia* group I has absent to rare concatenate conidia whereas *Embellisia* group II produces concatenate conidia abundantly. No notable differences are evident between *Embellisia* group III and group IV. *Nimbya* group I (*N. scirpicola*, *N. scirpinfestans*, *N. scirpivora*, and *N. caricis*) produce conidia with short rostra that are distoseptate followed by euseptate at maturity with no to rare longisepta. In contrast, *Nimbya* group II (*N. perpunctuata*, *N. alternantherae*, and *N. celosiae*) produce conidia with long tapering filamentous apical beaks that are distoseptate with a progression toward euseptate at maturity with few longisepta (Fig. 5, Table 3). Most importantly, these conidia are more similar to those in the porri species-group than to those in the *Nimbya* group I when compared side by side. Moreover, the molecular data strongly supports placement of these taxa well within the asexual *Alternaria* clade in all three loci studied. Thus, the distoseptate character that has been previously used as one of the defining characters of *Nimbya* is not of phylogenetic utility and taxonomic revision is required to resolve the systematic conflict.

Taxonomic revisions

Based upon results of both morphological and molecular data, nomenclatural revisions are proposed for taxa within *Nimbya* group II, herein referred to as the alternantherae species-group of *Alternaria*.

Alternaria celosiae (Simmons & Holcomb) Lawrence, Park, & Pryor, *comb. nov.*
Basionym: *Nimbya celosiae* E.G. Simmons (1995); Mycotaxon 55: 144.
Alternaria perpunctulata (Simmons) Lawrence, Park, & Pryor, *comb. nov.*
Basionym: *Nimbya perpunctulata* E.G. Simmons (2004); Studies in Mycology 50: 115.

The *comb. nov.* for *N. alternatherae* proposed by Simmons (1995) is not supported and the original taxonomy of *Alternaria alternantherae* Holcomb & Antonopoulos (1976) is re-established.

Discussion

This study describes the phylogenetic relationship among species of *Nimbya*, *Embellisia*, and closely related genera, based on nucleotide sequences of ITS, *Alt a1*, and *gpd*. Although some of these relationships have been previously evaluated (Pryor and Bigelow 2003; Hong et al. 2005), the exact phylogenetic placement of *Nimbya* and *Embellisia* within the Pleosporaceae remained uncertain. Thus, this study expanded the previous work by generating a dataset that included most recognized and commonly available species of each genus and provides the most comprehensive view of the phylogenetic relationship among these and related taxa.

Phylogenetic relationships of Embellisia

The morphological delimitation of *Embellisia* from closely related genera is currently based solely on conidial morphology. The thick and dark transverse conidial septa have been, in general, taxonomically useful characteristics for differentiation between *Embellisia* and related genera (Simmons 2007). However, phylogenetic analyses in this study show that *Embellisia* is not congruent with the previously established morphological taxonomy and does not form a monophyletic group in any analyses (Figs. 1, 2, 3, 4). Constrained analysis forcing *Embellisia* into a monophyletic group results in trees that are significantly poorer (longer tree length, lower consistency- and retention indices) than the unconstrained tree based on Kishino-Hasegawa test, Templeton Test, and Winning-Sites test ($P < 0.001$).

Table 3 Morphological characteristics of conidia for Nimbya, Embellisia, and closely realted genera

