

FULL PAPER

Lisa A. Castlebury · David F. Farr · Amy Y. Rossman
Walter Jaklitsch

Diaporthe angelicae comb. nov., a modern description and placement of *Diaporthopsis* in *Diaporthe*

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Abstract The genus *Diaporthopsis* was described for species that are similar to *Diaporthe* but have nonseptate ascospores. The type species of *Diaporthopsis* is *D. angelicae*, an earlier name for *D. nigrella*. Molecular analysis of the large subunit of the nuclear ribosomal DNA places *D. angelicae* within a group that includes the type and many other species of *Diaporthe*. In addition, *D. angelicae* is similar in stromatal, perithecial, and centrum morphology to species of *Diaporthe*. Based on morphological and molecular data, *Diaporthopsis angelicae* is transferred to *Diaporthe* and the genus *Diaporthopsis* is considered a synonym of *Diaporthe*. A description and illustrations of *D. angelicae* are presented, and an epitype specimen is designated.

Key words Apiaceae · Ascospore septation · *Diaporthe* · *Phomopsis* · Systematics

Introduction

The genus *Diaporthopsis* Fabre (1883) was described for species that resemble *Diaporthe* Nitschke except for nonseptate ascospores. Species of *Diaporthe* are considered to have one-septate ascospores (Barr 1978). Despite the morphological similarity of *Diaporthopsis* to *Diaporthe*, the separation of *Diaporthopsis* based on nonseptate ascospores has been generally accepted. Barr (1978) considered the blackened marginal zone at the stromatal surface to be another characteristic that separated *Diaporthopsis* from *Diaporthe*. According to Kirk et al. (2001), *Diaporthopsis* includes eight species and has a *Phomopsis*

anamorph. The type species of *Diaporthopsis* is *D. angelicae* (Berk.) Wehm., an earlier name for *D. nigrella* (Auersw.) Fabre. Internal transcribed spacer (ITS) and large subunit (LSU) nuclear ribosomal DNA (nrDNA) regions were sequenced to determine the generic affinities of two isolates of *D. angelicae*. In addition, specimens of *D. angelicae* were examined to determine if there were any morphological characteristics other than ascospore septation that distinguish *Diaporthopsis angelicae* from species of *Diaporthe*. A detailed description and illustrations of *D. angelicae* are presented.

Materials and methods

Morphological examination

For microscopic examination, material was rehydrated and mounted in 3% KOH. Ascomata were sectioned at ~10 µm thick using a freezing microtome. Sections were mounted in lactic acid with cotton blue. Observations of microscopic features were made using a Zeiss Axioplan 2 microscope with bright-field illumination. Photographs and measurements of microscopic features were taken using a Spot 2 digital camera (Diagnostic Instruments, Sterling Heights, MI, USA) and ImagePro software (Media Cybernetics, Silver Spring, MD, USA). Single ascospores were suspended in sterile water and plated on 1.7% Difco corn meal agar (CM) plus 0.2% dextrose and 0.005% each chlorotetracycline, streptomycin, and penicillin G (CMD). Cultures were transferred to plates of 3.9% Difco potato dextrose agar (PDA), CM, and 1.5% water agar (WA) plus an alfalfa stem (*Medicago sativa* L.) for observation. Color names were determined using Rayner (1970). Specimens and cultures examined are listed following the description.

DNA extraction, purification, and amplification

DNA extraction, amplification conditions, and sequencing of the large subunit (LSU) nuclear ribosomal DNA

L.A. Castlebury · D.F. Farr · A.Y. Rossman (✉)
Systematic Botany and Mycology Laboratory, U.S. Department of
Agriculture, Agricultural Research Service, Beltsville, MD 20705,
USA
Tel. +1-301-504-5364; Fax +1-301-504-5810
e-mail: arossman@nt.ars-grin.gov

W. Jaklitsch
Ratschkygasse, Vienna, Austria

(nrDNA) were carried out as detailed in Farr et al. (2001). The ITS regions 1 and 2 including the 5.8S rDNA gene were amplified under the same conditions as the LSU nrDNA fragments and sequenced using the primers ITS5 and ITS4 (White et al. 1990). Amplified products were sequenced with the BigDye terminator kit version 2.0 on an ABI 310 automated DNA sequencer (Applied Biosystems, Foster City, CA, USA).

