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The sections of *Alternaria*: formalizing species-group concepts

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Abstract: The systematics of *Alternaria* and allied genera traditionally has been based on the characteristics of conidia and the sporulation apparatus. This emphasis on morphology in the reconstruction of organismal relationships has resulted in taxonomic uncertainty and flux for a number of taxa in *Alternaria* and the related genera *Stemphylium*, *Embellisia*, *Nimbya* and *Ulocladium*. The present study used a molecular phylogenetic approach for systematic resolution and incorporated extensive taxon sampling (n = 176 species) representing 10 genera and analyses of 10 protein-coding loci. Phylogenetic analyses based on five of these genes revealed eight distinct asexual lineages of *Alternaria* that cluster as the sister group to the asexual paraphyletic genus *Ulocladium*, while taxa with known teleomorphs currently circumscribed as *Alternaria* (the infectoria species-group) cluster among genera that also have representatives with known teleomorphs. This work proposes to elevate the eight well supported asexual lineages of *Alternaria* to the taxonomic rank of section. Evolutionary relationships among *Alternaria* and closely related genera are discussed.

Key words: *Alternaria*, morphology, phylogenetics, species-groups, systematics

Introduction

Alternaria represents an ecologically diverse fungal genus recovered worldwide as ubiquitous agents of decay of natural and artificial substrates (Rotem 1994). Many species of *Alternaria* are important phytopathogens that cause plant diseases and postharvest rots of numerous agronomic and ornamental plants, and the genus ranks 10th among fungal genera in terms of the total number of host plants (more than 4000) it can infect (Farr et al. 1989, Thomma 2003). *Alternaria* spp. and related taxa are gaining prominence as emerging human pathogens, particularly in immunocompromised patients (Rossmann et al. 1996, de Hoog et al. 2000). Equally important, *Alternaria* spores are one of the most common and potent airborne allergens and sensitization to *Alternaria* allergens has been determined to be one of the most important factors in the onset of childhood asthma in arid regions (Peat et al. 1993, Halonen et al. 1997). In summary, few fungal taxa can match the global impact of *Alternaria* in native ecosystems and affecting humans and human activities.

The genus *Alternaria* first was described by Nees von Esenbeck in 1816 with *A. tenuis* as the type specimen but has suffered from considerable taxonomic uncertainty and flux since its inception. In 1832, Fries wrote his monumental work titled Systema Mycologicum in which he did not recognize Nees' description of *A. tenuis* and cited it as a synonym of *Torula alternata*, complicating the taxonomy of *Alternaria* from an early date. In 1912, Keissler reevaluated both Nees' and Fries' descriptions and synonymized both specimens with *Alternaria alternata*, which is now the recognized type for the genus. The defining characteristics of *Alternaria* include dark multicelled conidia with transverse and longitudinal septa (phaeodictyospores) that occur in chains (catenulate) or borne singly and possess an apical beak or tapering apical cells (Elliott 1917, Wiltshire 1933, Neergaard 1945, Joly 1964, Simmons 1967).

Alternaria species have been commonly identified and classified based on colony and conidial morphology. In 1917 Elliott undertook a comparative study of *Alternaria* and *Macrosporium* reference specimens in an attempt to classify them into morphological groups based on conidium morphology (size, shape, septation, beak). Six morphological *Alternaria* groups were proposed with a representative species typifying each group. Neergaard (1945) proposed three *Alternaria* species groupings, calling them sections, based upon conidial chain formation: *Longicatenatae* with 10 or more catenulate conidia, *Brevicatenatae* with short chains of 3–5 conidia and *Noncatenatae* with conidia borne singly or rarely producing a secondary conidium. Joly (1964) classified *Alternaria* species into three sections based largely on conidium

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color, rigidity or lateral symmetry: *Claroseminae*, with light or translucent yellowish colors; *Brunneoseminae*, with brown or reddish brown dark conidia; *Rigidae*, conidia with rare longitudinal septa and a rigid appearance. However, none of the above classification systems were widely adopted and are not in use today.

More recently, *Alternaria* species that share colony and conidial morphological characteristics have been classified into several morphological species-groups (Simmons 1992). Simmons and Roberts (1993) further divided the small-spored, catenulate taxa of *Alternaria* into six morphological groups. These include Group 1 with the production of short to moderate conidial chains (5–10) with modest spore size; Group 2 with short to moderate conidial chains (5–10) with larger, more robust spore size, with *Alternaria gaisen* as the group representative; Group 3 with short (50–70 μm) to exceptionally long (100–150 μm) conidiophores with extensive secondary sporulation via subconidium conidiophore elongation; Group 4 with short conidiophores in bushy clumps with abundant secondary sporulation patterns, with *Alternaria alternata* as the group representative; Group 5 with moderately long to long conidial chains, 10–15(–20) μm, with rare to no branching; Group 6 with clumps of branching chains with extended pseudorostrate connections between apically produced conidia with *Alternaria infectoria* as the group representative (Simmons and Roberts 1993). Simmons further developed the system of species groupings by describing the *A. infectoria* complex, the *A. alternata* species-group, the *A. tenuissima* species-group, the *A. cheiranthi* species-group and the *A. brassicicola* species-group (Simmons 1994, 1995).

Pryor and Gilbertson (2000) presented the first molecular phylogenetic analyses of *Alternaria* and demonstrated that *Alternaria* and *Ulocladium* cluster as a monophyletic group with internally nested phylogenetically distinct clades referred to as the alternata, radicina, porri, brassicicola and ulocladium species-groups. Of interest, a fifth *Alternaria* clade referred to as the infectoria species-group was phylogenetically more distant and more closely related to sexual genera such as *Macrospora* (*Nimbya* anamorph) and *Allewia* (*Embellisia* anamorph). Since this report, two additional *Alternaria* lineages have been discovered based on morphological and molecular analyses, including the sonchi species-group (Hong et al. 2005) and the alternantherae species-group (Lawrence et al. 2012). Continued phylogenetic studies of *Alternaria* and closely related genera using ITS and protein-coding genes (*gpd*, *Alt a1*, endopolygalacturonase) have demonstrated strong support for the species-groups described above, however these studies were unable to provide strong support for the monophyly of asexual *Alternaria* species or strong support for the order of divergence of the described species-groups (Pryor and Gilbertson 2000, Pryor and Bigelow 2003, Hong et al. 2005, Andrew et al. 2009, Lawrence et al. 2012).

The inability to resolve key phylogenetic nodes suggested that further phylogenetic analyses using a larger sample of taxa and a larger dataset might increase statistical support (Rokas and Carroll 2005). Thus, the objective of this study was to reconstruct phylogenetic relationships among species in the genus *Alternaria* by using increased taxon sampling and more phylogenetically informative loci. Specific goals for this study were to assess the phylogenetic relationships among *Alternaria* and closely related genera, identify undescribed species and/or phylogenetic lineages and more clearly resolve the relationships between asexual and sexual *Alternaria* lineages.

MATERIALS AND METHODS

Fungal taxa.—One hundred thirty-one species of *Alternaria*, 18 species of *Embellisia*, 10 species of *Ulocladium*, four species of *Nimbya*, three species of *Stemphylium*, two species of *Chalastospora*, two species of *Undifilum*, and *Brachycladium papaveris*, *Crivellia papaveracea*, *Sinomyces alternariae*, *Pleospora herbarum* and *Exserohilum pedicellatum* were used (TABLE I). These taxa were chosen to represent reference strains from established culture collections that have been carefully examined morphologically and to adequately encompass the morphological and ecological diversity of *Alternaria* and related genera.

DNA extraction and PCR amplification.—DNA extraction was performed according to protocols of Pryor and Gilbertson (2000). Sequences from 10 protein-coding genes initially were evaluated for phylogenetic utility and reconstruction. Amplification of fragments of genes *Alt a1* and *gpd* (glyceraldehyde-3-phosphate dehydrogenase) used primer pairs Alt-for/Alt-rev and gpd1/gpd2 respectively (Hong et al. 2005, Berbee et al. 1999 respectively). When PCR failed using Alt-for and Alt-rev, amplification of *Alt a1* was conducted with modified primers Alt-4for/Alt-4rev (Lawrence et al. 2012). Amplification of beta-tubulin used primer pairs Bt2a/Bt2b as described in Glass and Donaldson (1995). Novel primers were designed for the amplification of gene fragments from actin, plasma membrane ATPase, calmodulin, translation elongation factor 1-alpha (*TEF*), the second largest subunit of RNA polymerase II (*RPB2*), chitin synthase, and *Tsr1* (TABLE II) based on available genome sequences of three *Alternaria* species, *A. brassicicola*, *A. alternata* and *A. solani*, and a close relative within the Pleosporaceae, *Pyrenophora tritici-repentis*. Each PCR mixture contained 10 μM of each primer, 200 μM dNTP, 1 × *Taq* reaction buffer, 2 units of Ampli*Taq*-DNA polymerase, 2.5 mM $MgCl_2$ and 10 ng template DNA in a

