

***Bretziella*, a new genus to accommodate the oak wilt fungus, *Ceratocystis fagacearum* (Microascales, Ascomycota)**

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Abstract

Recent reclassification of the Ceratocystidaceae (Microascales) based on multi-gene phylogenetic inference has shown that the oak wilt fungus *Ceratocystis fagacearum* does not reside in any of the four genera in which it has previously been treated. In this study, we resolve typification problems for the fungus, confirm the synonymy of *Chalara quercina* (the first name applied to the fungus) and *Endoconidiophora fagacearum* (the name applied when the sexual state was discovered). Furthermore, the generic placement of the species was determined based on DNA sequences from authenticated isolates. The original specimens studied in both protologues and living isolates from the same host trees and geographical area were examined and shown to represent the same species. A lectotype was designated for *Chalara quercina* and *Endoconidiophora fagacearum* and an epitype linked to a living ex-epitype isolate was designated. Phylogenetic analyses confirmed that the species resides in a well-supported monophyletic lineage in the Ceratocystidaceae, distinct from all other genera in the family. The new genus *Bretziella* is described to accommodate the oak wilt fungus.

Keywords

Quercus, Ceratocystidaceae, Microascales, heterothallic

Introduction

Oak wilt is a serious disease of many *Quercus* spp. in the Midwestern and Eastern United States, as well as Texas (Juzwik et al. 2011). The disease was first described in the 1940's (Henry 1944, Bretz 1953) and sporadic, localized outbreaks occur frequently in the established range, although the disease is viewed by many as a manageable (Juzwik et al. 2011, Horie et al. 2013). However, with a growing global awareness of invasive alien species and their potential to cause destructive epidemics (Brasier 2008, Wingfield et al. 2015), oak wilt is considered one of several significant diseases that threaten the health of *Quercus* spp. worldwide (Gibbs 1981, 2003, Brasier 2001).

Oak wilt is caused by a fungus in the genus *Ceratocystis*, which is widely known as *Ceratocystis fagacearum* (Juzwik et al. 2008, 2011, Harrington 2009). The genus was originally described to accommodate the sweet potato pathogen, *C. fimbriata* (Halsted 1890). Since that time many morphologically similar species were described in or transferred to this genus, resulting in an aggregate genus incorporating more than 70 species a century later (Upadhyay 1981). DNA sequence analyses revealed that *Ceratocystis sensu* Upadhyay included two phylogenetically distinct groups (Hausner et al. 1993, Spatafora and Blackwell 1994). Several subsequent studies confirmed that the one group, including the type species of *Ophiostoma*, previously treated as *C. pilifera*, resides in the Ophiostomataceae (Ophiostomatales, Sordariomycetidae). The second group, including *C. fimbriata*, resides in the Ceratocystidaceae (Microascales, Hypocreomycetidae) (Réblová et al. 2011, De Beer et al. 2013a).

Generic boundaries within the Ceratocystidaceae were recently reconsidered based on DNA sequence data for three gene regions in 70 species (De Beer et al. 2014). Phylogenetic analyses showed that the family includes at least seven well-supported monophyletic lineages accepted as distinct genera, as well as four minor, unresolved lineages. De Beer et al. (2014) thus redefined *Ceratocystis s. str.* and *Ambrosiella*, re-instated and emended descriptions for *Chalaropsis*, *Endoconidiophora*, and *Thielaviopsis*, and described two new genera, *Davidsoniella* and *Huntiella*. The unresolved lineages included *Thielaviopsis basicola*, *Ceratocystis adiposa*, and *Ambrosiella ferruginea*. In a subsequent study, Mayers et al. (2015) re-instated the genus *Phialophoropsis* to accommodate *A. ferruginea* and *A. trypodendri*, and described an additional genus, *Meredithiella*.

The fourth unresolved lineage in the study of De Beer et al. (2014) included the single taxon, *Ceratocystis fagacearum*. The asexual state of the fungus was described first as *Chalara quercina* (Henry 1944). Bretz (1951) and Hepting (1951, 1952) soon discovered that the fungus was heterothallic and that the sexual state could be induced in culture by crossing isolates of opposite mating type. Bretz (1952) proceeded to describe the sexual state as *Endoconidiophora fagacearum*. However, Bretz was not aware that in the previous year, Bakshi (1951) reduced *Endoconidiophora* (Münch 1907) to synonymy with *Ceratocystis*, a treatment that soon gained wide acceptance (Moreau 1952, Moreau and Moreau 1952, Hunt 1956). In his monograph of *Ceratocystis*, Hunt (1956) transferred *E. fagacearum* to that genus.

During the course of the six decades following the Hunt (1956) monograph, the oak wilt fungus was treated as *Ceratocystis fagacearum*, with its asexual (anamorph) name, *Chalara quercina* as heterotypic synonym (Nag Raj and Kendrick 1975, Upadhyay 1981, Seifert et al. 1993, De Beer et al. 2013b). Following the dual nomenclature system, Paulin-Mahady et al. (2002) suggested that the asexual state of *C. fagacearum* should be treated as *Thielaviopsis quercina*. This was because the type species of the genus *Chalara*, *Chalara fusidioides*, was clearly different from the taxa related to *Ceratocystis* and was suggested to belong to the Leotiales.