Sequence editing, alignment, and analysis

Raw sequences were edited using Sequencher version 4.05 for Windows (Gene Codes, Ann Arbor, MI, USA). A BLAST search (Altschul et al. 1997) of the GenBank database with the ITS sequence of CBS 111591 (AR 3724) was performed to identify the most similar sequences in GenBank. The LSU nrDNA sequence alignment was manually adjusted using GeneDoc 2.6.001 (Nicholas et al. 1997). Large subunit nrDNA gene trees were inferred by neighbor joining (NJ) using the Kimura two-parameter distance as implemented in PAUP* 4.0b10 (Swofford 1998) and by maximum parsimony (MP) using the heuristic search option with the random addition sequence (1000 replications) with the MULTREES setting in effect, unlimited MAXTREES, and the branch-swapping (tree bisection-reconnection) option of PAUP* 4.0b10 (Swofford 1998). All molecular characters were unordered and given equal weight during the analysis. Gaps were treated as missing data.

Relative support for branches was estimated with 1000 bootstrap replications (Felsenstein 1985) for both NJ and MP analyses with MULTREES off, unlimited MAXTREES, and 10 random sequence additions for the MP bootstrap analysis. Sequences generated in this study were deposited in GenBank as *D. angelicae*: CBS 111591 (AR 3724) LSU = AY196780, ITS = AY196778; CBS 111592 (AR 3776) LSU = AY196781, ITS = AY196179; *D. arctii*: CBS 109490 ITS = AY196777. Sequences obtained from GenBank are listed by taxon name and GenBank accession number in the tree. The alignment was deposited in TreeBase.

Results

Results of the examination of specimens of *Diaporthopsis angelicae* suggest that this species has morphological features that are characteristic of *Diaporthe* except for ascospore septation. Given the similarity of the type species of *D. angelicae* to other species of *Diaporthe*, it is concluded

that this species belongs in *Diaporthe* and thus the genus *Diaporthopsis* should be considered a synonym of *Diaporthe*.

