

***Undifilum*, a new genus for endophytic *Embellisia oxytropis* and parasitic *Helminthosporium bornmuelleri* on legumes**

B.M. Pryor, R. Creamer, R.A. Shoemaker, J. McLain-Romero, and S. Hambleton

Abstract: Fungal endophytes of *Oxytropis kansuensis* Bunge from China, previously described as *Embellisia oxytropis* Q. Wang, Nagao & Kakish, and endophytes of *Oxytropis sericea* Nutt. and *Oxytropis lambertii* Pursh from the United States were compared and are reported here as conspecific members of a new genus in the Pleosporaceae, *Undifilum*, based on morphological and molecular analyses. Morphological comparisons revealed characters that are similar to those of the genus *Embellisia* including conidia ovate to obclavate to long ellipsoid, straight or slightly to decidedly inequilateral with occasionally one or two cells distinctly swollen, and transepta occasionally thickened, dark, and rigid in comparison with the exterior conidium wall. However, upon germination, conidia produced unique and diagnostic germ tubes that were wavy or undulating in their growth until branching. Moreover, all isolates were found to produce the toxic alkaloid swainsonine. Parsimony analysis of sequences from ITS1–5.8S–ITS2, glyceraldehyde-3-phosphate dehydrogenase gene, and mitochondrial small subunit rDNA data sets revealed that the *Oxytropis* endophytes formed a clade distinct from other *Embellisia* species and species in the genera *Alternaria*, *Ulocladium*, *Nimbya*, and *Crivellia*. A second taxon, *Helminthosporium bornmuelleri* P. Magnus, was reexamined and found to possess similar morphological features to those of the *Oxytropis* isolates, but lacked swainsonine production. Sequence analysis placed this second taxon in the same clade with high bootstrap support. The distinct morphology and genetics of these taxa demonstrates that these fungi, both recovered from legumes, represent a new genus, hereinafter described as *Undifilum*. The two species now placed in this genus are redescribed as *Undifilum oxytropis* and *Undifilum bornmuelleri*.

Résumé : Les auteurs ont comparé les endophytes fongiques de l'*Oxytropis kansuensis* Bunge de Chine préalablement décrits comme *Embellisia oxytropis* Q. Wang, Nagao & Kakish, et les endophytes de l'*Oxytropis sericea* Nutt. et de l'*Oxytropis lambertii* Pursh des Etats-Unis; on en fait des membres conspécifiques le nouveau genre *Undifilum* membre des Pleosporaceae, basé sur des analyses morphologiques et moléculaires. Les comparaisons morphologiques révèlent des caractères similaires à ceux du genre *Embellisia*, incluant des conidies ovées à obclavées ou ellipsoïdes, droites ou légèrement à nettement inéquilatérales, avec occasionnellement une ou deux cellules distinctes et enflées, munies de transepts occasionnellement épais, sombres, et rigides comparativement à la paroi externe des conidies. Cependant, en cours de germination, les conidies produisent des tubes germinatifs uniques et caractéristiques d'aspect sinueux ou à croissance ondulée jusqu'à leur ramification. De plus, on constate que tous les isolats produisent de la swainsonine, un alcaloïde toxique. Les analyses en parcimonie des ITS1–5.8S–ITS2, du gène de la déshydrogénase du glyceraldéhyde-3-phosphate, et les données de la petite sous unité de l'ADN mitochondrial montrent que les endophytes des *Oxytropis* forment un clade distinct différent des autres espèces d'*Embellisia* ainsi que les espèces des genres *Alternaria*, *Ulocladium*, *Nimbya* et *Crivellia*. Les auteurs ont réexaminé un second taxons, l'*Helminthosporium bornmuelleri* P. Magnus et ont constaté qu'il possède des caractéristiques morphologiques similaires à celles des isolats des *Oxytropis*, mais ne produit pas de swainsonine. Les analyses de séquences situent ce deuxième taxon dans le même clade avec un fort support bootstrap. La morphologie et la génétique distinctes de ce taxon démontrent que ces champignons, tous deux obtenus de légumineuses, représentent un nouveau genre, ici décrit comme *Undifilum*. Les auteurs redécrivent les deux espèces maintenant attribuées à ce genre comme *Undifilum oxytropis* et *Undifilum bornmuelleri*.

[Traduit par la Rédaction]

Introduction

Locoweed is the common name for a group of toxic plants in the genera *Oxytropis* and *Astragalus* (Fabaceae)

endemic to arid and semiarid regions of the western USA (Allred 1991). Recently, endophytic fungi were isolated from stems, seeds, and leaves from several species of locoweed, including *Oxytropis lambertii* Pursh and *Oxytro-*

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B.M. Pryor.¹ Department of Plant Sciences, University of Arizona, Tucson, AZ 85721, USA.

R. Creamer and J. McLain-Romero. Department of Entomology, Plant Pathology, and Weed Science, New Mexico State University, Las Cruces, NM 88003, USA.

R.A. Shoemaker and S. Hambleton. Agriculture and Agri-Food Canada, Ottawa, ON K1A 0C6, Canada.

¹Corresponding author (e-mail: bmpyor@u.arizona.edu).

pis sericea Nutt. (Ralphs et al. 2002; Braun et al. 2003; Gardner et al. 2003). The endophytes did not sporulate or produce external mycelium on their host plants, or cause obvious damage or disease, but were presumed to be intimately associated with locoweed, since they were recovered from both toxic plant species.

Consumption of locoweed by cattle, sheep, and horses results in the development of locoism, a neurological disease that can be fatal to livestock (James and Panter 1989). The cause of locoism is due to the presence in locoweed of swainsonine (Molyneux et al. 1995), a toxic alkaloid that inhibits α -mannosidase, resulting in lysosomal accumulation of mannose-rich oligosaccharide chains and cell vacuolization. Swainsonine was first discovered in Darling peas, *Swainsona canescens* (Benth.) F. Muell., an herbaceous legume native to Australia (Colgate et al. 1979). Consumption of Darling peas also produces a neurological disease in livestock known as swainsona poisoning, with symptoms similar to locoism.

The presence of swainsonine in locoweed appears to be solely a product of the resident endophytic fungi (Braun et al. 2003). Consumption of dried endophyte mycelia by rats induces symptoms identical to those caused by consumption of locoweeds (McLain-Romero et al. 2004), including vacuolization of hepatic, renal, and pancreatic tissues. Swainsonine production by locoweed endophyte strains in vitro is highly correlated with the swainsonine level of their host plant population from which they were recovered (Braun et al. 2003). The production of variable amounts of swainsonine by different endophyte isolates when grown in vitro under identical cultural conditions suggests that swainsonine production is genetically controlled by the endophyte.

In earlier studies, locoweed endophytes were referred to as *Alternaria* spp. or *Embellisia* spp. (Braun et al. 1997; Braun 1999; Pryor et al. 2004), and precise nomenclature was not yet established. Recently, the name *Embellisia oxytropis* Q. Wang, Nagao & Kakish was proposed for an endophyte recovered from *Oxytropis kansuensis* Bunge from Qinghai, China (Wang et al. 2006). The morphology attributed to *E. oxytropis* was very similar to that of endophytes recovered from *Oxytropis* spp. in the USA, and it was hypothesized that the two taxa were synonymous. However, the taxonomic study of *E. oxytropis* was based solely on morphology of isolates in pure culture and did not include examination of molecular characters, which would allow for a more robust phylogenetic placement.

The objective of this study was to further examine endophytes isolated from *Oxytropis* spp. from New Mexico, USA, and compare them with the type of *E. oxytropis* from China. In addition, an isolate of *Helminthosporium bornmuelleri* P. Magnus, which occurs as a pathogen of crown-vetch [*Securigera varia* (L.) Lassen (\equiv *Coronilla varia* L.)] in Austria and has morphology similar to that of the locoweed isolates and *E. oxytropis*, was included. The study encompassed morphological comparisons, studies in pure culture, and molecular comparisons based upon sequences of the ITS1–5.8S–ITS2 region (ITS), the glyceraldehyde 3-phosphate dehydrogenase gene (*gpd*), and the mitochondrial rDNA small subunit (mtSSU). Comparisons were made among the taxa in question and among other described species in several genera with possible affinities in an effort to establish the appropriate generic and specific placement.

Materials and methods

Isolates used in this study

Isolations of endophytic fungi from locoweed were performed by methods described by Braun et al. (2003). Hyphal tips were taken from initial isolates and transferred onto plates of potato dextrose agar (PDA, Difco Laboratories, Franklin Lakes, N.J.), maintained at 18 °C or room temperature, and transferred every few months. Two isolates were selected for further morphological and molecular characterization based upon differences in gross colony morphology, recovery location and host, and initial screening for swainsonine production (Table 1). The holotype specimen (HMJAU 10012) and three paratypes of *E. oxytropis* (HMJAU 10027, 10030, 10032) were obtained from Q. Wang, Jilin Agricultural University, Changchun, China, for examination. Re-isolations from dried paratypes (10030 and 10032) were used for morphological examinations and DNA extractions (Table 1). An isolate of *H. bornmuelleri* was obtained from C. Scheuer, Graz, Austria, and maintained in the same manner as the locoweed isolates and the paratypes of *E. oxytropis* (Table 1).

Cultural and morphological characterization

For morphological examination, isolates were cultured on PDA, potato carrot agar (PCA, Simmons 1992), and water agar with autoclaved alfalfa stems. Cultures were incubated in clear plastic boxes with the surface of each dish 40 cm beneath fluorescent lights (Sylvania cool-white (Sylvania Corporation, Danvers, Mass.), 10 h (light) : 14 h (dark) at 22 °C. Following 2 weeks incubation, isolates were examined for colony morphology and morphological characteristics of the conidia and conidiophores. Conidia and conidiophores were taken approximately 2 mm inside the colony margin, mounted in water on a microscope slide, and observed with an Olympus BX61 microscope at 640 \times . Conidium length and width measurements were taken from 40 randomly selected conidia, 10 in each of four categories corresponding to conidium septation (conidia with 2, 3, 4, or 5 transverse septa), and mean values were calculated. In addition, the number of transepta per conidium was determined for 20 randomly selected conidia in each of three fields of view (100 \times) as well as the maximum number of septa per isolate. Immature conidia (i.e., lacking pigmentation and septation) were not considered.

Analysis of swainsonine production

Isolates were grown on PDA or PCA for 3 weeks, and resulting mycelia mats from six to eight plates were scraped off and air dried at 50 °C for 18 h. Dried mycelium was ground in liquid nitrogen. Samples were assayed for swainsonine using the methods outlined in Gardner et al. (2001). Briefly, 100 mg of ground mycelia were extracted with chloroform and acetic acid, and the acetic acid fraction was passed through a cation exchange resin. The final extract was analyzed by liquid chromatography mass spectrometry (LC-MS).