De Beer et al. (2014) restricted *Ceratocystis* to species previously treated in the *C. fimbriata* complex. *Endoconidiophora* was confined to species previously treated in the *C. coerulescens* complex, with *E. coerulescens* as the type species. Based on the phylogenies presented by Mbenoun et al. (2014) and De Beer et al. (2014), *Thielaviopsis* with *T. ethacetica* as the type species, included species previously treated in the *C. paradoxa* complex. Consequently, none of the four genera (*Ceratocystis*, *Endoconidiophora*, *Thielaviopsis* or *Chalara*) are available to accommodate *C. fagacearum*, which resides in a lineage distinct from these genera (De Beer et al. 2014). Because the isolate representing *C. fagacearum* was not from a type specimen, De Beer et al. (2014) concluded a generic placement of the species could not be considered prior to resolving the typification of *E. fagacearum* and *Ch. quercina*. These authors also suggested that sequences of additional isolates should be included in such a study.

The aim of this study was firstly to consider the appropriate generic placement of the oak wilt fungus in the Ceratocystidaceae based on phylogenetic analyses of the three gene regions used by De Beer et al. (2014), and including additional isolates of the fungus. Secondly, all available materials used in the protologues of the two species were obtained to address unresolved typification issues. The synonymy of *Ch. quercina* and *E. fagacearum* and priority of the basionyms was also resolved against the backdrop of contemporary nomenclatural practices (McNeill et al. 2012, 2015).

Materials and methods

Herbarium specimens and isolates

Herbarium specimens labelled as *Chalara quercina* from the study of Henry (1944), and *Endoconidiophora fagacearum* from the study of Bretz (1952), were obtained respectively from the National Fungus Collections (BPI) (U.S. Department of Agriculture, Beltsville, Maryland) and the Forest Service (FP) (Center for Forest Mycology Research, Madison, Wisconsin). Each specimen included dried cultures and notes. In addition, four isolates of *Ceratocystis fagacearum* that were isolated from diseased oak trees in the USA, available from the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (University of Pretoria, Pretoria, South Africa), were included in the study (Table 1). The epitype for *C. fagacearum* was deposited in BPI.

Table 1. Isolates of *Bretziella fagacearum*[†] used in this study.

Culture numbers [‡]	Host	Locality	GenBank accession number		
			60S	LSU	MCM7
CMW 2039 = CBS 130770	<i>Quercus</i> sp.	Minnesota	=KM495518	=KM495341	=KM495430
CMW 2656 ^{EP} = CBS 138363	<i>Quercus rubra</i>	Iowa	KM495518 [§]	KM495341	KM495430
CMW 2658	<i>Quercus</i> sp.	Iowa	=KM495518	=KM495341	=KM495430
CMW 38759 = CBS 129241	<i>Quercus</i> sp.	Iowa	=KM495518	=KM495341	=KM495430

[†] Information on other species and isolates included in this study and their GenBank accession numbers are available in De Beer et al. (2014).

[‡] CMW = Culture collection of the Forestry and Agricultural Biotechnology Institute, University of Pretoria, Pretoria, South Africa, CBS = Westerdijk Fungal Biodiversity Institute, Utrecht, the Netherlands.

[§] Where DNA sequences of different isolates were identical, we only deposited one sequence representing each haplotype in GenBank. Identical sequences obtained from other isolates are indicated with '='

^{EP} = Ex-epitype.

PCR, DNA sequencing and phylogenetic analyses

Three gene regions, the nuclear ribosomal DNA large subunit (LSU), the 60S ribosomal protein RPL10 (60S), and mini-chromosome maintenance complex component 7 (MCM7), were amplified and sequenced for all four living isolates. These gene regions were the same as those selected and used by De Beer et al. (2014) to define generic boundaries in the Ceratocystidaceae. In addition to these, sequences were determined of the ribosomal internal transcribed spacer region (ITS) and translation elongation factor 1- α (TEF1 α), respectively the universal DNA barcode (Schoch et al. 2012) and secondary barcode (Stielow et al. 2015) for fungi, for isolate CBS 138363 = CMW 2656. Total genomic DNA was extracted with PrepMan[®] Ultra Sample Preparation Reagent (Applied Biosystems, Foster City, California) following the protocols used by Duong et al. (2012). Primers, PCR and PCR sequencing protocols used were the same as those described by De Beer et al. (2014).

Representative species of the dominant genera in the Ceratocystidaceae were included in the phylogenetic analyses. The recently described *Meredithiella* was not included because appropriate sequence data were not available for this taxon. Species of *Knoxdaviesia* and *Graphium* were included as outgroups. Datasets for each of the three gene regions were compiled and aligned separately with the online version of MAFFT v. 7 (Kato and Standley 2013) and concatenated into a single dataset for subsequent analyses. Maximum likelihood (ML) and Bayesian inference (BI) were carried out on the concatenated dataset. ML analysis was conducted using raxmlGUI v. 1.3.1 (Silvestro and Michalak 2012). Ten runs of a maximum likelihood search with the GTR+G model were performed, followed by 1000 bootstrap searches. BI analysis was conducted using MrBayes v. 3.2 (Ronquist et al. 2012). Ten parallel runs with the GTR+G model were performed for 5 million generations. Trees were sampled every 100th generation. The first 25 % of the tree samples were discarded as burn-in, and Bayesian posterior probabilities were computed from the remaining trees.