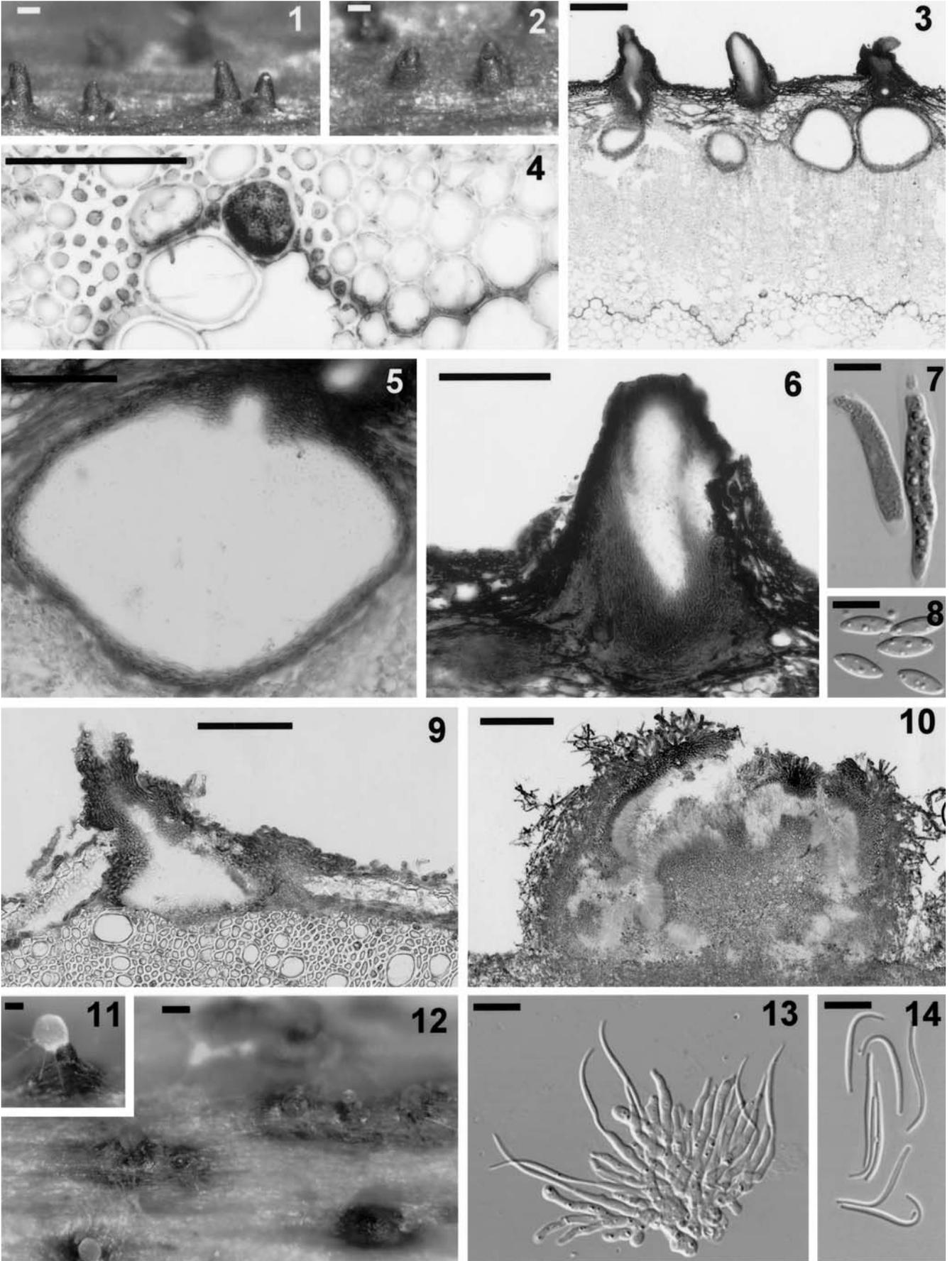
- Diaporthe angelicae*** (Berk.) Farr & Castlebury, comb. nov. Figs. 1–14
 = *Sphaeria angelicae* Berk., Mag. Zool. & Bot. 1837:28.
 = *Diaporthopsis angelicae* (Berk.) Wehm., The genus *Diaporthe* Nitschke, p. 228, 1933.
 = *Mazzantia angelicae* (Berk.) Lar. N. Vassiljeva, Pyrenomycetes of the Russia Far East. I. Gnomoniaceae p. 49, 1993.
 = *Leptosphaeria nigrella* Auersw., Mycol. Eur. Pyr. 5/6, pl. 12, fig. 163, 1869.
 = *Diaporthe nigrella* (Auersw.) Niessl, Beitr. p. 51, 1872.
 = *Diaporthopsis nigrella* (Auersw.) Fabre, Ann. Sci. Nat. Bot. Ser. 6 15:35, 1883.

Stroma on substratal surface effuse, extended over large areas, dotted by numerous, conical to cylindrical, separately erumpent, protruding ostioles, dorsal surface black with a violet or dark purple tinge, prosenchymatous to pseudoparenchymatous, several layers thick, cells 5–8 µm diameter, walls thick with lumen as small as 1 µm, frequently forming a collar around ostiole, ventral zone deep in host tissue; perithecia embedded in stroma composed of host and fungal hyphae; black line stroma beneath perithecia separating wood from pith, prosenchymatous, 1–2 layers, cells 2–3 µm wide. Perithecia black, globose to subglobose, 200–230 µm high × 140–320 µm wide, wall 20–25 µm thick, of elongated, thin-walled, black cells 10–20 × 2–5 µm, becoming hyaline toward interior, ostioles central, solitary, straight or bent parallel with surface before penetrating stroma, 80–140 (–220) µm high × 80–110 µm wide at apex, broader at base, cells forming textura porrecta. Paraphyses of broad, collapsing bands, unbranched, 50–80 × 5 µm, hyaline, absent at full maturity. Asci unitunicate, 39.0–51.0 (\bar{x} 45.2, SD = 3.2, n = 27) × 5.0–8.0 (\bar{x} 6.4, SD = 0.97) µm, clavate, tapering toward base at maturity, slightly constricted as apex, with a conspicuous refractive apical ring, cylindrical when young, at maturity detaching from base of centrum, ascospores biserial. Ascospores 9.0–13.0 (\bar{x} 10.7, SD = 1.08, n = 28) × 2.5–4.0 (\bar{x} 3.2, SD = 0.37) µm, fusiform-ellipsoid, narrowly rounded at apex, hyaline, unicellular, smooth, each with three guttules.

