SYSTEMATICS AND EVOLUTION OF BARK-INHABITING SPECIES OF THE GNOMONIACEAE (DIAPORTHALES, ASCOMYCOTA) WITH EMPHASIS ON THE GENERA *CRYPTOSPORELLA* AND *PLAGIOSTOMA*

By

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ABSTRACT OF THE DISSERTATION

SYSTEMATICS AND EVOLUTION OF BARK-INHABITING SPECIES OF THE GNOMONIACEAE (DIAPORTHALES, ASCOMYCOTA) WITH EMPHASIS ON THE GENERA *CRYPTOSPORELLA* AND *PLAGIOSTOMA*

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The Gnomoniaceae (Diaporthales, Ascomycota) comprise microfungi that grow on leaves and woody tissues of a range of plant families, mostly hardwood trees from temperate zones of the northern hemisphere. Many dominant endophytes of trees in North America and Europe are species of Gnomoniaceae. Several emerging and devastating diseases of forest trees are caused by pathogenic species of Gnomoniaceae. Despite their abundance and impact in forest ecosystems, the Gnomoniaceae have not received modern taxonomic review and phylogenetic study. Most morphologically defined genera in this family are polyphyletic when analyzed with molecular data, therefore new circumscription of genera is needed.

The objectives of this work are to: 1) define monophyletic genera and determine species limits for bark-inhabiting fungi in the Gnomoniaceae; and 2) infer the phylogeny of bark-inhabiting genera of Gnomoniaceae (e.g. *Cryptosporella*, and *Plagiostoma*). To

achieve these objectives fresh specimens were collected in locations in Europe, North, Central and South America, and China. Specimens from herbaria and living collections from culture repositories were included in the study. The methods integrate a comparison of morphological characters of specimens in natural substrates such as the arrangement, shape, and size of perithecia and the shape and size of asci and ascospores with molecular characters, i.e. DNA sequences from multiple loci (*β-tubulin*, ITS, LSU, *rpb2*, and *tef1-α*) analyzed by Bayesian inference, Maximum Likelihood, Neighbor Joining, and Parsimony.

This research resulted in the recircumscription of the genera *Cryptosporella* and *Plagiostoma* and the definition of a new genus *Occultocarpon* gen. nov. A total of 32 taxonomic novelties were defined. More specifically, 17 new species, a new genus of bark-inhabiting Gnomoniaceae, and 14 new name combinations were described. This project has shown that host identity is a better predictor than geographic location for finding species of Gnomoniaceae. By documenting species of Gnomoniaceae from the Neotropics, South America, and subtropical China, results from this project have changed the previous assumption that the Gnomoniaceae only occur in temperate zones of the Northern Hemisphere. Finally, the phylogenies obtained suggest a long evolutionary relationship between *Cryptosporella* and Betulaceae and a subclade of *Plagiostoma* with the Salicaceae.

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DEDICATION

This work is dedicated to the most special people in my life: my son Carlos Manuel and my wife Enith; my mom Elia Judith, my dad Carlos, my sister Sara, and my brothers Horacio and Isaac. Thanks for your patience and for being very supportive.

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Chapter 1

Review of the Diaporthales with emphasis on the Gnomoniaceae

Introduction to the Diaporthales

The Diaporthales is a highly supported monophyletic group of microscopic fungi in the Sordariomycetes (Ascomycota) and is mostly associated with plants either as pathogens or non-pathogenic endophytes (Rossman et al. 2007b; Zhang & Blackwell 2001; Zhang et al. 2006). The most infamous species in this order is probably the chesnut blight fungus *Cryphonectria parasitica* responsible for the annihilation of the American chestnut tree (*Castanea dentata*) populations. A major feature of the Diaporthales is a black perithecial fruiting body that develops in scattered groups or solitary in substrates, immersed or not in leaves or woody tissues of their hosts, and with or without stroma. Additionally their asci are unitunicate and float free at maturity, and in most lineages have a refractive apical ring (Barr 1978; Samuels & Blackwell 2001).

The number of families within the Diaporthales has varied through time depending on varying concepts by different authors, the set of characters used for circumscription of the group, and the number of taxa available (Barr 1978, 1990; Erikson et al. 2001, 2004; Kirk et al. 2001; Wehmeyer 1975; see Zhang & Blackwell 2001 for a summary). In these works up to eight families were recognized but none of the authors accepted all eight families within the order. In a comprehensive molecular overview of the Diaporthales (Castlebury et al. 2002), the previous morphological familial classifications of the order were tested and six distinct lineages were found. More recently, the Diaporthales were reviewed by Rossman et al. (2007b) and nine families

included. These nine families are accepted in the present study (see Figure 1.1). However, the work of Castlebury et al. (2002) was used as the framework for the initial questions that stimulated the present study and for the original hypotheses tested. One of the distinctive lineages recognized by Castlebury et al. (2002) was the Gnomoniaceae with the Melanconidaceae and Cryphonectriaceae (Gryzenhout et al. (2006) as sister lineages. Castlebury et al. (2002) presented evidence that the Gnomoniaceae was composed of genera that occur primarily on woody tissues, i.e. the bark of twigs and branches as well as genera that occur primarily on leaves. This evidence contrasted with previous concepts of the Gnomoniaceae based on morphology and habit of species, which considered the family to include almost exclusively genera that occur primarily in leaves (see Barr 1978 and Monod 1983). Castlebury et al. (2002) supported the inclusion of the type species of the bark-inhabiting genera *Amphiporthe*, *Cryptodiaporthe*, *Cryptosporella* (synonyms *Ophiovalsa* and *Winterella*), *Ditopella,* and *Phragmoporthe,* but whether these genera were monophyletic remained to be evaluated. Furthermore Castlebury et al. (2002) identified several cases of potential synonymy of generic names and highlighted the need to conduct more in-depth research on this family.

The need to clarify the relationships among fungi in the Gnomoniaceae became more evident because several pathogens responsible for emerging plant diseases especially on trees of ornamental or timber value belong in this family (Castlebury et al. 2002, 2003; Rossman et al. 2007; Zhang and Blackwell, 2001). For example, *Discula destructiva* has devastated native dogwood trees (*Cornus florida* and *C. nuttallii,* Cornaceae) in North America (Daughtrey et al. 1996; Redlin 1991), and the butternut canker fungus, *Sirococcus clavigignenti-juglandacearum* has caused a decrease of up to

90 percent on natural populations of butternut (*Juglans cinerea,* Juglandaceae) in some areas of USA (Harrison and Hurley, 2006). *Apiognomonia quercina* is responsible for outbreaks of oak anthracnose in North America (USDA Forest Service, 1979).

The Gnomoniaceae: Species richness and morphology

The Gnomoniaceae was reviewed recently by Sogonov et al. (2008) using morphological and molecular characters. The focus was the circumscription of leaf-inhabiting genera of Gnomoniaceae, but all genera traditionally included in the Gnomoniaceae and those recently supported by molecular data were treated. Nine teleomorph genera were accepted in the family. Table 1.1 summarizes the number of species described under these nine genera in *Index Fungorum* totaling 727 species or subspecies and the number of species supported by a combination of morphology based and molecular phylogenies totaling 99 species.

The Gnomoniaceae is currently characterized by black perithecia that are immersed in the mesophyll, sitting in the leaf epidermis, or immersed in the periderm of their hosts. Depending on the genera or species considered, the perithecia are solitary, aggregated, or in groups, and may develop stromatic tissue. The neck of the perithecia always protrudes from the host epidermis, may be short or relatively long (>300 μm), and may be oriented parallel, perpendicular or obliquely relative to the host surface (see Sogonov et al. 2008). In most of the genera each ascus has a distinctive apical ring. Species of the Gnomoniaceae have ascospores that are generally small i.e. less than 25 μm long, but some genera such as *Cryptosporella* and *Pleuroceras* include species in which the ascospores are longer, in the range of 25-110 μm in length (see Mejía et al

2008; Monod 1983). The ascospores can be non-septate, one-septate with median or eccentric septum, or multiseptate, and of various shapes, i.e. elliptical, oval, cylindrical, or filiform (see Sogonov et al 2008). The anamorphic states of species of Gnomoniaceae (*Diplodina*, *Discula*, *Disculina*, and *Sirococcus*) are characterized by the production of hyaline, aseptate conidia in acervular or pycnidial fruiting bodies (Monod, 1983).

The Gnomoniaceae: Life cycles and host associations

Many species of Gnomoniaceae are considered non-pathogenic fungal endophytes, but some serious pathogens do occur in the family. With few exceptions (e.g., Viret and Petrini 1994; Wilson et al. 1997; Wilson and Carroll 1994), the life cycle of species in the Gnomoniaceae have not been the subject of detailed studies. However compelling evidence based on time of the year when fruiting bodies are prevalent and surveys of endophytic flora suggest that most species are characterized by initially infecting their hosts by ascospores or conidia, followed by an endophytic stage as either pathogenic or non-pathogenic colonization and becoming saprobic as plant tissues die (see Belisario et al. 2008; Douglas 2008; Sogonov et al. 2007; Viret and Petrini 1994; Wilson et al. 1997; Wilson and Carroll 1994). Most of these observations have been conducted in the temperate zones of the Northern Hemisphere that are characterized by a marked seasonality. My observations suggest that these fungi tend to produce their fruiting bodies at the beginning of the spring on dead tissues that have over-wintered. This suggests that there may be a link between the availability of new leaves and buds for infection and the production of spores on over-wintered dead tissues.

Fungal endophytes associated with woody plants are taxonomically diverse belonging primarily to the Ascomycota (Stone et al. 2004). This fungal and host diversity has made evolutionary studies of endophytic fungi from woody plants difficult to resolve. However some patterns of fungal-host associations are starting to emerge. Stone et al. (2004) and Sieber (2007) have summarized information on the dominant endophytic species from woody plants. This latter author has proposed an evolutionary association of endophytic fungi from the order Diaporthales with some Angiosperm families. The present work, as well as others, has shown that several of the dominant endophytic fungi from woody plants of temperate zones of the Northern Hemisphere belong to the Gnomoniaceae (Castlebury et al. 2002; Mejía et al. 2008; Sogonov et al. 2007).

Species from the Gnomoniaceae have been found in a wide range of plant lineages principally in the core tricolpates clade, i.e. the Rosids and some Asterids, but also in the Gymnosperms. Within the Rosids, the Fagales, Rosales and Sapindales are the principal host orders. The Betulaceae, Fagaceae and Juglandaceae are important host families in terms of number of species infecting these families. Within the Asterids, the Cornales is an important host order (Barr 1978; Mejía et al. 2008; Monod 1983; Sogonov et al. 2006 a, b). Besides this wide range of host lineages, genera and species of the Gnomoniaceae have been observed to show some degree of host preference, i.e. differential association with certain families and genera of plants. In other words, a species of Gnomoniaceae generally infects a single host species, genus or a limited number of genera within the same family. However there are also generalist fungal species. Studies of the Gnomoniaceae and its host associations are of value for determining the host range of these fungi, especially for pathogens and closely related

species. Nevertheless, a first step to understanding the relationships of the Gnomoniaceae and their hosts is to develop a good phylogeny and taxonomy of the family.

Scope of this study and highlights of chapters

This study was conducted with the main goal of completing a systematic monograph of bark-inhabiting species of Gnomoniaceae associated with hardwood trees. Throughout this work when I refer to bark-inhabiting species I am referring to species that inhabit the bark of host twigs and branches. The initials LCM are used for specimens collected and cultures obtained by the author of this dissertation. This research complements a recent monograph of leaf-inhabiting genera of Gnomoniaceae (Sogonov et al. 2008). In this work, particular attention was paid to the identity and geographic distribution of both the hosts and species of Gnomoniaceae.

The present work is divided into six chapters. An updated phylogenetic tree of the Diaporthales is provided in this first chapter (Figure1.1). In the second chapter, a phylogeny of the Gnomoniaceae based on three genes is presented and a new genus of bark-inhabiting species is described. The second chapter also highlights the preference of some lineages of Gnomoniaceae for particular plant lineages and the importance of this in prospecting for new species in this family. The second chapter also presents evidence of Gnomoniaceae in regions previously not considered to harbor these species such as the mountain cloud forest in Central America and subtropical mountain forest in South Central China. In the third chapter, already published, the confused taxonomy of *Cryptosporella* is resolved, eight new combinations are established, and the genus is

recircumscribed within the Gnomoniaceae based on cultural observations, morphology, and multigene phylogeny. In chapter four, a revised phylogeny of *Cryptosporella* is presented to include eight species new to science and two new name combinations. The geographic distribution of the species is presented and the phylogeny is used to estimate the effect host taxa might have played in the diversification of *Cryptosporella*. The geographic distribution of *Cryptosporella* is expanded to mountain cloud forests of Central America (Panama) and South America (Argentina) and the total number of species accepted in the genus is increased to 19. Chapter five is a review of the genus *Plagiostoma* (synonym *Cryptodiaporthe*). This is a mixed-habitat genus in the sense that it contains species that grow exclusively on leaves, exclusively in the bark, or on both leafy and woody tissues of their hosts. Eight new species of bark-inhabiting *Plagiostoma* and four new name combinations are described and the total number of species accepted in the genus is expanded to 25. In chapter six, final remarks on bark-inhabiting species of Gnomoniaceae are presented including a brief discussion of *Amphiporthe* and *Ditopella*/*Phragmoporthe* and disposition of bark-inhabiting species previously considered congeneric with the genera included in this study but found to belong in other families of Diaporthales.

Table 1.1. Species richness in the Gnomoniaceae. Genera in bold are those well defined by both morphological and molecular data.

Genus	Species in Index Fungorum	Species confirmed as Gnomoniaceae
Ambarignomonia	1	1
Amphiporthe[b]	5	1
Apiognomonia	30	5
Apioplagiostoma[c]	4	2
Cryptosporella*/Ophiovalsa/Winterella*	65/16/22	18
Ditopella*/Phragmoporthe*	13/6	2
Gnomonia	278	15
Gnomoniella[d]	85	1
Gnomoniopsis	10	8
Ophiognomonia	16	17
Plagiostoma/*Cryptodiaporthe*	39/57	25
Pleuroceras/*Linospora*[e]	23/57	3
Occultocarpon gen. nov.		1
Discula destructiva[f]	1	1
Total teleomorph species	727	99
Total number of species		100

[a]Mejía et al. 2008, Sogonov et. al. (2008), present study.

[b] The type species *Amphiporthe hranicensis* belongs in the Gnomoniaceae. The other four species described in this genus belong outside the Gnomoniaceae but within the Diaporthales, see figure 1.1.

[c] The type species *Apioplagiostoma populi* is not included in the phylogenetic studies and remains to be confirmed as Gnomoniaceae by molecular data.

[d]The type species *Gnomoniella tubaeformis* is not included in phylogenetic studies but placement of the congeneric species *G. alnobetulae* based on molecular sequence data suggests this is a distinct genus in the Gnomoniaceae (see Sogonov et al 2008).

[e]The type species *Pleuroceras cryptoderis* is not included in phylogenetic studies.

[f]This is a species only know by its anamorph.

Table 1.2. Source of DNA sequences used in nrLSU phylogeny of the Diaporthales presented in figure 1.1. DNA sequences for cultures that do not appear in this table were obtained from Castlebury et al 2002.

Species and culture number	Source of DNA sequence
Ambarignomonia petiolorum CBS116866	Sogonov et al. 2008
Amphiporthe castanea LCM439.03	New sequence
Amphiporthe leiphaemia LCM398.03	New sequence
Amphiporthe raveneliana CBS142.27	New sequence
Chapeckia nigrospora AR3809	De Silva et al. 2009
Cryptodiaporthe acerina CBS121565	New sequence
Cryptodiaporthe aculeans CBS525.85	New sequence
Cryptodiaporthe aubertii CBS114196	New sequence
Cryptodiaporthe galericulata AR3811	New sequence
Cryptodiaporthe liquidambaris AR3648	New sequence
Cryptodiaporthe sp. LCM392.01	New sequence
Cryptodiaporthe vepris AR3559	New sequence
Dicarpella dryina ATCC76896	Castlebury et al. unpublished
Ditopella ditopa LCM94.02	New sequence
Gnomonia rostellata AR3533	Castlebury et al. unpublished
Hapalocystis berkeleyi AR3851	De Silva et al. 2009
Melanconiella sp. CBS109762	Castlebury et al. unpublished
Melanconis alni LCM569.01	New sequence
Melanconis stilbostoma LCM191.01	New sequence
Occultocarpon ailaoshanense sp. nov. LCM524.01	New sequence
Ophiognomonia melanostyla LCM389.01	New sequence
Pleuroceras tenellum LCM159.01	New sequence
Rossmania ukurunduense AR3484	De Silva et al. 2009
Sillia ferruginea AR3440	De Silva et al. 2009
Sydowiella depressula CBS813.79	De Silva et al. 2009
Sydowiella fenestrans AR3777	De Silva et al. 2009
Sydowiella sp. LCM519.02	New sequence
Winterella aurantiaca AR3564	Castlebury et al. unpublished
Togninia fraxinopennsylvanica ATCC 26664 (GB AY761083.1)	Réblová et al. 2004
Togninia minima CBS 6580 (GB AY761082.1)	Réblová et al. 2004
Togninia novae-zealandiae WIN 113BI (GB AY761081.1)	Réblová et al. 2004

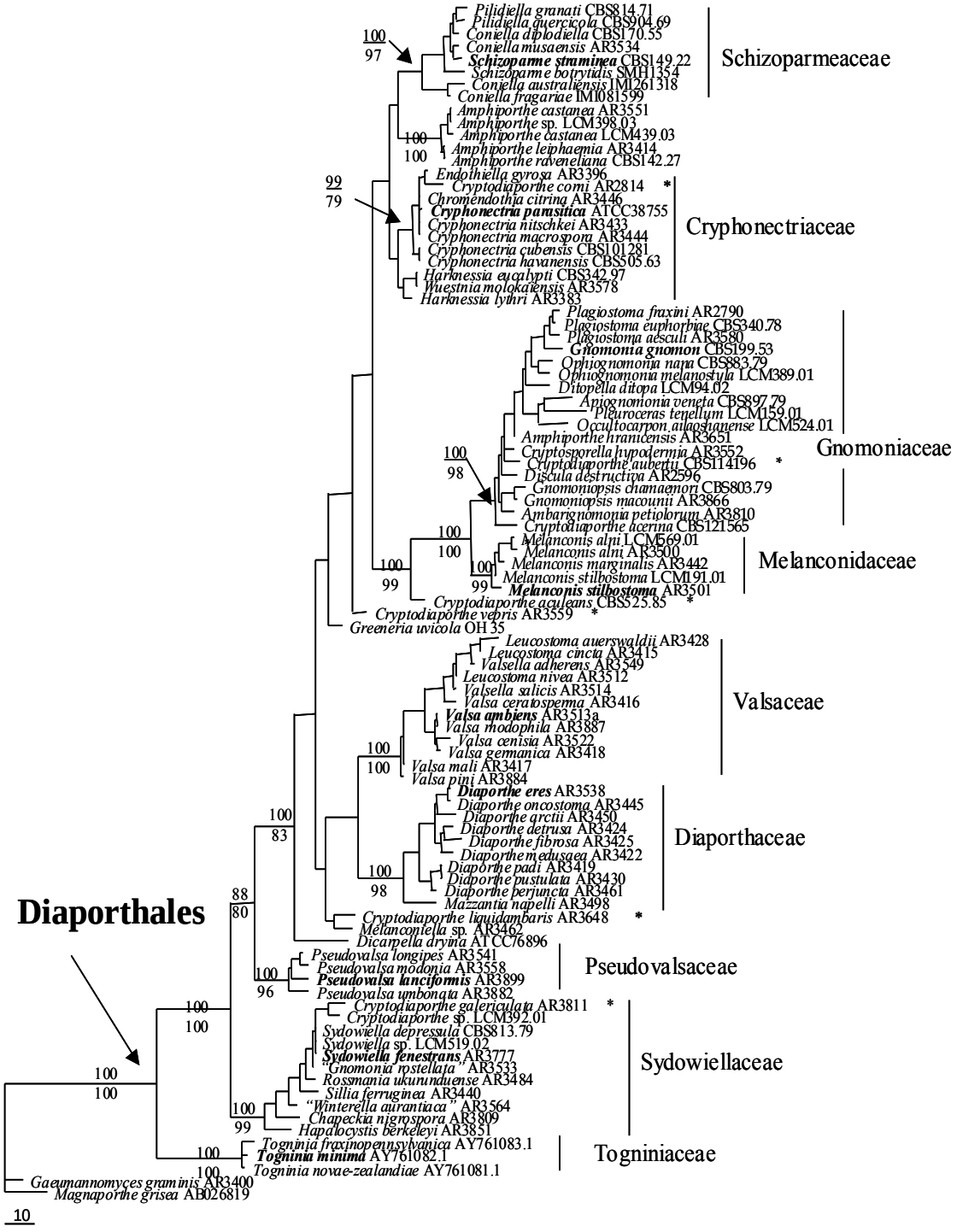

Figure 1.1. Phylogenetic analysis of the Diaporthales. One of 128 equally parsimonious trees based on analysis of 1225 bp from nrLSU gene of diaporthalean taxa

using *Gaeumannomyces graminis* and *Magnaporthe grisea* as outgroup (length 778, CI=.382, RI=.847). Trees and supports were generated as in Castlebury et al. (2002). Parsimony bootstrap support and Bayesian posterior probabilities are shown below and above branches respectively. Taxa in bold represent type species of the type genus of each family. Species of *Cryptodiaporthe* are marked with an asteric to highlight their polyphyly. Note that nine families are well supported as well as a clade containing three species of *Amphiporthe* that may be considered as new lineage of Diaporthales.

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Chapter 2

Occultocarpon, a new monotypic genus on *Alnus nepalensis* from China[1]

ABSTRACT

The new monotypic genus *Occultocarpon* and its species *O. ailaoshanense* are described. A phylogeny based on three genes (LSU, *rpb2*, and *tef1-α*) revealed that *O. ailaoshanense* belongs to the Gnomoniaceae (Diaporthales, Ascomycetes) and forms a distinct branch not strongly affiliated with any of the currently known genera. *Occultocarpon ailaoshanense* is characterized by perithecia with thin central to eccentric necks, in groups embedded in a stroma, and oblong elliptical-elongated, one-septate ascospores. *Occultocarpon ailaoshanense* occurs on the bark of branches of *Alnus nepalensis* (Betulaceae) in Yunnan, China.

INTRODUCTION

The Gnomoniaceae of China are poorly known, although some species of the genera *Apiognomonia*, *Gnomonia*, *Linospora*, and *Pleuroceras* have been reported from China (Eriksson & Yue 1988; Tai 1937; Teng 1996). China is considered the center of diversity for the Betulaceae, one of the core host families of Gnomoniaceae (see Chapter 1), and 89 species from this plant family including 56 endemic species occur there (Chen et al. 1999; Peiqiong and Skvortsov 1999). An exploratory trip for collecting Gnomoniaceae was conducted in Yunnan, China in July, 2008, by the author. This region was selected because it is considered a biodiversity "hot spot" (Myers et al. 2000, Xu &

[1] The new names included within this chapter are not accepted by the author as validly published in this dissertation (Botanical Code, Article 34.1[a]).

Wilkes 2004) and more than 40 species of Betulaceae, as well as species of other important host families of Gnomoniaceae such as Fagaceae, Juglandaceae, and Salicaceae occur in the this region (Anmin et al. 1999; Chengjiu et al. 1999; Fang et al. 1999; Peiqiong & Skvortsov 1999). Despite a lack of reports of Gnomoniaceae from Yunnan, fungi from this family were expected to occur there because the Gnomoniaceae have a strong association with certain plant families such as Betulaceae (see Chapters 4, 5, and 6 for more details). This chapter details the description of a new genus of Gnomoniaceae from *Alnus nepalensis* collections made in China during this trip.

METHODOLOGY

Specimens of dead branches of *A. nepalensis* with perithecia were placed in paper bags, air dried and transported to the laboratory for further processing. Observation and measurements of structures and culturing of specimens were done as in Mejía et al. (2008). New sequences generated for this study include *O. ailaoshanense* LCM522 and LCM524, which are detailed in the taxonomic section. Additional specimens sequenced in this study were *Ophiognomonia* sp. LCM367 (isolated from *Alnus acuminata* from Panama) and *O. melanostyla* LCM389.01 (isolated from *Tilia cordata* from Germany). The latter is the type species of *Ophiognomonia* and is included here for the first time in a multigene phylogeny of the Gnomoniaceae.

DNA extraction, amplification, and sequencing

Mycelium from approximately 1 cm^2 of the margin of a seven-day old cultures were harvested using a scalpel, transferred to 2-ml tubes of Lysing Matrix A (MP Biomedicals LLC, Irvine, CA, USA) and lysed using the Fast Prep FP120 (Thermo

Electron Corporation, Milford, MA, USA) for 20 seconds at speed 4. DNA was extracted using Puregene DNA Isolation kit (Gentra Systems, Minneapolis, MN, USA) following protocols provided by the manufacturer.

Three gene regions were amplified and sequenced. A region in the RNA polymerase second largest subunit (*rpb2*) was amplified with primers fRPB2-5F and fRPB2-7cR (Liu et al. 1999) and sequenced as in Mejía et al. (2008). A region of the translation elongation factor 1-α gene (*tef1-α*) was amplified and sequenced as in Sogonov et al. (2008) and sequenced with the PCR primers (EF1-728F, EF1-1567r) and the internal primer sequencing primer EF1-1199R (Carbone and Kohn 1999; Castlebury, unpublished data for primer 1199R 5' GGG AAG TAC CMG TGA TCA TGT 3'; Rehner 2001). Approximately 1200 base pairs of the 5' region of the nuclear ribosomal large subunit (LSU) were amplified and sequenced as in Castlebury et al. (2002).

Phylogenetic analyses

Editing of sequences and analyses of conflict among genes were done as described in Sogonov et al. (2008). The majority of the sequences used in the analyses of the Gnomoniaceae are from the study of Sogonov et al. (2008). The three genes were aligned individually and concatenated into a single alignment for subsequent phylogenetic analyses. Maximum parsimony (MP) analysis was conducted as described by Sogonov et al. (2008) using PAUP* v 4b10 (Swofford 2002). Support for branches was estimated with 1000 parsimony bootstrap replications (Felsenstein 1985), with MULTREES and TBR on and 10 random sequence additions per bootstrap replicate.

Bayesian analysis, using the program MrBayes v3.1.2 (Huelsenbeck and Ronquist 2001), was also performed. The best model for each gene was estimated using the

program MrModeltest v.2 (Nylander 2004). The Bayesian analysis was done as detailed in Sogonov et al. (2008) with 2000000 generations and burn-in=50000. *Cryphonectria parasitica* selected as the outgroup taxon based on the relatively close relationship of its family, the Cryphonectriaceae, with the Gnomoniaceae (see Castlebury et al. 2002 and Chapter 1 of this dissertation). A 50% majority rule consensus phylogram was computed using 7800 trees saved after the burn-in period (50000 generations).

RESULTS

Phylogenetic analyses

No conflicts among individual gene trees were observed and sequences from the three genes were concatenated into a single alignment containing LSU (1231bp), RPB2 (1061 bp), and *tef1-α* (443 bp). Maximum parsimony (MP) analysis resulted in 132 equally parsimonious trees (CI=.277, RI=0.663). A 50% majority rule consensus tree from the MP analysis was obtained and used for comparison with results from the Bayesian analysis. The model GTR + I + G (nst=6 rates=invgamma statefreqpr=dirichlet (1,1,1,1)) proved to be the best fitting model for each of the three genes and was applied for the Bayesian analysis. The same phylogeny was obtained by maximum parsimony and Bayesian analyses with clades representing 11 genera of Gnomoniaceae, including the new genus *Occultocarpon,* supported by both methods. The consensus phylogram obtained from the Bayesian analysis is presented in Figure 2 jointly with Bayesian posterior probabilities (PP) and MP bootstrap supports for nodes. *Occultocarpon ailaoshanense,* the only species known from this genus, forms a distinct branch in the Gnomoniaceae and is a part of a larger unsupported clade that includes *Plagiostoma*,

Apiognomonia and *Amphiporthe hranicensis.* Additionally, a clade containing seven genera, four of which are primarily on Betulaceae, (*Cryptosporella*, *Ditopella*, *Gnomonia* and *Occultocarpon)* was identified (see Fig. 2.1).

Taxonomy

Occultocarpon L. C. Mejía & Zhu L. Yang **gen. nov.**

Type *Occultocarpon ailaoshanense* L. C. Mejía & Zhu L. Yang

Perithecia black, in groups scattered in host branches, immersed in and pushing up the host periderm, with grey to brown scanty stroma on top of perithecia, with thin central to eccentric necks protruding from periderm of host branches and extending beyond surface, cream yellow mycelium at bottom of perithecia, perithecia collapsing from bottom when dry. Asci cylindrical elongated, apical ring visible as two slightly reniform bodies, with eight ascospores arranged obliquely parallel or biseriate. Ascospores hyaline, short, oblong elliptical-elongated, one-septate, multiguttulate.

Anamorph. Unknown

Etymology. Occultus - hidden, Gr. Karpos - fruit, referring to the hidden nature of the perithecia below the host surface.

Occultocarpon ailaoshanense L. C. Mejía & Zhu L. Yang **sp. nov**

Holotype. **China**, Yunnan, Jingdong county, Ailaoshan mountain, on the road, at 2381 meters above sea level, N 24° 31” 00.9” E 101° 00”47.1”, on dead, still attached branches of *Alnus nepalensis*, 14 Jul 2008, LCM524 (BPI879253, derived cultures LCM524.01 and LCM524.02).

Perithecia black, in groups of up to five, immersed in and pushing up the host periderm, with grey to brown stroma on top of perithecia, subglobose, diam×height = 471–480(–489)×(363–)364–369(–375) µm (mean = 477×367, SD 10, 6.4, n=3), with thin central to eccentric necks protruding from periderm and extending beyond surface, length (284–)386–496(–504) µm (mean = 425, SD 122, n=3), basal diameter (43.8–)44.6–53.4(–61.5) µm (mean = 50.2, SD 9.8, n=3), distal diameter (32.7–)35.7–43.1(–47.5) µm (mean = 39.6, SD 7.4, n=3), and hyaline ostiolar opening. Cream yellow mycelium at bottom of perithecia, perithecia collapsing from bottom when dry. Asci cylindrical, elongated, floating free in perithecia, (56.8–)64.2–71.8(–77.6)×(12.8–)14.7–16.9(–21.1) µm (mean = 67.3×16, SD 6.38, 1.92, n=22), apical ring 2–4 µm diam., visible as two slightly reniform bodies, with eight ascospores arranged obliquely parallel or biseriate. Ascospores hyaline, short, oblong elliptical-elongated, with rounded ends with many guttules, appearing granulated, one septate, often with one cell slightly wider than the other, slightly constricted at septum (16.1–)18.3–21.7(–29.4)×(3.2–)3.6–4.2(–4.8) µm (mean = 20.7×3.9 µm, SD 3.6, 0.4, n=32), l:w (3.8–)4.6–5.8(–7.6) (mean = 5.4, SD 1.0, n=32).

Etymology. *ailaoshanense* - from Ailaoshan, referring to the location where this species was first collected, Ailaoshan, Yunnan, China.

Other specimens examined: **China**, Yunnan, Jingdong county, Ailaoshan mountain, aprox. 100 m from the holotype location, on *A. nepalensis*, 14 Jul 2008, LCM522 (BPI879254, derived cultures LCM522.01 and LCM522.02); LCM561 (BPI879255, derived cultures LCM561.02, LCM561.04).

DISCUSSION

The monophyly of 10 teleomorphic genera in the Gnomoniaceae are well supported by both morphology and a multigene phylogeny as presented in Sogonov et al. (2008). The newly discovered *Occultocarpon ailaoshanense* is placed as a member of the Gnomoniaceae by a three gene phylogeny. *Occultocarpon ailaoshanense* contains features that are common to other species of the Gnomoniaceae such as black perithecia arranged in groups that collapse from the bottom when dry, upright perithecial necks that protrude through the host periderm, a refractive apical ring in the asci, and elliptic guttulate ascospores. Additionally *O. ailaoshanense* occurs on bark, a common habitat of Gnomoniaceae and its host is *Alnus nepalensis*, a plant species from one of the core host families of Gnomoniaceae.

A combination of morphological features makes *O. ailaoshanense* a distinctive species and genus. These include the combination of perithecia with thin (distal diameter < 50 μm) central to eccentric upright necks, arranged in groups, and embedded in a stroma within the bark of the host. Other genera and species of Gnomoniaceae such as *Amphiporthe hranicensis*, *Cryptosporella*, and *Plagiostoma* have grouped perithecia in bark; however, the necks of these taxa are generally greater than 50 μm, i.e., thicker than those of *Occultocarpon ailaoshanense*. *Plagiostoma exstocollum* L. C. Mejía sp. nov. (Chapter 5) is a species with thin necks and grows on *Corylus* (Betulaceae), however the necks of this species are marginal. The ascospore morphology of previously described bark-inhabiting species of Gnomoniaceae is different than that of *O. ailaoshanense* (see Barr 1975; Mejía et al. 2008; Monod 1983; Sogonov et al. 2008). When inside the ascus, the ascospores of *O. ailaoshanense* may appear cylindrical and resemble those of

Ditopella ditopa (Fr.) J. Schröt., the type species of *Ditopella* De Not. However, when outside the asci, the oblong elliptical-elongated shape of the ascospores and slight difference in the width of the two cells comprising the ascospores of *O. ailaoshanense* is evident. Additionally, asci of *O. ailaoshanense* contain eight ascospores per ascus in contrast to those of *Ditopella ditopa* that contain 32 ascospores per ascus. Perithecia of *Ditopella* are solitary and scattered in host tissue and not in groups as in *O. ailaoshanense*. Interestingly *Cryptosporella*, *Ditopella,* and *Occultocarpon* are all bark-inhabiting genera with species associated with Betulaceae. The finding of *O. ailaoshanense* in China supports the view of the Betulaceae as an important host family of Gnomoniaceae. This work will stimulate the search for more species of Gnomoniaceae on Betulaceae as well as on other hosts in China.

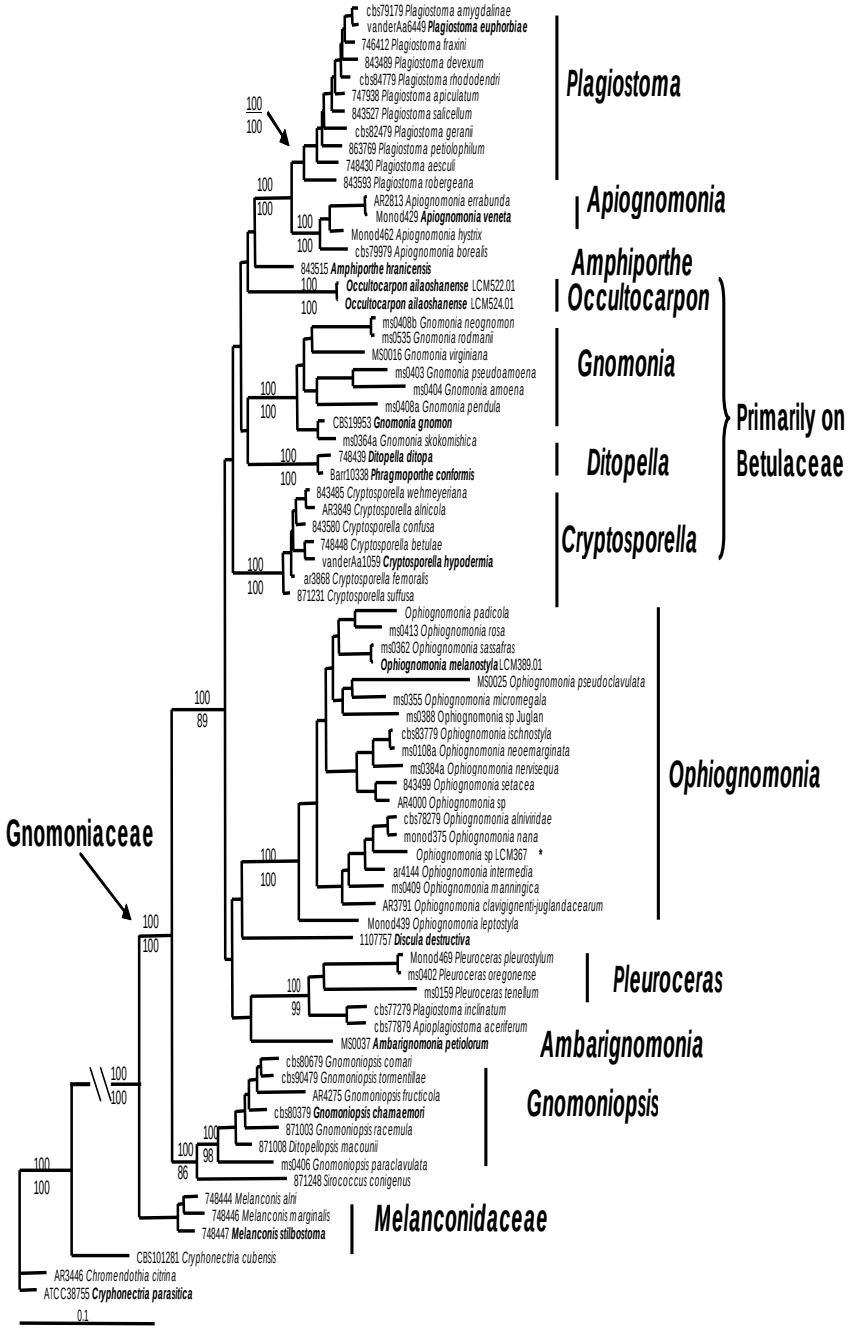

Figure 2.1. Fifty percent majority rule phylogram derived from Bayesian analysis of gnomoniaceous taxa using model GTR+I+G on gene regions *tef1-α*, *rpb2*, and nrLSU (total of 2730 characters). Species of Cryphonectriaceae and Melanconidaceae are included as outgroup taxa. Bayesian posterior probabilities and parsimony bootstrap

values appear above and below branches respectively. The type species of each genus is in bold. Notice that *Occultocarpon* forms a distinct branch within the Gnomoniaceae. Among the genera associated primarily with Betulaceae, only three species, *Cryptosporella hypodermia*, *C. tiliae*, and *C. wehmeyeriana* are not associated with Betulaceae.

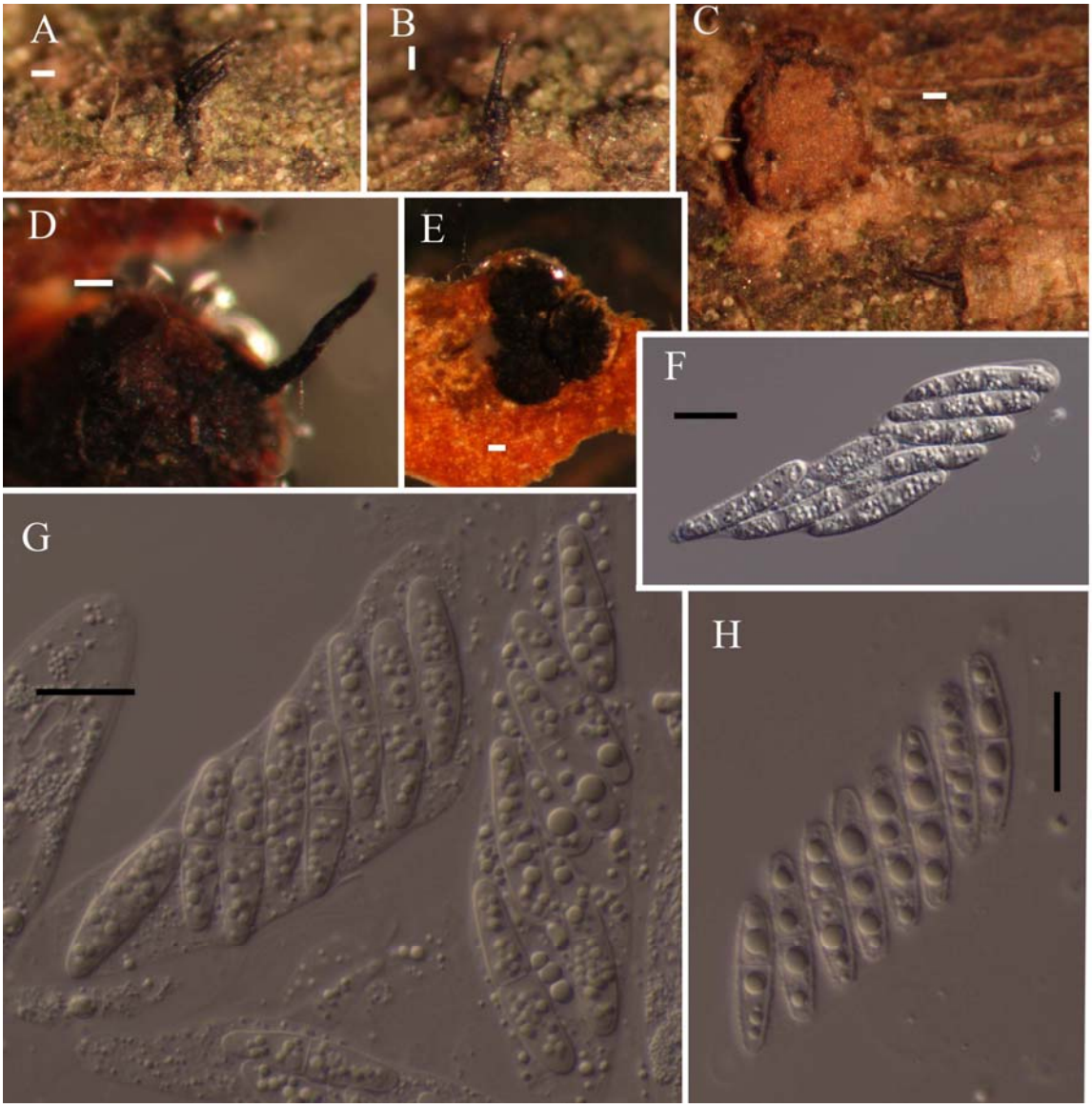

Figure 2.2 A–H Morphology on natural substrate. *Occultocarpon ailaoshanense.* A–C, G,H= BPI879253 (holotype), D–E=BPI979255, F=BPI879254. Scale: (A–E) 100 µm; (G–F) 10 100 µm.

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Chapter 3

Phylogenetic placement and taxonomic review of the genus *Cryptosporella* and its synonyms *Ophiovalsa* and *Winterella* (Gnomoniaceae, Diaporthales)[1].

ABSTRACT

The type species of *Cryptosporella* Sacc*., C. hypodermia* (Fr.) Sacc., and *Ophiovalsa* Petr*., O. suffusa* (Tul. & C. Tul.) Petr. as well as closely related species were studied using morphological, cultural, and DNA sequence characteristics. DNA sequence data from three different loci (ITS, LSU and RPB2) suggest that *C. hypodermia* (Fr.) Sacc. and *O. suffusa* (Fr.) Petr. are congeneric within the *Gnomoniaceae* (*Diaporthales*). This result is supported by similarities in perithecial, ascal and ascospore morphology and lifestyles characterized as initially endophytic, becoming saprobic as plant tissues die. Furthermore, both type species produce *Disculina* Höhn. anamorphs. A review of the literature indicates that the generic name *Cryptosporella* has priority over *Ophiovalsa* and its synonym *Winterella* (Sacc.) O. Kuntze sensu Reid & Booth (1987). A redescription of the genus *Cryptosporella* is included as well as a description of *C. hypodermia,* C. *suffusa*, the type species of *Ophiovalsa,* a brief account of the other seven species [2]accepted in *Cryptosporella*, and a key to species of *Cryptosporella*. Eight new combinations are established: *C. alnicola* (Fr.) L.C. Mejía & Castleb., **comb. nov**., *C. betulae* (Tul. & C. Tul.) L.C. Mejía & Castleb., **comb. nov**., *C. confusa* (Reid & Booth) L.C. Mejía & Castleb., **comb. nov.,** *C. corylina* (Tul. & C. Tul.) L.C. Mejía & Castleb.,

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comb. nov., *C. femoralis* (Peck) L.C. Mejía & Castleb., **comb. nov**., *C. suffusa* (Fr.) L.C. Mejía & Castleb., **comb. nov**., *C. tiliae* (Tul. & C. Tul.) L.C. Mejía & Castleb., **comb. nov.,** and *C. wehmeyeriana* (Reid & Booth) L.C. Mejía & Castleb., **comb. nov**.

Key words: *Disculina,* endophyte, pyrenomycetes, RNA polymerase, systematics

INTRODUCTION

Species in the genus *Cryptosporella* Sacc. as *Ophiovalsa* Petr. and *Winterella* (Sacc.) O. Kuntze (*Gnomoniaceae, Diaporthales*) are known throughout the temperate regions especially North America Europe, and Japan as saprobes, endophytes, and occasionally as pathogens on hardwood trees especially *Alnus*, *Betula*, *Corylus, Tilia* and *Ulmus* (Barr 1978; Chlebicki 2002; Glawe & Jensen 1986; Green *et al.* 2004; Kobayashi 1970; Reid & Booth, 1987, 1989; Spaulding 1961). Usually sporulating on small, overwintered branches, their fruiting bodies are inconspicuous, appearing as raised bumps as they develop underneath bark, eventually evident as short, black beaks erumpent through the bark surface. These species are also encountered as endophytes on their hardwood hosts, producing their *Disculina* anamorphic states in culture (Barengo *et al.* 2000; Ganley *et al.* 2004).

The genus *Cryptosporella* was described by Saccardo (1877) to distinguish fungi that were classified as *Cryptospora* Tul.& C. Tul. (1863), but differed in ascospore shape. At that time species of *Cryptospora* were distinguished by having one to several-celled, long cylindrical ascospores. Based on the type species *Cryptosporella hypodermia* (Fr.) Sacc., *Cryptosporella* was defined by having species with hyaline, one-celled, oval to fusoid ascospores. The distinction of these two genera based on shape and size of

ascospores was accepted by many mycologists (Arx and Müller 1954; Barr 1978, 1991; Berlese 1900; Dennis 1978; Höhnel 1917, 1918; Munk 1957; Traverso 1906; Wehmeyer 1926). Other scientists (Ellis and Everhart 1892; Winter 1887) recognized *Cryptosporella* as a subgenus of *Cryptospora* and considered that shared characteristics such as arrangement and position of perithecia, perithecial neck, habit, type of stroma, and asci were enough to retain these taxa in a single genus.

Petrak (1966) erected the genus *Ophiovalsa* Petr. based on *Cryptospora suffusa* Tul.& C. Tul. when he realized that *Cryptospora* Tul.& C. Tul. 1863 was a later homonym of *Cryptospora* Karelin & Kirilow 1842 in the *Brassicaceae.* Reid and Booth (1987, 1989) treated *C. hypodermia* as congeneric with *O. suffusa* and placed these two species and others in the genus *Winterella*. Discrepancies regarding the generic concepts and uncertainty as to which morphological traits to use for differentiating genera and species coupled with poor scientific communication during the 1800s and first half of the 1900s may have contributed to several nomenclatural and taxonomic problems related to these taxa that still persist today (see Discussion below).

A review of the order *Diaporthales* by Castlebury *et al.* (2002) based on large subunit ribosomal DNA sequences revealed that *O. suffusa* and *Cryptosporella hypodermia* are closely related within the *Gnomoniaceae*, but the details of this relationship were not resolved. Additional DNA sequence data and morphological observations suggest that several species described in these genera are congeneric. In order to determine the relationship of *Ophiovalsa* based on *O. suffusa* including *Winterella* sensu Reid & Booth (1987) with *Cryptosporella* based on *C. hypodermia,* both molecular and morphological evidence were obtained. A redescription of the genus

Cryptosporella and its type species, *C. hypodermia,* as well as the type species of *Ophiovalsa*, and an account of the genus as a whole is presented.

MATERIALS AND METHODS

Morphological observations

Specimens representing species of *Winterella* sensu Reid & Booth (1987) and the type species *Ophiovalsa suffusa* and *Cryptosporella hypodermia* were examined. Morphological observations included macroscopic appearance and microscopic characters such as size, shape, color and arrangement of asci, ascospores, perithecial wall and perithecial ostiolar tissues. Specimens were observed with a Zeiss SV 11 Apo (Carl Zeiss, New York, NY, USA) dissecting microscope and Zeiss Axiophot microscope (Carl Zeiss, New York, NY, USA) with conventional bright field or Nomarski differential interference contrast microscopy. Perithecia and pycnidia were placed in a drop of 3% aqueous KOH or water on a clean microscope slide. After rehydration perithecia were observed and photographed. Perithecia and pycnidia were crushed under a glass coverslip to release asci and conidia. Photographs were taken using a Nikon digital camera DXM1200F (Nikon, Instruments Inc., Melville, NY, USA). Microsoft Access 2000 (Microsoft Corporation, Bellevue, WA, USA) was used to store collection information and images and to measure specimen structures as described by Sogonov (2005).

Cultures derived from recent collections and collections made during the course of this study were obtained by means of single spore isolation on Corn Meal Agar (CMA, Sigma®, Sigma Chemical Co, St. Louis, MO, USA) supplemented with antibiotics (1%

solution 0.2% streptomycin sulfate and 0.2 % neomycin). Type specimens of *C. hypodermia* and *O. suffusa* were sectioned for detailed observation of perithecial structures. Small pieces of the substrata containing perithecia were excised and boiled in distilled water for hydration during 90 minutes and left overnight. For histological studies, tissue was prepared as in Torres *et al.* (2005). In brief, tissue was dehydrated in ethanol, embedded in LR White® acrylic resin, sectioned in slices of 4 µm using glass knives, and stained in aniline blue (0.1% aqueous) followed by toluidine blue (0.1% aque0us) for 20 seconds in each stain.

