TAXONOMY, SYSTEMATICS, ECOLOGY, AND EVOLUTIONARY BIOLOGY

OF THE GNOMONIACEAE (DIAPORTHALES), WITH EMPHASIS ON

GNOMONIOPSIS AND *OPHIOGNOMONIA*

by

DONALD MATTHEW WALKER

A Dissertation submitted to the

Graduate School-New Brunswick

Rutgers, The State University of New Jersey

in partial fulfillment of the requirements

for the degree of

Doctor of Philosophy

Graduate Program in Plant Biology

written under the direction of

Dr. James F. White, Jr.

and approved by

_____

_____

_____

_____

_____

New Brunswick, New Jersey

October, 2012

TAXONOMY, SYSTEMATICS, ECOLOGY, AND EVOLUTIONARY BIOLOGY

OF THE GNOMONIACEAE (DIAPORTHALES), WITH EMPHASIS ON

GNOMONIOPSIS AND *OPHIOGNOMONIA*

By

DONALD MATTHEW WALKER

Dissertation Director:

Dr. James F. White, Jr.

The Gnomoniaceae (Diaporthales, Sordariomycetes, Ascomycota) are a family of perithecial ascomycetes that occur as endophytes, pathogens, or saprobes on growing and overwintered leaves and twigs of hardwood trees, shrubs, and herbaceous host plants. Many species of Gnomoniaceae cause serious diseases in agricultural and ornamental plants. Despite their economic importance, the biodiversity, evolutionary biology, ecology, and host plant relationships are largely unknown.

The objectives of this study were to: 1) infer species level phylogenies of the genera *Gnomoniopsis* and *Ophiognomonia*; 2) design primers for newly identified single-copy protein-coding genes to be used as phylogenetic markers for species-level systematics in the Sordariomycetes; 3) integrate host association and environmental data with a phylogenetic analysis of *Ophiognomonia* to better understand speciation events in this genus. To achieve these objectives, herbarium and freshly collected specimens were compared using morphology, host association, and phylogenetic analyses of multiple molecular markers. Microscopic measurements of morphological characters such as

ascospore size and septation were integrated with molecular approaches to define species of *Gnomoniopsis* and *Ophiognomonia.* Fungal genome sequences were mined for single-copy orthologous genes, primers designed, gene regions sequenced, and phylogenetic informativeness of each marker assessed. The performance of the newly identified markers was then compared to other markers commonly used in fungal phylogenetics. In addition, the program Spatial Evolutionary and Ecological Vicariance Analysis (SEEVA) was used to study patterns of host plant specificity and ecological vicariance using a multi-gene phylogeny of *Ophiognomonia.*

This research resulted in the recircumscription of the genera *Gnomoniopsis* and *Ophiognomonia* in the Gnomoniaceae along with an account of each species in these genera. A total of 32 taxonomic novelties were defined including 26 new species and 6 new combinations. Primer sets for two newly identified markers were developed that should be useful for ascomycete systematists. Host specificity and environmental influences were hypothesized as mechanisms contributing to speciation patterns in *Ophiognomonia.* A visual and statistically solid understanding of evolutionary mechanisms influencing speciation events, host switches, and ecological divergence in the genus *Ophiognomonia* is presented and discussed. This a comprehensive report of the taxonomy, evolutionary biology, and ecology of the genus *Ophiognomonia.*

ACKNOWLEDGEMENTS

DEDICATION

This work is dedicated to the very special people who have supported, encouraged, and

believed in me over the last five years: my wife Lisa, my dad Donald, my mom Lynn,

and sisters Danielle and April; also to the late Donald E. Walker and Robert D. Hartinger,

you will be forever missed. Thank you for your love, patience, and support.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Chapter 1

Introduction to the taxonomy, systematics, ecology, and evolutionary biology

of the Gnomoniaceae (Diaporthales), with emphasis on

Gnomoniopsis* and *Ophiognomonia

The ascomycete order Diaporthales including the Gnomoniaceae are a highly

diverse group of microfungi that are largely understudied with respect to biodiversity,

host association, pathogenicity, and geographic distribution. Species of the Diaporthales

are known to be plant pathogens, endophytes and saprobes of hardwood trees, herbaceous

plants, and shrubs. Chestnut blight caused by *Cryphonectria parasitica* (Murrill) M.E.

Barr (Cryphonectriaceae, Diaporthales), is a particularly devastating disease that has

drastically altered the eastern North American forests (Anagnostakis 1988). Many species

in the Gnomoniaceae (Diaporthales) cause serious diseases to shade, nut, and lumber

producing trees, such as oak dieback (*Apiognomonia quercina* (Kleb.) Höhn.), cherry leaf

scorch (*A. erythrostoma* (Pers.) Höhn.), and sycamore canker (*A. veneta* (Sacc. & Speg.)

Höhn.) (Rossman et al. 2007). Species of the Gnomoniaceae are integral parts of

temperate and subtropical ecosystems existing as endophytes in living leaves,

herbaceous, or woody substrates producing fruiting bodies on overwintered leaves and

dead attached woody plant parts during cool moist spring conditions (Danti et al. 2002,

Rossman et al. 2007, Sieber and Hugentobler 1987, Stoykov 2005).

Species of the Gnomoniaceae have immersed, sometimes erumpent or superficial

perithecia, which are solitary or aggregated in an undeveloped stroma (Rossman et al.

2007, Sogonov et al. 2008). The perithecia are dark brown to black and

pseudoparenchymatous with central, eccentric, or lateral necks (Rossman et al. 2007, Sogonov et al. 2008). The asci detach from the base of the perithecium, floating free at maturity, and usually have an inconspicuous or distinct apical ring. The ascospores are generally small, hyaline, uniseptate, rarely with appendages at each end. Monod (1983) characterized the asexual states of Gnomoniaceae as acervular or pycnidial, phialidic, with non-septate conidia.

The genera of the Gnomoniaceae were recently revised based on a survey of leaf-inhabiting diaporthalean fungi (Sogonov et al. 2008). Sogonov et al. (2008) determined that traditional generic concepts that emphasized characters such as stromal development, position of the perithecium neck, and ascospore septation did not correlate with a multigene phylogeny (Barr 1978, Monod 1983). A single morphological character can no longer be used to define the genera of the Gnomoniaceae (Sogonov et al. 2008). Phylogenetic analyses of molecular markers is the primary methodology for systematic studies of the Gnomoniaceae, however, host specificity and morphology are also useful to confirm species concepts. For example, the genera *Cryptosporella* and *Gnomonia* associate almost exclusively with plants in the Betulaceae. Based on phylogenetic analyses of multiple molecular markers, ten genera are now recognized in the family Gnomoniaceae (Diaporthales) including the newly circumscribed genera *Gnomoniopsis* Berl. and *Ophiognomonia* (Sacc.) Sacc. (Mejía et al. 2011a, Sogonov et al. 2008). These genera served as the basis for my dissertation research.

The genus *Gnomoniopsis* based on the type species, *G. chamaemori* (Fr.) Berl., has been documented from a worldwide distribution in temperate climates and includes many economically significant pathogens of rosaceous crop plants such as strawberry,

raspberry, and blackberry (Bolay 1971, Maas 1998, Monod 1983). For example, *Gnomoniopsis fructicola* (Arnaud) Sogonov causes strawberry leaf blotch in Europe and Canada (Bolton 1954, Fall 1951, Maas 1998, van Adrichem and Bosher 1958). Sogonov et al. (2008) recognized eight species in *Gnomoniopsis* most of which are host genus specific on plants in the Fagaceae, Onagraceae, and Rosaceae.

Although Sogonov et al. (2008) included a number of species in *Gnomoniopsis*, only the type and two newly discovered species were fully described. In addition, the taxonomy for both known and novel species has yet to be resolved in the genus *Gnomoniopsis*. A multigene phylogeny, identification key including descriptions and illustrations, and taxonomic treatment of *Gnomoniopsis* was completed herein as chapter two (Walker et al. 2010). This account will be useful to plant pathologists for identification purposes, treatment, and quarantine decisions.

The taxonomy and species relationships in the genus *Ophiognomonia* have yet to be resolved for many of the known species. The genus *Ophiognomonia* was based on *Gnomoniella* subgenus *Ophiognomonia* Sacc. Sogonov et al. (2008) circumscribed this genus based on the type species, *O. melanostyla* (DC.:Fr.) Berl. *Ophiognomonia* includes species with elongate to fusiform and often septate ascospores that occur on plants in the families Betulaceae, Fagaceae, Juglandaceae, Lauraceae, Malvaceae, Platanaceae, Rosaceae, Salicaceae, and Sapindaceae (Sogonov et al. 2008). Species of *Ophiognomonia* are known to cause diseases on shade, lumber, and nut producing trees. For example, walnut anthracnose and leaf blotch are caused by virulent strains of *O. leptostyla* in the Eastern and Midwestern United States (Berry 1981, Juhasova et al. 2006, Neely and Black 1976). Four species were described and 15 included in the most recent revision of

Ophiognomonia (Sogonov et al. 2008). However, the species relationships within *Ophiognomonia* were not resolved in the latter study. A multigene phylogenetic approach, including an identification key and descriptions and illustrations for 45 species of *Ophiognomonia,* was completed in chapter four.

The use of molecular phylogenetics to study fungal systematics complements traditional morphological approaches. Phylogenetic analyses using complex algorithms allow scientists to quantify changes in DNA sequence data and infer relationships among taxa. Fungi are highly diverse but relatively understudied. To date only 100,000 species of fungi have been described (Blackwell 2011). The current estimate of fungal diversity ranges from 3.5 to 5.1 million species (O'Brien et al. 2005). Despite recent advances in sequencing technology and phylogenetic algorithms, the extant species remain incompletely documented (McLaughlin et al. 2009). The Deep Hypha Research Coordination Network and AFTOL1 (Assembling the Fungal Tree of Life 1) projects have resolved many higher-level relationships in fungi, leaving the terminal branches in question.

Advances in molecular biology have allowed for production of large amounts of DNA sequence data. For fungi, the majority of publicly available sequence data consists of the following markers: nuclear large and small subunits, 5.8S ribosomal RNA gene, subunits 1 and 2 of RNA polymerase II, elongation factor 1α (*tef-1α*), and mitochondrial ATP synthase (*e.g.,* Damm et al. 2007, Letcher et al. 2008, Mejía et al. 2011a, 2011b, Raja et al. 2008, Schoch et al. 2009, Spatafora et al. 2006, Walker et al. 2010). These genes are undeniably useful for fungal phylogenetics, however, problematic issues such as paralogs have been documented in several of these markers (Corradi et al. 2004a,

2004b, James et al. 2006, Ko and Jung 2002, Landvik et al. 2001, O'Donnell and Cigelnik 1997, O'Donnell et al. 1998). Development of additional markers appropriate for species-level systematics, which are useful across a broad range of fungi, are necessary to confirm and strengthen support in phylogenetic relationships.

The dataset from Aguileta et al. (2008, FUNYBASE; Marthey et al. 2008) consists of thousands of single-copy orthologous gene families among fungi and are an ideal resource for developing new phylogenetic markers. In chapter three, new markers were identified from the Aguileta et al. (2008) dataset to increase resolution and support species-level relationships within two genera in the Gnomoniaceae, *Gnomoniopsis* and *Ophiognomonia*. The phylogenetic performance of the newly developed markers was compared to other commonly used markers and primer utility demonstrated within a broad sampling of the Ascomycota.

Integrating evolutionary hypotheses within time and space including environmental, host, and geographical information provides evidence for how speciation occurred throughout evolutionary history and over a broad geographic range. Host plant specificity, ecological conditions, and the microclimate at the fungus/host interface are important factors to consider when exploring speciation of gnomoniaceous fungi. The niche is a fundamental concept in ecology and evolution since it describes a set of biotic and abiotic conditions that determine the presence of a species in a given habitat and ability to maintain a stable population (Hutchinson 1957). Species are influenced by both the fundamental niche, composed of abiotic conditions, and the realized niche that determines variables permitting species coexistence (Weins and Graham 2005). Diaporthalean fungi are dependent on their host plants to complete their life cycle.

Environmental conditions such as temperature and precipitation play an important role, as does the host, in the life cycle of these fungi.

Niche conservatism is the trend for species to retain their fundamental niche over evolutionary history (Harvey and Pagel 1991, Weins et al. 2010). Gradual shifts over long evolutionary periods can change ecological conditions and slowly alter a species niche. In general for gnomoniaceous fungi, as a new species evolves it is more likely to associate with a host closely related to the host of its most recent ancestor. However, host shifts to novel plant species are not uncommon for this family. Both niche conservatism and specialization in respect to host and the environment are important concepts necessary to understand influences on speciation in the genus *Ophiognomonia* (Weins and Donoghue 2004). In this research, speciation events in *Ophiognomonia* were linked with host switching or host conservatism at different taxonomic host ranks. Also, environmental data was utilized to determine if niche-based conservatism or specialization influences speciation events in this genus. A visual and statistically solid understanding of the evolutionary mechanisms influencing speciation in the genus *Ophiognomonia* is provided in chapters 5−6.

LITERATURE CITED

Aguileta, G., Marthey, S., Chiapello, H., Lebrun, M.-H., Rodolphe, F., Fournier, E., Gendrault-Jacquemard, A., Giraud, T., 2008. Assessing the Performance of Single-Copy Genes for Recovering Robust Phylogenies. Syst. Biol. 57, 613−627.

Anagnostakis, S.L., 1988. *Cryphonectria parasitica* cause of chestnut blight. Adv. Plant Pathol. 6, 123−136.