TABLE I. Species used for phylogenetic analyses

Species	Source[a]	Species	Source[a]	Species	Source[a]
Alternaria acalyphicola	CBS 541.94	*A. herbiphorbicola*	EGS 40-140	*A. tomato*	CBS 114.35
A. agerati	CBS 117221	*A. hordeiaustralica*	EGS 44-200	*A. tomatophila*	CBS 109156
A. agripestis	CBS 577.94	*A. hordeicola*	EGS 50-184	*A. toxicogenica*	PR320
A. alternarina	EGS 10-193	*A. humuli*	EGS 47-140	*A. triglochinicola*	EGS 41-070
A. alternantherae	EGS 52-039	*A. incomplexa*	EGS 17-103	*A. triticimaculans*	EGS 41-050
A. alternata	EGS 34-016	*A. infectoria*	EGS 27-193	*A. triticina*	EGS 17-061
A. anagallidis var. *anagallidis*	CBS 107.44	*A. intercepta*	EGS 49-137	*A. tropica*	CBS 631.93
A. angustiovoidea	EGS 36-172	*A. iridis*	CBS 101.26	*A. turkisafria*	EGS 44-159
A. aragakii	CBS 594.93	*A. japonica*	EGS 41-158	*A. vaccariae*	CBS 116533
A. arborescens	EGS 39-128	*A. limaciformis*	CBS 481.81	*A. vaccariicola*	EGS 46-003
A. arbusti	EGS 91-136	*A. limicola*	CBS 483-90	*A. ventricosa*	EGS 52-075
A. argyranthemi	EGS 43-033	*A. limoniasperae*	EGS 45-100	*A. viburni*	EGS 49-147
A. argyroxiphii	EGS 35-122	*A. lini*	CBS 106.34	*A. zinniae*	CBS 118.44
A. bataticola	CBS 531.63	*A. linicola*	CBS 103-46	*Brachycladium papaveris*	P351
A. blumeae	EGS 40-149	*A. longipes*	EGS 30-033	*Chalastospora cetera*	EGS 41-072
A. brassicae	EGS 38-032	*A. macrospora*	DGG Ams1	*C. gossypii*	CBS 135.31
A. brassicicola	EEB 2232	*A. malvae*	CBS 447.86	*Crivellia papaveracea*	P354.8
A. burnsii	CBS 107.38	*A. maritima*	CBS 126.60	*Embellisia abundans*	CBS 534.83
A. calendulae	CBS 224.76	*A. merytae*	EGS 46-153	*E. allii*	EGS 38-073
A. californica	EGS 52-082	*A. metachromatica*	EGS 38-132	*E. annulata*	CBS 302.84
A. calycipyricola	EGS 52-072	*A. mimicula*	EGS 01-056	*E. chlamydospora*	EGS 33-022
A. capsici	EGS 45-075	*A. molesta*	CBS 548.81	*E. conoidea*	CBS 132.89
A. carotiincultae	EGS 26-010	*A. mouchaccae*	EGS 31-061	*E. didymospora*	CBS 766.79
A. carthami	CBS 635.80	*A. multirostrata*	CBS 712.68	*E. eureka*	EGS 36-103
A. cassiae	CBS 477.81	*A. nelumbii*	EGS 12-135	*E. indefessa*	EGS 30-195
A. celosiae	EGS 42-013	*A. nitrimali*	CBS 109163	*E. hyacinthi*	EGS 49-062
A. cerealis	EGS 43-072	*A. noblis*	CBS 116490	*E. leptinellae*	EGS 40-187
A. cheiranthi	EGS 41-188	*A. novae-zelandiae*	EGS 48-092	*E. lolii*	EGS 43-054
A. chlamydospora	CBS 491.72	*A. oregonensis*	EGS 29-194	*E. novae-zelandiae*	EGS 39-099
A. cichorii	CBS 102.33	*A. panax*	EGS 29-180	*E. phragmospora*	EGS 27-098
A. cinerariae	EGS 33-169	*A. passiflorae*	CBS 113.38	*E. planifunda*	CBS 537.83
A. cirsinoxia	CBS 113261	*A. peglionii*	CBS 103.26	*E. proteae*	EGS 39-031
A. citriarbusti	SH-MIL-8s	*A. perangusta*	BMP 2336	*E. tellustris*	EGS 33-026
A. citrimacularis	BC2-RLR-17s	*A. perpunctulata*	EGS 51-130	*E. thlaspis*	EGS 45-069
A. colombiana	BMP 2337	*A petroselini*	EGS 09-159	*E. tumida*	CBS 539.83
A. conjuncta	EGS 37-139	*A. photistica*	EGS 35-172	*Exserohilum pedicellatum*	BMP 0384
A. crassa	DDG Acr1	*A. poonensis*	EGS 47-138	*Nimbya caricis*	EGS 13-094
A. cretica	CBS 109164	*A. porri*	ATCC 58175	*N. scirpicola*	EGS 19-016
A. cucumerina	BMP 0188	*A. postmessia*	EGS 39-189	*N. scirpinfestans*	EGS 49-185
A. cyphomandrae	CBS 109155	*A. protenta*	CBS 116696	*N. scirpivora*	EGS 50-021
A. danida	EGS 07-029	*A. pseudorostrata*	EGS 42-060	*Pleospora herbarum*	ATCC 11681
A. dauci	ATCC 36613	*A. radicina*	ATCC 96831	*Sinomyces alternariae*	BMP 0352
A. daucicaulis	EGS 36-1947	*A. resedae*	CBS 175.80	*Stemphylium botryosum*	ATCC 42170
A. destruens	EGS 46-069	*A. rhadina*	CBS 595.93	*S. callistephi*	EEB 1055
A. dianthicola	CBS 915.96	*A. ricini*	CBS 353.86	*S. vesicarium*	ATCC 18521
A. dichondrae	CBS 199.74	*A. rosae*	EGS 41-130	*Ulocladium atrum*	ATCC 18040
A. dumosa	EGS 45-007	*A. rostellata*	EGS 42-061	*U. botrytis*	ATCC 18043
A. eryngii	EGS 41-005	*A. scorzonerae*	CBS 478.83	*U. chartarum*	ATCC 18044
A. ethzedia	EGS 37-143	*A. selini*	EGS 25-198	*U. consortiale*	CBS 201-67
A. euphorbiicola	EGS 42-049	*A. sesami*	CBS 240.73	*U. cucurbitae*	EGS 31-021
A. frumenti	EGS 44-001	*A. smyrnii*	EGS 37-093	*U. dauci*	CBS 102062
A. gaisen	BMP 0243	*A. solani*	ATCC 58177	*U. multiforme*	CBS 102060
A. gossypina	CBS 104.32	*A. solani-nigri*	CBS 113403	*U. obovoideum*	CBS 101229
A. graminicola	EGS 41-139	*A. sonchi*	EGS 46-051	*U. septosporum*	CBS 109.38
A. grandis	CBS 109158	*A. steviae*	CBS 632.88	*U. tuberculatum*	CBS 202.67
A. grisea	CBS 107.36	*A. subcylindrica*	CBS 109161	*Undifilum bornmuelleri*	DAOM 231361

TABLE I. Continued

Species	Source[a]	Species	Source[a]	Species	Source[a]
A. grossulariae	CBS 100.23	*A. tagetica*	EGS 44-044	*U. oxytropis*	RC OlB9
A. gypsophilae	CBS 107.41	*A. tangelonis*	EV-MIL-2s		
A. hawaiiensis	CBS 630.93	*A. tenuissima*	EGS 34-015		

[a] Abbreviations for sources are: ATCC, American Type Culture Collection, Manassas, Virginia 20108; BMP, BM Pryor, School of Plant Sciences, University of Arizona, Tucson, Arizona 85721; DGG, DG Gilchrist, Department of Plant, Pathology, University of California, Davis, California 95616; EEB, EE Butler, Department of Plant Pathology, University of California, Davis, California 95616; EGS, EG Simmons, Mycological Services, Crawfordsville, Indiana 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 K1A0C8; P, P. Inderbitzin, Department of Plant Pathology, Cornell University, Ithaca, New York 14850; RC, Rebecca Creamer, Department of Entomology, Plant Pathology and Weed Science, New Mexico State University, Las Cruces, New Mexico 88003.

final reaction volume of 25 μL. PCR programs for novel primers are detailed (TABLE II).

Sequencing and alignment.—Nucleotide sequences of both forward and reverse DNA strands of PCR products were determined with FS DyeTerminator reactions (Applied Biosystems 3730 DNA Analyzer, Foster City, California) at the University of Arizona Genetics Core facility. Some sequences of *Alt a1* and *gpd* were obtained from Pryor and Bigelow (2003), Hong et al. (2005), Pryor et al. (2009), Lawrence et al. (2012). GenBank accession numbers are provided (SUPPLEMENTARY TABLE I). The sequences were proofread, edited and aligned in MacVector 12 (MacVector Inc., Cary, North Carolina). Nucleotide sequences were aligned manually where necessary with MacClade 4.08 (Maddison and Maddison 2003). All 10 nucleotide alignments were submitted to TreeBASE under accession number S12384.