Cultural studies

Cultures were plated in duplicate on three different media: Malt Extract Agar (MEA, Bacto ®, Becton, Dickinson & Co., Sparks, MD, USA), Potato Dextrose Agar (PDA, Difco™, Becton, Dickinson & Co., Sparks, MD, USA), and CMA. Agar plugs 5-mm in diameter from the edge of actively growing colonies were used as inocula for cultural studies. Cultures were grown at 23°C under 12 hours UV/white light and 12 hours of dark. Radial growth measurements and phenotypic character observations were made at 7, 14 and 21 days after plating. Two perpendicular colony diameter measurements were made for each culture replicate. The colony diameter presented represents the average of all measurements for a particular species. Colors assigned to colonies are based on the color chart by Rayner (1970)**.**

DNA extraction and PCR amplification

One square centimeter of mycelium was scraped from the surface of actively growing cultures (about one week old) and used for DNA extractions. Mycelium was lysed using Fast Prep FP120 (Thermo Electro Corporation) or liquid nitrogen. DNA was

extracted using DNAeasy Plant mini kit (Qiagen Inc., Valencia, CA, USA) or Puregene DNA Isolation kit (Gentra Systems, Minneapolis, MN, USA) following the manufacturers' instructions.

The internal transcribed spacer regions 1 and 2 including 5.8 S rDNA ITS DNA were amplified with primers ITS5 and ITS4 (White *et al* 1990). A region in the RNA polymerase second largest subunit (RPB2) was amplified with primers fRPB2-5F and fRPB2-7cR (Liu *et al.* 1999). Large subunit (LSU) ribosomal DNA was amplified using primers LR0R and LR7 (Vilgalys & Hester 1990; Rehner & Samuels 1994). Amplifications were carried out in 50-µl reactions on an iCycler™ thermal cycler (Bio-Rad Laboratories, Inc, Hercules, CA, USA) under the following reaction conditions: 5-15 ng of genomic DNA, 200 µM each dNTP, 2.5 units Amplitaq (Perkin Elmer), 2 µM of each primer and the supplied 10X buffer with 15 mM $MgCl_2$. The thermal cycler program for ITS was as follows: 2 min at 94°C followed by 35 cycles of 30 s at 94°C, 30 s at 55°C, 1 min at 72°C), with a final extension period of 10 min at 72°C. For RPB2 and LSU amplifications, Amplitaq Gold (Perkin Elmer) with an initial denaturation step of 10 min at 94°C and an annealing temperature of 58°C were used. The resulting PCR products were treated with ExoSAP-IT® (USB Corporation, Cleveland, OH, USA) following manufacturer instructions and sequenced with the BigDye version 3.1 (Applied Biosystems, Foster City, CA, USA) on an ABI 3100 automated DNA sequencer. PCR primers were used as sequencing primers for all three genes. Additionally for LSU, primers LR3R and LR5 (Vilgalys & Hester 1990, Rehner & Samuels 1994) were used as internal sequencing primers

Sequence Analysis

Sequences were edited using Sequencher version 4.2 for Windows (Gene Codes Corporation, Ann Arbor, MI, USA). Two datasets were prepared. Alignment 1 consisted of ITS, LSU and RPB2 sequences from representatives of the major lineages within the *Gnomoniaceae* with three species representing the *Cryptosporella/Ophiovalsa* group including both type species, and using species of *Melanconis* and *Cryphonectria* as outgroup taxa (Castlebury *et al.* 2002). This dataset was constructed to confirm the close relationship of *C. hypodermia* and *O. suffusa* with a multiple gene analysis. Alignment 2 consisted of ITS and RPB2 sequences from all available isolates of species of the *Cryptosporella/Ophiovalsa* group with outgroup species identified from the first dataset as closely related and easily alignable including *Amphiporthe hranicensis* (Petr.) Petr., *Discula destructiva* Redlin & Stack*, Gnomonia gnomon* (Tode: Fr.) J. Schröt, and *G. petiolorum* (Schwein.:Fr.) Cooke. Gene regions were aligned separately and concatenated into a single alignment. Sequences were initially aligned using ClustalX version 1.8 (Thompson *et al.* 1997) and manually adjusted in BioEdit Sequence Alignment Editor version 7.0.5.3 (Hall 1999).

Each gene was analyzed separately through the use of data partitions and a combined analysis was performed with PAUP 4.0b10 (Swofford 2002). Trees were inferred by the neighbor-joining (NJ) method (Kimura 2-parameter distance calculation) and by maximum parsimony (MP) using the heuristic search option with the random addition sequence (1000 replications) and the branch swapping (tree bisection-reconnection) option. For MP analyses, a limit of 1000 trees per random addition sequence was enforced with a MAXTREE limit of 10 000. For both types of analyses,

ambiguously aligned positions were excluded. All characters were unordered and given equal weight during the analysis. Gaps were treated as missing data in the parsimony analysis and the neighbor joining analysis with missing or ambiguous sites ignored for the affected pairwise comparison. Relative support for branches was estimated with 1000 bootstrap replications (Felsenstein 1985) and 100 random sequence additions per bootstrap replicate for the MP bootstrap analysis of alignment 1 (family tree, Fig 3.1) and 1000 bootstrap replications with MULTREES off and 10 random sequence additions per bootstrap replicate for alignment 2 (genus tree, Fig 3.2).

RESULTS

Molecular Results

The combined alignment 1, excluding PCR primer binding site regions, consisted of ITS (645 bp), LSU (1221 bp), and RPB2 (1102 bp) sequences for 29 isolates of gnomoniaceous taxa including the two type species, *Cryptosporella hypodermia* and *Ophiovalsa suffusa* and one additional species *O. betulae,* with 2968 total characters. Ninety-seven ambiguously aligned positions (1319-1415) were excluded from the ITS gene region, all positions were used for the LSU gene, and 40 ambiguously aligned positions (2779-2818) were excluded from the RPB2 gene encompassing a region with an insertion in the *Cryptosporella/Ophiovalsa* taxa. Of the remaining 2831 characters, 589 were parsimony informative, 2123 were constant and 119 were variable but not parsimony-informative. MP phylogenetic analysis of combined alignment 1 resulted in one parsimonious tree (length=2461, CI=0.427, RI=0.552, RC=0.236, HI=0.573). Figure 3.1 shows the MP tree generated for the combined alignment with MP bootstrap support

above and NJ bootstrap support below branches. Only bootstraps 70% or greater are shown.

Results show that the respective type species of *Cryptosporella* and *Ophiovalsa* and *O. betulae* form a monophyletic group (100 % MP and NJ bootstraps) within the *Gnomoniaceae* with *Amphiporthe hranicensis, Discula destructiva, Gnomonia gnomon, G. petiolorum, Linospora capreae,* and *Pleuroceras tenella* as a sister group, although bootstrap values do not support this as a strong relationship (< 70%, Fig 3.1). Based on these results *A. hranicensis, D. destructiva, G. gnomon,* and *G. petiolorum* were chosen as outgroup taxa in the analysis of all available isolates of *Cryposporella/Ophiovalsa*. Other monophyletic groups with 100 % bootstrap support include a group containing the type species of *Cryptodiaporthe* and *Plagiostoma* with *Apiognomonia errabunda.* The type species of *Gnomonia, G. gnomon,* is not supported as strongly grouping with any other currently accepted species of *Gnomonia*. A group of exclusively anamorphic taxa including *Sirococcus conigenus* and *Discula campestris* is supported as a monophyletic group (100 %).

The combined alignment 2 comprising all available isolates of *Cryptosporella* and excluding PCR primer binding site regions, consisted of ITS (553 bp), and RPB2 (1103 bp) sequences for 26 isolates of *Cryptosporella* and previously mentioned outgroup taxa with 1656 total characters. All positions were used for the two genes. Of the 1656 characters, 204 were parsimony informative, 1282 were constant and 170 were variable but not parsimony-informative. MP phylogenetic analysis of combined alignment 2 resulted in 104 equally parsimonious trees (length=607, CI=0.758, RI=0.792, RC=0.600, HI=0.242). Figure 3.2 shows one randomly chosen MP tree generated for the combined

alignment, with MP bootstrap supports above the branches. Only bootstraps 70% or greater are shown.

Results (Fig 3.2) show that *O. suffusa* (hereafter referred to as *C. suffusa*) is supported within a monophyletic group (100 %) containing *C. hypodermia* and five other species including *C. alnicola, C. betulae, C. confusa, C. femoralis,* and *C. wehmeyeriana* when analyzed with their closest known relatives as outgroup taxa. *Cryptosporella betulae* is supported (100 %) as most closely related to *C. hypodermia.* *Cryptosporella alnicola* and *C. wehmeyeriana* are strongly supported as sister species (100 %) with *C. confusa* moderately supported (70 %) with them in a monophyletic group. Although *C. suffusa* appears basal to the other taxa in the tree that is shown (Fig 3.2), bootstrap values do not support any taxa as basal to the others.

All species of *Cryptosporella* included in this analysis contain an insert of 14 bases in the RPB2 gene that is unique to the *Diaporthales* (position 2781-2798 of combined alignment 1 with a consensus sequence of CGSGCAARGASAAG). This is based on sampling of more than 30 species representing all accepted genera of *Gnomoniaceae*, plus type species of genera representing five other families of Diaporthales (unpublished data).

Winterella albofusca (Cooke & Ell.) J. Reid & Booth, *Winterella aurantiaca* (Wehm.) J. Reid & Booth, *Winterella aurantiaca* subsp. *valsoides* (Rehm) J. Reid & Booth (= *Ophiovalsa valsoides* (Rehm) D.A. Glawe & J.D. Jensen) and *Winterella cinctula* (Cooke & Peck) O. Kuntze are excluded from *Cryptosporella* based on morphological and molecular data. LSU data (not shown) indicate that *W. albofusca* and *W. aurantiaca* belong in the *Diaporthales*, although outside the *Gnomoniaceae.* The only

available isolate of *Winterella cinctula* (CBS 137.26) was sequenced and determined to be a representative of the *Diatrypales*. It is not known if this isolate is an authentic isolate of *W. cinctula* or a misidentified culture. The above-mentioned species are all characterized by multiseptate ascospores, a character state that is absent in species of *Cryptosporella* as defined in this study.

TAXONOMY

Cryptosporella Sacc., Fungi Veneti. Michelia 1: 30-31 (1877).

Synonyms: [*Cryptospora* Tul. & C. Tul., Selecta Fungorum Carpologia. 2: 144 (1863) non Karelin & Kirilow 1842.]

Winterella (Sacc.) O. Kuntze, Rev. Gen. Pl. 1. A. Felix, Leipzig. 1: 34 (1891).

Ophiovalsa Petr., Sydowia 19: 272 (1966).

Circinate arrangement of black perithecia, initially evident as a slight elevation in bark, later protruding through bark periderm either as a mass of perithecial necks or fused into a single ostiolar cavity; with or without a loosely developed stroma. Asci cylindric to clavate with thickened apex, with or without an apical apparatus distinguished by presence of one or two refractive bodies. Acospores hyaline, cylindric with rounded tips, tapering or slightly swollen toward their ends, fusiform, elliptic or femur-like, single-celled, except in *C. femoralis* that are two-celled when mature.

Habit: As endophytes or sporulating on dead, overwintered twigs or branches of trees in the *Betulaceae*, *Tiliaceae* and *Ulmaceae*.

Anamorph*: Disculina* Höhn. (Type species: *Disculina vulgaris* (Fr.) B. Sutton).

Type species:

Cryptosporella hypodermia (Fr.) Sacc., Michelia 1: 30 (1877). Figs 3.3A-J

Synonyms: *Sphaeria hypodermia* Fr. in Kunze & Schmidt, Mykol. Hefte 2: 49 (1823).

Valsa hypodermia Fr.:Fr., Summ. Veg. Scand. 2: 412 (1849).

Cryptospora hypodermia (Fr.) Fuckel, Sym. Mycol. 192 1869 (1870).

Winterella hypodermia (Fr.) J. Reid & C. Booth, Can. J. Bot. 67:879-908 (1989).

Sphaeria limminghii West., Bull. Sci. Bruxelles N.S. 7:89 (1859).

Valsa limminghii (West.) Kickx, Fl. Crypt. Fland. 323 (1867).

Cryptosporella limminghii (West.) Sacc., Syll. Fung. 1: 466 (1882).

Cryptosporella veneta Sacc., Michelia, 1: 31 (1877).

Cryptosporella compta var. *macrospora* Beeli, Bull. Soc. R. Bot. Belg. 56: 58 (1924).

Anamorph: *Disculina* sp.

Initially perithecia evident as elevations in bark up to 0.7 mm high. Later perithecia visible as a dry oval to fusoid pustule 0.7 mm long x 0.5 mm diam. Erumpent perithecia arranged in groups of 6-12, oriented at 45° angle toward surface. Perithecial necks converge centrally and, in many cases, emerge together as a column that protrudes through bark periderm. Perithecial neck column not extending much beyond rupture in bark. Mature perithecia black, shiny, flask-shaped and closely appressed but separable from one another, diam × height = (345–)362–619(–666) × (300–)333–551(–651) µm (mean = 504 × 469, SD 127, 121, n1=12, n2=12), perithecial necks (372–)492–650(–890) µm (mean = 591, SD 216, n=4) in length, basal diameter(144–)172–208(–241) µm (mean = 192, SD 27.8, n=12), and distal diameter (118–)163–213(–241) µm (mean = 182, SD 45.1, n=6). Ectostroma scanty, embedding neck column where perithecial beaks converge. Flared ostiolar openings, cup-like in appearance. No entostroma observed.

Perithecial wall tissue *textura angularis*. Venter wall bilaminate, outer region averaging 7.7–15 µm wide (mean=10.6, SD=1.7 µm, n=13), of one to two layers of brown, thick-walled cells, cells 15–25 µm diam (mean=19 µm, SD =3.84, n=9). Inner region 15–46 µm wide (mean= 25 µm, SD 11.36 µm, n=13), of 4 to 6 layers of hyaline cells. Neck cavity densely filled with periphyses, surrounded by seven to ten layers of thick-walled cells elongated to give appearance of *textura angularis* or *textura epidermoidea* when viewed in cross section. Asci cylindric-clavate, floating free at maturity, length×width = (115–)132–141(–200)×(19–)21.3–26.5(–31.3) µm (mean = 138×24.1, SD 17.7, 3.87, n1=24, n2=24). Ascus apex thickened when young, with elongate indistinct pore. Eight ascospores per ascus arranged biseriately or obliquely parallel. Ascospores one-celled, hyaline, fusiform, biguttulate, with thick cell walls, (21.5–)38.0–50.0(–69.5)×(5.5–)8.7–11.5(–16.5) µm (mean = 43.5×10.0, SD 8.5, 2.13, n1=139, n2=139), l:w (3–)4–5(–8) (mean = 4, SD 0.92, n=139) with cell walls appearing smooth at lower magnifications but at 1000X showing undulations or depressions. No color reaction with Meltzer reagent and KOH.

Cultural observations: (based on isolates AR 3552 and CBS 171.69)

On PDA after 7 days average colony diameter (a.c.d.) 1.7 cm (SD=0.25, n=8), after 14 days a.c.d. 3.7 cm (SD= 0.30 cm, n=8), and after 21 days a.c.d. 5.2 cm (SD= 0.73 cm, n=8). Colony appearance after 21 days on PDA smooth to regular margins, thin powdery with slimy pink drops containing conidia. Gray olivaceous (#107) rose colony with a dark halo 2.7 cm from center followed by a honey (#64) halo and hyaline to whitish marginal mycelium. Reverse with a radial growth pattern and a black depression in center of colony, surrounded by a gray olivaceous halo. Conidia hyaline, cylindrical to ellipsoidal,

aseptate, (29.0–)34.5–53.5(–64.0)×(9.0–)10.0–12.5(–14.5) µm (mean = 45.5×11.5, SD length 10.5, width 1.5, n=20). Conidiogenous cells holoblastic, annellidic, narrowly cylindrical, producing a conidium at apex.

On CMA after 7 days a.c.d. 0.7 cm (SD=0.26, n=8), after 14 days a.c.d. 1.5 cm (SD= 0.37 cm, n=8) and after 21 days a.c.d. 2.2 cm (SD= 0.58 cm, n=8). Colony appearance after 21 days on CMA with radial mycelium, hyaline to whitish, growing appressed to surface and smooth but irregular margins; reverse similar to above.

On 2% MEA after 7 days a.c.d. 0.7 cm (SD=0, n=8), after 14 days 1.1 cm (SD=0.19 cm, n=8) and after 21 days a.c.d. 1.4 cm (SD=0.35 cm, n=8). Colony appearance after 21 days on 2% MEA with smooth, regular to irregular margins, cartilaginous, greenish black (#124); iron gray (#122) with black inclusions in the colony reverse.

Lectotype of *Sphaeria hypodermia* designated by Reid and Booth, 1989: **Sweden:** Uppsala, as *Sphaeria hypodermia*, Scleromyc. Suec. Exs. 32 Herb. Fries (Lectotype designated by Reid and Booth, 1989).

Epitype of *Sphaeria hypodermia* designated herein: **Austria**: Vienna, 19th district, Lotheissengasse, grid square 7763/2, on *Ulmus minor*, 11 November 2000, W. Jaklitsch 1694 (BPI 748432, derived culture CBS122593 = AR 3552).

Specimens examined: **Austria:** Niederdonau, Donau-Auen near Klosterneuburg, as *Cryptosporella hypodermia* on *Ulmus* sp., April 1939, F. Petrak, (Dr. F. Petrak Mycotheca Generalis, BPI 601284); Vienna, 21st district. Marchfeldkanalweg, grid square 7764/2. as *C. hypodermia* on *U. minor* and *U. laevis*, 8 Jul 2000, W. Jaklitsch 1497 (BPI 748433, derived culture CBS109753= AR3566). **Belgium:** St. Georges Coutrai, as *Sphaeria limminghii* on *Ulmus campestris,* Herb. DeNotaris Rome, Shear

Study Collection Types and Rarities Series I (BPI 800140). **Hungary:** Pressburg, as *C. hypodermia*, on *Ulmus campestris*, J. A. Baumler (BPI 601289). **Germany:** Nassau, Briebrich, on *Ulmus* sp*.,* Fuckel 1894 (BPI 601287). **Netherlands:** Leiden, on *Ulmus sp*., 21 June 1923, C. L. Shear, determined by Petrak (BPI 601276).

Cryptosporella suffusa (Fr.) L.C. Mejía & Castleb., **comb. nov.** Figs 3.3K-P

MycoBank MB 510391

Synonyms: *Sphaeria suffusa* Fr., Syst. Mycol. 2: 399 (1823).

Valsa suffusa (Fr.) Fr., Summ. Veg. Scand. 412 (1846).

Cryptospora suffusa (Fr.) Tul. & C. Tul., Sel. Fung. Carpol. 2: 145 (1863).

Winterella suffusa (Fr.) O. Kuntze, Rev. Gen. Pl. 1: 34 (1891).

Ophiovalsa suffusa (Fr.) Petr., Sydowia, 19: 272, 1965 (1966).

Sphaeria cryptosporii Curr., Microsc. J. 3: 271 (1855).

Sphaeria rabenhorstii Berk & Broome, Ann. & Mag. Nat. Hist. Ser. 2. IX. 324. 1852.

Valsa commutata Fuckel, Fungi Rhen. 620 (1863).

Valsa rhabdospora de Not., Sfer. Ital. Cent. I: 39 (1863).

Cryptospora rhabdospora (de Not.) Sacc., Syll. Fung. 2: 362 (1883).

Anamorph: *Disculina vulgaris* (Fr.) B. Sutton, Mycol. Pap. 141: 75 (1977).

Ascomata evident as scattered elevations in bark with a plateau-like form with an average base of 2 mm diam and 0.7 mm high, with or without a darker circular area of 0.7 mm diam. Perithecia black, in groups of up to 11, oriented parallel or in angles of 45° toward bark surface with necks converging in center and fused to form a single cavity with a semi-biconic, flat tipped, protruding cone, 0.4×0.1 mm; diam×height = (458–)459–466(–

471)×(258–)264–342(–416) µm (mean = 463×314, SD 6.9, 89.0, n1=3, n2=3), Beak 806 µm high ×108 µm diam at base, 146 µm diam at apex. Perithecial wall tissue *textura angularis*. Asci oval to obovoid narrowing to the base and the apex and in some cases given the appearance of been constrained in the middle, length×width (52–)74.0–84.5(–100)×(17.5–)22.0–26.0(–30.0) µm (mean = 80.0×23.5, SD 8.5, 3.2, n1=31, n2=31), eight ascospores per ascus flexuous cylindrical twisted, interwoven, or parallel arranged, rounded at their tips (48.5–)57.5–66.0(–69.5)×(3.8–)4.0–4.56(–5.5) µm (mean = 61.5×4.38, SD 5.7, 0.4, n1=22, n2=22) l:w (10.5–)14.0–16.0(–16.5) (mean = 14.4, SD 1.7, n=22). No coloration in 3% KOH, no staining with Meltzer reagent.

Cultural observations (based on isolates AR3496 and AR3825):

On PDA after 7 days a.c.d. 1.5 cm (SD=0.3, n=8), after 14 days a.c.d. 3.5 cm (SD=0.8, n=8), after 21 days a.c.d. 4.3 cm (SD=0.9, n=8). Colony appearance after 21 days on PDA smooth and irregular margins, with many drops of water over mycelia. Smoke gray (#105) to honey and hazel in the margin. Reverse mycelia with concentric halos (isabelline # 65) with honey (#64) background and dark brown in the center. On CMA after 7 days a.c.d. 0.9 cm (SD=0.2, n=8), after 14 days a.c.d. 1.2 cm (SD=0.3, n=8), after 21 days a.c.d. 1.3 cm (SD=0.4, n=8). Colony appearance on CMA after 21 days with hyaline to salmon (#41) mycelium, growing appressed to the surface, with a slightly visible salmon halo, and smooth but irregular margin. Reverse similar to above with some dark inclusions appearing in the medium. On 2% MEA after 7 days a.c.d. 0.9 cm (SD=0.2, n=8), after 14 days 1.4 cm (SD=0.2, n=8), after 21 days 1.9 cm (SD=0.2, n=8). Colony appearance after 21 days in 2% MEA cartilaginous texture, color hazel with regular honey margins. Reverse breaking the agar and gray olivaceous in color.

Specimens examined: **Austria:** Tirol, Overtilliach an der Gail, grid square 924/4, on *Alnus incana*, 29 Aug. 2000, W. Jaklitsch 1556 as *Ophiovalsa suffusa* (BPI 748449, derived culture CBS109750 = AR3496); Vienna, Marchfeldkanalweg 7764/2, 21st district, on *Alnus incana,* 19 May 2002, W. Jaklitsch 1892 (BPI871231, derived culture CBS121077 = AR 3825).

The anamorph of *Crytosporella suffusa*, *Disculina vulgaris,* is the type species of the anamorph genus *Disculina* Höhn. (Sutton, 1980).

In addition to Cryptosporella suffusa*, the following new combinations are proposed. For descriptions, see Reid and Booth (1987).*

Cryptosporella alnicola (Höhn.) L.C. Mejía & Castleb. **comb. nov.** Figs 3.4A-E

MycoBank MB 510392

Synonyms: *Cryptospora alnicola* Höhn., Sitzungsber. Acad. Wiss. Wien, 123 (Abt.1): 107 (1914).

Winterella alnicola (Höhn.) J. Reid & C. Booth, Can. J. Bot. 65:1320-1342 (1987).

Cryptospora suffusa var. *nuda* Peck, New York State Mus. Rep. 46: 138, 1892 (1893).

Specimens examined: **USA:** Minnesota, Itasca State Park**,** Wilderness Drive, on *Corylus cornuta* Marsh, 15 August 2002, L. Vasilyeva as *Winterella alnicola* (BPI 872327). **Canada:** Ontario, Timmins. Highway 101, on *Alnus* sp., 24 Jun 1962, H. D. Griffin as *Cryptospora alnicola* (BPI 627094); British Columbia. Chancellor Mtn., Yoho National Park, on *Alnus* sp., 11 Aug 1962, R. F. Cain as *Cryptospora alnicola* (BPI 627095).

Cryptosporella betulae (Tul. & C. Tul.) L.C. Mejía & Castleb. **comb. nov.**

MycoBank MB 510393 Figs 3.4F-I

Synonyms: *Cryptospora betulae* Tul. & C. Tul., Sel. Fung. Carpol. 2: 148-150 (1863).

Winterella betulae (Tul. & C. Tul.) O. Kuntze, Rev. Gen. Pl. 1: 34 (1891).

Valsa tomentella Peck, New York State Mus. Rep. 35:144. 1881 (1884).

Cryptospora tomentella (Peck) Berl. & Vogl., Add. Syll. 1-4: 192 (1886).

Cryptospora betulae var. *tomentella* (Peck) Berl., Ic. Fung. 2: 157 (1889).

Ophiovalsa tomentella (Peck) Petr., Sydowia, 19: 275, 1965 (1966).

Anamorph: *Disculina betulina* (Sacc.) Höhn., Sitzungsber. Akad. Wiss. Wien, 125 (Abt. 1): 108 (1916).

Specimens examined: **USA**: New York, Adirondack, on *Betula* sp. 20 June 2002, L. Vasilyeva as *Ophiovalsa betulae* (BPI 843497, derived culture CBS121080 = AR3889); New York, on *Betula* sp., 20 Jun 2002, L. Vasilyeva as *Ophiovalsa betulae* (BPI 872328, derived culture CBS121073 = AR3863). **Austria**: Niederoesterreich, Losenheim, Laerchkogel. Mapping grid square 8261/1, on *Betula lenta*, 05 Jul 2003 W. Jaklitsch 2271 as *Winterella betulae* (BPI 843595)

Cryptosporella confusa (J. Reid & C. Booth) L.C. Mejía & Castleb. **comb. nov.**

MycoBank MB 510393 Fig 3.4 J-L

Synonym: *Winterella confusa* J. Reid & C. Booth, Can. J. Bot. 65: 1328. (1987).

Specimens examined: **USA**: Tennessee, Knoxville, waterfront downtown, between Calhoun's and University of Tennessee, on *Betula papyrifera,* 23 May 2003, W Jaklitsch

2208 as *Winterella confusa* (BPI 843580, derived cultures CBS121003 = AR3966 = AR3990).

Cryptosporella corylina (Tul. & C. Tul.) L.C. Mejía & Castleb. **comb. nov.**

MycoBank MB 510395 Figs 3.4M-O

Synonyms: *Valsa corylina* Tul. & C. Tul., Sel. Fung. Carpol. 2: 174. (1863).

Cryptospora corylina (Tul. & C. Tul.) Fuckel, Jahrb. Nassau. Ver. Naturkd. 23, 24: 192. 1869 (1870).

Winterella corylina (Tul. & C. Tul.) O. Kuntze, Rev. Gen. Pl. 1: 34 (1891).

Anamorph: *Disculina corylina* Höhn., Ann. Mycol. 16: 108 (1918).

Specimens examined: **Austria:** Kaernten, Kramer Strauch, St. Margareten im Rosental. Mapping grid square 9452/4, on *Corylus avellana,* 13 September 2001, W. Jaklitsch 1811 as *Winterella corylina* (BPI 843623).

Cryptosporella femoralis (Peck) L.C. Mejía & Castleb. **comb. nov.** Figs 3.4P-Q

MycoBank MB 510396

Synonyms: *Valsa femoralis* Peck, New York State Mus. Rep. 28. 74-75. 1874 (1879).

Cryptospora femoralis (Peck) Sacc., Syll. Fung. 2: 362 (1883).

Winterella femoralis (Peck) O. Kuntze, Rev. Gen. Pl. 1: 34 (1891).

Ophiovalsa femoralis (Peck) Petr., Sydowia, 19: 273. 1965 (1966).

Cryptospora humeralis Dearn. & House, Circ. N. Y. State Mus. 24: 41 (1940).

Specimens examined: **USA**: New York, Cranberry Lake, Adirondack mts., on *Alnus rugosa*, 13 June 2002, L. Vasilyeva as *Ophiovalsa femoralis* (BPI872326, derived culture CBS121076 = AR 3868); Michigan, Ludington State Park, on *Alnus rugosa*, 25 August 2006, coll. G. Adams, det. L. C. Mejia (BPI 872325, derived PCR products CF3 and CF4); Maine, North of New Portland, on *Alnus rugosa,* L. C. Mejía LCM 22 (BPI 872324, derived PCR product LCM22D).

Cryptosporella tiliae (Tul. & C. Tul.) L.C. Mejía & Castleb. **comb. nov.**

MycoBank MB 510399

Synonyms: *Cryptospora tiliae* Tul. & C. Tul., Sel. Fung. Carpol. 2: 150-151. 1863.

Ophiovalsa tiliae (Tul. & C. Tul.) Petr., Sydowia, 19: 274. 1965 (1966).

Winterella tiliae (Tul. & C. Tul.) O. Kuntze, Rev. Gen. Pl. 1: 34 (1891).

Cryptosporella wehmeyeriana (J. Reid & C. Booth) L.C. Mejía & Castleb. **comb. nov.**

MycoBank MB 510400 Figs 3.4S-U

Synonym: *Winterella wehmeyeriana* J. Reid & C. Booth, Can. J. Bot. 65: 1320-1342 (1987).

Specimens examined: **USA**: North Carolina, Great Smoky Mts. National Park, Cataloocheee vicinity, Caldwell Fork trail, on *Tilia* sp., 23 April 2002, L. Vasilyeva as *Winterella wehmeyeriana* (BPI 843485); District of Columbia, Washington. Dept. of Agriculture Grounds, on *Tilia* sp., 21 Feb 1903, C. L. Shear as *Cryptospora wehmeyeriana* (BPI 629315); New York. Lyndonville, on *Tilia* sp., June 1921, C. L. Shear as *Cryptospora wehmeyeriana* (BPI 629320); New York. Alcove, on *Tilia*

americana, June 1893, C. L. Shear as *Cryptospora wehmeyeriana* (BPI 629318); District of Columbia. Washington. Dept. of Agriculture Grounds, on *Tilia* sp., 21 February 1903, C. L. Shear 1419 as *Cryptospora wehmeyeriana* (BPI 629319). **Canada:** Ontario. London, on *Tilia* sp., June 1893, J. Dearness as *Cryptospora wehmeyeriana* (BPI 629317).

Key to species of *Cryptosporella::*

1. Ascospores with one median septum at maturity, ends swollen, thus appearing like a leg bone or femur, (24.0–)45.5–56.0(–74.0)×(3.0–)3.5–4.5(–5.5) µm, l:w (5–)12–14(–18); on *Alnus* spp. in North America……*Cryptosporella femoralis*

1. Ascospores non-septate……2

2. Ascospores ellipsoid to fusoid, acute ends, (22.0–)38.0–48.0(–69.5)×(5.5–)8.0–11.0(–16.5) µm, l:w (3–)4–5(–8); on *Ulmus* spp……...… *Cryptosporella hypodermia*

2. Ascospores mostly cylindrical…….3

3. Ascospores cylindrical with rounded ends…….…….4

3. Ascospores cylindrical slightly swollen at their ends……..5

4. Ascospores slightly curved, tapering toward rounded ends, (29.0–)38.0–50.0(–74.0)×(4.5–)5.0–6.0(–9.0) µm, l:w (5–)7–10(–13); on *Betula* spp…….

…….…..…*Cryptosporella betulae*

4. Ascospores flexuous, interwoven in asci, (49.0–)57.5–66.0(–69.5)×4.0–4.5(–5.5) µm, l:w (10–)14–16(–17); on *Alnus* spp…….*Cryptosporella suffusa*

5. Ascospores cylindrical to femuroid; 27-35 x 5-6.5 µm; on *Tilia* sp. in Europe

…….…..*Cryptosporella tiliae******

5. Ascospores cylindrical with slightly swollen ends, greater than 35 µm long……....6

6. On *Alnus* spp. and *Tilia* spp. in North America…….……7

6. On *Betula* spp. and *Corylus* spp. in North America and Europe……..……..8

7. Ascospores (53.5–)62.0–78.5(–99.0)×(3.0–)3.5–4.5 µm, l:w (12–)15–21(–28); on *Alnus* spp. in North America……..…*Cryptosporella alnicola*

7. Ascospores (49.5–)74.0–90.5(–109)×(4.0–)5.0–6.0(–7.0) µm, l:w (9–)14–16(–23); on *Tilia* spp. in North America…….…..…*Cryptosporella wehmeyeriana*

8. Ascospores (87.5–)88.5–89.5(–91.0)×3.0–3.5 µm, l:w (25–)26–28(–27); on *Betula* spp. …….. …….…….……..…*Cryptosporella confusa*

8. Ascospores (21.5–)26.5–75.0(–82.5)×(3.5–)4.0–4.5(–10) µm, l:w (5–)7–17(–23); on *Corylus* spp. ……..……..……..……..…*Cryptosporella corylina*

* Species not observed during the course of this study. Description and measurements from Reid and Booth (1987).

DISCUSSION

Here we show evidence from molecular data that *Ophiovalsa suffusa*, the type species of *Ophiovalsa,* and *Cryptosporella hypodermia*, the type species of *Cryptosporella,* belong to the same genus within the family *Gnomoniaceae* (*Diaporthales*) based on analysis of three different loci (LSU, ITS, and RPB2) (Fig 3.1). These data corroborate observations that *C. hypodermia* should be treated as congeneric with *O. suffusa* (Reid & Booth 1989), however, these authors placed these two species in *Winterella* (Reid & Booth 1987, 1989). Our review of the literature indicates that the correct name for taxa placed in *Winterella* sensu Reid & Booth (1987) is *Cryptosporella*.

As noted first by Kuntze (1891), the generic name *Cryptospora* Tul. & C. Tul. 1863 was antedated by *Cryptospora* Karelin & Kirilow 1842, a plant genus in the *Brassicaceae*. Thus, Kuntze (1891) recognized the name *Winterella* at the generic level as a replacement for the later homonym *Cryptospora* Tul. & C. Tul. The name *Winterella* was first used by Saccardo (1883) for a subgenus of *Cryptospora* Tul. & C. Tul. with *C. anthostomoides* Rehm as the type species. It is now known that *C. anthostomoides* is a loculoascomycete and possibly an older taxonomic synonym of *Montagnula* Berl. (Holm, 1992). Thus, the generic name *Winterella* cannot be used for diaporthalean species previously placed in *Cryptospora*. This conclusion is subject to interpretation about the meaning of the symbol used by Kuntze (1891) in his description of *Winterella.* Holm (1992) and later Rossman (2002) based on discussions with D. Nicolson (Smithsonian Institution, Washington, DC) argued that the symbol § used by Kuntze (1891) indicates a subgenus and thus Kuntze (1891) was raising Saccardo's *Cryptospora* subg. *Winterella* (1883) to generic status based on *Cryptospora suffusa*. However, Reid and Booth (1987) suggested that the generic name *Winterella* originated with Kuntze's (1891) publication with *W. suffusa* as the type species. In our view there is little evidence to support the latter interpretation. However, this disagreement becomes irrelevant because *Cryptosporella* is an older name for *Winterella* sensu Reid & Booth (1987) as well as *Ophiovalsa*. When establishing the genus *Ophiovalsa* for *Cryptospora* Tul. & C. Tul., Petrak (1966) did not mention the work of Kuntze (1891) and Petrak's proposed generic name *Ophiovalsa* has been used in the recent literature (Barr 1978; Kobayashi 1970; Glawe & Jensen 1986). *Cryptosporella* provides the oldest name for *Ophiovalsa* as well as *Winterella* sensu Reid & Booth (1987).

No single unique morphological characters distinguish species placed in *Cryptosporella* from other genera in the family *Gnomoniaceae.* However, these species share a suite of characters such as perithecia developing below the bark surface, aggregated with converging necks, similar perithecial wall morphology and structure, and ellipsoid to elongated, non-, rarely one-septate ascospores. The known anamorphs all belong to the genus *Disculina* Höhn. (Sutton 1980). Most species of *Cryptosporella* are endophytic in twigs of the hardwood trees, *Alnus, Betula, Corylus, Tilia* and *Ulmus,* with a saprobic stage following host tissue death.

Nine species are accepted in the genus *Cryptosporella,* seven of which are included in the RPB2, ITS and LSU DNA sequence analyses (Fig 3.2). Species in the genus *Cryptosporella* show a wide range of ascospore shapes and sizes, distinctive colony morphologies, and conidial formation and pigmentation. The species of *Cryptosphaerella* can be separated by a combination of host and ascospore size and shape. Most species have non-septate ascospores while those of *C. femoralis* become one-septate at maturity. The ascospores of *C. hypodermia* are elliptical to fusiform (Figs 3.3E, F, H, I), relatively short and broad compared to the other species of *Cryptosporella* that tend to be elongate to cylindrical with broadly rounded ends (Figs 3.3J, M; 4B-I, K-L, N-O, T, U). The ascospores of *C. femoralis* are distinctive in being swollen at each end thus appearing femuroid (Fig 3.4P). Ascospores of *C. tiliae* occasionally become femuroid but are much shorter than those of *C. femoralis* and are non-septate. Although Reid and Booth (1989) list hardwood hosts other than *Ulmus* spp. for *C. hypodermia* (as *Winterella hypoder*mia), most of their specimens examined were on *Ulmus* and this fungus was not found to occur on any other host genera.

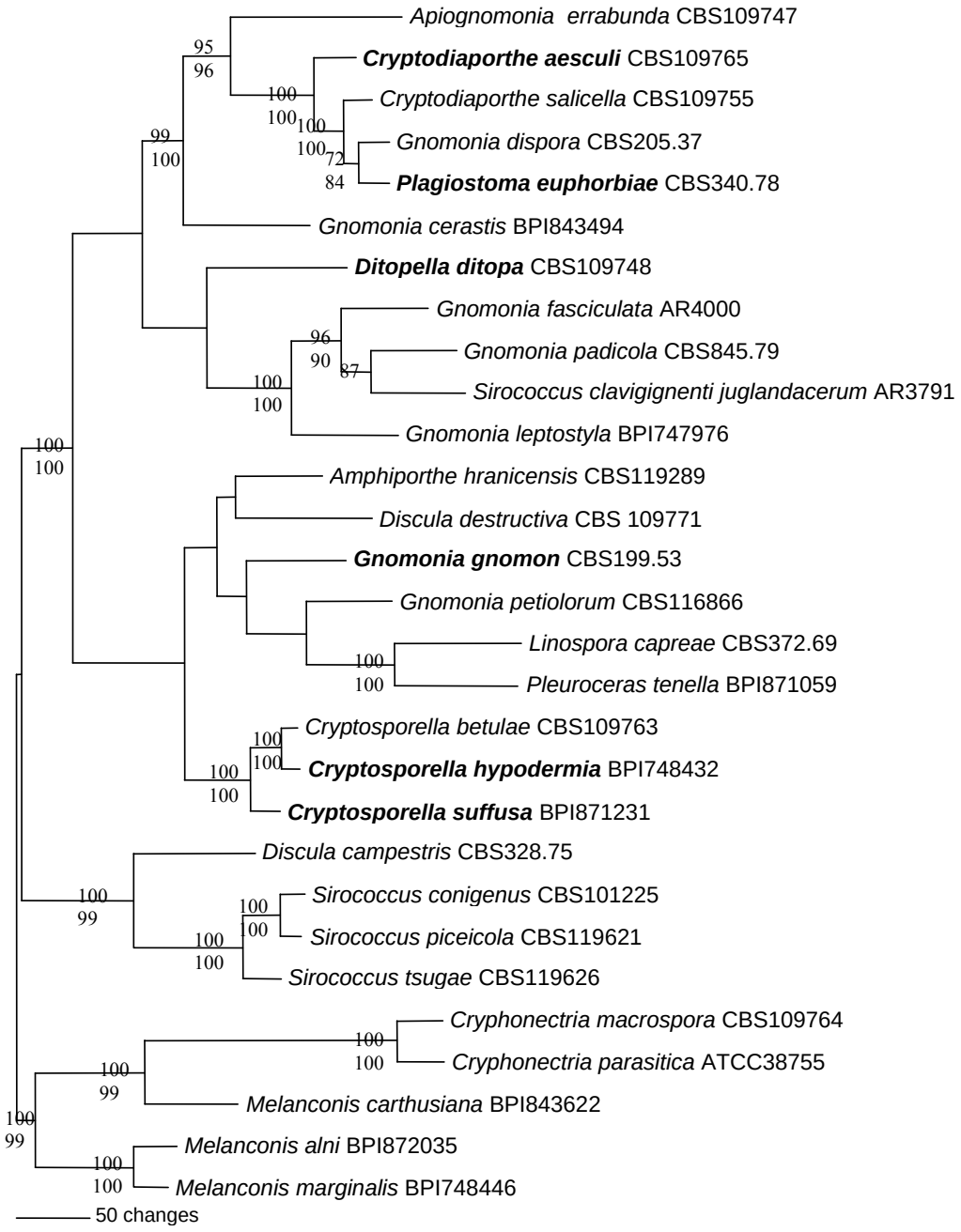

Figure 3.1 – Single most parsimonious tree based on analysis of 2831 bp representing a combination of three different loci (LSU, ITS and RPB2) of 24 gnomoniaceous taxa using species of *Melanconis* and *Cryphonectria* as outgroup taxa (CI = 0.246, RI = 0.552, RC = 0.236, HI = 0.573, length = 2461 steps). Bootstrap values greater than 70% are

shown above (MP) and below (NJ) each branch. Taxa in bold represent type species of their respective genera. Note that the type species of the genus *Ophiovalsa, O. suffusa*, here referred to as *Cryptosporella suffusa,* groups with the type species of the genus *Cryptosporella, C. hypodermia*.

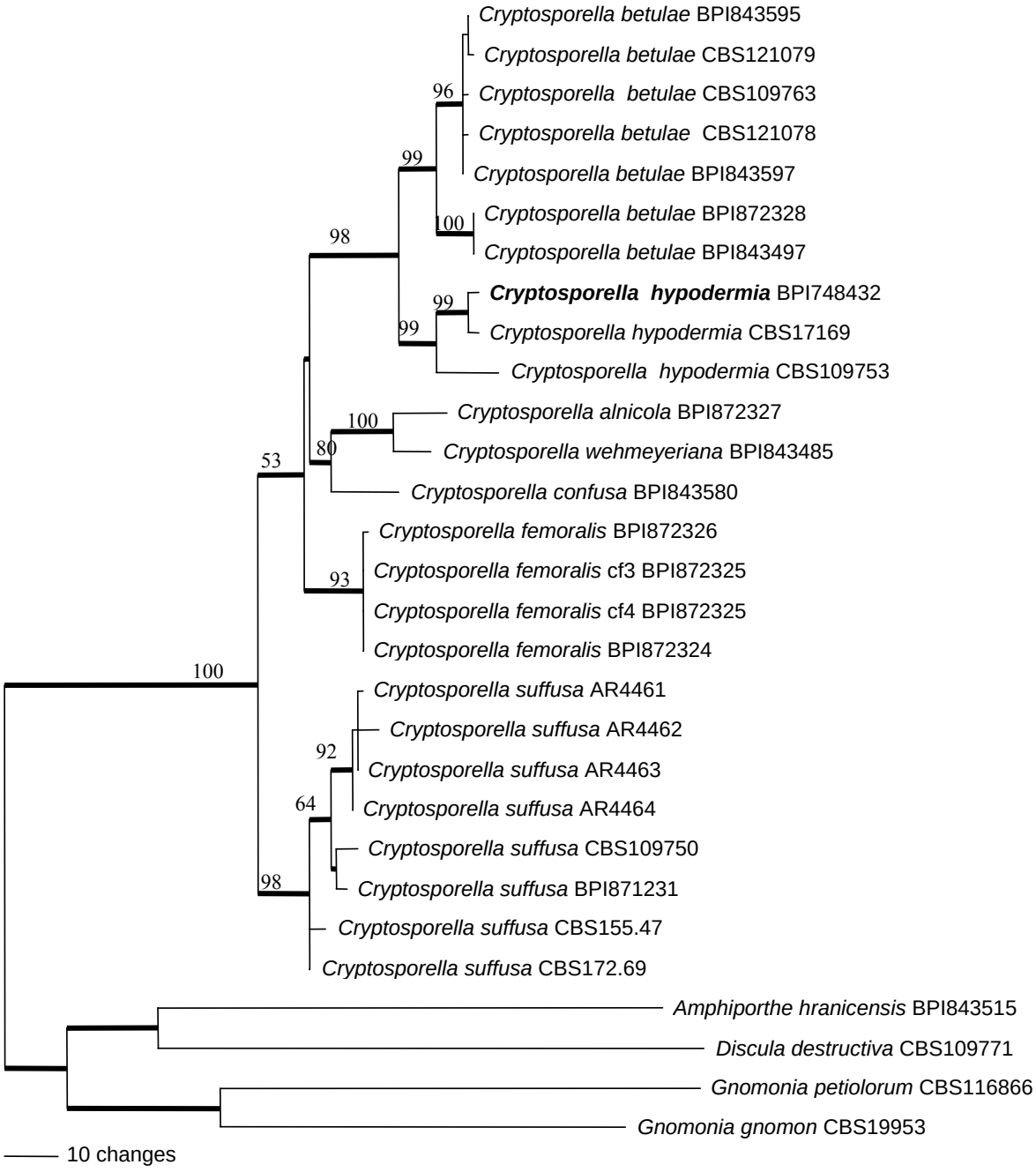

Figure 3.2 – One of 104 equally parsimonious trees based on analysis of combined alignment of ITS and RPB2 genes containing (CI = 0.758, RI = 0.792, RC = 0.600, HI = 0.242, length 607 steps) for 25 isolates of *Cryptosporella.* Bootstrap values above 70%

are shown above each branch. The isolate representing the epitype specimen and culture of the genus *Cryptosporella* is shown in bold. Thickened branches indicate that the particular branch appears in the strict consensus tree of the 104 trees.

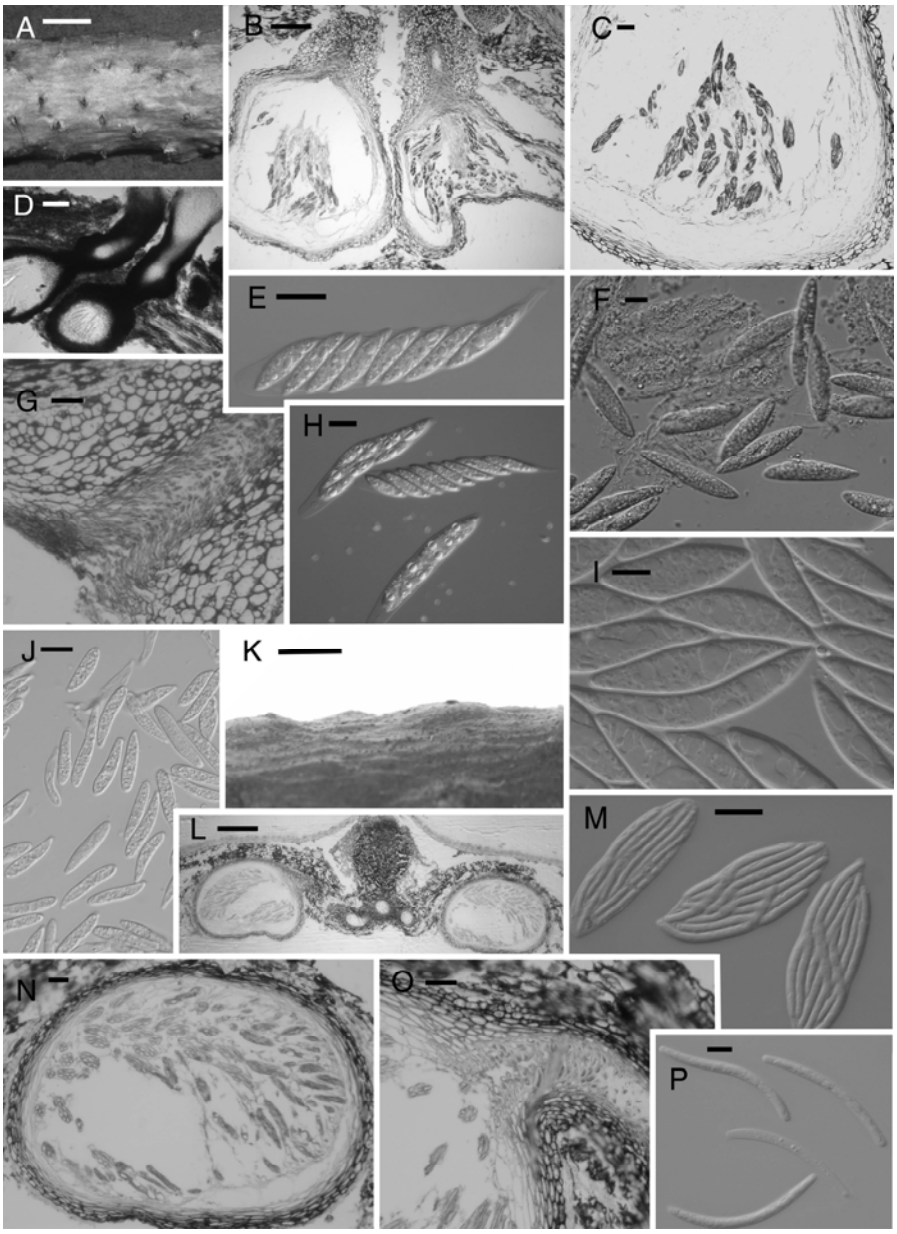

Figure 3.3 – A-J *Cryptosporella hypodermia*. Fig A – Evidence of ascomata on twigs of *Ulmus campestris*. Fig B – Perithecia. Fig C – Bilaminate venter wall, outer region one to two layers of cells, inner region 4-6 layer of cells. Fig D – Perithecia, appressed with converging necks but not fused and flared ostiolar openings. Fig E – Ascus. Fig F – Ascospores of the lectotype. Fig G – Perithecial neck densely filled with paraphyses. Fig H – Asci of the epitype. Fig I – Ascospores of the epitype. Fig J – Conidia. Figs K-P –

Cryptosporella suffusa. Fig K – Evidence of ascomata in twigs of *Alnus incana*. Fig L – Perithecia, note fusion of perithecial necks to form a single ostiolar cavity. Fig M – Asci. Figs N-O – Perithecia, note bilaminate venter wall as in *C. hypodermia*, and perithecial necks densely filled with periphyses. Fig P – Ascospores. Bars: A = 5mm; B, D, = 100 µm; C, L = 50 µm; E, G, H, M, N, O = 20 µm, F, I, J, P = 10 µm; K = 1mm.