Morphology

Morphological characters of sexual and asexual structures taken from the herbarium specimens and living isolates were compared with each other and with the original descriptions (Henry 1944, Bretz 1951, 1952). For morphological studies, isolates were grown on 2 % yeast malt agar (YMA). In an attempt to obtain sexual structures, the four isolates were crossed with each other in all possible combinations on 2 % water agar in the presence of sterilized oak twigs. The plates were incubated at room temperature under near UV light.

Microscopic structures taken from herbarium specimens were mounted and studied in 10 % KOH, and those from living cultures were mounted in water, later replaced with 85 % lactic acid in which they were then studied. Up to 50 measurements were made for each characteristic structure where possible. Microscopic structures were studied with a Nikon SMZ18 stereoscope and a Nikon Eclipse Ni compound microscope. Images were captured using a Nikon DS-Ri2 camera. Measurements were made using the Nikon Imaging Software (NIS) Elements (v. 4.3).

Results

Phylogenetic analyses

DNA sequences obtained for the LSU, 60S, and MCM7 regions of the four living isolates were used for phylogenetic analyses. These sequences, as well as the ITS and TEF1 α sequences for CBS 138363 = CMW 2656 (ex-epitype, see below), have been deposited in the RefSeq Targeted Loci (RTL) database in NCBI GenBank (Schoch et al. 2014).

A total of 39 isolates representing 35 species were included in the phylogenetic analyses. Alignment of the 60S dataset resulted in ambiguously aligned regions and long gaps that were a result of the inconsistency in the presence/absence of introns and highly variable intron sequences. Gap-containing positions from the 60S dataset were thus excluded from further analyses. After removing all gap positions, the 60S dataset consisted of 314 characters with 105 variable characters. The LSU dataset consisted of 875 characters with 173 variable characters. The MCM7 dataset consisted of 628 characters with 321 variable characters. The ML and BI analyses of the concatenated dataset of all three gene regions resulted in trees with almost identical topology. Monophyletic clades representing all genera included in the analyses could be identified and these clades were strongly supported in both ML and BI analyses.

The four *C. fagacearum* isolates included in this study formed a well-supported monophyletic clade (Figure 1) that was most closely related to, but distinct from, *Phialophopsis*. The only difference observed between the BI and ML trees was the positioning of *Thielaviopsis* in relation to other genera. In the ML tree, *Thielaviopsis* formed a sister clade to those of *Endoconidiophora* and *Davidsoniella*, but with no support. This was in contrast to the BI tree, where *Thielaviopsis* formed a clade basal to those of *Ceratocystis s. str.*, *Chalaropsis*, *Endoconidiophora* and *Davidsoniella* with high posterior probability values.