Genus	Conidiophore	Conidium shape	Condium beak	Conidial septa	Secondary sporulation	# of conidiogenous sites per conidophore
Nimbya I	Macronematous or mononematous	Elongated obclavate	Nonfilamentous beak	Transsepta distoseptate (longisepta rare)	Absent	Few
Nimbya II	Macronematous or mononematous	Elongated obclavate	Filamentous tapering beak	Transsepta distoseptate (longisepta infrequent)	Absent	Few
Embellisia I	Macronematous or mononematous	Ovate to ellipsoid and variously curved	No beak	Transsepta distinctly thickened and dark (longisepta rare)	Absent to rarely	Many
Embellisia II	Macronematous or mononematous	Ovate to ellipsoid and variously curved	No beak	Transsepta distinctly thickened and dark (longisepta common)	Abundant	Many
Embellisia III	Macronematous or mononematous	Ovate to ellipsoid and variously curved	No beak	Transsepta distinctly thickened and dark (longisepta common)	Rarely to abundant	Many
Embellisia IV	Macronematous or mononematous	Ovate to ellipsoid and variously curved	No beak	Transsepta distinctly thickened and dark (longisepta common)	Rarely to abundant	Many
Alternaria	Macronematous	Ovate to obclavate to elongated obclavate	No beak to elongate to filamentous	Transsepta and longisepta	Absent to abundant	Few to many
Ulocladium	Macronematous or mononematous	Obovate to ovate	No beak	Transsepta and longisepta	Absent to abundant	Many
Sinomyces	Macronematous or mononematous	Obovate to ovate	No beak	Transsepta and longisepta	Absent	Few
Undifilum	Macronematous or mononematous	Ovate to ellipsoid and variously curved	No beak	Transsepta distinctly thickened and dark (longisepta absent)	Absent	Many
Crivellia	Macronematous with stipe and head	Cylindrical to obclavate	No beak	Transsepta (longisepta absent)	Absent	Many

Phylogenetic analyses based on ITS, *gpd*, and *Alt a1* divides most *Embellisia* into four distinct groups. The type species, *E. allii*, forms a well-supported clade in all analyses as *Embellisia* group I with *E. tellustris* and *E. chlamydospora*, and this clade supports the previous establishment of the genus *Embellisia* based on morphological characters (Simmons 1971). In Hong et al. (2005) phylogenetic study using *gpd* and *Alt a1*, *E. allii* and *E. tellustris* formed a well-supported monophyletic group with *Nimbya* as the sister taxon in all analyses, and *E. novae-zelandiae* resolved either as a singleton or clustered separately with *A. eryngii*. In this study using a more robust dataset, *E. novae-zelandiae* is encompassed within *Embellisia* group III, which includes *E. hyacinthi*, *E. proteae*, *E. planifunda*, *E. tumida*, and *E. lolii*. This group was supported in a previous study (Pryor and Bigelow 2003) and formed a well-supported monophyletic group in all analyses. Two species, *E. phagmospora* and *E. thalaspis*, compose a strongly supported monophyletic group referred to as *Embellisia* group II. And finally, two species, *E. leptinellae* and *E. eureka*, form a strongly supported monophyletic *Embellisia* group IV sister to *Embellisia* group III.

Five other *Embellisia* species, however, do not group within these four clades. It is interesting that these species, *E. indefessa*, *E. conoidea*, *E. abundans*, *E. annulata*, and *E. dennisii* have typical *Embellisia* characteristics, but some also share a few or subtle morphological similarities to the taxa to which they do cluster. To summarize, our phylogenetic analyses are in agreement with a previous study that placed *E. indefessa* as distantly related to other *Embellisia* species and closely related to *A. cheiranthi* in the *Ulocladium* clade, and supports the current paraphyly of *Ulocladium* (Pryor and Bigelow 2003; Hong et al. 2005). *Embellisia indefessa* possesses similar catenulate arrangement of conidia with *U. chartarum*, with which it groups, in bearing conidia in chains 2–10 in length (Simmons 1967). However, *E. indefessa* is discriminated from *Ulocladium* by the absence of obovoid, non-beaked conidia, which are taxonomically useful characters delimiting the genus *Ulocladium* (Simmons 1997). *Ulocladium* encompasses the catenulate taxa *E. indefessa* and *U. chatarum*, as well as the unusual *A. cheiranthi* and *U. septosporum*. The separation of *Ulocladium* group II in Figs. 3 and 4, which contains non-*Ulocladium* taxa such as *E. indefessa*, from *Ulocladium* group I, which contains only *Ulocladium* spp., maintains the monophyly among a core of *Ulocladium* species. However, whether *E. indefessa* and all *Ulocladium* group II taxa have morphological similarities that link them together and clearly separates them from *Ulocladium* group I has not yet been revealed. Moreover, the final taxonomic disposition of *E. indefessa* and the remaining species in *Ulocladium* group II will require a more robust and unambiguous placement of this clade within the larger *Ulocladium-Alternaria* clade.