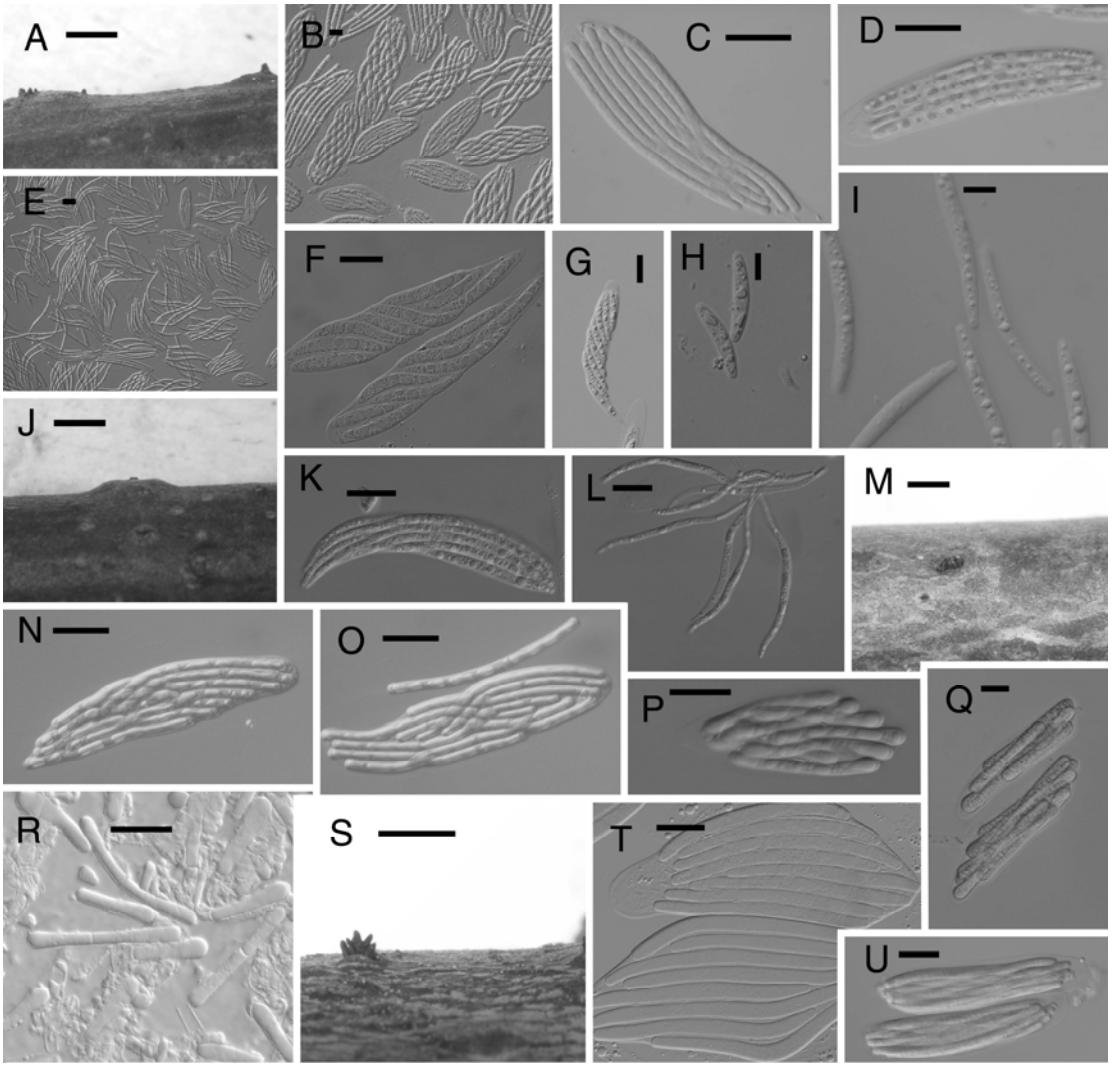

Figure 3.4 – A-E *Cryptosporella alnicola*. Fig A – Evidence of ascomata on twigs of *Corylus cornuta*. Figs B-E – Asci and ascospores. Figs F-I – *Cryptosporella betulae*. Figs F-G – Asci. Figs H-I – Ascospores. Figs J-L – *Cryptosporella confusa*. Fig J – Evidence of ascomata on twigs of *Betula papyrifera*. Fig K – Asci. Fig L – Ascospores. Figs M-O – *Cryptosporella corylina*. Fig M – Evidence of ascomata on twigs of *Corylus avellana* Figs N-O – Asci and ascospores. Figs P-R – *Cryptosporella femoralis***.** Figs P-Q – Asci. Fig R – Ascospores. Figs S-U – *Cryptosporella wehmeyeriana***.** Fig S – Evidence

of ascomata on twigs of *Tilia* sp. Figs T-U – Asci and ascospores. Bars: A, J, M, S = 1 mm; B, C, D, E, F, G, K, L, N, O, P, Q, R = 20 µm; H, I = 10 µm.

Table 3.1. Isolates in the phylogenetic analyses of *Cryptosporella* and related members of the *Diaporthales*, specimen and culture numbers, accession numbers for LSU, ITS, and RPB2 sequences, host, country, and collector. New DNA sequences are in bold.

Species	Specimen	Culture	LSU in Genbank	ITS in Genbank	RPB2 in Genbank	Host	Country	Collector
Amphiporthe hracinensis	BPI843515	CBS119289	**EU199122**	**EU199178**	**EU199137**	*Tilia platyphlylos*	Austria	W. Jaklitsch 1755
Apiognomonia errabunda	---	CBS109747	AF408334	DQ313525	EU212961	*Fagus sylvatica*	Switzerland	M. Monod
Cryphonectria macrospora	BPI 748428	CBS109764	AF408340	**EU199182**	**EU220029**	*Quercus mongolica*	Russia	L. Vasilyeva
Cryphonectria parasitica	---	ATCC 38755	**EU199123**	AY141856	DQ862017	*Castanea dentata*	USA	S. Anagnostakis
Cryptodiaporthe aesculi	BPI 748430	CBS109765	AF408342	**EU199179**	**EU199138**	*Aesculus hippocastanum*	Austria	W. Jaklitsch 1795
Cryptodiaporthe salicella	BPI 747938	CBS109755	AF408345	**EU199183**	**EU199141**	*Salix* sp.	Austria	W. Jaklitsch 1463
Cryptosporella alnicola	BPI872327	CBS 121074	---	**EU199204**	**EU199160**	*Corylus cornuta*	USA, MN	L. Vasilyeva
Cryptosporella betulae	BPI748448	CBS109763	AF408375	**EU199180**	**EU199139**	*Betula pendula*	Austria	W. Jaklitsch 1610
Cryptosporella betulae	BPI843595	CBS 121075	---	**EU199214**	**EU199170**	*Betula pendula*	Austria	W. Jaklitsch
Cryptosporella betulae	BPI843597	CBS 121351	---	**EU199215**	**EU199171**	*Alnus alnobetula*	Austria	W. Jaklitsch
Cryptosporella betulae	---	CBS 121078	---	**EU199213**	**EU199169**	*Betula pendula*	Scotland	S. Green
Cryptosporella betulae	---	CBS 121079	---	**EU199216**	**EU199172**	*Betula pendula*	Scotland	S. Green
Cryptosporella betulae	BPI872328	CBS 121073	---	**EU199217**	**EU199173**	*Betula* sp.	USA, NY	L. Vasilyeva
Cryptosporella betulae	BPI 843497	CBS 121080	---	**EU199218**	**EU199174**	*Betula* sp.	USA	L. Vasilyeva
Cryptosporella confusa	BPI 843580	CBS121063	---	**EU199219**	**EU199175**	*Betula papyrifera*	USA, TN	W. Jaklitsch
Cryptosporella femoralis	BPI872326	CBS 121076	---	**EU199220**	**EU199176**	*Alnus rugosa*	USA, NY	L. Vasilyeva
Cryptosporella femoralis	BPI872325	cf3	---	**EU199221**	---	*Alnus rugosa*	USA, MI	G. Adams
Cryptosporella femoralis	BPI872325	cf4	---	**EU199222**	---	*Alnus rugosa*	USA, MI	G. Adams
Cryptosporella femoralis	BPI872324	lcm22D	---	**EU199223**	---	*Alnus rugosa*	USA, ME	L. C. Mejia
Cryptosporella hypodermia	BPI 748432	AR3552	AF408346	**EU199181**	**EU199140**	*Ulmus minor*	Austria	W. Jaklitsch 1694
Cryptosporella hypodermia	BPI 748433	CBS109753	**---**	**EU199224**	**EU199177**	*Ulmus* sp.	Austria	W. Jaklitsch
Cryptosporella hypodermia	---	CBS 171.69	DQ862028	**EU199225**	DQ862018	*Ulmus* sp.	Netherlands	H. van der Aa
Cryptosporella suffusa	BPI748449	CBS109750	---	**EU199207**	**EU199163**	*Alnus incana*	Austria	W. Jaklitsch 1556
Cryptosporella suffusa	BPI871231	CBS 121077	**EU199124**	**EU199184**	**EU199142**	*Alnus incana*	Austria	W. Jaklitsch 1892
Cryptosporella suffusa	---	CBS155.47	---	**EU199206**	**EU199162**	*Alnus glutinosa*	Netherlands	S. Truter

Cryptosporella suffusa	---	AR4461	---	**EU199208**	**EU199164**	*Alnus sinuata*	USA, WA	S. Lattomus, L. Mejia
Cryptosporella suffusa	---	AR4462	---	**EU199209**	**EU199165**	*Alnus sinuata*	USA, WA	S. Lattomus, L. Mejia
Cryptosporella suffusa	---	AR4463	---	**EU199210**	**EU199166**	*Alnus sinuata*	USA, WA	S. Lattomus, L. Mejia
Cryptosporella suffusa	---	AR4464	---	**EU199211**	**EU199167**	*Alnus sinuata*	USA, WA	S. Lattomus, L. Mejia
Cryptosporella suffusa	---	CBS172.69	---	**EU199212**	**EU199168**	*Alnus glutinosa*	Netherlands	H. van der Aa 1068
Cryptosporella wehmeyeriana	BPI 843485	CBS 121085	---	**EU199205**	**EU199161**	*Tilia* sp.	USA, NC	L. Vasilyeva
Discula campestris	---	CBS 328.75	**EU199125**	**EU199185**	**EU199143**	*Acer pseudoplatanus*	Germany	A. John
Discula destructiva	BPI 107757	CBS109771	AF408359	**EU199186**	**EU199144**	*Cornus nuttallii*	USA	M. Daughtrey
Ditopella ditopa	BPI748439	CBS109748	**EU199126**	**EU199187**	**EU199145**			
Gnomonia cerastis	BPI 843494	CBS 121084	**EU199127**	**EU199188**	**EU199146**	*Acer* sp.	USA, NY	L. Vasilyeva
Gnomonia dispora	---	CBS 205.37	**EU199128**	**EU199189**	**EU199147**	*Carya illinoensis*		M. Wilcox
Gnomonia fasciculata	BPI 872323	AR4000	**EU199129**	**EU199190**	**EU199148**	*Quercus mongolica*	Russia	L. Vasilyeva
Gnomonia gnomon	---	CBS 199.53	AF408361	AY818956	EU219295	*Corylus avellana*	Italy	M. Ribaldi
Gnomonia leptostyla	BPI747976	CBS 110136	AF408362	**EU199191**	**EU199149**	*Juglans nigra*	USA	D. Neely
Gnomonia padicola		CBS 845.79	AF277134	**EU199192**	**EU199150**	*Prunus padus*	Switzerland	M. Monod 508
Gnomonia petiolorum	BPI843530	CBS116866	AY818963	**EU199193**	**EU199151**	*Liquidambar styraciflua*	USA, TN	A. Rossman
Linospora capreae	---	CBS 372.69	AF277143	**EU199194**	**EU199152**	*Salix caprea*	Netherlands	H. van der Aa
Melanconis alni	BPI872035	AR3748	**EU199130**	**EU199195**	**EU199153**	*Alnus viridis*	Austria	W. Jaklitsch 1796
Melanconis carthusiana	BPI843622	CBS 121083	**EU199131**	**EU199196**	**EU199154**	*Juglans regia*	Austria	W. Jaklitsch 1450
Melanconis marginalis	BPI748446	CBS 109744	AF408373	**EU199197**	EU219301	*Alnus rubra*	Canada	M. Barr 1021A
Plagiostoma euphorbiae	---	CBS 340.78	AF408382	**EU199198**	EU219292	*Euphorbia palustris*	Netherlands	H. van der Aa
Pleuroceras tenella	BPI871059	CBS 121082	**EU199132**	**EU199199**	**EU199155**	*Acer rubrum*	USA, NC	M. Sogonov 0159
Sirococcus clavigignenti juglandacearum	---	CBS 121081	**EU199133**	**EU199200**	**EU199156**	*Juglans cinerea*	USA, MN	M. Ostry
Sirococcus conigenus	BPI871248	CBS 101225	**EU199134**	**EU199201**	**EU199157**	*Picea abies*	Austria	H. Anglberger
Sirococcus piceicola	BPI 871166	CBS119621	**EU199135**	**EU199202**	**EU199158**	*Picea abies*	Switzerland	O. Holdenreider
Sirococcus tsugae	BPI 871167	CBS119626	**EU199136**	**EU199203**	**EU199159**	*Tsuga martensiana*	Alaska	G. Stanosz

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Chapter 4

New species, phylogeny, host-associations, and geographic distribution of the genus *Cryptosporella* (Gnomoniaceae, Diaporthales)[1,2]

ABSTRACT

The phylogeny of *Cryptosporella* is revised to include recently discovered species. The host association of each species of *Cryptosporella* and its geographic distribution is examined. The phylogeny and species delimitation are based on analyses of DNA sequences from three genes (*β-tubulin*, ITS, and *tef1-α*) by comparative morphology of sexual structures of the species on their host substrate and by host associations. Eight species new to science are described and two new combinations are proposed, raising the total number of species accepted in *Cryptosporella* to 19. The inferred phylogeny suggests that *Cryptosporella* has speciated primarily on Betulaceae with 16 species occurring exclusively on Betulaceae. The host range of most species is very narrow with nine species reported only from a single host species and ten species occurring on a few, usually congeneric, host species. The data suggest a geographic structure among *Cryptosporella* species, potentially due to speciation events resulting from host jumps to both distantly and more closely related host species within the same geographic area. The known distribution range of *Cryptosporella* is expanded to mountain cloud forests of the provinces of Chiriquí in Panama and Tucumán in Argentina.

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[1] This chapter is intended for publication with the following authors Mejía, L.C., Rossman, A.Y., Castlebury, L. A., White, J.F., Jr.

[2] The new names included within this chapter are not accepted by the author as validly published in this dissertation (Botanical Code, Article 34.1[a]).

INTRODUCTION

The genus *Cryptosporella* Sacc. (synonym *Ophiovalsa*, anamorph *Disculina*) has recently been treated taxonomically (Mejia et al. 2008); however, little is known of the biology, ecology and evolution of these species. Because of the cryptic nature of these species, they may exist largely asymptomatically within their host plants. Even when produced, their microscopic reproductive structures are difficult to detect. However, species of *Cryptosporella* can be quite abundant in hosts from temperate forests, and some species of *Cryptosporella* have been reported as the dominant endophyte on branches and twigs of alders and birches (Betulaceae). For example, *Cryptosporella suffusa* (or its anamorph *Disculina vulgaris*) have been reported as the most frequently isolated endophyte species on the bark of *Alnus glutinosa* in Europe and *A. rubra* in North America (Fisher and Petrini 1990; Sieber et al. 1991). Similarly *C. betulae* has been reported as the most frequently isolated endophyte on branches of *Betula pendula* and *B. pubescens* in Europe (Barengo et al. 2000; Kowalski and Kehr 1992).

Sixty species have been described as *Cryptosporella.* However, most of these species have been excluded from the genus and transferred principally to *Wuestneia* but also to other genera such as *Botryosphaeria*, *Diaporthe*, *Kapooria*, *Keinstirschia*, *Kensinjia*, *Mebarria*, and *Wehmeyera* (see Reid and Booth 1989). Recently Mejía et al. (2008) recircumscribed *Cryptosporella,* accepting nine species in the genus, suggesting that species of *Cryptosporella* are primarily associated with hosts in the Betulaceae. Castlebury et al. (2002) determined that the genus *Cryptosporella* belongs in the Gnomoniaceae (Diaporthales). This contrasted with previous classification schemes that considered *Cryptosporella* (or its synonyms) to belong in the Diaporthaceae (Höhnel

1917), in the Cryptosporellaceae (Arx and Müller, 1954), or in the Melanconidaceae or Valsaceae (see Barr 1978). Similarly, other species in the Diaporthales have been reported as frequently present or dominant endophytes in broad-leaf trees (e.g. Aceraceae, Betulaceae, Fagaceae, Salicaceae, and Tiliaceae) in temperate forests (see Sieber 2007; Stone et al. 2004). Furthermore, Sieber (2007) has proposed that endophyte communities associated with angiosperms are dominated by species of Diaporthales and that this association may date to near the time of the origin of angiosperms.

Recent phylogenetic studies have shown that several of the dominant diaporthalean endophyte species belong in genera of the Gnomoniaceae such as *Apiognomonia*, *Ditopella*, *Gnomonia*, *Ophiognomonia*, and *Plagiostoma* (synonym *Cryptodiaporthe*) (Castlebury et al. 2002, Sogonov et al. 2008, present work, see Figure 2.1). Therefore fungi from the Gnomoniaceae are an important component of the endophytic mycobiota in temperate forests. With regard to host associations, it has been observed during the course of this work and elsewhere that species of *Cryptosporella* and other Gnomoniaceae have narrow host ranges, sometimes specific to a single host species (Barr 1978; Mejía et al. 2008; Monod 1983; Sogonov et al. 2008). The association is often between an entire genus or subclade of Gnomoniaceae and a particular genus or family of plants (see Chapter 5 and Figs. 2.1 and 5.1). Multiple levels of association between species and genera of Gnomoniaceae and their hosts make it an attractive system for investigations on the effects of host plant evolution on the diversification of endophytes. Certainly the effects of host on the diversification of bark-inhabiting endophytes such as *Cryptosporella* are not documented. In this work, new species of *Cryptosporella* are described based on morphological characters and host associations

and a multigene phylogeny of the genus is presented. Additionally the inferred phylogeny is used to estimate the effects that hosts may have played in the diversification of this genus.

MATERIALS AND METHODS

Collection of specimens, culture preparation, and morphological observations

Specimens were collected from known hosts of *Cryptosporella* species as well as closely related hosts with no record of association with species of *Cryptosporella.* Specimens consisted of dead twigs and branches with perithecia of *Cryptosporella* and were collected in Argentina (Tucumán), France (Deux-Sèvres Department), Germany (Frankfurt), Panama (Chiriquí), and the United States (Maryland, New Hampshire, New York, Oregon, Washington) in 2007 and 2008. Specimens were placed in paper bags, which were left open overnight at room temperature to reduce moisture content. For longer term storage, paper bags containing specimens were placed within tightly sealed plastic bags and stored in the dark at 8-10 °C, remaining viable for up to six months. Isolation of cultures, morphological observations and digital imaging were as described in Mejía et al. (2008). Specimens were deposited in the U.S. National Fungus Collections (BPI). Fungal cultures were deposited at the Central Bureau Voor Schimmelcultures (CBS, The Netherlands).

DNA extraction and sequencing

DNA was extracted as specified in Mejía et al. (2008). Three genes were sequenced in this study: *β-tubulin*, ITS, and *tef1-α*. The conditions and primers employed for amplification of the ITS and *tef1-α* genes were as described by Sogonov et al. (2008). When necessary, the *tef1-α* gene was amplified as two overlapping fragments with the

primer combinations EF1-728F / EF1-1199R and EF1-983F / EF1-1567R and sequenced with the PCR primers (Carbone and Kohn 1999; Castlebury, unpublished data for primer 1199R 5' GGG AAG TAC CMG TGA TCA TGT 3'; Rehner 2001). The *β-tubulin* gene fragment was amplified using the primers T1 and T22 as described by O'Donnell and Cigelnik (1997), using primers T1, T2, T12, and T22 for sequencing. DNA sequencing methods were as described by Mejía et al. (2008).

Phylogenetic analyses

Editing and alignment of sequences are described in Mejía et al. (2008). Individual alignments of the genes were concatenated into a single alignment composed of ITS (560 bp), *β-tubulin* (1645 bp), and *tef1-α* (1169 bp) for a total of 50 isolates. The taxa included in this alignment represent 17 of the 19 species of *Cryptosporella* accepted in this work with *Ditopella ditopa* and *Plagiostoma petiolophillum* as outgroup taxa. Outgroup taxa were selected based on the close relationships of the genera *Ditopella* and *Plagiostoma* with *Cryptosporella* (Sogonov et al. 2008). The concatenated alignment was partitioned by gene and conflict among genes was analyzed using a reciprocal bootstrap test (Reeb et al. 2004) as described in Sogonov et al. (2008).

Maximum parsimony (MP) analysis and MP bootstrap analysis were performed as described in Mejía et al. (2008). Bayesian analysis was performed as specified in Sogonov et al. (2008) using MrModeltest v.2 (Nylander 2004) to determine the best model for each gene region. A consensus phylogram was constructed from 7800 trees saved after the burn-in period of 50000 generations. The resulting Bayesian posterior probabilities (PP) for individual nodes are presented in Figure 4.1. Finally, a Maximum Likelihood (ML) analysis was performed as detailed in Sogonov et al. (2008).

RESULTS

Phylogenetic analyses

The likelihood parameters obtained for each gene for the reciprocal bootstrap analyses were as follows: *β-tubulin*: Base=(0.1867 0.3358 0.2535) Nst=2 TRatio=1.7644 Rates=gamma Shape=0.4684 Pinvar=0; ITS: Nst=6 Rmat=(1.0000 3.2902 1.0000 1.0000 7.9560) Rates=gamma Shape=0.8158 Pinvar=0.5986; *tef1-α*: Base=(0.2103 0.3169 0.2435) Nst=6 Rmat=(1.0000 1.9032 1.0000 1.0000 3.7089) Rates=gamma Shape=0.3413 Pinvar=0. The reciprocal bootstrap analyses indicated no conflict among the genes employed in this work; however no single gene resolved all the species as terminal monophyletic clades with bootstrap support >70%. Although ITS trees resolved most species of *Cryptosporella*, only four species were supported with bootstrap support >70%. The *β-tubulin* and *tef1-α* trees resolved clades for most species analyzed with 12 species of *Cryptosporella* supported as monophyletic clades with bootstrap support >70% in individual analyses of each. In general *β-tubulin* and *tef1-α* trees supported well-resolved clades of closely related species such as the subclade containing *C. pacifica*, *C. suffusa* and *C. multicontinentalis* and the subclade containing *C. betulae*, *C. tomentella*, *C. corylina*, and *C. hypodermia*. The gene tree topologies were similar for *β-tubulin*, and *tef1-α* and both differed slightly from the ITS tree. Nonetheless the topological differences observed were not supported by bootstrapping analysis.

The following models were estimated and applied to the gene partitions in the Bayesian analyses: HKY + G for β-tubulin, GTR + I + G for ITS, GTR + G for tef1-α. The model TrN+G was estimated to be the best for the entire alignment and employed in the ML analysis. The likelihood parameters for this model were as follows Base=(0.2133

0.3105 0.2415) Nst=6 Rmat=(1.0000 2.5629 1.0000 1.0000 4.0229) Rates=gamma Shape=0.2924 Pinvar=0. Maximum parsimony analyses of the combined data resulted in 1212 most parsimonious trees (length=1117, CI=0.830, RI=0.902). The same topology resulted from Bayesian and ML analyses of the concatenated alignment. Maximum likelihood analysis of the concatenated alignment resulted in one tree –lnL score of 9746.37704 that is presented here as the inferred phylogeny of *Cryptosporella* (see Fig. 4.1).

All species of *Cryptosporella* included in the concatenated alignment were supported by MP bootstraps and Bayesian posterior probabilities (Fig. 4.1). The inferred phylogeny based on three genes supports the recognition of eight new species of *Cryptosporella*, which are described in the taxonomy section of this work. No specimens of *C. rabenhorstii* and *C. tiliae* suitable for DNA extractions were available for this study and therefore were not included in the phylogeny. *Cryptosporella tiliae* was collected only one time by the author of this species. During this project, an unsuccessful attempt was made to collect this species in the type locality (Meudon, France). Both *C. rabenhorstii* and *C. tiliae* are accepted here on the basis of their morphology.

Three major clades supported by Bayesian analysis and MP bootstrapping can be observed in the phylogeny (Fig. 4.1). One clade (100% MP, PP) contains seven species that occur exclusively on *Alnus* spp. Contained within is a subclade (100% MP, PP) of three species characterized by having necks fused and forming a single ostiolar cavity at the center of the perithecial group: *C. multicontinentalis*, *C. suffusa*, and *C. pacifica*. Each of the other four species included in this major clade is specific to one host species. These four species are split in two subclades. One subclade (97% MP, 100% PP) includes

two species whose hosts co-occur in the Pacific Northwest (USA): *C. alni-rubrae* on *A. rubra* and *C. alni-tenuifolia* on *A. tenuifolia*. The other subclade (100% MP, PP) contains two species with hosts that may overlap in northern USA or whose geographic distribution boundaries are close: *C. alni-sinuatae* on *A. sinuata* and *C. jaklitschi* on *A. serrulata*.

A second major clade (<70% MP, 77% PP) includes six species of which five (*C. wehmeyeriana*, *C. alnicola*, *C. confusa*, *C. femoralis*, and *C. marylandensis*) occur in eastern North America and one (*C. amistadensis*) occurs in Central and South America. Except for *C. wehmeyeriana* (*Tilia* spp.), all the other species grow on host species in the family Betulaceae (*Alnus*, *Betula*, and *Corylus*). *Cryptosporella amistadensis* is found exclusively on *Alnus acuminata*. *Cryptosporella wehmeyeriana*, *C. alnicola*, and *C. confusa* are characterized by having long cylindrical ascospores and *C. femoralis*, *C. marylandensis* and *C. amistadensis* have femuroid ascospores.

A third major clade (100% MP, PP) includes four species: *C. betulae*, *C. tomentella*, *C. corylina*, and *C. hypodermia*. *Cryptosporella betulae* and *C. tomentella* had been considered to be the same species because the two species are morphologically similar and both occur on species of *Betula* (see Reid and Booth 1987, Mejía et al. 2008). However observation of type specimens indicate the two species are distinctive and supported as distinct species by the phylogeny. Additionally, *C. betulae* is restricted to Europe and *C. tomentella* to North America. Another species included in this major clade is *C. hypodermia* (type species of the genus) that grows on *Ulmus* spp. in Europe and North America. Immature ascospores of *C. betulae* and *C. tomentella* resemble those of *C. hypodermia*; however, when mature, ascospores of *C. hypodermia* are elliptical and

can be distinguished readily (see key to species below). *Cryptosporella corylina,* the sister species to *C. betulae* plus *C. tomentella,* has long cylindrical ascospores and a host association with the genus *Corylus* that is distinctive.

On a global scale, the geographic distribution of *Cryptosporella* is here expanded to Central and South America and regionally to more localities in North America and Europe, including eight new species. *Cryptosporella* has been reported from Japan; however specimens were not available for inclusion in the multigene phylogeny. Sequences of the ITS rDNA region for isolates from surveys of endophytic fungi conducted in China and deposited in Genbank were compared with sequences from this work and confirmed to be *Cryptosporella,* with a potential new species on *Betula platyphilla* (trees not shown).

TAXONOMY

Key to species of *Cryptosporella:*

1. Ascospores with one median septum at maturity, ends swollen, thus appearing like a leg bone or femur; on *Alnus* spp. in North America……..…*Cryptosporella femoralis*

1’. Ascospores non-septate……..…2

2. Ascospores ellipsoid to fusoid, acute ends; on *Ulmus* spp…*Cryptosporella hypodermia*

2’. Ascospores cylindrical to cylindrical femuroid, with or without swollen ends..……...3

3. Perithecial necks fused forming a single ostiolar cavity……..4

3’. Perithecial necks erumpent as a mass or closely appressed, but not forming a single ostiolar cavity……...……7

4. Ascospores cylindrical, relatively thick, on *A. tenuifolia* and *A. sinuata* in the Pacific Northwest (USA)..…….....*Cryptosporella pacifica*

4’. Ascospores cylindrical; on hosts other than *A. tenuifolia* and *A. sinuate*, and not in the Pacific Northwest (USA)..……...……5

5. Ascospores wider at center, slightly tapering toward ends..*Cryptosporella rabenhorstii*

5’. Ascospores not wider at center, nor tapering toward ends……...….6

6. Dark to black spot on surface of host and on top of group of perithecia, whitish to cream stromatic tissue delimited by a black halo surrounding central ostiolar cavity; on Alnus in Europe…….……...*Cryptosporella suffusa*

6’. Dark to black spot absent, no stromatic tissue delimited by a black halo; on *Alnus* in Europe and Eastern North America…….....*Cryptosporella multicontinentalis*

7. Ascospores cylindrical with rounded ends…….…….8

7’. Ascospores cylindrical, femuroid, or cylindrical with slightly to pronouncedly swollen at ends……...……..9

8. Ascospores slightly curved, tapering toward rounded ends; on *Betula* sp. in Europe …….…..…*Cryptosporella betulae*

8’. Ascospores slightly curved, tapering toward rounded ends, with whitened tomentum at base of perithecia; on *Betula* in North America……..*Cryptosporella tomentella*

9. Ascospores cylindrical to femuroid, 27-35 x 5-6.5 µm; on *Tilia* sp. in Europe …….……...*Cryptosporella tiliae*

9’. Ascospores cylindrical, with slightly or pronounced swollen ends, or femouroid, greater than 35 µm long…….....10

10. On *Alnus* spp. and *Tilia* spp. in the New World…….11

10’. On *Betula* spp. and *Corylus* spp. in North America and Europe……..…..12

11. On *Tilia americana*, in North America. ……...…..…*Cryptosporella wehmeyeriana*

11’. On *Alnus* spp. in the New World……....13

12. Ascospores (87.5–)88.5–89.5(–91.0)×3.0–3.5 µm, l:w (25–)26–28(–27); on *Betula* spp. in North America or Europe ……..…….*Cryptosporella confusa*

12’. Ascospores (21.5–)26.5–75.0(–82.5)×(3.5–)4.0–4.5(–10) µm, l:w (5–)7–17(–23); on *Corylus* spp. in Europe……..…….…...……...…*Cryptosporella corylina*

13. Ascospores femuroid or swollen at ends……...……..14

13’. Ascospores cylindrical, not swollen at ends……...…16

14. Ascospores elongated with pronounced swollen ends; on *A. rubra* in the Pacific Northwest (USA)……*Cryptosporella alni-rubrae*

14’. Ascospores femuroid with slightly swollen ends…….…..15

15. On *A. serrulata* or *A. maritima* in Maryland and near areas ……...*Cryptosporella marylandensis*

15’. On *A. acuminata* in Central and South America…...…...*Cryptosporella amistadensis*

16. Ascospores cylindrical to slightly wider at center and slightly narrowing at ends; on *A. tenuifolia*……..….*Cryptosporella alni-tenuifolia*

16’. Ascospores cylindrical……...…….……17

17. Grey stroma surrounding perithecial necks, on *A. sinuata* in northern USA…….*Cryptosporella alni-sinuatae*

17’. No grey stroma surrounding perithecial necks, on different hosts ……...18

18. Ostiolar opening papillated; ascospores (64.2–)73.9–79.7(–107)×(3.7–)4.2–5.0(–5.6) µm, l:w (14–)15–18(–29); on *A. serrulata* in northern USA……*Cryptosporella jaklitschi*

18’. Ostiolar opening not papillated; ascospores (53.5–)62.0–78.5(–99.0)×(3.0–)3.5–4.5 µm, l:w (12–)15–21(–28); on *Alnus* spp. in North America…...…*Cryptosporella alnicola*

DESCRIPTIONS OF SPECIES

New species of Cryptosporella

Cryptosporella alni-rubrae L. C. Mejía **sp. nov.** Fig. 4.2 A-J.

Latin Diagnosis: Not included.

Etymology: The name refers to *Alnus rubra*, the only known host of this species.

Evidence of the fungus as scattered elevations in bark up to 0.7 mm high and 2 mm diam at base, each elevation composed of multiple rounded bumps that result from perithecia pushing up host periderm. Perithecia arranged circularly in groups of up to eight, with necks parallel to host surface and oriented toward a central point, necks closely appressed but not fused, bent, projecting perpendicularly, penetrating through host periderm at center of group; often with black halo surrounding mass of protruding perithecial necks. Mature perithecia black, subglobose, diam × height = **(**382–)466–703(–792)×(374–)499–584(–651) µm (mean = 585×528, SD 163, 96, n=6), perithecial necks (165–)387–595(–774) µm (mean = 487, SD 211, n=6) long, basal diam (124–)140–158(–188) µm (mean = 153, SD 22, n=6), distal diam (147–)168–188(–188) µm (mean = 174, SD 17, n=5) . Asci elliptical with rounded apex and acute base, with no apical ring or bodies, floating free at maturity, (79.4–)79.5–87.4(–92.5)×(17.7–)21.7–27.4(–33.3) µm (mean = 84.4×25.1, SD 5.6, 5.9, n=5), with eight ascospores arranged in a parallel pattern or interwoven. Ascospores one-celled, hyaline, femuroid, with moderately expanded to greatly swollen ends, narrow at central point, (39.7–)43.9–50.7(–67)×(3.4–)4.2–4.7(–5.7)

µm (mean = 48.4×4.4, SD 6.6, 0.5, n=38) l:w (8–)10–13(–16) (mean = 11.1, SD 1.9, n=38), with multiple circular guttules varying in size. No color reaction with Meltzer reagent and KOH.

Host species and habitat. In the bark of dead and still attached branches of *Alnus rubra* (Betulaceae).

Distribution. USA: Oregon, Washington.

Holotype. **USA**,Washington, Jefferson County, Route 101 near Queets, in *Alnus rubra*, 26 May 2008, LCM499 (BPI879199, derived culture LCM499.01).

Other specimens observed. **USA.** Oregon, Lane County, Route 58, approx. one mile west of Salt Creek Tunnel, from *Alnus rubra*. 22 May 2008, LCM466 (BPI879204, derived culture LCM466, LCM466.01); Lane County Salmon Creek campground, close to Lowell, from *Alnus rubra*, 22 May 2008, LCM407 (BPI879205, derived cultures LCM407), LCM408b (BPI879206, derived culture LCM408b.01); Alder Dune Campground, close to Florence, from *Alnus rubra*, 24 May 2008, LCM487 (BPI879207, derived culture LCM487.01); Lincoln County, Rocky Creek scenic view point, from *Alnus rubra*, 24 May 2008, LCM486 (BPI879208, derived culture LCM486.01); Cape Foulweather, from *Alnus rubra*, 24 May 2008, LCM496 (BPI879209, derived culture LCM496.01); Washington, Grays Harbor County, Humptulips, in *Alnus rubra*, 25 May 2008, LCM 489 (BPI879200, derived culture LCM489.01), LCM488 (BPI879201, derived culture LCM488.01); Jefferson County, Intersection of route 101 and Hoh river, close to Cottonwood, from *Alnus rubra*, 26 May 2008, LCM498 (BPI879202, derived culture LCM498.01); Clallam County, Olympic National Park, Heart O' Hill

Campground, from *Alnus rubra*, 29 May 2008, LCM411 (BPI879203, derived cultures LCM411 and 411.02).

Cryptosporella alni-sinuatae L. C. Mejía **sp. nov.** Fig. 4.2 K-P.

Latin Diagnosis: Not included.

Etymology: The name refers to *Alnus sinuata*, the only host known for this species.

Perithecia evident as scattered elevations in bark up to 0.4 mm high; often with an oval, dark brown spot, up to 0.7 cm diam on top of elevations. Perithecia black, in groups, up to eight, oriented parallel or in angles of 45° toward bark surface, with necks converging in center, fused to form a single, thick-walled cavity, with a semi-biconic, flat-tipped, protruding rounded cone of 340 µm at base × 175 µm high. Mature perithecia black, globose, diam × height = (253–)272–374(–403)×(251–)339–389(–457) µm (mean = 323×360, SD 63, 69, n=6), perithecial necks (224–)242–265(–347) µm (mean = 266, SD 43, n=6) long, basal diameter (83–)95–100(–102) µm (mean = 96., SD 7.2, n=6), and distal diameter (72.3–)78.3–103(–108) µm (mean = 92.1, SD 15.8, n=6). Asci obovoid, with no apical ring or bodies, floating free at maturity, (76.7–)80.2–93.5(–103)×(23.1–)26.4–30.7(–31.7) µm (mean = 87.9×28.1, SD 13.7, 4.45, n1=3, n2=3), with eight ascospores parallel or interwoven. Ascospores one-celled, hyaline, cylindrical, slightly curved), tapering toward rounded ends, with up to 8 circular guttules, (57.2–)66.3–70.5(–78.8)×(4.5–)5.3–5.8(–6.6) µm (mean = 68.8×5.5, SD 5.1, 0.5, n1=36, n2=36), l:w (10.7–)11.8–12.7(–15) (mean = 12.5, SD 1.1, n=36). No color reaction with Meltzer's reagent and KOH.

Host species and habitat. In the bark of branches of *Alnus sinuata* (Betulaceae).

Distribution. USA: Washington.

Holotype. **USA.** Washington, Clallam County, Olympic National Park, Hurricane Ridge, from *Alnus sinuata*, 28 May 2008. LCM412 (BPI879210, derived culture LCM412).

Other specimens observed. **USA.** Washington, Yakima County, along Rimrock Lake, from *Alnus sinuata*, 2 Aug 2005, A. Y. Rossman (BPI 878446, derived culture AR4200).

Notes: The dark brown spot visible in the host surface is grey stromatic tissue that developd on top of perithecia and surrounds the main perithecial neck cavity.

Cryptosporella alni-tenuifoliae L. C. Mejía **sp. nov.** Fig. 4.3 A-G.

Latin Diagnosis: Not included.

Etymology: The name refers to *Alnus tenuifolia*, the only known host of this species.

Perithecia evident as scattered small elevations in bark up to 0.3 mm with two to three hyaline ostiolar openings slightly protruding from center. Perithecia black, in groups of up to eight, arranged circularly, flattened and oriented parallel to bark surface or botryosely arranged, with necks converging at center of group, either bending or oriented vertically toward surface, or merged to form thick-walled ostioles oriented vertically toward and protruding through host surface. Mature perithecia black, subglobose, diam × height = (399–)414–434(–438)×(269–)285–308(–315) µm (mean = 422×295, SD 20.4, 23.8, n1=3, n2=3), perithecial necks (401–)414–476(–524) µm (mean = 451, SD 65, n=3) in length, basal diameter (100–)105–121(–132) µm (mean = 114, SD 16.1, n=3), and distal diameter (105–)106–116(–124) µm (mean = 112, SD 10.3, n=3). Asci cylindrical to elliptical with rounded apex and acute base, with no apical ring, floating free at maturity, (52.6–) 69.2–88.7(–103)×(11.6–)13.4–18.3(–25.6) µm (mean =

80.9×16.6, SD 14.5, 3.74, n=16), with eight ascospores arranged parallel or interwoven. Ascospores one-celled, hyaline, cylindrical, slightly tapering toward rounded ends, with multiple globose guttules that differ in size, (32.9–)45.6–52.4(–63.4)×(3.8–)4–4.7 (–5.9) µm (mean = 49.3×4.42, SD 6.85, 0.6, n1=41, n2=41), l:w (8.2–)10–12.3(–13.2) (mean = 11.2, SD 1.3, n=41). No color reaction with Meltzer's reagent and KOH.

Host species and habitat. In the bark of still attached branches of *Alnus tenuifolia* (Betulaceae).

Distribution. USA: Oregon.

Holotype. **USA.** Oregon, Jackson County, Rogue River National Forest, Upper Rogue River trail close to River Bridge campground, in *Alnus tenuifolia*, 21 May 2008. LCM480 (BPI879211, derived culture LCM480.01).

Other specimens observed. **USA.** Oregon, Jackson County, Rogue River National Forest, Upper Rogue River trail close to River Bridge campground, in *Alnus tenuifolia*, 21 May 2008. LCM475 (BPI879212); Rogue River National Forest, Upper Rogue River trail close to River Bridge campground, in *Alnus tenuifolia*, 21 May 2008, LCM481 (BPI879213).

Cryptosporella amistadensis L. C. Mejía **sp. nov.** Fig. 4.3 H-M.

Latin Diagnosis: Not included.

Etymology: Name refers to the type locality and place where this species was first found, La Amistad International Park, in Chiriquí, Panama.

Perithecia evident as slight elevations in bark periderm usually up to 0.3 mm high, with perithecial necks of two to three or up to eight protrude extending from center ca. 0.5 mm

above host epidermis. Perithecia arranged in groups of up to eight, with necks oriented at 45° angle towards center, closely appressed but not fused, protruding vertically or pushing each other away from central point. Mature perithecia black, shiny, flask-shaped, diam × height = (385–)412–466(–601)×(291–)365–404(–465) µm (mean = 457×386, SD 85.5, 64, n=5, perithecial necks (473–)507–570(–645) µm (mean = 542, SD 66.9, n=5) in length, basal diameter (114–)122–155(–178) µm (mean = 139, SD 26.4, n=5), distal diameter (110–)112–141(–174) µm (mean = 134, SD 25.9, n=5). Peridial cells textura angularis. Ostiolar openings cone-shaped, hyaline or paler in color. Asci (71.5–)88.7–101(–112)×(22.3–)23.7–26.1(–28.5) µm (mean = 93.6×25.5, SD 11.6, 2.0, n=9), cylindrical with rounded apex to slightly obovoid. The asci might get other shapes because ascospores expand ascal wall. Apical ring not observed; eight ascospores per ascus arranged obliquely parallel or interwoven. Ascospores one-celled, hyaline, guttulated, cylindrical, thick, slightly swollen at rounded ends, (33.7–)40.7–49(–57.3)×(3.5–)4.3–5.3(–6.5) µm (mean = 45.3×4.9, SD 6.1, 0.7, n=45), l:w (7.2–)8.6–10.3(–11.5) (mean = 9.4, SD 1.1, n=45).

Host species and habitat. In the bark of branches of *Alnus acuminata* (Betulaceae).

Distribution. Panama (province of Chiriquí) and Argentina (Tucumán).

Holotype. **Panama**, Chiriquí, Las Nubes, Parque Internacional La Amistad, from *Alnus acuminata*, 22 Dec. 2006, LCM27 (BPI879214, derived cultures LCM27.03=extype, LCM27.01, LCM27.02, LCM27.04, and LCM27.05).

Additional specimens examined: **Panama.** Chiriquí, Las Nubes, Parque Internacional La Amistad, from *Alnus acuminata,* 21 Dec 2006, LCM 25(BPI879215), LCM 26 (BPI879248), LCM28 (BPI879216); 29 Dec. 2007, LCM342 (BPI879217), LCM 357

(BPI879249, derived culture LCM357). **Argentina.** Tucumán, Villa Nougués, in *Alnus acuminata*, 16 Nov. 2008, LCM 617 (BPI879218, derived culture LCM 617.01), LCM 618 (BPI879219, derived cultures LCM618.01 and LCM 618.03); LCM 619 (BPI879220, derived culture LCM619.01); LCM621 (BPI879221).

Cryptosporella jaklitschi L. C. Mejía **sp. nov.** Fig. 4.4 A-E.

Latin Diagnosis: Not included.

Etymology: The species is named after Walter Jaklitsch in recognition of his contributions to the systematics of Diaporthales.

Initially perithecia evident as scattered elevations in bark up to 0.4 mm high. Erumpent perithecial necks protrude from the periderm at central area of elevations either in a row or as a mass; from host surface few perithecial necks seen (3-4) but when host periderm is peeled off up to 10 often visible; perithecia arranged circularly with necks projected toward center of group. Perithecial necks closely appressed in center, appearing to fuse, protruding the periderm through few thick necks; only ca. 0.2 mm of distal part of perithecial necks extends beyond rupture in bark. Perithecia black at maturity, globose, shiny, diam × height = (384–)404–417(–435)×(402–)406–419(–426) µm (mean = 410×413, SD 20.6, 10.6, n1=4, n2=4), perithecial necks (530–)539–584(–608) µm (mean = 564, SD 35.3, n=4) long, basal diam (114–)123–131(–137) µm (mean = 126, SD 9.8, n=4), and distal diam (127–)127–138(–164) µm (mean = 137, SD 18, n=4). Stromatic tissue scanty grey flat, below host epidermis, on top of perithecia and surrounding necks. Ostiolar openings appearing like a dome-shaped papilla of approximately 50 µm high and 70 µm base seated on top of distal part of neck with an area of 35 µm surrounding base of

dome, appearing like a rounded lip. Asci oblong elliptical, with no apical ring, floating free at maturity, (74.1–)81.2–85.4(–91.9)×(13.2–)16.8–17.8(–21.5) µm (mean = 83.7×17.3, SD 4.7, 2.0, n=21), with eight ascospores slightly twisted to interwoven. Ascospores sigmoid, one-celled, hyaline, with multiple rounded guttules, (64.2–)73.9–79.7(–107)×(3.7–)4.2–5.0(–5.6) µm (mean = 78.4×4.61, SD 8.6, 0.5, n=27), l:w (14.1–)14.9–18.2(–29.2) (mean = 17.3, SD 3.4, n=27). No color reaction with Meltzer's reagent and KOH.

Host species and habitat. In the bark of branches of *Alnus serrulata* (Betulaceae).

Distribution. USA: New York.

Holotype. **USA**, New York, Essex County, Adirondack High Peaks Region, Marcy Dam, from *Alnus serrulata*, 11 Jun. 2007, LCM112 (BPI879231, derived cultures LCM112.04=extype and LCM112.01)

Cryptosporella marylandensis L. C. Mejía **sp. nov.** Fig. 4.4 F-J.

Latin Diagnosis: Not included.

Etymology: The species name refers to the state of Maryland, USA, where this species was found.

On *Alnus maritima* scattered groups or rows of ostiolar openings exposed through slits in host periderm approximately 0.8 mm long, level with host surface. On *Alnus serrulata* forming circularly arranged swellings in host periderm around a central point approximately 1.5 mm diam×0.5 mm high where distal part of perithecial necks protrude slightly beyond host epidermis. Perithecia black, globose, with rounded ostiolar openings, in groups of up to 8, with necks oriented toward a central point, closely

appressed and protruding vertically as a column or parallel in rows through host periderm, diam×height = (370–)405–467(–495)×(339–)371–422(–472) µm (mean = 438×394, SD 39.2, 42, n=11), necks (330–)350–458(–530) µm (mean = 410, SD 68.1, n=11) in length, basal diameter (104–)112–125(–145) µm (mean = 119, SD 13.8, n=11), distal diameter (101–)116–136(–158) µm (mean = 126, SD 17.8, n=11). Asci obovoid with rounded apex and acute base or looking like a parallelogram when ascospores get fully develop and extend ascus wall, (68.7–)76.2–82.6(–93.5)×(22.1–)24.4–33.7(–39.4) µm (mean = 80.1×29.2, SD 6.74, 5.7, n1=11, n2=11), with eight ascospores arranged in a parallel manner along ascus base-apex axis or slightly interwoven. No apical bodies. Ascospores one cell, hyaline, thick, bacillar, usually straight, with broadly rounded ends that are slightly wider than in the central area of the cell, (39.1–)46.2–51.2(–58.4)×(3.3–)4.8–5.34(–6.83) µm (mean = 48.8×5.08, SD 4.37, 0.62, n1=69, n2=69), l:w (6.39–)8.65–10.8(–15.1) (mean = 9.77, SD 1.71, n=69). No color reaction with Meltzer's reagent and KOH.

Host species and habitat. In the bark of branches of *Alnus maritima* and *Alnus serrulata* (Betulaceae).

Distribution. USA: Maryland.

Holotype. **USA**, Maryland, Dorchester county, Marshyhope Creek, Richard Henson Scout Reserve, from *Alnus maritima*, 11 June 2008, LCM386 (BPI879232, derived cultures LCM386.05=extype and LCM386.04).

Additional specimens examined: USA, Maryland, Prince George's County, Beltsville, Little Paint Branch Park, on *Alnus serrulata*, 2 March 2008, LCM359 (BPI879233, derived cultures LCM359, LCM359.01, and LCM359.02); LCM385 (BPI879234); 28

April 2008, LCM631 (BPI879235); 15 June 2008, LCM580 (BPI879236, derived cultures LCM580.01 and LCM580.02); LCM581 (BPI879250, derived cultures LCM581.01 and LCM581.02); Dorchester County, Richard Henson Scout Reserve, from *Alnus maritima*, 11 June 2008, LCM387 (BPI879237); LCM388 (BPI879238).

Notes. The arrangement of the perithecia varies with the host. In collections on *Alnus serrulata*, ascospore length may be greater than on *A. maritima*. Despite these slight morphological differences, molecular data indicate that these specimens comprise a single species.

Cryptosporella multicontinentalis L.C. Mejía **sp. nov.** Fig. 4.3 N-Q.

Latin Diagnosis: Not included.

Etymology. The name refers to the geographic distribution of this species on at least two continents (Europe and North America).