Phylogenetic analyses.—Maximum parsimony (MP) analyses were performed in PAUP* 4.0b10 (Swofford 2002). Gaps were treated as missing data. Analyses were conducted by heuristic searches consisting of 1000 stepwise random addition replicates with branch swapping by the tree-bisection-reconnection algorithm. Branch stability for individual datasets and the concatenated dataset were evaluated by 1000 bootstrap replications using a heuristic search with simple sequence addition to produce a majority rule consensus tree with nodal support values. Concordance among datasets ($P \geq 0.010$) was evaluated with the partition homogeneity test (PHT) implemented in PAUP* 4.0b10 (Swofford 2002).

Bayesian analyses were performed in MrBayes 3.1.1 (Huelsenbeck and Ronquist 2001). The Akaike information criterion implemented in the program Modeltest 3.7 (Posada and Crandall 1998) and was used to estimate the best-fit model of nucleotide evolution for each dataset (HKY + I + G for *gpd* and *Alt a1*, and GTR + I + G for actin, plasma membrane ATPase and calmodulin). The combined analysis used an independent model of nucleotide evolution for each locus. Bayesian analyses were performed on individual datasets and the combined dataset consisted of two independent runs of four chains each for 10 000 000 generations, sampling every 1000 generations. Convergence was estimated based on plots of −lnL values and examination of the standard deviation of split frequencies < 0.01, which stabilized after approximately 5 000 000 generations. The first 5 000 000 generations were discarded as burn-in. Majority rule consensus trees with Bayesian posterior probabilities (BPP) were estimated in PAUP* 4.0b10 (Swofford 2002). Sequences of *Exserohilum* or *Stemphylium* served as outgroup taxa based on results from Pryor and Gilbertson (2000), Pryor and Bigelow (2003), Hong et al. (2005).

Substitution saturation test.—To investigate differences among topologies estimated from the 10 datasets, sequences from all alignments were used to assess the hypothesis of nucleotide substitution saturation based on MP reconstructions. The observed transitions/transversions of first, second and third codon positions were plotted against F84 corrected distance using the program DAMBE 5.2.68 to produce substitution scatter plots (Xia and Xie 2001, Xia 2009). Substitution saturation of each codon position was considered saturated if the point on the substitution scatter plot appears to level off with an increase in sequence divergence. To corroborate the substitution scatter plots, the I_{ss} statistic, which is a measurement of substitution saturation in nucleotide datasets developed by Xia et al. (2003), also was implemented in DAMBE. Loci that possessed substantial substitution saturation were not included in the phylogenetic analyses of the combined dataset.

Phylogenetic informativeness.—To investigate variance in phylogenetic signal among datasets, tests of phylogenetic informativeness (PI) as proposed by Townsend (2007) were implemented for each dataset with the program PhyDesign (López-Giráldez and Townsend 2011). The PI of each locus was calculated with one of the most parsimonious trees obtained from the analysis of the final five-gene concatenated dataset. This phylogeny was used to construct an ultrametric tree with the program PATHd8 (Britton et al. 2007) for implementation along with alignments as in Townsend (2007) to obtain net PI for each gene fragment. Loci that possess low phylogenetic informativeness were not included in the phylogenetic analyses of the concatenated dataset.

TABLE II. Novel primers and PCR programs

Locus	Primer name	Primer sequence (5′–3′)	Initial denaturation	Denaturation	Annealing	Extension	Final extension
Actin	ACTDF1	TTCGGGTATGTGCAAGGC	95 C for 5 min	95 C for 30 s	58 C for 30 s	72 C for 75 s	72 C for 5 min
	ACTDR1	ATACCGGGGTACATGGTGG					
Plasma membrane ATPase	*ATPDF1*	*ATCGTCTCCATGACCGAGTTCG*	*94 C for 4 min*	*94 C for 30 s*	*59 C for 30 s*	*72 C for 1 min*	*72 C for 5 min*
	ATPDR1	TCCGATGGAGTTCATGATAGCC					
Calmodulin	CALDF1	AGCAAGTCTCCGAGTTCAAGG	95 C for 4 min	95 C for 30 s	58.5 C for 30 s	72 C for 1 min	72 C for 5 min
	CALDR1	CTTCTGCATCATCAYCTGGACG					
Translation elongation factor 1-alpha	TEF1	AGCCGCCGAACTCGGTAAGG	94 C for 5 min	94 C for 30 s	60 C for 30 s	72 C for 1 min	72 C for 10 min
	TEF2	TCTTGGAGTCACCGGCAACG					
RPB2	RPB2DF	ACCGACACACAAATGCTGGAGC	95 C for 5 min	95 C for 40 s	58 C for 30 s	72 C for 1 min	72 C for 10 min
	RPB2DR	CAAGACCCCAATGAGAGTTGTG					
Tsr1	Tsr1F	GAGCGCAAGGGTGTTCTACT	95 C for 4 min	95 C for 30 s	57.5 C for 30 s	72 C for 80 s	72 C for 5 min
	Tsr1R	CCTTGAAGTATCCATGCGTACC					
Chitin synthase	CHSDF1	TGGGARATGATYGGYTGGATG	95 C for 5 min	95 C for 30 s	58 C for 30 s	72 C for 1 min	72 C for 5 min
	CHSDR1	TAGTATAGCTGGCCGCTGATGAG					

RESULTS

Phylogenetic analysis, RPB2.—PCR amplification of the *RPB2* locus resulted in 573 bp fragments and the alignment resulted in a 573 character dataset (387 characters were constant, 27 characters were parsimony uninformative and 159 characters were parsimony informative). Maximum parsimony analysis of an initial screen of sequences from 77 taxa produced trees (SUPPLEMENTARY FIG. 1) with topologies that were incongruent with Pryor and Gilbertson (2000), Pryor and Bigelow (2003), Hong et al. (2005), Lawrence et al. (2012) and other loci (*gpd*, *Alt a1*, actin, plasma membrane ATPase, calmodulin) examined in this study. Previously described *Alternaria* species-groups and closely related genera did not cluster into monophyletic groups as in Pryor and Gilbertson (2000), Pryor and Bigelow (2003), Hong et al. (2005) and Lawrence et al. (2012) (SUPPLEMENTARY FIG. 1). Extensive intermixing of species-groups and related genera was evident in the tree, although there was no support for any node except moderate support for the sister relationship of *Embellisia annulata* and *Stemphylium botryosum*. Due to the potential introduction of systematic error the *RPB2* locus was not used in additional phylogenetic analyses.

Phylogenetic analysis, Tsr1.—PCR amplification of the *Tsr1* locus for most taxa resulted in 1100 bp fragments and the alignment resulted in a 1107 character dataset (602 characters were constant, 110 were parsimony uninformative, 395 were parsimony informative). Maximum parsimony analysis of an initial screen of sequences from 121 taxa resulted in most parsimonious trees (SUPPLEMENTARY FIG. 2) that were incongruent with Pryor and Gilbertson (2000), Pryor and Bigelow (2003), Hong et al. (2005), Lawrence et al. (2012) and additional loci sampled in this study. Several previously described lineages including the alternantherae, infectoria, and brassicicola species-groups, the paraphyletic genus *Ulocladium* and a clade of *Embellisia* species circumscribed as *Embellisia* group III clustered into distinct monophyletic lineages with moderate to strong support (≥ 85%), although the phylogenetic position was inconsistent with previous work. The porri species-group clustered on the backbone of the tree sister to *Stemphylium vesicarium* and remaining taxa. The alternata species-group was strongly supported (100%) but was paraphyletic due to the inclusion of *Sinomyces alternariae*. Two previously undescribed *Alternaria* species-groups, gypsophilae and panax, were strongly to moderately supported (99% and 79% respectively) in the analysis of *Tsr1*. Due to the potential introduction of systematic error the *Tsr1*

locus was not used in additional phylogenetic analyses.

Phylogenetic analysis, chitin synthase.—PCR amplification of the chitin synthase locus for most species produced 647 bp fragments. Alignment of the chitin synthase fragments resulted in a 676 character dataset (340 characters were constant, 53 were parsimony uninformative, 283 were parsimony informative). Maximum parsimony analysis of sequences from 176 taxa resulted in most parsimonious trees (SUPPLEMENTARY FIG. 3) that reveal strong structure for previously described species-groups of *Alternaria*, however the phylogenetic relationship of most species-groups was unsupported. Several topological inconsistencies were obvious, which included the early divergence of the brassicicola species-group and the paraphyletic genus *Ulocladium*. Additionally, *Embellisia* group II is nested within the asexual lineage of *Alternaria* albeit with low support. Other described genera such as *Crivellia*, *Undifilum* and *Nimbya* clustered as independent lineages as reported previously. Because of topological conflicts, this locus was not used in the analysis of the combined dataset.