Conidiomata on alfalfa twigs in culture pycnidial, black, scattered, uniloculate or multiloculate, 3–8 mm long, with one or two layers of black hyphae above epidermis, a thicker prosenchymatous to pseudoparenchymatous layer of four to many layers of brown, more or less thick-walled

Figs. 1–14. *Diaporthe angelicae* BPI 842091. **1, 2** Perithecial ostioles protruding through black ectostroma on host surface. **3, 4** Perithecia and ostioles showing thin ventral edge of black ectostroma on substratal surface and black line stroma beneath perithecia and fungal–host entostromatal tissue, cross section. **5, 6** Perithecial ostiole, cross section. **7** Asci each with a conspicuous apical ring. **8** Ascospores.

9 Conidiomata developing on alfalfa twigs in culture, cross section. **10** Conidiomata developing on potato dextrose agar (PDA), cross section. **11, 12** Conidiomata on alfalfa twigs with beta conidia oozing from ostiole. **13** Conidiogenous cells producing beta conidia. **14** Beta conidia. Bars **1–6** 100 µm; **7, 8** 10 µm; **9–12** 100 µm; **13, 14** 10 µm



cells separating the cortex from vascular tissue, pycnidia globose to subglobose, 150–225 µm high × 150–220 µm wide, ostiolate, cells at bottom of locule hyaline, thin-walled, cells on side hyaline to pale brown, often thick-walled, ostiole short, straight, 70–130 µm high, inner cells hyaline, outer black, thick-walled. Alpha conidia not observed. Beta conidia sinuous, hooked, lunate, apex rounded, base truncate, tapering toward apex, hyaline, aseptate, 19.9–31.3 (\bar{x} 25.4, SD = 2.2, n = 32) × 1.3–2.1 (\bar{x} 1.6, SD = 0.19) µm.

In culture: On PDA after 7 days in light: dark at 25°C, colony 8 cm diameter, white fluffy to pale lavender gray, white, wavy at margin, reverse white or white at margin to amber, bright luteous or darker in center. On CMD after 7 days in light: dark at 25°C, 6.8–8 cm diameter, thin, white to pale isabelline, white at margin, slightly stringy, reverse white to pale isabelline, white at margin. Pycnidia developing on alfalfa twigs after 10 days, mycelium none or slightly fluffy, thin, aerial, pycnidia subepidermal, beta conidia only.

Specimens examined: Austria: Kaernten, Wograda, St. Margareten im Rosental, on *Heracleum sphondylium*, Nov. 2, 2001, collected by W. Jaklitsch WJ 1842, isolated by A. Rossman AR 3776 = CBS 111592 (BPI 842091, designated EPITYPE herein); Niederoesterreich, Ottenstein, at Dobra-Stausee, mapping grid square 7458/1, on *Heracleum sphondylium*, Aug. 18, 2001, collected by W. Jaklitsch WJ 1773, isolated by A. Rossman AR 3724 = CBS 111591 (BPI 842090); Salzburg, Lofer, on stems of *Angelica* sp., collected by G. Niessl, possibly 1884, Rabenhorst-Winter, Fungi europaei 3356 as *Diaporthe nigrella* (BPI 616595; BPI 616596). Germany: near Islebiam, on stems of *Eryngium campestris*, collected by J. Kunze, May 1875, Johannes Kunze, Fungi selecti exsiccati 135 as *Diaporthe nigrella* (BPI 616594, also in bound set). Switzerland: near Zurich, on stems of *Daucus carota*, August 1878, collected by G. Winter, Johannes Kunze, Fungi selecti exsiccati 354 as *Diaporthopsis angelicae* (BPI 601782, BPI 601783). United States: Dane Co., Madison, Wisconsin, on overwintered stems of *Angelica atropurpurea*, Apr. 1, 1958, collected by H.C. Greene, detected by L.E. Wehmeyer (BPI 601784 as *Diaporthe apiculosa*).