Barr, M.E., 1978. The Diaporthales in North America with emphasis on *Gnomonia* and its segregates. Mycologia Memoir 7, 1−232.

Berry, F.H., 1981. Walnut Anthracnose Forest Insect & Disease Leaflet 85. U.S. Department of Agriculture, Forest Service, Northern Area State & Private Forestry, Broomall, PA.

Bolay, A., 1971. Contribution a la connaissance de *Gnomonia comari* Karsten (syn. *G. fructicola* [Arnaud] Fall). Etude taxonomique, phytopathologiqueet recherches sur sa croissance in vitro. Berichte der Schweizerischen Botanischen Gesellschaft 81, 398−482.

Bolton, A.T., 1954. *Gnomonia fructicola* on Strawberry. Can. J. Bot. 32, 172−181.

Corradi, N., Hijri, M., Fumagalli, L., Sanders, I.R., 2004. Arbuscular mycorrhizal fungi (Glomeromycota) harbour ancient fungal tubulin genes that resemble those of the chytrids (Chytridiomycota). Fungal Genet. Biol. 41, 1037−1045.

Corradi, N., Kuhn, G., Sanders, I.R., 2004. Monophyly of B-tubulin and H+-ATPase gene variants in *Glomus intraradices*: consequences for molecular evolutionary studies of AM fungal genes. Fungal Genet. Biol. 41, 262−273.

Damm, U., Crous, P.W., Fourie, P.H., 2007. Botryosphaeriaceae as potential pathogens of *Prunus* species in South Africa, with descriptions of *Diplodia africana* and *Lasiodiplodia plurivora* sp. nov. Mycologia 99, 664−680.

Danti, R., Sieber, T.N., Sanguineti, G., 2002. Endophytic mycobiota in twigs of healthy and declining *Fagus sylvatica* in the Apennines. Mycol. Res. 106, 1343−1348.

Fall, J., 1951. Studies on fungus parasites of strawberry leaves in Ontario. Can. J. Bot. 29, 299−315.

Harvey, P.H., Pagel, M.R., 1991. The comparative method in evolutionary biology. Oxford University Press.

Hutchinson, G.E., 1957. Concluding remarks. Cold Spring Harbor Symp. 22, 415−427.

James, T.Y., Kauff, F., Schoch, C.L., Matheny, P.B., Hofstetter, V., Cox, C.J., Celio, G., Gueidan, C., Fraker, E., Miadlikowska, J., Lumbsch, H.T., Rauhut, A., Reeb, V., Arnold, A.E., Amtoft, A., Stajich, J.E., Hosaka, K., Sung, G.-H., Johnson, D., O'Rourke, B., Crockett, M., Binder, M., Curtis, J.M., Slot, J.C., Wang, Z., Wilson, A.W., Schuszler, A., Longcore, J.E., O'Donnell, K., Mozley-Standridge, S., Porter, D., Letcher, P.M., Powell, M.J., Taylor, J.W., White, M.M., Griffith, G.W., Davies, D.R., Humber, R.A., Morton, J.B., Sugiyama, J., Rossman, A.Y., Rogers, J.D., Pfister, D.H., Hewitt, D., Hansen, K., Hambleton, S., Shoemaker, R.A., Kohlmeyer, J., Volkmann-Kohlmeyer, B., Spotts, R.A., Serdani, M., Crous, P.W., Hughes, K.W., Matsuura, K., Langer, E., Langer, G., Untereiner, W.A., Lucking, R., Budel, B., Geiser, D.M., Aptroot, A., Diederich, P., Schmitt, I., Schultz, M., Yahr, R., Hibbett, D.S., Lutzoni, F., McLaughlin, D.J., Spatafora, J.W., Vilgalys, R., 2006. Reconstructing the early evolution of Fungi using a six-gene phylogeny. Nature 443, 818−822.

Juhasova, G., Ivanova, H., Spisak, J., 2006. Biology of fungus *Gnomonia leptostyla* in agro-ecological environments of Slovakia. Mikologica Fitopatologie 40, 538−547.

Ko, K.S., Jung, H.S., 2002. Three nonorthologous ITS1 types are present in a polypore fungus *Trichaptum abietinum*. Mol. Phylogenet. Evol. 23, 112−122.

Landvik, S., Eriksson, O.E., Berbee, M.L., 2001. *Neolecta* - a fungal dinosaur? Evidence from beta-tubulin amino acid sequences. Mycologia 93, 1151−1163.

Letcher, P.M., Powell, M.J., Viusent, M.C., 2008. Rediscovery of an unusual chytridiaceous fungus new to the order Rhizophydiales. Mycologia 100, 325−334.

Maas, J.L., 1998. Compendium of Strawberry Diseases. 2nd edn. The American Phytopathological Society. U.S.A.

Marthey, S., Aguileta, G., Rodolphe, F., Gendrault, A., Giraud, T., Fournier, E., Lopez-Villavicencio, M., Gautier, A., Lebrun, M.-H., Chiapello, H., 2008. FUNYBASE: a FUNgal phYlogenomic dataBASE. BMC Bioinformatics 9, 456.

McLaughlin, D.J., Hibbett, D.S., Lutzoni, F., Spatafora, J.W., Vilgalys, R., 2009. The search for the fungal tree of life. Trends Microbiol. 17, 488−497.

Mejía, L., Rossman, A., Castlebury, L., Yang, Z., White, J., 2011a. *Occultocarpon*, a new monotypic genus of Gnomoniaceae on *Alnus nepalensis* from China. Fungal Divers. 52, 99−105.

Mejía, L.C., Rossman, A.Y., Castlebury, L.A., White, J.F., Jr., 2011b. New species, phylogeny, host-associations, and geographic distribution of the genus *Cryptosporella* (Gnomoniaceae, Diaporthales). Mycologia 103, 379−399.

Monod, M., 1983. Monographie taxonomique des Gnomoniaceae (Ascomycétes de l'ordre des Diaporthales I). Beihefte zur Sydowia 9, 1−315.

Neely, D., Black, W.M., 1976. Anthracnose of black walnuts in the Midwest. Plant Dis. Reporter 60, 519−521.

O'Donnell, K., Cigelnik, E., 1997. Two divergent intragenomic rDNA ITS2 types within a monophyletic lineage of the fungus *Fusarium* are nonorthologous. Mol. Phylogenet. Evol. 7, 103−116.

O'Donnell, K., Cigelnik, E., Nirenber, H.I., 1998. Molecular systematics and phylogeography of the *Gibberella fujikuroi* species complex. Mycologia 90, 465−473.

Raja, H.A., Miller, A.N., Shearer, C.A., 2008. Freshwater ascomycetes: *Aquapoterium pinicola*, a new genus and species of Helotiales (Leotiomycetes) from Florida. Mycologia 100, 141−148.

Rossman, A.T., Farr, D.F., Castlebury, L.A., 2007. A review of the phylogeny and biology of the Diaporthales. Mycoscience 48, 135−144.

Schoch, C.L., Sung, G.-H., Lopez-Giraldez, F., Townsend, J.P., Miadlikowska, J., Hofstetter, V., Robbertse, B., Matheny, P.B., Kauff, F., Wang, Z., Gueidan, C., Andrie, R.M., Trippe, K., Ciufetti, L.M., Wynns, A., Fraker, E., Hodkinson, B.P., Bonito, G., Groenewald, J.Z., Arzanlou, M., Sybren de Hoog, G., Crous, P.W., Hewitt, D., Pfister, D.H., Peterson, K., Gryzenhout, M., Wingfield, M.J., Aptroot, A., Suh, S.-O., Blackwell, M., Hillis, D.M., Griffith, G.W., Castlebury, L.A., Rossman, A.Y., Lumbsch, H.T., Lucking, R., Budel, B., Rauhut, A., Diederich, P., Ertz, D., Geiser, D.M., Hosaka, K., Inderbitzin, P., Kohlmeyer, J., Volkmann-Kohlmeyer, B., Mostert, L., O'Donnell, K., Sipman, H., Rogers, J.D., Shoemaker, R.A., Sugiyama, J., Summerbell, R.C., Untereiner, W., Johnston, P.R., Stenroos, S., Zuccaro, A., Dyer, P.S., Crittenden, P.D., Cole, M.S., Hansen, K., Trappe, J.M., Yahr, R., Lutzoni, F., Spatafora, J.W., 2009. The Ascomycota Tree of Life: A Phylum-wide Phylogeny Clarifies the Origin and Evolution of Fundamental Reproductive and Ecological Traits. Syst. Biol. 58, 224−239.

Sieber, T., Hugentobler, C., 1987. Endophytische Pilze in Blättern und Ästen gesunder und geschädigter Buchen (*Fagus sylvatica* L.). Eur. J. Forest Pathol. 17, 411−425.

Sogonov, M.V., Castlebury, L.A., Rossman, A.Y., Mejía, L.C., White, J.F., 2008. Leaf-inhabiting genera of the Gnomoniaceae, Diaporthales. Stud. Mycol. 62, 1−77.

Spatafora, J.W., Sung, G.-H., Johnson, D., Hesse, C., O'Rourke, B., Serdani, M., Spotts, R., Lutzoni, F., Hofstetter, V., Miadlikowska, J., Reeb, V., Gueidan, C., Fraker, E., Lumbsch, T., Lucking, R., Schmitt, I., Hosaka, K., Aptroot, A., Roux, C., Miller, A.N., Geiser, D.M., Hafellner, J., Hestmark, G., Arnold, A.E., Budel, B., Rauhut, A., Hewitt, D., Untereiner, W.A., Cole, M.S., Scheidegger, C., Schultz, M., Sipman, H., Schoch, C.L., 2006. A five-gene phylogeny of Pezizomycotina. Mycologia 98, 1018−1028.

Stoykov, D.Y., 2005. New records of Diaporthales in Bulgaria. I. Mycologia Balcanica 2, 69−74.

van Adrichem, M.C.J., Bosher, J.E., 1958. Leaf blotch and petiole blight of strawberry caused by *Gnomonia fructicola*. Plant Dis. Reporter 42, 772−775.

Walker, D.M., Castlebury, L.A., Rossman, A.Y., Sogonov, M.V., White, J.F., 2010. Systematics of genus *Gnomoniopsis* (Gnomoniaceae, Diaporthales) based on a three gene phylogeny, host associations and morphology. Mycologia 102, 1479−1496.

Wiens, J.J., Ackerly, D.D., Allen, A.P., Anacker, B.L., Buckley, L.B., Cornell, H.V., Damschen, E.I., Jonathan Davies, T., Grytnes, J.-A., Harrison, S.P., Hawkins, B.A., Holt,

R.D., McCain, C.M., Stephens, P.R., 2010. Niche conservatism as an emerging principle in ecology and conservation biology. Ecol. Lett. 13, 1310−1324.

Wiens, J.J., Donoghue, M.J., 2004. Historical biogeography, ecology and species richness. Trends Ecol. Evol. 19, 639−644.

Wiens, J.J., Graham, C.H., 2005. Niche conservatism: integrating evolution, ecology, and conservation biology. Ann. Rev. Ecol. Evol. Syst. 36, 519−539.

Chapter 2

Systematics of genus *Gnomoniopsis* (Gnomoniaceae, Diaporthales) based on a three gene phylogeny, host associations, and morphology[1]

ABSTRACT

Species of *Gnomoniopsis* are leaf- and stem-inhabiting pyrenomycetes that infect plants in the Fagaceae, Onagraceae, and Rosaceae. Morphology, and analyses of DNA sequences from three ribosomal DNA and protein coding regions, namely *β-tubulin*, translation elongation factor 1α (*tef-1α*) and the ITS region including ITS1, 5.8S rDNA and ITS2, were used to define species in *Gnomoniopsis*. Secondary structural alignment of the ITS region across four genera in the Gnomoniaceae was used to increase the potential number of homologous positions in the ITS alignment. Ascospore isolates were grown from newly collected specimens. Type specimens were compared with these specimens to determine their identity. In this paper a recent concept of *Gnomoniopsis* is confirmed with phylogenetic resolution of additional species. Four new combinations and one new species are proposed. Nine species are described and illustrated and a key is provided to the thirteen species currently recognized in *Gnomoniopsis*.

INTRODUCTION

Members of the Diaporthales including the Gnomoniaceae are well documented as plant pathogens. *Cryphonectria parasitica* (Murrill) M.E. Barr causes chestnut blight

---

[1] This chapter was published as: Walker, D.M., Castlebury, L.C., Rossman, A.Y., Sogonov, M.V., White, J.F., Jr. 2010. Systematics of genus *Gnomoniopsis* (Gnomoniaceae, Diaporthales) based on a three gene phylogeny, host associations and morphology. Mycologia. 102, 1479−1496.

that drastically altered the eastern North American forests (Anagnostakis 1988). Species in the Gnomoniaceae cause serious tree diseases such as cherry leaf scorch (*A. erythrostoma* (Pers.) Höhn.), oak dieback (*Apiognomonia errabunda* (Roberge) Höhn.), and sycamore canker (*A. veneta* (Sacc. & Speg.) Höhn.) (Rossman et al. 2007a). Members of the Gnomoniaceae also exist as endophytes in leaves, herbaceous, or woody substrates and produce fruiting bodies on overwintered leaves and attached woody plant parts during the spring months (Danti et al. 2002, Rossman et al. 2007a, Sieber and Hugentobler 1987, Stoykov 2005).