Phylogenetic analysis, Beta-tubulin.—PCR amplification of the beta-tubulin locus for most species generated 347 bp fragments. Alignment of the beta-tubulin locus resulted in a 352 character dataset (203 characters were constant, 56 were parsimony uninformative, 93 were parsimony informative). Maximum parsimony analyses of sequences from 151 taxa resulted in most parsimonious trees (SUPPLEMENTARY FIG. 4) that generally conformed to topologies as reported by other authors and other loci in this study. However, many previously described *Alternaria* species-groups were unresolved and existed as polytomies on the backbone of the tree. Additionally, well studied genera including *Ulocladium* and *Nimbya* did not cluster into strongly supported independent lineages as in analyses performed by other authors and other loci in this study. The beta-tubulin locus was not included in the analysis of the combined dataset because of the inability to resolve species-group and genus relationships.

Phylogenetic analysis. TEF.—PCR amplification of the *TEF* gene for most species generated 778–835 bp fragments. Alignment of the *TEF* locus resulted in an 1120-character dataset. The large intron was not alignable and therefore excluded from the analysis. The analyzed dataset consisted of a 696 character dataset (561 characters were constant, 38 were parsimony uninformative, 97 were parsimony informative). Maximum parsimony analyses of sequences from 114 taxa resulted in most parsimonious trees (SUPPLEMENTARY FIG. 5) that generally conformed to topologies as reported by other authors and other loci in this study. However, divergences of most of the previously described species-groups of *Alternaria* including the genus *Ulocladium* were unresolved and existed as polytomies on the backbone of the tree. Additionally, *Embellisia*, *Nimbya* and *Crivellia* species were nested within the asexual lineages of *Alternaria* with low support. Similar to the beta-tubulin analyses *TEF* was not included in the analysis of the combined dataset because of the inability to resolve species-group and genus relationships.

Phylogenetic analyses. gpd.—PCR amplification of the *gpd* gene for most species generated 500–576 bp fragments. PCR products from *A. brassicicola*, *A. mimicula* and *E. conoidea* were smaller by approximately 26 bp, the infectoria species-group was 54 bp smaller and *E. abundans*, *Chalastospora cetera*, and *C. gossypii* were 76 bp smaller (data not shown). Alignment of the *gpd* gene sequences resulted in a 623 character dataset (339 characters were constant, 42 characters were parsimony uninformative, 242 characters were parsimony informative).

The alternata, alternantherae, porri and radicina species-groups clustered into independent clades with strong to moderate MP bootstrap and Bayesian posterior probability (BPP) support $\geq$ 88%/1.0 respectively (SUPPLEMENTARY FIG. 6). The sonchi species-group (*A. sonchi*, *A. cinerariae*, and *A. brassicae*) was weakly supported (<70%/<0.95). The brassicicola species-group including *A. brassicicola*, *A. mimicula*, *E. conoidea* and *A. japonica* formed a weakly supported monophyletic group (73%). The infectoria species-group was well defined with strong support (98%/1.0) and was sister to the clade that consists of *E. abundans*, *C. cetera* and *C. gossypii* that was phylogenetically distant to asexual *Alternaria* lineages. In addition, two new species-groups were resolved in the analysis of *gpd* and were circumscribed as the panax and gypsophilae species-groups respectively. The panax species-group, which included *A. panax*, *A. eryngii* and *A. calycipyricola*, was strongly supported (98%/1.0), and the gypsophilae species-group, which included *A. gypsophilae*, *A. vaccariae*, *A. noblis* and *A. vaccariicola*, was weakly supported in the bootstrap analysis but strongly supported by BPP (< 70%/1.0). Many other closely related genera were strongly supported as in previous studies.

Phylogenetic analyses, Alt a1.—PCR amplification of the *Alt a1* gene generated 412–472 bp fragments. PCR products from *Stemphylium vesicarium*, *S. callistephi*, *Pleospora herbarum* and *E. eureka* were smaller by approximately 60 bp (data not shown). No amplification of the *Alt a1* locus could be obtained

with DNA from *Exserohilium pedicellatum*, *Embellisia annulata*, *A. triglochinicola*, *A. triticimaculans*, *A. dianthicola*, *A. hordeiaustralica*, *A. peglionii*, *A. arbusti*, *A. humuli*, *A. daucicaulis* and *A. merytae* despite repeated attempts and repeated primer redesign. Alignment of the *Alt a1* gene sequences resulted in a 497 character dataset (96 characters were constant, 56 characters were parsimony uninformative and 345 characters were parsimony informative).

The porri, alternata, alternantherae, radicina and gypsophilae species-groups clustered into monophyletic groups with strong support (≥94%/1.0) (SUPPLEMENTARY FIG. 7). The alternantherae and sonchi species-groups clustered as sister groups to the porri species-group, albeit with weak support. The radicina and gypsophilae species-groups formed sister clades with moderate to strong support (70%/0.98). The panax species-group, *A. panax* and *A. calcipyricola* clustered as sister species (86%/0.88), while *A. eryngii* clusters with low support as an independent lineage sister to the radicina/gypsophilae clade. The brassicicola species-group formed a monophyletic group that includes *A. brassicicola*, *A. mimicula* and *E. conoidea* with strong support basal to all *Alternaria* species-groups except the infectoria species-group. *Alternaria japonica* and *E. eureka* clustered with the brassicicola species-group with weak support. The infectoria species-group clustered with moderate to strong support (88%/1.0) at the tip of the clade that contains species of *Embellisia*, *Undifilum*, *Crivellia*, *Sinomyces* and *Nimbya* and was phylogenetically distant to other species-groups of *Alternaria*.

Phylogenetic analyses. Actin.—PCR amplification of the actin gene generated 915–939 bp fragments. Alignment of the actin gene sequences resulted in a 999 character dataset (668 characters were constant, 85 characters were parsimony uninformative, 246 characters were parsimony informative). The porri, alternantherae, gypsophilae and radicina species-groups were strongly supported (≥89%/1.0), however the alternata and panax species-groups were moderately supported (SUPPLEMENTARY FIG. 8). The brassicicola species-group including *A. brassicicola*, *A. mimicula* and *E. conoidea*, was strongly supported (100%/1.0), however *A. japonica* did not cluster within this group. The sonchi species-group consisted of *A. sonchi* and *A. cinerariae* and was strongly supported (sequence fragment from actin was not obtained for *A. brassicae*). The infectoria species-group was strongly supported (88%/1.0) as a monophyletic lineage and was distantly related to other *Alternaria* clades.

Phylogenetic analyses. Plasma membrane ATPase.—PCR amplification of the plasma membrane ATPase gene for most species generated 1188–1201 bp fragments. Alignment of the plasma membrane ATPase gene sequences resulted in a 1441 character dataset (718 characters were constant, 147 characters were parsimony uninformative, 576 characters were parsimony informative). The porri, alternantherae, radicina, sonchi and panax species-groups were strongly supported (≥99%/1.0) by the analyses of the plasma membrane ATPase (SUPPLEMENTARY FIG. 9). The alternata and brassicicola species-groups were moderately to strongly supported (≥79/1.0). The gypsophilae species-group was split into two strongly supported (100%/0.93) grades. The infectoria species-group was strongly supported (100%/1.0) and was sister to the clade that contains *C. cetera*, *C. gossypii* and *E. abundans* and was phylogenetically distant to the asexual *Alternaria* lineage.

Phylogenetic analyses. Calmodulin.—PCR amplification of the calmodulin gene generated 566–763 bp fragments. PCR products from *A. triglochinicola*, *E. leptinellae*, *C. cetera*, *A. tomato* and *A. burnsii* were smaller by approximately 180 bp, and taxa in the infectoria species-group were smaller by approximately 110 bp (data not shown). Alignment of the calmodulin gene sequences resulted in a 978 character dataset (408 characters were constant, 120 characters were parsimony uninformative, 450 characters were parsimony informative).

Most *Alternaria* species-groups described in other analyses were moderately to strongly supported by the calmodulin analyses (SUPPLEMENTARY FIG. 10). The alternata, alternantherae, porri, panax, radicina and sonchi species-groups clustered into independent clades with strong support (≥92%/≥0.95). The gypsophilae species-group was moderately supported (72%), whereas the brassicicola species-group (*A. brassicicola*, *A. mimicula*, *E. conoidea*) was strongly supported (97%/1.0). The infectoria species-group clustered into a clade (100%/1.0) and formed a well resolved sister relationship with the *Chalastospora* clade, which together were more closely related to sexual genera than to asexual lineages of *Alternaria*.