The LC-MS system consisted of an HP 1100 binary solvent pump and autosampler, a Betasil C18 reversed-phase HPLC column (100 \times 2 mm), and a Finnigan LCQ mass spectrometer (Thermo Fisher Scientific, Waltham, Mass.).

Table 1. Source of fungal cultures assessed for swainsonine.

Isolate	Host plant	Location	Collection date	Swainsonine production ^a
RC OIB9	<i>Oxytropis lambertii</i>	Grants, NM, USA	1997	0.03
RC OsL12	<i>Oxytropis sericea</i>	Colfax, NM, USA	1997	0.17
DAOM 237696 ^b	<i>Oxytropis kansuensis</i>	Qinghai, China	2004	0.25
DAOM 237697 ^b	<i>O. kansuensis</i>	Qinghai, China	2004	0.25
DAOM 231361 ^c	<i>Securigera varia</i>	Austria	2002	None detected

^aValues are in mg/mg dry mass mycelium.

^b*Embellisia oxytropis*: DAOM 237696 = HMJAU 10030, DAOM 237697 = HMJAU 10032.

^c*Helminthosporium bornmuelleri*.

Swainsonine was eluted using an isocratic mixture of 5% methanol in 20 mmol/L ammonium acetate at a flow rate of 0.5 mL/min. Sample injection size was 20 µL. Ionization was achieved using an atmospheric pressure chemical ionization source with a vaporizer temperature of 450 °C and corona discharge current of 5 µA. The mass spectrometer was run in an MS2 mode, scanning product ions over a mass range of 70–300 amu. The swainsonine peak area was measured from the reconstructed ion chromatogram (*m/z* 156) and quantitation based on an external calibration standard. The resulting swainsonine concentration (mg/mL) of the injected sample was converted to percent dry mass of the original plant material.

DNA extraction, PCR amplification, and sequencing

Phylogenetic analyses were based upon sequences amplified from the ITS rDNA region, the glyceraldehyde-3-phosphate dehydrogenase (*gpd*) gene, and the mitochondrial small subunit (mtSSU) rDNA region. DNA from the two locoweed isolates was extracted from 0.2 g mycelia. Samples were ground with a mortar and pestle in liquid nitrogen. Lysis buffer (700 µL of 50 mmol/L Tris-HCl, 50 mmol/L EDTA, and 3% SDS) was added to the samples, which were mixed and incubated for 1 h at 65 °C. An equal volume of phenol-chloroform (1:1, *v/v*) was added to each sample and mixed vigorously. The samples were centrifuged at 11 750g, after which the aqueous phase was removed. Organic solvent extraction of the aqueous phase was repeated again, followed by a final extraction with an equal volume of chloroform and a final centrifugation. DNA was precipitated from the aqueous phase by the addition of 1/10 volume 3 mol/L sodium acetate and 6/10 volume isopropanol, centrifuged for 10 min at 11 750g, washed with 70% ethanol, and dried. The resulting DNA pellets were resuspended in 100 µL TE (0.5 mol/L Tris-HCl, 10 mmol/L EDTA). DNA from *E. oxytropis* and *H. bornmuelleri* was extracted using the UltraClean Microbial DNA Isolation kit (Biocompare, Inc., South San Francisco, Calif.) according to the manufacturer's protocol.

PCR amplification was performed using ITS primers ITS4 and ITS5 (Braun et al. 2003), *gpd* primers *gpd1* and *gpd2* (Berbee et al. 1999), and mtSSU primers NSM1 and NMS2 (Li et al. 1994). PCR procedures and conditions for target loci were as described in a previous study (Pryor and Bigelow 2003). Amplification was carried out in a Perkin-Elmer model 480 thermal cycler (Norwalk, Conn.). Sequencing of amplicons was done by Big Dye Terminator methods (Applied Biosystems, Foster City, Calif.) using an ABI 3100 capillary sequencer. Primers used for sequencing were those

used in the original PCR amplification. Both DNA strands were sequenced for nucleotide confirmation.

Phylogenetic analysis

DNA sequences from each isolate and those from other *Embellisia* spp. and species of *Alternaria* (encompassing five species groups (Pryor and Gilbertson 2000)), *Ulocladium*, *Nymphaea*, *Crivellia*, and *Stemphylium* (Table 2) were aligned with the PILEUP program of the GCG Sequence Analysis software package. For all amplified regions, manual adjustments of sequence alignments were performed with the data editor program of MacClade Phylogenetic software. Phylogenetic analyses were performed with programs contained in PAUP Phylogenetic software. Sequences of *Exserohilum pedicellatum* (Henry) Leonard & Suggs were included as the outgroup in all analyses. Parsimony analysis heuristic searches for the most-parsimonious trees were conducted with random stepwise addition and branch swapping by tree bisection-reconnection (TBR). Sequence gaps were treated as missing data. To assess the effect of ambiguous regions in the alignment, analyses were repeated after removing ambiguous sections and (or) introns. For each analysis, 1000 bootstrap replicates were performed to assess the statistical support for each tree topology. Concordance between data sets was evaluated based upon the partition-homogeneity test (PHT) implemented in PAUP. For combined data sets, parsimony analyses were run as previously described.

Results

All *Oxytropis* endophytes were very slow growing on all media tested, but growth was slowest on PDA, often requiring more than 30 d incubation to attain colony diameter of at least 5 mm (Figs. 1 and 2; Table 3). After colonies reached 5–6 mm in diameter, continued radial growth was extremely slow or arrested. Colony color ranged from dark grey to black on all media tested. Isolates produced colonies composed of two hyphal types: a basal layer of densely packed, short, torulose, and intertwined hyphal segments, and a surface layer of more typical elongated filamentous aerial hyphae (Figs. 3 and 4). Some hyphal segments contained an abundance of guttulate cells (Figs. 5 and 6). Chlamydospore production was evident, particularly RC OsL12 (Fig. 7). Sporulation occurred on geniculated conidiophores (Figs. 8–13 and 15). Upon germination, conidia produced distinctive and diagnostic germ tubes that were wavy or undulating in their growth until branching (Fig. 14), then continuing as typically contorted, torulose vegetative growth.

Table 2. Isolates used for phylogenetic analyses, their sources, and GenBank accession numbers.

Species	Source ^a	GenBank accession		
		ITS	<i>gpd</i>	mtSSU
<i>Alternaria alternata</i> ^b	EGS 34–016	AF347031	AY278808	AY278849
<i>A. arborescens</i>	EGS 39–128	AF347033	AY278810	AY278851
<i>A. brassicicola</i>	BMP 0325	AF229462	AY278813	AF229652
<i>A. carotiincultae</i>	EGS 26–010	AF229465	AY278798	AF229654
<i>A. cheiranthi</i>	EGS 41–188	AF229457	AY278802	AF229655
<i>A. crassa</i>	BMP 0172	AF229464	AY278804	AF229656
<i>A. dauci</i>	ATCC 36613	AF229466	AY278803	AF229657
<i>A. destruens</i>	EGS 46–069	AY278836	AY278812	AY278853
<i>A. ethzedia</i>	EGS 37–143	AY278833	AY278795	AY278847
<i>A. infectoria</i>	EGS 27–193	AF347034	AY278793	AY278846
<i>A. japonica</i>	ATCC13618	AF229474	AY278814	AF229661
<i>A. longipes</i>	EGS 30–033	AY278835	AY278811	AY278852
<i>A. macrospora</i>	BMP 0173	AF229469	AY278805	AF229663
<i>A. mimicula</i>	EGS 01–056	FJ266477 ^c	AY562415	FJ357321 ^c
<i>A. petroselini</i>	EGS 09–159	AF229454	AY278799	AF229664
<i>A. porri</i>	ATCC 58175	AF229470	AY278806	AF229667
<i>A. radicina</i>	ATCC 96831	AF229472	AY278797	AF229668
<i>A. selini</i>	EGS 25–198	AF229455	AY278800	AF229673
<i>A. smyrnii</i>	EGS 37–093	AF229456	AY278801	AF229674
<i>A. solani</i>	ATCC 58177	AF229475	AY278807	AF229675
<i>A. tenuissima</i>	EGS 34–015	AF347032	AY278809	AY278850
<i>A. triticina</i>	EGS 41–050	AY278834	AY278796	AY278848
<i>Brachycladium papaveris</i>	P351	FJ357310 ^c	FJ357298 ^c	FJ357322 ^c
<i>Crivellia papaveracea</i> ^b	P354.8	FJ357311 ^c	FJ357299 ^c	FJ357323 ^c
<i>Embellisia allii</i> ^b	EGS 38–073	AY278840	AY278827	AY278857
<i>E. conoidea</i>	CBS 132.89	FJ348226 ^c	FJ348227 ^c	FJ357324 ^c
<i>E. didymospora</i>	CBS 766.79	FJ357312 ^c	FJ357300 ^c	FJ357325 ^c
<i>E. indefessa</i>	EGS 30–195	AY278841	AY278828	AY278858
<i>E. lolii</i>	EGS 43–054	FJ357313 ^c	FJ357301 ^c	FJ357326 ^c
<i>E. novae-zelandiae</i>	EGS 39–099	AY278844	AY278831	AY278861
<i>E. phragmospora</i>	EGS 27–098	FJ357314 ^c	FJ357302 ^c	FJ357327 ^c
<i>E. planifunda</i>	CBS 537.83	FJ357315 ^c	FJ357303 ^c	FJ357328 ^c
<i>E. proteae</i>	EGS 39–031	AY278842	AY278829	AY278859
<i>E. hyacinthi</i>	EGS 49–062	AY278843	AY278830	AY278860
<i>E. telluster</i>	EGS 33–026	FJ357316 ^c	FJ357304 ^c	FJ357329 ^c
<i>E. tumida</i>	CBS 589.83	FJ266481 ^c	FJ266493 ^c	FJ357330 ^c
<i>Exserohilum pedicellatum</i>	BMP 0384	AF229478	AY278824	AF229660
<i>Lewia infectoria</i> ^b	ATCC 12054	AF229480	AY278794	AF229666
<i>Nimbya caricis</i>	EGS 13–094	AY278839	AY278826	AY278856
<i>N. scirpicola</i> ^b	EGS 19–016	AY278838	AY278825	AY278855
<i>Pleospora herbarum</i> ^b	ATCC 11681	AF229479	AY278823	AF229665
<i>Stemphylium botryosum</i> ^b	ATCC 42170	AF229481	AY278820	AF229671
<i>S. callistephi</i>	BMP 0377	AF229482	AY278822	AF229672
<i>S. vesicarium</i>	ATCC 18521	AF229484	AY278821	AF229677
<i>Ulocladium atrum</i>	ATCC 18040	AF229486	AY278818	AF229680
<i>U. botrytis</i> ^b	ATCC 18043	AF229487	AY278817	AF229681
<i>U. chartarum</i>	ATCC 18044	AF229488	AY278819	AF229682
<i>U. consortiale</i>	BMP 3151001	AY278837	AY278816	AY278854
<i>Undifilum bornmuelleri</i> ^b	DAOM 231361	FJ357317 ^c	FJ357305 ^c	FJ357331 ^c
<i>U. oxytropis</i>	RC OsL12	AY228650	FJ357309 ^c	FJ357335 ^c
<i>U. oxytropis</i>	RC OIB9	FJ357320 ^c	FJ357308 ^c	FJ357334 ^c
<i>U. oxytropis</i>	DAOM 237696	FJ357318 ^c	FJ357306 ^c	FJ357332 ^c
<i>U. oxytropis</i>	DAOM 237697	FJ357319 ^c	FJ357307 ^c	FJ357333 ^c

^aAbbreviations for sources are as follows: ATCC, American Type Culture Collection, Manassas, VA 20108; BMP, B.M. Pryor, Department of Plant Pathology, University of Arizona, Tucson, AZ 85721; DAOM, Department of Agriculture, Ottawa, Mycological Collection, Ottawa, Ontario K1A 0C6; EGS, E.G. Simmons, Mycological Services, Crawfordsville, IN 47933; P, P. Inderbitzin, Department of Plant Pathology, Cornell University, Ithaca, NY 14850; RC, Rebecca Creamer, Department of Entomology, Plant Pathology, and Weed Science, New Mexico State University, Las Cruces, NM 88003.