A recent monograph of the genera of the Gnomoniaceae was based on an extensive survey of leaf-inhabiting diaporthalean fungi (Sogonov et al. 2008). In this monograph revised concepts of several genera of the Gnomoniaceae were determined to be contrary to historical concepts that emphasized such characters as stromal development, position of the neck, and ascospore septation (Barr 1978, Monod 1983). Genera of the Gnomoniaceae can no longer be defined based on one or two morphological characters (Sogonov et al. 2008). However, host identity in combination with certain morphological characters is often useful in delineating genera and species in the Gnomoniaceae (Sogonov et al. 2008). For example, the genus *Gnomonia* appears host specific on plants in the Betulaceae whereas species of *Apiognomonia* have a diverse host range. Based on a multigene phylogeny Sogonov et al. (2008) recognized nine genera in the family Gnomoniaceae including the resurrected genus *Gnomoniopsis* Berl.

The genus *Gnomoniopsis,* based on the type species *G. chamaemori* (Fr.) Berl., had previously been considered to be a synonym of *Gnomonia* (Barr 1978, Monod 1983). According to Sogonov et al. (2008) *Gnomoniopsis* is characterized by having small, black

perithecia immersed in the host tissue. The perithecia are solitary lacking a stroma or in groups aggregated in a minimal stroma. The perithecia have a single central, marginal, or lateral neck. The asci are oval to fusiform and contain eight spores with a visible apical ring. The ascospores are one-septate, oval to fusiform, and without appendages except *G. tormentillae,* which has short appendages (Barr 1978). The known asexual states of *Gnomoniopsis* have subglobose pycnidia with hyaline, one-celled conidia that are oval, oblong, globose, or femur shaped. Sogonov et al. (2008) recognized eight species in *Gnomoniopsis*. Most species of *Gnomoniopsis* are host genus specific on plants in the Fagaceae*,* Onagraceae*,* and Rosaceae.

Several species now placed in *Gnomoniopsis* were synonymized under the name *Gnomonia comari* P. Karst. by previous workers (Bolay 1971). The taxonomy and pathology of *G. comari* was studied by Bolay (1971) whose observations were based on morphological and cultural data. Because of the overlapping morphological characters he recognized *G. comari* in a broad sense including six synonyms. Some of these synonyms, specifically *Gnomonia agrimoniae* Bref. & Tavel*, G. fructicola* (Arnaud) Fall*, Gnomoniella guttulata* Starbäck, and *Gnomonia occulta* Kirschst., are reconsidered here. *Gnomonia fragariae* Kleb., which had also been synonymized with *G. comari* because of morphological similarities*,* causes a disease on strawberry (Maas 1998). Moročko and Fatehi (2007) determined that isolates of *G. fragariae* belonged outside the Gnomoniaceae in a diaporthalean family now recognized as the Sydowiellaceae (Rossman et al. 2007a).

Additionally Sogonov et al (2008) identified three species of the asexually reproducing genus *Sirococcus* Preuss including the type species *S. conigenus* (DC.) P.F.

Cannon & Minter that are closely related to *Gnomoniopsis. Sirococcus conigenus* is a pathogen of *Cedrus*, *Larix*, *Picea*, and *Pinus* in Europe and North America and causes severe seedling death and shoot blight disease of seedlings, saplings, and mature trees in Europe and eastern North America (Rossman et al. 2007b, Sanderson and Worf 1986, Shahin and Claflin 1978, Smith 1973).

The genus *Gnomoniopsis* has a worldwide distribution and contains economically significant pathogens of rosaceous crop plants including blackberry, raspberry, and strawberry (Bolay 1971, Maas 1998, Monod 1983). *Gnomoniopsis fructicola* (Arnaud) Sogonov causes leaf blotch and petiole blight of strawberry in Europe and Canada (van Adrichem and Bosher 1958, Bolton 1954, Fall 1951, Maas 1998). *Gnomoniopsis clavulata* (Ellis) Sogonov, mistakenly identified as *Discula umbrinella* (Berk. & Broome) M. Morelet, causes oak anthracnose on hardwood trees such as *Quercus alba* and *Q. rubra* (Cohen 2004, Sogonov et al. 2007). A taxonomic system that documents key morphological, cultural, and molecular characters in *Gnomoniopsis* is needed by plant pathologists in order to identify causal organisms and treat diseases caused by species of *Gnomoniopsis* as well as to make quarantine decisions.

The primary objective of this study is to provide an account of the species in *Gnomoniopsis*. Morphology and analyses of sequences from three ribosomal DNA and protein coding regions, including: *β-tubulin*, translation elongation factor 1α (*tef-1α*) and the ITS region including ITS1, 5.8S rDNA and ITS2 regions (ITS), were used to define species in *Gnomoniopsis*. Sogonov et al. (2008) described three species of *Gnomoniopsis*, including *G. chamaemori*, *G. clavulata*, and *G. paraclavulata.* Barr (1978) and Monod (1983) described *G. tormentillae* as *Plagiostoma tormentillae.* Descriptions and

illustrations are presented herein for nine of the remaining species of *Gnomoniopsis* and a key is provided to the thirteen species currently recognized in *Gnomoniopsis*.

MATERIALS AND METHODS

Morphological observations

Specimens were rehydrated in water and mounted in water for observation. Macroscopic and microscopic characters were observed with a Zeiss Discovery v20 stereoscope and Zeiss Axiophot compound microscope (Carl Zeiss, Thornwood, New York) with differential interference contrast microscopy. Digital images were captured with a Zeiss AxioCam HRc camera and AxioVision version 4.6 software (Carl Zeiss, Thornwood, New York). Measurements were obtained with an ocular micrometer or with the AxioVision 4.6 software. Perithecia were prepared for microtome sectioning according to Torres et al. (2005). Freshly collected specimens were air dried, then preserved in an airtight plastic bag at 4 C. Perithecia from these newly collected specimens were crushed in a drop of sterile water on a glass slide to release the perithecial contents. Single asci or ascospores were transferred with a micropipette onto corn meal agar (CMA, Sigma, St. Louis, Missouri) plates in a 30 µl drop of antibiotic solution (aqueous solution of 0.025g/25 ml each of tetracyclin, erythromycin, penicillin G, streptomycin, ampicillin). Cultures were grown up on Potato Dextrose Agar (PDA, Difco, Becton, Dickinson & Co., Sparks, Maryland) for one week for observations of colonies. Colony colors were standardized according to Watling and Turnbull (1998). Cultures were deposited at Centraalbureau voor Schimmelcultures (CBS) in the Netherlands (Table 2.1).

DNA Extraction, Amplification, and Sequencing

Cultures were incubated on PDA for 5-7 days. Mycelial scrapings (approximately 50 mg) were harvested and lysed using the Fast Prep FP120 (Fischer Scientific Inc, Waltham, Massachusetts). Genomic DNA was extracted using the Qiagen DNeasy Plant Mini Kit (Qiagen Inc, Chatsworth, California, USA) according to the manufacturer's instructions. The *β-tubulin*, ITS, and *tef-1α* were amplified in 25 µL reactions on a GeneAmp 9700 thermal cycler (Applied Biosystems, Foster City, California) under the following reaction conditions: 10-15 ng of genomic DNA, 12.5 µl Taq 2x Master Mix (New England BioLabs, Ipswich, Massachusetts), and 1 µl of 10 µM each primer. The ITS regions were amplified with primers ITS5 and ITS4 (White et al. 1990). An approximately 750 base pair (bp) region of the *β-tubulin* gene was amplified with primers T1 and T2 (O'Donnell and Cigelnik 1997). An approximately 1000 bp region of the *tef-1α* gene was amplified in one or two reactions with the primer pairs EF1-728F/EF1-1567R or EF1-728F/EF1-1199R and EF1-983F/EF1-1567R (Carbone and Kohn 1999, Rehner 2001). The primer sequence for EF1-1199R was GGGAAGTACCMGTGATCATGT designed for use in this project based on an existing alignment of gnomoniaceous taxa. The thermal cycler program for amplification of *β-tubulin*, ITS, and *tef-1α* was as follows: 10 min at 95 C, 35 cycles of 30 sec at 94 C, 30 sec at 55 C, 1 min at 72 C, extension period of 10 min at 72 C. The PCR products were purified with ExoSAP-IT (USB Corporation, Cleveland, Ohio) according to the manufacturer's instructions. Amplified products were sequenced with the BigDye Terminator v3.1 cycle sequencing kit (Applied Biosystems, Foster City, California) on an

Applied Biosystems 3130*xl* Genetic Analyzer. The respective PCR primers were used as sequencing primers for each gene.

Sequence Data Analyses

Raw sequences were edited and assembled into contigs with Sequencher version 4.9 for Windows (Gene Codes Corporation, Ann Arbor, Michigan). Four alignments were prepared for the analyses. Alignment one consisted of the ITS regions for 31 isolates, representing 14 species in *Gnomoniopsis,* three species in *Sirococcus* and the two outgroup taxa *Apiognomonia veneta* (Sacc. & Speg.) Höhn. and *Plagiostoma euphorbiae* (Fuckel) Fuckel in the Gnomoniaceae. Secondary structure stems and loops were inferred in a diagrammatic representation using Mfold (Zuker 2003) and aligned according to Kjer (1995) using the Mfold diagram as a guide. Alignments two and three correspond to MUSCLE (Edgar 2004) alignments for *β-tubulin* and *tef-1α* respectively. Each alignment was composed of the DNA sequences for 29 isolates, representing 13 species of *Gnomoniopsis* and the additional isolates mentioned above. For *tef-1α,* only the 394 bp intron region was available for the *Sirococcus* isolates. The three individually aligned gene regions were concatenated into a single file to form alignment four. Because *β-tubulin* and *tef-1α* could not be amplified for *G. guttulata,* this species was excluded from alignment four. All alignments have been deposited in TreeBase.

Potential conflicts among data sets were assessed in two ways. An incongruence length-difference test (ILD) was implemented in PAUP* as the partition homogeneity test with 1000 heuristic search replicates, TBR, and MAXTREE at 1000 (Farris et al. 1995). Comparisons were made among all three genes at a threshold of $P < 0.05$. We also

assessed congruence among the three gene trees using a conditional comparison test (Johnson and Soltis 1998, Kellogg et al. 1996, Mason-Gamer and Kellogg 1996) using maximum parsimony bootstrap (BS) values of ≥70 %.

Phylogenetic trees were inferred with maximum parsimony (MP), maximum likelihood (ML) and Bayesian analyses. For all analyses rooting was accomplished by the outgroup method (Nixon and Carpenter 1993) based on evidence from Sogonov et al. (2008). Each single gene alignment was analyzed individually and then together in a three gene combined alignment using PAUP 4.0b10 (Swofford 2002). Phylogenetic trees were inferred using the maximum parsimony heuristic search option with 1000 random addition replicates and the tree bisection-reconnection (TBR) branch swapping option with MULTREES on. MAXTREE was set at 1000 with a limit of 1000 trees per random addition replicates. Gaps were treated as missing data. All characters were given equal weight and designated as unordered. Relative branch support was estimated with 1000 BS replications with ten random addition replicates per BS replicate (Felsenstein 1985).

Phylogenetic trees were also inferred using the Markov chain Monte Carlo (MCMC) algorithm as implemented in MrBayes v. 3.1.2 (Huelsenbeck and Ronquist 2001, Ronquist and Huelsenbeck 2003). ModelTest v. 3.7 was used to determine the appropriate model (Posada and Crandall, 1998). A GTR+I+G model was used for both the combined and ITS analyses with base frequencies calculated from the sample. Three hot and one cold chains were run in the MCMC analysis in MrBayes v. 3.1.2 (Huelsenbeck and Ronquist 2001, Ronquist and Huelsenbeck 2003). Five million generations were run with a sampling frequency of every 2000 generations. The first 500 000 generations were eliminated as the "burn in" period. Two individual runs were

performed for both the combined and ITS datasets. The results from the two runs were pooled and the majority rule consensus tree was calculated in PAUP 4.0b10.

The program PAUP 4.0b10 was used to calculate the most likely tree in a maximum likelihood analysis. ModelTest v. 3.7 estimated the model parameters for the combined analysis as follows: base=(0.2164, 0.2925, 0.2316, 0.2595), nst=6, rmat=(1.0606, 3.0941, 1.3794, 0.9034, 3.8452), rates=gamma, shape=1.5222, pinvar=0.4449 (Posada and Crandall 1998). The likelihood settings for the ITS analysis were as follows: base=(0.2504, 0.2340, 0.2218, 0.2939), nst=6, rmat=(2.4036, 3.5867, 1.4918, 1.0941, 5.0381), rates=gamma, shape=0.5967, pinvar=0.5292. A heuristic search with 100 random addition replicates, TBR, and MULTREES on was conducted for both alignments.

The program GARLI v0.96b8 was used to calculate maximum likelihood bootstrap (ML BS) support values (Zwickl 2006) since likelihood bootstraps did not run to completion in PAUP. ModelTest v. 3.7 calculated the GTR+I+G as the appropriate model for the analyses (Posada and Crandall 1998). Branch support was estimated under the default settings with 700 ML BS replications with ten random addition replicates per BS replicate. Bootstrap consensus trees for the combined and ITS datasets were calculated in PAUP*. A threshold of ≥70 % was used as the cutoff for significantly supported nodes.