Tests of substitution saturation.—Because *RPB2*, *Tsr1* and chitin synthase exhibited topologies inconsistent with previously hypothesized relationships, tests for substitution saturation were performed. For all loci, there was no evidence of first or second codon position substitution saturation. However, for the *RPB2*, *Tsr1* and chitin synthase loci, analysis of the third codon position showed significant substitution saturation as indicated by the plateau of the third base position in the substitution saturation scatter plots of these three gene fragments (FIG. 1, *RPB2*; SUPPLEMENTARY FIGS. 11–12, *Tsr1* and chitin synthase respectively). Using the test of Xia et al. (2003) suggested that the first and second codon positions for all amplified gene fragments was not saturated,

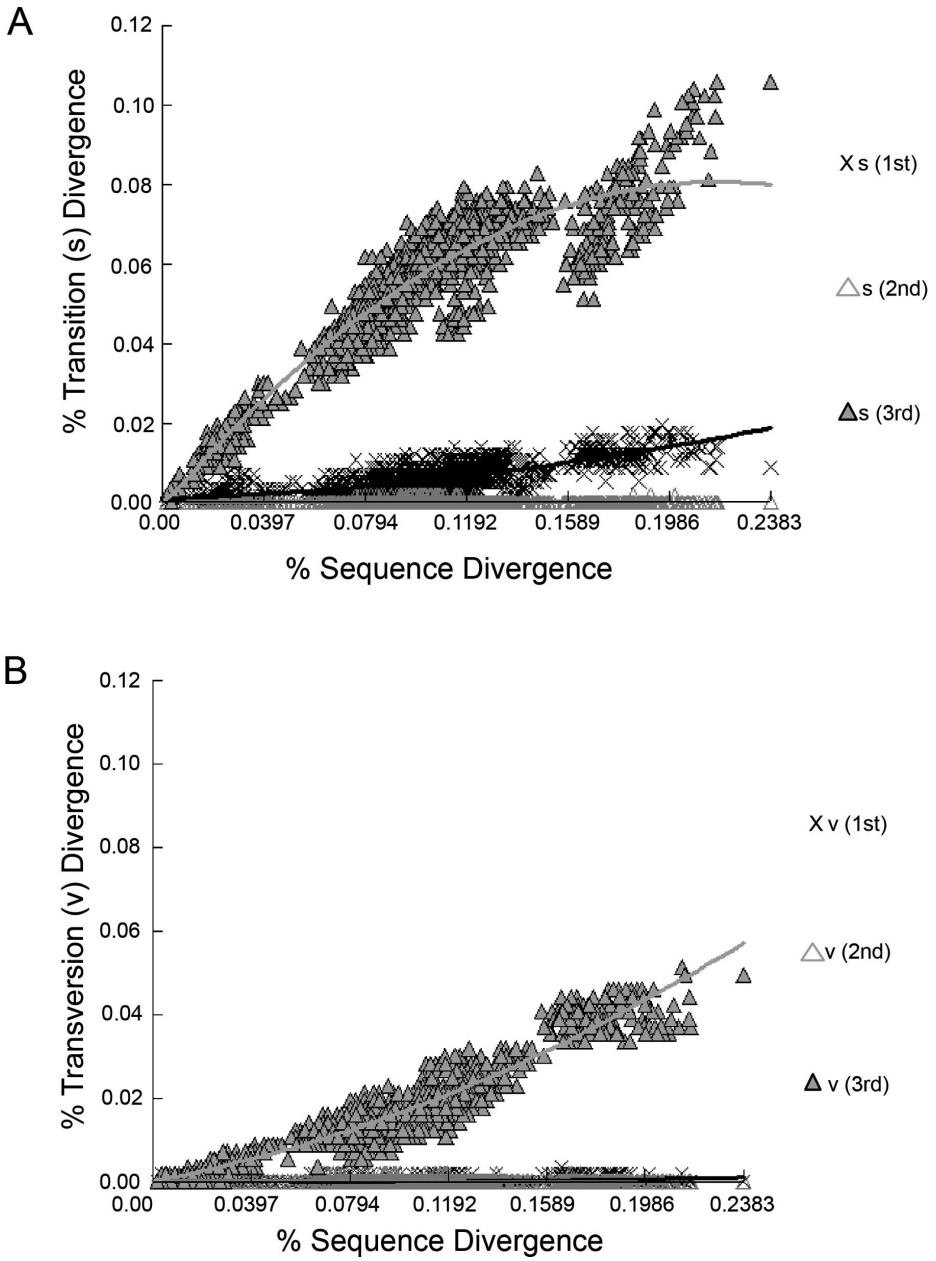

FIG. 1. Nucleotide substitution saturation plots for each codon position for *RPB2*. The proportion of transitions (s) and transversions (v) were plotted against the percent sequence divergence with the F84 genetic distance algorithm in DAMBE.

while the third codon position in fragments from *RPB2*, *Tsr1* and chitin synthase have experienced substantial substitution saturation (TABLE III). This statistical test corroborated the substitution saturation scatter plots revealing that the third codon position from *RPB2*, *Tsr1* and chitin synthase was not phylogenetically informative. Because of the possibility of introducing systematic error *RPB2*, *Tsr1* and chitin synthase were excluded from the analysis of the combined dataset. The seven remaining loci showed no evidence of substitution saturation at any codon position (TABLE III).

Phylogenetic informativeness.—The net PI profiles were derived for the remaining seven loci using PhyDesign (López-Giráldez and Townsend 2011). This approach provided an empirical metric for directing and prioritizing marker selection (Raja et al. 2011). The plasma membrane ATPase and calmodulin loci produced the highest net pulse of PI followed by a moderate net pulse of PI for *Alt a1*, *gpd* and actin and poor net pulse of PI for beta-tubulin and *TEF* (FIG. 2). Because of the low net PI, beta-tubulin and *TEF* also were excluded from the analysis of the combined dataset.

TABLE III. Substitution saturation test statistics for third codon position

Locus	I_{ss}	$I_{ss.c}$	*P*
RPB2	0.303	0.383	0.0157
Tsr1	0.288	0.356	0.0041
Chitin synthase	0.315	0.378	0.0451
Beta-tubulin	0.205	0.609	<0.0001[a]
TEF	0.196	0.362	<0.0001[a]
gpd	0.209	0.475	<0.0001[a]
Alt a1	0.243	0.502	<0.0001[a]
Actin	0.123	0.355	<0.0001[a]
Plasma membrane ATPase	0.204	0.354	<0.0001[a]
Calmodulin	0.174	0.490	<0.0001[a]

[a] Indicates significant differences (given alpha = 0.001), which is indicative of little to no saturation.

*Phylogenetic analyses. Five-gene combined dataset (*gpd, Alt a1, *Actin, Plasma membrane ATPase, Calmodulin).*—These five loci were combined because they do not possess substitution saturation at any codon position and have the greatest net PI of the 10 loci examined for phylogenetic utility. Test for concordance between datasets using PHT revealed that these data (*gpd*, *Alt a1*, actin, plasma membrane ATPase, calmodulin) were not significantly inconcordant ($P = 0.014$), and were combined and analyzed as above. Alignment of the combined dataset resulted in 4538 characters (2229 constant, 450 parsimony uninformative, 1859 parsimony informative). Maximum parsimony and Bayesian analyses of the concatenated dataset produced phylogenetic trees that contained four strongly supported clades (≥85%/1.0) (FIG. 3, clades A–D). Clade A consists of a large, strongly supported (100%/1.0) monophyletic group of asexual *Alternaria* (86%/1.0) that was sister to a well supported (100%/1.0) paraphyletic assemblage of *Ulocladium* spp. that included *E. indefessa* and *Alternaria cheiranthi*. The large *Alternaria* clade (clade A) consisted of eight distinct phylogenetic lineages in which the order of divergence was moderately to strongly supported for all groups. The brassicicola species-group was the earliest diverging asexual *Alternaria* lineage and encompassed four species, *A. brassicicola*, *A. mimicula*, *E. conoidea* and *A. japonica*. The following diverging lineage of *Alternaria* consisted of three monophyletic groups with the earliest diverging lineage composed of a previously undescribed species-group that consisted of three distinct phylogenetic species (*A. panax*, *A. calycipyricola*, *A. eryngii*) now referred to as the panax species-group. There was a close and strongly supported (86%/1.0) evolutionary relationship between the radicina species-group and the sister group that consisted of four *Alternaria* species (*A. gypsophilae*, *A. vaccariae*, *A. noblis*, *A. vaccariicola*) that comprised a previously unnamed clade now circumscribed as the gypsophilae species-group. The subsequent diverging lineage was well supported and consisted of members of the sonchi species-group (*A. sonchi*, *A. cinerariae*, *A. brassicae*). The terminal four-fifths of the *Alternaria* clade consisted of three closely related and strongly supported (100%/1.0) species-groups, with the porri species-group (46 species) sister to the alternata species-group (31 species) and the recently described alternantherae species-group (three species).