^bIndicates type species for each genus. Type for *Brachycladium* is *B. penicillatum* and type for *Exserohilum* is *E. turcicum*.

^cSequences that were determined in this study.

Figs. 1–15. Figs. 1 and 2. *Undifilum oxytropis*. Growth of two isolates on PDA, 22 °C after 45 d. Scale bar = 10 mm. Fig. 1. RC OsL12. Fig. 2. OIB9. Figs. 3 and 4. Two types of hyphae produced by RC OsL12. Fig. 3. Linear fungal filaments. Scale bar = 35 µm. Fig. 4. Torulose cells. Scale bar = 35 µm. Fig. 5. Guttulate filaments. Scale bar = 13 µm. Fig. 6. Guttulate torulose cells. Scale bar = 13 µm. Fig. 7. Chlamydospore of RC OsL12. Scale bar = 35 µm. Figs. 8–13. Conidia of RC OsL12. Scale bar = 10 µm. Fig. 14. Wavy germ tube on V8 24 h. Scale bar = 10 µm. Fig. 15. Poric conidiogenesis in RC OsL12. Scale bar = 5 µm.

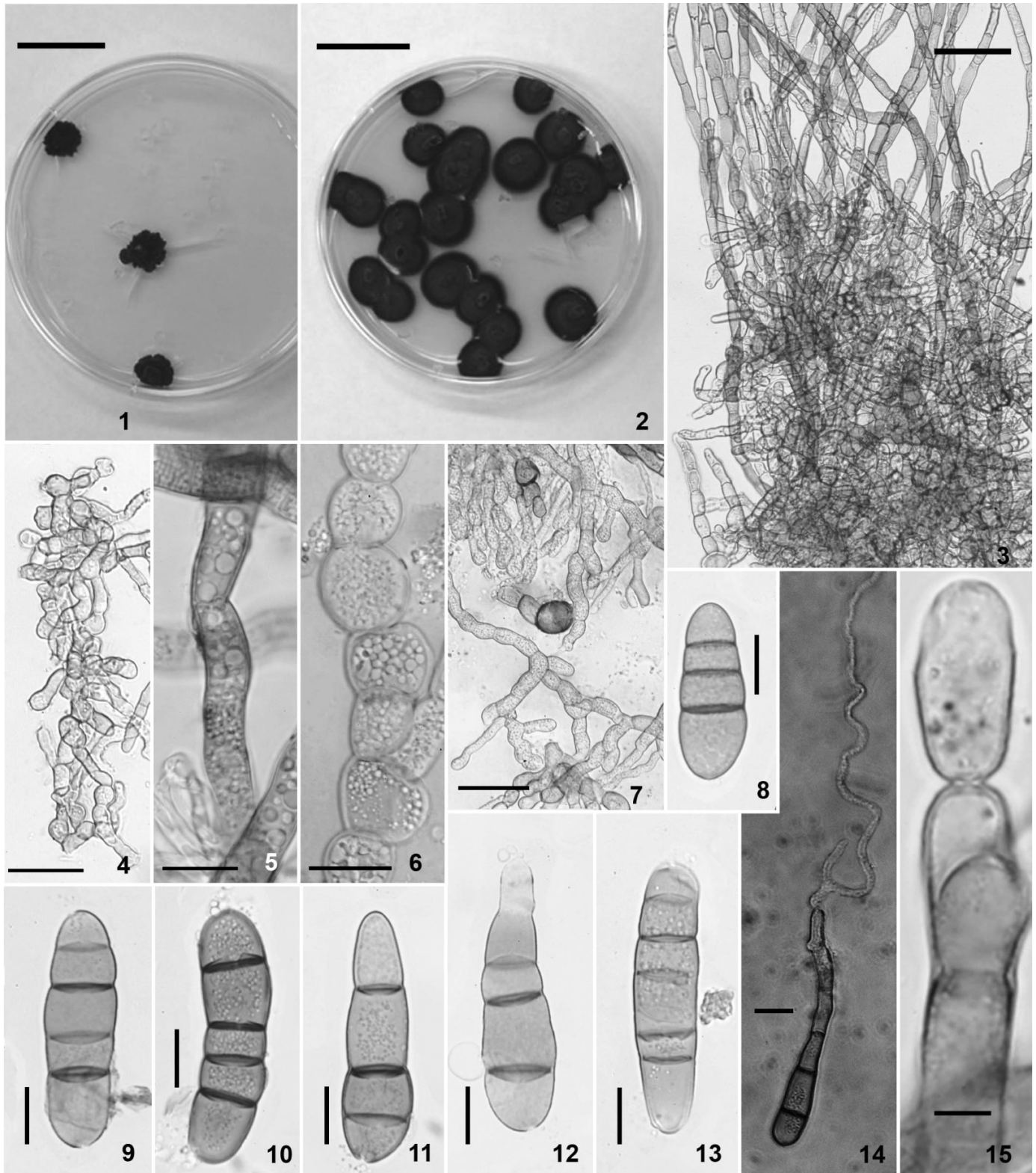


Table 3. Comparisons of growth rate and conidia among isolates of *Undifilum* recovered from locoweed in the USA, plus data from *Embellisia oxytropis* and *Helminthosporium bornmuelleri*.

Isolate	Medium	Colony growth ^b	Conidium length/no. of transepta ^d				Transepta	
			2 septa	3 septa	4 septa	5 septa	Avg. no. ^c	Max. no.
RC OsL12	APDA	6.3	32.4	41.0	46.8	50.8	3.1	7
RC OIB9	APDA	6.6	33.9	42.7	46.1	51.7	3.0	7
E.o. 10012 ^d	Dried PDA	na	37.8	46.0	56.3	55.0	3.1	6
E.o. 10032 ^e	PCA	9.0	35.0	41.2	51.0	55.9	2.9	5
H.b. 57132 ^f	Dried host	na	30.9	39.0	44.3	52.8	3.6	6
H.b. 231361 ^g	PCA	47.0	34.0	37.7	53.7	53.3	3.9	7

^aValues represent mean conidium length (μm) for 40 randomly selected conidia from four conidium categories based upon number of transepta.

^bValues for RC OsL12 and RC OIB9 represent mean colony diameter in millimetres for four colonies after 45 d on PDA. Values for cultures derived from E.o. 10032 and H.b. 231361 represent mean colony diameter in millimetres for four colonies after 35 d on PDA. Values for E.o. 10012 and H.b. 57132 were not available (na).

^cThe number of transepta per conidium was determined for 40 randomly selected conidia.

^d*Embellisia oxytropis* holotype HMJAU 10012 dried on PDA obtained from Dr. Qi Wang.

^eCulture derived from *E. oxytropis* HMJAU 10032 on PCA (DAOM 237697).

^f*Helminthosporium bornmuelleri* 57132 dried on host. Though collected later than the type, this specimen is from the same general locality on the same host and determined by Bornmüller.

^gCulture derived from *H. bornmuelleri* IMI 390145 on PCA (DAOM 231361).

Considerable variation in conidium size and shape was noted in cultures for both *Oxytropis* and *Securigera* isolates, but a comparison of conidium dimensions revealed no significant difference in length or width for each of the four categories of conidia (Table 3).

Isolates of *H. bornmuelleri* from crownvetch shared characteristics with those of the *Oxytropis* isolates. However, conidium size and septation were influenced by culture conditions. The longest and more frequently septate conidia were observed in cultures on sterilized alfalfa stems in water agar when compared with conidia from other media or the original description from the host plant. Both RC OsL12 and DAOM 231361 developed very well on the alfalfa medium.

The dimensions given by Magnus (1899) and Wang et al. (2006) are in agreement with those reported in this study. However, on alfalfa medium, RC OsL12 and DAOM 231361 gave conidia with more numerous septa and greater length. The character that does separate these taxa appears to be the basal cell of the conidia of *H. bornmuelleri*, which is generally wider than long or isodiametric, whereas in *E. oxytropis* the basal cell is usually longer than wide. The other cells tend to be short and slightly wider in the former.

Analysis of swainsonine production

All locoweed isolates and isolates of *E. oxytropis* recovered from *Oxytropis* species were found to produce the alkaloid swainsonine. Production varied from 0.03 to 0.25 mg swainsonine/mg dried mycelium (Table 1). Swainsonine was not detected in dry mycelium from the isolate of *H. bornmuelleri* recovered from crownvetch.