Perithecia in scattered groups immersed in bark of host branches; each group containing nine to ten perithecia, evident as elevations in bark that appear as a circle of bumps with a single ostiolar cavity in center that protrudes through a central elevation of periderm; alternatively perithecia may be closer to center of group causing an elevation of periderm that appears cone-shaped with a flattened apex. Ostiolar opening single, wide, appearing labiated. No black spot on host surface. White mycelium may develop at base of perithecial group. Perithecia black, with thin necks oriented parallel to host surface toward center of group. Central ostiole surrounded by a whitish to cream stromatic tissue. Perithecia diam×height = (318–)345–411(–557)×(284–)346–392(–455) µm (mean = 393×370, SD 70.9, 46.4, n=9), perithecial necks (156–)247–382(–483) µm (mean = 310,

SD 111, n=9) in length, basal diam (66.9–)82.5–89.7(–107) µm (mean = 86.3, SD 10.9, n=9), distal diam (68.9–)72.2–86.4(–108) µm (mean = 83.3, SD 13.1, n=9). Asci oval to obovoid narrowing to base and apex. (61.5–)77–93.8(–98)×(16.3–)19.4–25.3(–38.4) µm (mean = 82.9×24.1, SD 11.2, 6.2, n=21), with eight ascospores. Ascospores cylindrical, flexuous commonly narrowing toward ends, (46.3–)54.7–67(–73.3)×(4.3–)4.7–5.6(–6.2) µm (mean = 60.1×5.1, SD 7, 0.6, n=49), l:w (9.2–)10.4–12.3(–16.8) (mean = 11.8, SD 1.9, n=49).

Host species and habitat. In the bark of dead, still attached branches of *Alnus* spp. (Betulaceae): *A. glutinosa*, *A. hirsuta-siberica* (one specimen was collected on this host species in an arboretum in France, see below), and *A. rugosa*.

Distribution. Europe (France, Germany), North America (USA).

Holotype. **France.** Deux-Sèvres department, Amure, Port Le Goron, from *Alnus glutinosa*, 15 Apr 2008, LCM401 (BPI879226, derived culture LCM401.01).

Other specimens observed. **France**, Deux-Sèvres department, Melle, from *A. glutinosa,* Apr. 2008, LCM 406 (BPI879227, derived culture LCM 406.01); Melle Arboretum, from *Alnus hirsuta-siberica,* 15 Apr. 2008, LCM394 (BPI879258, derived culture LCM394.01, LCM394.02, LCM394.04). **Germany**, Frankfurt, Naturschutzgebiet, from *A. glutinosa*, 20 Apr 2008, LCM427 (BPI879228, derived culture LCM427.01); **USA**, New York, Anondaga County, Syracuse, Heiberg Memorial Forest, Kochanek pond, from *Alnus rugosa,* 06 Jun. 2007, LCM93 (BPI879229, derived culture LCM93.01), LCM93b (BPI879230, derived culture LCM93b.02).

Cryptosporella pacifica L.C. Mejía **sp. nov.** Fig. 4.4 K-N.

Latin Diagnosis: Not included.

Etymology. The name refers to the geographic distribution of this species in the Pacific Northwest (USA).

Perithecia in groups, of up to nine, scattered in the bark of the host branches. Groups of perithecia commonly arranged in circles, with necks oriented toward the center and merging to form a single thick ostiole that vertically protrude the periderm and become exposed at level with the host epidermis. A white stromatic mycelium develops surrounding the single ostiolar opening. Perithecia diam×height = (339–)344–423(–470)×(312–)322–351(–394) µm (mean = 390×342, SD 61.3, 35.9, n1=4, n2=4), necks length (212–)287–360(–376) µm (mean = 313, SD 72.8, n=4), basal diameter (87.8–)89.4–95.2(–105) µm (mean = 93.7, SD 7.91, n=4), distal diameter (82.1–)85.7–98.4(–108) µm (mean = 93.2, SD 11.5, n=4). Asci oval to obovoid with rounded apex and narrowing toward the base, (87.1–)88.6–92.9(–104)×(25.7–)25.8–28.2(–29.2) µm (mean = 92.4×27.2, SD 7.98, 1.67, n1=4, n2=4), with eight ascospores and no apical ring. Ascospores relatively thick, cylindrical, with rounded ends, **(**68.7–)74.6–83.9(–94)×(5.54–)5.85–6.3(–6.47) µm (mean = 79.2×6.07, SD 8.71, 0.31, n1=10, n2=10), l:w (11.6–)12.2–14(–15) (mean = 13, SD 1.19, n=10).

Host species and habitat. In branches of *Alnus sinuata* and *A. tenuifolia* (Betulaceae).

Distribution. USA (California, Oregon, Washington).

Holotype. **USA**, California, Lassen County, Lassen National Forest, Lassen Campground, from *A. tenuifolia*, 18 May 2008, LCM461 (BPI879239, derived culture LCM461.01).

Other specimens observed. **USA**, California, Plumas county, Little Last Chance campground, from *A. tenuifolia*, 17 May 2008, LCM453 (BPI879240, derived culture

LCM453.01); **Oregon**, Jackson county, Upper Rogue River trail near River Bridge campground, 20 May 2008, from *A. tenuifolia*, LCM 420 (BPI879241, derived culture LCM420.01); **Washington**, Yakima Co., near Rimrock Lake, isolated from healthy branches of *A. sinuata*, 2006, coll. S. Lattomus, isol. LCM (cultures CBS122311, CBS 122312, CBS 122313).

Species with new name combinations or reviewed taxonomy

Cryptosporella betulae (Tul. & C. Tul.) L.C. Mejía & Castleb., Mycol. Res.112: 32. (2008).

≡ *Cryptospora betulae* Tul. & C. Tul., Sel. Fung. Carpol. 2: 148-150 (1863).

≡ *Winterella betulae* (Tul. & C. Tul.) O. Kuntze, Rev. Gen. Pl. 1: 34 (1891).

Specimens examined: **Austria**: Niederoesterreich, Losenheim, Laerchkogel. Mapping grid square 8261/1, from *Betula lenta*, 05 Jul 2003 W. Jaklitsch 2271 as *Winterella betulae* (BPI 843595). **Russia**, Nizhniy Novgorod Oblast Piliha, from *Betula pendula,* 30 Jun. 2008, M. V. Sogonov (BPI879251=LCM477, derived culture LCM477.01).

Cryptosporella femoralis (Peck) L.C. Mejía & Castleb. Mycol. Res.112: 23-35 (2008).

≡ *Valsa femoralis* Peck, New York State Mus. Rep. 28. 74-75. 1874 (1879).

≡ *Cryptospora femoralis* (Peck) Sacc., Syll. Fung. 2: 362 (1883).

≡ *Winterella femoralis* (Peck) O. Kuntze, Rev. Gen. Pl. 1: 34 (1891).

≡ *Ophiovalsa femoralis* (Peck) Petr., Sydowia, 19: 273. 1965 (1966).

=*Cryptospora humeralis* Dearn. & House, Circ. N. Y. State Mus. 24: 41 (1940).

Specimens examined. Lectotype, **USA** New York, West Albany, from *Alnus*, C.H. Peck (NYS-F1166, as *Valsa femoralis)*; New York, Greenbush, from *Alnus*, C.H. Peck (NYS-

F1167, as *Valsa femoralis*). New York, Essex county, head trail, Adirondack Loj, Adirondack High Peaks region, from *Alnus rugosa*, 12 Jun 2007, LCM103 (BPI879224, derived cultures LCM103.01 and LCM103.02); New York, Essex county, Adirondack High Peaks region, from *Alnus rugosa*, LCM196 (BPI879223, derived cultures LCM196.02 and LCM196.04). Other specimens observed are in Mejía et al. (2008).

Cryptosporella rabenhorstii (Berk & Broome) L.C. Mejía **comb. nov.** Fig. 4.5 A-C.

≡ *Sphaeria rabenhorstii* Berk & Broome, Ann. & Mag. Nat. Hist. Ser. 2, 9: 324. 1852.

Notes. This species has previously been considered a synonym of *C. suffusa* (Reid & Booth 1987). A characteristic feature of *C. suffusa* is the fusion of perithecial necks to form a single ostiolar cavity. However, examination of the holotype of *Sphaeria rabenhorstii* (K(M) 163853) showed non-fused perithecial necks and the asci and ascospores differed from those of *C. suffusa*. The asci of *S. rabenhorstii* are cylindrical to clavate and longer than those of *C. suffusa,* which are ovate to obovoid. The ascospores of *S. rabenhorstii* are wider at the center and longer than those of *C. suffusa*. *Cryptosporella suffusa* is a species associated with the genus *Alnus* and the holotype of *S. rabenhorstii* was collected on *Betula*. Therefore, *S. rabenhorstii* is considered a species distinct from *C. suffusa*.

Specimen examined. *Holotype*, **England** Wiltshore, Spye Park. From *Betula* sp., on bark, Mar 1859, coll. Ex. Herb. Berkeley, K(M) 163853.

Cryptosporella suffusa (Fr.) L.C. Mejía & Castleb. Mycol. Res.112: 23-35 (2008).

Fig. 4.5 D-G.

≡*Sphaeria suffusa* Fr., Syst. Mycol. 2: 399 (1823).

≡*Valsa suffusa* (Fr.) Fr., Summ. Veg. Scand. 412 (1846).

≡*Cryptospora suffusa* (Fr.) Tul. & C. Tul., Sel. Fung. Carpol. 2: 145 (1863).

≡*Winterella suffusa* (Fr.) O. Kuntze, Rev. Gen. Pl. 1: 34 (1891).

≡*Ophiovalsa suffusa* (Fr.) Petr., Sydowia, 19: 272, 1965 (1966).

=*Sphaeria cryptosporii* Curr., Microsc. J. 3: 271 (1855)

=*Valsa rhabdospora* de Not., Sfer. Ital. Cent. I: 39 (1863).

≡*Cryptospora rhabdospora* (de Not.) Sacc., Syll. Fung. 2: 362 (1883).

Anamorph: *Disculina vulgaris* (Fr.) B. Sutton, Mycol. Pap. 141: 75 (1977).

Type specimen examined: Sweden: on *Alnus*, Fries (Scleromycetae Sueciae 229 BPI Sbarbaro collection, type of *Sphaeria suffusa*.

Other specimens examined: **Austria**: Tirol, Overtilliach an der Gail, grid square 924/4, on *Alnus incana*, 29 Aug. 2000, W. Jaklitsch 1556 as *Ophiovalsa suffusa* (BPI 748449, derived culture CBS 109750); Vienna, Marchfeldkanalweg 7764/2, 21st district, on *Alnus incana,* 19 May 2002, W. Jaklitsch 1892 (BPI871231, derived culture CBS121077 = AR 3825). **England**, as *Cryptospora suffusa*, syntype of *Sphaeria cryptosporii*, West Kent, Eltham K(M) 163855, 10 Jan. 1855, from *Alnus* sp., ex. Herb. F. Currey; as *Cryptospora suffusa*, syntype of *Sphaeria cryptosporii*, West Kent, Chislehurst, Petts Wood, K(M)16385417 Sep. 1855, on *Alnus* sp., ex. Herb. F. Currey. **Germany**: Frankfurt, Botanical Garden of Johann Wolfgang Goethe Universit***ät***, 22 Apr. 2008, from *Alnus* sp., LCM 576 (BPI879242, derived cultures LCM576.01, LCM576.03). **Hungary:**

Prope Ungarisch, Altenburg, in ramis aridis *Alni incanae* DC, April 1885 leg. Linhart, Rabenhorst-Winter Fungi europaeie 3458 as *Cryptosporella suffusa* (BPI); an abgestorbenen Aesten von *Alnus glutinosa* beim Kloster Zella unweit Nossen um Pfingsten 1877 mit reifen sclauchen gesammelt von W. Krieger, Rabenhorst Fungi europaei 2322 as *Cryptospora suffusa* (BPI).

Notes. Valsa commutata Fuckel, Fungi Rhen. 620 (1863) has been considered a synonym of *Cryptosporella suffusa* (Reid and Booth 1987). Images of the exsiccati with the type specimen of *V. commutata* (Fungi Rhenani 620, Germany, on *Betula*) from the Swedish Museum of Natural History suggest that this specimen represents a species of *Melanconis* as the ascospores are unlike those of *Cryptosporella*. Therefore we do not consider *Valsa commutata* to be a synonym of *C. suffusa*.

Cryptosporella tomentella (Peck) L.C. Mejía **comb. nov.**

≡ *Valsa tomentella* Peck, New York State Mus. Rep. 35:144. 1881 (1884).

≡ *Cryptospora tomentella* (Peck) Berl. & Vogl., Add. Syll. 1-4: 192. 1886.

≡ *Cryptospora betulae* var. *tomentella* (Peck) Berl., Icones Fung. 2:157. 1889.

≡ *Ophiovalsa tomentella* (Peck) Petr., Sydowia 19: 275, 1965(1966).

Host species and habitat. On bark of branches of *Betula* spp. (Betulaceae).

Distribution. USA (New York).

Specimens observed. **USA**, *Type* New York, West Albany, from *Betula populifolia*, May, C.H. Peck (NYS-F3608, as *Valsa* (*Cryptospora*) *tomentella)*; New York, West Albany, from *Betula populifolia,* May, C.H. Peck (NYS-F3197, as *Valsa tomentella*); New York, Adirondack, from *Betula* sp. 20 June 2002, L. Vasilyeva as *Ophiovalsa betulae* (BPI

843497, derived culture CBS121080); New York, from *Betula* sp., 20 Jun 2002, L. Vasilyeva as *Ophiovalsa betulae* (BPI 872328, derived culture CBS 121073); New York, Essex County, North Pole, White Face Mountain, from *Betula alleghaniensis*, 9 Jun 2007, LCM184B (BPI879243, derived culture LCM184B.01).

DISCUSSION

In this study the known species diversity of the genus *Cryptosporella* is expanded. A robust phylogeny for the genus based on three genes was obtained. Nineteen species of *Cryptosporella* are accepted, 16 of which occur on Betulaceae. The rationale for prospecting for new species of *Cryptosporella* was based on the assumption of a long evolutionary association between species of *Cryptosporella* and their hosts. This approach proved valuable in discovering the eight new species of *Cryptosporella* here described. New species of *Cryptosporella* were found on betulaceous hosts previously not reported to harbor *Cryptosporella* but congeneric with known hosts: *C. amistadensis* on *Alnus acuminata*, *C. marylandensis* on *A. maritima*, and *C. jaklitschi* on *A. serrulata.*

Although the other new species of *Cryptosporella* described here occur on hosts already known to harbor *Cryptosporella*, the host range attributed to previously described species may have been considered too broad. For example, in the past all collections of *Cryptosporella* with a fused perithecial neck forming a single ostiolar cavity were identified as *C. suffusa*, a species considered capable of colonizing more than five species of *Alnus* (Mejía et al. 2008, Reid and Booth 1987). This work shows that specimens considered *C. suffusa* in the previous works comprise three species, each with a characteristic geographic distribution and host association. These three species are: *C.*

pacifica restricted to *A. sinuata* and *A. tenuifolia* in the Pacific Northwest region of North America; *C. multicontinentalis* associated with three species of *Alnus* in Europe and North America, and *C. suffusa* on *Alnus glutinosa* and *A. incana*, restricted to Europe. Another example is *Cryptosporella femoralis*, a species reported from Asia and North America with a host range on several species of *Alnus*. This work recognizes three additional species with ascospores that are moderately to prominently swollen similar to *C. femoralis* and associated with *Alnus*: *C. alni-rubrae*, *C. amistadensis*, and *C. marylandensis*.

Nine species of *Cryptosporella* are known to occur on a single host species and the remaining known species are associated with a few usually congeneric hosts (see Table 4.2). A single host species may harbor one or two species of *Cryptosporella*. No host was found to have two exclusive species of *Cryptosporella.* When hosts with two *Cryptosporella* species were found, it does not seem the fungal speciation event was the result of within host speciation, i.e., derived from a common recent ancestor. Instead, the phylogeny obtained (Fig. 4.1) shows that in these cases the two species sharing the same host are not sister species. Examples of this are *C. pacifica* and *C. alni-tenuifolia* on *Alnus tenuifolia* and *C. pacifica* and *C. alni-sinuatae* on *Alnus sinuata*. In the host species reported to harbor two species, one of the species occurs exclusively on that particular host with the other species occurring on two, often congeneric, host species in the Betulaceae (see Table 4.2). In these cases, hosts that shared a single species of *Cryptosporella* were not necessarily sister species but species in the same genus or family sharing a geographic area or whose ancestral species may have shared the same area (see phylogeny of Betulaceae, Chen et al. 1999).

Species of *Cryptosporella* occur in America, Asia, and Europe (Kobayashi et al. 1978, Mejía et al 2008). This broad geographic distribution of *Cryptosporella* associated with Betulaceae, the finding of *Cryptosporella* species known to occur exclusively on one host species, and the number of *Cryptosporella* species associated with Betulaceae support evidence of a long evolutionary association between *Cryptosporella* and the Betulaceae. The finding of new species of *Cryptosporella* in each of three *Alnus* species that co-occur in the Pacific Northwest supports the idea of hosts promoting diversification and speciation in *Cryptosporella*. The same is also true for species of *Cryptosporella* on co-occuring host species in eastern North America. Geographic isolation also might have played a role. Species of *Cryptosporella* in western North America are not found in eastern North America. In the cases of species occurring in Europe and North America, these species are restricted to eastern North America.

Determining the timing of the association of *Cryptosporella* with the Betulaceae was not attempted but fossils of *Gnomonia*-like fungi co-occurring with betulaceous hosts date back to the early Miocene (Sherwood Pike & Gray 1988). The Betulaceae is well documented in the fossil record, is a family of Laurasian origin, and appears to have originated during the Cretaceous and early Tertiary in China (Chen et al. 1999). Betulaceous species could have migrated between Eurasia and North America through the Bering and North Atlantic land bridges (Chen et al. 1999). By the Oligocene 36.6-23.7 million years ago (mya) all extant genera of Betulaceae had been differentiated (Chen et al. 2009). The movement appears to be from Laurasia to North America, and later to South America. The only extant species of Betulaceae in South America is *Alnus acuminata* and its range is from Mexico to Argentina. Based on fossil evidence *Alnus*

appears to have moved from north to south, passing through Panama and arriving in Colombia one mya and later to its southernmost area, Argentina (see Bush et al. 2007, Graham 1999). One species of *Cryptosporella, C. amistadensis,* was found associated with *A. acuminata* in Argentina and Panama. Populations of *A. acuminata* in Panama and Argentina are greatly separated spatially and temporally with forests that harbor populations of *A. acuminata* separated by extensive grasslands (see Bush et al. 2007) and tropical rain forest. Therefore connection between populations of *A. acuminata* from Panama and Argentina is unlikely and it would appear that *Cryptosporella* may have moved through South America with *Alnus* during the Pleistocene.

The genus *Cryptosporella* appears to have a long but not exclusive evolutionary association with the Betulaceae. A few species of *Cryptosporella* have colonized and speciated on hosts other than Betulaceae. This is not surprising considering that species of *Cryptosporella* have a stage in their life cycles where millions of ascospores and conidia are released with a high probability of landing on other hosts and occasionally being able to infect those hosts. Despite this, there does seem to be a high fidelity of *Cryptosporella* for Betulaceae and it appear that diversification of this plant family promoted diversification of *Cryptosporella*. The inferred phylogeny of *Cryptosporella* does not appear to mirror the phylogeny of Betulaceae (compare to phylogeny of Betulaceae by Chen et al. 1999 and of *Alnus* by Navarro et al. 2003). Therefore co-speciation appears not to be the mechanism for diversification of *Cryptosporella*. Species of *Cryptosporella* would seem more likely to infect closely related hosts, in this case host plants in the Betulaceae. However host jumps to phylogenetically distantly related hosts, i.e., *C. hypodermia* on *Ulmus* and *C. tiliae* on *Tilia* may occur. Sampling for

Cryptosporella on more betulaceous hosts, as well as other host families, will likely yield new species of *Cryptosporella* and will be required to address questions concerning the factors promoting diversification and maintenance of host preferences as well as the age of host associations.

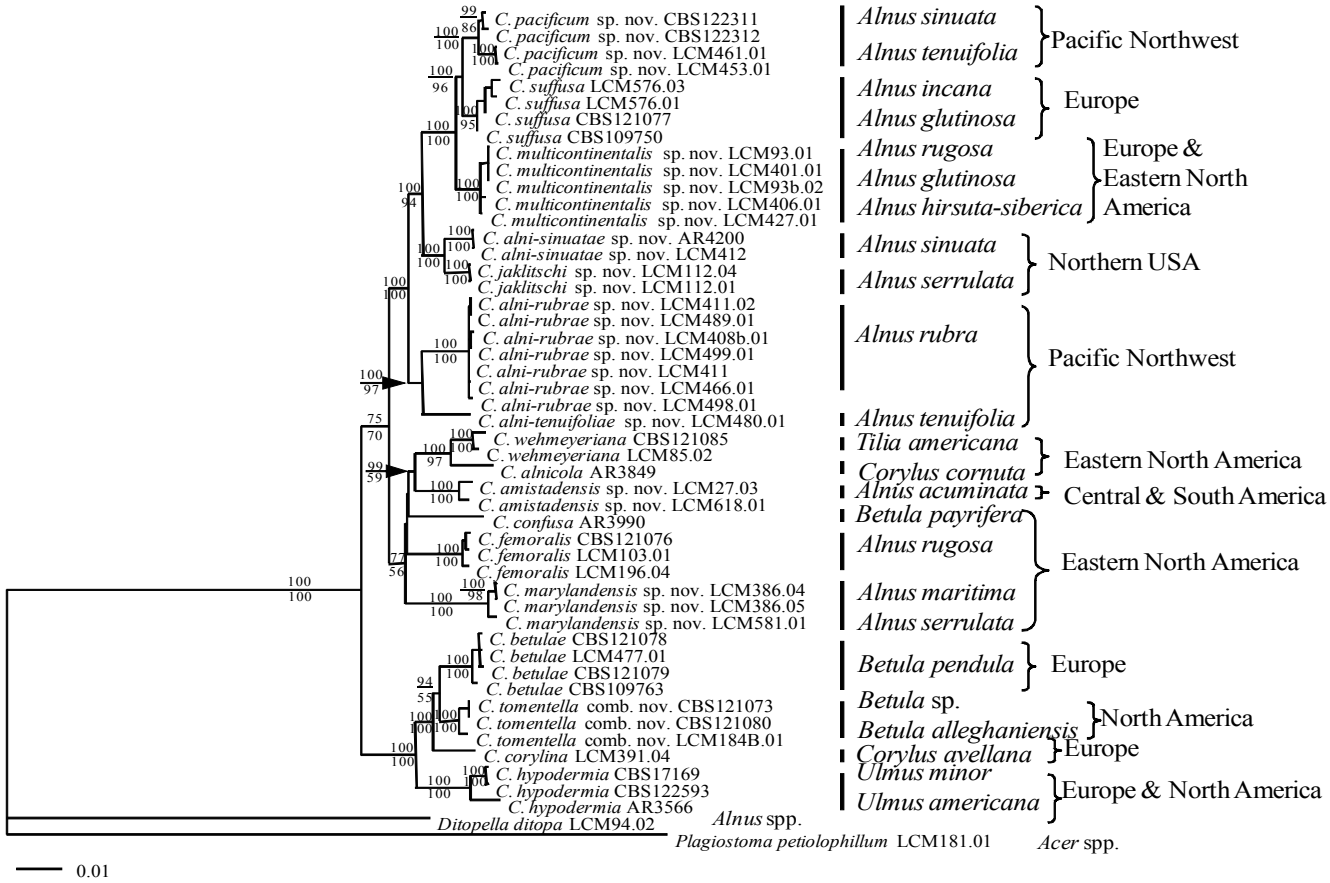

Figure 4.1. ML phylogenetic analysis (ML score = -lnL 9746.38) of sequences for the ITS, *β-tubulin*, and *tef1-α* multigene analysis of *Cryptosporella* species with *Ditopella ditopa* and *Plagiostoma petiolophilum* as outgroup. Bayesian posterior probabilities and maximum parsimony bootstrap support appear above and below branches respectively.

Table 4.1. Source of isolates and specimens used in phylogenetic analyses. DNA sequences generated in this work are labeled as new.

Taxon	Specimen	Culture	Country	Host	Collector	*β-tubulin*	ITS	*tef1-α*
Cryptosporella alni-rubrae	BPI879199	LCM499.01	U.S.A.	*Alnus rubra*	L.C. Mejía	new	new	new
Cryptosporella alni-rubrae	BPI879200	LCM489.01	U.S.A.	*Alnus rubra*	L.C. Mejía	new	new	new
Cryptosporella alni-rubrae	BPI879201	LCM488.01	U.S.A.	*Alnus rubra*	L.C. Mejía		new	
Cryptosporella alni-rubrae	BPI879202	LCM498.01	U.S.A.	*Alnus rubra*	L.C. Mejía	new	new	new
Cryptosporella alni-rubrae	BPI879203	LCM411	U.S.A.	*Alnus rubra*	L.C. Mejía	new	new	new
Cryptosporella alni-rubrae	BPI879203	LCM411.02	U.S.A.	*Alnus rubra*	L.C. Mejía	new	new	new
Cryptosporella alni-rubrae	BPI879204	LCM466	U.S.A.	*Alnus rubra*	L.C. Mejía		new	
Cryptosporella alni-rubrae	BPI879204	LCM466.01	U.S.A.	*Alnus rubra*	L.C. Mejía	new	new	new
Cryptosporella alni-rubrae	BPI879205	LCM407	U.S.A.	*Alnus rubra*	L.C. Mejía		new	
Cryptosporella alni-rubrae	BPI879206	LCM408b	U.S.A.	*Alnus rubra*	L.C. Mejía	new	new	new
Cryptosporella alni-rubrae	BPI879207	LCM487.01	U.S.A.	*Alnus rubra*	L.C. Mejía		new	
Cryptosporella alni-rubrae	BPI879208	LCM486.01	U.S.A.	*Alnus rubra*	L.C. Mejía		new	
Cryptosporella alni-rubrae	BPI879209	LCM496.01	U.S.A.	*Alnus rubra*	L.C. Mejía		new	
Cryptosporella alni-sinuatae	BPI879210	LCM412	U.S.A.	*Alnus sinuata*	L.C. Mejía	new	new	new
Cryptosporella alni-sinuatae	BPI 878446	AR4200	U.S.A.	*Alnus sinuata*	A.Y. Rossman	new	new	new
Cryptosporella alni-tenuifoliae	BPI879211	LCM480.01	U.S.A.	*Alnus tenuifolia*	L.C. Mejía	new	new	new
Cryptosporella alni-tenuifoliae	BPI879212	LCM475	U.S.A.	*Alnus tenuifolia*	L.C. Mejía			
Cryptosporella alni-tenuifoliae	BPI879213	LCM481	U.S.A.	*Alnus tenuifolia*	L.C. Mejía			
Cryptosporella amistadensis	BPI879214	LCM27.01	Panama	*Alnus acuminata*	L.C. Mejía		new	
Cryptosporella amistadensis	BPI879214	LCM27.02	Panama	*Alnus acuminata*	L.C. Mejía		new	
Cryptosporella amistadensis	BPI879214	LCM27.03	Panama	*Alnus acuminata*	L.C. Mejía	new	new	new
Cryptosporella amistadensis	BPI879214	LCM27.04	Panama	*Alnus acuminata*	L.C. Mejía		new	
Cryptosporella amistadensis	BPI879214	LCM27.05	Panama	*Alnus acuminata*	L.C. Mejía		new	
Cryptosporella amistadensis	BPI879215	LCM25	Panama	*Alnus acuminata*	L.C. Mejía			
Cryptosporella amistadensis	BPI879216	LCM28	Panama	*Alnus acuminata*	L.C. Mejía			
Cryptosporella amistadensis	BPI879217	LCM342	Panama	*Alnus acuminata*	L.C. Mejía			
Cryptosporella amistadensis	BPI879218	LCM617.01	Argentina	*Alnus acuminata*	L.C. Mejía		new	
Cryptosporella amistadensis	BPI879219	LCM618.01	Argentina	*Alnus acuminata*	L.C. Mejía	new	new	new
Cryptosporella amistadensis	BPI879219	LCM618.03	Argentina	*Alnus acuminata*	L.C. Mejía		new	
Cryptosporella amistadensis	BPI879220	LCM619.01	Argentina	*Alnus acuminata*	L.C. Mejía		new	

Cryptosporella amistadensis	BPI879221	LCM621	Argentina	*Alnus acuminata*	L.C. Mejía		new		
Cryptosporella amistadensis	BPI879248	LCM26	Panama	*Alnus acuminata*	L.C. Mejía				
Cryptosporella amistadensis	BPI879249	LCM357	Panama	*Alnus acuminata*	L.C. Mejía		new		
Cryptosporella betulae	BPI879251	LCM477	Russia	*Betula pendula*	M. V. Sogonov	new	new	new	
Cryptosporella betulae		CBS121078	Scotland	*Betula pendula*	S. Green	new	EU199213	new	
Cryptosporella betulae		CBS121079	Scotland	*Betula pendula*	S. Green	new	EU199216	new	
Cryptosporella betulae	BPI748448	CBS109763	Austria	*Betula pendula*	W. Jaklitsch	new	EU199180	new	
Cryptosporella corylina	BPI879222	LCM391.02	France	*Corylus avellana*	L.C. Mejía				
Cryptosporella corylina	BPI879222	LCM391.04	France	*Corylus avellana*	L.C. Mejía	new	new	new	
Cryptosporella femoralis	BPI872326	CBS121076	U.S.A.	*Alnus rugosa*	L. Vasilyeva	new	EU199220	new	
Cryptosporella femoralis	BPI879223	LCM196.04	U.S.A.	*Alnus rugosa*	L.C. Mejía	new	new	new	
Cryptosporella femoralis	BPI879223	LCM196.02	U.S.A.	*Alnus rugosa*	L.C. Mejía		new		
Cryptosporella femoralis	BPI879223	LCM196.01	U.S.A.	*Alnus rugosa*	L.C. Mejía	new	new	new	
Cryptosporella femoralis	BPI879224	LCM103.01	U.S.A.	*Alnus rugosa*	L.C. Mejía	new	new	new	
Cryptosporella femoralis	BPI879224	LCM103.02	U.S.A.	*Alnus rugosa*	L.C. Mejía		new		
Cryptosporella hypodermia	BPI879225	LCM92.01	U.S.A.	*Ulmus americana*	L.C. Mejía	new	new	new	
Cryptosporella hypodermia	BPI879225	LCM92.02	U.S.A.	*Ulmus americana*	L.C. Mejía		new		
Cryptosporella hypodermia	BPI 748432	CBS122593	Austria	*Ulmus minor*	W. Jaklitsch	new	EU199181	new	
Cryptosporella hypodermia	BPI748433	CBS 109753	Austria	*Ulmus minor*	W. Jaklitsch	new	EU199224	new	
Cryptosporella hypodermia		CBS 171.69	Netherlands	*Ulmus* sp.	H. van der Aa	new	EU199225	new	
Cryptosporella jaklitschi	BPI879231	LCM112.01	U.S.A.	*Alnus serrulata*	L.C. Mejía	new	new	new	
Cryptosporella jaklitschi	BPI879231	LCM112.04	U.S.A.	*Alnus serrulata*	L.C. Mejía	new	new	new	
Cryptosporella marylandensis	BPI879232	LCM386.04	U.S.A.	*Alnus maritima*	L.C. Mejía	new	new	new	
Cryptosporella marylandensis	BPI879232	LCM386.05	U.S.A.	*Alnus maritima*	L.C. Mejía	new	new	new	
Cryptosporella marylandensis	BPI879233	LCM359	U.S.A.	*Alnus serrulata*	L.C. Mejía		new		
Cryptosporella marylandensis	BPI879233	LCM359.01	U.S.A.	*Alnus serrulata*	L.C. Mejía		new		
Cryptosporella marylandensis	BPI879233	LCM359.02	U.S.A.	*Alnus serrulata*	L.C. Mejía		new		
Cryptosporella marylandensis	BPI879234	LCM385	U.S.A.	*Alnus serrulata*	L.C. Mejía		new		
Cryptosporella marylandensis	BPI879235	LCM631	U.S.A.	*Alnus serrulata*	L.C. Mejía		new		
Cryptosporella marylandensis	BPI879236	LCM580.01	U.S.A.	*Alnus serrulata*	L.C. Mejía		new		
Cryptosporella marylandensis	BPI879236	LCM580.02	U.S.A.	*Alnus serrulata*	L.C. Mejía		new		
Cryptosporella marylandensis	BPI879237	LCM387	U.S.A.	*Alnus maritima*	L.C. Mejía				
Cryptosporella marylandensis	BPI879238	LCM388	U.S.A.	*Alnus maritima*	L.C. Mejía				
Cryptosporella marylandensis	BPI879250	LCM581.01	USA	*Alnus maritima*	L.C. Mejía	new	new	new	
Cryptosporella multicontinentalis	BPI879226	LCM401.01	France	*Alnus glutinosa*	L.C. Mejía	new	new	new	
Cryptosporella multicontinentalis	BPI879227	LCM406.01	France	*Alnus glutinosa*	L.C. Mejía	new	new	new	

Cryptosporella multicontinentalis	BPI879228	LCM427.01	Germany	*Alnus glutinosa*	L.C. Mejía	new	new	new
Cryptosporella multicontinentalis	BPI879229	LCM93.01	U.S.A.	*Alnus rugosa*	L.C. Mejía	new	new	new
Cryptosporella multicontinentalis	BPI879230	LCM93b.02	U.S.A.	*Alnus rugosa*	L.C. Mejía		new	
Cryptosporella multicontinentalis	BPI879258	LCM394.01	France	*Alnus hirsuta-siberica*	L.C. Mejía		new	
Cryptosporella multicontinentalis	BPI879258	LCM394.02	France	*Alnus hirsuta-siberica*	L.C. Mejía		new	
Cryptosporella multicontinentalis	BPI879258	LCM394.04	France	*Alnus hirsuta-siberica*	L.C. Mejía		new	
Cryptosporella pacifica	BPI879239	LCM461.01	U.S.A.	*Alnus tenuifolia*	L.C. Mejía	new	new	new
Cryptosporella pacifica	BPI879240	LCM453.01	U.S.A.	*Alnus tenuifolia*	L.C. Mejía	new	new	new
Cryptosporella pacifica	BPI879241	LCM420.01	U.S.A.	*Alnus tenuifolia*	L.C. Mejía		new	
Cryptosporella pacifica		CBS122311	U.S.A.	*Alnus sinuata*	S. Lattomus & LCM	new	new	new
Cryptosporella pacifica		CBS122312	U.S.A.	*Alnus sinuata*	S. Lattomus & LCM	new	new	new
Cryptosporella suffusa	BPI871231	CBS121077	Austria	*Alnus incana*	W. Jaklitsch	new	EU199184	new
Cryptosporella suffusa	BPI748449	CBS109750	Austria	*Alnus incana*	W. Jaklitsch	new	EU199207	new
Cryptosporella suffusa	BPI879242	LCM576.01	Germany	*Alnus* sp.	L.C. Mejía	new	new	new
Cryptosporella suffusa	BPI879242	LCM576.03	Germany	*Alnus* sp.	L.C. Mejía	new	new	new
Cryptosporella tomentella	BPI879243	LCM184b.01	U.S.A.	*Betula alleghaniensis*	L.C. Mejía	new	new	new
Cryptosporella tomentella	BPI843595	CBS 121075	U.S.A.	*Betula* sp.	L. Vassilyeva	new	new	new
Cryptosporella tomentella	BPI872328	CBS 121073	U.S.A.	*Betula* sp.	L. Vassilyeva	new	new	new
Cryptosporella wehmeyeriana	BPI879244	LCM85.01	U.S.A.	*Tilia americana*	L.C. Mejía		new	
Cryptosporella wehmeyeriana	BPI879244	LCM85.02	U.S.A.	*Tilia americana*	L.C. Mejía	new	new	new
Cryptosporella wehmeyeriana	BPI879244	LCM85.03	U.S.A.	*Tilia americana*	L.C. Mejía		new	
Cryptosporella wehmeyeriana	BPI879245	LCM137.01	U.S.A.	*Tilia americana*	L.C. Mejía		new	
Cryptosporella wehmeyeriana	BPI879245	LCM137.02	U.S.A.	*Tilia americana*	L.C. Mejía		new	
Cryptosporella wehmeyeriana	BPI879246	LCM139.01	U.S.A.	*Tilia americana*	L.C. Mejía		new	
Cryptosporella wehmeyeriana	BPI879246	LCM139.02	U.S.A.	*Tilia americana*	L.C. Mejía		new	
Cryptosporella wehmeyeriana	BPI843485	CBS121085	U.S.A.	*Tilia* sp.	L. Vasilyeva	new	EU199205	new
Ditopella ditopa	BPI879247	LCM94.02	U.S.A.	*Alnus rugosa*	L.C. Mejía	new	new	new
Plagiostoma petiolophillum	BPI879252	LCM181.01	U.S.A.	*Acer spicatum*	L.C. Mejía	new	new	new

Table 4.2. Summary of *Cryptosporella* species and their host associations.

Species of *Cryptosporella*	Host	Distribution	Host preference
C. alni-rubrae L.C. Mejía sp. nov.	*Alnus rubra*	Pacific Northwest (OR, WA)	one host species
C. alni-sinuatae L.C. Mejía sp. nov.	*A. sinuata*	Pacific Northwest (WA)	one host species
C. alni-tenuifoliae L.C. Mejía sp. nov.	*A. tenuifolia*	Pacific Northwest (OR)	one host species
C. amistadensis L.C. Mejía sp. nov.	*A. acuminata*	Central and South America	one host species
C. corylina (Tul. & C. Tul.) L.C. Mejía & Castleb.	*Corylus avellana*	Europe	one host species
C. femoralis (Peck) L.C. Mejía & Castleb.	*A. rugosa*	Eastern North America	one host species
C. jaklitschi L.C. Mejía sp. nov.	*A. serrulata*	Eastern North America (NY)	one host species
C. tiliae (Tul. & C. Tul.) L.C. Mejía & Castleb.	*Tilia cordata*	Europe	one host species
C. wehmeyeriana (Reid & Booth) L.C. Mejía & Castleb.	*T. amistadensis*	Eastern North America	one host species
C. alnicola (Fr.) L.C. Mejía & Castleb.	*Alnus* spp. & *Corylus* sp.	Eastern North America	generalist, on *Alnus* and *Corylus*
C. betulae (Tul. & C. Tul.) L.C. Mejía & Castleb.	*Betula* spp.	Europe	generalist, only on *Betula* spp.
C. confusa (Reid & Booth) L.C. Mejía & Castleb.	*B. alba* & *B. papyrifera*	Europe (morphology) and Eastern North America (DNA)	generalist, on two species of *Betula*
C. hypodermia (Fr.) Sacc.	*Ulmus* spp.*	Europe & North America	generalist, on *Ulmus* spp.
C. multicontinentalis L.C. Mejía sp. nov.	*A. rugosa, A. glutinosa, A. hirsuta-siberica*	Europe & North America, Japan?	generalist on *Alnus* spp.
C. marylandensis L.C. Mejía sp. nov.	*A. maritima* & *A. serrulata*	Eastern USA (MA)	generalist on two species of *Alnus*
C. pacifica L.C. Mejía sp. nov.	*A. sinuata* & *A. tenuifoliae*	Pacific Northwest (CA, OR, WA)	generalist on two species of *Alnus*
C. suffusa (Fr.) L.C. Mejía & Castleb.	*A. incana & Alnus* spp.	Europe	generalist, only on *Alnus*
C. tomentella (Peck) L.C. Mejía comb. nov.	*B. papyrifera*, *Betula* sp.	Eastern North America	generalist? on *Betula*
C. rabenhorstii L.C. Mejía comb. nov.	*Betula* sp.	Europe	generalist? on *Betula*
Cryptosporella sp. (NCBI deposited DNA sequences)	*B. platyphylla*	Beijing, China	generalist? on *Betula*

* *Cryptosporella hypodermia* has been reported in hosts other than *Ulmus*. In three cases the original report was done under another species name that later was synonymized with *C. hypodermia*: as *Cryptosporella compta-macrospora* on *Fagus sylvatica*; as *Cryptosporella veneta* on *Populus tremula*; as *Sphaeria limminghii* on *Platanus orientalis.* Three other reports exist originally as *Cryptospora corylina* on *Corylus avellana*; as *C. hypodermia* on *Acer* sp. and *Alnus incana*. All the *C. hypodermia* specimens sequenced in this study were collected on species of *Ulmus*. Fresh specimens with *C. hypodermia* morphology from the above mentioned hosts are needed to confirm this unusually broad host range.

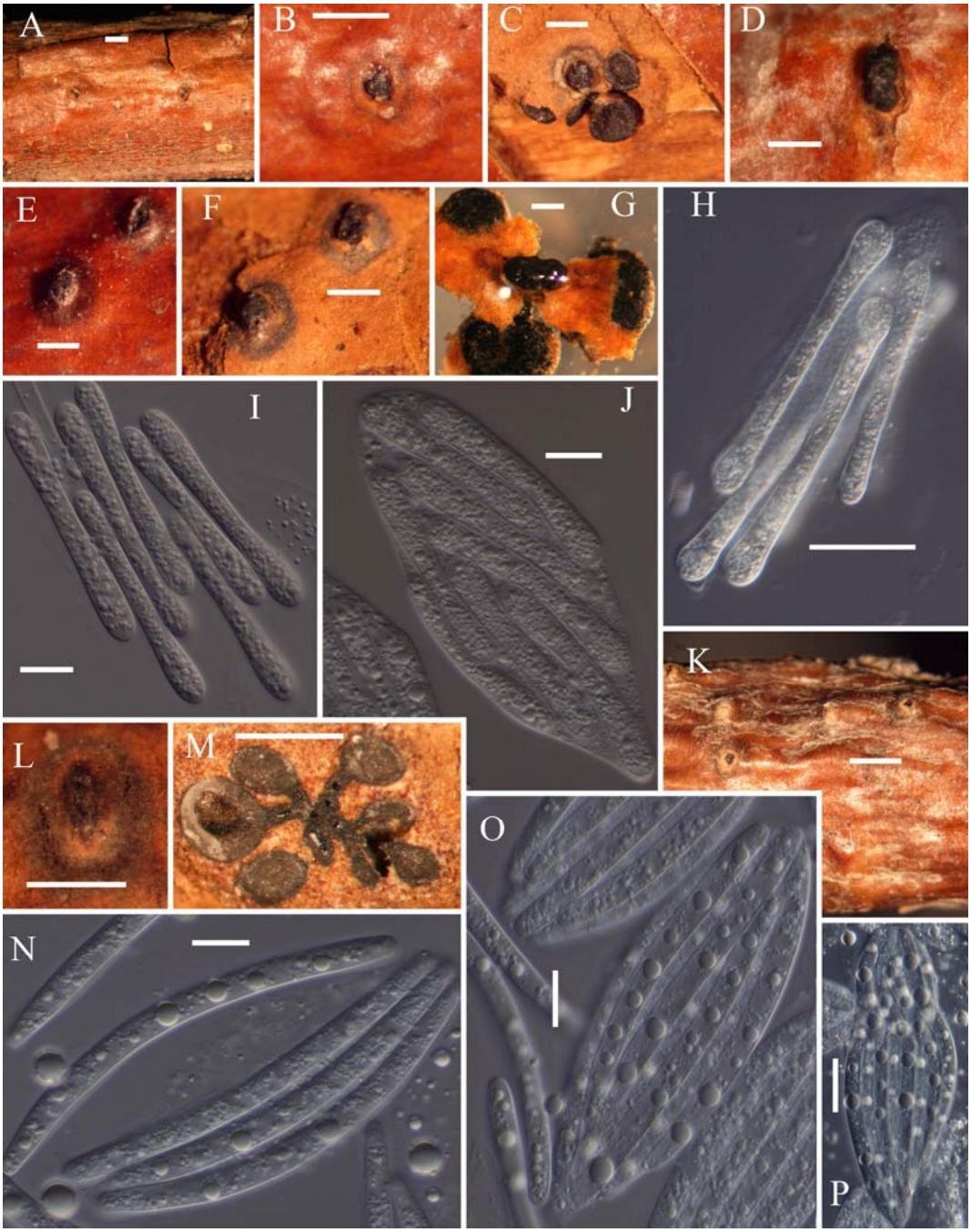

Figure 4.2. Morphology on natural substrate. A-J: *Cryptosporella alni-rubrae,* A: BPI879203, B-J: BPI879199 (holotype). K-P: *C. alni- sinuatae* BPI879210 (holotype). Bars = (A-B, K-L) 1 mm; (C-F, M) 500 µm; (G) 200 µm; (H, N-O) 20 µm; (I-J, P) 10 µm.

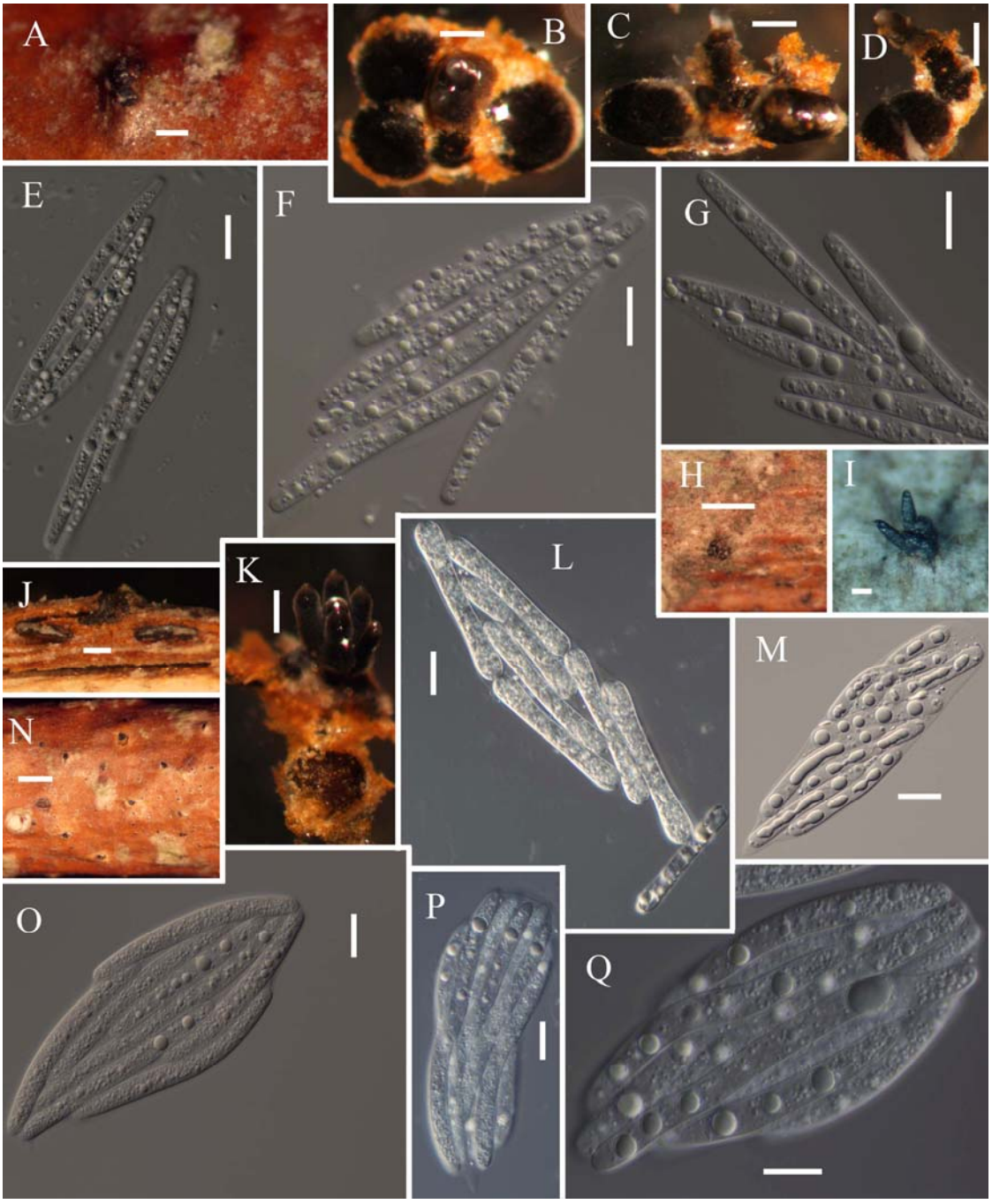

Figure 4.3 – Morphology on natural substrate. A-G: *Cryptosporella alni-tenuifoliae* BPI879211 (holotype). H-M: *C. amistadensis,* H,J,L=BPI879218, I=BPI879249, K,M=BPI879214 (holotype). O-Q, *C. multicontinentalis* BPI879226 (holotype). Bars = (H, N) 1 mm; (I) 300 µm; (A-D, J-K) 200 µm; (E, L-M, O-Q) 20 µm; (F-G) 10 µm.