RESULTS

The ITS alignment consisted of 546 total characters: 417 constant, 38 non-parsimony informative and 91 parsimony informative. A maximum parsimony (MP)

heuristic search produced two most parsimonious trees with a length of 244 steps (CI=0.676, RI=0.814, RC=0.551, HI=0.324). The *β-tubulin* alignment consisted of 819 total characters: 422 constant, 83 non-parsimony informative and 314 parsimony informative. The *tef-1α* alignment consisted of 1138 total characters: 671 constant, 101 non-parsimony informative and 366 parsimony informative. The combined three gene alignment consisted of 2,503 total characters: 1,510 constant, 222 non-parsimony informative and 771 parsimony informative. A MP heuristic search produced a single most parsimonious tree with a length of 2,286 steps (CI=0.661, RI=0.780, RC=0.515, HI=0.339). Only an ITS sequence represents *Gnomoniopsis guttulata* and therefore it was not included in the combined alignment. No difference in topology or support values was observed for the parsimony analysis when excluding the 744 bp region of the *tef-1α* gene that was unavailable for the *Sirococcus* isolates.

The partition homogeneity test (ILD) of the combined three gene alignment showed no significant differences among the three genes ($P = 0.820$). The conditional comparison test at ≥70 % MP BS values showed no conflict among significantly supported nodes present in each gene tree (Johnson and Soltis 1998, Kellogg et al. 1996, Mason-Gamer and Kellogg 1996) indicating the data could be combined for analyses.

Model selections for the Bayesian analyses were determined individually for each gene and the combined alignment. The GTR+I+G model was determined as the best model for the combined and each individual gene alignment. The Bayesian distribution of likelihood scores for the combined analysis was -lnL 14288.4–14340 and ITS analysis -lnL 2065.51–2149.48.

The ML analysis in PAUP* for the ITS data matrix resulted in one tree with a -lnL score of 2032.538 (Fig. 2.1). The maximum likelihood analysis in PAUP* for the combined data matrix resulted in one tree with a -lnL score of 14272.26 (Fig 2.2). The ITS and combined analyses both resolved all included species of *Gnomoniopsis* and *Sirococcus*. One major clade (100% PP, ML, MP) is strongly supported and contains 11 species of *Gnomoniopsis* that occur on the host plant families Onagraceae and Rosaceae (Fig. 2.2). Within this clade are isolates representing *G. idaeicola* (100% PP, ML, MP)*, which occur exclusively on *Rubus* spp. Also within this major clade are the two closely related species *G. comari* and *G. tormentillae* (100% PP, ML; 99% MP), which are host specific on *Comarum palustre* and *Potentilla* sp. respectively. *Gnomoniopsis alderdunense, G. chamaemori,* and *G. occulta* also form a closely related group of species (100% PP, ML, MP). *Gnomoniopsis sanguisorbae* and a closely related unidentified species on *R. parviflorus* each produce asci with four small and four large ascospores. This character is unique to these two isolates.

DISCUSSION

In this paper nine of the thirteen currently recognized species of *Gnomoniopsis* are redescribed and illustrated based on recently collected and type specimens. Three of the remaining species, *G. chamaemori, G. clavulata*, and *G. paraclavulata*, were described by Sogonov et al. (2008). *Gnomoniopsis tormentillae* was described by Barr (1978) and Monod (1983) as *Plagiostoma tormentillae*. Monod's isolate was sequenced for this study.

Species of *Gnomoniopsis* are known throughout the Northern Hemisphere. Production of perithecia occurs in spring on recently dead branches or twigs still attached to the plant as well as on overwintered dead leaves. Some species such as *Gnomoniopsis fructicola* are pathogenic, causing leaf blotch and petiole blight on strawberries (Bolton 1954, van Adrichem and Bosher 1958). Similar to other Gnomoniaceae, species of *Gnomoniopsis* exist as endophytes in healthy host plants (Danti et al. 2002, Rossman et al. 2007a, Sieber and Hugentobler 1987).

Most species of *Gnomoniopsis* show host preference or potentially limited host specificity to genera in the Fagaceae, Onagraceae and Rosaceae. The two closely related species, *G. clavulata* and *G. paraclavulata,* occur primarily on *Quercus* spp. in the Fagaceae. *Gnomoniopsis racemula* occurs on *Chamerion angustifolium* in the Onagraceae. The remaining species of *Gnomoniopsis* show host genus preference to plants in the Rosaceae. Three taxa, *G. alderdunense, G. idaeicola,* and the type species of the genus *G. chamaemori,* have been found only on *Rubus*. Both *G. occulta* and *G. tormentillae* occur on *Potentilla* spp. *Gnomoniopsis guttulata* was collected on the herbaceous plant *Agrimonia. Gnomoniopsis fructicola* is found on living and overwintered leaves and petioles of wild and cultivated species of *Fragaria*. Collections of *Gnomoniopsis comari* are restricted to Europe on *Comarum palustre*. *Gnomoniopsis macounii* occurs on woody tissue of *Spiraea* spp. Most species of *Gnomoniopsis* are known either from North America or Europe although some species such as *G. fructicola, G. idaeicola*, *G. occulta,* and *G. tormentillae* are found on both continents.

Morphological characters that give insight into species identification in *Gnomoniopsis* are perithecial grouping and ascospore length, width, and septation.

Perithecia of *Gnomoniopsis* are immersed and usually solitary although, for some species such as *G. idaeicola*, *G. macounii*, and *G. racemula,* they may be aggregated up to 11 perithecia, and, for *G. macounii* and *G. racemula*, a minimal stroma exists. Perithecial arrangement can be useful in distinguishing *G. idaeicola,* in which they may be solitary or aggregated, from *G. alderdunense* in which they are always solitary. Perithecial necks are central to lateral except for an unknown *Gnomoniopsis* sp. (BPI 879154) on *Rubus,* which has distinctive flared perithecial necks (Fig. 2.5H). Ascospores of species of *Gnomoniopsis* are one-septate with a median to submedian septum that may or may not be slightly constricted. Ascospores are generally fusiform, although *G. clavulata* and *G. paraclavulata* have pyriform ascospores. Unlike many species in the Gnomoniaceae, ascospores of species of *Gnomoniopsis* lack appendages except *G. tormentillae* for which Barr (1978) illustrates a short appendage at each end. Ascospore size is relatively constant within each species. While most species have eight, similarly sized ascospores, *G. sanguisorbae* and the unidentified *Gnomoniopsis* sp. have four large and four small ascospores in each ascus (Fig. 2.5I-M).

Previously, *Gnomoniopsis comari*, including all synonyms,was considered to occur on six different rosaceous genera (Bolay 1971, Monod 1983). *Gnomoniopsis comari* is herein recognized in a narrow sense occurring only on *Comarum palustre*. In 1977 and 1979 three isolates of *G. comari* were collected by Monod from the type locality of Finland on *Comarum palustre* (Monod 1983) and these isolates were used in this study. DNA sequences show these isolates to be distinct from isolates of *G. comari sensu lato* on other hosts. Three of the synonyms of *G. comari sensu lato* are herein recognized as distinct species based on host preference and DNA sequence data.

Gnomoniopsis occulta was described on *Potentilla* sp. from Germany. Fresh specimens on *Potentilla* from the Pacific Northwest in North America were determined to be identical to the type specimen of *G. occulta* and distinct from *G. comari.* The holotype specimens representing *Gnomoniella guttulata* and *Gnomonia agrimoniae* were examined and these names were determined to be synonyms with *G. guttulata* having priority. *Gnomoniella guttulata* shares host (*Agrimonia eupatoria*), continent (Europe), and morphological characters with the representative isolate used in this study and was determined to be distinct from *G. comari*. This species is recognized as distinct and placed in the genus *Gnomoniopsis*. Arnaud and Arnaud (1931) described *Gnomonia fragariae* f. *fructicola* on *Fragaria* sp. from France and deposited a derived isolate CBS 208.34 as representative of this taxon. Fall (1951) recognized this entity as *Gnomonia fructicola*. Sogonov et al. (2008) recognized *Gnomonia fructicola* in *Gnomoniopsis*. In our multigene analysis the original French isolate (CBS 208.34) grouped with an isolate derived from a specimen on *Fragaria* collected in New Jersey. Based on the ITS sequence, an isolate reported by Moročko and Fatehi (2007) as *Gnomonia comari* on *Fragaria* in Latvia was also determined to be *Gnomoniopsis fructicola*.

Two additional species are placed in *Gnomoniopsis* based on the molecular analyses and distinguishing host and morphological characteristics. *Gnomoniopsis idaeicola,* based on *Calosphaeria idaeicola* P. Karst., was originally collected in Finland on *Rubus idaeus.* The newly described *Gnomoniopsis alderdunense* also occurs on *Rubus* spp. and is morphologically similar to *G. idaeicola*. However, *G. alderdunense* produces only solitary perithecia and has slightly larger ascospores than *G. idaeicola,* which has aggregated or solitary perithecia. *Gnomoniopsis alderdunense* is currently known from

Oregon in the Pacific Northwest of the United States, whereas *G. idaeicola* has a much broader geographical distribution (Europe, U.S.A).

The relationships of *G. racemula* and *G. macounii* to other species of *Gnomoniopsis* remain unsupported. Both of these species are somewhat anomalous in *Gnomoniopsis* in having individual perithecia surrounded by a rudimentary stroma. The type specimen of *G. racemula* was not available from the New York State Museum (NYS); however, Barr (1978) examined this specimen when she re-described *Gnomoniopsis racemula* as *Ditopellopsis racemula.* Our isolate is similar in geography (eastern North America), host (*Chamerion angustifolium*) and minimal stroma formation.

This study presents an account of the known species of *Gnomoniopsis*; however, sampling from a more diverse geographic range including eastern Asia and the southern hemisphere will likely result in the discovery of additional species in *Gnomoniopsis.*

KEY TO SPECIES OF *GNOMONIOPSIS*

1. On *Quercus* spp. In U.S.A…………………………………………......………….2

1'. On Onagraceae or Rosaceae. In Europe and North America.…....................................3

    2. Ascospores pyriform, inequilateral, (8–)9–10(–11) × (3–)3.5–4 μm. Septum at mean 34% of ascospore length..*Gnomoniopsis clavulata*

    2'. Ascospores pyriform, inequilateral, (5–)8.5–9.5(–11) × (2–)3.5–4(–5.5) μm. Septum at mean 40% of ascospore length...................*Gnomoniopsis paraclavulata*

    These two species are difficult to distinguish based on morphology alone. See Sogonov et al (2008) for full descriptions of these two species.

3. Asci each with four small (ca. 6.5 µm long) and four large (10–12 µm long) ascospores. …………………...………………………..….*Gnomoniopsis sanguisorbae*

3'. Ascus with eight ascospores of equal size…………...…………………………………...4

4. On *Chamerion angustifolium* (Onagraceae)..................*Gnomoniopsis racemula*

4'. On Rosaceae...5

5. Perithecia aggregated, 2-5 in minimal stroma. On *Spiraea* spp. ....*Gnomoniopsis macounii*

5'. Perithecia aggregated, 2-6 in minimal stroma, or solitary, lacking stroma. On other rosaceous hosts…………………………………….......................................6

6. On *Rubus* spp…………………………………………...…………..…….…7

6'. On other rosaceous hosts……………………………….......…….………9

7. On *Rubus chamaemorus.* Ascospores (10–)10.5–11.5(–13) × (2–)2.5(–3) µm………………………………………………………...…*Gnomoniopsis chamaemori*

7'. On other species of *Rubus.* Ascospores smaller……………......…………………..……...8

8. Perithecia solitary or aggregated, 2-6; ascospores (6.5–)7–10.5(–11) × (2–)2.5–3(–3.5) µm. In U.S.A. and Europe……………………....*Gnomoniopsis idaeicola*

8'. Perithecia solitary; ascospores (9–)9.5–10.5(–11) × (2–)2.5–3.5(–4) µm. In U.S.A..*Gnomoniopsis alderdunense*

These species are difficult to distinguish based on morphology except that *G. idaeicola* usually has grouped perithecia.

9. On *Potentilla.* In Europe and U.S.A..10

9'. On other rosaceous hosts…………………………………………….........……………...11

10. Perithecia necks central; ascospores fusiform, (8–)8.5–9.5(–10) × (1–)2–2.5 µm, lacking appendages...*Gnomoniopsis occulta*

10'. Perithecial necks lateral; ascospores fusiform, 6–10 × 1.5–3 µm fide Barr (1978 as *Plagiostoma tormentillae*), with minute appendages…………………………………….…*Gnomoniopsis tormentillae*

11. On *Agrimonia eupatoria.* In Europe. Ascospores fusiform, (6.5–)7–9.5(–10.5) × 2–2.5 µm...*Gnomoniopsis guttulata*

11'. On *Comarum palustre* in Europe or on *Fragaria* spp. in North America and Europe...12

12. Perithecia immersed on stems, superficial on leaves; ascospores 8–10 × 2–3 µm. On *Comarum palustre.* In Europe...................................*Gnomoniopsis comari*

12'. Perithecia always immersed; ascospores (8–)8.5–11.5(–12) × 2–3 µm. On *Fragaria* sp. and *F. vesca.* In North America and Europe. ....*Gnomoniopsis fructicola*

TAXONOMY

GNOMONIOPSIS Berl., Icon. Fung. 1:93. 1894.

Type species: *Gnomoniopsis chamaemori* (Fr.) Berl.

Perithecia on overwintered canes, leaves, and twigs, solitary, lacking stroma, or in groups of up to 11, aggregated in minimal stroma, black, globose to subglobose, concave from base when dry. Neck central to lateral, straight or slightly curved to curved, short to long, sometimes almost absent, in cross section circular to oval or flattened. Asci ellipsoid,

ovoid, obovoid, to fusiform, with conspicuous apical ring, eight ascospores arranged mostly uniseriate and biseriate but also irregularly multiseriate. Ascospores fusiform, obovoid or pyriform, one-septate, median to submedian, non- to constricted at septum, hyaline, lacking appendages except *G. tormentillae* with slight appendages at each end according to Barr (1978).