DNA extraction, PCR amplification, and sequencing

PCR resulted in the successful amplification of fragments ranging in size from 550 to 588 bp using primers ITS5 and ITS4, fragments ranging in size from 523 to 582 bp using primers *gpd1* and *gpd2*, and fragments ranging in size from 588 to 699 using primers NMS1 and NMS2 for the loco-

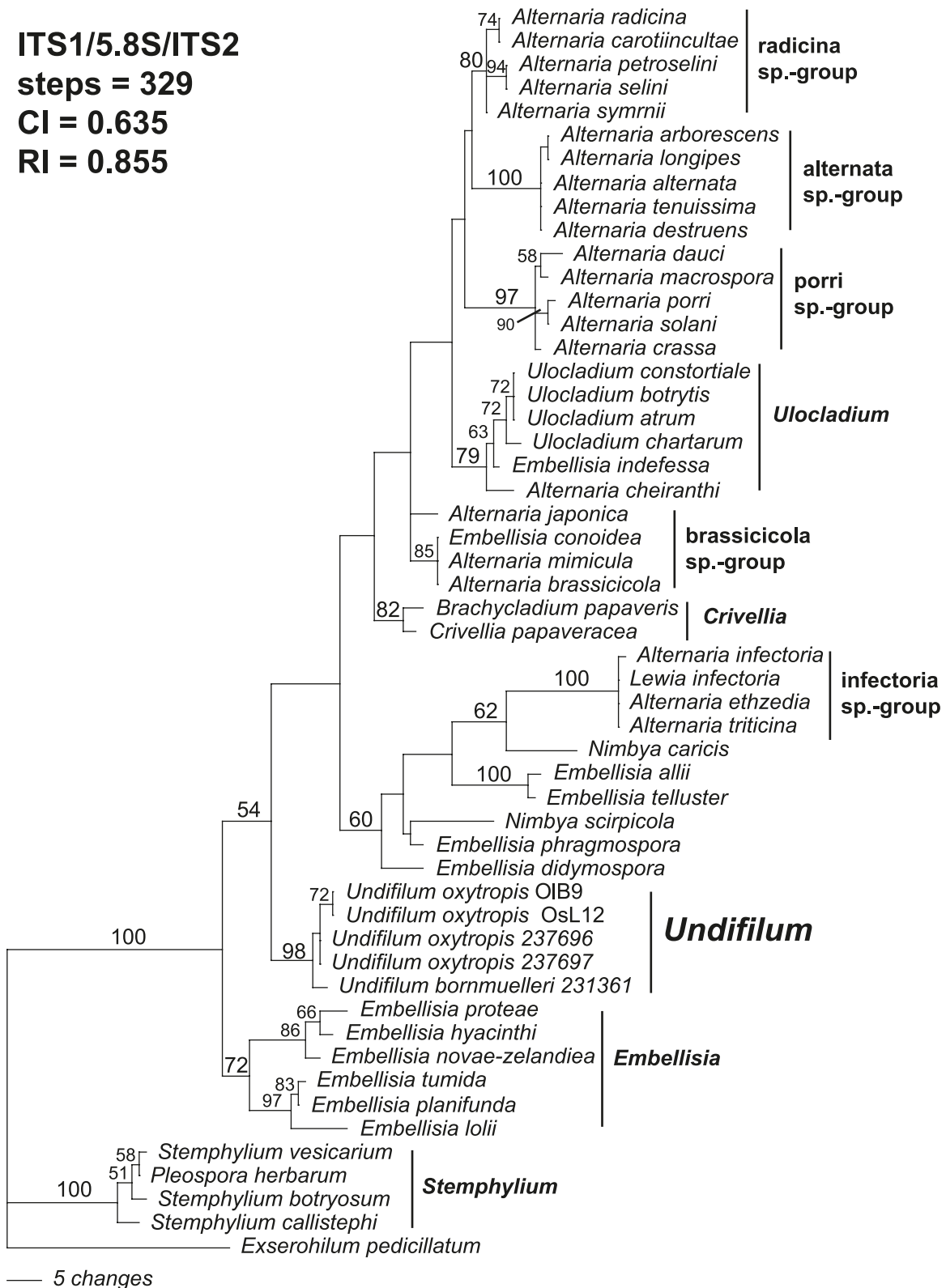
weed isolates *E. oxytropis* and *H. bornmuelleri*. All sequences determined in this study have been submitted to GenBank, and accession numbers are listed in Table 2.

Phylogenetic analysis

Alignment of the ITS sequences with those of *Alternaria*, *Nimbya*, *Embellisia*, *Ulocladium*, *Crivellia*, *Stemphylium*, and *Exserohilum* spp. resulted in a 634-character data set of which 161 characters (25.4%) were variable and 55 characters (8.8%) were parsimony informative. In the aligned data set, five regions of numerous indels were apparent, and the alignment of characters within these regions was variable. Variable region 1 (ITS-VR1) spanned characters 55–126. Within this region, a notable insertion (characters 75–106) was present in sequences from locoweed isolates, *E. oxytropis*, *H. bornmuelleri*, *Crivellia*, and *Brachycladium* spp., *Nimbya* spp., several *Embellisia* spp., members of the infectoria species-group, and members of the brassicicola species-group but not in sequences from the other isolates. Variable regions 2, 3, 4, and 5 (ITS-VR2, ITS-VR3, ITS-VR4, and ITS-VR5, respectively) spanned characters 194–210, 236–256, 445–472, and 551–591, respectively.

Maximum-parsimony analysis of the ITS data set yielded 216 equally most-parsimonious trees (steps = 329, CI = 0.635, RI = 0.855), which differed only in minor changes in the relationship among members within the principal clades and relationships between some of the basal principal clades (Fig. 16). Ten principal clades were evident, 9 of which corresponded with previously established monophyletic groups: the *Stemphylium* group, the *Embellisia* group, the infectoria species-group, the *Crivellia* group, the brassicicola species-group, the *Ulocladium* group, the porri species-group, the alternata species-group, and the radicina species group, (Pryor and Gilbertson 2002; Inderbitzin et al. 2006). Most of these groups were supported strongly by bootstrap values of > 80%, with the exception of the *Embellisia* group and the *Ulocladium* group, which were moderately supported by bootstrap values of 72% and 79%, respectively. Two *Nim-*

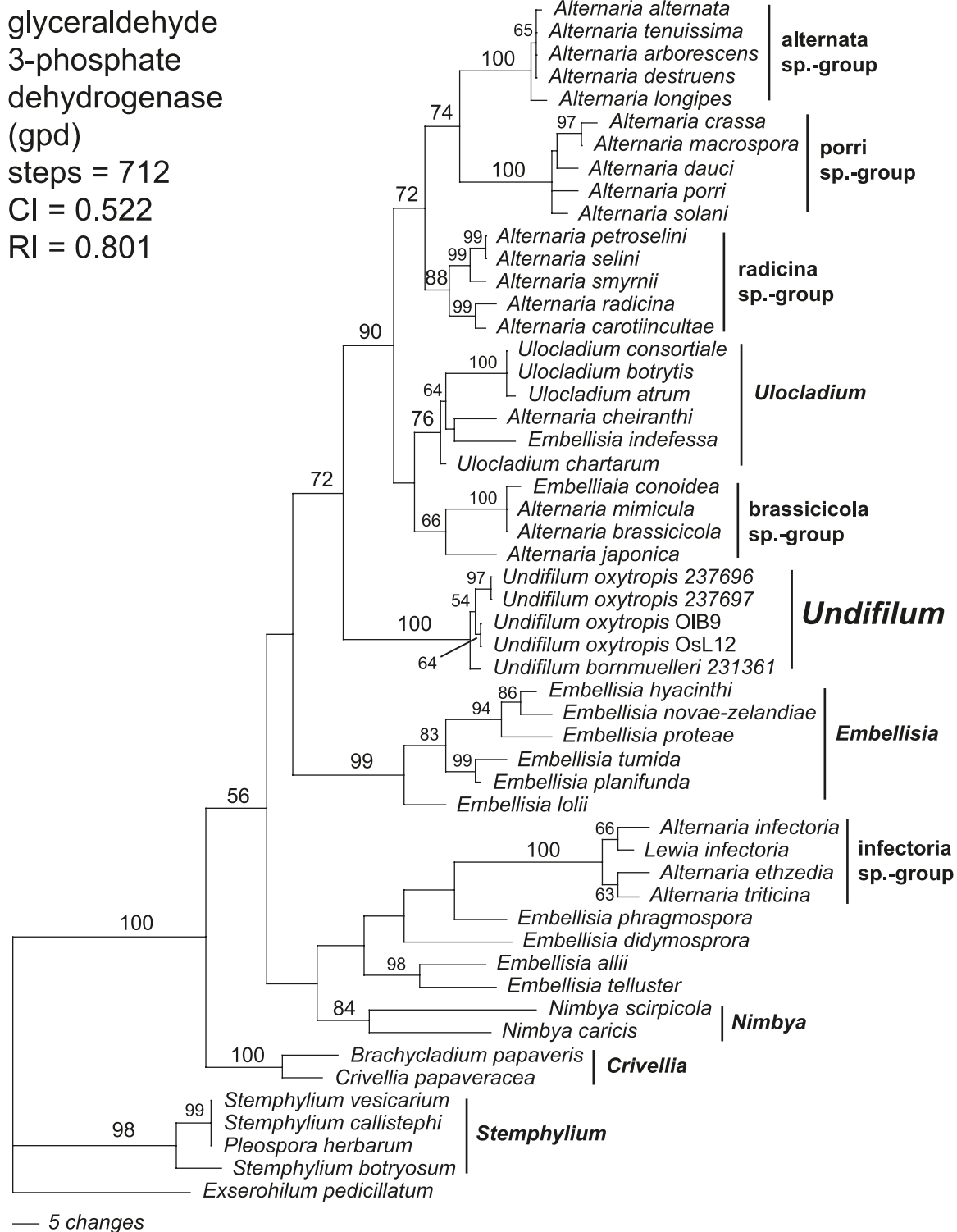
Fig. 16. One of 270 most parsimonious trees resulting from analysis of ITS sequence data. Bootstrap confidence values over 50% from 1000 bootstrap replicates are presented at each corresponding node.



bya spp. and four *Embellisia* spp., including the type for *Embellisia*, *E. allii*, were closely related to the infectoria species-group, but were not supported as distinct clades. The 10th distinct clade consisted of the locoweed isolates,

E. oxytropis and *H. bornmuelleri*, which clustered together in a strongly supported monophyletic group (98%) that was basal to all groups except the *Embellisia* and *Stemphylium* groups.

Fig. 17. One of 359 most parsimonious trees resulting from analysis of the glyceraldehyde-3-phosphate dehydrogenase (*gpd*) gene sequence data. Bootstrap confidence values over 50% from 1000 bootstrap replicates are presented at each corresponding node.