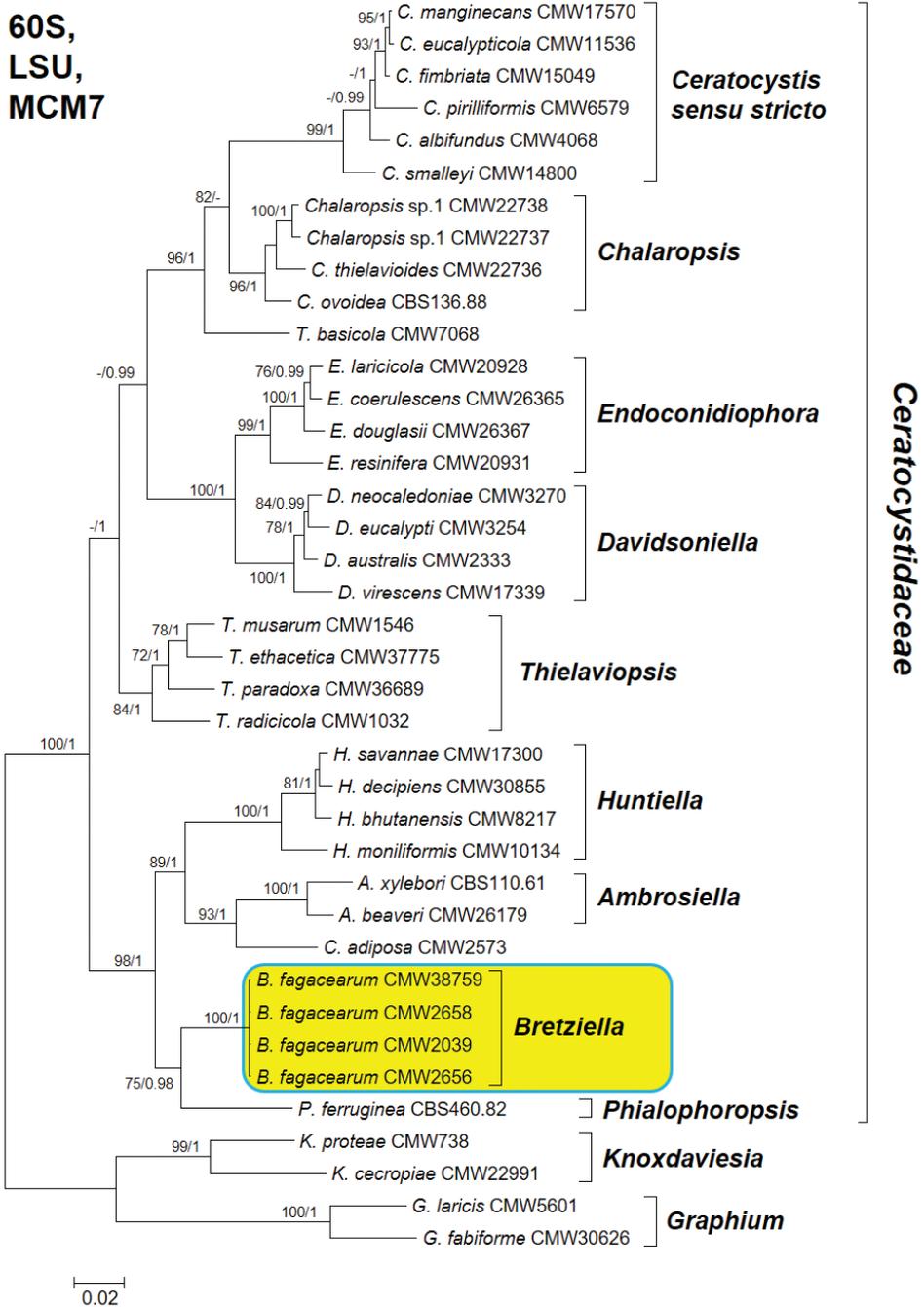


Figure 1. Bayesian phylogram derived from the analyses of the concatenated dataset (60S, LSU, MCM7). Maximum likelihood bootstrap values ($\geq 70\%$, 1000 replicates) and Bayesian posterior probabilities values (≥ 0.95) are indicated at nodes. “-” indicated no phylogenetic support or the support values are below 70% for ML and 0.95 for BI.

Morphology

The herbarium specimen of *Chalara quercina* (BPI 595712) from study of Henry (1944) consisted of a dried culture with dark brown to grey clumps of aerial hyphae present. Only asexual structures were obtained from this specimen (Figure 2A, E, F, K).

Description: *Conidiophores* cylindrical tapering towards the apex, single, upright, straight or slightly curved, occasionally branched, pale to dark brown, becoming paler to the apex, 3–9 septate, up to 140 μm long including conidiogenous cells, 3–5 μm wide at the base. *Conidiogenous cells* cylindrical, tapering towards the apex, slightly pigmented to hyaline, 20–32 μm long, 2.5–3.5 μm wide at the base, 2–3 μm wide near the apex. *Conidia* endogenous, hyaline, rectangular shaped, 4–8.5 \times 2–3 μm , produced in chains. *Aleuriiconidia* not observed.

The herbarium specimen of *Endoconidiophora fagacearum* (FP 97476) from the study of Bretz (1952) consisted of a few broken pieces of dried agar covered with a thick, grey to dark brown mycelial mat. A few ascomatal necks were observed with their bases completely embedded in the mycelial mats, which also contained asexual structures (Figure 2B, C, D, G, H, L).

Description: *Ostiolar hyphae* observed in a single ascomatal neck hyaline, divergent. *Ascospores* recovered from broken ascoma hyaline, ellipsoidal, occasionally curved, 4.5–9.5 \times 2–3.5 μm , embedded in gelatinous sheath. *Conidiophores* cylindrical tapering towards the apex, single, upright, straight or slightly curved, occasionally branched, pale to dark brown, becoming paler towards the apex, 2–6 septate, up to 100 μm long including conidiogenous cells, 3.5–5 μm wide at the base (these measurements reflect a limited number of intact conidiophores due to the brittle condition of the specimen). *Conidiogenous cells* cylindrical, tapering towards the apex, slightly pigmented to hyaline, 19–35 μm long, 2.5–3.5 μm at the base, 2–3.5 μm near the apex. *Conidia* endogeneous, hyaline, rectangular-shaped, 3–6.5 \times 2–3 μm , produced in chains. *Aleuriiconidia* not observed.

Laboratory crosses between the living isolates (Table 1) treated to date as *Ceratocystis fagacearum*, did not yield sexual structures and produced only asexual structures (Figure 2I, J, M).

Description: On 2 % YMA with oak sticks mycelia fluffy, pale to dark grey. Spore-bearing structures hidden in mycelial mat. *Conidiophores* cylindrical, tapering towards the apex, single, upright, straight or slightly curved, occasionally branched or reduced to conidiogenous cells, pale to dark brown, becoming paler towards the apex, 3–9 septate, up to 155 μm long including conidiogenous cells, 3–5 μm wide at the base, often constricted at septum. *Conidiogenous cells* cylindrical tapering towards the apex, slightly pigmented to hyaline, 25–35 μm long, 2.5–4.5 μm wide at the base, 2.5–3.5 μm wide near the apex. *Conidia* endogenous, rectangular shaped, hyaline, 3.5–9 \times 1.5–3.5 (avg. 5.9 \times 2.5 μm), produced in chains. *Aleuriiconidia* not observed.