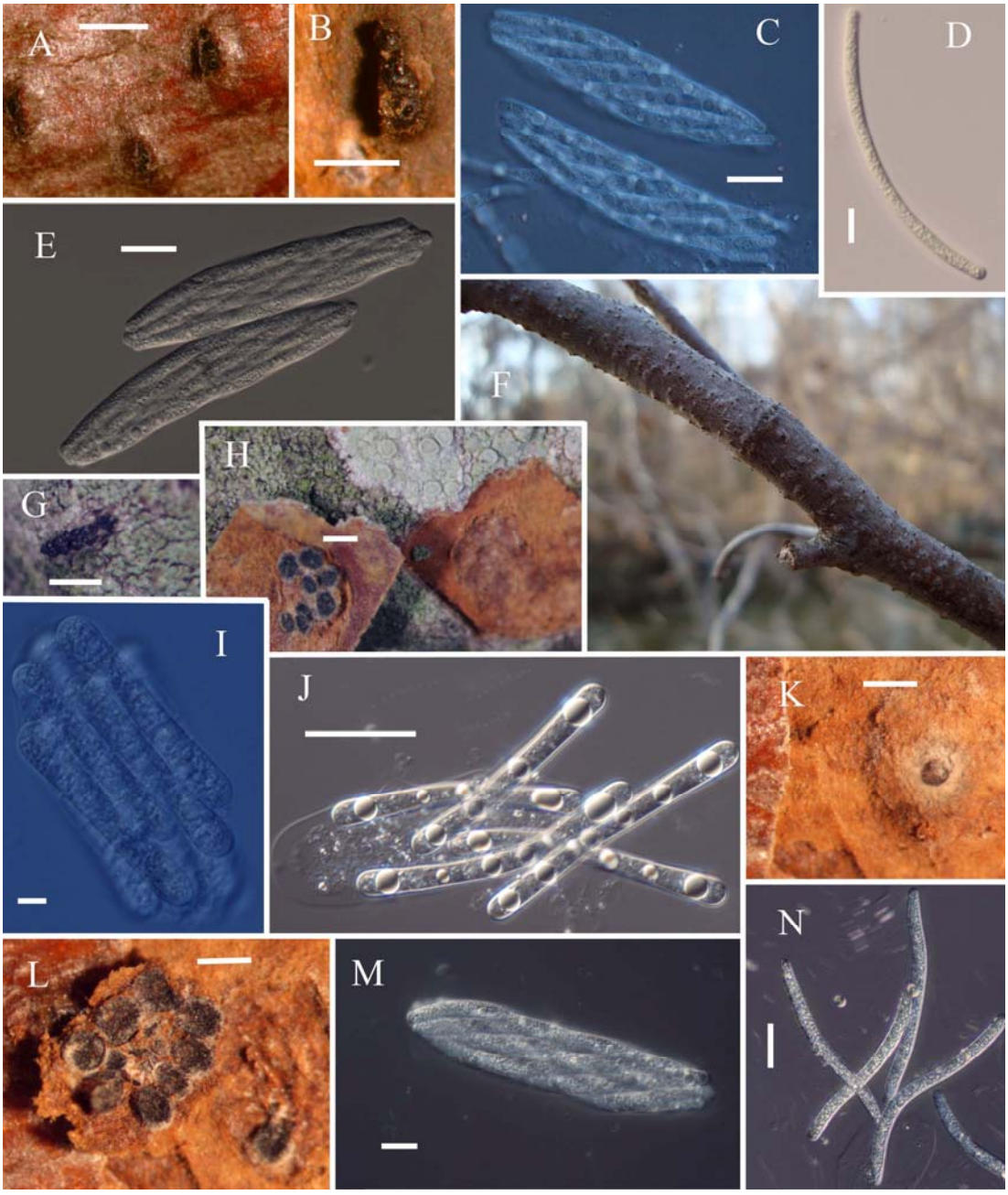

Figure 4.4 – Morphology on natural substrate. A-E: *Cryptosporella jaklitschi* BPI879231 (holotype). F-J: *C. marylandensis*, F=branch of *Alnus serrulata* with perithecia of this species in the field,G-I= BPI879232 (holotype), J= BPI879236. K-N, *C. pacifica*, K-L= BPI879240, M= BPI879241, N= BPI879239 (holotype). Bars = (A, G-H) 1mm; (B, K-L) 500 µm; (J, M-N) 20 µm; (C-E, I) 10 µm.

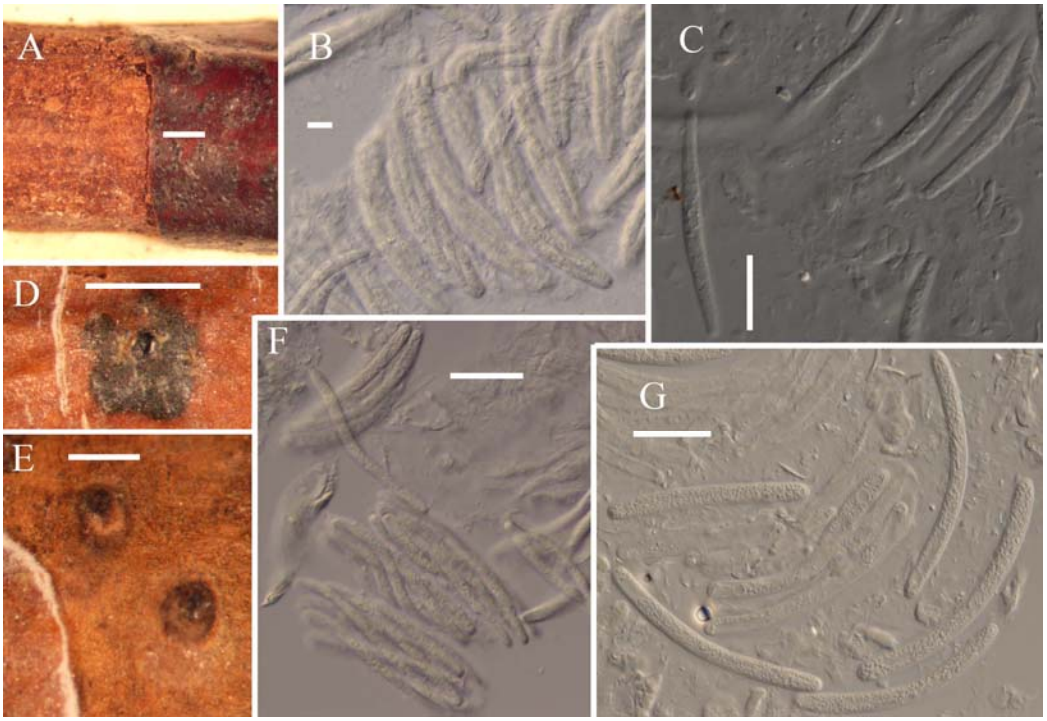

Figure 4.5 – Morphology on natural substrate. A-C, holotype (K(M) 163853) of *C. rabenhorstii*. D-G, Scleromyceti Sueciae 229, type of *C. suffusa*. Bars = (A, D) 1 mm; (E) 500 µm; (C, F-G) 20 µm; (B) 10 µm.

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Chapter 5

Systematic review of the genus *Plagiostoma* Fuckel (Gnomoniaceae) based on morphology, host-associations, and four-gene phylogeny[3,4]

ABSTRACT

The genus *Plagiostoma* is monographed based on analyses of morphological, cultural, and molecular data and review of the literature. The morphological data included shape and size of perithecia, asci, and ascospores and overall arrangement of the ascomata in the host. Cultural studies included the comparison of growth rate and pigmentation of the species in culture media. The molecular data included DNA sequences from four genes (*β-tubulin*, ITS, *rpb2*, and *tef1-α*). This work includes the recircumscription of the genus *Plagiostoma*, the recognition and description of eight new species, the recircumscription of four species, and the proposal of four new name combinations. A total of 25 species of *Plagiostoma* are accepted here of which 24 are included in a four-gene phylogeny. A key to all species of *Plagiostoma* is provided.

INTRODUCTION

The genus *Plagiostoma* Fuckel (Gnomoniaceae, Diaporthales) includes microscopic fungi that inhabit the leaves and branches of a diverse range of woody and herbaceous plant families (e.g. Aceraceae, Betulaceae, Euphorbiaceae, Geraniaceae,

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[4] The new names included within this chapter are not accepted by the author(s) as validly published in this dissertation (Botanical code, article 34.1[a])

Hippocastanaceae, Oleaceae, Polygonaceae, Salicaceae, Staphylaceae) in temperate zones of the Northern Hemisphere (see Sogonov et al. 2008). Although pathogenic species of *Plagiostoma* occur, most species appear to be asymptomatic on their hosts and only become noticeable when sexually reproducing through production of perithecia and ascospores on dead tissues of their hosts. Since Fuckel (1870) described *Plagiostoma*, the concept of this genus based exclusively on morphological characters has varied but not considerably. However, recent phylogenetic studies using molecular data (Mejía et al. 2008; Sogonov et al. 2008) have shown a highly supported monophyletic clade consisting of the type species of *Plagiostoma*, *P. euphorbiae,* and the type species of *Cryptodiaporthe*, *C. aesculi.* These two species represent genera not considered closely related in traditional classification schemes of the Diaporthales (see Barr 1978). Based on molecular phylogenetic studies, Sogonov et al. (2008) synonymized *Cryptodiaporthe* with *Plagiostoma* as the genus with nomenclatoral priority, i.e. described first. Sogonov et al. (2008) also created new species combinations for the type and three former species of *Cryptodiaporthe* under *Plagiostoma*.

Traditionally *Cryptodiaporthe* and *Plagiostoma* have been considered distinct genera, each with a specific morphology and arrangement of reproductive structures and occurring on certain hosts. Species of *Plagiostoma* were characterized by not forming a stroma, having single perithecia, and occurring primarily on leaves. Species of *Cryptodiaporthe* were characterized by forming a rudimentary stroma, having grouped perithecia, and occurring primarily in the bark of their host branches. Furthermore the differences between *Cryptodiaporthe* and *Plagiostoma* have been emphasized so much that some authors have placed them in different families or other suprageneric ranks (see

below). The results of the recent molecular studies and the synonymization of *Plagiostoma* with *Cryptodiaporthe* suggest a major change in the concept of *Plagiostoma* and have raised several questions that stimulated the present study. Among these are: whether other species of *Cryptodiaporthe* should be treated as congeneric with *Plagiostoma*, what is the full range of morphological, biological, and ecological characters and host associations that define *Plagiostoma,* and what is the species richness of *Plagiostoma*? Furthermore, recent collections representing newly discovered species of *Plagiostoma* are described.

In the latest monographic treatment of the Gnomoniaceae, Sogonov et al. (2008) included 12 species of *Plagiostoma* in a multilocus phylogenetic study. In that work, *Plagiostoma* was determined to form a highly supported clade within the Gnomoniaceae, however, relationships among species of *Plagiostoma* were not addressed. Currently a number of species formerly defined under *Cryptodiaporthe* remain to be included in molecular phylogenetic studies. Several taxonomic issues persist in closely related species formerly treated as *Cryptodiaporthe,* such as the extensive synonymies under *Cryptodiaporthe salicella* (Fr.) Wehm. and *Cryptodiaporthe salicina* (Curr.) Wehm. At present, 49 and 54 species names have been listed under *Plagiostoma* and *Cryptodiaporthe,* respectively, as recorded in Mycobank (Crous et al. 2004; Robert et al. 2005).

The following is a brief account of the major taxonomic treatments of *Plagiostoma* and *Cryptodiaporthe* that illustrate the different views of these genera through time. Fuckel (1870) created the genus *Plagiostoma* to group sphaericeaous species characterized by having flattened perithecia (oriented horizontally) in the

substrate and lateral, short, and erumpent ostioles. Fuckel (1870) included the genera *Ceratostoma, Gnomonia*, *Linospora*, *Melanospora,* and *Rhaphidospora* together with *Plagiostoma* in the tribe Ceratostomeae of the so-called Sphaeriacei. In his original description of *Plagiostoma*, Fuckel included four species *P. euphorbiae*, *P. petiolicola*, *P devexa*, and *P. suspecta*. The Fuckelian concept of *Plagiostoma* was followed by von Höhnel (1917) and von Arx (1951) who, like Fuckel, considered *Plagiostoma* to be relatively closely related to *Gnomonia* Ces. & De Not., the genus name on which the Gnomoniaceae is based. These authors differentiated *Gnomonia* from *Plagiostoma* mainly by orientation of the perithecial neck. *Gnomonia* was characterized by having central upright perithecial necks in contrast to species of *Plagiostoma* with eccentric and laterally oriented perithecial necks. In her treatment of the order Diaporthales, Barr (1978) followed Fuckel's concept of *Plagiostoma* and considered *Gnomonia* and *Plagiostoma* in the same suborder Gnomoniineae but in different families, *Gnomonia* in the Gnomoniaceae and *Plagiostoma* in the Valsaceae. Valsaceae was defined based on the horizontal orientation of the perithecia. Barr (1978) made nine new combinations under *Plagiostoma* and thereby greatly expanded the number of species in the genus. In his monograph of the Gnomoniaceae Monod (1983) accepted most species treated by Barr (1978). However, Monod considered the type species of *Plagiostoma, P. euphorbiae,* not to be representative of *Plagiostoma* because the perithecial neck of this species is central or eccentric and not lateral as conceived by Fuckel, the original author of the genus. Based on his observations, Monod (1983) transfered *P. euphorbiae* to the genus *Gnomonia* and re-typified *Plagiostoma* with *P. devexum*. This typification by Monod does not follow the International Code of Botanical Nomenclature (McNeill et al.

2006) and was not accepted by Barr (1991) on the basis of morphology nor by Sogonov et al. (2008) on the basis of morphology and molecular data. Petrak (1921) described *Cryptodiaporthe* for species with an euvalsoid arrangement of perithecia and, in contrast to *Diaporth*e, with no black marginal zone surrounding the perithecia. In describing *Cryptodiaporthe,* Petrak included *C. aesculi*, *C. hystrix*, and *C. populina* and reported *Septomyxa* as the anamorphic stage. Later, Wehmeyer (1933) recircumscribed *Cryptodiaporthe*, emphasized the lack of blackened marginal zones within the substratum, and made 17 new combinations under this genus for species previously included in *Diaporthe* expanding the genus to 19 species. Additionally, Wehmeyer reported several anamorphs in addition to *Septomyxa* and considered *Cryptodiaporthe* as a "heterogeneous group of species".

In addition to the morphological and molecular characters, it has been observed by us and others that species of *Plagiostoma* and Gnomoniaceae generally have a marked host preference or specificity that in many cases is useful for identifying species, observing that some plant families such as the Betulaceae, Fagaceae, and Salicaceae are particularly favored hosts of Gnomoniaceae (see Barr 1978; Mejía et al. 2008; Monod 1983; Sogonov et al. 2008). Based on our own observations and literature review on host associations of Gnomoniaceae species and as part of this work, we asked the question of whether there are specific associations between lineages (genera or groups of species) of Gnomoniaceae with specific lineages (genera, family, or orders) of plant hosts. Moreover we predicted that new species of Gnomoniaceae are likely to be found on plant species congeneric to known host species and in localities where these host species naturally occur. An assumption behind this prediction is that lineages of Gnomoniaceae have a

long evolutionary relationship with and have followed the same geographic distribution of host plant genera and/or families.

Here we provide phylogenetic analyses to address the relationships of species of *Plagiostoma*, a taxonomic revision of known species, descriptions of new species, and information on the species distribution and their host associations. We also recircumscribe the genus *Plagiostoma* since several species treated by Wehmeyer (1933), Barr (1978) and Monod (1983) are here excluded from *Plagiostoma* based on our phylogenetic analyses.

MATERIAL AND METHODS

Collection of specimens, culture preparation, and morphological observations

Hosts known to harbor species of *Plagiostoma* and other Gnomoniaceae were sampled as well as hosts congeneric with known hosts in the same localities. Known and potential hosts were also sampled in localities where Gnomoniaceae had not previously been collected. Collections (Table 5.1) were made in the following countries mainly during the springs and summers of 2007–2008: Argentina (Tucumán), China (Yunnan), France (Deux-Sèvres Department), Germany (Frankfurt), and the United States of America (California, Maryland, New York, Oregon, Washington). Specimens consisted of overwintered dead attached or fallen twigs and branches with perithecia of the *Plagiostoma* type. Specimens were placed in paper bags, air-dried to remove excess moisture and stored at 8-10 °C in sealed plastic bags. All specimens were deposited in the U.S. National Fungus Collections (BPI). Additional specimens for comparisons were obtained from various herbaria, including BPI and NYS.

Observations, measurement and digital image capture of morphological characters and isolation of cultures were performed using the same equipment and procedures as in Mejía et al. (2008). AxioVision version 4.7.2.0 (Carl Zeiss Image Solutions, Carl Zeiss, New York, NY, USA) was used in conjunction with those methods to measure structures. Measurements of perithecial neck length in species with expanded perithecia included the expanded area and not just the portion above the expanded area. Only structures from exsiccati were mounted in 3% potassium hydroxide for photographs. Cultural characteristics were observed on Potato Dextrose Agar (PDA, Difco™, Becton, Dickinson & Co., Sparks, MD, USA) seven days after plating as described in Mejía et al. (2008). Representative cultures of new species described in this study were deposited at the Central Bureau Voor Schimmelcultures (CBS, The Netherlands).

DNA extraction and PCR amplification

DNA extractions were done as described by Mejía et al. (2008), using a Fast Prep FP 120 with Lysing Matrix 'A' (Thermo Electron Corporation, Milford, MA) for mechanical lysis. Four gene fragments were amplified and sequenced for the phylogenetic analyses: the complete nuclear ribosomal internal transcribed spacer regions 1 and 2 including 5.8 S rDNA (ITS) and regions of the RNA Polymerase second largest subunit (*rpb2*), Beta Tubulin gene (*β-tubulin*) and Translation Elongation Factor 1-alpha gene (*tef1*-α). The ITS and *rpb2* genes were amplified and sequenced as described in Mejía et al. (2008) in 25 µl reactions with two internal sequencing primers designed specifically for species of *Plagiostoma*: RPB2 Plag-F (5' CGT CGC TGC ATY ATC TCR CA 3') and RPB2 Plag-R (5' TGY GAG ATR ATG CAG CGA CG 3'). *β-tubulin* was amplified using primers T1 and T22 and sequenced with the PCR primers and the

internal primers T2 and T12 from O'Donnell & Cigelnik (1997). For some isolates it was necessary to amplify the *tef1*-α region in two fragments using the following primer combinations: EF1-728F /EF1-1199R and EF1-983F/ EF1-1567R (Carbone and Kohn 1999; Castlebury, unpublished data for primer 1199R 5' GGG AAG TAC CMG TGA TCA TGT 3'; Rehner 2001). For the purpose of determining taxonomic affinities of species previously described as *Cryptodiaporthe* or *Plagiostoma,* but not congeneric with *P. euphorbiae* (type species), a region of the nuclear ribosomal large subunit (LSU) was amplified as described in Castlebury et al. (2002).

Phylogenetic analyses

Editing and alignment of DNA sequences was performed as described in Mejía et al. 2008. Individual genes were aligned separately and concatenated into a single alignment. Table 5.1 includes detailed information on the source of individual gene sequences. The concatenated sequence alignment included *β-tubulin* (1619 bp) ITS (625 bp), *rpb2* (1212 bp), and *tef1-α* (1149 bp) for a total of 4605 bp and 45 taxa. The taxa included in this alignment represent 24 of the 25 species of *Plagiostoma* accepted in this work with *Apiognomonia veneta* and *A. hystrix* as outgroup taxa. Outgroup selection was based on the sister relationship of the genus *Apiognomonia* with *Plagiostoma* as recently inferred by a three-gene phylogeny of the family Gnomoniaceae (Sogonov et al. 2008). Positions with ambiguous alignment were excluded from all analyses.

The concatenated alignment was partitioned by gene and by codon position for *rpb2*, *β-tubulin*, and *tef1-α* using PAUP* (Swofford 2002). The gene partitions were analyzed for conflict with the partition homogeneity test (PHT) as implemented in PAUP*(Swofford 2002) using the following settings: 100 homogeneity replicates, 10

random sequence addition replicates and MULTREES off. Additionally conflict among gene partitions was assessed by reciprocal bootstrap analyses (Reeb et al. 2004) using distance settings for each partition as determined by Modeltest v.3.7 (Posada & Crandall 1998) following the Bayesian Information Criterion (BIC).

Genes were first analyzed individually and then as a combined alignment using Maximum Parsimony, Bayesian, and Maximum Likelihood analyses. Trees and bootstrap supports of branches were estimated by Maximum Parsimony (MP) analysis as in Sogonov et al. (2008) with all characters considered unordered with equal weight and an additional analysis with unordered characters weighted as follows: weight=3 for first and second codon positions and weight=1 for third codon position. Additionally, trees were estimated using Bayesian analysis with the program MrBayes v3.1.2 (Huelsenbeck & Ronquist 2001) as described in Sogonov et al. (2008) with sampling every 500 generations. Model settings for each gene were determined using the program MrModeltest v.2 (Nylander 2004) and selected based on the Akaike Information Criterion (AIC). The first 50000 generations were discarded (burn-in period) based on comparison of tree likelihood scores. A 50% majority rule consensus tree and a consensus phylogram were constructed from the trees saved after the burn-in period. The Bayesian posterior probabilities (PP) of nodes of the consensus trees are presented in Figure 5.1. Trees were also estimated by Maximum Likelihood analysis using the program PAUP* (Swofford 2002) as described in Sogonov et al. (2008) with Modeltest v.3.7 (Posada & Crandall 1998) employed to estimate the best model for the concatenated alignment. Maximum likelihood bootstrap analysis was not conducted.

RESULTS

Collection of specimens

Species of *Plagiostoma* were collected from plant species reported as hosts of Gnomoniaceae as well as from a number of new plant species congeneric with known hosts. The following plant species are reported as new hosts for species of *Plagiostoma*: *Alnus tenuifolia, Salix dasyclados*, *S. humboldtiana, S. irorata, S. lucida*, and *S. sitchensis* (Table 1). Additionally, several species of *Plagiostoma* were isolated from *Salix* trees that could not be identified to species (Table 5.1). Of these, the hosts of *P. ovalisporum* sp. nov. and *P. yunnanense* sp. nov. are likely to represent unreported hosts for *Plagiostoma* as they appear to be different from *Salix* species known to host species of *Plagiostoma*. Additionally, *P. yunnanense* (southwestern China) and *P. pulchellum* (Argentina) were collected in regions where species of *Plagiostoma* had previously not been formally reported.

Phylogenetic analyses

The partition homogeneity test suggested incongruency among the four genes sequenced in this study: ITS, *rpb2*, *β-tubulin*, and *tef1-α* (P=0.01). However this was only the case when *rpb2* was included in the comparisons. For combinations of the remaining three genes, no incongruence was detected: ITS, *β-tubulin*, and *tef1-α* (P=0.09); ITS and *β-tubulin* (P=0.07); and ITS and *tef1-α* (P=0.24). The following are the likelihood settings estimated for each gene for the reciprocal NJ bootstrap analyses: ITS: Base=equal Nst=2 TRatio=2.5434 Rates=equal Pinvar=0.8337; *rpb2*: Base=equal Nst=6 Rmat=(1.0000 4.6961 1.0000 1.0000 13.3827) Rates=gamma Shape=0.2029 Pinvar=0; *β-tubulin*: Base=(0.2006 0.3249 0.2505) Nst=2 TRatio=2.1757 Rates=gamma

Shape=0.5017 Pinvar=0; and *tef1-α*: Base=(0.1918 0.3110 0.2229) Nst=2 TRatio=1.8586 Rates=gamma Shape=0.6109 Pinvar=0. There was no conflict among gene partitions as determined by the reciprocal bootstrap analyses and species delimitations were not contradicted by bootstrap values (trees not shown).

The ITS, *β-tubulin*, and *tef1-α* trees individually resolved terminal clades for most of the species analyzed (trees not shown). However, no single gene analysis resolved all the species of *Plagiostoma* with bootstrap support higher than 70%. The following numbers of species were resolved by gene with bootstrap > 70%: ITS=11, *rpb2*=9, *β-tubulin*=12, and *tef1-α*=11. In general, *rpb2* was not as useful for resolving clades of closely related species as the other three genes. The ITS gene resolved and supported all terminal clades except *P. amygdalinae* and *P. euphorbiaceae* for which the sequences were nearly identical. However, it did not support backbone nodes at levels greater than 70%. In contrast, bootstrap support greater than 90% for all backbone nodes containing two or more species was obtained in the *rpb2*, *β-tubulin*, and *tef1-α* trees. The topology of the individual gene trees differed slightly. However, only one topological conflict supported by bootstrap values greater than 70% was observed between individual gene trees. In this instance the *β-tubulin* analysis resulted in a clade (97%) that included all species of *Plagiostoma* on Salicaceae and the *rpb2* analysis resulted in a clade (72%) that included some but not all the species that grow on Salicaceae and some species that grows on other hosts.

Trees resulting from the combined four-gene alignment (ITS, *β-tubulin*, *rpb2*, and *tef1-α*) were compared with those from the alignment of the three genes found to be congruent by the PHT (ITS, *β-tubulin*, and *tef1-α*). Maximum parsimony analyses of the

four-gene combination resulted in 114 equally parsimonious trees (length=1713, CI=0.689, RI=0.809) and 42 equally parsimonious trees (length= 2062, CI=0.689, RI=0.807) for the un-weighted and weighted analyses, respectively. Fifty percent majority rule consensus trees computed for each analysis did not differ in the identification of terminal species clades but higher bootstrap support was obtained for several clades in the weighted analysis. Maximum parsimony analysis of the three-gene combination composed of ITS, *β-tubulin*, and *tef-α* resulted in eight equally parsimonious trees (length=1275, CI=0.707, RI= 0.817). The tree topologies obtained by MP analyses of the two alignments did not contradict each other; however, bootstrap support for several nodes increased in analyses of the four-gene combination. Therefore subsequent analyses were performed on the four-gene combination.

The following models were the best estimates for each gene and were applied during the Bayesian analyses: HKY + I + G for ITS and *tef1-α*; SYM + G for *rpb2*; and HKY + G for *β-tubulin.* The model TrN+G was estimated to be the best for the entire alignment by both hLRT and BIC and those settings were applied to the maximum likelihood analysis: Base=(0.2245 0.2859 0.2454) Nst=6 Rmat=(1.0000 3.5234 1.0000 1.0000 5.8336) Rates=gamma Shape=0.2849 Pinvar=0. Bayesian, ML, MP and weighted parsimony (WP) analyses of the four-gene alignment all resulted in the same topology. High MP bootstrap supports and Bayesian PP were obtained for all species of *Plagiostoma* included in the analyses. Maximum likelihood analysis of the concatenated alignment of four genes resulted in one tree –lnL score of 13921.12887 and is presented here as the inferred phylogeny of *Plagiostoma* (Fig. 5.1). Bayesian PP and MP bootstraps are shown above and below the branches. The inferred phylogeny of *Plagiostoma*

supports the recognition of eight new species from this genus, which are described in the taxonomic section of this work. Bayesian PP and MP bootstrap supports greater than 90% were obtained for all the species of *Plagiostoma* in this multigene phylogeny. *Plagiostoma euphorbiae-verrucosae* is not included in the multigene phylogeny only the ITS could be sequenced from this species. This species was confirmed as *Plagiostoma* by analysis of ITS sequences (tree not shown).

Both Bayesian analysis and MP bootstrapping supported a clade containing 11 species that occurs exclusively on hosts of the family Salicaceae. All of these 11 species occur on the bark of their host twigs and branches with one species, *Plagiostoma versatile* sp. nov., also occurring in the leaf midvein and petioles. Within the species on Salicaceae, one clade consists of four closely related species characterized by having an expanded perithecial neck: *P. apiculatum*, *P. dilatatum* sp. nov., *P. imperceptibile* sp. nov., and *P. pulchellum*. Cryptic morphological features such as perithecial size, ascospore size and length-to-width (l:w) ratio, and hyphal color in culture help differentiate these species. See key and taxonomic section for details. In brief, *P. imperceptibile* is characterized by having ascospores longer than 18 µm but l:w less than five. *Plagiostoma pulchellum* is characterized by having ascospores with l:w greater than five and by producing rosy colored hyphae that become dark green on PDA. *Plagiostoma apiculatum* and *P. dilatum* are similar but the average size of perithecia and ascospores of *P. dilatatum* are larger than *P. apiculatum*. Highly supported (>95% MP, PP) as basal to these four species is *P. convexum* with a moderately expanded perithecial neck. The pathogenic species *P. populeum* comb. nov. and *P. ovalisporum* sp. nov. are closely related and contained within a larger clade including the five species previously

mentioned (100% MP, PP). However, their relationship to one another is not supported. The remaining species on Salicaceae form a sister clade (<70% MP, 80% PP) to the seven species mentioned above. This clade contains three species with cylindrical, usually elongated perithecial necks and elongated ascospores: *P. salicellum*, *P. versatile*, and *P. yunnanense*. The remaining member of this clade, *P. oregonense,* is characterized by short, expanded perithecial necks with non-elongated ascospores.

Bayesian PP and MP bootstrapping support a clade (83% MP, 100% PP) of eight species which hosts represent a wide range of woody and herbaceous plant families. This clade includes subclades with *P. devexum* from *Polygonum* as basal. One of the subclades in this group is composed of three species that grows on Euphorbiaceae: *P. amygdalinae*, *P. euphorbiae,* which is the type species of the genus, and *P. euphorbiaceum*. Basal to these species is *P. fraxinum* on *Fraxinum pennsylvanicum*. The second subclade contains *P. exstocollum* and *P. samuelsii* both on betulaceous hosts and *P. rhododendri* on *Rhododendron*. The rest of the species included in the phylogeny, namely *P. aesculi*, *P. barriae*, *P. geranii*, *P. petiolophillum,* and *P. robergeanum,* are relatively distant from eacht other and the species previously mentioned. *Plagiostoma robergeana,* a species that grows on *Staphylea,* was placed as basal to all other species of *Plagiostoma*. Sequenced specimens of *P. pulchellum* were collected in Europe, North America (USA) and South America (Argentina). This species is recognized as the most widely distributed species of *Plagiostoma* included in this study and presented here as the first report of Gnomoniaceae for South America. *Plagiostoma yunnanense* is the first report of *Plagiostoma* for China.

TAXONOMY

Key to species of *Plagiostoma*

1. Ascospores non-septate……..……..…….2

1’.Ascospores one septate…….……..….4

2. Ascospores oval, (12–)14–16(–17) × 7–8(–9) µm. On twigs of *Salix* sp., in North America (USA, ID)…….……..*Plagiostoma ovalisporum*

2’.Ascospores elliptic fusiform. Not on *Salix*. In Europe and North America……..…..……3

3. Ascospores 20–25.5 × 5.3–6 µm *fide* Monod (1983), with pointed ends. On *Euphorbia,* in Europe……..….*Plagiostoma euphorbia-verrucosae*

3’.Ascospores (7.7–)8.6–12.7(–13.8) × (2.2)2.8–5.9(–6.6) µm *fide* Redlin & Stack (1988). On *Chionanthus* and *Fraxinus* (*Oleaceae*), in Canada and U.S.A…….…*Plagiostoma fraxini*

4. On *Salicaceae*…….….5

4’.On hosts other than the *Salicaceae*……...…….14

5. Perithecia neck cylindric. On woody substrates except *P. versatile* that occurs on both leaf and woody substrates……6

5’. Perithecia neck dilated i.e. with an expanded area that looks like a disc when seen from the top usually appearing with a black halo or black spot in the host surface where perithecial necks protrude. On woody substrates…….……....10

6. Perithecial neck surrounded by a whitish stroma. On *Salix,* in Europe……..……*Plagiostoma salicellum*

6’.Perithecial neck without whitish stroma. On *Salix* or *Populus,* in Europe and other places…..……..……..…7

7. On twigs and branches of *Populus,* in Europe and North America (USA). Ascospores 14–16 × 6–9 µm *fide* Butin (1958)……..……...*Plagiostoma populeum*

7’.On twigs and branches of *Salix,* in China, Europe, and North America. Ascospores greater than 16 µm long……...……8

8. Ascospores elliptic fusiform, constricted, curved, tapering to acute ends, (16–)18–20 (–22) × 4–5 µm. In Europe and North America (USA, NY)…....*Plagiostoma convexum*

8’. Ascospores elliptic elongated, slightly constricted, straight to slightly curved, rounded ends. In China or North America…….9

9. Perithecial neck slightly twisted in upper half of neck and of constant length. On *Salix* sp., in China (Yunnan). Ascospores (19–)23–26(–27) × 3–4 µm…….*Plagiostoma yunnanense*

9’.Perithecial neck straight, of variable length, very short on twigs, longer in leaves. On *Salix* spp., in North America (Pacific Northwest region). Ascospores (18–)20–23(–25) × 3–4 µm …….…….....*Plagiostoma versatile*

10. Ascospores elliptic, constricted, tapering to narrowly rounded ends. Ascospores (16–)17–19(–22) × (4–)6(–7) µm. On *Salix*, in North America (USA, OR)……..…….*Plagiostoma oregonense*

10’. Ascospores oblong elliptic to reniform, not or slightly constricted, rounded ends. Ascospore size different than above. On *Populus* and *Salix*, in North America and elsewhere……..11

11. Ascospores usually straight, sometimes slightly curved, l:w > 5, (17–)18–22(–27) × (5–)6–7(–7.5) µm. On *Populus* and *Salix*, in Europe, North and South America (Argentina)……..…….*Plagiostoma pulchellum*

11'.Ascospores slightly curved, l:w < 5. On *Salix* spp., in Europe and North America……..12

12. Asci ovoid elongated, with long usually persistent stalk. Ascospores slightly constricted at septum, (18–)19–20(–21)×(5–)6–7(–8) µm, l:w (2.5–)2.9–3.1(–3.8), on *Salix* sp. In North America (USA-California)…….....*Plagiostoma neodilatatum*

12'. Asci cylindric, often with long but not persistent stalk. Ascospores slightly depressed to slightly constricted at septum, less than 18 µm long. In Europe and North America……..13

13. Ascospores slightly constricted, (12–)13–15(–22) × 4–5(–7) µm, mean = 15 × 5 µm, l:w (2.6–)3.0–3.3(–3.8). On *Salix*., in Europe (France) …….*Plagiostoma dilatatum*

13'. Ascospores slightly depressed to slightly constricted (12–)16–18.5(–21) × (3–)5–6(–7) µm, mean = 17 × 6 µm, l:w (2.43–)2.87–3.17(–4.03). On *Salix*, in Europe and North America…..... *Plagiostoma apiculatum*

14. On hosts in the *Euphorbiaceae*……..15

14'.On hosts other than *Euphorbiaceae*……...17

15. On leaves of *Euphorbiaceae*, specifically *Euphorbia amygdaloides* and *E. stepposa*. Perithecial neck cylindrical, longer than 100 µm. Ascospores 13–15.5 × 2.3–3 µm *fide* Monod (1983), with a thin appendage at each end……...*Plagiostoma amygdalinae*

15'.On twigs, branches or stems of the *Euphorbiaceae*. Perithecial neck cylindrical, more or less than 100 µm. Ascospores without appendages……..…….16

16. Perithecial neck shorter than 100 µm. Ascospores (12–)13–13.5(–15.5) × (3–)3.5(–4) µm *fide* Sogonov et al. (2008)…….*Plagiostoma euphorbiae*

16'.Perithecial neck longer than 100 µm. Ascospores 14–17.5 × 3.5–4.5 µm *fide* Monod (1983)…….…….*Plagiostoma euphorbiaceum*

17. On hosts in the *Aceraceae*……..…..18

17'.On hosts other than *Aceraceae*……..…..19

18. On leaves, twigs, and branches of *Acer* spp., in the Pacific Northwest region of USA. Ascospores (11.5–)14–15.5(–17.5) × (2.5–)3.5–4(–4.5) µm *fide* Sogonov et al. (2008)…….....*Plagiostoma barriae*

18'.On leaves, twigs, and branches of *Acer saccharum* and *A. spicatum*, in eastern USA and Canada. Ascospores 7–12 × 1–2.5 µm *fide* Barr 1978……*Plagiostoma petiolophilum*

19. Ascospores with thin deliquescent appendages……..……..…20

19'.Ascospores without thin deliquescent appendages……..……21

20. Ascospores (10–)11–12(–19) × 3–4 µm. Necks eccentric, stout, cone-shaped, surrounded by a whitish stroma. On *Alnus* spp., in the Pacific Northwest region of U.S.A…….……....*Plagiostoma samuelsii*

20'. Ascospores 8–10 × 2–3 µm *fide* Monod (1983). Necks marginal, cylindrical, without whitish stroma. On *Persicaria* and *Polygonum,* rarely on *Rumex* and *Vitis*,.in Europe and U.S.A. (NY)…….…….…..*Plagiostoma devexum*

21. Ascospores with one cell rounded and the other conical, 13–16 × 4–5 µm. In pedicels and branches of *Rhododendron* spp., in Europe…….…….*Plagiostoma rhododendri*

21'.Ascospores not as above…….……22

22. On dead stems of herbaceous plants, specifically *Geranium* spp., in Europe. Ascospores 13–18 × 1.8–2.5 µm *fide* Monod (1983)…….*Plagiostoma geranii*

22’.On twigs and branches of woody plants,.in Europe or North America. …….23

23. Perithecia in groups, with necks closely appressed as a mass emerging together or in a row, and surrounded by a white stroma. On *Aesculus hippocastanum,* in Europe.……..*Plagiostoma aesculi*

23. Perithecia in groups or solitary, with necks emerging together or not, surrounded or not by a pale brown stroma. On hosts other than *Aesculus hippocastaneum,* in Europe or North America…….…..24

24. Perithecia arranged in groups with necks emerging as a group but oriented in different directions and protruding outside host epidermis. Stroma pale brown covering perithecia but not surrounding the necks. On *Corylus californica*…….…….*Plagiostoma exstocollum*

24’. Perithecia arranged in groups or solitary, with necks oriented toward a central point where they slightly protrude outside host epidermis. No stroma observed. On *Staphylea* ……....*Plagiostoma robergeanum*

DESCRIPTIONS OF SPECIES

Plagiostoma Fuckel, Jb. Nassau Ver. Naturk. 23–24: 118. 1870.

Lectotype designated by Höhnel (1917): *Plagiostoma euphorbiae* Fuckel

= *Cryptodiaporthe* Petr., Ann. Mycol. 19: 118. 1921. Lectotype designated by Clements and Shear (1931): *Cryptodiaporthe aesculi* Fuckel now *Plagiostoma aesculi* (Fuckel) Sogonov, Stud. Mycol. 62: 69. 2008.

= *Rostrocoronophora* Munk, Dansk Bot. Arkiv 15: 98. 1953. Type: *R. geranii* Munk, now *Plagiostoma geranii* (Hollos) Sogonov, Stud. Mycol. 62: 72. 2008.

Anamorph: *Diplodina* Westend., Bull. Acad. R. Belg. Cl. Sci. nat., II, 2: 562. 1857. Type species: *Diplodina microsperma* (Johnst.) B. Sutton, Mycol. Pap. 141: 69. 1977. See Sutton 1980, pp. 604–606.

Type species of *Plagiostoma* (synonym *Cryptodiaporthe*)

Plagiostoma euphorbiae (Fuckel) Fuckel, Jb. Nassau Ver. Naturk. 23–24: 118. 1870.

≡ *Sphaeria euphorbiae* Fuckel, Enumeratio fungorum Nassoviae: p. 69. 1860

≡ *Gnomonia euphorbiae* (Fuckel) Sacc., Michelia 2: 312. 1881.

≡ *Gnomoniella euphorbiae* (Fuckel) Sacc., Syll. Fung. 1: 418. 1882

= *Gnomoniella tithymalina* Sacc. & Briard, Revue mycol. 7:209. 1885 *fide* Monod 1983.

New species of Plagiostoma

Plagiostoma dilatatum L. C. Mejía **sp. nov.** Figs. 5.3 A–D; 5.7 I–L.

Latin Diagnosis: Not included.

Diagnosis: Perithecia collapsed from base when dried, globose when moist, (382–)475–572(–642) µm diam ×(277–)320–442(–502) µm high. Perithecial neck short, with ostiole tip punctuate and appearing as a small pale yellowish papilla, (152–)257–308(–327) µm longum, dilated with an expanded area (217–)352–401(–452) µm diam that look like a disc when seen from the top and like a vaulted thick collar when longitudinally sectioned, apice (92–)95–108(–122) µm. Ascus cylindrical. Ascospores (12–)13–15(–22)×(4–)4–5(–7) µm, l:w (2.61–)3.03–3.33(–3.75). Distinctive DNA sequences.

Etymology: dilatatum-dilate; referring to the dilated or expanded area of the perithecial necks that look like a disc when seen from the top and like a vaulted thick collar when longitudinally sectioned.

Perithecia immersed in bark, solitary or aggregated, appearing initially as slight elevation of periderm, initially appearing as a black halo, later developing into a black circular spot, ascomatal apex protrudes through a tiny slit; globose, collapsed from base when dry, (382–)475–572(–642) µm diam × (277–)320–442(–502) µm high (mean = 515 × 383, SD 78.5, 77.8, n1=10, n2=11), each with one neck. Neck central to eccentric, relatively short compared to other species of *Plagiostoma*, with ostiolar opening punctuate, looking like a papilla that protrudes above the epidermis through a tiny slit, with an expansion of the perithecial neck, expanded, initially below epidermis appearing disk-like when seen from top, in longitudinal section, appearing like a vaulted collar, becoming exposed, expansion results in black halo or circular area through epidermis or directly when epidermis gone, sometimes two necks share this expanded area. Above perithecia an intricate black mycelium that may be part of the conidioma develops. This mycelium appears black to the naked eye and dark brown when seen under the microscope and in some pustules this mycelium and the tip and expanded area of the neck become exposed, neck length (152–)257–308(–327) µm (mean = 263, SD 61, n=10), basal diam (217–)352–401(–452) µm (mean = 367, SD 67, n=10), distal diam (92–)95–108(–122) µm (mean = 103, SD 10, n=9); asci cylindrical, (48–)54–62(–77)×(8–)12–14(–18) µm (mean = 58×13, SD 7.2, 2.5, n=15), long stalked, with a rectangular, apical ring, 2.1–4.3 µm diam, with eight ascospores arranged obliquely parallel to multiseriate. Ascospores reniform to oblong elliptical, one-septate, constricted

median to submedian septum, slightly curved and slightly tapering to rounded ends, (12–)13–15(–22) × 4–5(–7) µm (mean = 14.8×4.7, SD 2.5, 1.0, n=48), l:w (2.6–)3.0–3.3(–3.8) (mean = 3.2, SD 0.3, n=48), granular appearance.

Cultures: Moderate to fast growth on PDA, after 7 days a.c.d. 5.2 cm (SD 0.2, n=8). Thin aerial mycelium of velvety to granular texture, whitish to vinaceous buff 86, becoming olivaceous 48 toward the margin. Fasiculate mycelium buff 45 developing from concave central area. Reverse same; 7 days a.c.d. denser mycelium hazel 88 in center, with vinaceous 86, black droplets on surface, with immersed mycelium dark, reverse dark, with a lighter halo and whitish to translucent margin.

Habitat and host: On dead still attached twigs of *Salix irorata* and *S. caprea*

Distribution: **France** (Melle)

Holotype: **France**, Deux-Sèvres Department, Melle, Melle Arboretum, on *Salix irorata*, 15 Apr. 2008, LCM402 (BPI878959, derived cultures LCM402.02= CBS124976, LCM402.01).

Additional specimens examined: **France**, Deux-Sèvres Department, Foret del' Hermitain, on *Salix caprea*, 17 Apr. 2008, LCM403 (BPI878958, derived cultures LCM403.01, LCM403.02).

Notes: The intricate mycelium that develops on top of the perithecia in some pustules resemblea the conidioma of *Diplodina*, the known anamorph for species of *Plagiostoma*.

Plagiostoma exstocollum L. C. Mejía **sp. nov.** Figs. 5.3 E–H; 5.7 M–P.

Latin Diagnosis: Not included.

Etymology: exsto – standing out; collus – neck, referring to the neck on each perithecium that emerges outside the host periderm.

Perithecia immersed in bark, aggregated in groups up to twelve, joined by a scanty brownish to cream stroma, occasionally solitary, appearing as elevations in bark where perithecia push up periderm, making a slit in the periderm, usually elliptical in shape when seen from top, black, suboblate, collapsed from bottom when dried, (219–)269–336(–341) µm diam × (186–)194–227(–278) µm high (mean = 293 × 216, SD 48.6, 30.5, n=9), each with one neck. Necks marginal, relatively long compared to other species of *Plagiostoma*, protruding through slit or crack in substrate, (197–)247–281(–382) µm long (mean = 270, SD 53.9, n=9), base (50–)53–63(–67) µm diam (mean = 58.8, SD 6.08, n=9), apex (39–)44–49(–50) µm diam (mean = 46.1, SD 3.7, n=9), ostiole slightly sulcate. Asci cylindric to clavate, (15–)39–57(–76) × (3.3–)6.7–11(–13) µm (mean = 49.6×8.7, SD 15.1, 2.6, n=26), with a conspicuous refractive apical ring 1.8–3.6 µm diam, with eight ascospores arranged biseriately. Ascospores ellipsoid, two celled, constricted at submedian septum, tapering to rounded ends, (9–)10–15(–16) × 2–3(–4) µm (mean = 12.7×3.0, SD 2.4, 0.7, n=49), l:w (3–)4–4.5(–6) (mean = 4.3, SD 0.4, n=49), usually with at least four refractive circular bodies in each ascospore, two large ones on each side of septum, one smaller one at end of each cell.

Cultures: Moderate to fast growth on PDA, after 7 days a.c.d. 4.3 cm (SD 1, n=16). Thin aerial mycelium appearing velvety, margin fringed, stringy. whitish to buff 45 or vinaceous buff 86 from top, with a slightly to pronounced halo of thick, white mycelium extending about 2 cm from center. Reverse whitish to buff 45.

Habitat and host: On dead, still attached, overwintered twigs of *Corylus californica* (A. DC.) Rose (*Betulaceae*).

Distribution: **U.S.A.** (Oregon)

Holotype: **U.S.A.** Oregon, Jackson Co., Upper Rogue River, River Bridge Campground, on *Corylus californica*, 20 May 2008, LCM468 (BPI878961, derived culture LCM468.01)

Specimens examined: **U.S.A.** Oregon, Jackson Co., River Bridge Campground, Upper Rogue River, on *Corylus californica*, 20 May 2008, LCM469 (BPI878962); on *Corylus californica*, 21 May 2008, LCM422 (BPI878959, derived culture LCM422.02); on *Corylus californica*, 21 May 2008, LCM472 (BPI878963, derived culture LCM472.01); Upper Rogue River trail, on *Corylus californica*, 21 May 2008, LCM473 (BPI878964, derived culture LCM473.01); Oregon, Lane Co., Willamette National Forest, Salmon Creek, 22 May 2008. LCM483 (BPI878965, derived culture LCM483.01); on *Corylus californica*, 23 May 2008, LCM464 (BPI878960, derived culture LCM464).

Plagiostoma imperceptibile L. C. Mejía **sp. nov.** Figs.5.3 I–M; 5.7 Q–R.

Latin Diagnosis: Not included.

Etymology: imperceptibile referring to the hard to find or perceive this species in natural substrate.

Perithecia immersed in bark, solitary, appearing as slight elevations of periderm, that have a central area paler in color and delimited by a black halo in which center just the tip of the perithecial neck protrude, collapsed from base when dried, globose when moist, (385–)412–462(–504) µm diam × (289–)309–356(–414) µm high (mean = 437×338, SD

41.2, 44.2, n=7), each with one neck. Necks central to eccentric, short compared to other species of *Plagiostoma*, with ostiole tip punctate looking like a papilla, protruding above the epidermis through a tiny slit, with an expansion of the perithecial neck initially located below the epidermis that is circular or look like a disc when seen from the top and look like a vaulted thick collar when longitudinally sectioned, becoming exposed later in time, this expansion being responsible for the black halo or circular black spot that can be seen through the epidermis or directly when the epidermis is gone, length (136–)175–211(–225) µm (mean = 189, SD 38.4, n=4), diameter at the most dilated point or expanded area (251–)301–318(–351) µm (mean = 307, SD 36.3, n=5), distal diam (87–)89–100(–113) µm (mean = 97.5, SD 10.5, n=5). Asci ovoidal elongated, often with long, persistent stalk, fertile part of the asci with length×width (67–)76–80(–87)×(13–)18–21(–24) µm (mean = 77.6×19.6, SD 4.9, 3.1, n=11), with an apical ring seen as two refractive bodies, 3.07–4.74 µm diam, with eight ascospores arranged obliquely parallel to multiseriate. Ascospores reniform to oblong elliptical, two celled, constricted at septum, slightly curved and slightly tapering to rounded end, (18–)19–20(–21)×(5–)6–7(–8) µm (mean = 19.6×6.6, SD 0.85, 0.6, n=45), l:w (2.5–)3–3(–4) (mean = 3, SD 0.3, n=45), granular appearance.

Cultures: Moderate growth on PDA, after 7 days a.c.d. 4 cm (SD 0.4, n=4). Thin aerial mycelium of velvety powdery texture, margin stringy, color grey becoming vinaceous buff 86 from the top, reverse isabelline 65.

Habitat and host: On twigs of *Salix* sp.

Distribution: U.S.A. (CA)

Holotype: U.S.A. California, Shasta Co., Cow Creek, close to Old Station, on *Salix* sp., 18 May 2008, L. C. Mejía 456 (BPI878967, derived cultures LCM456.01, and LCM456.02)

Notes: The expansion of the perithecial neck is considered to be the "*coronatum dilatatis*" in the original description of *Sphaeria apiculata* by Wallroth (1833). This morphological character is not a stromatic disc or ectostromatic disc in the sense of Ruhland (1900); it is just an expansion or dilation of the neck. This perithecial neck character is conserved in a clade containing *P. apiculatum* (Wallr.) L.C. Mejía comb. nov., *P. convexum* (Preuss) L.C. Mejía comb. nov., *P. dilatatum* sp. nov., *P. neodilatatum* sp. nov., and *P. pulchellum* (Sacc.) L.C. Mejía comb. nov. (see Fig. 5.1).

Plagiostoma oregonense L. C. Mejía **sp. nov.** Figs. 5.4 A–C; 5.7 S–T.

Latin Diagnosis: Not included.

Etymology: *oregonense* – from Oregon, referring to the only US state where it has been collected.

Perithecia immersed in the bark, solitary, evidenced by the conic shape elevation of the periderm that produce, in which center protrude the upper part of the perithecial neck, black, collapsed from base when dried, globose to subglobose when moist, (369–)381–400(–407) µm diam × (261–)270–326(–373) µm high (mean = 389×304, SD 18.8, 60.1, n=3), each with one neck; neck expanded, with the expanded area usually attached to periderm, eccentric or lateral, length (156–)168–182(–185) µm (mean = 173, SD 15.5, n=3), basal diam (176–)182–204(–221) µm (mean = 195, SD 23.2, n=3), distal diam (119–)119–120(–121) µm (mean = 120, SD 1.0, n=3). Asci cylindric, length×width =

(74–)78–92(–95) × (12–)15–17(–19) µm (mean = 85.8 × 16.2, SD 7.8, 2.13, n=10), with a refractive apical ring that look like a stretched hexagon, 2.82–4.01 µm diam, and eight ascospores arranged obliquely parallel or biseriately. Ascospores broadly elliptical to elliptical, two celled and thick walled, appearing granulated, constricted at median to submedian septum, with rounded ends, (16–)17–19(–22)×(4.5–)5.5–6(–7) µm (mean = 18.2 × 6.0, SD 1.4, 0.5, n=36, n), l:w (2.6–)2.9–3.2(–4.0) (mean = 3.1, SD 0.3, n=36). *Cultures*: Moderate growth on PDA, after 7 days a.c.d. 4.6 cm (SD 0.1, n=2)**.** Thin aerial mycelium of felty texture, margin fringed and stringy, central area white, with a halo of aerial mycelium 1.5 cm from center, marginal area buff 45, reverse with a central circular area of 2 cm diam fawn 87.