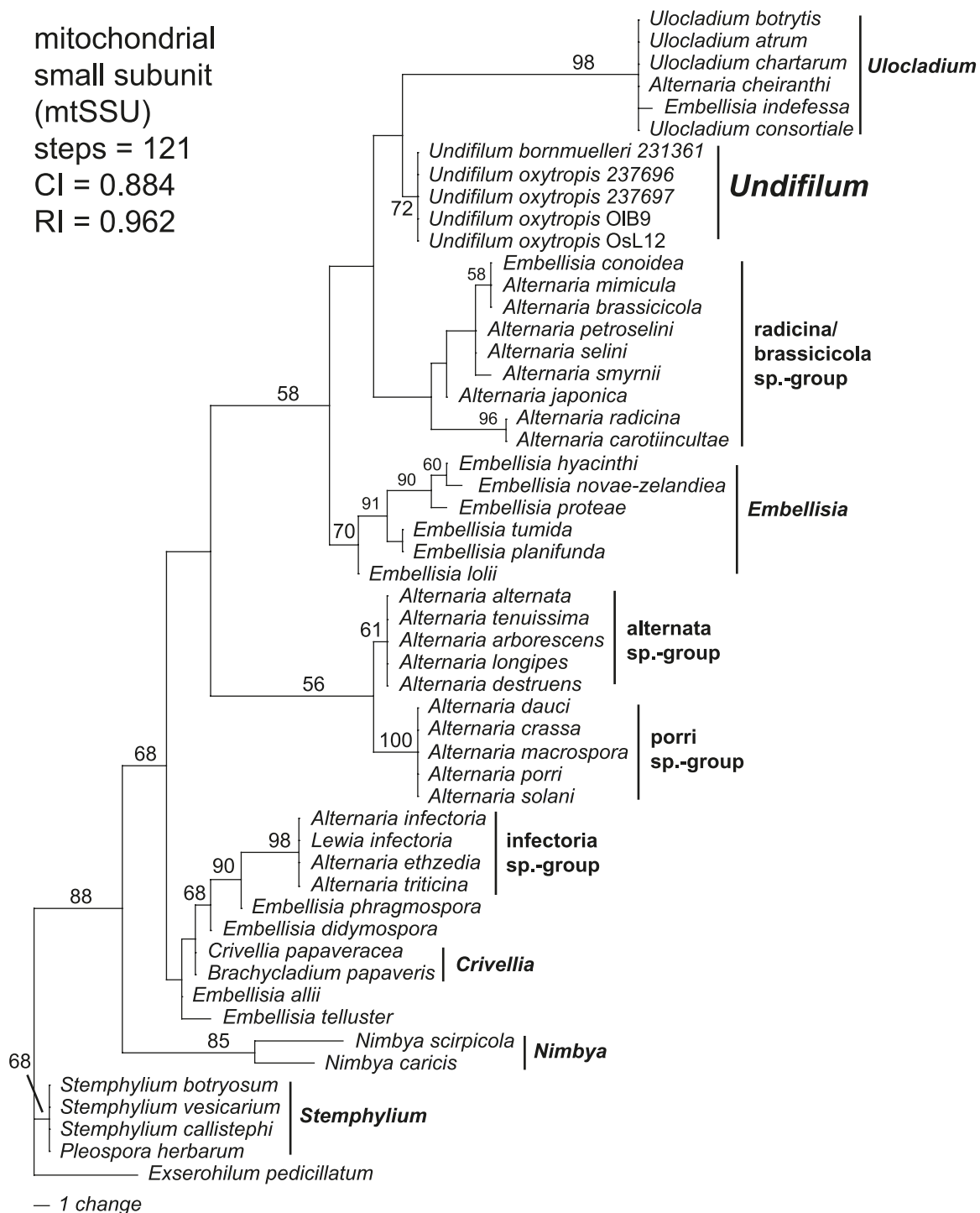


Alignment of the *gpd* sequences resulted in a 595-character data set, of which 242 characters (40.4%) were variable and 51 characters (8.6%) were parsimony informative. In the aligned data set, two introns were present. Intron 1 (*gpd*-int1) spanned characters 23–81 and intron 2 (*gpd*-int2) spanned characters 146–275. Alignment of sequences within these re-

gions was variable. Interestingly, intron 1 was absent from taxa in the infectoria species-group.

Maximum-parsimony analysis of the *gpd* data set yielded 40 equally most-parsimonious trees (steps = 712, CI = 0.522, RI = 0.801), which differed only in minor changes in the relationship among members within the principal clades

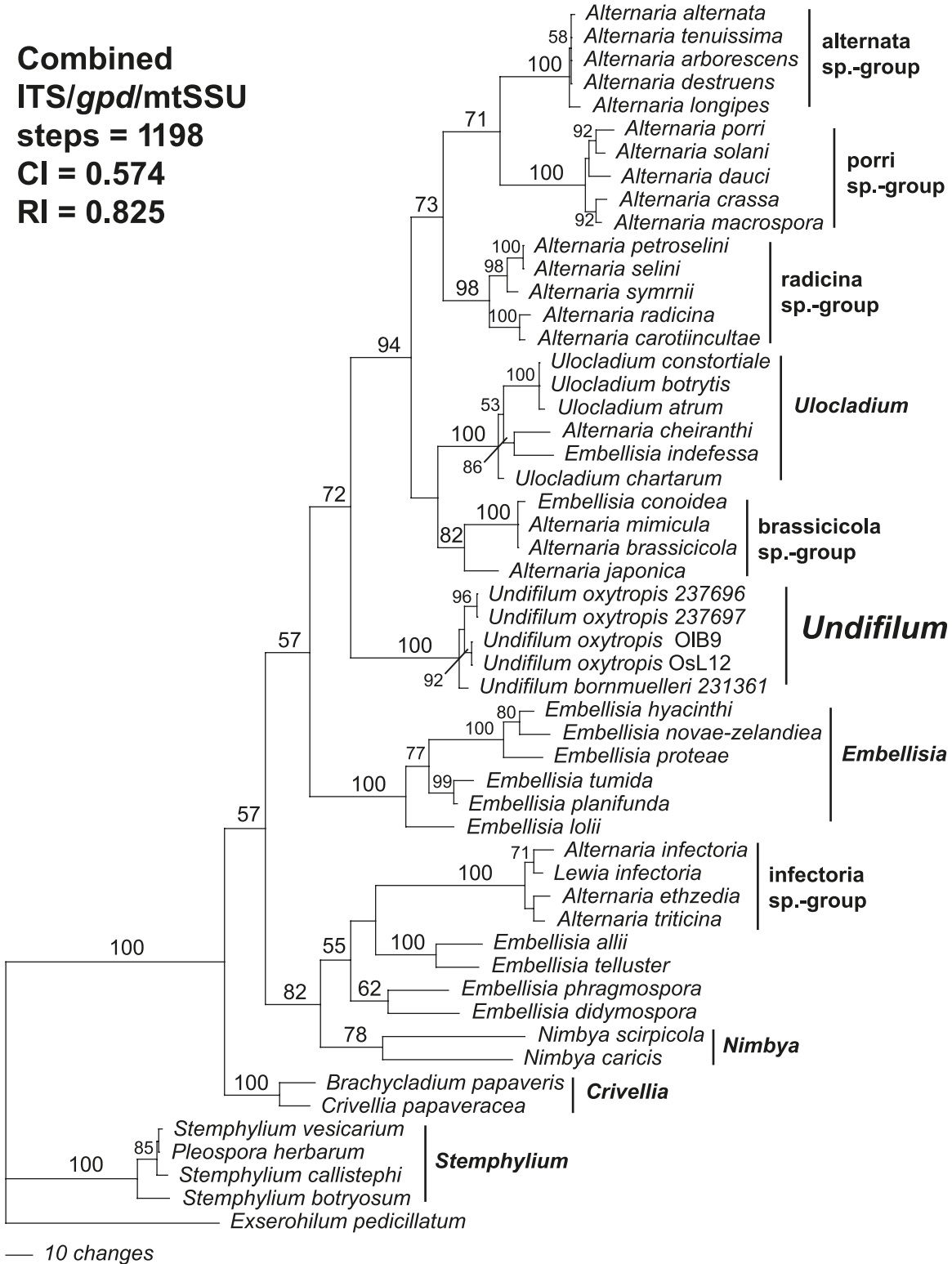
Fig. 18. One of >20 000 most parsimonious trees resulting from analysis of the mitochondrial small subunit (mtSSU) rDNA region sequence data. Bootstrap confidence values over 50% from 1000 bootstrap replicates are presented at each corresponding node.



(Fig. 17). Similar to the ITS data set, 11 principal clades were evident, 10 of which corresponded with previously established monophyletic groups: the *Stemphylium* group, the *Crivellia* group, the *Nimbya* group (not strongly supported with the ITS data set), the infectoria species-group, the *Embellisia* group, the brassicicola species-group, the *Ulocladium* group, the radicina species-group, the porri species-group, and the alternata species-group (Pryor and Bigelow 2003; Inderbitzin et al. 2006). Most of these groups were

supported strongly by bootstrap values of > 80%, with the exception of the brassicicola species-group and the *Ulocladium* group, which were supported by bootstrap values of 66% and 76%, respectively. As in the ITS data set, four *Embellisia* spp. were related to the infectoria species group, but were not supported as a distinct clade. The 11th distinct clade consisted of the locoweed isolates, *E. oxytropis* and *H. bornmuelleri*, which clustered together in a strongly supported monophyletic group (100%) that was sister to a large

Fig. 19. One of 36 most parsimonious trees resulting from analysis of combined ITS, glyceraldehyde-3-phosphate dehydrogenase (*gpd*) gene, and mitochondrial small subunit (mtSSU) rDNA region sequence data. Bootstrap confidence values over 50% from 1000 bootstrap replicates are presented at each corresponding node.