Hosts: On decaying stems of plants in the Apiaceae, reported from *Angelica sylvestris* L., *Angelica* sp., *A. atropurpurea* L., *Anthriscus sylvestris* (L.) Hoffm. (Eriksson 1992), *Anthriscus* sp., *Daucus carota* L., *Eryngium campestre* L., *Heracleum sphondylium* L., *Oenanthe crocata* L. (Kirk and Spooner 1984), *Pastinaca sativa* L. (Munk 1957), and *Peucedanum oreoselinum* (L.) Moench (Wehmeyer 1933).

Distribution: Austria, Belgium (Wehmeyer 1933), Canada (British Columbia; Barr 1978), Czech Republic (Sukova 2001), France (Wehmeyer 1933), Germany, Holland (Steketee 2002), Ireland (Muskett and Malone 1983), Portugal (Wehmeyer 1933), Sweden (Eriksson 1992), Switzerland, United Kingdom (England; Dennis 1995; Kirk and Spooner 1984; Hebrides; Dennis 1986); United States (Wisconsin).

Diaporthe angelicae is reported on members of the Apiaceae from throughout Europe and North America.

Molecular results

A total of 1364 bases of the 5'-LSU nrDNA were sequenced for both isolates of *D. angelicae*. The LSU nrDNA alignment consisted of 50 taxa and 1284 positions of the 5'-end of the LSU nrDNA, with 173 parsimony informative characters. A total of 539 bases including the complete ITS1, 5.8S nrDNA, and ITS2 regions with partial 18S and 28S nrDNA were sequenced for *D. angelicae*, and 528 bases of the same regions were sequenced for *Diaporthe arctii* CBS 109490.

The two isolates of *D. angelicae* differed by one substitution in the ITS2 region and one substitution in the LSU nrDNA. A BLAST search of GenBank using the ITS sequence of *D. angelicae* (CBS111591) returned sequences from three strains of *Diaporthe helianthi* Muntañola-Cvetkovic et al. (GenBank accessions AJ312348, AJ312351, AJ312366) with 96% identities (520/540, 523/540, and 520/540 identities, respectively, with 6/540 gaps for each). The next best matches (95% identity) were five sequences from strains of *Phomopsis longicolla* Hobbs (GenBank accessions AF000207–AF000211) each with 519/542 identities and 5/542 gaps. The ITS sequences of both isolates of *D. angelicae* differed from *D. arctii* CBS 109490 at 13 positions of the 528 (2.5%) available for all isolates, whereas the LSU nrDNA differed at 3 positions of the 1301 (0.2%) available for all isolates.