Another unique *Embellisia* species is *E. conoidea* isolated from dry latex on a wounded stem of *Hevea* spp. *Embellisia conoidea* shares morphological characters with more typical *Embellisia* species, except for the production of secondary sporulation (similar to *E. indefessa*) and conoid or ovoid conidia with 1–2 transverse septa. In this study, *E. conoidea* groups within the brassicicola species-group along with *A. brassicicola* and *A. mimicula* in all analyses (≥96%/≥.98). Similar to *E. conoidea*, *A. brassicicola* and *A. mimicula* also produce a percentage of conidia with septa characteristic of *Embellisia* in general. Thus, these taxa reveal that the thickened and distinct septa characteristic of *Embellisia* may not be a genus-specific feature. Moreover, the final taxonomic disposition of *E. conoidea* and the remaining species in the brassicicola species-group will also require a more robust and unambiguous placement of this clade relative to both *Ulocladium* the remaining *Alternaria* clades.

Embellisia abundans and *A. cetera* form a well-supported monophyletic group, whose sister group is the infectoria species-group in all analyses except for the ITS dataset. In a previous phylogenetic study, *A. cetera* and the infectoria species-group formed a well-supported monophyletic group (Hong et al. 2005). However, with the inclusion of *E. abundans* into the dataset, these two taxa cluster separately revealing the impact of taxon inclusion/exclusion on groupings from phylogenetic analyses. Although they cluster together in a strongly supported clade in the combined dataset, *Embellisia abundans* differs from *A. cetera* considerably in having 3–6 transverse, long ovoid or obclavoid conidia borne on 3–4 geniculate conidiophores compared to the much reduced conidium morphology of *A. cetera*. As with the previously mentioned singleton *Embellisia* species, the exact taxonomic placement of *E. abundans* will require a more robust phylogenetic analysis and unambiguous placement of this clade relative to its sister clades. This analysis will also likely resolve the status of the infectoria species-group relative to the primary asexual *Alternaria* clade.

The phylogenetic placement of *E. annulata* based on phylogenetic analysis of ITS, *gpd*, and *Alt a1* sequences is also incompatible with previous morphological classification. The unique swollen conidiophore terminus and particular mode of proliferation of *Stemphylium* are taxonomically useful characteristics for differentiation between this genus and other closely related genera such as *Alternaria* and *Ulocladium* (Simmons 1967). However, *E. annulata* does have conidiophores with an inflated apical cell and relatively dark pigmentation at the region below the inflated apical cell (de Hoog et al. 1985), which fits with the concept of *Stemphylium* as proposed by Simmons (1967). Moreover, phylogenetic analyses also groups *E.*

annulata with *Stemphylium* species in a strongly supported clade basal to all other *Alternaria*, *Undifilum*, *Ulocladium*, *Embellisia*, *Sinomyces*, *Crivellia*, and *Nimbya* species suggesting an agreement between molecular and certain morphological characters. However, the mode of conidium proliferation in *E. annulata* is quite distinct from *Stemphylium* (multi-poric vs precurrent proliferation, respectively), suggesting that *E. annulata* is simply sister to *Stemphylium*, which would resolve more definitively with further analyses. Finally, *Embellisia dennisii* consistently resolves as a singleton whose phylogenetic placement fluctuated between being placed basal to *Ulocladium* and basal to all taxa excluding the *Stemphylium-Pleospora* clade. Evidently, the correct phylogenetic placement of this problematic taxon will likely require additional loci sampling and the inclusion of additional taxa as well.