Hosts: In the Fagaceae, Onagraceae, and Rosaceae.

Notes: This generic description differs slightly from Sogonov et al (2008). Perithecia were found to be aggregated in groups of up to 11 instead of five. A minimal stroma was observed for *G. macounii* and *G. racemula.* According to Barr (1978) appendages are present on ascospores of *G. tormentillae;* no type or other material of this species was available for examination.

Gnomoniopsis alderdunense D.M. Walker, sp. nov.   Fig. 2.4I–K

MycoBank MB 515714.

Etymology. Refers to the locality of Alder Dunes State Park in Oregon where the holotype was collected.

        Similis *Gnomoniopsis idaeicola*, sed peritheciis solitariis et ascosporis fusiformibus (9–)9.5–10.5(–11) × (2–)2.5–3.5(–4) µm differt.

Perithecia immersed, on leaves and canes, cause swelling in host tissue that exposes base, solitary, black, subglobose, (280–)350–373(–375) µm high × (327–)339–455(–490) µm diam (mean = 345 × 404, S.D. 45, 52, n1 = 4, n2 = 9). Necks differ on leaves and canes: on leaf veins, central, straight to curved slightly contorted, longer than those on canes, apex cream colored; on canes, central, straight, short to medium length, black, (80–)81–

202(–475) μm long (mean = 177, S.D. 139, n = 7), (75–)76–102(–135) μm diam at base

(mean = 96, S.D. 20, n = 7), (42–)44–73(–80) μm diam at apex (mean = 54, S.D. 16, n =

7). Asci fusiform, oval to slightly obovoid, (28–)30–44(–47) × (7–)8–9 μm (mean = 38 ×

8, S.D. 7.5, 1, n = 6), ascospores arranged uniseriate, biseriate to irregularly biseriate.

Ascospores fusiform to slightly obovoid, straight to slightly curved, one-septate,

submedian, slight to no constriction at septum, multiple small guttules, (9–)9.5–10.5(–11)

× (2–)2.5–3.5(–4) μm (mean = 10 × 3, S.D. 0.5, 0.5, n1 = 26, n2 = 24).

Habitat. On overwintered leaves and canes of *Rubus parviflorus* Nutt. and canes of *R.*

pedatus Sm.

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

Specimens examined: UNITED STATES. OREGON: Alder Dunes State Park, on canes

of *Rubus parviflorus,* 24 May 2008, *D.M. Walker* (HOLOTYPE, BPI 879186, ex-type

cultures DMW 72.1, DMW 72.5 = CBS 125680); OREGON: Alder Dunes State Park, on

leaves of *Rubus parviflorus,* 24 May 2008, *D.M. Walker* (BPI 879187, culture DMW

81.1 = CBS 125681); OREGON: Alder Dunes State Park, on leaves of *Rubus parviflorus,*

24 May 2008, *D.M. Walker* (culture DMW 69.1 only); OREGON: River Bridge

Campground, Upper Rouge River, on canes of *Rubus pedatus,* 21 May 2008, *D.M.*

Walker (BPI 879188, culture DMW 115.2).

Gnomoniopsis comari (P. Karst.) Sogonov, Stud. Mycol. 62:47. 2008.   Fig. 2.3F–I

    ≡ *Gnomonia comari* P. Karst., Mycoth. Fenn. (Helsinki) 2:122. 1873.

    ≡ *Gnomoniella comari* (P. Karst.) Sacc., Syll. Fung. 1:415. 1882.

Perithecia immersed on herbaceous stems or covered with thin layer of host tissue on leaf veins and blades, hypophyllous and epiphyllous, solitary, scattered, glossy black, globose to subglobose, (112–)125–300(–330) μm high × (125–)130–450(–500) μm diam (mean = 206 × 278, S.D. 72, 106, n1 = 11, n2 = 23). Necks central to lateral, short, some barely emerging from host tissue, (75–)85–125(–175) μm long (mean = 109, S.D. 28, n = 10), (50–)71–110(–120) μm diam at base (mean = 80, S.D. 22, n = 16), (25–)33–75(–80) μm diam at apex (mean = 42, S.D. 18, n = 14). In culture necks extremely long, occasionally contorted, sometimes with multiple necks per perithecium, (240–)250–2100(–2450) μm long (mean = 1034, S.D. 787, n = 14). Perithecial cell walls on substratum not observed; cell wall in culture of 6–7 layers, cells decreasing in size from outer to inner layers, irregular oval-shaped cells, 30–52 μm wide (mean = 40, S.D. = 7.5, n = 6). Asci fusiform to ellipsoid, (27–)30–40(–43) × (5–)6–9(–10) μm (mean = 33.5 × 7.5, S.D. 4.3, 1.5, n1 = 30, n2 = 26), ascospores arranged irregularly biseriate or obliquely uniseriate. Ascospores fusiform, straight to slightly curved, one-septate, submedian, not constricted or slightly constricted at septum, each cell with 2–4 distinct guttules, 8–10 × 2–3 μm (mean = 9.1 × 2.7, S.D. 0.9, 0.4, n1 = 30, n2 = 24).

Cultures. Pycnidia pink, (350–)450–1050(–1089) μm high × (300–)600–800(–825) μm diam (mean = 699 × 660, S.D. 292, 164.5 n1 = 7, n2 = 8). Conidia variably shaped, fusiform, globose, to femur-shaped, hyaline, no guttules, 5–8 × 2–3 μm (mean = 6.3 × 2.1, S.D. 0.9, 0.2, n1 = 13, n2 = 9). Pycnidia produced in CBS 807.79 and CBS 809.79. Cultural observations based on isolates CBS 806.79, CBS 807.79, and CBS 809.79. On PDA after 7 d colony diam (c.d.) 6.9–8.5 cm (S.D. = 0.8 n = 4), after 15 d, c.d. 8.5 cm (S.D. = 0.0, n = 4), and after 21 d, c.d. 8.5 cm (S.D. = 0.0, n = 4). After 21 d at 26 C on

PDA colony center fuscous black, apricot, and dark red, diffusing red and orange pigments with luteous yellow margin. Surface appearing "rubbery" with a layer of saffron hyphae. Reverse similar, black, deep red, orange and yellow.

Habitat. On overwintered leaves and petioles of *Comarum palustre* L. (Rosaceae).

Distribution. Europe (Finland, Germany, Switzerland).

Specimens examined. FINLAND: Lami, on *Comarum palustre*, 5 Jul 1977, *M. Monod 366* (CBS H–12977 dried culture, culture CBS 806.79); Iso Evo, on *C. palustre*, 7 Jul 1977, *M. Monod 353* (CBS H–12978 dried culture, culture CBS 807.79); QVARKEN, near Krakkkloefverns, on leaves of *C. palustre*, Jul 1859, *Karsten*. (LECTOTYPE designated here, Fl. Fenn. Exs. 869, K(m):157111). GERMANY: Pirna, on petioles of *C. palustre,* Jul 1903, *W. Krieger, Krieger Fungi Saxonici 1980* (BPI 596303; RUTPP). SWITZERLAND: Vaud: Tourbière du Sentier, on *C. palustre*, 21 May 1979, *M. Monod 540* (culture CBS 809.79).

Gnomoniopsis fructicola (Arnaud) Sogonov, Stud. Mycol. 62:47. 2008. Fig. 2.3A–E

≡ *Gnomonia fragariae* forma *fructicola* Arnaud, Traité de Pathol. Veg. Encycl. mycol. (Paris) p. 1558. 1931.

≡ *Gnomonia fructicola* (Arnaud) Fall, Can. J. Bot. 29:309. 1951.

Perithecia immersed, on leaves and petioles, hypophyllous, only neck visible protruding through host cortex, solitary, shiny black, globose to subglobose, (150–)225–450(–475) μm high × (200–)225–425(–475) μm diam (mean = 313 × 318, S.D. 85, 77, n1 = 13, n2 = 17). Necks central, straight to curved, oval to flattened in cross section, protruding at perpendicular angle to host epidermis or flat against petioles, apex cream to pale brown,

(230–)325–375(–400) μm long (mean = 343, S.D. 45.5, n = 12), (50–)60–75(–100) μm diam at base (mean = 70, S.D. 15, n = 12), (25–)35–40(–50) μm diam at apex (mean = 39, S.D. 9.5, n = 10). In culture necks elongated, (865–)890–1312(–1519) μm long (mean = 1147, S.D. 322, n = 4). Outer region of perithecium cell wall of three layers, large, irregular oval shaped cells, inner region composed of 3–4 layers, thin, rectangular cells, 55–71 μm wide (mean = 62, S.D. = 7, n = 4). Asci fusiform, oval to obovoid, (22–)25–34(–35) × (5–)6.5–7.5(–10) μm (mean = 30 × 7.5, S.D. 3.6, 1.1, n1 = 21, n2 = 12), ascospores arranged obliquely uniseriate, irregularly biseriate or irregularly multiseriate. Ascospores fusiform, straight to curved, one-septate, submedian, not constricted or constricted at septum, 4–6 guttules, (8–)8.5–11.5(–12) × 2–3 μm (mean = 10 × 2, S.D. 1.1, 0.5, n1 = 28, n2 = 21).

Cultures. Pycnidia cream colored, mustard yellow to light brown (100–)150–400(–450) μm high × (150–)250–750(–800) μm diam (mean = 295 × 414, S.D. 128, 227, n1 = 10, n2 = 11). Conidia oval, spherical to ellipsoidal or femur-shaped, hyaline, 2–3 guttules, (4–)5–10(–11) × 2–3 μm (mean = 6.8 × 2.1, S.D. 1.7, 0.3, n1 = 25, n2 = 22). Cultural observations: based on isolates AR 4275, CBS 275.51, CBS 208.34, CBS 255.61. On PDA after 7 d, colony diam (c.d.) 1.3–6.5 cm S.D. = 2, n = 4), after 15 d, (c.d.) 3–8 cm (S.D. = 2, n = 4), and after 21 d, (c.d.) 3.7–8.5 cm (S.D. = 2, n = 4). After 21 d at 26 C on PDA, colony center tan to grey diffusing to cream, olive or dark grey, grey to olive margin. Reverse of darker tones, sepia, olivaceous grey, and hazel, similar pattern as surface. CBS 121226 producing yellow pycnidia, others clear to black exudates, felty texture, creases radiating from center to margin, minimal aerial hyphae.

Habitat. On fruits, leaves, and petioles of *Fragaria* sp. and *Fragaria vesca* L. (Rosaceae).

Distribution. Canada (Ontario), Europe (Belgium, France, Latvia, Netherlands, Switzerland), and U.S.A. (Maryland, New Jersey, New York).

Lectotype designated here. Fig. 606 in Arnaud, Traité de Pathol. Veg. Encycl. mycol. (Paris) p. 1561. 1931. This figure is designated the iconotype.

Specimens examined. BELGIUM: On *Fragaria* sp., 1961, J.A. von Arx (CBS 255.61). CANADA. ONTARIO: Ottawa, on leaves of *Fragaria* sp., Jun 1950, *coll. A. Bolton, det. J.W. Groves* (CBS 275.51). FRANCE. SEINE & OISE: Chevreuse, on *Fragaria* sp., Dec 1934, *G. Arnaud* (CBS 208.34). NETHERLANDS: On fruit of *Fragaria* sp., Aug 1961, *J.A. von Arx* (CBS H–12975 dried culture, IMI 101573, culture CBS 254.61). UNITED STATES. MARYLAND: Beltsville, on diseased leaves of *Fragaria vesca,* May 2006, *B. Turechek* (BPI 877447, culture AR 4275 = CBS 121226); NEW JERSEY: Skillman, Dutchtown and Zion Road, on leaves of *Fragaria* sp., 1 Mar 2008, *coll. L. Struwe, det. D.M. Walker* (BPI 879155, culture DMW 61 = CBS 125671); NEW JERSEY: Skillman, Dutchtown and Zion Road, on leaves of *Fragaria* sp., 1 Mar 2008, *coll. L. Struwe, det. D.M. Walker* (BPI 879156, culture DMW 63); NEW YORK: Sullivan Co., Roscoe, area around Campbell Inn, on dead petioles of *Fragaria* sp., Jul 2005, *M.V. Sogonov* (BPI 877446). SWITZERLAND: Miex Vs., on petioles of *Fragaria* sp., 23 Aug 1961, *A. Bolay* (BPI 611431).

Gnomoniopsis guttulata (Starbäck) D.M. Walker, comb. nov.   Fig. 2.5D–G

MycoBank MB 515710.

> ≡ *Gnomoniella guttulata* Starbäck, Bih. K. Svenska Vetensk Akad. Handl. 15(3):10. 1889. Basionym.

≡ *Gnomonia guttulata* (Starbäck) Kirschst., Ann. Mycol 33:219. 1935.

≡ *Apiognomonia guttulata* (Starbäck) Wehm., Can. J. Research, Sec. C
20:585. 1942.

= *Gnomonia agrimoniae* Bref. & Tavel, Untersuch. Gesammtgebiete Mykol.
10:232. 1891.