Features of the conidiophores were almost identical between the two herbarium specimens and the living isolates (Figure 2E–J). Conidial dimensions, however, showed some variability between the original descriptions and our observations. Henry (1944)

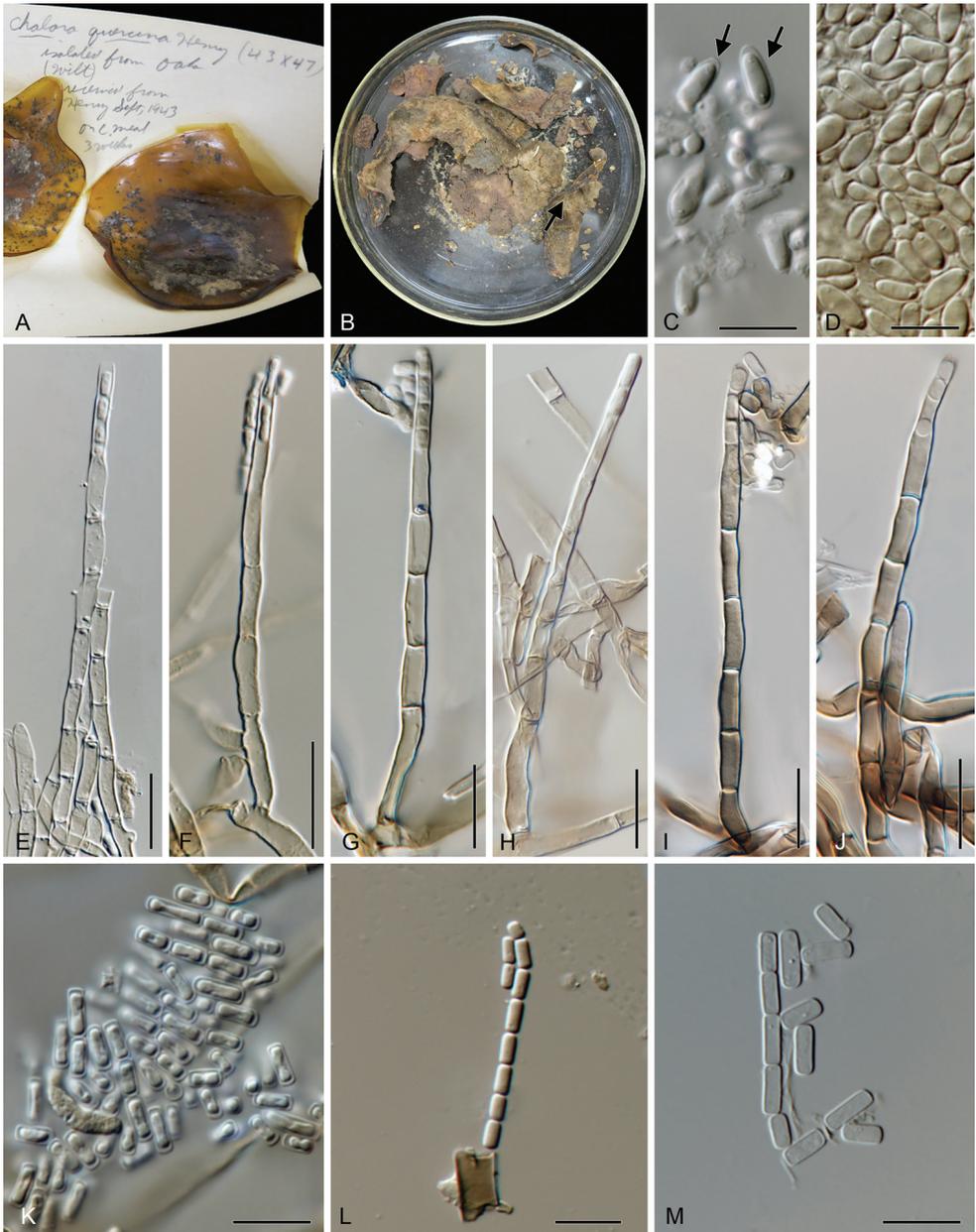


Figure 2. Morphological features of herbarium specimens and a living isolate of the oak wilt fungus. **A, E, F, K** *Chalara quercina* (BPI 595712, Lectotype) **B, C, D, G, H, L** *Endoconidiophora fagacearum* (FP 97476, Lectotype) **I, J, M** Living isolate treated as *Ceratocystis fagacearum* (CMW 2656 = CBS 138363, ex-epitype) **A, B** Dried cultures (arrow in **B** indicates the piece where ascomata were found) **C, D** Ascospores with sheaths (arrows) **E–J** Conidiophores **K–M** Conidia. Scale bars: **C–J** = 20 µm, **K–M** = 10 µm.

described conidia in the range of $4\text{--}22 \times 2\text{--}4.5 \mu\text{m}$, whereas in this study the size range of conidia from his specimen (BPI 595712) was $4\text{--}8.5 \times 2\text{--}3 \mu\text{m}$. Bretz's (1952) description of conidia reflected a mixture of endogenous conidia and aleurioconidia (see Mbenoun et al. 2014), described as 'thick-walled, olivaceous to brown, polymorphic spores, $3.5\text{--}5.5 \mu\text{m}$ wide to 5 to 20 μm long, formed endogenously and intercalarily in hyphae, which may also produce hyaline endoconidia'. We observed only hyaline, endogenous conidia in the range of $3\text{--}6.5 \times 2\text{--}3 \mu\text{m}$ from the Bretz specimen (FP 97476). This concurs with the description of Henry (1944), and observations based on the holotype specimen of Bretz (now lost, see below) by Nag Raj and Kendrick (1975) and Upadhyay (1981). It is also consistent with more recent observations that aleurioconidia do not occur in this species (Paulin-Mahady et al. 2002, Harrington 2009). The conidial dimensions taken from the living isolate (CMW 2656) were in the range of $3.5\text{--}9 \times 1.5\text{--}3.5 \mu\text{m}$, and corresponded with those on both herbarium specimens.