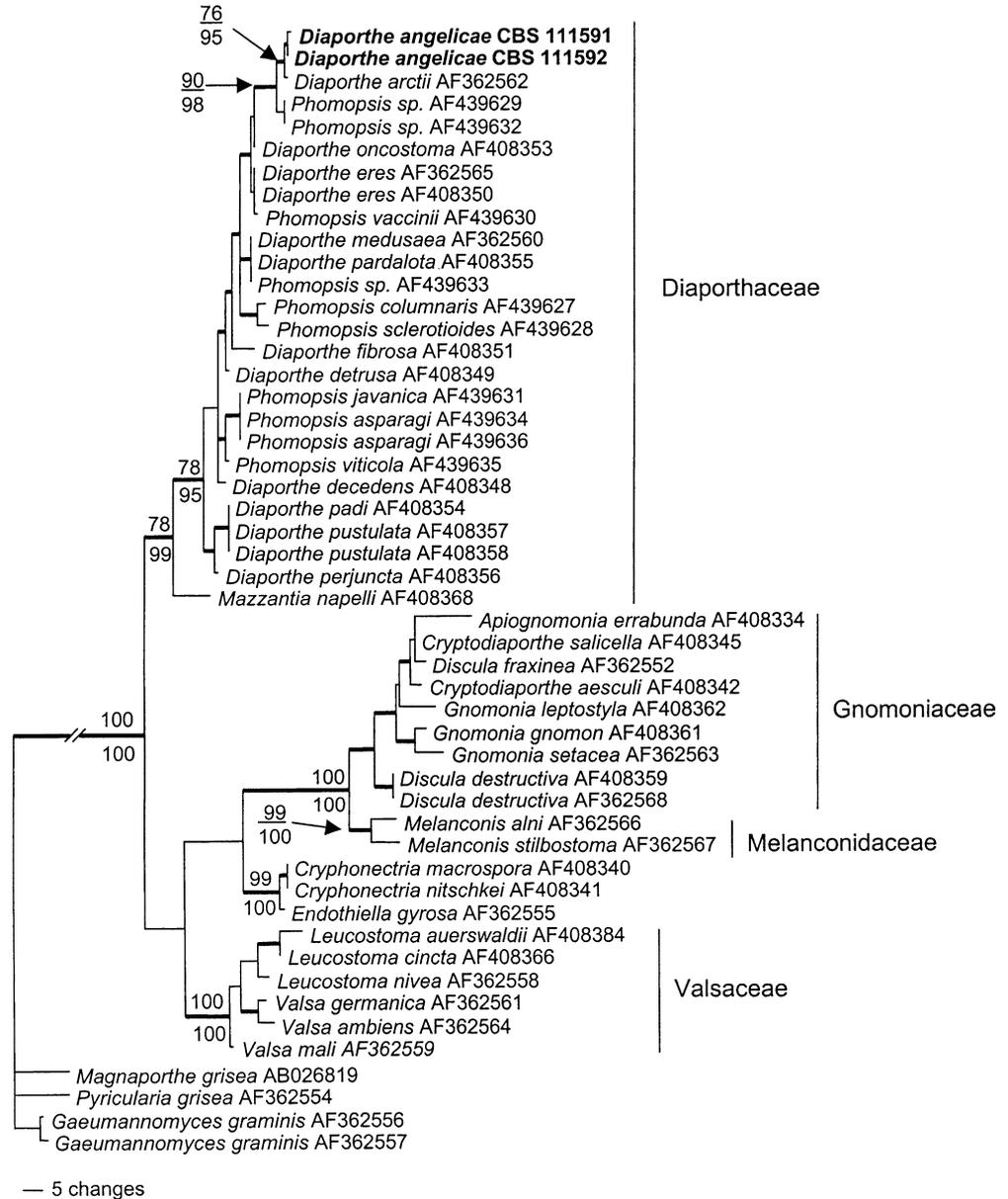
Maximum-parsimony sequence analysis of the LSU nrDNA for representative members of the Diaporthales resulted in 28 equally parsimonious trees with the following statistics: length, 400; consistency index (CI), 0.568; retention index (RI) 0.894; rescaled consistency index (RC), 0.507; and homoplasy index (HI), 0.432. One arbitrarily chosen tree is shown in Fig. 15 with thickened lines indicating branches present in the strict consensus of all 28 trees. Maximum-parsimony bootstrap supports for branches are shown above and NJ bootstrap supports are shown below the branches. Both isolates of *D. angelicae* group with *D. arctii* (CBS 109490) isolated from *Ambrosia trifida* L. (bootstrap = 76% MP and 95% NJ). Other closely related isolates in this tree include two isolates of *Phomopsis* (Sacc.) Bubák from *Vaccinium* L.

Discussion

The genus *Diaporthopsis* was established for species of *Diaporthe* having continuous, i.e., nonseptate ascospores (Fabre 1883). *Diaporthe nigrella* was listed as the only species in the new genus and thus serves as the type. Wehmeyer (1933) determined that *D. nigrella* is a synonym of *Sphaeria angelicae*, which provides an older epithet. This synonymy is confirmed here based on examination of the historical specimens listed above.

Examination of specimens of *Diaporthe angelicae* reveals morphological characteristics that are typical for species of *Diaporthe* except for ascospore septation. Within the genus *Diaporthe*, the stroma in which perithecia are embedded can be variable in development, especially when

Fig. 15. One of 28 equally parsimonious trees resulting from analysis of 1284 bp of 5'-large subunit nuclear rDNA of some diarthalean fungi, including *Diaporthe angelicae*. Length, 400; consistency index (CI), 0.568; retention index (RI), 0.894; rescaled consistency index (RC), 0.507; homoplasy index (HI), 0.432. Accession numbers follow taxon names for sequences obtained from GenBank. Numbers above branches indicate maximum-parsimony (MP) bootstrap support expressed as a percentage; numbers below indicate neighbor-joining (NJ) bootstrap support. Thickened lines indicate branch was present in the strict consensus tree