This study revealed that regardless of the locus used for phylogenetic analysis, most *Embellisia* species cluster within 3–4 different clades and this result is in agreement with previous studies (Pryor and Bigelow 2003; Hong et al. 2005). This suggests that the remarkably thick and dark transverse conidial septa which are considered important in distinguishing *Embellisia* from *Alternaria* is homoplasious and is not a phylogenetically informative character. Although the clade *Embellisia* group I contains the type of the genus and, thus, is properly *Embellisia*, the taxonomic disposition of the remaining clades in relation to *Embellisia* cannot yet be unambiguously resolved without stronger support for their positions. Regarding the five taxa that did not cluster within *Embellisia* groups I-IV, despite the fact that these taxa are well-supported in their placement in non-*Embellisia* clades, the positions of these clades in relation to *Alternaria*, *Ulocladium*, and other *Embellisia* groups is not strongly supported and, thus, their taxonomic status cannot be definitively established in this work.

Phylogenetic relationships of Nimbya

In this study, phylogenetic placement of the genus *Nimbya* based on ITS, *gpd*, and *Alt a1* gene is not congruent with the morphological taxonomy established by Simmons and the genus *Nimbya* does not form a monophyletic group. Rather, *Nimbya* forms two independent monophyletic groups each with distinctive conidium morphologies. The conspicuously disto-pharagmoseptate conidia delimited by pseudoseptum material are useful in delimiting the genus as formerly circumscribed. However, in constrained analyses, the hypothesis that *Nimbya* species are monophyletic was rejected by Kishino-Hasegawa test, Templeton Test, and Winning-Sites test ($P<0.001$). Previous studies also failed to resolve the monophyly of *Nimbya* in sequence analysis of ITS, mtSSU, *gpd*, and combined analyses (Pryor and Bigelow 2003). *Nimbya scirpicola*, *N. caricis*, and *E. allii* formed a weakly supported *Nimbya* group in all analyses in a previous study (Pryor and Bigelow 2003). Phylogenetic study by Hong et al. (2005), however, has shown that the *Nimbya* group, comprising *N. scirpicola* and *N. caricis*, formed a monophyletic group based on sequence analysis of *Alt a1* and combined analyses and suggests that the difference between these results was due to the difference in taxon number and choice of loci. The addition of six different *Nimbya* species in this study reveals that *Nimbya* is not only divided into two distinct monophyletic groups, but clearly differs from *Embellisia* and other taxa.

The type species *N. scirpicola* forms a well-supported group with *N. caricis*, *N. scirpivora*, and *N. scirpinfestans* and is circumscribed as *Nimbya* group I. This group was supported in a previous study (Hong et al. 2005) and is supported by the morphological taxonomy established by Simmons (1992). *Nimbya alternantherae*, *N. celosiae*, and *N. perpunctulata* form a well-supported *Nimbya* group II in all analyses with the alternata species-group as the sister group in all analyses except for the ITS analysis. This group is also supported by the morphological taxonomy established by Simmons (1992). However, all taxa in this second group have long-beaked conidia and are very similar to *Alternaria* species in the porri species-group and differ from the species of *Nimbya* group I that have relatively short-beaked conidia. The type and all recognized species of *Nimbya* were recovered from the Juncaceae, the Cyperaceae, and the Amaranthaceae (Simmons 1989; Zhao and Zhang 2005). It is interesting that host specificity of *Nimbya* correlates with the two groups resolved in this study with *Nimbya* group I originating from the Juncaceae and the Cyperaceae and *Nimbya* group II originating from the Amaranthaceae. This further supports molecular data which reveals that *Nimbya* group I and *Nimbya* group II have independent evolutionary origins. We have revealed that the strikingly disto-pharagmoseptate conidium morphology is not useful in understanding the phylogenetic relationship among the species in the genus *Nimbya*. These data suggest that *Nimbya* should be restricted to species with short-beaked and strikingly disto-pharagmoseptate conidia and species isolated from Juncaceae and Cyperaceae. On the basis of morphological and unambiguous phylogenetic characterization, this work proposes that the species of *Nimbya* group II be transferred to the genus *Alternaria.* Several earlier-described species of *Nimbya* as well as several recently described species were not available for this study and a more comprehensive study of *Nimbya* with additional *Nimbya* taxa may be needed to reveal perhaps an even more diverse taxonomy.

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