Culture characteristics of the fresh isolates were similar to those of the Bretz specimen (FP 97476), forming fluffy, thick mycelial mats containing the sexual structures (Figure 2A, B). The morphology of the dried culture of Henry (BPI 595712) differed from the other two specimens. However, the original description (Henry 1944) reads as follows: 'mycelial mat fluffy, 1–3 mm high, white, becoming gray to olive-green with occasional patches of tan', and is consistent with the morphology of the cultures examined in this study as well as that for the Bretz specimen.

Only a few broken ascomata were removed from the Bretz specimen (FP 97476) for this study. The shape of the ascomata was similar to those described by Bretz (1952). Diverging ostiolar hyphae were observed on the specimen and corresponded to Bretz's description of 'a cluster or fringe of hyaline filaments' that terminated in the 'long, black beaks'. The ascospores were $4.5\text{--}9.5 \mu\text{m}$ long and $2\text{--}3.5 \mu\text{m}$ wide, consistent with those reported by Bretz (1952) that were $5\text{--}10 \times 2\text{--}3 \mu\text{m}$. Bretz (1952) described the ascospores as 'elliptical and slightly curved', but did not specifically mention a sheath; a feature also not mentioned by Hunt (1956) when he provided the new combination for *Endoconidiophora fagacearum* in *Ceratocystis*. However, Upadhyay (1981) described ascospores from the lost holotype of Bretz (see below) as 'elongate ellipsoid or elongate orange section shaped in side view, cylindrical to elliptical in face view, end view not seen, surrounded by a uniform hyaline gelatinous sheath, $5\text{--}11 \times 2.5\text{--}3.5 \mu\text{m}$ including sheath'. Our observations of the ascospores (Figure 2C, D) included the presence of sheaths surrounding the ascospores, consistent with the description of Upadhyay (1981).

Taxonomy and nomenclature

Morphological comparisons with herbarium specimens representing *Chalara quercina* and *Endoconidiophora fagacearum*, confirmed that the four living isolates included in this study represented the same taxon. Unresolved typification and nomenclatural issues relating to this taxon are considered below. Phylogenetic analyses including

DNA sequences showed that the four isolates grouped in a well-supported clade in the Ceratocystidaceae (Figure 1), distinct from all other genera recently defined by De Beer et al. (2014) and Mayers et al. (2015). The lineage clearly represents an undescribed, at present monotypic genus in the *Ceratocystidaceae*, described as follows:

***Bretziella* Z.W.deBeer, Marinc., T.A.Duong & M.J.Wingf., gen. nov.**

MycoBank MB822520

Etymology. Named after Theodore W. Bretz who first discovered and described the sexual state of the type species of this genus (Bretz 1951, 1952).

Diagnosis. The genus is distinguished from all other genera of the *Ceratocystidaceae* based on the mycelial mats that it forms on infected oak trees. These mats form pressure cushions or pads that push the bark away from the underlying sapwood. This causes cracks in the bark, exposing the mats to fungal-feeding arthropod vectors, primarily nitidulid beetles.

Type species. *Bretziella fagacearum* (Bretz) Z.W.deBeer, Marinc., T.A.Duong & M.J.Wingf.

Description. *Ascomatal bases* black, globose, with undifferentiated ornamental hyphae, often embedded in mycelial mat. *Ascomatal necks* elongated, black at base, lighter at apex. *Ostiolar hyphae* present. *Asci* dehiscent. *Ascospores* one-celled, hyaline, ellipsoidal, occasionally curved, embedded in hyaline sheath. *Conidiophores* arise laterally from vegetative hyphae, occasionally branched. *Conidiogenous cells* phialidic, cylindrical, pale to dark brown. *Conidia* unicellular, cylindrical with flattened ends, hyaline, borne in chains of varying length. *Aleurioconidia* not present.

Ecology and distribution. The only known species in the genus causes vascular wilt on various oak species in North America.

***Bretziella fagacearum* (Bretz) Z.W.deBeer, Marinc., T.A.Duong & M.J.Wingf., comb. nov.**

MycoBank MB822521

Figures 2, 3

Bas.: *Endoconidiophora fagacearum* Bretz, *Phytopathology* 42: 436. 1952; *Ceratocystis fagacearum* (Bretz) Hunt, *Lloydia* 19: 21. 1956.

TYPES: USA. Dry culture resulting from a cross between two isolates, locations unknown, from *Quercus* sp., 26 Feb 1952, T.Bretz (Lectotype designated here: FP 97476, MycoBank typification number: MBT 378423). USA. Iowa, on *Quercus rubra*, 1991, S.Seegmueller (Epitype designated here: BPI 893238, MycoBank typification number: MBT 378424; ex-epitype culture CBS 138363 = CMW 2656). Representative sequences from epitype: 60S = KM495518, LSU = KM495341, MCM7 = KM495430, ITS = KU042044, TEF1 α = KU042043. See Notes 1, 2 and 3 below.