viewed superficially. Species of *Diaporthe* have “stromatic tissues composed of prosenchymatous hyphae mixed with substrate tissues in entostromatic regions, often forming dark marginal zones above in epidermal tissues and frequently deep in wood beneath perithecia or groups of perithecia” (Barr 1978). Many species of *Diaporthe* may appear to be in a well-developed stroma that is pustulate and extends considerably above the surface of the substratum. Ostioles may extend in dense clusters or individually to a greater or lesser extent above the surface of the stroma. At the other end of the spectrum are *Diaporthe angelicae* and related taxa in which the stroma at the substratal surface is relatively thin, developing as a blackened layer above non-clustered perithecia (see Figs. 1–3). However, in all species of *Diaporthe*, the entostromatic tissue consists of a mixture of host and fungal cells. The margin at the sides and base of the entostroma is delineated by a black line well

below the perithecia (Figs. 4, 5). Septation of the ascospores is not a useful taxonomic character for distinguishing *Diaporthopsis* from *Diaporthe* and may not be useful in distinguishing genera in the Diarthraceae.

Diaporthe angelicae is placed firmly within the genus *Diaporthe* using LSU nrDNA sequence analysis, and a close relationship to *D. arctii* (CBS109490) is indicated. Comparison of ITS sequences confirms this close relationship and also suggests close relationships to isolates identified as *Diaporthe helianthi*, causal agent of a stem canker on sunflower, and *Phomopsis longicolla*, causal agent of a seed decay of soybeans.

Both *D. angelicae* and *D. arctii* have an extensive thin, black, ectostromatic layer covering the host tissue through which extend the individual ostioles of the perithecia (Figs. 1–3). The perithecia are immersed in the endostroma that consists of mixed host–fungal tissue. At the margin of this

tissue is a distinct black line often well below the perithecia that separates the fungal stromatic tissue from the host (Figs. 4, 5). Wehmeyer (1933) stated that this distinct black line was lacking in *D. angelicae*. In the specimens examined for this article, the black line that delimits the edge of the fungal–host stroma from host tissue is distinct although occasionally difficult to find. In both *D. angelicae* and *D. arctii* the ostioles are solitary and separate extending above the black, superficial ectostroma (Figs. 1–3). The height of the ostiole is variable, ranging from 80 to 220 µm. *D. angelicae* differs from *D. arctii* mainly by nonseptate, non-constricted, three-guttulate ascospores, somewhat longer ostioles, and a violet tinge of the stroma surface (Wehmeyer 1933). Both species have a *Phomopsis* anamorph. A close relationship between *D. angelicae* and *D. arctii* is suggested by morphological similarities of the stroma and occurrence on herbaceous stems, as noted previously by Munk (1957) and Wehmeyer (1993). In fact, Munk (1957) transferred *Diaporthopsis angelicae* to *Diaporthe* as a new combination, but this transfer is not considered valid because he did not cite the basionym (Greuter et al. 2000).

Vasilyeva (1993) placed *D. angelicae* in the genus *Mazzantia* Mont. and thus considered *Diaporthopsis* to be a synonym of *Mazzantia*, a diaporthalean genus characterized by a sharply delimited, strongly melanized, sclerotial clypeal ectostroma, a whitish entostroma, and nonseptate ascospores (Höhnelt 1918; Petrak 1940). *Mazzantia* is typified by *M. galii* (Fr.) Mont., a species that has not been obtained in culture or sequenced. Analysis of LSU nrDNA sequences of a number of species of *Diaporthe* including *D. angelicae*, and a non-type species of *Mazzantia*, *M. napelli* (Fr.) Sacc., does not support a relationship between *D. angelicae* and *M. napelli* (see Fig. 15). This analysis indicates that *M. napelli* is a species closely related to but distinct from *Diaporthe* in the Diaporthaceae, as reported in Castlebury et al. (2002).

A number of additional species have been described in *Diaporthopsis*; however, these species have not been examined to determine if they belong in *Diaporthe* as well. Although 17 fungal names have been placed in *Diaporthopsis*, Kirk et al. (2001) stated that the genus includes 8 species. Wehmeyer (1933) considered *Diaporthe foeniculacea* Niessl to be a synonym of *D. angelicae*; however, Phillips (unpublished data) suggests that this species is a distinct entity.

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