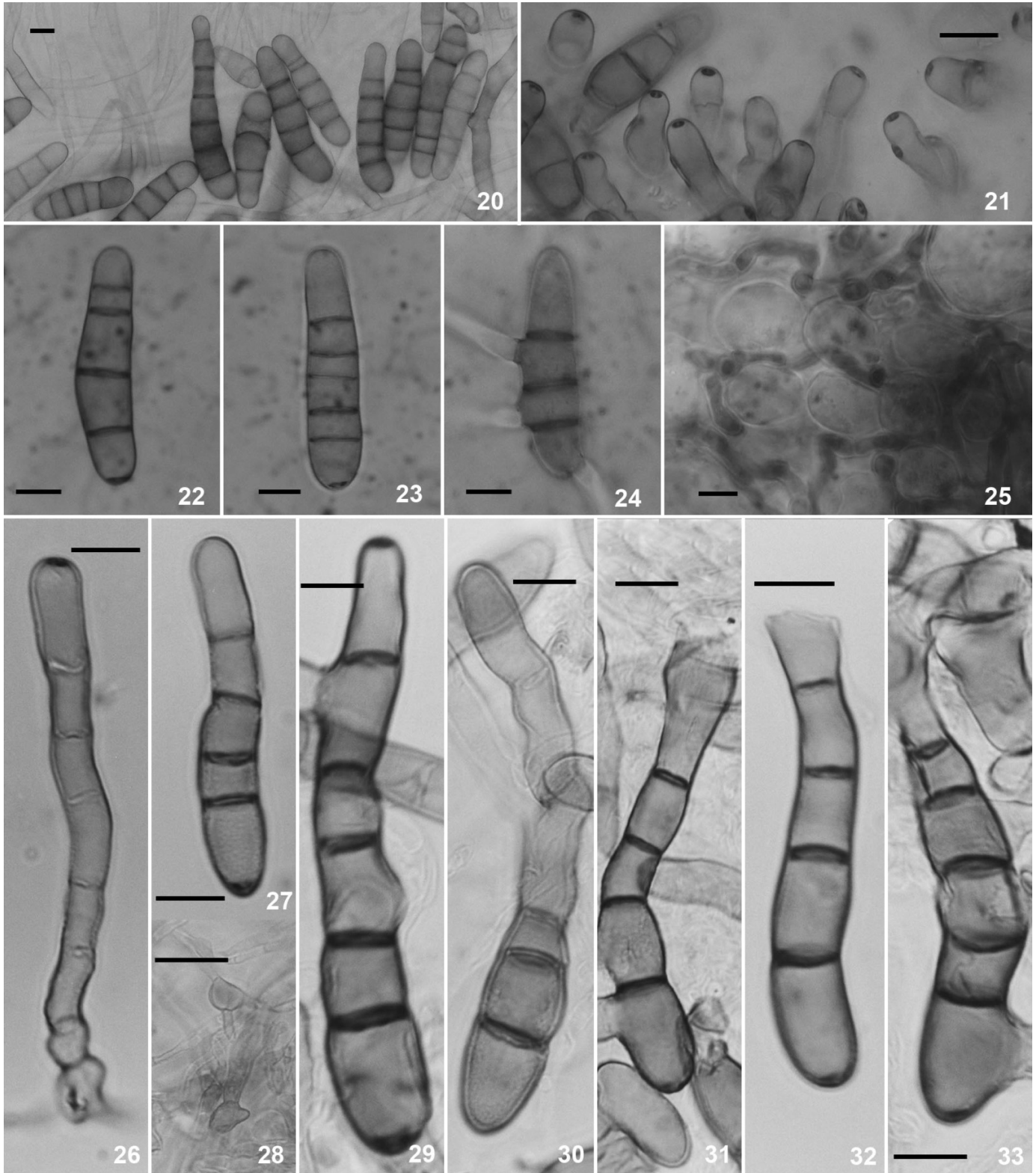


group comprising the *Ulocladium* clade and most *Alternaria* species-groups.

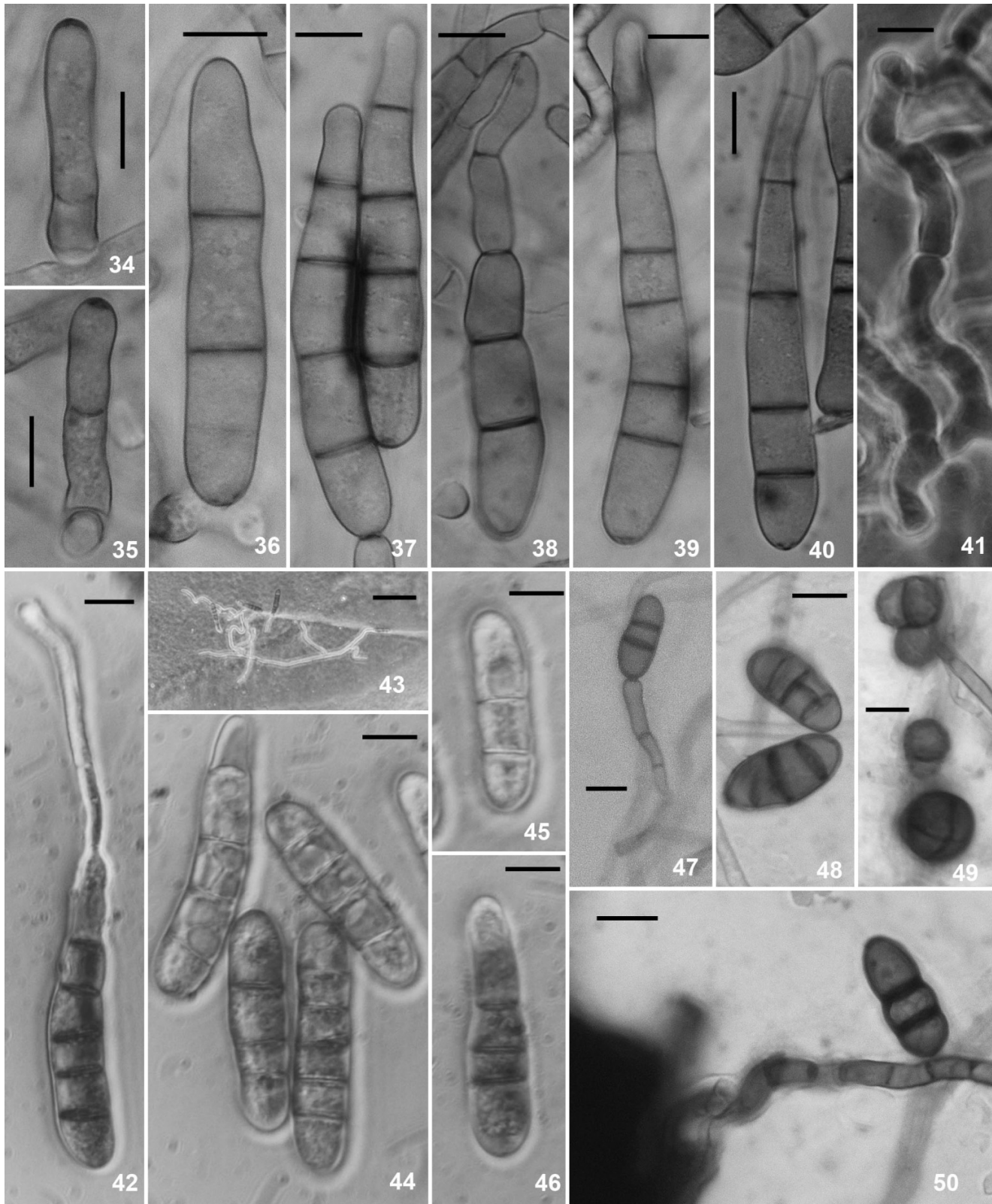
Alignment of the mtSSU sequences resulted in a 739-character data set, of which 87 characters (11.8%) were var-

iable and 19 characters (2.6%) were parsimony informative. In the aligned data set, numerous introns were present between bp 353 and 511, and global alignment within this region was quite variable. However, alignment of taxa into

Figs. 20–33. Figs. 20–25. *Undifilum bornmuelleri*. Fig. 20. Conidia on PCA. Scale bar = 10 μ m. Fig. 21. Conidiophores on leaf. Fig. 22. Conidium. Fig. 23. Conidium atrium hilum. Fig. 24. Germinated conidium with slight flange around germ tubes. Fig. 25. Intracellular hyphae in host, cotton blue stain. Figs. 26–33. *Undifilum oxytropis*. Fig. 26. Conidiophore HMJAU 10027. Fig. 27. HMJAU 10027. Fig. 28. Chlamydospore HMJAU 10032. Figs. 29–33. Conidia HMJAU 10012. Scale bar = 10 μ m unless otherwise indicated.



Figs. 34–50. Figs. 34–41. *Undifilum oxytropis* RC OsL12, DAOM 234034, on alfalfa stem agar. Figs. 34 and 35. Conidiophores. Figs. 36–40. Mature conidia. Fig. 41. Wavy mycelium. Figs. 42–46. *Undifilum oxytropis* RC OIB9, DAOM 236562, on V8. Figs. 42 and 44–46. Conidia. Fig. 43. Wavy germ tubes 24 h on V8. Scale bar = 40 μm . Figs. 47–49. *Embellisia chlamydospora* DAOM 75737, conidiophores, conidia, and one- to four-celled chlamydospores. Fig. 50. *Embellisia chlamydospora* DAOM 113803, conidiophore, conidium, and large multicellular chlamydospore. Scale bar = 10 μm unless otherwise indicated.



groups within this region was unambiguous, as the indels appeared as diagnostic signatures for groupings established by other loci. Alignments of the mtSSU, *gpd*, and ITS data sets have been submitted to TreeBASE for review (SN3426, reviewer pin 30601).

Maximum-parsimony analysis of the mtSSU data set yielded > 20 000 equally most-parsimonious trees (steps = 121, CI = 0.884, RI = 0.962), which differed in minor changes in the relationship among members of the principle clades as well as relationships between nearly all of the principle clades (Fig. 18). Similar to the ITS data set, nine principal clades were evident, seven of which corresponded to previously established monophyletic groups: the *Stemphylium* group (68%), the *Nimbya* group (85%), the infectoria species-group (98%), the porri species-group (100%), the alternata species-group (61%), the *Embellisia* group (70%), and the *Ulocladium* group (98%) (Pryor and Bigelow 2003). A combined radicina-brassicicola clade was evident but did not resolve with statistical support. The *Crivellia* group and four *Embellisia* species did not resolve as distinct clades but were immediately basal to the infectoria species-group. The ninth distinct clade consisted of the locoweed isolates, *E. oxytropis* and *H. bornmuelleri*, which clustered together in a moderately supported monophyletic group (72%) that was sister to the *Ulocladium* group.

Results from the PHT test revealed that the *gpd* and mtSSU data sets were not significantly inconcordant ($P = 0.50$), whereas the ITS data set was significantly inconcordant with both the *gpd* and mtSSU data sets ($P = 0.01$ for both comparisons). However, the PHT test is prone to type-I error (wrongly rejecting the null hypothesis of congruence) (Sullivan 1996; Darlu and Lecointre 2002). Others view a significance threshold of 95% ($P = 0.05$) or even 99% ($P = 0.01$) for the PHT to be insufficient for establishing significant inconcordance (Cunningham 1997). Moreover, total evidence analysis, whereby as much data as possible are included to increase signal/noise ratio for increasingly robust analysis, is widely supported providing that there is not strong statistical supported (bootstrap) for conflicts in topologies resulting from separate analyses (de Queiroz 1993; Huelsenbeck et al. 1996; Rokas, et al. 2003). Therefore, all three data sets were combined in an additional analysis to provide supplementary insight as to the relationships among taxa. Maximum-parsimony analysis of the combined ITS-*gpd*-mtSSU data set yielded 6 equally most-parsimonious trees (steps = 1198, CI = 0.574, RI = 0.825), which differed primarily in minor changes in the relationship among members of the *Ulocladium* group (Fig. 19). Eleven principle clades were evident, all of which were previously revealed in analysis of the *gpd* data set. All of these groups were supported strongly by bootstrap values of > 80%, with the exception of the *Nimbya* group, which was supported by bootstrap values of 78%. Additionally, the *Nimbya* group, the infectoria species-group, and four *Embellisia* species clustered together with a bootstrap support of 82%. As with the other single-locus data sets, the locoweed isolates *E. oxytropis* and *H. bornmuelleri* clustered together in a strongly supported monophyletic group (100%) that was sister to the large asexual clade encompassing the *Ulocladium* group and most *Alternaria* species-groups.