= *Chalara quercina* Henry, *Phytopathology* 34: 633. 1944; *Thielaviopsis quercina* (Henry) A.E.Paulin, T.C.Harr. & McNew, *Mycologia* 94: 70. 2002.

TYPE: USA. Dry culture, Wisconsin, Madison, on *Quercus* sp., Sept. 1943, B.Henry (Lectotype designated here: BPI 595712, MycoBank typification number: MBT 378425). See Note 4 below.

Descriptions. Henry (1944, pp. 631–635, Figure 1); Bretz (1951, p. 298, Figure 1); Bretz (1952, p. 436–437, Figure 1); Stessel and Zuckerman (1953, pp. 65–67, Figure 1); Hunt (1956, p. 21); Nag Raj and Kendrick (1975, pp. 94, 131, figure 32A); Upadhyay (1981, p. 66).

Note 1. Based on the one fungus one name principles adopted in the Melbourne Code (Hawksworth 2011, McNeill et al. 2012), the older basionym of the oak wilt pathogen, *Chalara quercina* (Henry 1944), has nomenclatural priority over *Endoconidiophora fagacearum*, the name Bretz (1952) assigned to the sexual state of the fungus. However, since Hunt (1956) treated the fungus as *Ceratocystis fagacearum*, the latter name were given preference under the dual nomenclature system in all major taxonomic works on the genus to date (Griffin 1968, De Hoog 1974, Nag Raj and Kendrick 1975, Upadhyay 1981, Seifert et al. 1993, Paulin-Mahady et al. 2002, Harrington 2009, De Beer et al. 2013b, 2014, Wingfield et al. 2013, Mayers et al. 2015). During the course of the past approximately 60 years, the name *Ceratocystis fagacearum* has also been adopted by plant pathologists and mycologists working on all aspects of the important disease known as oak wilt and the biology of the fungus (e.g. Shigo 1958, Cobb et al. 1965, Peplinski and Merrill 1974, Gibbs and French 1980, Juzwik and French 1983, Appel et al. 1990, Kile 1993, Gibbs 2003, Juzwik et al. 2008, 2011). A search on 26 August 2017 for *C. fagacearum* in Google Scholar and Google respectively yielded 1940 and 119000 hits, while the name *Ch. quercina* yielded only 431 and 3330 hits respectively. This provides strong evidence that *C. fagacearum* is the more ‘widely used’ name (see Hawksworth 2012).

In the present study, we have shown that the oak wilt fungus does not belong in *Ceratocystis s. str.*, *Endoconidiophora*, *Thielaviopsis* or any of the other genera currently accepted in the Ceratocystidaceae (De Beer et al. 2014, Mayers et al. 2015). We have consequently suggested that it is treated in a novel genus for which we have provided the name *Bretziella*. Based on the widespread use of the name *C. fagacearum*, we submitted a formal proposal that its basionym, *Endoconidiophora fagacearum*, is conserved against *Chalara quercina* (= *Thielaviopsis quercina*), to enable the new combination, *Bretziella fagacearum*, proposed above.

Note 2. In the protologue of *E. fagacearum*, Bretz (1952) specified the location of the holotype as ‘Type, For. Path. 97476, deposited in the Mycological Collections of the Bureau of Plant Industry, Soils and Agricultural Engineering’. In subsequent studies, the holotype specimen was referred to as ‘BPI-FP 97476’ (Hunt 1956, Nag Raj and Kendrick 1975, Upadhyay 1981). BPI has confirmed to us that this specimen had been lost. Fortunately, another specimen with the same number (FP 97476) as the one used in the protologue, was recently discovered in the Centre for Forest Mycology

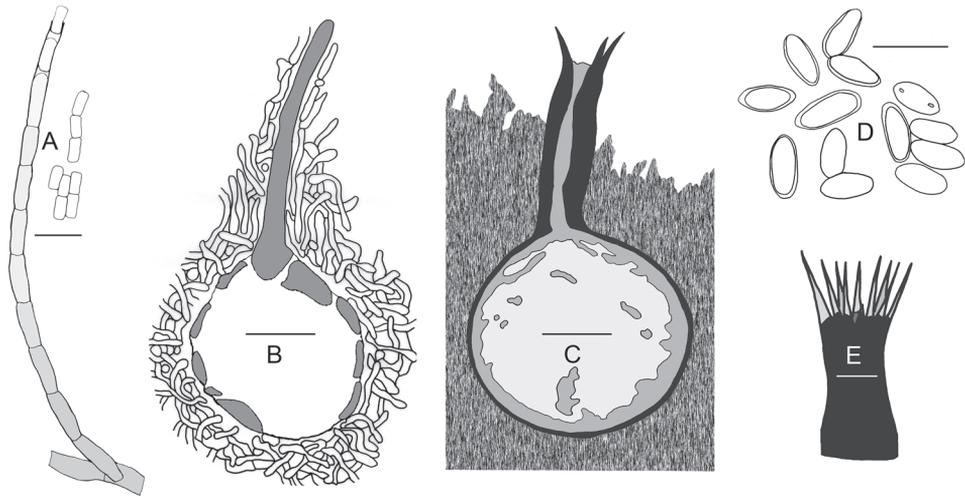


Figure 3. Line drawings of the oak wilt fungus. These illustrations are based on previously published line drawings and observations of the herbarium specimens (BPI 595712, FP 97476) in the present study. **A** Conidiophore and conidia in 10 % KOH (BPI 595712) **B** Ascomatal primordium re-drawn from Wilson (1956) **C** Median, histological section through ascoma embedded in the mycelial mat, re-drawn from Bretz (1952) **D** Ascospores in 10 % KOH (FP 97476) **E** Ostiolar hyphae (FP 97476). Scale bars: **A, D** = 10 μm , **E** = 50 μm , **B, C** = 100 μm .