Clade B was a large strongly supported (85%/1.0) group that consisted of several nested monophyletic genera, which included *Crivellia*, *Undifilum* and *Nimbya* in addition to a strongly supported monophyletic lineage of *Embellisia* that contained three species (*E. allii*, *E. telluster*, *E. chlamydospora*) and paraphyletic lineages of *Embellisia* that were closely related to *Sinomyces* and polyphyletic lineages of *Alternaria*. The infectoria species-group consisted of a monophyletic lineage that was strongly supported and was sister to the paraphyletic genus *Chalastospora*. Clade C was well resolved and composed a paraphyletic group that consisted of eight species of *Embellisia* and one *Alternaria* species. Clade D was strongly supported and consisted of three species of *Stemphylium* and one species of *Pleospora*, with *E. annulata* as the sister taxon.

TAXONOMY

Based on results of extensive molecular phylogenetic analyses of five phylogenetically informative loci and previous morphological data, this work proposes to elevate all currently and newly described asexual species-groups of *Alternaria* to the taxonomic status of section. The infectoria species-group is not supported phylogenetically as *Alternaria*; therefore we do not propose to formalize this species-group as a section within *Alternaria* but instead suggest that further taxonomic revision of this group is necessary to eliminate the current polyphyly of the genus *Alternaria*.

***Section* Alternaria** Lawrence, Gannibal, Peever & Pryor, sect. nov.

MycoBank MB802304.

On PCA primary conidiophores are straight or curved, simple or branched, short to very long. Conidiophores bear one or several conidiogenous loci at the apex. Conidial chains are moderately long or long, simple or branched. Young conidia are short ovoid, ellipsoid or obclavate. Mature conidia are

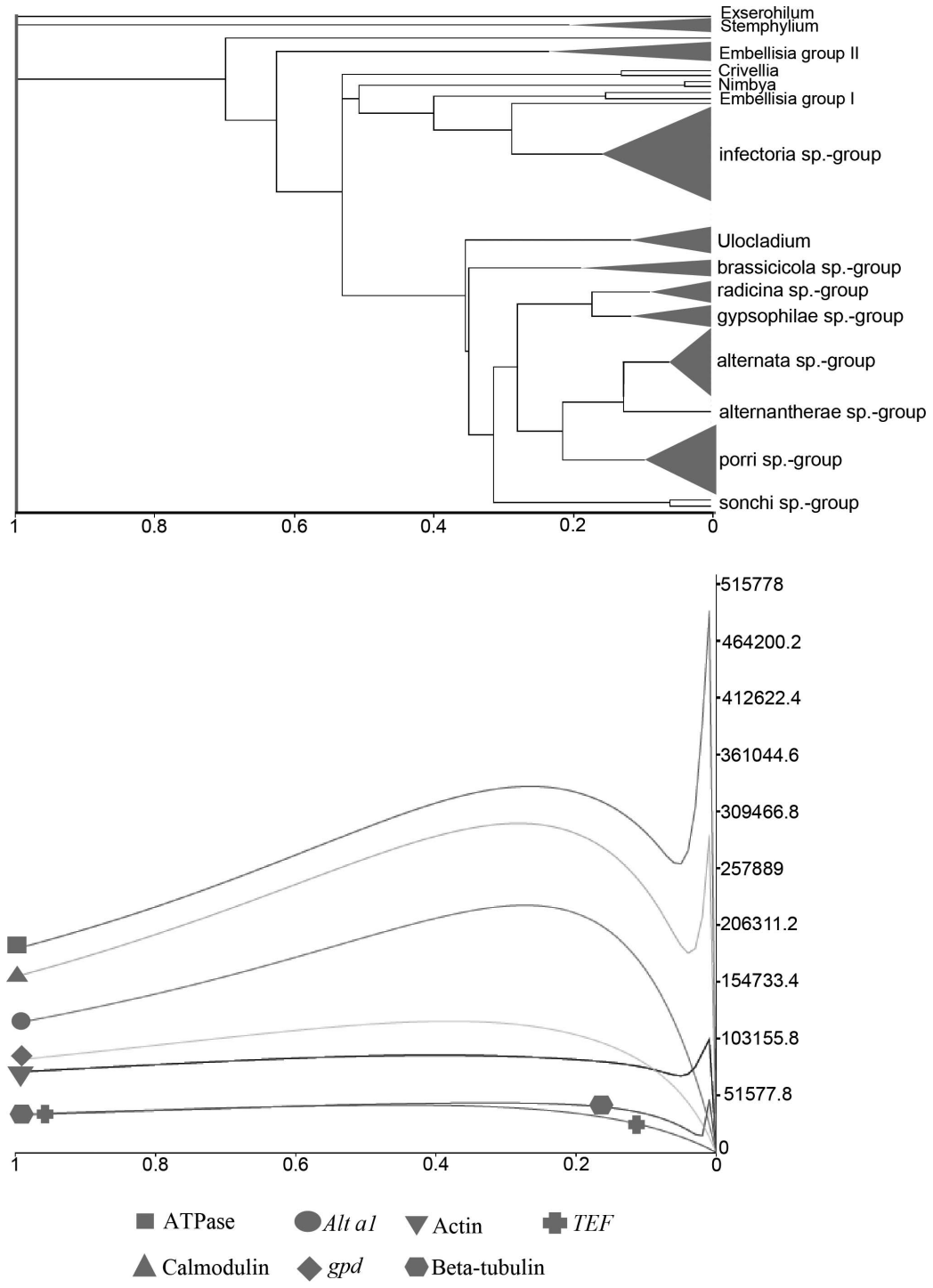

FIG. 2. Phylogenetic informativeness profiles for seven genes: plasma membrane ATPase (1441 bp), calmodulin (978 bp), *Alt a1* (497 bp), *gpd* (623 bp), actin (999 bp), beta-tubulin (351 bp) and *TEF* (696 bp).

obclavate, long ellipsoid or ellipsoid, small or moderate, septate, with a few longitudinal septa. Conidia are slightly constricted near some septa. Most conidia narrow gradually into a tapered beak or secondary conidiophore. Beak is no longer than conidial body. Apical secondary conidiophores are short or moderately long, with one or a few conidiogenous loci. Some conidia produce short solitary lateral secondary conidiophores with one or a few conidiogenous loci.