Perithecia immersed, on leaves and stems, solitary or aggregated 2–4, due to close
proximity, ascomatal walls contiguous but stroma lacking, black, subglobose, (68–)125–
175(–200) µm high × (82–)220–336(–350) µm diam (mean = 144 × 266, S.D. 45.5, 67,
n1 = 6, n2 = 15). Necks central, short to medium, straight to curved, oval in cross section,
some barely emerging from host epidermis, aggregated perithecia protruding through a
single opening in host tissue, some with cream colored apex, (50–)66–81(–125) µm long
(mean = 79, S.D. 25, n = 6), 41–93(–100) µm diam at base (mean = 78, S.D. 32, n = 3),
(19–)26–50(–53) µm diam at apex (mean = 37, S.D. 17, n = 4). Outer region of
perithecial cell wall of two layers, large, irregularly circular to oval cells; inner region of
two layers, thin, compacted, oval cells, 22.5–30 µm wide (mean = 27, S.D. = 3, n = 5).
Asci fusiform, (24–)25–35(–36) × (5–)6–7.5(–8) µm (mean = 29.5 × 7, S.D. 4, 1, n1 =
12, n2 = 11), ascospores arranged uniseriate, biseriate, to irregularly biseriate. Ascospores
fusiform, straight to slightly curved, one-septate, submedian, with distinct to slight
constriction at septum, no guttules observed, (6.5–)7–9.5(–10.5) × 2–2.5 µm (mean = 8.3
× 2.2, S.D. 1, 0.5, n1 = 27, n2 = 21).

Habitat. On overwintered canes and leaves of *Agrimonia eupatoria* L. (Rosaceae).

Distribution. Europe (Bulgaria, Sweden, Switzerland).

Specimens examined. BULGARIA. SREDNA GORA MT (western): Lozenska Planina, above Pancharevo lake, near the track from 'Stenata' locality to VEC Kokaljane, on *Agrimonia eupatoria,* 21 May 2005, *D. Stoykov* (BPI 877452, GenBank EU254812). SWEDEN. OELANDIA: Morbylanga, on canes of *A. eupatoria,* 19 Jun 1888, *K. Starbäck* (HOLOTYPE of *Gnomonia guttulata* S #F65659). SWITZERLAND. ALLEMAGNE: Münster, Westfalien. *A. eupatoria,* 21 Apr 1888, *F. von Tavel* (HOLOTYPE of *Gnomonia agrimoniae* ZT).

Gnomoniopsis idaeicola (P. Karst.) D.M. Walker, comb. nov.   Fig. 2.4E–H
MycoBank MB 515711.

≡ *Calosphaeria idaeicola* P. Karst., Fungi Fenniae 856. 1869. Basionym.

≡ *Diaporthe idaeicola* (P. Karst.) Vestergr. Bot. Notis. 156. 1899.

Perithecia immersed, on overwintered canes, covered by thin swollen layer of host epidermis, solitary or aggregated 2–6, lacking stroma, black, subglobose, (191–)197–295(–290) μm high × (228–)236–391(–402) μm diam (mean = 230 × 290, S.D. 40, 47, n1 = 6, n2 = 17). Necks central to marginal, straight, short to medium length, rounded to oval, flattened in cross section, multiple necks often converge centrally and protrude through a single break in host epidermis causing swelling in host tissue, (55−)65–130(–145) μm long (mean = 94, S.D. 27, n = 14), (40–)45–101.5(–117) μm diam at base (mean = 67.5, S.D. 23, n = 12), (26–)31–61(–93) μm diam at apex (mean = 42.5, S.D. 19, n = 11). Asci fusiform, oval to obovoid, (30–)33.5–47(–48.5) × (5–)5.5–8(–10) μm (mean = 39 × 7, S.D. 5, 1, n1 = 30, n2 = 30), ascospores arranged uniseriate to biseriate. Ascospores fusiform to slightly obovoid, straight to slightly curved, one-septate,

submedian, no constriction to distinct constriction at septum, 1–4 guttules, (6.5–)7–10.5(–11) × (2–)2.5–3(–3.5) μm (mean = 9 × 3, S.D. 1, 0.5, n = 30).

Habitat. On overwintered canes of *Rubus idaeus* L.*, R. nivalis* Howell*, R. pedatus* Sm.*, R. procerus* Boulay, *Rubus* sp., *R. spectabilis* Pursh. (Rosaceae).

Distribution. Europe (Finland, France), U.S.A. (California, Oregon, Washington).

Specimens examined: FINLAND: Mustiala, on canes of *Rubus idaeus,* May 1886, *P. Karsten* (HOLOTYPE, Karsten Fungi Fenniae 856, FH). FRANCE. FORET HERMITAIN: Melle. *Rubus* sp., 17 Apr 2008, *coll. L.C. Mejía, det. D.M. Walker* (EPITYPE designated here, BPI 879157, culture DMW 103.5 = CBS 125674); LAS MURO'. Ariege, Riront, on *Rubus* sp.*,* 23 May 2008, *coll. L.C. Mejía, det. D.M. Walker* (BPI 879158, culture DMW 100.3). UNITED STATES. CALIFORNIA: Cow Creek past Shingle town, on *Rubus* sp., 18 May 2008, *D.M. Walker* (BPI 879159, culture DMW 91.6); CALIFORNIA: Cow Creek past Shingle town, on *Rubus* sp., 18 May 2008, *D.M. Walker* (BPI 879160, culture DMW 92.1); CALIFORNIA: Ellery Campground, McCloud River. *R. nivalis,* 19 May 2008, *D.M. Walker* (BPI 879161, culture DMW 83.3); CALIFORNIA: Ellery Campground, McCloud River. *R. nivalis,* 19 May 2008, *D.M. Walker* (BPI 879162, culture DMW 129.1); CALIFORNIA: Ellery Campground, McCloud River, on *R. nivalis,* 19 May 2008, *D.M. Walker* (BPI 879163, culture DMW 130.2); CALIFORNIA: Hirz Bay Campground, on *Rubus* sp.*,* 19 May 2008, *D.M. Walker* (BPI 879164, culture DMW 147.1 = CBS 125672); CALIFORNIA: Hirz Bay Campground, on *Rubus* sp.*,* 19 May 2008, *D.M. Walker* (BPI 879165, culture DMW 176.1); OREGON: on *R. procerus,* 24 May 2008, *D.M. Walker* (BPI 879170, cultures DMW 190.1 = CBS 125675); OREGON: Alder Dunes State Park. *Rubus* sp., 23 May

2008, *D.M. Walker* (BPI 879166, culture DMW 194.1); OREGON: Cape Foul Weather, on *Rubus* sp., 24 May 2008, *D.M. Walker* (BPI 879169, culture DMW 95.3); OREGON: Lost Creek State Park, on *R. spectabilis,* 24 May 2008, *D.M. Walker* (BPI 879167, culture DMW 105.2); OREGON: Lost Creek State Park, on *R. spectabilis,* 24 May 2008, *D.M. Walker* (BPI 879168, culture DMW 106.4); OREGON: McGregor and Casey Park, on *R. pedatus,* 20 May 2008, *D.M. Walker* (BPI 879171, culture DMW 150.2); OREGON: McGregor and Casey Park, on *R. pedatus,* 20 May 2008, *D.M. Walker* (BPI 879172, culture DMW 154.1); OREGON: McGregor and Casey Park, on *Rubus* sp.*,* 20 May 2008, *D.M. Walker* (BPI 879173, culture DMW 175.1); OREGON: Rt. 62 and Rt. 234 near Shady Cove, on *Rubus* sp., 19 May 2008, *D.M. Walker* (BPI 879174, culture DMW 152.2); OREGON: Waldo Lake off Rt. 58, on *R. pedatus,* 22 May 2008, *D.M. Walker* (BPI 879175, culture DMW 153.1); OREGON: Waldo Lake off Rt. 58, on *R. pedatus,* 22 May 2008, *D.M. Walker* (BPI 879176, cultures DMW 167.2 = CBS 125673); WASHINGTON: Crescent Lake, on *R. procerus,* 27 May 2008, *D.M. Walker* (BPI 879177, culture DMW 138.1); WASHINGTON: Rt. 101, on *Rubus* sp., 29 May 2008, *D.M. Walker* (BPI 879178, cultures DMW 139.1, DMW 139.2, DMW 139.3); WASHINGTON: Rt. 101, on *Rubus* sp., 29 May 2008, *D.M. Walker* (BPI 879179, culture DMW 140.1); WASHINGTON: Fort Columbia, on *R. procerus*, 28 May 2008, (BPI 879180, cultures DMW 98.2 = CBS 125676); WASHINGTON: Hoh Oxbow, near entrance to Cottonwood campground, Rt. 101, on *R. pedatus,* 26 May 2008, *D.M. Walker* (BPI 879181, culture DMW 189.1); WASHINGTON: Humptulips River, on *R. procerus,* 25 May 2008, *D.M. Walker* (BPI 879182, culture DMW 208.1); WASHINGTON: Kitsap Memorial Park, on *Rubus* sp., 29 May 2008, *D.M. Walker* (BPI 879183, culture DMW

162.1); WASHINGTON: Kitsap Memorial Park, on *Rubus* sp., 29 May 2008, *D.M. Walker* (BPI 879184, culture DMW 164.2); WASHINGTON: Kitsap Memorial Park, on *Rubus* sp., 29 May 2008, *D.M. Walker* (BPI 879185, culture DMW 165.1).

Gnomoniopsis macounii (Dearn.) Sogonov, Stud. Mycol. 62:47. 2008.　　　Fig. 2.3J–M

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

　　≡ *Cryptodiaporthe macounii* (Dearn.) Wehm., The Genus *Diaporthe* and its Segregates p. 191. 1933.

Perithecia immersed beneath periderm, on twigs causing swelling and splitting of host tissue, aggregated in minimal stroma 2–5, black, subglobose, 100–133 µm high × 125–350 µm diam (mean = 117 × 245, S.D. 23, 73, n1 = 2, n2 = 14). Necks central to marginal, short and rounded, protrude through single split in host epidermis, (50–)60–100(–150) µm long (mean = 77, S.D. 32, n = 10), 50–75 µm diam at base (mean = 55, S.D. 11, n = 10), 25–50 µm diam at apex (mean = 31, S.D. 12, n = 8). Outer region of perithecial cell wall 2–3 layers, large, irregular oval or rectangular shaped cells; inner region of 3–4 layers, thin, elongate, oval cells, 15–37 µm wide (mean = 30.5, S.D. = 13.6, n = 5). Asci fusiform, (30–)35–38 × (4–)7–8(–8.5) µm (mean = 35 × 7, S.D. 4, 2, n1 = 4, n2 = 4), ascospores arranged irregularly biseriate. Ascospores fusiform, straight to slightly curved, one-septate, median to submedian, distinct constriction to no constriction at septum, (8–)8.5–11(–12.5) × (2.5–)3–4 µm (mean = 9.5 × 3, S.D. 1, 0.5, n1 = 23, n2 = 20).

Anamorph. Pycnidia outer walls carbonaceous black, exude cream to yellow conidial mass, solitary or aggregated 4–6, (150–)175–200(–225) µm high × (225–)250–500(–550)

µm diam (mean = 178 × 348, S.D. 31, 109, n1 = 8, n2 = 11). Conidia variable-shaped, oval, oblate, fusiform, straight to curved, hyaline, 2–3 guttules, (4–)5–7(–7.5) x (2–)2.5–3 µm (mean = 5.8 x 2.4, S.D. 1, 0.5, n1 = 21, n2 = 19).

Habitat. On overwintered branches of *Spiraea douglasii* Hook. var. *mensiesii* (Hook.) C. Presl. and *Spiraea* sp. (Rosaceae).

Distribution. Canada (British Columbia) and U.S.A. (New Hampshire, New York).

Specimens examined. CANADA. BRITISH COLUMBIA: Sidney, W. Saanich Road, in *Spiraea douglasii*, 3 Jul 1990, *M.E. Barr 7203* (DAOM 213496); Vancouver Island, in *S. douglasii* Hook. var. *mensiesii,* May 1915, *J. Macoun* (HOLOTYPE, Ex Herbarium L.E. Wehmeyer 3295, DAOM 121038). UNITED STATES. NEW YORK: On *Spiraea* sp., 26 Jun 2002, *L. Vasilyeva* (EPITYPE designated here, BPI 871008**,** culture ex-type AR 3866 = CBS 121468).

Gnomoniopsis occulta (Kirschst.) D.M. Walker, comb. nov.   Fig. 2.5A–C

MycoBank MB 515712.

≡ *Gnomonia occulta* Kirschst., Verh. Bot. Ver. Prov. Brandenb. 48:58. 1906.

Basionym.

Perithecia immersed in leaves, hypophyllous, causing host tissue to swell, often exposing perithecial base under a thin layer of epidermal tissue, solitary, black, subglobose, (129–)140–300(–340) µm high × (147–)174–350(–428) µm diam (mean = 231 × 276, S.D. 79, 97, n1 = 8, n2 = 9). Necks central, straight, medium to long, upright and rounded or apex curled over at approximately 90 angle and pale tan to cream colored, (86.5–)88–230(–346) µm long (mean = 153, S.D. 64.5, n = 17), (45–)56–128(–133) µm diam at base

(mean = 84.5, S.D. 29, n = 12), (26–)30–58.5(–60) μm diam at apex (mean = 41, S.D. 12, n = 12). Asci fusiform, (24–)25–32(–32.5) × (4–)5–7(–8) μm (mean = 27.5 × 6, S.D. 2, 1 n1 = 30, n2 = 28), ascospores arranged uniseriate to irregularly biseriate, often overlapping. Ascospores fusiform, straight to slightly curved, one-septate, submedian, no constriction at septum, 1-4 guttules, (8–)8.5–9.5(–10) × (1–)2–2.5 μm (mean = 9 × 2, S.D. 0.5, 0.5, n = 30).