Habitat and host: *Salix* sp. (*Salicaceae*)

Distribution: **U.S.A.** (Oregon)

Holotype: **U.S.A.**, Oregon, Lincoln Co., Fogarty Creek, on *Salix* sp., 24 May 2008, LCM597 (BPI878968 derived culture LCM597.01).

Plagiostoma ovalisporum L. C. Mejía **sp. nov.** Figs. 5.4 D–H; 5.7 U–V.

Latin Diagnosis: Not included.

Etymology: ovalis- oval; sporum- spore, referring to the oval shape of the ascospores.

Perithecia immersed in bark, solitary, growing close to each other, or in groups up to five usually in a row, scattered, erumpent, appearing as raised elevations of bark periderm, conic in shape, making a slit or hole where perithecial necks protrude, black, globose when moist**,** collapsed from base when dried, (394–)403–414(–427) µm diam × (246–)277–363(–385) µm high (mean = 409 × 322, SD 11.3, 57.2, n=6), each with one neck.

Necks lateral, relatively short and thick, with ostiole tip cupulate, length (131–)146–159(–162) µm (mean = 151, SD 11.9, n=6), basal diam (125–)136–153(–194) µm (mean = 150, SD 24.3, n=6), distal diam (113–)117–160(–168) µm (mean = 139, SD 24.4, n=6). Asci cylindric to obclavate, length×width (63.3–) 68.4–75.7(–87.7) × (12.5–)14.4–17(–17.9) µm (mean = 72 × 15.7, SD 6.4, 1.7, n=19) with a conspicuous refractive apical ring 3.43–4.49 µm diam, with eight ascospores arranged obliquely parallel to biseriately; Ascospores oval, non-septate, appearing double-walled, more evident when stained with cotton blue lactophenol or Melzer's, (12–)14–16(–17) × 7–8(–9) µm (mean = 14.8×7.6, SD 1.2, 0.5, n=35), l:w (1.6–)1.8–2(–2.2) (mean = 1.9, SD 0.1, n=35).

Cultures: Moderate growth on PDA, after 7 days a.c.d. 4.2 cm (SD 0.1, n=2). Thin aerial mycelium of felty texture, margin fringed and like roots, whitish, with denser mycelium in center within a radius of 1 cm., reverse buff 45 becoming dark grey and whitish in the margin.

Habitat and host: On dead twigs of *Salix* sp. (Salicaceae)

Holotype: **U.S.A.** Idaho, Idaho Co., near Burgdorf, Burgdorf Rd. FR246, parking area at camping site at 3 Mile Creek, (approx. GPS: N45º 18.139 W 115º 55.782), elevation 6309 ft, on dead twigs of *Salix* sp., 05 Sep. 2008 (NAMA Annual Foray, Orson K. Miller Jr. Memorial Foray), A. M. Minnis*, s.n.* (BPI878969, derived culture CBS124977 = LCM458.01)

Notes: This species differs from other species of *Plagiostoma* by having oval, non-septate ascospores. The other two known species of of *Plagiostoma* with non-septate ascospores *P. euphorbia-verrucosae* and *P. fraxini* occur on hosts other than *Salix* and their

ascospores are elliptic fusoid. Unlike in *P. dilatatum*, no circular black halo or spot was observed at the point where perithecia necks emerge through the periderm.

Plagiostoma samuelsii L. C. Mejía **sp. nov.** Figs. 5.5 I–O; 5.8 M–P.

Latin Diagnosis: Not included.

Etymology: in honor of distinguished mycologist Gary J. Samuels for his outstanding contributions to the systematics of Pyrenomycetes.

Perithecia immersed in bark, solitary or in groups up to five, scattered on substrate, evident as conical shaped elevations of host periderm with necks protruding slightly through small hole in periderm, black, subglobose, collapsed from base when dried, (295–)302–327(–334) µm diam × (192–)204–258(–305) µm high (mean = 313×239, SD 16.3, 43, n=6), each with one neck; necks eccentric to lateral, surrounded by a whitish stroma, cone shaped with rounded apex, length (114–)128–161(–170) µm (mean = 145, SD 23, n=6) basal diam (69–)72–74(–81) µm (mean = 73.8, SD 3.9, n=6), distal diam (58–)62–73(–78) µm (mean = 67.5, SD 8.0, n=6). Asci cylindric to clavate, (32–)42–62(–79) × (6–)7–11(–12) µm (mean = 52.8×8.8, SD 13, 2.1, n=24), with a conspicuous refractive apical ring 1.8–3.6 µm diam, with eight ascospores arranged biseriately or obliquely parallel. Ascospores elliptical, one-septate, constricted median to submedian septum, with two deliquescent appendages, one at end of each cell, narrowly filiform, usually twice the length of ascospores, (10–)11–12(–19) × 3–4 µm (mean = 11.8×3.5, SD 1.4, 0.2, n=48), l:w (2.8–)3.2–3.5(–4.8) (mean = 3.4, SD 0.4, n=48), with four refractive bodies in each cell, two big ones on each side of septum, one smaller one at end of each cell.

Cultures: Fast growth on PDA, after 7 days reaching edge of petri plates of 5.3 cm diam (n=8), thin aerial mycelium of felty to very granular texture, fringed plus stringy margin, with concentric halo of denser mycelium 1.4 cm from center, buff 45 inside halo or central region, whitish to white toward margin, some cultures making a depression at concentric halo, reverse honey 64 developing a halo fawn 87 near margin**.**

Habitat and host: Teleomorph on dead still attached twigs and branches of *Alnus* spp. (Betulaceae).

Distribution: **U.S.A.** (CA, OR, WA)

Holotype: **U.S.A.**, California, Plumas Co., Little Last Chance Creek, Chilcook Campground, on *Alnus tenuifolia*, 17 May 2008, LCM454 (BPI878977, derived LCM454.04).

Specimens examined: **U.S.A.** Oregon, Jackson Co., Upper Rogue River, on *Alnus (tenuifolia*?), 21 May 2008, LCM419 (BPI878976, derived culture LCM419.01 and LCM419.02), Upper Rogue River trail on *Alnus* sp. 21 May 2008, LCM474 (BPI878978, derived culture LCM474.01), Washington, Clallam Co., Crescent Lake, on *Alnus rubra* (branch on soil), 27 May 2008, LCM596 (BPI878979, derived culture LCM596.01).

Notes: In contrast to *P. samuelsii*, *P. jensenii* Barr, a species that occurs in leaves of *Alnus rubra*, is not stromatic and has longer, wider ascospores (20–30 × 4–6 μm) with very short pulvinate appendages.

Plagiostoma versatile L. C. Mejía & Sogonov **sp. nov.** Figs. 5.6 A–C; 5.8 Q–V.

Latin Diagnosis: Not included.

Etymology: *versatile* – versatile, referring to the occurrence of this species on different plant organs, twigs, branches and leaves; and to the variable nature of the perithecia that grow with very short necks on twigs and branches and with medium to long necks on leaves.

Perithecia immersed in the bark of twigs or in the midvein and petioles of the adaxial and abaxial side of leaves, solitary or in pairs, scattered, black, on twigs evident as slight elevations of the periderm that may look black because the upper part of the perithecia is just few cell layers below the epidermis and can be seen through, on leaves producing very swollen raised areas on midvein, becoming highly erumpent, cracking periderm and leaving an ellipsoidal cavity when gone, with longer neck than on twigs, in both organs collapsed from base when dried, subglobose when moist, (232–)264–378(–444) µm diam × (178–)194–317(–345) µm high (mean = 323×248, SD 78.1, 68.6, n=8), each with one neck; necks eccentric to lateral, very short compared to other species of *Plagiostoma*, with ostiolar opening sulcate with four grooves, length (60–)77–137(–226) µm (mean = 115, SD 59.7, n=8), basal diam (51–)56–80(–87) µm (mean = 68.7, SD 13.4, n=8), distal diam (37–)49–60(–76) µm (mean = 55.1, SD 11.8, n=8). Asci cylindric to clavate, length×width = (49–)54–66(–71)×(11–)13–16(–20) µm (mean = 60×15, SD 7.66, 2.29, n=15), with a conspicuous apical ring 2.27–3.35 µm diam, with eight ascospores arranged biseriately. Ascospores two celled, elliptical elongated, slightly tapering toward rounded ends, (18–)20–23(–25)×(3–)3–4(–4) µm (mean = 21.5×3.5, SD 2.05, 0.4, n=36), l:w (4.88–)5.57–6.81(–8.63) (mean = 6.24, SD 1.0, n=36), constricted at the median to submedian septum, usually with four big refractive circular bodies, two close to the

septum, one on each cell, and two towards the distal part of the ascospore, one on each cell.

Cultures: Fast growth on PDA, after 7 days reaching the edge of petri plates of 5.3 cm diam (n=12). Thin aerial mycelium of felty to granular texture, margin fringed and like roots, buff 45 with clumps of white mycelium, with a halo of higher elevated mycelium located at 1.5 cm from the center, reverse buff 45 getting dark, with the halo visible from reverse too.

Habitat and hosts: twigs of *Salix scouleriana* and *Salix* sp., overwintered leaves of *Salix* sp. (Salicaceae).

Distribution: **U.S.A.** (OR, WA); **Canada** (BC).

Holotype: **U.S.A.** Washington, Jefferson Co., Intersection of Upper Hoh River Road & Route 101, on *Salix scouleriana*, 27 May 2008, L. C. Mejía 594 (BPI878980, derived culture CBS124978 = LCM594.01).

Additional specimens examined: **Canada**, British Columbia, Vancouver, on overwintered dead leaves of *Salix* sp., 12 May 2006, M. V. Sogonov 379 (BPI877702, derived culture CBS121251 = AR4294); **U.S.A**., Oregon, Lane Co., Willamette Pass, on *Salix* sp., 22 May 2008, LCM598 (BPI878982, derived culture LCM598.01), Washington, Jefferson Co., Hoh River Campground, on *Salix scouleriana*, 27 May 2008, LCM595 (BPI878981, derived culture LCM595.01).

Notes: The ascospores of this species are similar to the ones for *Plagiostoma salicellum* (Fr.) Sogonov but the perithecia on twigs have shorter necks and do not have a stroma surrounding the necks.

Plagiostoma yunnanense L. C. Mejía & Zhu L. Yang **sp. nov.** Figs. 5.6 D–F; 5.8 W–X.

Latin diagnosis: Not included.

Etymology: referring to the place where this species was first collected: Yunnan, China.

Perithecia immersed, solitary or in groups, numerous, appearing as slight conical elevations of host periderm where perithecial necks protrude slightly, black, globose, collapsed from base, (282–)312–352(–362)µm diam × (231–)267–311(–318) µm high (mean = 328×284, SD 41.6, 46.8, n=3), each with one neck; necks eccentric, convoluted, length (315–)318–321(–322) µm (mean = 319, SD 3.78, n=3), basal diam (77–)78–81(–82) µm (mean = 79, SD 2.4, n=3), distal diam (57–)59–63(–66) µm (mean = 61, SD 4.8, n=3). Ascospores elliptic elongated, one-septate, slightly or not constricted at median to submedian septum, with rounded ends, granulated, (19–)23–26(–27) × 3–4 µm (mean = 24×3.3, SD 2.7, 0.4, n=6), l:w (6.6–)6.8–7.9(–8.2) (mean = 7.3, SD 0.7, n=6).

Cultures: moderate growth on PDA, after 7 days a.c.d. 3.4 cm (SD 0.2, n= 4). Thin aerial mycelium of granular texture, margin stringy, whitish with granules becoming grey or vinaceous buff. Reverse with dark inclusions near the center, most of colony whitish.

Habitat and host: On dead still attached branches of *Salix* sp. (Salicaceae)

Distribution: **China** (Yunnan)

Holotype: **China**, Yunnan, Ailoshan, on *Salix* sp., 14 Jul. 2008, LCM513 (BPI878983, derived cultures LCM513.02, and CBS124979 = LCM513.03).

Additional species of Plagiostoma**.**

Plagiostoma aesculi (Fuckel) Sogonov, Stud. Mycol. 62: 69. 2008.

≡ *Cryptospora aesculi* Fuckel, Jb., Nassau Ver. Naturk. 23–24:193. 1870.

≡ *Cryptosporella aesculi* (Fuckel) Sacc., Michelia 1:30. 1877.

≡ *Diaporthe aesculi* (Fuckel) Höhn., Ann. Mycol. 16:116. 1918.

≡ *Cryptodiaporthe aesculi* (Fuckel) Petr., Ann. Mycol. 19:119. 1921

Note: Sogonov et al. (2009) provided a description and illustrations of this species. Illustrations of cultures appear in Figs. 5.7A–B in this work.

Plagiostoma amygdalinae (Fuckel) Sogonov, Stud. Mycol. 62: 70, 2008.

≡*Gnomonia amygdalinae* Fuckel Jb. Nassau Ver Naturk., 23–24: 121, 1870.

≡*Gnomoniella amygdalinae* (Fuckel) Sacc. Syll. Fung. 1:418.1882.

=*Gnomoniella amygdalinae* (Fuckel) Sacc. f. *euphorbiae-stepposae* Sanduville, Studii Cerc. Biol., Bot. 18:18 1966 *fide* Monod 1983.

Note: Monod (1983) provided a detailed description of this species as *Gnomonia amygdalinae*.

Plagiostoma apiculatum (Wallr.) L.C. Mejía **comb. nov.** Figs. 5.2 A–J; 5.7 C–F.

Basionym: *Sphaeria apiculata* Wallr., Fl. Crypt. Germ. II p. 778, 1833.

[= *Sphaeria apiculata* Fuckel, Jb. Nassau Ver. Naturk. 23:24: 115. 1870]

≡ *Metasphaeria apiculata* (Wallr.) Sacc., Syll. 2: 166. 1883.

≡ *Gnomonia apiculata* (Wallr.) G. Winter, Rabenh., Krypt.- Fl. 1(2): 589. 1887.

≡ *Cryptodiaporthe apiculata* (Wallr.) Petr., Ann. Myc. 19: 177, 1921.

Perithecia immersed in bark, black, solitary, aggregated, appearing initially as slight punctiform elevation of periderm surrounded by a black halo with tip of neck protruding through slit, usually with three short radiating slits and paler halo in some collections,

later becoming completely black, globose, collapsed from base when dry, (349–)370–476(–477) µm diam × (223–)252–364(–440) µm (mean = 429×314, SD 59, 77, n=8), each with one neck. Neck central to eccentric, straight to oblique, with a pale brown papilla, with an expanded area that appears disk-like from surface, initially below epidermis, becoming exposed, this expansion being responsible for the black halo that can be seen in the surface, length including expanded area (115–)159–256(–351) µm (mean = 208, SD 77.5, n=8), diameter of expanded area (187–)224–340(–389) µm (mean = 284, SD 73.6, n=8), distal diam (62.5–)81–128(–134) µm (mean = 104, SD 28.8, n=7). Asci cylindrical (45–)51–80(–86)×(4–)10–16(–18) µm (mean = 67.5×12.7, SD 15, 3.8, n=19), apical ring 2.5–4.8 µm diam, variable in shape e.g. elongated as two bodies or hexagonal, with eight ascospores arranged biseriately to multiseriately. Ascospores oblong elliptical, with rounded ends, one median to submedian septum, not constricted, slightly tapering, straight to slightly curved, (12–)16–18.5(–21)×(3–)5–6(–7) µm (mean = 16.7×5.6, SD 2.5, 1.0, n=106), l:w (2.4–)2.9–3.2(–4) (mean = 3.0, SD 0.3, n=106), granular appearance.

Cultures: moderate to fast growth on PDA, after 7 days a.c.d. 5 cm (SD 0.4, n=4). Thin aerial mycelium of velvety granular texture, central area vinaceous buff 45, with scattered black mycelial clumps of 0.5 mm diam in central area, margin white, stringy. Reverse similar but slightly darker.

Habitat and host: On dead twigs and branches of *Salix* spp., *Salix alba, S. dasyclados, S. sitchensis,* and *S. vitellina* (Salicaceae).

Distribution: Europe and North America

Lectotype designated here: BPI799092, as *Sphaeria apiculata* Wallr., ex. Herb. Strasburg. This specimen contains an authograph attributed to Wallroth.

Epitype designated here: **Austria**, Vienna, 21st district, Marchfeldkanalweg, MTB 7764/1, as *Cryptodiaporthe salicella* on *Salix* sp., 20 May 2000, W. Jaklitsch 1463 (BPI747938, derived culture CBS109775 = AR3455).

Exsiccati examined. Fungi Rehnani 918, as *Sphaeria apiculata*, from *Salix vitellina*.

Additional specimens examined: **Austria**, Vienna, St. Margareten im Rosental, Kaernten, Drau-Auen. 9452/1, as *Cryptodiaporthe salicella* on *Salix alba*, 2 May 2002, W. Jaklitsch 1890 (BPI843511, derived culture AR3826), St. Margareten im Rosental, Drau-Auen, Kaernten. 9452/2, as *C. salicella* on *Salix alba*, 14 Apr. 2001, W. Jaklitsch 1741 (BPI872037); **France**, Deux-Sèvres Department, Melle, Melle Arboretum, 15 April 2008, on twigs of *Salix dasyclados*, LCM393 (BPI878951, derived cultures LCM393.01 and CBS124974=LCM393.03); **U.S.A.**, Washington, Kitsap County, Kitsap Memorial State Park, on twigs of *Salix sitchensis*, 28 May 2008, LCM436 (BPI878952, derived culture LCM436.01).

Notes: Fuckel (1870) recircumscribed *Sphaeria apiculata* Wallr. based on Fungi Rehnani 918. This specimen agrees with Wallroth's description of *S. apiculata* and with the specimen of *S. apiculata* autographed by Wallroth. Fungi Rhenani 918 specimen represents *S. apiculata* Wallr. In some collections the perithecia do not present the characteristic disc-shaped expansion of the neck, but instead have a thick neck with a diameter similar to the disc shaped expanded area.

The taxonomy and morphology associated with the name *Sphaeria apiculata* Wallr. have changed several times. Höhnel (1917), Petrak (1921) and later authors

considered this species to have narrowly elongated ascospores while Wehmeyer (1933) recognized this species as a synonym of *Cryptodiaporthe salicina* and hence to have broad elliptic ascospores. The protologue of *S. apiculata* by Wallroth (1833) did not describe the ascospores. The following is an account of why this species has been confused and the results of our study of the original description, type specimen, and relevant later specimens.

The Latin description of *Sphaeria apiculata* Wallr. (1833) includes morphological characters of the perithecia such as apiculate papilla i.e. "*coronatum dilatatis*" here considered to be the disk-shaped expansion of the perithecial neck, and "*nucleo atro*" at the apex. These morphological characters are present in a portion of the type specimen BPI799092 of *S. apiculata* autographed by Wallroth. The fungus on this specimen contains broadly ellipsoid ascospores. This species is distinctive and different from *S. salicella* Fr. as discussed under *Plagiostoma salicellum*. The portion of the type specimen at BPI799092 is herein designated the lectotype and BPI747938 with the derived culture CBS109775 is designated the epitype of *Sphaeria apiculata* Wallr. The new combination *Plagiostoma apiculatum* (Wallr.) L.C. Mejía is formally presented here.

Höhnel (1917) treated *Sphaeria apiculata* Wallr. sensu Fuckel based on the redescription in Fuckel (1870) and argued that this species and *Diaporthe spina* Fuckel (1870) were the same species. He based his arguments on observations of specimens made by Rehm, Krieger, and his own. He acknowledged differences in perithecial neck length among collections of these two species. To review the synonymy of these two species we compared the original description of *D. spina* by Fuckel with the original description and recircumscriptions of *S. apiculata* by Wallroth (1833) as well as Fuckel

(1870). In his original description of *D. spina*, Fuckel (1870) provided a drawing of this species that does not agree with the original description of *S. apiculata* or the recircumscription by Fuckel (1870). Additionally we studied the exsiccati specimen at BPI of C. Roumeguere, Fungi selecti 7019, labelled *D. spina* Fuckel f. *salicis-capreae* F. Fautrey, Epoisses (Côte-d'Or), Avril 1896. The fungus in this exsiccati has a black marginal zone characteristic of *Diaporthe* and its overall appearance is completely different than that of *S. apiculata* Wallr. Based on the comparison of descriptions and the specimen observed, we consider that *S. apiculata* and *D. spina* are not synonyms. In agreement with Fuckel (1870) who treated *S. apiculata* and *D. spina* as two different species, we also consider *S. apiculata* Wallr. and *D. spina* Fuckel to be different species. The synonymy of these two species proposed by von Höhnel (1917) and accepted by Petrak (1921) when he published the combination *Cryptodiaporthe apiculata* (Wallr.) Petrak may be in part the reason later authors considered *S. apiculata* to be characterized by narrow, elongated ascospores. It is not clear from Petrak's writings which specimens of *S. apiculata* he examined, but he described *C. apiculata* (Wallr.) Petrak as having elongated narrow ascospores, as described and observed for *D. spina*. The black marginal zone and ascospore characteristics in the exsiccati of *D. spina* suggest that *D. spina* is not a synonym of *Cryptodiaporthe salicella* sensu Wehmeyer (1933). Additionally *Sphaeria apiculata* Wallr. has been confused with *S. salicella* Fr. as discussed under *P. salicellum*.

Plagiostoma barriae Sogonov, Stud. Mycol. 62: 69, 2008.

Note: Sogonov et al. (2008) provided a description and illustrations of this species. Pictures of cultures appear in Figs. 5.7G–H in this work.

Plagiostoma convexum (Preuss) L.C. Mejía **comb. nov.** Figs. 5.2 K–R.

Basionym: *Sphaeria convexa* Preuss, Linnea 26: 714. 1853.

≡ *Diaporthe convexa* (Preuss) Sacc., Syll. Fung. 1: 630. 1882.

[= *Sphaeria salicina* Curr., Trans. Linn. Soc. Lond., 22: 157, tab. 48, fig. 149, 1858, non *Sphaeria salicina*, Pers., 1796.]

= *Cryptodiaporthe salicina* Wehm. as (Curr.) Wehm., The Genus *Diaporthe* Nitschke and Its Segregates p. 193. 1933.

= *Diaporthe salicis* Nitschke in Fuckel, Fungi Rehnani 1987. 1867. *Fide* Wehmeyer 1933

= *Diaporthe salicella* Sacc., Myc. Ven. Spec. 135. 1873. *Fide* Höhnel, 1917.

= *Diaporthe santonensis* Sacc., Fung. Gall., Ser. 5, 2163. 1884. *Fide* Wehmeyer 1933.

= *Valsa punctata* Cooke, Grevillea 14: 47. 1885. *Fide Wehmeyer* 1933

≡ *Diaporthe punctata* (Cooke) Berl. & Vogl., Syll. Fungorum, Add. 108. 1886.

= *Gnomonia salicella* J. Schröt., Pilze Schles. 3, 2: 392.1897. *Fide* Wehmeyer 1933.

= *Chorostate salicella* Traverso, Fl. Ital. Cryp.t. 2: 203. 1906. *Fide* Wehmeyer 1933.

= *Diaporthe salicella* Sacc. *var. populi-tremulae* Feltg., Vorst. Pilz. Lux. Nachyr. 4:87, 1905. *Fide* Wehmeyer, 1933.

Perithecia immersed in bark, black, solitary or in groups of up to four, appearing initially as slight conic elevation of periderm with apex protruding through a small hole, globose, collapsed from base when dry, (282–)303–352(–415) diam × (180–)213–258(–326) µm high (mean = 329×238, SD 38, 44, n=13), each with one neck. Neck central to eccentric, cylindrical, thickened compared to other species of *Plagiostoma*, being most specimens thicker toward apex and other thicker at other parts of the neck. Necks upright or

diagonally straight or curved, closely appressed when in groups, length (82–)161–204(–222) µm (mean = 176, SD 36, n=13) µm, basal diam (71–)82–104(–121) µm (mean = 95, SD 16, n=13), distal diam (64–)78–108(–128) µm (mean = 93, SD 21, n=13), apex usually paler. Asci clavate, (54–)60–63(–69) × (14–)15–18(–20) µm (mean = 61×17, SD 4.5, 2.2, n=8) apical ring 2.9–3.8 µm diam, with eight ascospores arranged obliquely parallel to multiseriate. Ascospores elliptic fusiform, curved or straight, tapering toward rounded ends, one median to submedian septum, constricted, (16–)18–20(–22) × 4–5 µm (mean = 18.7×4.5, SD 1.2, 0.4, n=51), l:w (3.2–)3.9–4.5(–4.9) (mean = 4.2, SD 0.4, n=51), with four refractive bodies of various shapes, often globose.

Lectotype specimen designated herein: *Sphaeria convexa* Preuss, in Linnea, without other data, ex. Herb. Brussels in Shear study collection types and rarities (BPI799418).

Epitype specimens designated herein: **U.S.A.** New York, Tompkins Co., near Ithaca, Arnot Forest, on *Salix* sp., as *Cryptodiaporthe salicina*, 12 Jul 2002, L. Vasilyeva (BPI 843490, derived culture CBS 123206).

Notes: Wehmeyer (1933) listed 28 synonyms of *Cryptodiaporthe salicina*. Among specimens that Wehmeyer (1933) recognized under that name, Butin (1958) elaborated differences in ascospore morphology, conidial state, host, and ecological characteristics. Within Wehmeyer concept of *C. salicina,* Butin (1958) distinguished three species: *C. apiculata* (Wallr.) Petr., *C. populea* (Sacc.) Butin, and *C. pulchella* (Sacc.) Butin but he did not consider the basionym *Sphaeria salicina* Curr. to be a synonym of any of these three species. On the contrary he listed *Cryptodiaporthe salicina* based on *Sphaeria salicina* Curr. as a synonym of *Cryptodiaporthe salicella* (Fr.) Petr. The three species

distinguished by Butin (1958) are here accepted as *Plagiostoma apiculatum*, *P. populeum* (Sacc.) L.C. Mejía comb. nov., and *P. pulchellum* (Sacc.) L.C. Mejía *comb. nov.*

Here we also recognize another species from *C. salicina* (Curr.) Wehm. This fourth species has ascospores that agree with those drawn by by Wehmeyer (1933 as *C. salicina,* plate XIII, figs. 3, 4, 5). The protologue of *Sphaeria salicina* Curr. (1858), basionym of *C. salicina,* was used to verify its morphology. Based on Currey's (1858) drawing of *Sphaeria salicina,* it is not clear to which species this name should be attributed. In his description of *S. salicina*, Currey (1858) mentions that the septum in the sporidia (ascospores) is "often very difficult to make out", but the ascospores in his drawing have a septum. The rest of his description agrees with the description of another taxon, *S. convexa* Preuss, synonym of *Diaporthe convexa* (Preuss) Sacc. Further it also agrees with a portion of the type specimen of *S. convexa* Preuss (BPI799418 ex. Herb. Brussels with a note on the label saying apparently from Preuss). This evidence suggests that *S. salicina* Curr. (Currey 1858) and *S. convexa* Preuss 1852 represent the same species. Because *S. salicina* Curr. is a later homonym of *Sphaeria salicina* Pers. (1796), the epithet *salicina* cannot be used and the oldest known epithet is *S. convexa;* hence the correct name for this taxon is the new combination *Plagiostoma convexum* (Preuss) L.C. Mejía. The type specimen at BPI799418 is here designated the lectotype and the specimen BPI 843490 with the ex-epitype culture CBS123206 is designated the epitype of *Sphaeria convexa* Preuss.

Butin (1958) considered that the drawing made by Currey represented *Cryptodiaporthe salicella* (Fr.) Petrak (= *Cryptodiaporthe salicina* (Curr) Wehm.). Although Wehmeyer (1933) listed *Sphaeria sphingiophora* Oudem. (1873) (= *Diaporthe*

sphingiophora (Oudem.) Sacc.) as a synonym of *C. salicella*, *S. sphingiophora* occurs on *Cornus*, so it is unlikely to be the same species as *P. convexum*. *Diaporthe cupulata* Berl. & Destrée was considered a synonym of *Sphaeria convexa* by Wehmeyer (1933), however, the ascospore sizes of these species are different. We do not consider them to be synonyms. The specimen at BPI of *Sphaeria salicina* Pers., Scleromyceti Sueciae 10 was examined and determined to be a species of *Valsa*.

Plagiostoma devexum (Desm.) Fuckel, Jb. Nassau Ver. Naturk. 23–24: 119. 1870.

≡ *Sphaeria devexa* Desm., Cryptog. De France, Edit. II, Ser. II, No. 367. 1856

≡ *Gnomonia devexa* (Desm.) Auersw. in Gonn. & Rabenh., Mycol. Europ. 5/6: 23. 1869.

≡ *Gnomoniella devexa* (Desm.) Sacc., Syll. Fung. 1: 417. 1881.

≡ *Gnomoniopsis devexa* (Desm.) Moesz & Smarods, Bot. Közl. 38: 68. 1941.

= *Sphaeria euphorbiae* f. *polygoni* Fuckel, Fungi Rhenani 864. 1864 *fide* Monod 1983.

= *Sphaeria excentrica* Cooke & Peck, Ann. Rep. New York State Museum 25: 105. 1873 *fide* Monod 1983.

≡ *Gnomoniella excentrica* (Cooke & Peck) Sacc., Syll. Fung. 1: 418. 1882.

= *Diaporthe sechalinensis* Sacc., Atti. Del Congr. Bot. Di Palermo 1902: 52. 1902 *fide* Monod 1983.

= *Ceriosporella polygoni* A. L. Sm. & Ramsb., Trans. Br. Mycol. Soc. 4: 325. 1914 *fide* Monod 1983.

Note: Barr (1978) and Monod (1983) provided detailed descriptions of this species.

Plagiostoma euphorbiaceum (Sacc. & Briard) Sogonov, Stud. Mycol. 62: 72. 2008.

≡ *Gnomonia euphorbiacea* Sacc. & Briard, Revue Mycol. 7: 208. 1885.

Note: Monod (1983) provides a detailed description of this species.

Plagiostoma euphorbiae-verrucosae (M. Monod) Sogonov, Stud. Mycol. 62: 72. 2008

≡ *Gnomoniella euphorbiae-verrucosae* M. Monod, Beih. Sydowia 9:42. 1983.

Note: Monod (1983) provided a detailed description of this species.

Plagiostoma fraxini (Redlin & Stack) Sogonov, Stud. Mycol. 62: 72. 2008.

≡ *Gnomoniella fraxini* Redlin & Stack, Mycotaxon 32:185. 1988.

Note: Redlin & Stack (1988) provided a detailed description of this species as *Gnomoniella fraxini*.

Plagiostoma geranii (Hollós) Sogonov, Stud. Mycol. 62: 72. 2008.

≡ *Gnomonia geranii* Hollos, Annls. Mus. Nat. Hung. 7: 52. 1909.

Note: Müller & Arx (1962) and Monod (1983) provided detailed descriptions of this species as *Gnomonia geranii*.

Plagiostoma petiolophilum (Peck) Sogonov, Stud. Mycol. 62: 72. 2008.

≡ *Sphaeria petiolophila* (Peck) Berl. & Voglino, Syll. Fung. Addit. 1–4: 90. 1886.

≡ *Cryptodiaporthe petiolophila* (Peck) Barr, Mycol. Mem. 7: 136. 1978.

Note: Barr (1978) provided a detailed description of this species as *Cryptodiaporthe petiolophila*.

Plagiostoma populeum (Sacc.) L. C. Mejía **comb. nov.**

≡ *Diaporthe populea* Sacc. in Mouton, Bull. Soc. Roy. De Bot. Belg. 26 174. 1887.

≡ *Cryptodiaporthe populea* (Sacc.) Butin, Sydowia 11 (1–6): 31. 1958 [1957]

≡ *Cryptodiaporthe populea* (Sacc.) Butin, Nachrictenblatt des Deutschen Pflanzenschutzdienstes 9: 70. 1957.

= *Chorostate populea* Traverso, Fl. Ital. Cryptogam. 2: 204. 1906.

Moderate to fast growth on PDA. After 7 days a.c.d. 3.3 cm (SD 1.2, n=8). Thin aerial mycelium of velvety or felty texture, whitish to buff 45 or rosy buff 6 in the central area and isabeline 65 in the margin, with some droplets (honey 64) in the center, with fringed margin appearing like roots. Reverse whitish to fawn 87 or honey 64, in some cultures becoming dark and with a concentric halo light. Cultures illustrated here in Figs. 5.8A–D.

Note: Butin (1958) presents a full description with illustrations of this species under *Cryptodiaporthe populea.*

Plagiostoma pulchellum (Sacc.) L. C. Mejía **comb. nov.** Figs. 5.4 I–M; 5.8 E–J.

Basionym: *Diaporthe pulchella* Sacc., Atti Istit. Veneto Sci. 2, Ser. 6, 437. 1884.

≡ *Cryptodiaporthe pulchella* (Sacc.) Butin, Phytop. Z. 32: 407. 1958.

= *Diaporthe recedens* Sacc., Ann. Mycol. 12, 290 1914 *fide* Butin 1958.

Perithecia immersed in bark, black, solitary, often growing close together, appearing initially as slight elevation of periderm, with black halo or black spot where perithecia apex protrudes through a small hole, globose, collapsed from base when dry, (467–)483–

642(–660) µm diam × (311–)371–473(–613) µm high (mean = 563×435, SD 99, 128, n=4), each with one neck. Neck central to eccentric, straight to oblique, with an expanded disk-like area, initially below epidermis, becoming exposed with time, producing black halo or spot in surface, length of expanded area (169–)173–319(–388) µm (mean = 257, SD 105, n=4), diam of expanded area (153–)209–256(–306) µm (mean = 231, SD 62.5, n=4), distal diam (93–)99–160(–212) µm (mean = 137, SD 54, n=4). Asci ovoidal elongated, (75–)85–107(–117) × (15–)17–21(–24) µm (mean = 95×19, SD 13.4, 2.9, n=15), apical ring 4–4.8 µm diam, very thick, with eight ascospores arranged obliquely parallel to multiseriate. Ascospores oblong elliptic-elongated, with rounded ends, one median to submedian septum, not constricted, slightly tapering, straight to slightly curved, (17–)18–22(–27) × (5–)6–7(–7.5) µm (mean = 20.3×6.3, SD 2.9, 0.6, n=39), l:w (2.5–)2.9–3.4(–4.4) (mean = 3.2, SD 0.4, n=39), granular appearance.

Cultures: Moderate growth on PDA, after 7 days a.c.d. 3.9 cm (0.8 n=6), thin aerial mycelium whitish to rosy vinaceous 58 color, of velvety, granular texture due to mycelial clumps ca. 500 um diam, isabeline 65, produced in central area of 2.4 cm diam, central area appearing often moist, margin translucent to buff 45, with hyphae extending radially, stringy, becoming fringed toward margin. Reverse whitish to rosy vinaceous 58 or olivaceous. At 14 days small black and dark green slimy droplet surrounded by a second halo rosy vinaceous 58 with white greysh margin, reverse same color pattern.

Specimens examined: **Argentina,** Tucumán, vicinity of Villa Nougués, on twigs of *Salix humboldtiana*, 16 Nov. 2008; LCM623 (BPI878974, derived cultures LCM623.01 and 623.03); **Netherlands,** CBS170.69 as *Cryptodiaporthe pulchella* on *Populus balsamifera*; **U.S.A.**, Maryland, Prince George's Co., Beltsville, USDA-BARC, outside

of B011A, on twigs of *Salix babylonica*, 03 March 2008, Amy Y. Rossman & L. C. Mejía 365 (BPI878971, derived culture LCM365.04), Maryland, Prince George's Co., Greenbelt, Lake Artemisia, on twigs of *Salix babylonica*; 15 March 2008, LCM371 (BPI878972, derived culture LCM371.02), Washington, Kitsap Co., Kitsap Memorial State Park, on twigs of *Salix lucida*, 28 May 2008, LCM438 (BPI878973, derived cultures LCM438.03 and LCM438.04).

Notes: Butin (1958) refers to this species as saprobic on *Populus* spp. The evidence presented in this study shows this species also infects *Salix* spp. and its geographic distribution is wider than previously thought.

Plagiostoma rhododendri (Auersw.) Sogonov, Stud. Mycol. 62: 72. 2008.

≡ *Gnomonia rhododendri* Auersw. in Gonn. & Rabenh., Mycol. Europ. 5/6: 26. 1869.

≡ *Apiognomonia rhododendri* (Auersw.) Remler, Biblioteca Mycologica 68: 74. 1979.

Note: Remler (1959 as *A. rhododendri*) and Monod (1983 as *G. rhododendri*) present a full description of this species.

Plagiostoma robergeanum (Desm.) Sogonov, Stud. Mycol. 62: 73. 2008.

≡ *Sphaeria robergeana* Desm., Ann. Sci. Nat., ser. 3, 16: 306. 1851.

≡ *Diaporthe robergeana* (Desm.) Niessl. In Rabenh., Fungi Europ. 2222. 1882.

≡ *Cryptodiaporthe robergeana* (Desm.) Wehm., The Genus *Diaporthe* Nitschke and Its Segregates p. 200. 1933.

Note: Wehmeyer (1933) provides a description of this species as *Cryptodiaporthe robergeana.*

Plagiostoma salicellum (Fr.) M. V. Sogonov, Stud. Mycol. 62: 73. 2008. Figs. 5.5 A–H.

Basionym: *Sphaeria salicella* Fr., Syst. Myc. II, 377, 1823.

≡ *Cryptodiaporthe salicella* (Fr.) Wehm., The Genus *Diaporthe* Nitschke and Its Segregates p. 193. 1933.

= *Cryptodiaporthe populina* Petr., Ann. Myc. 19: 117. 1921 *fide* Wehmeyer, 1933.

Diagnostic features: Perithecial neck cylindrical and surrounded by a whitish "stroma". Ascospores elliptical elongated (some looking cylindrical), slightly tapering towards rounded ends, one septate, slightly constricted. Asci obliquely parallel. A very short "appendage" 1.5 – 2 µm can be seen in some ascospores.

Perithecia immersed in bark, solitary or in groups up to five, scattered, black, evident as slight elevations of periderm, subglobose, collapsed from base when dried, (339–)372–410(–507) µm diam × (157–)208–308(–331) µm (mean = 397×257, SD 43, 60, n=11), each with one neck; necks cylindrical, eccentric to lateral, surrounded by a whitish stroma, length (96–)147–202(–308) µm (mean = 177, SD 67, n=11), basal diam (61–)80–84(–95) µm (mean = 81, SD 8.8, n=11), distal diam (54–)74–85(–91) µm (mean = 79, SD 10, n=11). Asci cylindric to clavate, (40–)51.5–59(–63) × (11–)13–14(–15) µm (mean = 55×13, SD 5.8, 1.4, n=15), with apical ring 2.1–3.3 µm diam, with eight ascospores obliquely parallel or irregularly seriate. Ascospores elliptic elongated, slightly tapering toward rounded ends, one-septate, often with short appendages 1.5–2 µm, slightly constricted at median to submedian septum, (14–)17–20(–27) × 2.8–3.8(–5) µm (mean = 18.7×3.3, SD 2.3, 0.5, n=57), l:w (3.2–)5.2–6.6(–8.7) (mean = 5.9, SD 1.1, n=57), granular appearance.

Lectotype of Sphaeria salicella *designated here:* Scleromyceti Sueciae 188 issued 1821, Sbarbaro Collection (BPI exsiccati).

Epitype designated here: **Austria** St. Margareten im Rosental, Kaernten, Drau-Auen. 9452/1, as *Cryptodiaporthe apiculata* on *Salix alba*, 02 May 2002, Jaklitsch, W. 1889 (BPI843527, derived culture CBS121466), Genbank ITS EU254996.

Notes: The application of the name *Sphaeria salicella* Fr. has been the source of confusion and the subject of taxonomic studies since the 1800s. It was clearly specified by Fries (1823) that this name is typified by to Fries: Scleromyceti Sueciae 188 published in 1823. Fries (1823) did not include a description of the ascospores of *S. salicella*. According to Wehmeyer (1933) and Butin (1958) confusion with regard to which species this name should be applied was in part due to the fact that different portions of exsiccati Scleromyceti Sueciae 188 contain one or two different species. One of these species has elliptic, elongated i.e. narrow ascospores and the other has broad ellipsoidal ascospores. While not recognizing the confusion regarding the ascospores of the two species of Scleromyceti Sueciae 188, Petrak (1921) suggested that *S. salicella* Fr. was characterized by having broadly elliptic ascospores and made a new combination *Cryptodiaporthe salicella* (Fr.) Petrak. In the same publication under his treatment of *Gnomonia apiculata* (Wallr.) G. Winter, Petrak (1921) made the new combination *C. apiculata* (Wallr.) Petr. based on *S. apiculata* Wallr. The latter species was considered to have elongated, elliptic ascospores. This is the other ascospore morphology that has been observed in Scl. Suec. 188 and has been confused with the broad, elliptic ascospores. No type specimen was designated for *S. apiculata* and it is not clear if Petrak (1921) looked at specimens of *S.*

apiculata or if he based his conclusions on the description of *S. apiculata* by Wallroth (1833). Neither Fries' description of *S. salicella* nor Wallroth's description of *S. apiculata* contains a good description of the ascospores. Wehmeyer (1933) arrived at a conclusion different from that of Petrak (1921). Wehmeyer studied the exsiccati Scleromyceti Sueciae. 188 at the Farlow Herbarium and determined that this number and hence *S. salicella* Fr. was characterized by having elliptic elongated ascospores. To use his words, *S. salicella* represents "the narrow-spored species". He synonymized *S. salicella* Fr. with *C. apiculata* (Wallr.) Petrak, and created the combination *C. salicella* (Fr.) Wehm. (1933) non Petrak (1921). In addition, Wehmeyer made the new combination *C. salicina* (Curr.) Wehm. based on *S. salicina* Curr. for species having broad elliptic ascospores (see notes under *P. convexum*). Later Butin (1958) studied species of *Cryptodiaporthe* on *Populus* and *Salix,* examined Scl. Suec. 188 at Uppsala Herbarium, and determined that *S. salicella* Fr. should be understood as a species with broad elliptic ascospores and followed Petrak's concept of *S. salicella* Fr.

As part of the present monographic work, we studied the Scleromyceti Sueciae 188 (Sbarbaro collection) available at the BPI Herbarium as well as other exsiccati from taxa that have been synonymized with *S. salicella* Fr. and *C. salicina* (Curr.) Wehm. including *S. apiculata* Wallr. In doing that we paid close attention to the Latin descriptions of *S. salicella* Fr. and *S. apiculata* Wallr. Neither Wallroth (1833), Petrak (1921), Wehmeyer (1933), or Butin (1958) refer to a type specimen of *S. apiculata* Wallr. in their studies of this species. We found and studied a specimen of *S. apiculata* Wallr. authographed by Wallroth as discussed under *Plagiostoma apiculata*. While neither Fries' description of *S. salicella* nor Wallroth's description of *S. apiculata* contain

information on ascospores, their Latin descriptions contain detail that that previous authors may have been overlooked that helps to identify the species to which the original authors were referring. In referring to Scleromyceti Sueciae 188 Fries (1823) describes *S. salicella* as having aggregated perithecia with erumpent, cylindrical ostioles. He states that this species is characterized by having a powdery "albicant" (whitish) stroma and that multiple ostioles are "erumpent simultaneously". The specimen of Scleromyceti Sueciae 188 at BPI contains all of these morphological characters; furthermore its ascospores are elliptic elongated (see Fig. 5.5 A, B, D, and G for images of this specimen). These observations of *S. salicella* Fr. agree with the concept of Wehmeyer (1933) for this species as *C. salicella* (Fr.) Wehm. but disagree with the interpretations made by Petrak (1921) and accepted by Butin (1958).

In their treatment of *Plagiostoma*, Sogonov et al. (2008) made the combination *Plagiostoma salicellum* (Fr.) Sogonov. This species is based on the original description of *S. salicella* by Fries and the type specimen Scleromyceti Sueciae 188 that matches his description, which have elliptic elongated ascospores. We herein designate the specimen of Scleromyceti Sueciae 188 at BPI (Sbarbaro collection) as the lectotype of *S. salicella* and BPI 843527 (W. Jaklitsch 1889 and derived culture CBS121466) as the epitype of *P. salicellum* Fr. (see Fig. 5.5 A–H for illustrations). In our study of this species we noticed that ascospore length and width can be quite variable, even within an ascus, but with a prevalence of the elongated ascospores (See Figures 5.5 A–H). During the course of this study, we collected a specimen (BPI 878975=LCM449) here identified as *P. salicellum* (Fr.) Sogonov on *Salix repens* in Frankfurt, Germany. This specimen matches the original description by Fries of *P. salicellum* and DNA data from four genes place it as

conspecific with the epitype of *P. salicellum* designated here and within a major clade containing two other species also characterized by having cylindrical ostioles and elliptic elongated ascospores. However the ascospores of this specimen are elliptic but not elongated. In spite of this difference in ascospore morphology from the type specimen designated by the original author, we conclude that the specimen from Germany is *P. salicellum* because of the shared character of cylindrical perithecial neck surrounded by a whitish stroma and the multigene phylogeny.

In summary *P. salicellum* (Fr.) Sogonov is characterized by having cylindrical perithecial necks surrounded by a whitish stroma and ascospores predominantly elliptic-elongated, less commonly elliptic tapering to slightly acute rounded ends, unlike *P. apiculatum* that has oblong elliptical to reniform, broadly rounded ascospores.

Species excluded from *Cryptodiaporthe* and *Plagiostoma*

Cryptodiaporthe acerinum J. Reid & Cain, Canad. J. Botany 40:839. 1962.

A fresh specimen determined to be this species was cultured and sequenced. Analyses of LSU and RPB2 sequences place this species in a basal branch in the Gnomoniaceae. See Figure 1.1.

Specimen examined: USA: New York, Adirondacks, Cranberry Lake, on dead branch of Acer sp., 13 Jun 2002, coll. L. Vasilyeva (BPI 870989, ex culture CBS 121465).

Cryptodiaporthe aculeans (Schwein.) Wehm., The Genus *Diaporthe* Nitschke and its Segregates 212. 1933.

≡ *Sphaeria aculeans* Schwein., Trans. Am. Phil. Soc., New Series **4**(2): 204. 1832. [1834]

The only available culture of this species was sequenced. Analyses of LSU sequences place this species in a clade sister to the Melanconidaceae. See Figure 1.1.

Specimen examined: JAPAN: on branch of *Rhus javanica*, isol. G. Okada (CBS 525.85).

Cryptodiaporthe aubertii (Westend.) Wehm., The Genus *Diaporthe* Nitschke & its Segregates 202. 1933.

≡ *Sphaeria aubertii* Westend., Bull. Acad. R. Sci. Belg., Cl. Sci., sér. 2: tab. 7, no. 5 (1859)

An culture of this species was sequenced. Analyses of LSU sequences suggest it is related to the genus *Cryptosporella* within the Gnomoniaceae; however, this relationship is not well supported. See Figure 1.1.

Specimen examined: SWEDEN: Småland, on *Myrica gale*, 14 Apr 1989, coll. K. & L. Holm, isol. O. Constantinescu, 89–53 (CBS 114196).

Cryptodiaporthe galericulata (Tul. & C. Tul.) Wehm., The Genus *Diaporthe* Nitschke & its Segregates 211. 1933.

≡ *Valsa galericulata* Tul. & C. Tul., Select. Fung. Carpol. (Paris) 2:203. 1863.

A fresh specimen determined to be this species was cultured and sequenced. Analyses of LSU sequences suggest this species belongs in the Sydowiellaceae. See Figure 1.1.

Specimen examined: USA: Tennessee, Great Smoky Mts. National Park, near Cosby, Horse Trail, on *Fagus grandifolia*, 25 Mar 2002, coll. L. Vasilyeva (BPI 863767 ex culture AR 3811).

Cryptodiaporthe liquidambaris Petr., Sydowia 5: 236. 1951.

A fresh specimen determined to be this species was cultured and sequenced. Analyses of LSU sequences place this species within the Diaporthales but not within any described family. See Figure 1.1.

Specimen examined: USA: Maryland, on overwintered twig of *Liquidambar styraciflua*, 15 May 2001, coll. M. Barr, isol. A. Rossman AR 3648 (BPI 749123).

Cryptodiaporthe macounii (Dearn.) Wehm., The Genus *Diaporthe* Nitschke & its Segregates 191. 1933.

≡ *Diaporthe macounii* Dearn., Mycologia 8: 100. 1916.

This species was recently included in the genus *Gnomoniopsis* (Gnomoniaceae) by Sogonov et al. (2008). See Figure 2.1.

Cryptodiaporthe vepris (Delacr.) Petr., Ann. Mycol. 32: 445. 1934.

≡ *Sphaeria vepris* Delacr., Fungi europ. 443. 1859.

A fresh specimen determined to be this species was cultured and sequenced. Analyses of LSU sequences place this species within the Diaporthales but not within any described family (tree not shown).

Specimen examined: AUSTRIA: Wograda, St. Margareten, Kaernten, on *Rubus idaeus*, 27 Oct 2000, coll. W. Jaklitsch 1661, isol. A. Rossman AR 3559 (BPI 749132).

Plagiostoma acerophilum (Dearn. & House) Barr, Mycol. Mem. 7: 113. 1978.

≡ *Gnomoniopsis acerophila* Dearn. & House, Bull. New York State Mus. 233–234: 36. 1921.

A fresh specimen determined to be this species was cultured and sequenced. This species is *incerta sedis* within the Gnomoniaceae according to analyses of LSU sequences. The perithecial neck of this species is lateral, upright, and slightly curved in the tip.

Plagiostoma alneum (Fr.) Arx is now *Gnomonia alnea* (Fr.) Sogonov.

This species is described and illustrated in Sogonov et al. (2008).

Plagiostoma arnstadtiense (Auersw.) M. Monod, Beihefte Sydowia 9: 143. 1983.

≡ *Gnomonia arnstadtiensis* Auersw. In Gonnerm. & Robenh., Mycol. Europ. 5/6: 22. 1869.