Removal of ITS and mtSSU variable regions and *gpd* in-

trons had only minor effects on tree topology. Removal of all variable regions resulted in an 861-character data set, of which 155 (18.0%) characters were variable and 102 (11.8%) were parsimony informative. A heuristic search of this data set resulted in more than 744 most-parsimonious trees (steps = 288, CI = 0.625, RI = 0.821), which varied primarily in the relationships among members within several primary clades and an exchanging of clade positions among the *Embellisia* group, the *Nimbya* group, and the *Crivellia* group (data not shown). The position of the locoweed/*E. oxytropis* – *H. bornmuelleri* clade was immediately basal to the *Nimbya*, *Embellisia*, and the *Crivellia* clades. Bootstrap analysis of the truncated data set had only minor effects on the statistical support of the primary clades with the exception of the *Nimbya* group, the brassicicola species-group and the radicina species-group, for which bootstrap support was less than 50%. However, most intermediate nodes were poorly supported, that is, little resolution of relationships among primary clades (data not shown).

In summary, regardless of the sequences used, whether a single locus, combined loci, or truncated data sets (minus variable regions), the locoweed isolates *E. oxytropis* and *H. bornmuelleri* clustered together with high bootstrap support. Moreover, this clade was always distinct and generally basal to all other *Alternaria* and *Ulocladium* clades (with the exception of the outlying infectoria species-group), and appeared to be closely related to, but distinct from, *Embellisia* lineages examined. As such, sequence analysis corroborates morphological analyses and supports the designation of the locoweed isolates *E. oxytropis* and *H. bornmuelleri* as a distinct lineage and new genus, hereinafter described as *Undifilum*.

Taxonomy

Undifilum gen. nov

Hyphae sinuatae vel strictae, septatae, hyalinae vel brunneae, laeviae vel verruculosae, tenui-tunicatae, cylindricae vel inflatae, lente aut parce crescens; in vivo intercellulares. Conidiophorum solitarium vel aggregatum, strictum vel sympodialis ramosum, brunneum, septatum; in vivo curtum, fasciculatum vel nullum; in vitro longum, solitarium. Conidia terminales, euseptata, cylindrica vel protracta, solitaria vel pauci-catenata, brunnea; septa brevis, primum septum basalis; hilum atrium simile; cellulae totae germinans.

Hyphae sinuate or straight, septate, hyaline to brown, smooth or verruculose, thin walled, cylindrical to globose, growing slowly or moderately; on the host intercellular. Conidiophores solitary or aggregated, simple to sympodially branched, brown, septate, hilum poroid, terminal, becoming subapical; in vivo short, fasciculate or absent; in vitro long and solitary. Conidia terminal, euseptate, cylindrical to narrowed to apex, solitary or in very short chains, brown, septa thin, first-formed septum nearest base, hilum internal (atrium type, Alcorn 1983), germination from any cell.

ETYMOLOGY: unda L wave, and filum L thread alluding to the sinuous germ hyphae.

TYPE SPECIES: *Undifilum bornmuelleri* comb. nov. \equiv *Helminthosporium bornmuelleri* P. Magnus Hedwigia 38 (Beiblatt): 73 + tav. V. 1899.

PROTOLOGUE: *Helminthosporium bornmuelleri* on *Coronilla*

montana Scop. Blättern und Blattfiedern auf "Rosenberg" und "Hexenberg" bei Berka a.d. Ilm in Thüringen. J. Bornmüller Juli 1898.

IN VIVO: Conidiophores foliicolous, amphigenous, abundant, clustered in groups of up to 10, rarely solitary, widest at base, basal cell slightly longer than wide and tapered in from base, stalk cylindrical and slightly narrowed to hemispherical apex, producing first terminal hilum at about 20 μm , with 1 or 2 thin septa, hilum atrium type (within contour, centrally perforated and extending inward), 2–3 μm in diameter, soon becoming lateral from sympodial growth, hila 5–10 μm apart, stalks yellowish brown, smooth, erect, slightly bent, (10)20–50 μm \times 10–12 μm (basally) 6–8 μm (apically). Conidia cylindrical, slightly narrowed towards apex of longer conidia, widest at second cell from base, broadly rounded at both ends, thin walled with thin eusepta concolorous with the wall but divided by a dark brown middle lamella and easily fragmenting along the midline when pressed, first-formed septum basal, basal cell wider than long or isodiametric (Figs. 20–24), not constricted at the septa, yellowish brown, basal and apical cells sometimes pale colored, hilum internal, within the inner contour of the basal wall, centrally perforated, 2–3(4) μm in diameter, about 0.5 μm thick near perforation, and tapered to outer edge, (2)3–4(5) septate (29)39–50(55) μm \times 10–12(15) μm . Germination was prompt, overnight, from conidia 6 months old. Germ tubes emerged from any cell and seemed to burst out the apparently thin wall and often leave an evident ring (Fig. 24); on potato–carrot agar plates at 20 °C exposed to January daylight, the colonies from single conidia are slow growing, of radiating, hyaline, appressed to slightly erect, mycelium, and 4–5 mm in diameter after 7 d and 45–50 mm diam after 35 d. Conidiation was abundant over the small colony after 10 d. Conidia were like those described from leaf material, but some had an elongated apical region with one terminal hilum or even two hila from sympodial growth. Catenate conidia did form but not in long chains. The vegetative mycelium was hyaline and narrower than the conidiophores, which ranged from short, as on the host, to elongated; on autoclaved alfalfa stem on water agar at 20 °C in room light 3–12 November 2004 from inoculum placed next to the stem. Mycelial growth slow. Conidiophores and a crop of conidia visible on stem only near the inoculum. Mycelium hyaline, sparingly septate, often arched or wavy, 2–5 μm wide. Conidiophores intercalary or terminal, from warted hyphae, pale brown, smooth, broadly rounded at apex, 6–8 μm wide, with atrium type hilum, 15–50(60) μm long, closely sympodially branched. Conidia solitary, rarely in short chains, cylindrical to usually narrowed to the apex, widest below, at, or just above basal septum, smooth, euseptate, septa thin without detectable central pores, 40–80 μm \times 9–11 μm (avg. 60.6 μm \times 10.4 μm) L/W 5.8, 3–7 septate (avg. 4.1), mean cell length 11.9 μm .

Germination of conidia from alfalfa stems placed on water agar was from any cell. First tube was longitudinal from apex, less often oblique from basal cell, sometime from central cell with a "fringe" of brown wall around the hyaline germ tube. Multiple germ tubes from one conidium were not seen. An unusual feature was a wavy mycelium extending for some distance before branching and then a continuation of wavy hyphae and some coiled hyphae.

SPECIMENS EXAMINED: DAOM 231361 (Figs. 20–25), *Helminthosporium bornmuelleri* Magn. on *Securigera varia* (L.) Lassen (\equiv *Coronilla varia* L.) Leguminosae. vom Blaettchenrand gleichmaessig fortschreitende Nekrosen. Austria: Steiermark: Bezirk Andritz: Admonter Kogel (ESE vom Kanzelkogel) ca. 450 m a.s.l. 47°06'75"N, 15°23'78"E. 17 June 2002. Coll. G. Heber. det. Scheuer No. 3244 ex GZU and derived cultures = MTB 8858/3 = E.G. Simmons 51–28 = IMI 390145. Exsiccatae: Sydow, Mycotheca germanica 293 on *Coronilla montana*, Thüringen, G. Oertel, 10.6.1904 ex WIS (DAOM 548300); Sydow, Mycotheca germanica 294 on *Coronilla vaginalis*, Thüringen, 22.6.1904, H. Diederich ex WIS (DAOM 54831); Jaap, Fungi selecti exsiccati 297 on *C. vaginalis*, Sud Tirol, Otto Jaap, 13.vii.1907 ex WIS (DAOM 54832); Jaap, Fungi selecti exsiccati 798 on *C. coronata*, Thüringen, Otto Jaap, 3.vii.1915 ex WIS (DAOM 54833); Flora Thuringiaca Berka a. d. Ilm: Müfling, on *C. montana*, J. Bornmüller, 8.viii.1901 ex FH ex Herb. F. Bucholtz (DAOM 57132). The last specimen, though collected later than the type, is from the same general locality on the same host and determined by Bornmüller.

Magnus (1899) gave a detailed description and illustrations. His measurements of the conidia are summarized: (32)38–54 (62) μm long and averaged 43 μm \times 12 μm with an average cell length of 10 μm , the basal cell being longer, 13–18 μm . The recent measurements accord well with the original description.

Undifilum oxytropis comb. nov. \equiv *Embellisia oxytropis* Q. Wang, Nagao & Kakish Mycotaxon 95: 257–258, 2006

IN VITRO: on autoclaved alfalfa stem on water agar at 20 °C under ambient light 3–19 November 2004 from inoculum placed next to the stem. Mycelial growth slow. Abundant conidiophores and conidia visible on stem near the inoculum. Mycelium hyaline, sparingly septate, often arched or wavy, 2–5 μm wide. Conidiophores solitary from warted surface mycelium, intercalary or terminal, base slightly swollen, 5–7(9) μm wide, an isodiametric cell, yellow, smooth, sparingly septate, slightly swollen at apex, (11)30–50(130) μm \times 6–7 μm (at apex), with atrium type hilum 2.5–3.5 μm , closely sympodially branched but often simple. Conidia solitary, rarely with secondary conidiophore, cylindrical to usually narrowed to the apex, widest below, at, or just above basal septum, smooth, euseptate, septa thin without detectable central pores, hilum 2.5–3.5 μm , atrium type, 53–88 μm \times 8–11 μm (avg. 65.5 μm \times 10.3 μm) L/W 6.4, 3–8 septate (avg. 4.8), mean cell length 11.3 μm , basal cell longer than wide, apical cells slender and elongated.

Colony on agar either near seeds of *Oxytropis sericea* or stem of alfalfa slow growing, restricted with numerous short, deep penetrating, hyaline hyphae closely appressed, and eventually forming a mound of brown interwoven hyphae resembling a black ball about 5 mm in diameter. Germ-tube growth reasonably rapid, wavy and unbranched for three or four spore lengths. Older hyphae often sinuous and may bear a conidiophore at the bend. Mycelium growth habit is very distinctive.

SPECIMENS EXAMINED: HMJAU 10012 Holotype *Embellisia oxytropis* Q. Wang, Nagao & Kakish. Dried culture speci-

men isolated from *Oxytropis kansuensis* Bunge, Mt. Daming, Qinghai, China, 20 July 2004. The Q. Wang and paratypes 10027, 10030, 10030 are labeled identically. Also, new isolations made in Ottawa, dried and deposited in DAOM, from 10030 (DAOM 237696) and 10032 (DAOM 237697) from agar cultures dried 2 years previously (Figs. 26–33). Also examined were *Embellisia* spp. RC OsL12 (DAOM 234034) and RC OIB9 (DAOM 236562) (Figs. 34–41 and Figs. 42–46, respectively); cultures from Braun et al. (2003).