Habitat. On overwintered leaves and petioles of *Potentilla anserina* L., *P. canescens* Spreng., and *Potentilla* sp. (Rosaceae).

Distribution. Europe (Germany, Russia), U.S.A. (Oregon).

Specimens examined. GERMANY. RATHENOW: Havelufer, Göttling, on leaves and petioles of *Potentilla anserina,* 21 Aug 1905, *Kirschstein* (HOLOTYPE, B 700014205). RUSSIA. NOVGOROD PROVINCE: Kholm, on *P. anserina,* 7 Jun 2005, *M.V. Sogonov* (BPI 877455, GenBank EU254811). UNITED STATES. OREGON: Rocky Creek State Park, on *Potentilla* sp., 24 May 2008, *D.M. Walker* (EPITYPE designated here, BPI 879191, cultures DMW 206.1 = CBS 125678). OREGON: Rocky Creek State Park, on *Potentilla* sp., 24 May 2008, *D.M. Walker* (BPI 879189, cultures DMW 113.1 = CBS 125677); OREGON: Rocky Creek State Park, on *Potentilla* sp., 24 May 2008, *D.M. Walker* (BPI 879190, culture DMW 199.1).

Gnomoniopsis racemula (Cooke & Peck) Sogonov, Stud. Mycol. 62: 47. 2008.
Fig. 2.4A–D

≡ *Sphaeria racemula* Cooke & Peck in Peck, New York State Museum Rep. 29: 65. 1878.

≡ *Diaporthe racemula* (Cooke & Peck) Sacc., Syll. Fung. 1:691. 1882.

≡ *Ditopellopsis racemula* (Cooke & Peck) M.E. Barr, Mycologia Memoir 7:91.
1978.

Perithecia immersed, on stalks, solitary or aggregated 2–11 in a minimal stroma,
sometimes difficult to distinguish between individual bases, black, globose to
subglobose, (112–)150–275(–300) μm high × (130–)140–325(–350) μm diam (mean =
221 × 239, S.D. 56.8, 67.2, n1 = 11, n2 = 19). Necks central to lateral, short to medium
length, multiple necks fuse together centrally and emerge together, each neck apex
sometimes distinguishable when protruding through host epidermis, (50–)100–200(–250)
μm long (mean = 138, S.D. 54, n = 15), 50–100 μm diam at base (mean = 70, S.D. 27, n
= 5), (25–)50–75(–100) μm diam at apex (mean = 52, S.D. 22, n = 13). Outer region of
perithecium cell wall of 3–4 layers, large irregular oval, circular, or rectangular-shaped
cells; inner region of 4–6 cell layers of broad to thin, oval-shaped cells, 12–75 μm wide
(mean = 28, S.D. = 19, n = 14). Asci fusiform, oval to obovoid, (38–)40–45(–48) × (8–
)9–10(–11) μm (mean = 43 × 9, S.D. 3, 1.0, n = 12), ascospores arranged uniseriate,
biseriate to irregularly biseriate. Ascospores fusiform to oval, straight to slightly curved,
one-septate, median to submedian, distinct constriction to no constriction at septum, 3–4
guttules, (7.5–)9–10(–12) × (2–)2.5–3(–3.5) μm (mean = 10 × 3, S.D. 1.0, 0.5, n1 = 18,
n2 = 19).

Habitat: On overwintered stalks of *Chamerion angustifolium* L. (Onagraceae).

Distribution: Canada (British Columbia) and U.S.A. (Minnesota, New York)

Specimens examined: CANADA, BRITISH COLUMBIA: On dead stalks of *Chamerion
angustifolium,* 5 Jun 2001, *M.E. Barr* (BPI 748021A & B). UNITED STATES.

MINNESOTA: On dead stalks of *C. angustifolium,* 14 Jul 2002*, L. Vasilyeva* (EPITYPE designated here, BPI 871003, culture AR 3892 = CBS 121469). NEW YORK: Lower Ausable, Adirondack Mts., on *C. angustifolium,* Jul 1873, *C.H. Peck* (HOLOTYPE, NYS specimen not available).

Gnomoniopsis sanguisorbae (Rehm) D.M. Walker, comb. nov.　　　　　　　Fig. 2.5H–N
MycoBank MB 515713.

≡ *Gnomonia tithymalina* var. *sanguisorbae* Rehm, Ann. Mycol. 3:229. 1905. Basionym.

≡ *Gnomonia sanguisorbae* (Rehm) E. Müll., Beitr. Kryptfl. Schweiz 11(2):744. 1962.

≡ *Septomazzantia sanguisorbae* (Rehm) Lar. N. Vassilyeva, Pyrenomycetes of the Russian Far East 1. Gnomoniaceae (Vladivostok): 52. 1993.

Perithecia immersed, solitary or two together, no stroma, black, subglobose, 100–200 μm high × (150–)175–250(–400) μm diam (mean = 125 × 225, S.D. 50, 84, n1 = 4, n2 = 7). Necks central, short to medium length, straight to curved, apex tan to cream-colored, (50–)75–100(–300) μm long (mean = 98, S.D. 72, n = 10), 75–100(–150) μm diam at base (mean = 87.5, S.D. 24, n = 10), (50–)55–70(–75) μm diam at apex (mean = 59, S.D. 10, n = 15). Asci fusiform, (30.5–)33–35(–38) × (5–)6–8(–10) μm (mean = 34.5 × 6.5, S.D. 2, 1.5, n1 = 15, n2 = 13), ascospores arranged irregularly biseriate. Ascospores fusiform, straight to slightly curved, one-septate, submedian, slightly to distinctly constricted at septum, 1-4 guttules, two ascospore sizes, four large (10–12 μm) and four

small (6.5 μm) spores per ascus, (6.5–)7–12(–12.5) × 2–3 μm (mean = 10 × 2.5, S.D. 2.4, 0.5, n1 = 27, n2 = 26).

Cultures. Observations based on CBS 858.79. On PDA after 7 d at 22 C, colony diam (c.d.) 9 cm (S.D. = 0.0, n = 6), after 15 d (c.d.) 9 cm (S.D. = 0.0, n = 6), and after 21 d (c.d.) 9 cm (S.D. = 0.0, n = 6). After 21 d at 22 C on PDA, colony center smoke grey to hazel, diffusing to sepia or mouse grey, margin of same shades with minor mycelial roping, faint ring pattern. Reverse violaceous black to sepia. Slight to medium density felt texture of smoke grey hyphae covering entire colony.

Habitat. On overwintered canes of *Sanguisorba officinalis* L.

Distribution. Europe (Germany).

Specimens examined. GERMANY. KÖNIGSTEIN: Elbe (Sachsen.), on canes of *Sanguisorba officinalis,* Jun 1904, *W. Krieger*, (LECTOTYPE designated here, Rehm Ascomyceten 1597, BPI exsiccati).

ACKNOWLEDGEMENTS

Fig. 2.1. ML phylogenetic analysis (ML score = -lnL 2032.538) of ITS sequences of 14 species in *Gnomoniopsis,* 3 species of *Sirococcus,* and 2 outgroup taxa all within the Gnomoniaceae. Bayesian posterior probabilities ≥ 95 % are displayed above each branch. GARLI ML bootstrap values ≥70 % are displayed to the bottom left and MP bootstrap

values ≥ 70 % are displayed to the bottom right of each branch. Taxa in bold are new

combinations or new species.
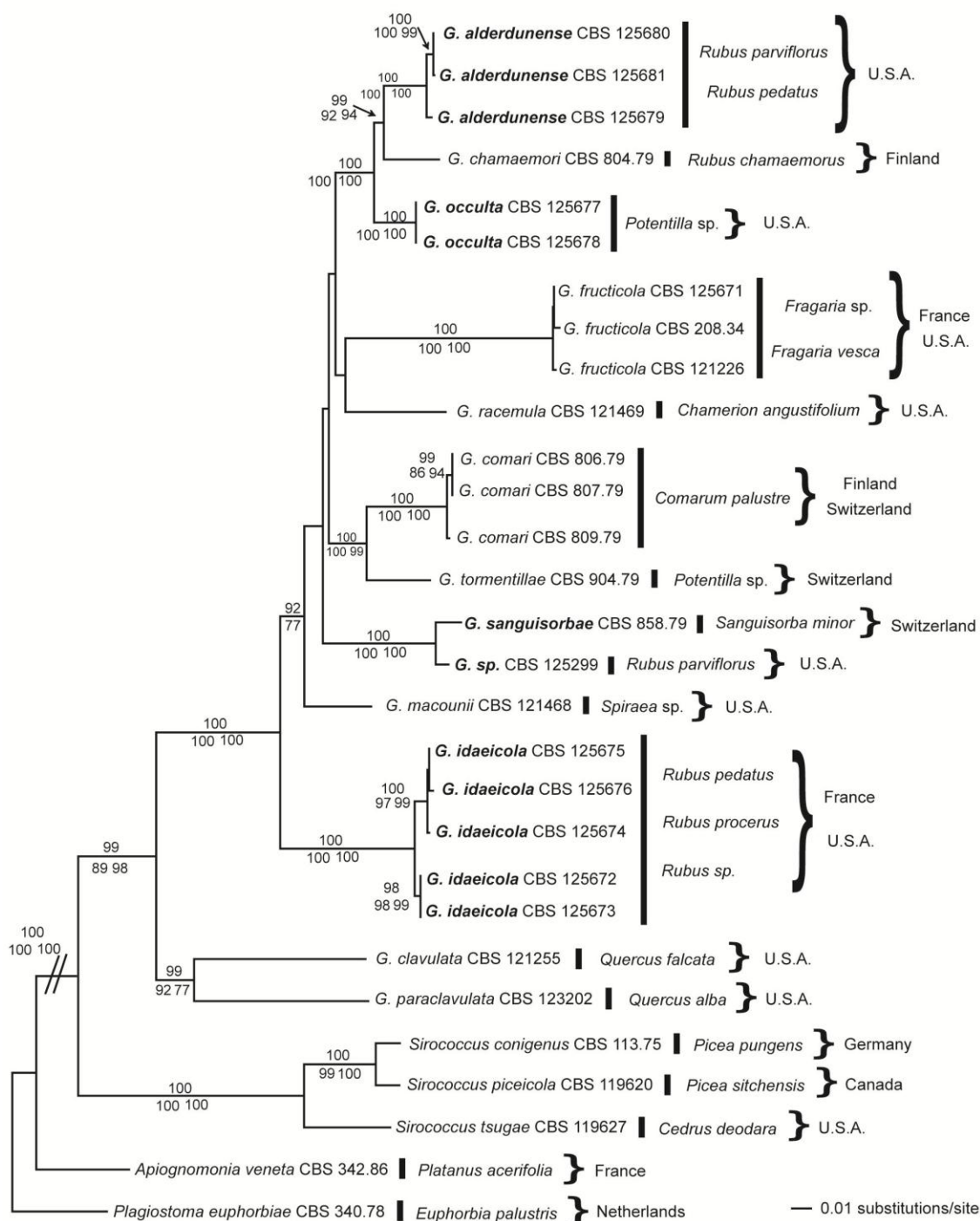
Fig. 2.2. ML phylogenetic multigene analysis (ML score = -lnL 14272.26) of *β-tubulin*,

ITS, and *tef1-α* sequences of 13 species in *Gnomoniopsis,* 3 species of *Sirococcus,* and 2