The classification of this species is *incerta sedis.*

Plagiostoma bavaricum (Rehm) M.E. Barr, Mycol. Mem. 7: 112. 1978.

≡ *Hypsopila bavarica* Rehm, Ann. Mycol. 6:322. 1908.

The classification of this species is *incerta sedis* within the Gnomoniaceae.

Specimen sequenced: SWITZERLAND, on *Acer opalus*, M. Monod (CBS772.79)

Plagiostoma conradii (Ellis) M.E. Barr, Mycol. Mem. **7**: 107. 1978.

≡ *Diaporthe conradii* Ellis, Am. Nat. 17: 316. 1883.

A fresh specimen determined to be this species was cultured and sequenced. Analyses of LSU suggest this species is close related to *Cryptodiaporthe aubertii* and to the genus *Cryptosporella* but *incerta sedis* within the Gnomoniaceae. It is reported on *Hudsonia* spp. in North America. The perithecial neck of this species is lateral upright.

Specimen examined: USA: New Jersey, on living stems of *Hudsonia tomentosa*, coll. G. Bills (BPI 746482, culture CBS 109761 = AR 3488).

Plagiostoma inclinatum (Desm.) M.E. Barr, Mycol. Mem. 7: 115. 1978.

≡ *Sphaeria inclinata* Desm., Ann. Sci. Nat. Bot. III, 16: 315. 1851.

This species was recently determined to be closely related to a clade containing several species of but not the type of *Pleuroceras* within the Gnomoniaceae (tree not shown).

Plagiostoma lugubre (P. Karst.) Bolay, Ber. Schweiz. Bot. Ges. 81: 436. 1972.

≡ *Gnomonia lugubris* P. Karst., Mycol. Fenn. 2: 121. 1873.

The classification of this species is *incerta sedis.*

Plagiostoma magnoliae (Ellis) M.E. Barr, Mycol. Mem. 7: 117. 1978.

≡ *Gnomonia magnoliae* Ellis, Amer. Nat. 17: 318. 1883.

The classification of this species is *incerta sedis*. This species has been reported in leaves of *Magnolia virginiana* in North America. The perithecial neck of this species is lateral, and obliquely upright, as drawn by Barr (1978).

Plagiostoma micromegalum (Ellis & Everh.) M.E. Barr, Mycol. Mem. 7: 112. 1978.

≡ *Diaporthe micromegala* Ellis & Everh., Proc. Acad. Nat. Sci. Philadelphia 1893: 449. 1894.

The classification of this species is *incerta sedis*. This species has been reported on *Carya* spp. in North America. The perithecial neck of this species is lateral, proyected upright, elongated, and terminally curved.

Plagiostoma petrakii (E. Müll.) M. Monod, Beihefte Sydowia 9: 146. 1983.

≡ *Plagiostigme petrakii* E. Müll., Sydowia 18:90. 1965.

The classification of this species is *incerta sedis*.

Plagiostoma pseudobavaricum M. Monod, Beihefte Sydowia 9: 151. 1983.

Analyses of ITS sequences suggest this species is close related to *Apiognomonia* and *Plagiostoma* but representing a different genus within the Gnomoniaceae (tree not shown).

Plagiostoma robertiani (Petr.) M.E. Barr, Mycol. Mem. 7: 113. 1978.

≡ *Gnomonia robertiana* Petr., Ann. Mycol. 23: 122. 1925.

The classification of this species is *incerta sedis*.

Plagiostoma tormentillae (Lind) Bolay is now *Gnomoniopsis tormentillae* (Lind) Sogonov.

DISCUSSION

The present study provides: 1) for the first time a robust multigene phylogeny for species of *Plagiostoma*; 2) a broad set of characters that define this genus and its species; 3) descriptions for eight new species; 4) four new combinations; and 5) an association of a subclade of *Plagiostoma* with members of the Salicaceae. As part of this research, the extensive and controversial synonymy under *C. salicella* (Fr.) Wehm. and *C. salicina* (Wall.) Wehm. was reviewed and clarified. In addition to the three species identified by Butin (1958) under *C. salicina*, namely *P. apiculatum*, *P. populeum*, and *P. pulchellum,* the multigene phylogeny also supports a fourth species, *P. convexum*. *Plagiostoma apiculatum* is here recircumscribed to follow the original author's description and concept of this species based of an examination of a portion of the type specimen. The recognition of these four species formerly described under *Crytodiaporthe salicina* broadens the range of morphological, biological, and ecological traits of the genus *Plagiostoma*. First *P. populeum* is a pathogen of poplars in contrast with the non-pathogenic behavior of most species of *Plagiostoma*. Second, *P. apiculatum* as well as *P. pulchellum* and other species described here for the first time are characterized by having an expanded perithecial neck, a morphological trait not previously recognized in species of *Plagiostoma.* Third, *P. pulchellum* is identified here as the first species of Gnomoniaceae reported to occur in South America as well as the first species of this family from the southern hemisphere to be confirmed by molecular studies.

Morphological characters that are phylogenetically informative for subclades of *Plagiostoma* include the expanded neck characteristic of species with broad ellipsoid ascospores and cylindrical necks characteristic of species with narrowly ellipsoidal

ascospores. Presence of stromatic tissues is diagnostic in species such as *P. aesculi*, *P. salicellum*, and *P. samuelsii*.

In contrast to previous concepts of *Plagiostoma* by Barr (1978), Fuckel (1870), Monod (1983), and von Arx (1951) but in agreement with Sogonov et al. (2008), the genus now includes species with aseptate ascospores. Species with aseptate ascospores were included in *Gnomoniella* by Monod (1983). Monod (1983) emphasized ascospore characteristics in differentiating genera and species in the Gnomoniaceae. Barr (1978) emphasized perithecia morphology and position in substrate as well as ascospore morphology. Furthermore DNA sequence data from multiple genes also support the inclusion of species that develop stroma, most of which were formerly considered species of *Cryptodiaporthe*.

Monod's (1983) concept of *Plagiostoma* differed significantly from the concept presented here. Monod (1983) excluded *P. euphorbiae* from the genus and considered this species to be a *Gnomonia*. In doing that he proposed *P. devexum* as the type species because he considered this species to be a better representative of the genus and because it was simultaneously described with *P. euphorbiae* by Fuckel (1870) when described the genus. Thirteen species were treated by Monod (1983) under *Plagiostoma*, nine species from Europe and four species from North America. Among these thirteen species, only *Plagiostoma devexum* is accepted here as *Plagiostoma*. Two species, *P. alneum* and *P. tormentillae,* were transferred to *Gnomonia* and *Gnomoniospsis* respectively by Sogonov et al. (2008). Five species, *P. acerophilum*, *P. bavaricum*, *P. conradii*, *P. inclinatum*, and *P. pseudobavaricum,* are here recognized as Gnomoniaceae by LSU sequences but cannot yet be placed in a genus (see Figure 1.1).

Six species formerly considered under *Cryptodiaporthe* by Wehmeyer (1933) are placed in *Plagiostoma* based on the multigene phylogeny presented in Figure 5.1. Among these, *P. aesculi* and *P. salicellum* were formerly proposed in a previous study (Sogonov et al. 2008) and the new combinations *P. apiculatum*, *P. convexum*, *P. populeum*, and *P. pulchellum* are proposed here. One of the species treated by Wehmeyer as *Cryptodiaporthe*, *C. macounii*, was transferred to *Gnomoniopsis* (Sogonov et al. 2008). Six other species treated by Wehmeyer under *Cryptodiaporthe*: *C. acerinum*, *C. aculeans*, *C. aubertii*, *C. galericulata*, *C. liquidambaris*, and *C. vepris* are not supported as *Plagiostoma* on the basis of ITS and LSU and their close relatives were listed in the taxonomic section.

Sogonov et al. (2008) accepted a total of 13 species of *Plagiostoma*. The name "*Plagiostoma apiculatum*" appears in the multigene tree of that publication, however, was not included in the taxonomic section nor described or formally proposed as a new combination. The combination *P. apiculatum* is formally presented here. Of all the species of *Plagiostoma* accepted only *P. euphorbiae* and *P. devexum* were originally considered *Plagiostoma*. *Plagiostoma barriae* was described as a new species in Sogonov et al. (2008). Data from ITS sequences suggest that culture CBS791.79 (*P. amygdalinae*) and culture MS196 (= CBS121241 *P. euphorbiaceum*) belong to the same species. In his discussion of *P. amygdalinae* Sogonov et al. (2008) emphasizes the similarity between *P. amygdalinae* and *P. euphorbiaceum* and did not provide features to distinguish them. They refer to Monod (1983) for a description of *P. amygdalinae* and *P. euphorbiaceum* and the ascospore sizes provided for these two species overlap.

This study has shown a clear association between a clade composed of 11 species of *Plagiostoma* and the host family Salicaceae (Fig 5.1). This is in line with our suggestion of specific associations between lineages of Gnomoniaceae and lineages of plants. Furthermore eight new species were found on species congeneric with known hosts of Gnomoniaceae. This supports our prediction that new species of Gnomoniaceae are likely to be found on plant species congeneric with known host species and in localities where these known host species naturally occur. The broad geographic distribution of species of *Plagiostoma* in association with specific plant lineages suggests a long evolutionary relationship between Gnomoniaceae and some plant families, certainly with the Salicaceae. Long evolutionary associations between fungal endophytes that inhabit bark as do species of *Plagiostoma* and their hosts have been examined but have not been fully documented. This work is a first step toward such documentation.

Species of *Plagiostoma* occurs in a broad range of plant families within the core eudicots. However, most species are associated with Rosids. Some genera or subgeneric clades of species of Gnomoniaceae appear to have speciated primarily on specific genera within plant families such as *Cryptosporella* on *Alnus* and *Betula* (Betulaceae) (Mejía et al 2008, and Chapter 4), *Gnomonia* on the Coryiolidae (Betulaceae) (Sogonov et al. 2008), and a clade of *Plagiostoma* on the Salicaceae (the present study). Considering the number of plant species that are both congeneric with known hosts of *Plagiostoma* and that have not been sampled for *Plagiostoma*, the number of species in this genus is most certainly much larger than presented here. Therefore we predict that new species of *Plagiostoma* have yet to be found.

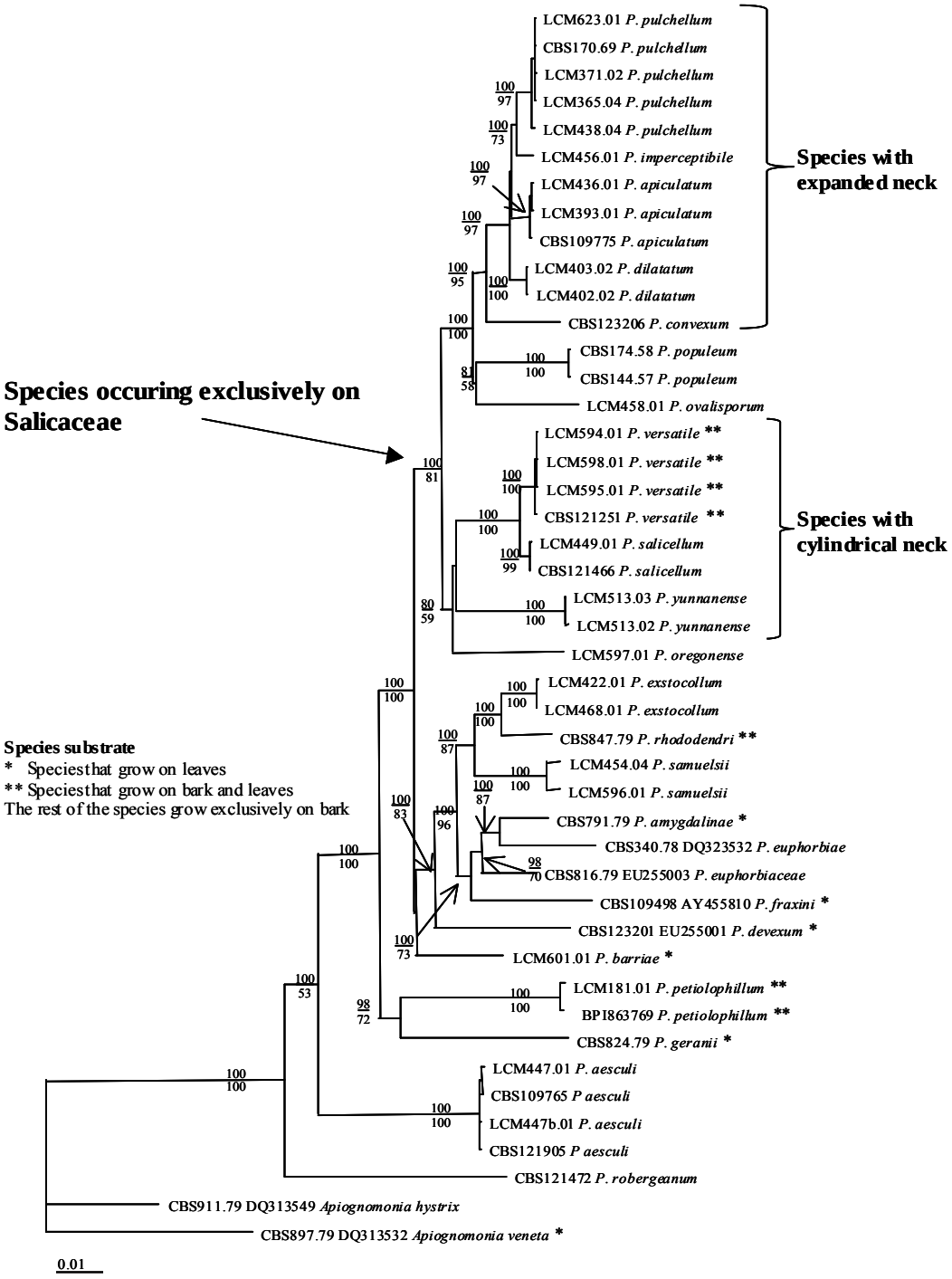

Figure 5.1. Maximum likelihood tree (ML score = - lnL 13921.12887) estimated from sequences of the *β-tubulin*, ITS, *rpb2*, and *tef1-α* genes for 24 species of *Plagiostoma* and

two *Apiognomonia* species as outgroup. Bayesian posterior probabilities are shown above each branch and maximum parsimony bootstrap values greater than 70% are shown below branches.

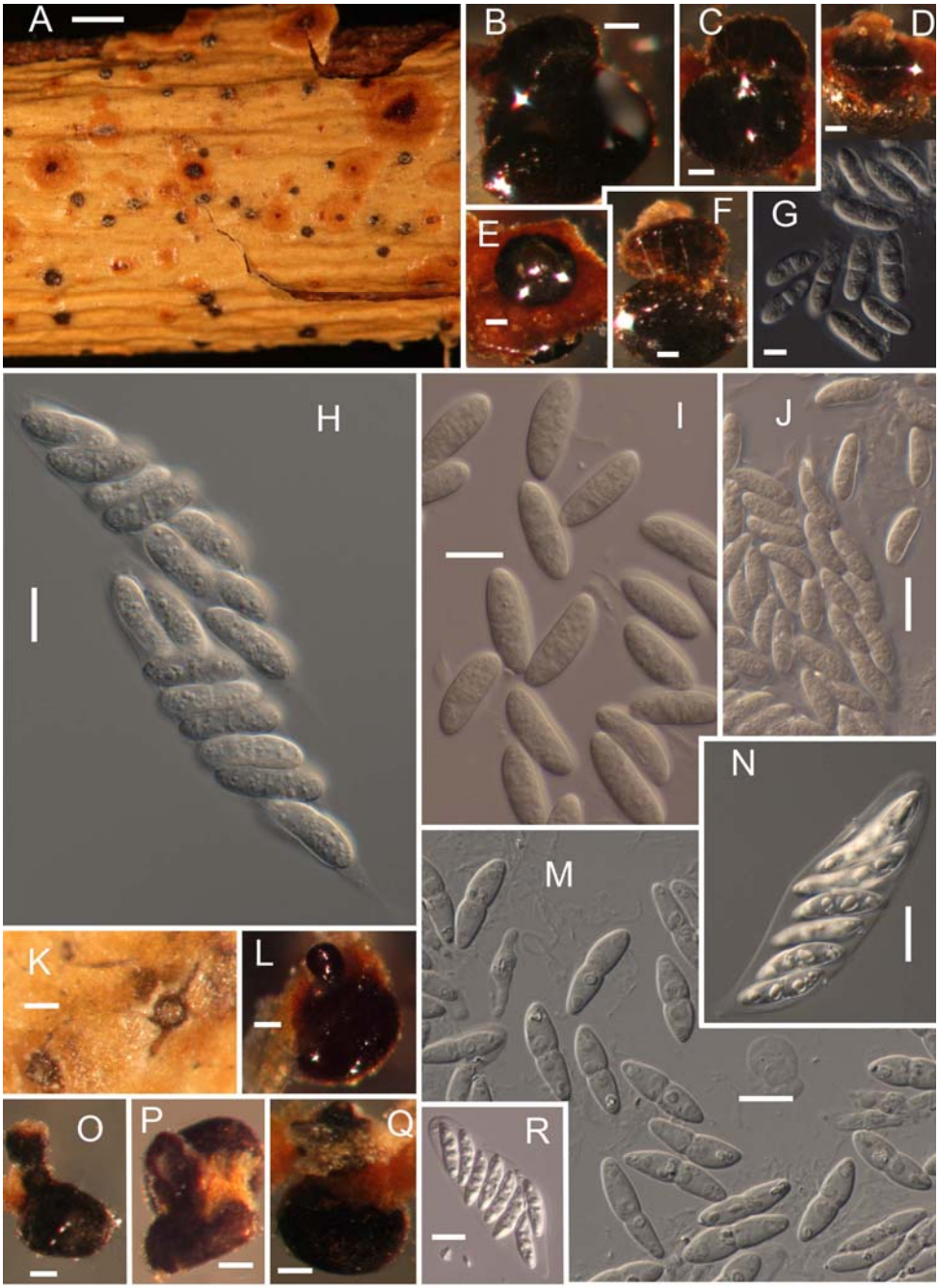

Figure 5.2. Morphology on natural substrate. A–J: *Plagiostoma apiculatum*: A,B,I,J = BPI799002 (lectotype), C–G = BPI747938 (epitype), H=BPI 878952. K–R. *P. convexum*: K–M = BPI799418 (lectotype), L–R = BPI843490 (epitype). Bars = (A, K) 1mm; (B-F, L, O-Q) 100 µm; (G-J, M-N, R) 10 µm.

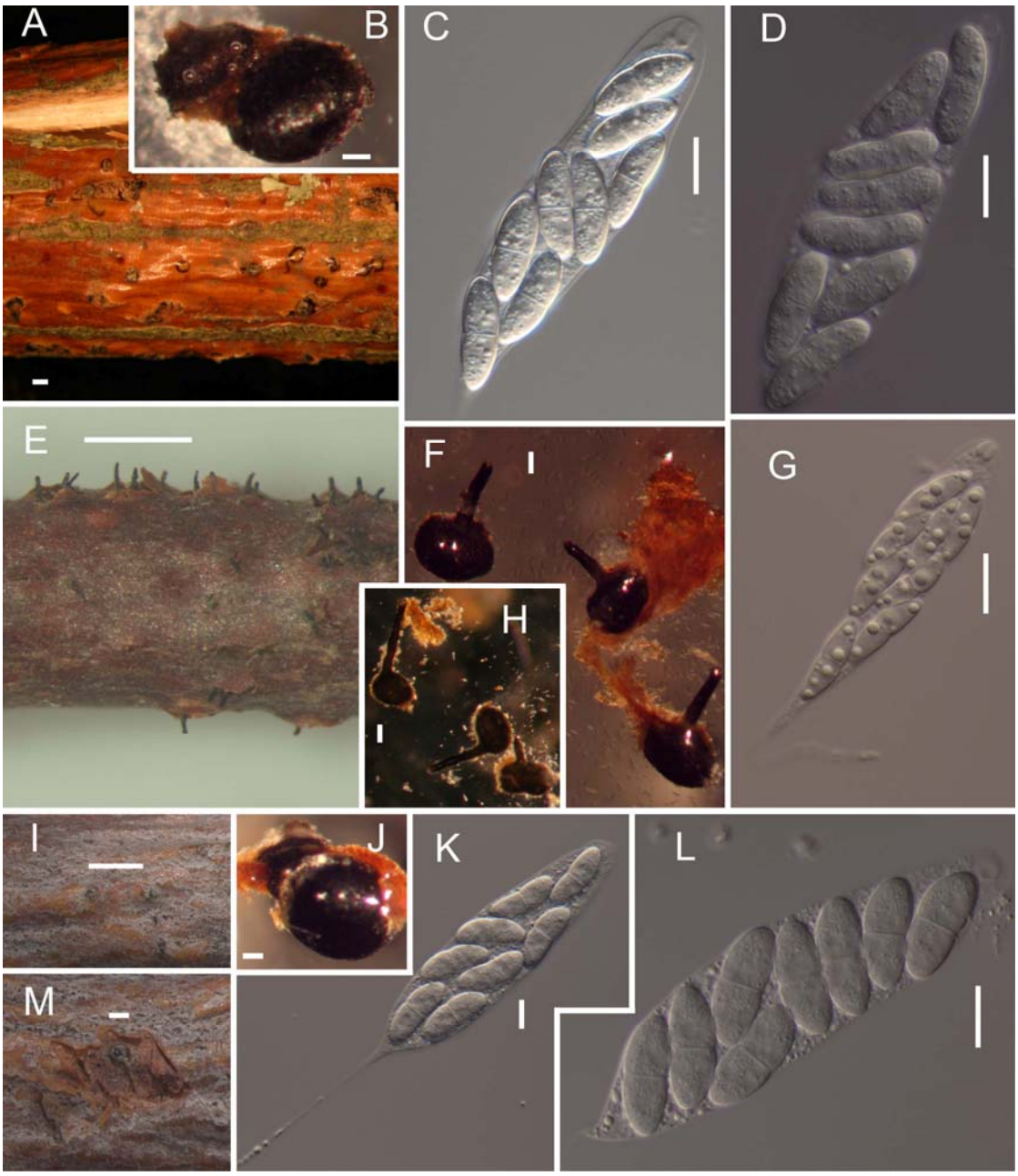

Figure 5.3. Morphology on natural substrate. A–D: *Plagiostoma dilatatum*: A–C = BPI878959 (holotype), D = BPI878958. E–H: *P. exstocollum*: E–G = BPI878961 (holotype), H = BPI878964. I–M: *P. imperceptibile* BPI878967 (holotype). Bars = (A, E, I) 1mm; (M) 200 µm; (B, F, H, J) 100 µm; (C-D) 20 µm; (G, K-L) 10 µm.

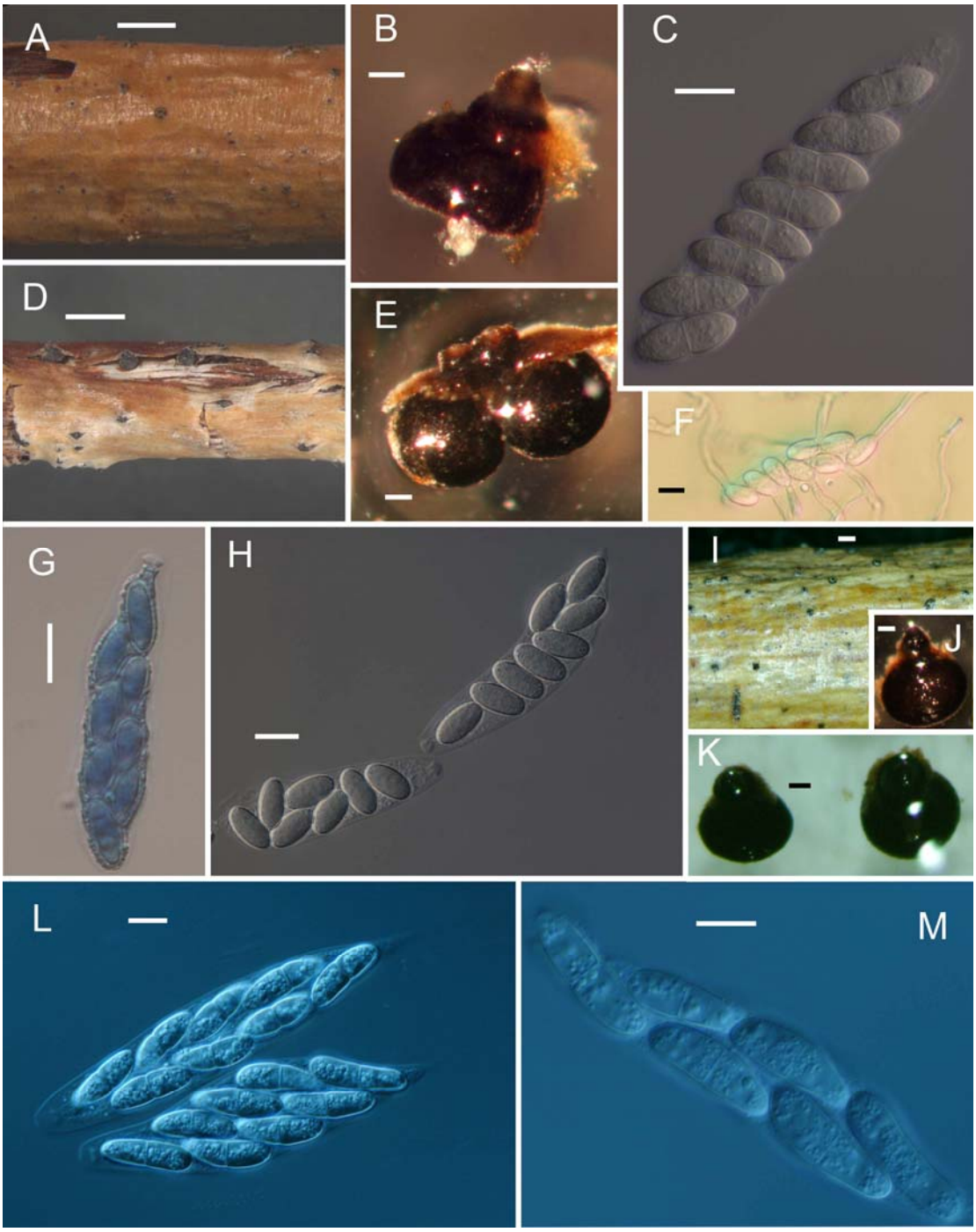

Figure 5.4. Morphology on natural substrate. A–C: *Plagiostoma oregonense* BPI878968 (holotype). D–H. *P. ovalisporum*: BPI878969 (holotype). I–M. *P. pulchellum*: I, M = BPI878971, J = BPI878974, K–L = BPI878972. Bars = (A, D, I) 1 mm; (K) 300 µm; (B, E, J) 100 µm; (H, L-M) 20 µm; (C, F-G) 10 µm.

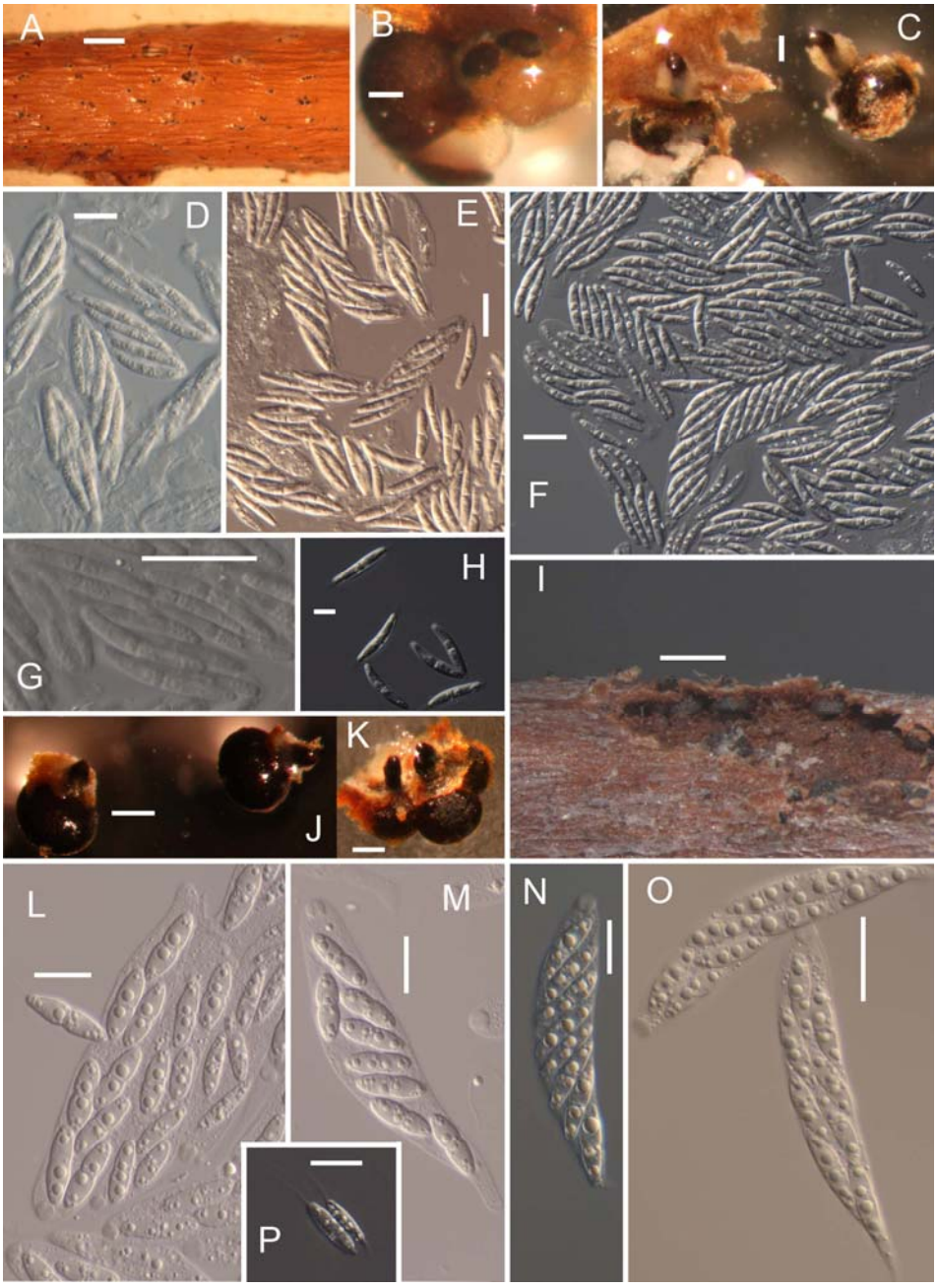

Figure 5.5 Morphology on natural substrate. A–H: *Plagiostoma salicellum*: A, B, D, G = Scleromyceti Sueciae 188 (lectotype), C, E, F, H = BPI843527 (epitype); note whitish stromatic tissue surrounding perithecial neck in Figures 5.5 B and C. I–O: *P. samuelsii*: I, M, P = BPI878977 (holotype), N–O = BPI878979. Bars = (A) 1 mm; (I) 500 µm; (C, J) 200 µm; (B, K) 100 µm; (D-G) 20 µm; (H, L-P) 10 µm.

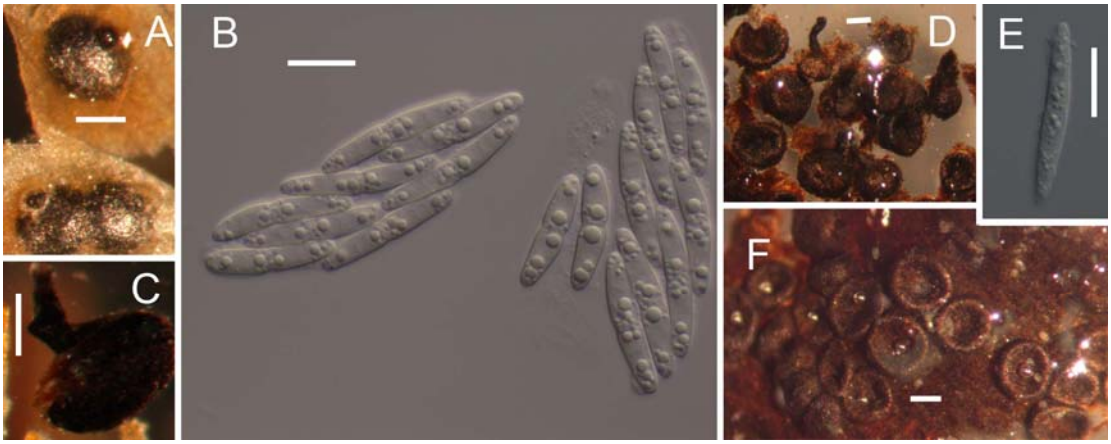

Figure 5.6. Morphology on natural substrate. A–C. *Plagiostoma versatile*: A–B = BPI878980 (holotype), C = BPI877702. D–F. *P. yunnanense* BPI878983 (holotype). Bars = (A, C-D, F) 200 µm; (B, E) 10 µm.

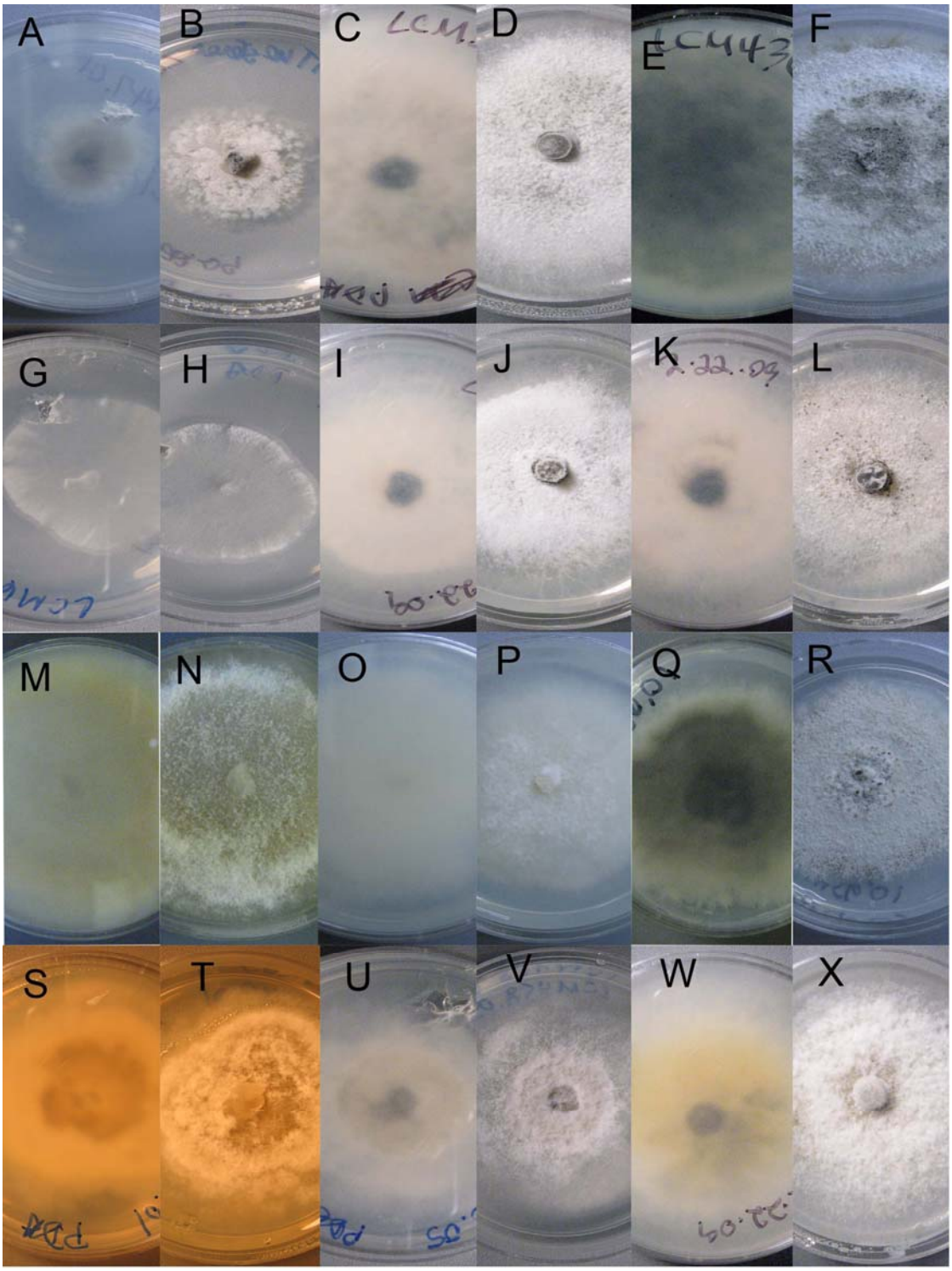

Figure 5.7. Culture morphology. A–B. *P. aesculi*. C–F. *P. apiculatum*. G–H. *P. barriae*. I–L. *P. dilatatum*. M–P. *P. exstocollum*. Q–R. P. *imperceptibile*. S–T. *P.oregonense*. U–V. *P. ovalisporum.*

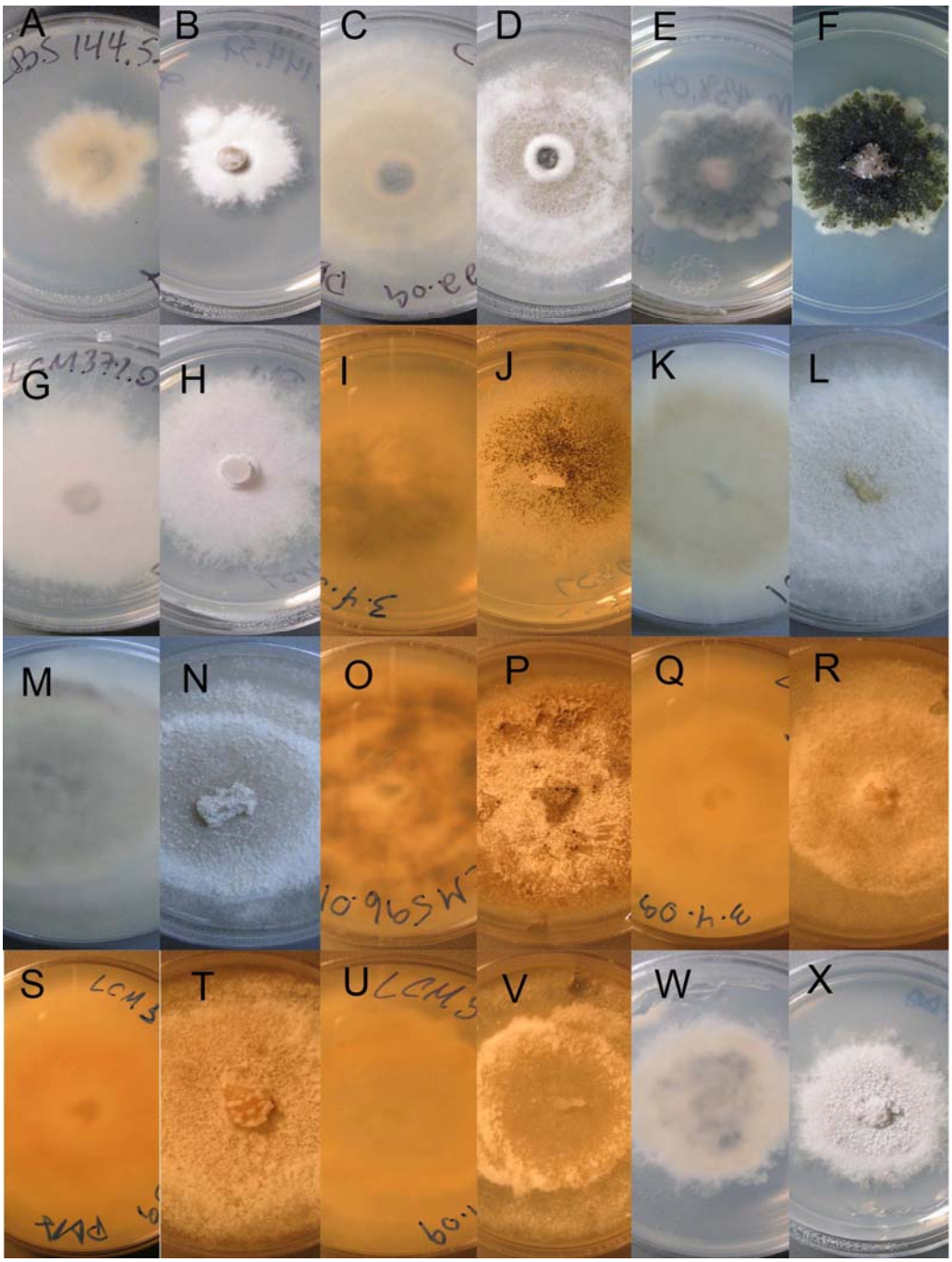

Figure 5.8. Culture morphology. A–D, *P. populeum*. E–J, *P. pulchellum*. K–L, *P. salicellum* (epitype CBS121466). M–P, *P. samuelsii*. Q–V, *P. versatile*. W–X, *P. yunnanense*.

Table 5.1. Isolates included in the phylogenetic analyses of *Plagiostoma*

Taxon	Specimen	Culture	Country	Host	Collector	*β-tubulin*	ITS	*rpb2*	*tef1-α*
Apiognomonia hystrix	CBSH 11343	CBS 911.79	Switzerland	*Acer pseudoplatanus*	M. Monod		DQ313549		
Apiognomonia veneta	NA	CBS 897.79	Switzerland	*Platanus orientalis*	M. Monod		DQ313532		
Plagiostoma aesculi	BPI878950	447.01	Germany	*Aesculus hippocastaneum*	L. C. Mejía				
Plagiostoma aesculi	BPI878950	447b.01	Germany	*Aesculus hippocastaneum*	L. C. Mejía				
Plagiostoma aesculi	BPI 748430	CBS 109765	Austria	*Aesculus hippocastaneum*	W. Jaklitsch		DQ323530		
Plagiostoma aesculi		CBS 121905					EU254994		
Plagiostoma amygdalinae	NA	CBS 791.79	Switzerland	*Euphorbia amygdaloides*	M. Monod		EU254995		
Plagiostoma apiculatum comb. nov.	BPI878951	393.01	France	*Salix dasyclados*	L. C. Mejía				
Plagiostoma apiculatum comb. nov.	BPI878951	CBS 124974	France	*Salix dasyclados*	L. C. Mejía				
Plagiostoma apiculatum comb. nov.	BPI878952	436.01	U.S.A:WA	*Salix sitchensis*	L. C. Mejía				
Plagiostoma apiculatum comb. nov.	BPI 747938	CBS 109775	Austria	*Salix* sp.	W. Jaklitsch		DQ323529		
Plagiostoma barriae	BPI878953	484.01	U.S.A:OR	*Acer* sp.	L. C. Mejía				
Plagiostoma barriae	BPI878954	601.01	U.S.A.:WA	*Acer macrophyllum*	L. C. Mejía				
Plagiostoma barriae	BPI878954	CBS 124975	U.S.A.:WA	*Acer macrophyllum*	L. C. Mejía				
Plagiostoma barriae	BPI 877717B	CBS 121249	U.S.A.:WA	*Acer macrophyllum*	M. V. Sogonov		EU254997		
Plagiostoma convexum comb. nov.		CBS 123206	U.S.A.:NY	*Salix* sp.	L. Vasilyeva		EU255047		
Plagiostoma devexum	BPI 843489	CBS 123201	U.S.A.:NY	*Polygonum* sp.	L. Vasilyeva		EU255001		
Plagiostoma dilatatum sp. nov.	BPI878957	402.01	France	*Salix irorata*	L. C. Mejía				
Plagiostoma dilatatum sp. nov.	BPI878957	402.02	France	*Salix irorata*	L. C. Mejía				
Plagiostoma dilatatum sp. nov.	BPI878958	403.01	France	*Salix caprea*	L. C. Mejía				
Plagiostoma dilatatum sp. nov.	BPI878958	403.02	France	*Salix caprea*	L. C. Mejía				
Plagiostoma dilatatum sp. nov.	BPI878958	403	France	*Salix caprea*	L. C. Mejía				
Plagiostoma euphorbiaceum		CBS 816.79					EU255003		
Plagiostoma euphorbiae		CBS 817.79					EU255005		
Plagiostoma euphorbiae	NA	CBS 340.78	The Netherlands	*Euphorbia palustris*	W. Gams		DQ323532		
Plagiostoma euphorbiae verrucosae	BPI877685						EU255006		
Plagiostoma fraxini	BPI 746412	CBS 109498	U.S.A.:MD	*Fraxinus pennsylvanica*	S. Redlin		AY455810		

Plagiostoma geranii	NA	CBS 824.79	Switzerland	*Geranium sylvaticum*	M. Monod	EU255009
Plagiostoma exstocollum sp. nov.	BPI878959	422.01	U.S.A.:OR	*Corylus californica*	L. C. Mejía	
Plagiostoma exstocollum sp. nov.	BPI878959	422.02	U.S.A.:OR	*Corylus californica*	L. C. Mejía	
Plagiostoma exstocollum sp. nov.	BPI878960	464	U.S.A.:OR	*Corylus californica*	L. C. Mejía	
Plagiostoma exstocollum sp. nov.	BPI878961	468.01	U.S.A.:OR	*Corylus californica*	L. C. Mejía	
Plagiostoma exstocollum sp. nov.	BPI878961	468.02	U.S.A.:OR	*Corylus californica*	L. C. Mejía	
Plagiostoma exstocollum sp. nov.	BPI878962	469.01	U.S.A.:OR	*Corylus californica*	L. C. Mejía	
Plagiostoma exstocollum sp. nov.	BPI878963	472.01	U.S.A.:OR	*Corylus californica*	L. C. Mejía	
Plagiostoma exstocollum sp. nov.	BPI878964	473.01	U.S.A.:OR	*Corylus californica*	L. C. Mejía	
Plagiostoma exstocollum sp. nov.	BPI878965	483.01	U.S.A.:OR	*Corylus californica*	L. C. Mejía	
Plagiostoma exstocollum sp. nov.	BPI878966	495.01	U.S.A.:OR	*Corylus californica*	L. C. Mejía	
Plagiostoma imperceptibile sp. nov.	BPI878967	456.01	U.S.A.:CA	*Salix* sp.	L. C. Mejía	
Plagiostoma imperceptibile sp. nov.	BPI878967	456.02	U.S.A.:CA	*Salix* sp.	L. C. Mejía	
Plagiostoma oregonense sp. nov.	BPI878968	597.01	U.S.A.:OR	*Salix* sp.	L. C. Mejía	
Plagiostoma ovalisporum sp. nov.	BPI878969	CBS 124977	U.S.A.:ID	*Salix* sp.	L. C. Mejía	
Plagiostoma ovalisporum sp. nov.	BPI878969	458.05	U.S.A.:ID	*Salix* sp.	L. C. Mejía	
Plagiostoma petiolophillum	BPI878970	181.01	U.S.A.:NY	*Acer spicatum*	L. C. Mejía	
Plagiostoma petiolophillum	BPI878970	181.02	U.S.A.:NY	*Acer spicatum*	L. C. Mejía	
Plagiostoma petiolophillum		CBS 121254				EU255050
Plagiostoma petiolophillum	BPI863769	AR 3821	U.S.A.:NY	Acer sp.	L. Vasilyeva	EU255039
Plagiostoma populeum comb. nov.		CBS 144.57	The Netherlands	*Populus trichocarpa*		
Plagiostoma populeum comb. nov.		CBS 174.58	The Netherlands	*Populus canadensis*		
Plagiostoma populeum comb. nov.		CBS 175.58	The Netherlands	*Populus canadensis*		
Plagiostoma populeum comb. nov.		CBS 227.51	The Netherlands	*Populus* sp.		
Plagiostoma pulchellum comb. nov.	BPI878971	365.04	U.S.A.:MD	*Salix babylonica*	L. C. Mejía	
Plagiostoma pulchellum comb. nov.	BPI878972	371.02	U.S.A.:MD	*Salix babylonica*	L. C. Mejía	
Plagiostoma pulchellum comb. nov.	BPI878973	438.03	U.S.A.:WA	*Salix lucida*	L. C. Mejía	
Plagiostoma pulchellum comb. nov.	BPI878973	438.04	U.S.A.:WA	*Salix lucida*	L. C. Mejía	
Plagiostoma pulchellum comb. nov.	BPI878974	623.01	Argentina	*Salix humboldtiana*	L. C. Mejía	

Plagiostoma pulchellum comb. nov.	BPI878974	623.03	Argentina	*Salix humboldtiana*	L. C. Mejía	
Plagiostoma pulchellum comb. nov.		CBS 170.69				EU255043
Plagiostoma rhododendri	NA	CBS 847.79	Switzerland	*Rhododendron hirsutum*	M. Monod	EU255044
Plagiostoma robergeanum	BPI 843593	CBS 121472	Austria	*Staphylea pinnata*	W. Jaklitsch	EU255046
Plagiostoma salicellum	BPI878975	449.01	Germany	*Salix repens*	L. C. Mejía	
Plagiostoma salicellum		CBS 121466				EU254996
Plagiostoma samuelsii sp. nov.	BPI878976	419.01	U.S.A.:OR	*Alnus tenuifolia*	L. C. Mejía	
Plagiostoma samuelsii sp. nov.	BPI878976	419.02	U.S.A.:OR	*Alnus tenuifolia*	L. C. Mejía	
Plagiostoma samuelsii sp. nov.	BPI878977	454.04	U.S.A.:CA	*Alnus tenuifolia*	L. C. Mejía	
Plagiostoma samuelsii sp. nov.	BPI878978	474.01	U.S.A.:OR	*Alnus* sp.	L. C. Mejía	
Plagiostoma samuelsii sp. nov.	BPI878979	596.01	U.S.A.:WA	*Alnus* sp.	L. C. Mejía	
Plagiostoma versatile sp. nov.	BPI878980	CBS 124978	U.S.A.:WA	*Salix scouleriana*	L. C. Mejía	
Plagiostoma versatile sp. nov.	BPI878981	595.01	U.S.A.:WA	*Salix scouleriana*	L. C. Mejía	
Plagiostoma versatile sp. nov.	BPI878982	598.01	U.S.A.:OR	*Salix* sp.	L. C. Mejía	
Plagiostoma versatile sp. nov.		CBS 121251	Canada	*Salix* sp.	M. V. Sogonov	EU255059
Plagiostoma yunnanense sp. nov.	BPI878983	513.02	China	*Salix* sp.	L. C. Mejía	
Plagiostoma yunnanense sp. nov.	BPI878983	CBS 124979	China	*Salix* sp.	L. C. Mejía	

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Chapter 6

Final remarks about bark-inhabiting species of Gnomoniaceae

Bark-inhabiting species of Gnomoniaceae do not represent a monophyletic group; therefore, a division between leaf- and bark-inhabiting species is purely artificial. Here the two genera of Gnomoniaceae with the largest number of species that inhabit the bark of their hosts, *Cryptosporella* and *Plagiostoma*, were recircumscribed and monographed on the basis of multigene phylogenies and comparisosn of morphological characters and host associations. A broader range of morphological and biological features were revealed that define these genera and more generally the family. This work uncovered patterns of fungus-host associations apparently phylogenetically conserved (see Chapter 4 and below).