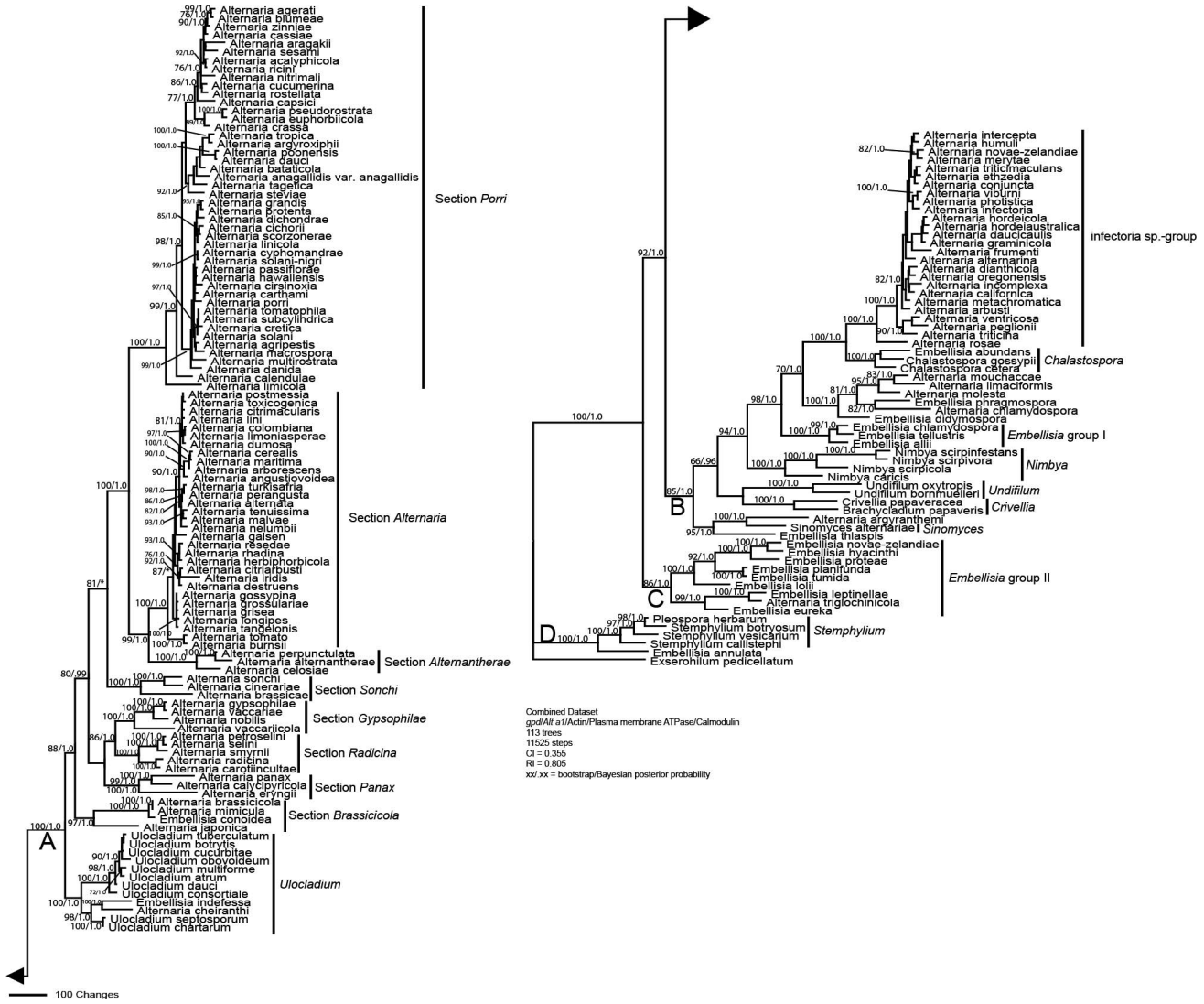

FIG. 3. One of 113 most parsimonious trees generated from maximum parsimony analysis of the five-gene combined dataset. Number in front of slash represents parsimony bootstrap values from 1000 replicates, and number after slash represents Bayesian posterior probabilities. Values represented by an asterisk were less than 70% for bootstrap or less than 0.95 for Bayesian posterior probability respectively. The scale bar indicates the number of nucleotide substitutions.

Type species: Alternaria alternata (Fr.) Keissl., Beih. Bot. Centralbl. 29:433 (1912).

***Section* Alternantherae** Lawrence, Gannibal, Peever & Pryor, sect. nov.

MycoBank MB802305

On PCA primary conidiophores are short or moderately long with one or a few conidiogenous loci; conidiogenous tip can be slightly enlarged. Conidia are solitary or paired. Juvenile conidia are ellipsoid, subcylindrical, rarer narrow ellipsoidal, lancet or near obclavate. The conidial body is narrow ellipsoid or ovoid sometimes subcylindrical, disto- and euseptate. Transverse divisions have no or one longitudinal or oblique septum. Conidium is dilute to medium golden tan. Lumina in the transverse divisions are octagonal or almost rounded, rarer almost rectangular. Fully developed conidia are slightly constricted near some septa. Conidial beak is long filiform unbranched, the same length as body or longer, sometimes terminates in swelling. Beak is septate or aseptate; swellings delimited by a septum can be present in the beak. The wall of young conidia is smooth, minutely ornamented or densely punctate.

Type species: Alternaria alternantherae (Holcomb & Antonop.) Simmons & Alcorn Mycotaxon 55: 142 (1995). Lawrence, Park & Pryor Mycological Progress 11:3 (2012).

***Section* Brassicicola** Lawrence, Gannibal, Peever & Pryor, sect. nov.

MycoBank MB802306

On PCA primary conidiophores are simple or branched, short or moderately long bearing one or a few conidiogenous loci. Conidial chains are moderately long or long, simple or branched. Mature conidia are ellipsoid, ovoid or somewhat obclavate, septate, usually with solitary apical and seldom with solitary lateral secondary conidiophores that are not longer than the length of the body and have only one conidiogenous locus. Conidia are slightly or strongly constricted at most of their transverse septa. Conidia have no or a few or many longitudinal septa. The apical cell of the terminal conidium in a chain is wide and rounded. Conidia are medium yellowish to dark brown; the spore wall is smooth, punctulate or ornamented. Chlamydospores present or absent.

Type species: Alternaria brassicicola (Schwein.) Wiltshire Mycol. Papers 20, p. 8. (1947).

***Section* Gypsophilae** Lawrence, Gannibal, Peever & Pryor, sect. nov.

MycoBank MB802307

On V-8 primary conidiophores are short or long, simple or occasionally branched, with one or a few conidiogenous loci. Conidia form simple chains of several units or moderately long branched chains. Juvenile conidia are ovoid or short-ellipsoid with a rounded conidium apex. Fully developed conidia are broadly or narrowly ovoid, ellipsoid or globose, dilute yellow-brown to dark brown; primary (basal) conidia tend to be obclavate. Conidia are small or relatively large, densely septate with large numbers of transverse and longitudinal septa, conspicuously constricted near some transverse septa. Conidial apex can be rounded, cylindrical, conical or produce a secondary conidiophore. Apical secondary conidiophores are short or longer than body with one or a few conidiogenous loci. Conidia have no or single short lateral secondary conidiophores.

Type species: Alternaria gypsophilae Neerg. Danish Species of *Alternaria* and *Stemphylium*, p. 207. (1945).

***Section* Panax** Lawrence, Gannibal, Peever & Pryor, sect. nov.

MycoBank MB802308

On V-8 primary conidiophores are short or moderately long, simple or branched, with one or a few conidiogenous loci. Conidia are in short or moderately long simple or branched chains. Juvenile conidia are narrow ovoid or ellipsoid with rounded or conical apical cell. Fully developed conidia are obclavate, long obclavate or ellipsoid to ovoid, medium sized, septate and constricted near several septa. Some mature conidia are deeply constricted and asymmetrical. Conidia have several or many longitudinal septa. Conidial apex consists of a rounded conical or cylindrical cell or short or long secondary conidiophore with one or several conidiogenous loci. Some conidia have one or several lateral secondary conidiophores. Conidia are yellow-tan to medium brown; the wall is smooth or minutely punctulate.

Type species: Alternaria panax Whetzel USDA BPI Bulletin No. 250, p 11. (1912).

***Section* Porri** Lawrence, Gannibal, Peever & Pryor, sect. nov.

MycoBank MB802309

On V-8 primary conidiophores are short to long, simple or branched, with one or a few conidiogenous loci. Conidia are solitary or in short or moderately long chains, simple or with solitary lateral conidia. Mature conidia are moderately large, broad ovoid, obclavate, ellipsoid, subcylindrical or obovoid, disto- and euseptate, slightly constricted near septa. Some mature conidia have one or a several filiform beaks. The beak apex is subtle or conversed into secondary conidiophores. Conidia can have lateral or apical secondary conidiophores with no filiform portion. Conidia have several transverse and longitudinal septa. Some conidia have one or a few lateral secondary conidiophores appearing from the body or beak. Conidia are pale yellow-tan to dark olivaceous brown; the wall is smooth or punctulate.

Type species: Alternaria porri (Ellis) Cif. Journal Porto Rico Dept. Agric. 14:30 (1930).

***Section* Radicina** Lawrence, Gannibal, Peever & Pryor, sect. nov.

MycoBank MB802310

On PCA primary conidiophores are straight, geniculate, knobby or hook-like, simple or branched, short or long, with one or a few conidiogenous loci at the apex of each conidiophore branch. Conidiogenous loci usually are successive, aggregated at the upper part of conidiophore. Sporulation resembles clumps or clusters of conidia. Conidia are solitary or in short chains. Juvenile conidia are ovoid or short ellipsoid, dilute yellow-tan. Mature conidia wide ovoid to narrowly ellipsoid, moderate in size, medium or dark brown, with several transverse and longitudinal septa. Apical cell is rounded, hemispherical or conical. Some conidia have a solitary short apical secondary conidiophore. Wall is smooth or punctulate.

Type species: Alternaria radicina Meier, Drechsler & E.D. Eddy Phytopathology 12:164 (1922).

Section *Sonchi* Lawrence, Gannibal, Peever & Pryor, sect. nov.

MycoBank MB802311

On PCA primary conidiophores are moderately long or long, simple or branched, with one or a few conidiogenous loci. Conidia are solitary or in short chains. Juvenile conidia are ovoid to narrowly ellipsoid, subcylindrical or obclavate with a rounded or broadly tapered apical cell. Mature conidia are subcylindrical, broadly ovoid, broadly ellipsoid or obclavate, moderately large or large, dilute straw-colored or medium yellow-tan, with several transverse and longitudinal septa, slightly or sufficiently constricted near the septa. The conidium apex is blunt, consisting of a broadly tapered apical cell or short sturdy beak or secondary conidiophore. Secondary conidiophore is short one-celled or as long as or longer than the spore body. Wall is smooth or punctulate.

Type species: Alternaria sonchi J.J. Davis, in J.A. Elliott, Bot Gazette 62:416 (1916).

ADDITIONAL ACCEPTED SPECIES

Based on its established morphological characters and phylogenetic analyses presented in this work, we hereby propose the transfer of *Embellisia conoidea* to *Alternaria* sect. Sonchi.

Alternaria conoidea (Simmons) Lawrence, Gannibal, Peever & Pryor, *comb. nov.*

MycoBank MB802303.

Basionym: Embellisia conoidea E.G. Simmons, Mycotaxon 17:226 (1983).