Braun et al. (2003), from material from USA, recorded conidial dimensions as 14–60 $\mu\text{m} \times 6$ –13 μm with a mean length 35 μm with (1)2–4(5) septa. Braun's illustrations rescaled to $\times 1000$ gave the measurements 48–65 $\mu\text{m} \times 11$ –13 μm , L/W 4.6, mean cell length 12.1 μm , the basal cell slightly longer than the width at the basal septum, and the apical cell much longer than the width of the apical septum. Wang et al. (2006) recorded conidial dimensions as 50–60 $\mu\text{m} \times 8$ –10 μm and (2–)3–4 septate. This compares with recent measurements: 53–88 $\mu\text{m} \times 8$ –11 μm (avg. 65.5 $\mu\text{m} \times 10.3 \mu\text{m}$) L/W 6.4, 3–8 septate (avg. 4.8), mean cell length 11.3 μm , basal cell longer than wide, apical cells slender and elongated. Previous measurements of less fully developed conidia were as follows: 40–80 $\mu\text{m} \times 9$ –11 μm (avg. 60.6 $\mu\text{m} \times 10.4 \mu\text{m}$) L/W 5.8, 3–7 septate (avg. 4.1), mean cell length 11.9 μm . Images of *E. chlamydospora* DAOM 113803 are provided for comparative purposes (Figs. 47–50).

Discussion

This study describes the morphological characters of a new endophytic fungus isolated from locoweed (*Oxytropis* spp.) from the USA, and compares these features with those of a newly described *Embellisia* species from *Oxytropis kansuensis* from China and a previously described *Helminthosporium* sp. from crownvetch from Austria. In addition, this study presents phylogenetic relationships between these fungi and related *Alternaria*, *Ulocladium*, *Nimbya*, *Crivellia*, and *Embellisia* spp.

All phylogenetic analyses placed the *Undifilum* isolates into a strongly supported monophyletic clade, which in the combined analysis was sister to a previously established and strongly supported clade encompassing *Ulocladium* and most *Alternaria* species groups (Pryor and Bigelow 2003). Most importantly, *Undifilum* was distinct from other clades containing *Embellisia* species, including basal clades containing the type, *E. allii*. Although the molecular circumscription of *Embellisia* to date has been problematic and the genus appears to be polyphyletic (Pryor and Bigelow 2003), the resolution of *Undifilum* as a strongly supported clade distinct from others that contain *Embellisia* refutes the previous circumscription of *Oxytropis* isolates as *Embellisia*. The placement of *Undifilum* in relation to *Alternaria*, *Ulocladium*, and *Embellisia* was only moderately resolved in this study, and a more robust resolution of relationships among these genera will likely require more comprehensive analyses using a number of additional phylogenetically informative genetic loci.

The search for allied genera suitable for inclusion was restricted to those with the following features that in combina-

tion are diagnostic for the taxa examined in this study: simple or sparingly branched, micronematous, brown conidiophores with terminal conidiogenous cell, with porogenous production of conidia and sympodial regrowth and conidia with atrium-type hilum, euseptate, phragmosporous, cylindrical to slightly narrowed towards apex, smooth, concolorous, rarely producing secondary conidia, germinating from any cell, and hyphae becoming sinuous. Some generic names that were considered on the basis of morphological similarities to members of the new genus are listed in alphabetical order. The features mentioned apply to the type species but not necessarily to all species in the particular genus. The new genus is distinct from all of the genera considered, listed below:

- (i) *Acroconidiella* Lindquist & Alippi, typified by *A. tropaeoli* (T.E.T. Bond) Lindquist & Alippi, differs by the presence of distinctly verrucose conidia and development of straight germ hyphae (Davis 1952).
- (ii) *Alternaria* Nees: Fr., typified by *Alternaria alternata* (Fr.: Fr.) Keissler, differs by the presence of dictyosporous ovate to obclavate beaked euseptate conidia.
- (iii) *Brachycladium* Corda (see teleomorph *Crivellia* Inderbitzin et al.), typified by *Brachycladium penicillatum* Corda, differs by the presence of macronematous conidiophores in vivo (Inderbitzin et al. 2006).
- (iv) *Dendryphiella* Bubak & Ranojevic, typified by *Dendryphiella interseminata* (Berk. & Rav.) Bub. & Ran., differs by the presence of macronematous conidiophores with nodose conidiogenous cells (Ellis 1971).
- (v) *Dendryphion* Wallroth, typified by *Dendryphion comosum* Wallr., differs by the presence of apically much-branched macroconidiophores, mono- or polytretic conidiogenous cells bearing euseptate, catenate, echinulate conidia (Ellis 1971).
- (vi) *Dendryphiopsis* Hughes, typified by *Dendryphiopsis atra* (Corda) Hughes, differs by the presence of dichotomously branched conidiophores (up to four branchings), monotretic conidiogenous cells bearing euseptate, solitary, two- to six-septate phragmoconidia.
- (vii) *Drechslera* (S. Ito) Shoemaker typified by *Drechslera tritici-vulgaris* (Nisikado) S. Ito, differs by the presence of simple or sparingly branched conidiophores, sympodial conidiogenous cells with small atrium-type hilum (Alcorn 1983) bearing distoseptate, phragmosporous, brown, solitary to short-chained conidia.
- (viii) *Embellisia* Simmons (1971), typified by *Embellisia allii*, differs by the presence of conidiophores supporting a succession of conidia from terminal pores by sympodial growth, and conidia phragmosporous, euseptate with thick septa up to 2 μm , but without distinctive germ tubes that are wavy or undulating in their growth until branching.
- (ix) *Heterosporium* Klotsch in Cooke (1877), typified by *Heterosporium ornithogali* (Klotsch) Cooke, differs by the presence of verrucose conidia with a small, slightly exerted hilum.
- (x) *Marielliottia* Shoemaker, typified by *Marielliottia biseptata* (Sacc.) Shoemaker (1998) differs by the presence of clavate conidia that germinate only from the basal cell.

(xii) *Nimbya* Simmons, typified by *Nimbya scirpicola* (Fuckel) Simmons, differs by the presence of conidiophores simple or branched, hilum prominent, with phragmosporous, tapered, solitary or catenate, distoseptate conidia with a few eusepta.

(xiii) *Ulocladium* Preuss typified by *Ulocladium botrytis* Preuss differs by the presence of branched or unbranched conidiophores, brown, polytretic conidigenous cells, bearing brown, dictyosporous, euseptate, ovate to obovate, verrucose conidia that are solitary or in short chains.

Although the initial report on the recovery of the locoweed endophyte suggested it may be a species of *Alternaria* (Braun et al. 1997), the more comprehensive morphological examination conducted in this study revealed that it is not. Whereas *Alternaria* species produce conidia that are fundamentally ovate in shape and generally possess both longitudinal and transverse septa, the isolates from locoweed produce conidia that are fundamentally ellipsoid in shape (infrequently ovate) and produce only phragmospores. Later work based upon both morphology and ITS sequence analysis suggested the endophyte was a species of *Embellisia* (Braun et al. 2003). The original description of the genus *Embellisia* states that conidia are usually phragmosporic and occasionally produce longisepta (Simmons 1971). Moreover, in subsequent descriptions of additional *Embellisia*, it is stated that for some species longiseptum number is zero, or very rarely, one (e.g., *E. telluster* and *E. phragmospora* (Simmons 1983). Such description applies to the genus *Nimbya* as well (Simmons 1989). Thus, the presence or absence of longisepta may not be an absolute character by which to base genus inclusion or exclusion, at least for some of these monophyletic clades basal to the primary groups of *Alternaria*.

One character that appears to be particularly diagnostic for the genus *Embellisia* is the nature of the conidium septum, which tends to be relatively thick, dark, and rigid in contrast with the exterior conidium wall (Simmons 1971). Transepta for locoweed isolate RC OsL12 were nearly all thicker and darker than the exterior conidium wall. In addition, conidia were straight or variously curved, and often enlarged near the base or near the apex; characters presented in descriptions of *Embellisia* (Simmons 1983). However, even some *Alternaria* species may have one or more transepta that may be darker and thicker than others in the same conidium (e.g., *A. tenuissima*, *A. brassicicola*; Simmons 1995), so this also may not be a definitive criterion for genus designation. Although locoweed isolates also produced some transepta that were distinctively darker and thicker, most transepta were morphologically undifferentiated from the conidium wall. Thus, the case previously made for placement of these isolates and those from *O. kansuensis* into *Embellisia* was not particularly robust. Indeed, Simmons states that “*Embellisia* is recognized on the basis of a combination of characters, no one of which is firmly diagnostic” (Simmons 1983).

Toxin production also varied among *Oxytropis* isolates. In contrast, no swainsonine production was detected for *U. bornmuelleri*. Moreover, growth rate was notably different between the *U. oxytropis* and *U. bornmuelleri* with the former being slow and the later being much faster. Wang et al. (2006) gave good illustrations of *U. oxytropis* on a vari-

ety of media and noted that some were more suitable for sporulation while others promoted growth of mycelium, but on all growth was slow. In contrast, *U. bornmuelleri* formed relatively large colonies on all media tested. A similar relationship between toxin production and growth rate has been reported for two related fungi pathogenic on carrot, *A. radicina* and *A. carotiincultae* (Pryor and Gilbertson 2002). In that report, *A. radicina* produced the toxin radicinin and was noted to have slow-growing colonies with irregular or torn margins. In contrast, *A. carotiincultae* produced little to no toxin and produced rapidly growing colonies with even margins. Most importantly, it was shown that radicinin negatively affected colony growth. Whether there is a similar relationship between swainsonine production and growth rate of *Undifilum* has yet to be definitively concluded.

The endophytic nature of *U. oxytropis* is distinct, as is the production of a mammalian-affecting toxin. This distinctive ecology is a notable character, because additional distinctions between the two *Undifilum* species described in this work in microscopic morphology or in cultural habit are not dramatically evident. *Undifilum bornmuelleri* is a leaf parasite, sporulating on *Securigera* (\equiv *Coronilla*) and spreading by airborne conidia. This taxon does not produce swainsonine. *Undifilum oxytropis* is an endophyte occurring in most parts of *Oxytropis* spp., is not known to sporulate on the host plant, and is dispersed via the host seeds. Its ability to produce swainsonine has been comprehensively tested, with variation among isolates documented. Continued studies on morphological, molecular, toxicological, and ecological characteristics of *U. oxytropis* and other *Undifilum* spp. will likely advance knowledge on the systematics of *Alternaria* and related genera in general, as well as understanding relationships between secondary metabolite production, fungal morphology, and endophytic ecology.

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