Results from this work support the inclusion in the Gnomoniaceae of the bark-inhabiting genera *Amphiporthe*, *Cryptosporella*, and *Occultocarpon* gen. nov. as well as several bark-inhabiting species formerly described in *Cryptodiaporthe* and here accommodated in *Plagiostoma*. The bark-inhabiting Gnomoniaceae comprise 45 species distributed in the following genera: *Cryptosporella* (19 spp.), *Occultocarpon* (1 sp.), *Plagiostoma* (20 spp., including three species that grow on both leaves and bark), *Ditopella ditopa*, *Phragmoporthe conformis*, *Gnomoniopsis macounii*, *Cryptodiaporthe acerinum* and *C. aubertii*. All species of *Amphiporthe*, *Cryptosporella* and *Occultocarpon* as well as several of the species of *Cryptodiaporthe* recently or here transferred to *Plagiostoma* are characterized by perithecia formed in groups (valsoid arrangement) in the bark of their hosts. The formation of perithecia in groups is a feature

that broadens the range of morphological characters that define the Gnomoniaceae and contrasts with previously accepted concepts of the family. Previously accepted concepts of the Gnomoniaceae considered the production of solitary perithecia as one of the unifying characters in the family (see Barr 1978 and Monod 1983). The molecular phylogenies presented here also confirm *Ditopella ditopa*, a bark-inhabiting species that is the type of *Ditopella*, in the Gnomoniaceae. *Ditopella ditopa* produces solitary perithecia and, despite being considered Gnomoniaceae (Monod 1983), this species was considered atypical for this family in being polysporic and colonizing the bark of its host twigs and branches. Before this work, the Gnomoniaceae was considered to be associated mostly with leaves and now it is clear that this family has evolved on both leaves and bark of branches.

Results of this research support early observations by Wehmeyer (1933) that the bark-inhabiting genus *Cryptodiaporthe* was composed of a heterogeneous group of species. Species of *Cryptodiaporthe* are polyphyletic and belong in different families or lineages of Diaporthales (Fig. 1.1). The type species of *Cryptodiaporthe*, *C. aesculi,* is monophyletic with species of the older genus *Plagiostoma* as typified by *P. euphorbiae* and was recently synonymized with *Plagiostoma* (Sogonov et al. 2008). Of species formerly described under *Cryptodiaporthe,* six are now accommodated in *Plagiostoma* (Chapter 5), and one in *Gnomoniopsis,* i.e., *G. macounii* (Sogonov et al. 2008; Figs. 1.1 and 2.1). Analyses of LSU sequences (Fig. 1.1) support the inclusion of two other species of *Cryptodiaporthe* (*C. acerinum* and *C. aubertii*) in the Gnomoniaceae; however, their generic placement within this family remains to be defined.

The type species of the bark-inhabiting genus *Amphiporthe*, *A. hranicensis*, belongs in the Gnomoniaceae (Fig. 2.1). However three of the other four described species of *Amphiporthe* (*A. castanea*, *A. leiphaemia*, and *A. raveneliana*) form a distinctive and highly supported lineage outside the Gnomoniaceae but within the Diaporthales (Fig. 1.1). This lineage is likely a new family of the Diaporthales. The other known species of *Amphiporthe*, *A. aculeans* (synonym *Cryptodiaporthe aculeans*) appear to be closely related to the Melanconidaceae, but does not group with any of the currently accepted nine families of Diaporthales (Fig. 1.1). Results from this work also suggest that *Phragmoporthe conformis* should be treated as congeneric with *D. ditopa*. Both of these species occur in *Alnus* in Europe and *D. ditopa* also occurs on *Alnus* in North America (Fig. 2.1)

Although the capacity to infect and colonize bark tissue seems to have been gained and lost multiple times within lineages of Gnomoniaceae, species from this family tend to be specific to a plant organ or tissue and few species are known to be capable of exploiting more than one organ or tissue. Examples of species capable of colonizing both leaf and bark tissues are *Plagiostoma petiolophilum*, *P. rhododendri*, and *P. versatile* sp. nov. (Fig. 5.1).

Two close fungus-host associations that appear to have a long evolutionary history were found in this work: the association of *Cryptosporella* with Betulaceae (*Alnus*, *Betula*, and *Corylus*) (see Chapters 3 and 4) and the association of a subclade of *Plagiostoma* with Salicaceae (*Populus* and *Salix*) (see Chapter 5). Similar associations appear to exist between the genus *Gnomonia* and the Coryloideae (Betulaceae) (see Sogonov et al. 2008 and Fig. 2.1) and between subclades of *Ophiognomonia* and

Fagaceae and Betulaceae (personal observations). Additionally, genera and species of Gnomoniaceae exhibit different levels of host association or host preference (see Fig. 2.1, Chapters 4 and 5). These host associations show several variations including: 1) one fungal species associated specifically with one host species as is frequent in *Cryptosporella*; 2) one fungal species associated with a few congeneric host species, e.g., *Cryptosporella* and *Plagiostoma*; 3) one fungal species associated with different genera of the same family, e.g., *Ophiognomonia*; 4) one species associated with genera from different families (not frequent in the Gnomoniaceae; 5) subgeneric clades of Gnomoniaceae associated primarily with a plant genus or family as occurs in *Cryptosporella*, *Gnomonia*, and *Plagiostoma*.

In this work, the observed marked affinity of Gnomoniaceae for certain genera from some plant families, e.g., Betulaceae, Fagaceae, and Salicaceae was used as a baseline to prospect for new species of Gnomoniaceae in host species congeneric to known hosts. Using this approach new species were found in all regions sampled: eastern North America, the Pacific Northwest of USA, and, very interestingly, for the first time in forests in subtropical China and mountain cloud forests of Central and South America.

The global distribution of the Gnomoniaceae in association with particular plant families across their geographic distribution, e.g., *Cryptosporella* on Betulaceae, and *Plagiostoma* on *Salix* suggests a long evolutionary relationship between Gnomoniaceae and their hosts. Furthermore the frequently found association of one species of Gnomoniaceae with one host species suggests that species of Gnomoniaceae have speciated upon the availability of and capability to colonize new host species. More likely but not exclusively, this type of speciation might have occurred on closely related hosts

(Chapter 4). The phylogeny and host associations of *Cryptosporella* strongly suggest that the availability of diverse host species in the Betulaceae appear to be a primary source of diversification in *Cryptosporella* and that few species from this genus have speciated through jumps to distantly related but co-occuring hosts in the same area (Fig. 4.1).

With the available data it is difficult to infer the true evolutionary history of the association between *Cryptosporella* and its core host family, the Betulaceae. The phylogenies presented here does not support cocladogenesis between genera of Gnomoniaceae and their hosts, at least not between the most detailed association studied here of *Cryptosporella* and their hosts. Neither the multigene phylogeny of *Cryptosporella* nor single gene trees from this genus mirror the available phylogenies of their betulaceous hosts (compare to Chen 1999 and Navarro et al. 2003). The global distribution of *Cryptosporella* in strong association with the widely distributed Betulaceae suggests that this genus might have moved or migrated jointly with their main hosts. However, the apparent absence of cocladogenesis supposes no contemporary speciation between these associates. If cospeciation is ruled out, an alternative explanation could be a scenario in which *Cryptosporella* evolved after the extant taxa of Betulaceae evolved. An alternative mechanism could be host tracking coevolution (see Roy 2001) whereby an initial association with a betulaceous host facilitated *Cryptosporella* to later infect, colonize, and speciate on new hosts that share similar resources (species of Betulaceae). Similar explanations can be offered for the associations of a *Plagiostoma* subclade with Salicaceae, *Gnomonia* with Coryloideae and clades of *Ophiognomonia* on Betulaceae and Fagaceae.

Although some bark-inhabiting species of Gnomoniaceae cause diseases on their hosts, e.g., *Plagiostoma apiculatum* on *Salix* and *P. populeum* on *Populus*, most of the bark-inhabiting species of Gnomoniaceae appear to be asymptomatic endophytes. Moreover, several bark-inhabiting species of Gnomoniaceae have been reported as dominant species in surveys of endophytic mycoflora based on cultural approaches. The dominance reported for some species of Gnomoniaceae seems obvious by direct observation when fruiting bodies of these fungi are massively produced on hosts (see Fig. 4.4 F). For example, bark-inhabiting species are commonly found covering large areas of host tissue and forming what Mejia et al. (2009) called "pure microstands" when referring to sporulation and dominance of *Cryptosporella* through production of antimicrobial compounds on host branches.

From the current distribution of extant taxa of Gnomoniaceae in association with particular plant families it can be said that the Betulaceae, Fagaceae, and Salicaceae have not migrated alone through their evolutionary history but that they moved with their symbiont endophytes, including bark-inhabiting species of Gnomoniaceae. The bark-inhabiting species of Gnomoniaceae may have "caught up" with their hosts in new regions because their spreading strategies (via spores) are comparable to those of their hosts (via seeds). Tracking with hosts does not seem to have been a limitation for bark-inhabiting species of Gnomoniaceae. Interestingly, although having ample probability to land on the surfaces of many diverse hosts, genera and species of Gnomoniaceae show high fidelity for particular plant host lineages. Future research with the aim of timing and determining what factors might have promoted the maintenance of this fidelity and

different levels of host associations remains to be fully addressed and promises to be rewarding.

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Appendix

Fungal endophytes: defensive characteristics and its agricultural applications[5]

Introduction

Endophytes are the subject of intensive research, in part because of the potential they hold in agriculture as a source of beneficial effects to their host plants such as increased vigor and tolerance to a range of abiotic and biotic stresses (Backman & Sikora, 2008; Kuldau & Bacon, 2008). They are defined as organisms that asymptomatically infect internal tissues of plants during at least part of their life cycle (see Petrini, 1991; Saikkonen et al., 1998; Stone et al., 2000; Wilson, 1995). In particular, fungal endophytes have been reported from all plant species surveyed, including representatives from all ecosystems; In addition, these fungi can be isolated from different plant organs and tissues, including roots, stems, branches, leaf, flowers, and fruits (Arnold, 2007; Rodriguez et al., 2004; Saikkonen et al., 1998; Stone et al., 2000; 2004). Commonly found fungal endophytes (excluding mycorrhizal associations) belong to diverse classes of Ascomycota, mostly Dothidiomycetes, Leotiomycetes, and Sordariomycetes, although Basidiomycota endophytes have been observed to be common in some hosts (Crozier et al., 2006; Sieber, 2007; Stone et al., 2004; Thomas et al., 2008).

One type of fungal endophyte–host association that has been frequently examined is that found in fungal endophyte-grass associations, specifically referring to endophytic

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fungi from the family Clavicipitaceae (tribe Balansiae) in cool-season grasses (Pooideae) (White et al., 2000). Two well known examples of this type of association are between *Neotyphodium coenophialum* and tall fescue (*Festuca arundinaceae)* and between *Neotyphodium lolii* and perennial ryegrass (*Lolium perenne).* In this association the fungal partner can confer some protection to their host against herbivores or an enhanced ability to overcome abiotic stresses (e.g. drought, heavy metals) through production of mycotoxins or other fungal derived molecules, while the host provide nutrients and a stable environment (Arechavaleta et al., 1989; Clay, 1988; Clay & Schardl, 2002). The symbiotic relationship between clavicipitalean endophytes and their grass hosts have been exploited commercially in the turf and forage grass industry specifically by the production of endophyte-infected grass varieties with enhanced tolerance to abiotic and biotic stresses (e.g. MaxQ®, AR37 endophyte)

A second type of host-endophyte interaction that also often exhibits similar mutualistic benefits with agricultural potential is that between non-Clavicipitaceous endophytic fungi (see Schulz & Boyle, 2005) and woody plants (see Table 1). In this group, different studies have shown that fungal endophytes can make substantial contributions to their host's capacity to tolerate or avoid adverse abiotic and biotic factors, ranging from drought stress to herbivory to pathogens (Alvarez et al., 2008; Arnold et al., 2003; Campanile et al., 2007;Carroll, 1988; Faeth & Hamon, 1997; Herre et al., 2007; Miller et al., 2002; Saikkonen et al., 1996; see Table 1). In particular, some field trials have shown that inoculation with particular endophyte strains can benefit their hosts by limiting damage by pests or reducing dispersal capabilities of their pathogens (Miller et al., 2008; Mejía et al., 2008b; Narisawa et al., 2000).

The host-endophyte properties of agriculturally important crops (e.g. vegetables, cereals, fruits, ornamental flowers) as well as other plants that do not fit neatly into the two major host categories previously mentioned (i.e. cool-season grasses and woody plants) require intensive study to determine their relevance for crop protection and production. Studies have been conducted on these plants and some fungal endophytes have been determined to increase plant productivity and resistance to diseases (see D'Amico et al., 2008; Narisawa et al., 2002; Rodriguez et al. 2005; Sieber et al., 1988; Sutton et al., 2008; Waller et al., 2005). In some cases the benefits attributed to fungal endophytes to their hosts may involve additional partners. This is the case with the tripartite interaction involving a plant, a virus, and a fungal endophyte. In this case, the fungus *Curvularia protuberata* provide thermotolerance to its wild host *Dichanthelium lanuginosum* only when infected with a specific virus (Márquez et al., 2007; Redman et al., 2002). In some cases the beneficial effects have been reproduced in hosts other than the original source of the endophytic isolate. However in these studies the effect of endophytes appear to be greater in their wild hosts (Márquez et al., 2007).

Currently, some general attributes of fungal endophytes such as their transmission mode, colonization pattern, and species diversity have been determined for both the grass and the woody plant-fungal endophyte associations. However, specific characteristics that likely vary for particular plant-fungal endophyte species interactions need to be considered for successful application to agriculture. For instance, target plant tissues and organs, plant life cycle (annual vs. perennial), crop production condition (greenhouse vs. open field system), and fungal endophyte life cycles are likely to be crucial for determining suitable matches between endophyte species and their hosts.

In this chapter we highlight the importance of some general attributes of plant-fungal endophyte symbioses and their implications for practical uses in agriculture. Additionally, we will consider a case study of *Cryptosporella* (synonym *Ophiovalsa*, Mejía et al. 2008a), a fungal genus with dominant species in assemblages of endophytes from several hosts in hardwood forests of the Northern Hemisphere (Sieber, 2007; Stone et al., 2004). In particular, we will focus on the growth inhibitory activity of *Cryptosporella wehmeyeriana* on the plant pathogenic bacterium *Xanthomonas campestris* pv. *campestris*, and provide a preliminary identification of the compounds responsible for this activity.

1. Fungal Endophytes: Diversity, Transmission Mode, and Dominance

1.1. Diversity and transmission mode

Some common features have been found between the association of cool-season grasses and species of *Neothypodium* and the associations between non-Clavicipitaceous endophytes and woody plants. However, marked differences have been observed between these two types of associations, which hold important implications for practical application of endophytes in agriculture. Species of *Neotyphodium* are transmitted vertically from mother to offsprings through seeds, except when producing the sexual stage or when conidia are produced epiphytically (see Tadych et al. 2007). In these last two cases the transmission is horizontally (i.e. laterally from plant to plant). Additionally, *Neotyphodium* spp. and other clavicipitaceous endophytes associated with cool-season grasses establish systemic and non-organ specific colonization of aboveground tissues, and are generally considered host specific and to have a long evolutionary history of

relationship with their hosts (Clay & Schardl, 2002; Schardl et al., 1997). In some grasses the diversity of endophyte species can be high (see Sánchez Márquez et al., 2007). However in cool-season grasses, the norm is the occurrence of one or few fungal endophyte species per host individual, usually with one species, able to establish systemic colonization of above ground tissues of their hosts (e.g. *N. coenophialum* on tall fescue *F. arundinaceae*). The long-term evolutionary relationships between clavicipitalean endophytes and cool-season grasses, their low fungal endophyte species diversity per host individuals, their systemic colonization pattern, and vertical transmission mode, are factors that may contribute to the persistence of desirable effects from *Neotyphodium* species when this association is artificially manipulated. For instance, high levels of tissue colonization can be maintained for long periods of time in grasses after plants are artificially inoculated with Clavicipitaceous endophyte species and placed either under greenhouse or field conditions (see Clay & Holah, 1999). Additionally, while the frequencies of infection of these fungi varies depending on abiotic and biotic factors, these frequencies are expected to be high in nature (see Shelby & Dalrymple, 1993; Wäli et al., 2007).

In contrast, the associations between fungal endophytes and woody plants are characterized by a high diversity of fungal species, usually exceeding more than 30 species per host (see Stone et al., 2004). These endophytes are transmitted horizontally, and little information exists regarding their evolutionary histories or symbiotic interactions with their hosts (Saikkonen et al., 1998; but see Sieber, 2007). In addition, fungal endophytes from leaves of woody plants tend to form localized infection (Stone, 1986; Wilson and Carroll, 1994). Thus, maintaining high densities of a particular fungal

endophyte strain or species in a given host seems more challenging in woody plants and vegetables crops than in the Clavicipitaceous endophytes-grass system. This high diversity of endophyte species represents a challenge to answer the major question of what are the roles of fungal endophytes in general and particularly in woody plants and other non-grass hosts. Specifically determining which endophyte strains or species have a positive effect on their hosts?, which are latent pathogens?, and which ones are just there? Recent studies have been conducted with the aim of identifying the role of fungal endophytes in woody plants and other non-grass hosts and to specifically test defensive mutualism hypotheses (see Clay 1988; Carroll 1988). Specifically addressing the questions of whether these fungi help their hosts to tolerate herbivore and pathogen damage. Because of the high diversity of endophyte species in woody plants and their horizontal transmission mode it is not simple to reconcile this association with current mutualism theory. It has been hard to simplify this complex system of multiple species interactions and to clearly determine the effects that these symbionts have on their hosts. It is poorly understood whether an assemblage of multiple fungal endophyte species work synergistically in a given woody plant host, or whether different species or strains perform different roles (see Arnold et al., 2003; Herre et al., 2007). For example, based on the high diversity of fungal taxa associated with woody plants and vegetable crops, the number of fungal derived molecules with direct or indirect effects on their hosts in this association could be expected to be much more diverse than that observed in the Clavicipitaceous endophyte-grass systems (See Schulz & Boyle, 2005). Nonetheless, some woody hosts and their associated endophytic mycoflora have been experimentally manipulated to address questions on the roles of these fungi and beneficial effects have

been observed to be provided by fungal endophytes (see Arnold et al., 2003; Sumarah et al., 2008; Wilson, 1996; Wilson & Faeth, 2001).

Horizontal transmission of endophytes as observed in woody plants and vegetables crops increases the likelihood that many different species colonize plants. With many encounters some of the fungi are able to get into the plants and persists for multiple generations. When the host plant encounters stresses that are a threat to its survival (plant diseases, insect herbivores or environmental factors), those endophytes that enable hosts to overcome the stresses will increase in frequency during the stress and persist within plants with varying frequencies in the future. Those that do not improve host fitness in the face of stress will move to a new host or become extinct. It is also important to notice that for the plants, keeping multiple fungal endophytes species may be a faster way of evolving extrinsic defense mechanisms than what their host could do because these symbionts have shorter life cycle or because they have similar rates of evolution compared to plant pest and pathogens (see Carroll, 1988; Herre et al., 2007). While perennial plants may not generate new sources of defenses as quickly as their pest and pathogens, their symbionts may keep track of their host' enemies.

1.2. Dominance of endophyte species in particular host assemblages

Besides the diversity of endophyte species associated with woody hosts, in these plants there is usually a set of species that dominate the assemblage in a given host. While the endophyte species determined to be dominant in a particular host or host organ can be an artifact of the method used for isolating or detecting them (e.g. endophyte isolates grow differentially on different media and uncultivable endophyte species may occur); it is also

likely that dominant species are good at colonizing their host. It has been shown experimentally that fungal endophytes from woody plants determined to be dominant in a given host based on surveys using culturing methods, are good colonizers of the organs they were isolated from.

The pattern of dominance of one or few endophyte species over an assemblage of species in a given host has been reproduced in simplified form experimentally (see Mejía et al., 2008b; Wille et al., 2002). Additionally these dominant species are better colonizers than rarely found or singleton species for a given host (Mejía et al., 2008b; Wilson, 1996; Wilson & Carroll, 1994). Mechanistically, this dominance of one or few endophyte species can be explained by the specialization of some species at degrading specific compounds produced by their hosts (see Saunders & Kohn, 2008). Other possibility is that dominant endophyte species produce toxic compounds to other endophytes occupying the same niche. Alternatively dominant species bring an advantage to the host under some stresses, so they have been naturally selected and became dominant over time. Moreover evidence suggests that some mutualistic symbioses between fungal endophytes and their hosts are the results of specific adaptations to stresses following a habitat-specific manner (Rodriguez et al., 2004).

Knowing what endophyte species are dominant in a particular host is of great relevance when the particular host is intended to be inoculated with a selected endophyte strain that has shown some promise at benefiting the host. In some hosts good endophyte colonizers are not necessarily the ones with more toxic or antibiotic potential on host pathogens and pests. It has been observed that in woody plants there is apparently a tradeoff between production of compounds with antibiotic properties and mycelial

growth in fungal endophytes (Mejía et al., 2008b), so endophyte species with antibiotic or toxic capabilities on plant pathogens and pests in a given host are not necessarily good *in planta* colonizers.

2. Fungal Endophytes: General Life Cycle

Observation on fungal endophyte life cycles may help in the search for good fungal endophyte candidates for agricultural applications (e.g. for biocontrol, growth improvement, etc.). Studies conducted in temperate broad leaf and particularly in evergreen tropical rain forests, suggest that a particular host is constantly receiving the arrival of fungal spores from the environment, and a subset of these spores are able to germinate in a given host, infect, and colonize. Some fungal endophyte species will infect one or few hosts (endophytes with specific or limited host range) while others will infect several hosts (generalist endophytes). Most of these endophytes will establish localized infections (Petrini, 1991; Stone, 1986). On these hosts, fungal endophyte colonization goes usually from undetectable or low levels in very young tissues such as recently emerged leaves and shoots to high levels in mature tissues and to full colonization in old ones. Jointly with this pattern of colonization there is generally an increase in the diversity of endophyte species in host tissues through time. In some cases this diversity reaches a peak in mature tissues that is later followed by a decrease in the diversity in older tissues, but with a group of few species that tend to be preferentially associated, or specific with a particular host (Faeth & Hammon, 1997; Herre et al., 2007; Wilson & Carroll, 1994; Wilson et al., 1997). Some of these endophytes will sporulate on the dead tissue and the cycle begins again (See Herre et al., 2007; Promputtha et al., 2007).

3. Relevance of Ecological Studies

To appropriately address questions on the roles of fungal endophytes we consider important to conduct ecological studies designed to determine the identities of fungal endophyte species associated with a particular host under different growth conditions and environments (e.g. sampling of host in their natural distribution area, exotic environments, and agricultural systems), seasons, and tissues (see Table 2). Studies on the chocolate tree *Theobroma cacao* and associated endophyte mycoflora may help to illustrate the complexity of endophyte-woody plant interactions, determining their roles, and the challenges of making a practical application of the effects that these fungi have in their hosts. *Theobroma cacao* and some congeneric species as well as co-occurring plant species, have been recently surveyed for its endophytic mycoflora. The surveys have been conducted in or near the center of origin of *T. cacao*, in exotic environments, and under a wide range of conditions and seasons. While the number of fungal endophyte species and morphospecies reported in this host is extremely high, in the order of 1000, studies suggest that there are a group of species preferentially associated with it and that a subset of species or genera tend to be localized in particular tissues (e.g., leaf vs. trunk; Arnold et al., 2003; Crozier et al., 2006; Evans et al., 2003; Herre et al., 2007; Rojas et al., 2008; Samuels et al., 2006; Van Bael et al., 2005). In this host a major goal is to find endophyte species that can help the trees to better tolerate or resist damage by pests and pathogens. Similar surveys and approaches to find endophytes antagonistic to plant pests have been conducted for coffee plants (Posada et al., 2007; Santamaría and Bayman, 2005; Vega et al., 2008). These crops can be manipulated under laboratory and greenhouse conditions, so that plants with (E+) and without (E-) fungal endophytes can

be compared under different conditions and against particular pests and pathogens; Importantly it has been shown that fungal endophytes can limit pathogen damage to host (table 1). For example, strains of *Clonostachys* and *Trichoderma* show promise for their antagonistic activity to important cacao pathogens (Arnold et al., 2003; Evans et al., 2003; Mejía et al., 2008b, Posada and Vega, 2006; Rubini et al., 2005; Samuels et al., 2006; Tondje et al., 2006). However a major challenge is ensuring that the chosen endophyte strains remain viable and active within host plants for extended periods. Towards that end, information on the colonization ability, persistence, and activity of a given endophyte within a given host is critical.

Importantly, when attempting to use a particular endophyte to directly antagonize a pest or a pathogen, it is not only important to find evidence for antagonistic activity in vitro. What is probably more important is to identify endophytes that are good colonizers of the host and remain active within it. This is especially important because woody plants can accumulate many endophyte species over time that will compete for the same habitat. Dominant endophyte species in a given host may out compete selected endophyte species under field conditions. Extensive endophyte surveys on particular host to determine patterns of species dominance and their capacity to colonize target organs or tissues can be tedious but is likely to be fruitful, if not essential, in the long term.

4. Fungal Endophytes: Defensive Characteristics

Fungi including fungal endophytes are known for their ability to produce a diverse range of molecules (e.g., antibiotics, toxins, peptides) that positively or negatively affect other organisms (Gunatilaka, 2006; Petrini et al., 1992; Tan & Zou, 2001; Zhang et al., 2006).

Further, many of those molecules are believed to play an integral role in fungal development and survival in specific environments. To better appreciate the effects of these molecules in relation to fungal endophyte niches, it is important to understand the nature of the endophytic habitat and of the particular plant-fungal endophyte association. The endophytic habitat (i.e., the internal tissues of the host), can be rich in nutrients and in endophyte species (Arnold et al., 2000; Kuldau & Bacon, 2008; White et al., 2000). Endophytic fungi tend to be localized in extracellular spaces however some are located intracellularly and multiple species can occur within a small area (Herre et al., 2005, 2007; Lodge et al., 1996; Stone, 1986; Stone et al., 2000).

Independent of the endophyte-plant association, it is likely in the interest of a particular fungal endophyte species not to be displaced by other species, and to efficiently exploit the available resources provided by the host (see Herre et al. 2007). It is plausible to think that general ecological tenets of species interactions would apply to endophytic communities as they do to the host species. This would imply competition for resources among endophytes with particular endophyte species being better at exploiting specific resources and at colonizing particular plant tissues and organs. Fungal endophyte species that produce compounds antagonistic to other endophytes, pathogens, and pests that occupy or depend on the same habitat (the inner plant) will have an advantage at colonizing particular host tissues. Identifying fungal endophyte species that can help their host to neutralize specific pathogens or pests (directly via inhibitory or toxic compounds produced *in planta* or indirectly via induction of host defense mechanisms) is the quest for the 'holy grail' of applied fungal endophyte research in crop protection and relevant to developing clean technologies for pest management in agriculture.

There is ample evidence from *in vitro* studies showing that fungal endophytes inhibit the growth of plant pests and pathogens (White & Cole, 1985; Evans et al., 2003; Holmes et al., 2004; Tunali and Marshal, 2000; data presented here).The inhibitory compounds have been identified in multiple cases (Aneja et al., 2005; Calhoun et al., 1992; Daisy et al., 2002; Schwarz et al., 2004; Schulz et al., 1995; Strobel et al., 2001; Wang et al. 2007; Wicklow et al., 2005). These compounds can be defensive in its nature for the own fungus in the endophytic habitat. However, with exception of the alkaloids produced by clavicipitaceous endophytes (see Siegel et al. 1990; Kuldau and Bacon 2008), there is little evidence for *in planta* production of inhibitory compounds. When the toxic or inhibitory compounds have been detected *in planta*, their levels have been too low to actually stop the progress of a pathogen (see Schulz & Boyle, 2005). Nevertheless a recent study under an open nursery condition found that levels of Rugulosin in needles of *Picea glauca* infected with a Rugulosin producing fungal endophyte were at the concentration necessary to reduce the weight of the budworm *Choristoneura fumiferana* (Miller et al., 2008).

Often biological activities by fungal endophytes have been tested to known standard organisms *in vitro*. However it is not a prerequisite that inhibitory compounds need to be produced *in planta* to be useful for practical agricultural applications. Research on treatments of some crop plants with fungal endophyte derived compounds has been shown to have beneficial effects compared to controls in terms of protection against pests and pathogens (Daisy et al., 2002; Lacey & Neven, 2006). Other studies have shown that inoculation of plants with fungal endophytes help protect the treated plants against a wide variety of plant enemies (Table 1). While a general mechanism of

action has been proposed in most of these cases, the details of the mechanisms are largely unknown (see Herre et al., 2007).

5. Antagonistic Activity of Cryptosporella wehmeyeriana *on* Xanthomonas campestris *p.v.* campestris

In temperate broadleaf forests spore production of some fungal endophyte species have a marked seasonality. For instance, fungal endophyte species from the family Gnomoniaceae (Diaporthales) have marked seasonality. While conidia of these fungi can be found through the growing season, ascospores of most fungi from this family can be found more likely during the spring in dead and over wintered leaf and twigs (Sogonov et al. 2008; Wilson et al., 1997; Wilson & Carroll, 1994). Furthermore genera and species from this family are dominant in assemblages of fungal endophytes associated with particular hosts in temperate broadleaf forests. (Stone et al., 2004; Sieber 2007). For example, species of *Cryptosporella* and their anamorphs are dominant endophytes on twigs from trees of the family Betulaceae. *Apiognomonia* spp. are dominant on leaves of Fagaceae in temperate broadleaf forests (see Sieber et al., 2007; Fisher & Petrini, 1990) and we have observed a species of *Ophiognomonia* to be dominant on leaves of *Alnus acuminata* (Betulaceae) in tropical cloud mountain forests in central america (Mejía & White unpublished). Moreover when fungi such as *Ophiognomonia* sporulate, they cover large tissue area. In species of *Cryptosporella* perithecia are found covering big patches (several cm^2) on dead twigs. We have observed that some of these species also produce inhibitory compounds to plant pathogenic fungi and bacteria (see Fig. 1). As has been suggested by Fisher et al. (1984a, 1984b), the primary function of these inhibitory

compounds maybe competition against antagonists. We suggest that the dominance of *Cryptosporella* species in extensive area of their hosts is facilitated by production of compounds antagonistic to other species that occupy the same habitat. Furthermore there is usually no evidence of growth of other fungi co-occurring with fruiting bodies of *Cryptosporella*. Based on these observations we hypothesized that relatively "pure microstands" of *Cryptosporella* could be due to the production of antagonistic compounds to the growth of potentially co-occurring species (e.g. bacteria or fungi). To test this hypothesis an to evaluate the antimicrobial potential of a group of dominant fungal endophytes including *C. wehmeyeriana*, we have done *in vitro* assays of these fungi to evaluate their capacity to inhibit the growth of common plant pathogenic bacteria, including *Xanthomonas campestris* p.v. *campestris*.

These *in vitro* assays have been conducted following similar methodology as in Peláez et al. 1998. From these assays we have found that *C. wehmeyeriana* (isolated from *Tilia americana*) has strong inhibitory activity on the growth of *X. campestris* p.v. *campestris* (Fig.1). Our preliminary chemical analyses using HPLC-PDA indicate that major types of bioactive compounds from *C. wehmeyeriana* extracts are phenolic acids and their derivatives, flavonoids. Some representative compounds identified in this study are shown in figure 2. Based on HPLC retention time and UV spectra, the compounds were characterized as phenolic acid derivatives, quercetin derivatives. Phenolic compounds are well known to occur in plant tissues and they have been implicated in plant disease resistance. Specially some flavonoids have been reported as phytoalexins that help in the defense response against insect, fungi, and bacteria (McNally et al., 2003; Nicholson & Hammerschmidt, 1992; Pereira et al., 2007; Xu & Lee, 2001). Furthermore

it has been observed that flavonoids are involved in the defense and hypersentive reaction of cotton against *Xanthomonas campestris* pv. *malvacerum* (Dai et al., 1996). Here we report that *C. wehmeyeriana* produces compounds that could benefit its host, specifically by inhibiting plant pathogenic bacteria. These results emphasize previous observations that fungal endophytes produce compounds with antibacterial activity including phenolic compounds and that this activity may be significant for host protection from natural enemies (see Yang et al., 1994). To what extent could be the antibacterial compounds produced by *C. wehmeyeriana*, synthesized *in planta* and beneficial for its host remain to be determined. Certainly *T. americana* twigs with these compounds will be less hospitable for pathogenic bacteria.

Conclusions

Endophytes are symbiotic organisms that spend part or their entire life asymptomatically within plants. Some fungal endophytes have been observed to benefit their hosts by helping them to tolerate stressful abiotic and biotic conditions. Several lines of evidence support a defensive mutualism for some plant-endophytic fungi interactions. In these interactions the fungal partner protect their host in two major ways: 1) directly via production of compounds with antagonistic activity on host natural enemies or 2) indirectly activating host defense responses to plant pest and pathogens.

Most benefits provided by fungal endophytes seems agriculturally exploitable. Some of these benefits have been exploited agriculturally only in the association between few fungi from the family Clavicipitaceae and cool season grasses. Crop cultivated in nurseries or greenhouses could be easier systems to control than open field systems. For

example focusing protection efforts on fruits only during the relatively short period that they develop might prove to be a tractable and efficient strategy for using fungal endophytes to prevent production losses.

Ecological studies aimed at assessing the diversity and species composition of endophyte assemblages for a given host are important when selecting for fungal endophytes with potential agricultural applications. Attributes of particular plant-fungal endophyte interactions such as transmission mode, species diversity, and endophytes life cycle are important factors to be considered when practical applications of the effects endophytes have in their hosts are intended.

We have found that a fungus that live asymptomatically inside plant branches inhibit the growth of a common plant pathogenic bacterium (*Xanthomonas*) *in vitro*. This fungus (*C. wehmeyeriana*) produce phenolic compounds, quercetin derivatives flavonoids, which may be responsible for this activity. These compounds may represent an extrinsic source of defense against bacterial infection for the host of *C. wehmeyeriana.*

The observed cases of defensive mutualism between endophytic fungi and their host present the question of why plants get damaged by pests and pathogens if they are already infected by endophytes in nature? While pest and pathogens are common in natural plant ecosystems the norm is a balance whereby plant populations usually does not get completely eliminated by the effects of pest and pathogens. Plant infection by multiple endophyte species that may perform different roles as have been discussed here could be good for plant species in the long term. Frequencies of infection by endophyte species that could bring an advantage to their host do not need to be high. Endophyte

species found at low frequencies in contemporary time may have performed better under the stressful conditions in the past. For plants that harbor multiple species is like keeping an extra arsenal of weapons ready for when the stressful conditions arrive.

It is important to notice that fungal endophytes can also negatively affect the physiology of the host and it is important to determine to what extend this occur and to know the cost and benefits of fungal endophyte infections under stress and not stress conditions (see Arnold & Engelbretch, 2007; Santos Rodriguez et al., 2000, E. A. Herre unpublished). Based on the evidence provided (Table 1 and text), overall it is plausible to think that fungal endophytes jointly with other endophytic organisms boost the capacity of the plant to tolerate adverse abiotic and biotic factors. Additionally it is tentative to think that in some cases they may perform a work similar to what the microflora of mammals do for the immune system of their hosts.

A review of the research literature on fungal endophytes made by Saikkonnen et al., (2006) suggest that it is more likely that mutualistic effects of fungal endophytes occur in agroecosystems. In agroecosystems, plant crops may get depauperate in their symbiotic endophytes specially if the crop have been moved far away from its center of origin (i.e. where their potentially co-evolved symbionts and natural enemies are more likely to be found, see Evans 1999) or if its genotype have received extensive artificial selection (breeding). While agroecosytems may be not good promoter of fungal endophyte diversity, inoculating and keeping good endophytes inside target tissues may be handier compared to the wild ecosystems.

Whether or not the major role of the majority of fungal endophyte species is to protect their hosts against natural enemies, multiple studies in a range of plant lineages

indicate that they can confer several advantages including survival or tolerance to specific adverse factors spanning a wide range of abiotic and biotic stressful conditions. The studies reviewed here encourage deepening research on prospection of these fungi for its applicability in agricultural systems. The promise of practical use of endophytes for crop protection and production is starting to be realized.

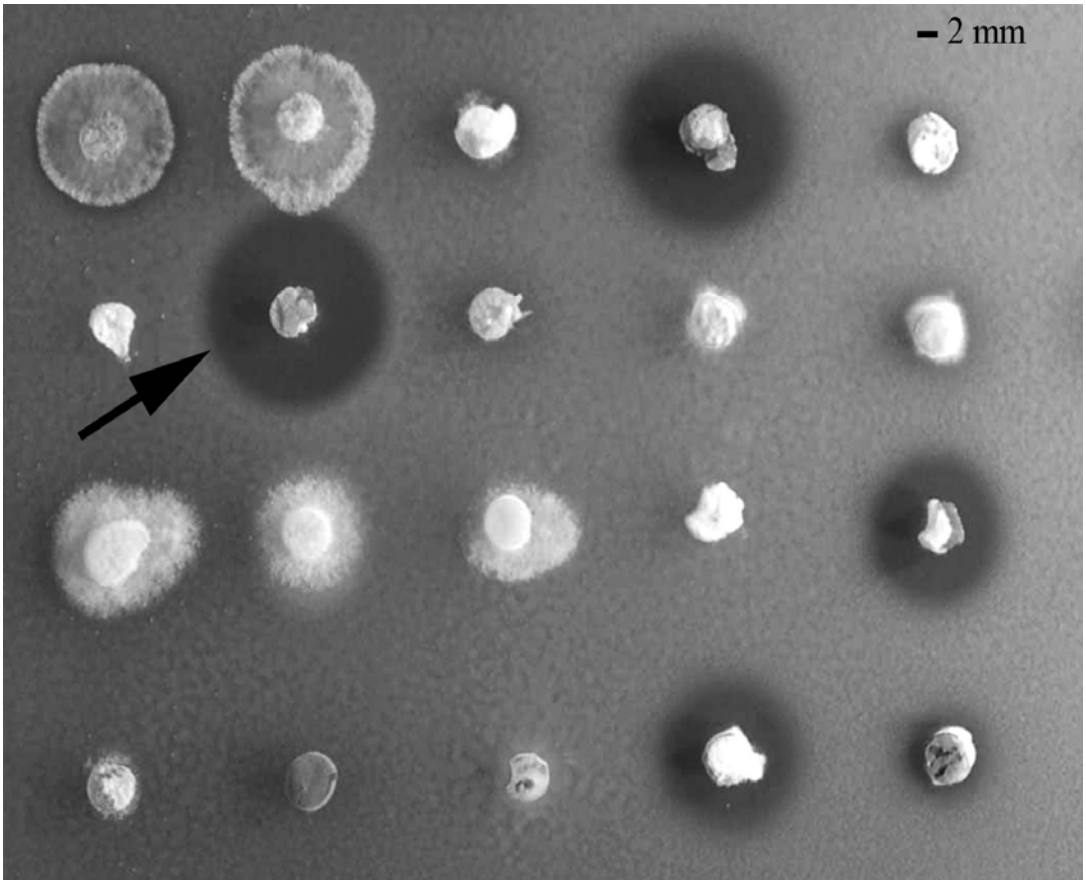

Figure 1. *In vitro* assay testing fungal endophytes activity on the growth of *Xanthomonas campestris campestris*. Plugs of agar with mycelia of fungal endophytes were plated almost at the same time with the bacterium on Potato Dextrose Agar (see methodology in the text). The arrow shows inhibition on the bacterium growth due to a diffusible compound coming from the agar plug with mycelium of *Cryptosporella wehmeyeriana*.

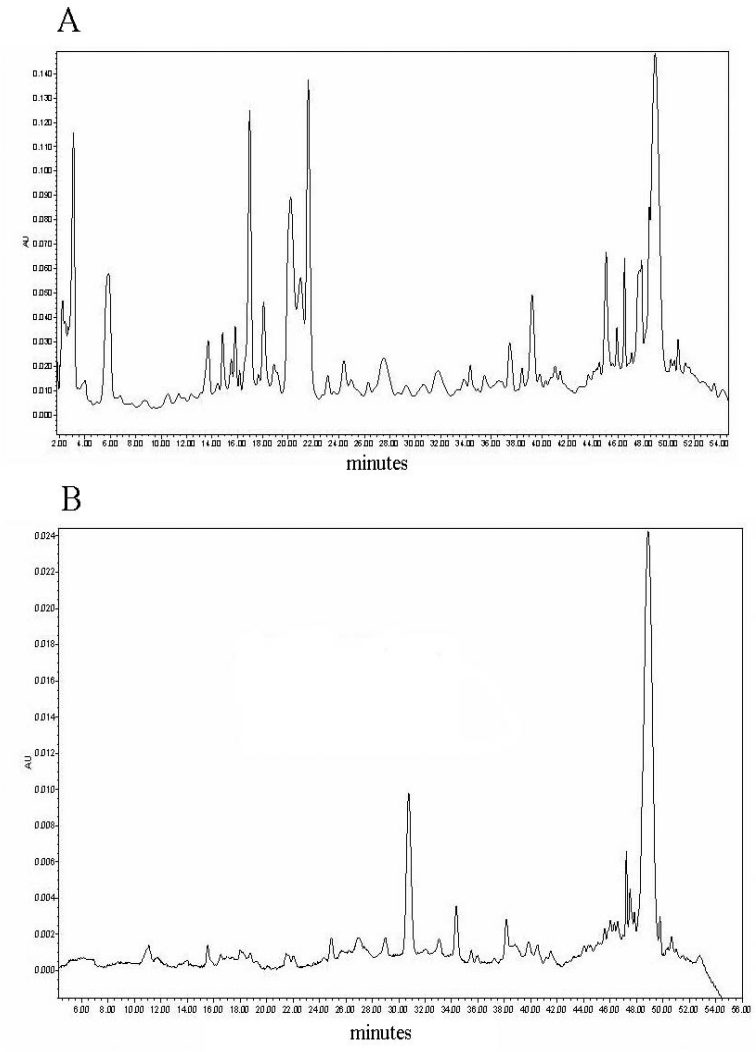

Figure 2. Representative bioactive compounds produced by *Cryptosporella wehmeyeriana*. Phenolic acids (A), and quercetin derivatives (B), as detected by HPLC-PDA at 280 and 366 nm respectively.

Table 1. Representative studies conducted *in planta* on host of agricultural importance showing that fungal endophytes can enhance increased plant productivity and resistance against pathogens. For fungal endophyte effects on plant insect deterrence or control see Azevedo et al 2000, Breen 1994, Kuldau and Bacon 2008, and Rowan and Latch 1994.

Host	Host family	Endophyte	Benefit confered by endophyte	Reference
Brassica campestris	Brassicaceae	*Heteroconium chaetospira*	Suppresion of clubroot and *Verticillium* yellows	Narisawa et al. 2000
Cucumis sativus	Cucurbitaceae	*Clonostachys rosea*	Growth enhancement and productivity	Sutton et al. 2008
Quercus cerris, *Q. pubescens*	Fagaceae	*Fusarium tricinctum* and *Alternaria alternata*	Reduce seedling mortality due to *Diplodia corticola* pathogen	Campanile et al. 2007
Geranium sp.	Geraniaceae	*Clonostachys rosea*	Growth enhacement and productivity	Sutton et al. 2008
Theobroma cacao	Malvaceae	Mix of different fungal species	Limit leaf damage due to *Phytophthora palmivora*	Arnold et al. 2003
Theobroma cacao	Malvaceae	*Clonostachys rosea*	Limit reproduction of the fungal pathogen *Moniliophthora roreri*	Mejía et al. 2008b
Theobroma cacao	Malvaceae	*Colletotrichum gloeosporioides*	Control incidence of *Phytopthora* spp.	Mejía et al. 2008b
Theobroma cacao	Malvaceae	*Gliocladium catenulatum*	Reduce incidence of *Crinipellis perniciosa* (Witche's Broom disease of cacao)	Rubini et al. 2005
Musa	Musaceae	*Fusarium* spp.	Reduce number of *Rhadopolus similis*/g of roots	Pocasangre et al. 2001

Musa AAA	Musaceae	*Fusarium oxysporum* & *Trichoderma atroviride*	Control of the nematode *Rhadopolus similis*	zum Felde et al. 2006
Brachiaria brizantha	Poaceae	*Acremonium implicatum*	Reduce number and size of lesions by *Dreschlera* sp.	Kelemu et al. 2001
Festuca arundinacea	Poaceae	*Acremonium coenophialum*	Drougth tolerance	Arechavaleta et al. 1989
Festuca arundinacea	Poaceae	*Acremonium coenophialum*	Control nematode	West et al. 1988
Festuca arundinacea	Poaceae	*Acremonium coenophialum*	Limit nematode reproduction	Kimmons et al. 1990
Festuca arundinaceae	Poaceae	*Acremonium coenophialum*	Reduce seedling loss due to *Rhizoctonia zeae* seedling disease	Gwinn and Gavin 1992
Festuca spp.	Poaceae	*Epichloe festucae*	Suppression of Red threat (*Laetisaria fusiformis*)	Bonos et al. 2005
Festuca spp.	Poaceae	*Epichloe festucae*	Suppression of Dollar Spot disease by *Sclerotinia homoeocarpa*	Clarke et al. 2006
Lolium perenne	Poaceae	*Acremonium lolii*	Reduction of galls caused by *Meloidogyne naasi*	Stewart et al. 1993
Hordeum vulgare	Poaceae	*Piriformospora indica*	Tolerance to salt stress, increase in yield and resistance to pathogens	Waller et al. 2005
Oryza sativa	Poaceae	*Fusarium*	Reduce galling severity by *Meloidogyne graminicola*	Sikora et al. 2008

Triticum aestivum	Poaceae	*Chaetomium* spp. and *Phoma* sp.	Reduce density of pustules of the rust pathogen *Puccinia recondita*	Dingle and Mcgee 2003
Zea mays	Poaceae	*Acremonium zeae*	Interfere with *Aspergillus flavus* infection	Wicklow et al. 2005
Rosa sp.	Rosaceae	*Clonostachys rosea*	Growth enhacement and productivity	Sutton et al. 2008
Lycopersicon esculentum	Solanaceae	*Fusarium oxysporum*	Reduce infection by the nematode *Meloidogyne incognita*	Hallman and Sikora 1995
Lycopersicon esculentum	Solanaceae	*Fusarium oxysporum*	Induce resistance toward *Meloidogyne incognita*	Dababat and Sikora 2007
Solanum melongena	Solanaceae	*Heteroconium chaetospira*, *Phialocephala fortinii*	Suppresion of *Verticillium* wilt	Narisawa et al. 2002

Table 2. Summary of recommendations for selection and application of fungal endophytes to agricultural plants.

1. Survey and collect fungal endophytes from the target host species and organs, including sampling of organs at different ages. Sampling is recommended under both cultivated and wild conditions, in particular at the center of origin of the host. Mature organs close to senescence are likely to harbor dominant or better adapted fungal endophyte strains. Dominant endophyte species are likely to outcompete rare ones, and thus be more easily administered to the host and remain within tissues.

2. Classify fungal endophyte strains by morphology and molecular methods. Strains accurately determined to belong to species known as pathogenic on target and co-occurring plant species should be avoided for application in the field. Test pathogenicity of selected endophyte strains on nontarget hosts. Co-occuring crops should be tested.

3. In vitro screening for bioactivity on target pathogens and pests should be combined with comparison of endophyte colonized (E+) and noncolonized (E-) plants to determine likely mechanism of beneficial function. This work also evaluates Koch's postulates and determines the pathogenicity of the endophytes to be used. Small scale testing should be conducted under a range of nursery or field conditions.

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Curriculum Vita
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Education

Ph.D., Plant Biology/ Plant Pathology, Rutgers University, NJ, October 2009.
B.Sc., Biology/ Microbiology & Parasitology, University of Panama, Panama, 2002.

Research Experience

- 2004-present. Graduate Research Assistant. Dept. Plant Biology and Pathology. Rutgers University. New Brunswick NJ, USA. Project: Phylogenetic and taxonomic studies of microfungi from the Gnomoniaceae (Diaporthales, Ascomycetes).

- 2005-present. Visiting Scientist. Systematic Mycology & Microbiology Laboratory, USDA-ARS, Beltsville, MD, USA. Systematics of Diaporthales (Ascomycetes).

- 1998-2004 Research Assistant, Smithsonian Tropical Research Institute. Project: Ecology and biocontrol potential of fungal endophytes associated with the chocolate tree.

- 1998 Research Technician, Smithsonian Tropical Research Institute. Project: Physiological regulation of water flux in lianas of Barro Colorado Island.

- 1997 Volunteer, Central Laboratory for Plant Disease Diagnosis. Agricultural Research Institute of Panama (IDIAP). First report of *Tilletia barclayana* in Panama.

- 1997 Research Aid. Smithsonian Tropical Research Institute. Project task. Isolation and culture maintenance of oomycetes (*Pythium* spp. and *Phytophthora* spp.) pathogenic to tropical seedlings.

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