DISCUSSION

This study continues the ongoing systematic revision of the genus *Alternaria* with a specific goal of organizing this group of fungi at the infrageneric level. Several fungal genera have benefited greatly from this type of additional taxonomic resolution (e.g. sections, series) including *Aspergillus*, *Penicillium*, *Fusarium* and *Xylaria* (Pitt 1979, Guadet et al. 1989, Lee et al. 2000, Peterson et al. 2008, Moretti 2009). Some of the proposed sections within each of these genera are in agreement with prior classifications based on morphological characters, while others conflict with traditional classifications. However, the advent of molecular analyses has overturned some of these groupings, thus advancing understanding of the genetic basis of morphology. Ultimately, systematists seek agreement between morphological and molecular data leading to phylogenetic clades. Subgeneric taxonomic status such as sections or series may be achieved for a group of taxa when morphological features and molecular data are congruent with robust support.

This study describes the phylogenetic relationships among species of *Alternaria* and closely related genera in *Ulocladium*, *Embellisia*, *Nimbya*, *Crivellia*, *Undifilum*, *Sinomyces*, *Chalastospora*, *Stemphylium* and *Exserohilum* based on nucleotide sequences from five phylogenetically informative nuclear loci, *gpd*, *Alt a1*, actin, plasma membrane ATPase and calmodulin. Results from each individual locus (*gpd*, *Alt a1*, actin, plasma membrane ATPase, calmodulin) and the combined dataset support the grouping of previously described species-groups based on molecular data and morphological characteristics. This work also revealed two additional phylogenetic *Alternaria* species-groups, the gypsophilae and panax species-groups nested within the asexual *Alternaria* clade. Given strong phylogenetic and morphological support for all eight asexual species-group designations this work supports the elevation of each asexual species-group of *Alternaria* to the taxonomic status of section.

Phylogenetic results from partial datasets of three loci (*RPB2*, *Tsr1*, chitin synthase) produced topologies that were inconsistent with previous studies and additional loci studied in this work. It was hypothesized that these inconsistencies were systematic error resulting from codon substitution saturation, based on examination of the nucleotide alignments. Subsequent statistical analysis confirmed that the third codon position had experienced substantial saturation for these loci, and this result was corroborated by plotting the proportion of substitutions against sequence divergence using F84 evolutionary distance. In this analysis, a codon site is considered saturated if a plateau of substitution is reached with an increase in sequence divergence. The finding of this type of systematic error in phylogenetic study of *Alternaria* and related genera was not unusual in that saturation at the third codon position appears to be a commonly encountered obstacle among protein-coding loci used to infer evolutionary relationships among fungi, particularly when the study's evolutionary scope includes several genera (Hansen et al. 2005, Miller and Huhndorf 2005, Sung et al. 2007).

The best approach is hotly debated for dealing with genes that exhibit substitution saturation in phylogenetic analyses. Presently, there are two opinions regarding the exclusion or inclusion of these highly variable sites. The first states that these fast evolving saturated sites should be excluded from phylogenetic analyses based on the signal/noise ratio that can produce positively misleading phylogenetic hypotheses (Swofford et al. 1996, Blouin et al. 1998, Xia et al. 2003). The competing opinion states that the

inclusion of highly variable sites increases the ability to redice stochastic errors and increase statistical support for branching nodes (Edwards et al. 1991, Källersjö et al. 1998, Müeller et al. 2006, Simmons et al. 2006). More practically, Björklund (1999) suggests that third codon position should be included in phylogenetic analyses unless there is a priori knowledge that suggests that it is significantly misleading. This is consistent with Hennig's auxiliary principle that states ''… always assume homology in the absence of contrary evidence'' (Hennig et al. 1966). Based on all previous analyses phylogenetic relationships and tests of substitution saturation, these three loci have experienced substantial substitution saturation at the third codon position and therefore were excluded from additional analyses to avoid introducing systematic error.

Genes that evolve at different rates possess different abilities to effectively discriminate different phylogenetic scales, for example slowly evolving genes such as *RPB2* and *TEF* have been routinely used to resolve early divergences within high level phylogenetic studies (Baldauf and Palmer 1993, Stiller and Hall 1997, Hirt et al. 1999, Cheney et al. 2001), while faster evolving sequences (i.e. *Alt a1*) have been used to infer evolutionary relationships at lower phylogenetic scales (Hong et al. 2005). Phylogenetic analyses and test of phylogenetic informativeness have revealed that beta-tubulin and *TEF* do not possess phylogenetic utility to resolve recent divergences among *Alternaria* and closely related genera because of their slow molecular evolution (Peever et al. 2004). However, the five remaining loci (*gpd*, *Alt a1*, actin, plasma membrane ATPase and calmodulin) possess high phylogenetic utility and produce a strongly supported hypothesis regarding the evolutionary relationship among *Alternaria* and closely related genera.

The systematics of *Alternaria* and closely related genera has been plagued with erroneous taxonomic placement of morphologically similar taxa. Lawrence et al. (2012) demonstrated that morphological characters of closely related genera may be positively misleading and are not phylogenetically informative in certain cases. Previously described morpho groups of *Alternaria* first were proposed by means of morphological characters of conidia and subsequent molecular phylogenetic analyses have provided support for many of the morpho groups resulting in phylogenetic species-groups (Pryor and Gilbertson 2000, Hong et al. 2005, Lawrence et al. 2012), while other morpho groups such as the *Alternaria cheiranthi* species-group (Simmons 1995) does not form a phylogenetic clade (Pryor and Gilbertson 2000).

The use of single-gene phylogenies routinely yields poorly supported trees due to limited number of informative sites and stochastic noise that leads to inaccurate phylogenetic hypotheses (Brinkmann et al. 2005). This study is the most comprehensive phylogenetic analysis of *Alternaria* and closely related fungal genera to date based on the number of taxon sampled and the number of loci examined. This research examined the phylogenetic utility of 10 nuclear protein-coding loci from 176 species spanning 10 genera. Results from this study demonstrate that five (*gpd*, *Alt a1*, actin, plasma membrane ATPase, calmodulin) of the 10 loci possess substantial phylogenetic utility to produce a strongly supported robust phylogeny for the sexual and asexual lineages of *Alternaria* and allied genera.

This research is also the first to provide strong statistical support for all previously described asexual phylogenetic lineages of *Alternaria* and elucidates two newly described phylogenetic lineages, the gypsophilae species-group with four species and panax species-group with three species. Phylogenetic results provide strong statistical support for nine phylogenetically distinct lineages of *Alternaria*. Eight of the *Alternaria* phylogenetic lineages form a strongly supported monophyletic group that is sister to the asexual paraphyletic genus *Ulocladium*. Pryor and Gilbertson (2000) suggested expanding the morphological description of *Alternaria* to include *Ulocladium* based on their phylogenetic analyses. This study has revealed that *Alternaria* and *Ulocladium* are two distinct and strongly supported phylogenetic lineages. The analyses performed here fully resolved the evolutionary relationships within six of the nine phylogenetic lineages of *Alternaria*.

Results from this study demonstrate that conidial morphological characters are useful for delimitation of most sections of *Alternaria*, however substantial morphological homoplasy is evident. Phylogenetically distant sections, *Brassicicola* and *Alternaria*, and the infectoria species-group are hypothesized as having experienced convergent evolution by producing morphologically similar chains of small multicellular, darkly pigmented spores. Conidia from section *Brassicicola* are readily distinguished from section *Alternaria* and the infectoria species-group by producing extremely small mostly phragmosporic conidia with heavily melanized transverse septa. Additionally, section *Alternaria* and the infectoria species-group produce chains of phaeodictyospores that are virtually identical even though these two groups are phylogenetically divergent.

Conclusions of this study demonstrate that eight asexual phylogenetic lineages of *Alternaria* are strongly supported by the analysis of the five-gene

combined dataset. All sections of *Alternaria*, which includes *Brassicicola*, *Panax*, *Gypsophilae*, *Radicina*, *Sonchi*, *Alternantherae*, *Alternaria* and *Porri*, cluster as a strongly supported monophyletic group sister to the asexual paraphyletic genus *Ulocladium*. *Alternaria* as currently circumscribed is polyphyletic because the sexual *Alternaria* (*Lewia* teleomorph) in the infectoria species-group do not cluster with the asexual *Alternaria* lineage but instead clusters near other sexual taxa such as *Allewia* (*Embellisia* anamorph), *Macrospora* (*Nimbya* anamorph) and *Crivellia* (*Brachycladium* anamorph). The current polyphyly of *Alternaria* is due to the inclusion of non-phylogenetic *Alternaria* from the infectoria clade as a result of morphological delimitation based on spore character homoplasy. This study clearly reveals that caution must be used when including taxa into previously described genera based on potentially homoplastic morphological characters in the absence of molecular data. Phylogenetic analyses based on recognized informative loci need to be conducted, and these data either corroborate the inclusion of the taxa into the described genus in question or provide evidence of a unique phylogenetic lineage.

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