Research Herbarium USDA-FS-NRS (FP) and was made available for this study. This specimen included a note by T. Bretz dated 26 Feb. 1952, marked as ‘type’. It is thus clear that this specimen represents an isotype of *E. fagacearum*. Based on Art. 9.12 (McNeill et al. 2012), we designate FP 97476 as lectotype for *E. fagacearum*.

Note 3. The lectotypes designated here for *Ch. quercina* and *E. fagacearum* both consist of dried specimens for which DNA sequence data are not available. However, based on careful microscopic comparisons between these two specimens and a living isolate from Iowa (Figure 2), we have concluded that the specimens and isolate all represent the same species. Although Bretz (1951, 1952) did not specify the host and location of the (now) lectotype of *E. fagacearum*, he stated that ascomata were obtained from multiple crosses between isolates from several *Quercus* spp. and Chinese chestnut (*Castanea mollissima*) occurring in Missouri, Arkansas, Ohio, Michigan, Pennsylvania, West Virginia, Kentucky, Tennessee, North Carolina, and Virginia. The specimen of Henry (1944) came from an unnamed *Quercus* sp. in Wisconsin, but he also included isolates from several *Quercus* spp. in Illinois, Iowa, and Minnesota in his study. Thus, although our living isolates do not come from the same host species and location as the lectotypes, they originate from the same host genus and geographical area (Midwest and Eastern States) from where isolates have been included in the studies of Henry (1944) and Bretz (1951, 1952). Based on the morphology, host, and origin, we have designated a dried culture of one of our isolates as epitype for *E. fagacearum* to enable the inclusion of the oak wilt fungus in DNA based studies.

Note 4. Henry (1944) lodged the original specimens of *Chalara quercina* in two collections but did not designate either as the holotype. One of these specimens (BPI 595712 = FP 94260) was included in the present study and is designated here as lectotype.

Discussion

The oak wilt fungus is an economically important pathogen in the USA, with the potential to become a serious, alien invasive if it was ever introduced into other countries having oak forests. It is listed as a quarantine organism by the European and Mediterranean Plant Protection Organization (EPPO) and the European Union (EU) (<http://www.q-bank.eu/>). Making a change to the name of a species having this level of importance must clearly be done responsibly and with care (Crous et al. 2015). Once the Ceratocystidaceae had been revised by De Beer et al. (2014) it became inevitable that *C. fagacearum* would require taxonomic revision, but it was felt that additional data were required to support a name change. In this study, we have shown, based on robust phylogenetic data, that the oak wilt fungus clearly requires a new genus in the Ceratocystidaceae, distinct from all four of the genera (*Ceratocystis*, *Endoconidiophora*, *Chalara* and *Thielaviopsis*) in which it has previously been treated. The alternative of retaining this important pathogen in *Ceratocystis* would be confusing to plant pathologists (Wingfield et al. 2012), phylogenetically incorrect and inconsistent with its unique biology.

In addition to phylogenetic data, the unusual biology of the oak wilt fungus supports the description of the new genus, *Bretziella*, to accommodate this species. After infection of healthy trees through wounds or root grafts, the fungus forms pressure pads under the bark that lead to cracks in the bark, exposing mats of mycelium and fruiting structures, attractive to fungus-feeding arthropods such as nitidulid beetles that then act as vectors of the fungus (Juzwik and French 1983, Harrington 2009, Juzwik et al. 2011). These insects move to fresh wounds on trees perpetuating the infection cycle. There are no other species in the Ceratocystidaceae that share this unique biology.

The choice of an epithet for the new species name in *Bretziella* was problematic. If we were to follow the Melbourne Code strictly, the unknown basionym of the asexual morph, *Ch. quercina*, would have priority over *E. fagacearum*, the basionym for *C. fagacearum* and the name that has been widely used. A formal proposal has thus been submitted to conserve the better known basionym against one that would be unfamiliar to most plant pathologists and mycologists. In this way, it is possible to ensure that even though the species has to be treated in a new genus, the epithet will remain familiar to those working with the fungus.

Subsequent to careful morphological comparisons, two lectotypes and an epitype have been designated for the two basionyms, *Chalara quercina* and *Endoconidium fagacearum*. These procedures ensure that the basionyms are now permanently linked to specimens. Sequences obtained from the epitype have been deposited in the RefSeq Targeted Loci (RTL) database in NCBI GenBank to enable accurate and reliable

identifications when BLAST searches are conducted (Schoch et al. 2014). In addition, a draft genome sequence for the ex-epitype culture has already been generated and is publicly available (Wingfield et al. 2016). The typifications together with the formal proposal will serve to stabilize the nomenclature of the oak wilt fungus. It is also hoped that they will prevent a need for further name changes for *B. fagacearum* in the future.

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