outgroup taxa all within the Gnomoniaceae. Bayesian posterior probabilities ≥ 95 % are displayed above each branch. GARLI ML bootstrap values ≥70 % are displayed to the bottom left and MP bootstrap values ≥ 70 % are displayed to the bottom right of each branch (single value of 92 % PP = 77 % BS are displayed). Taxa in bold are new combinations or species.
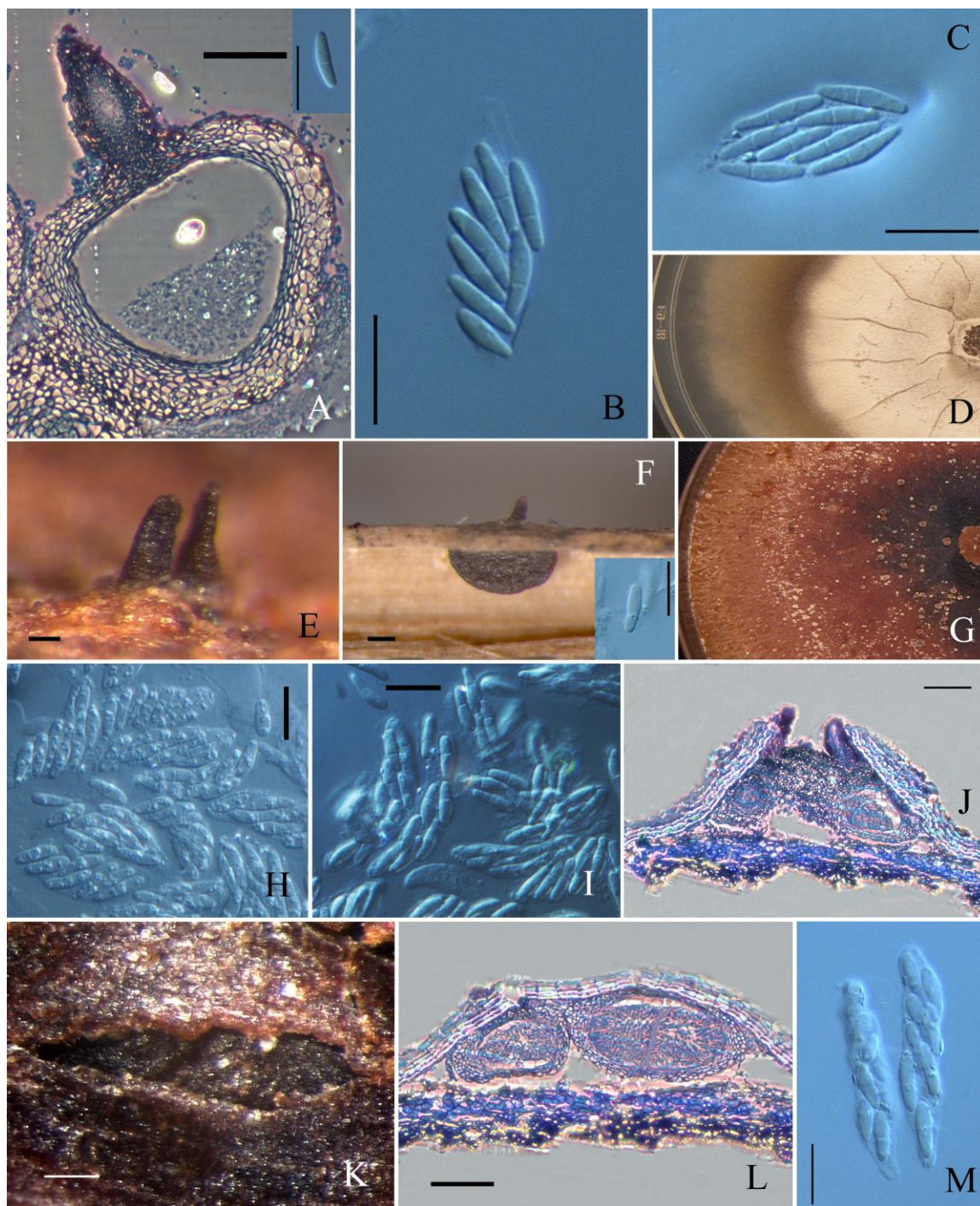
Fig. 2.3. Morphology of perithecia, asci, ascospores, and cultures. A–E. *Gnomoniopsis fructicola.* A–B. CBS 121226. C. BPI 879155. D. CBS 255.61 on PDA at 26 C, 28d. E. BPI 611431. F–I. *G. comari.* F. BPI 596303. G. CBS 809.79 on PDA at 26 C, 28d. H–I. Lectotype Karsten 869/ (K(m):157111). J–M. *G. macounii.* J, L. BPI 121468. K, M.

Holotype DAOM 121038/ Wehmeyer 3295. Scale bars of all asci and ascospores 10 μm.

Scale bars of all perithecia and cross sections 100 μm.
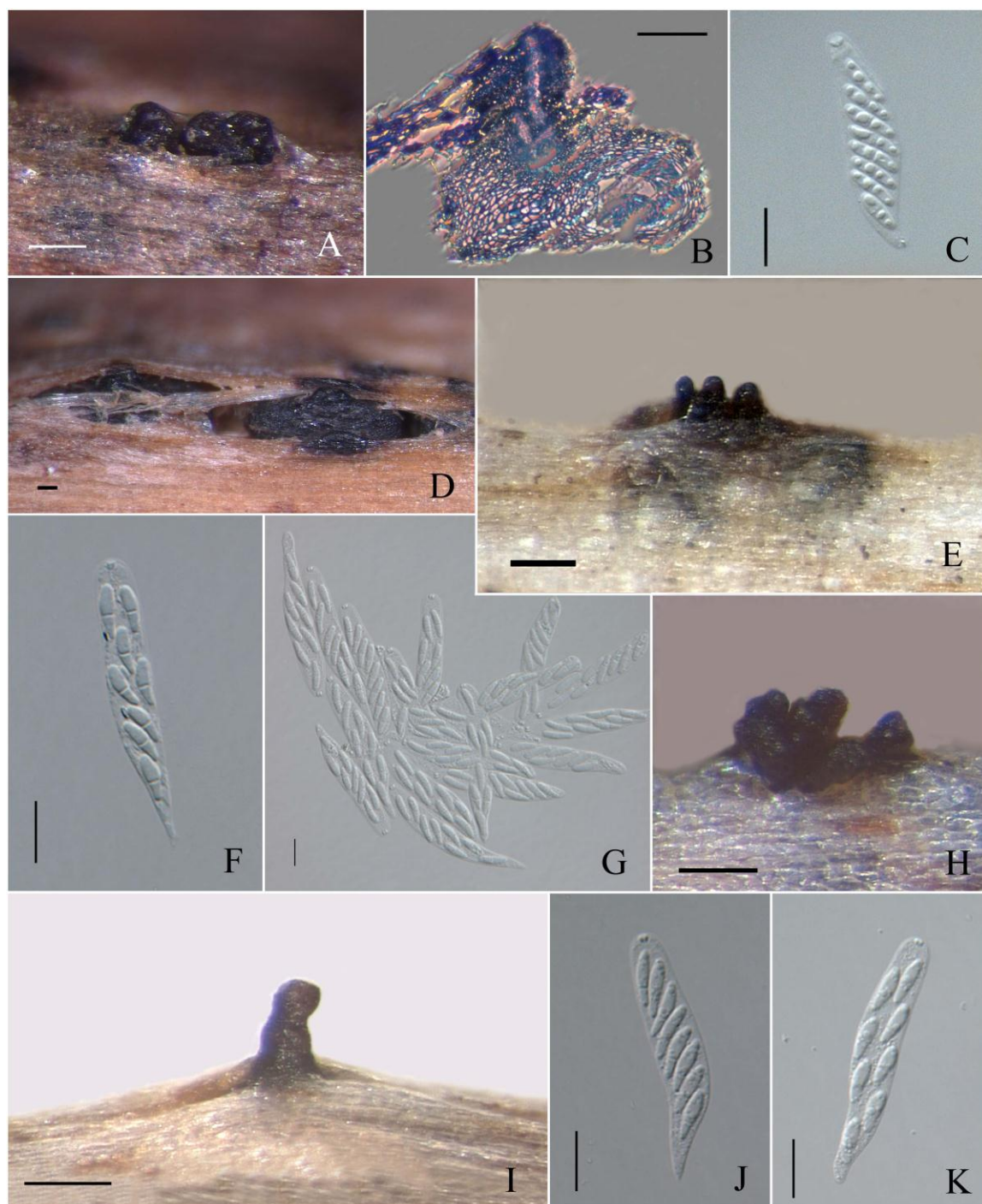
Fig. 2.4. Morphology of perithecia, asci, and ascospores on natural substrate. A–D. *Gnomoniopsis racemula,* BPI 748021. E–H. *G. idaeicola.* E–F. BPI 879162. G. BPI

879157. H. BPI 879182. I–K. *G. alderdunense.* I. Holotype BPI 879186. J–K. BPI

879188. Scale A: 50 μm. Scale bars of all asci and ascospores 10 μm. Scale bars of all

other perithecia and cross sections 100 μm.
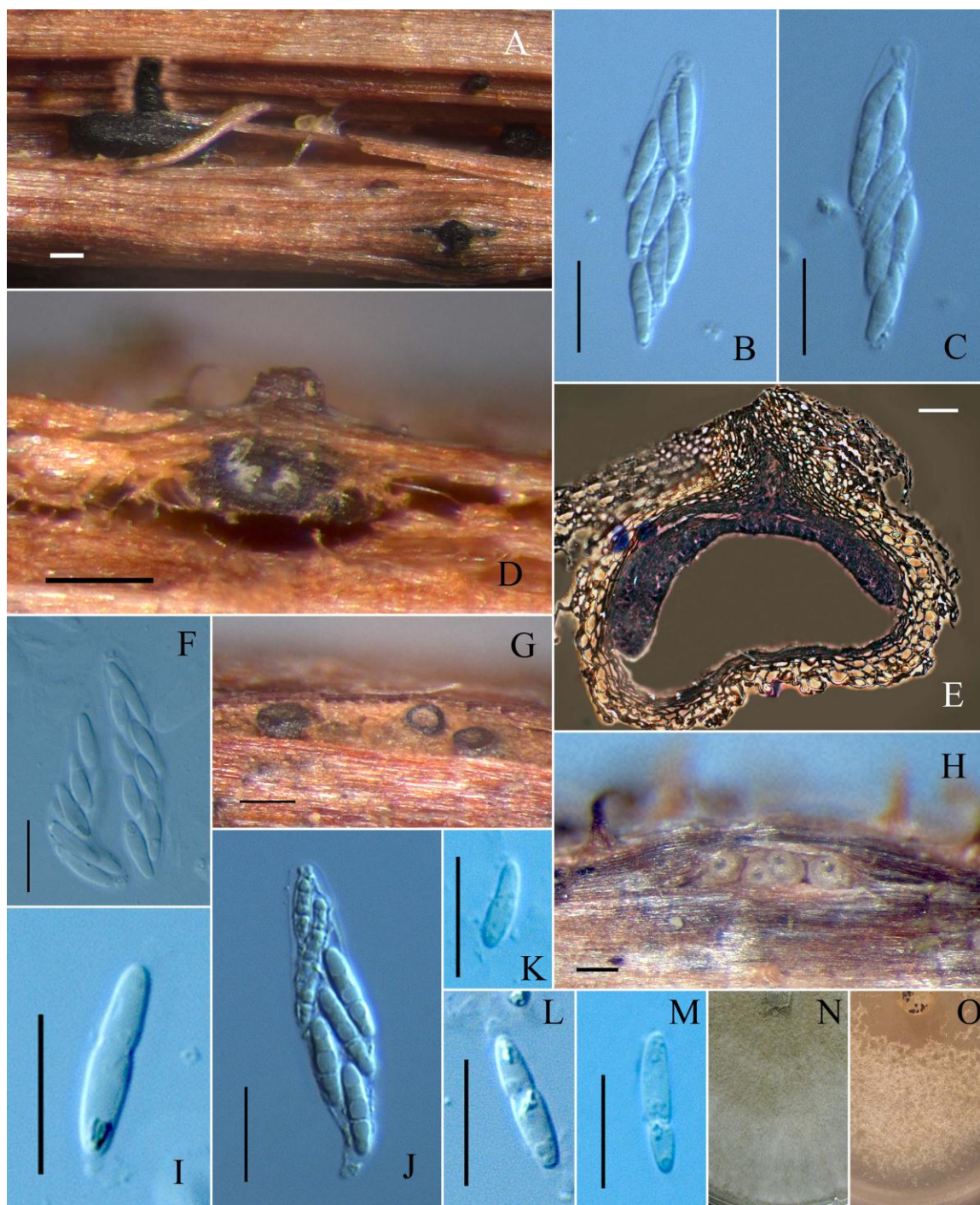
Fig. 2.5. Morphology of perithecia, asci, ascospores, and cultures. A–C. *Gnomoniopsis*

occulta. A. BPI 879189. B–C. BPI 879191. D–G. *G. guttulata*, F65659. I, K–M. *G. sanguisorbae.* Rehm Ascomyceten 1597. H, J, N. *Gnomoniopsis* sp. H, J. BPI 879154. N. CBS 125299 on PDA at 22 C, 7d. O. *G. tormentillae* on PDA at 26 C, 28d. Scale bars of all asci and ascospores 10 μm. Scale bars of all perithecia and cross sections 100 μm.

Table 2.1. Specimens and cultures of Gnomoniaceae sequenced for this study.

Species	CBS #	Isolate	Specimen	ITS	β-tubulin	tef1-α	Locality	Host	Collector, Year
Apiognomonia veneta	CBS 342.86	CBS 342.86	na	DQ313531	EU219235	DQ318036	France	*Platanus occidentalis*	H.A. van der Aa, 1986
Gnomoniopsis alderdunense	CBS 125679	DMW 115.2	BPI 879188	GU320826	GU320788	GU320813	USA, OR	*Rubus pedatus*	D.M. Walker, 2008
Gnomoniopsis alderdunense	CBS 125680	DMW 72.1	BPI 879186	GU320825	GU320787	GU320801	USA, OR	*Rubus parviflorus*	D.M. Walker, 2008
Gnomoniopsis alderdunense	CBS 125681	DMW 81.1	BPI 879187	GU320827	GU320789	GU320802	USA, OR	*Rubus parviflorus*	D.M. Walker, 2008
Gnomoniopsis chamaemori	CBS 804.79	CBS 804.79	na	GU320817	GU320777	GU320809	Finland	*Rubus chamaemorus*	M. Monod, 1977
Gnomoniopsis clavulata	CBS 121255	MS 0397	BPI 877439	EU254818	EU219211	GU320807	USA, MD	*Quercus falcata*	M.V. Sogonov, 2006
Gnomoniopsis comari	CBS 806.79	CBS 806.79	CBS-H12997	EU254821	EU219156	GU320810	Finland	*Comarum palustre*	M. Monod, 1977
Gnomoniopsis comari	CBS 807.79	CBS 807.79	CBS-H12978	EU254822	GU320779	GU320814	Finland	*Comarum palustre*	M. Monod, 1977
Gnomoniopsis comari	CBS 809.79	CBS 809.79	na	EU254823	GU320778	GU320794	Switzerland	*Comarum palustre*	M. Monod, 1979
Gnomoniopsis fructicola	CBS 121226	AR 4275	BPI 877447	EU254824	EU219144	GU320792	USA, MD	*Fragaria vesca*	B. Turechek, 2006
Gnomoniopsis fructicola	CBS 208.34	CBS 208.34	na	EU254826	EU219149	GU320808	France	*Fragaria sp.*	G. Arnaud, 1934
Gnomoniopsis fructicola	CBS 125671	DMW61	BPI 879155	GU320816	GU320776	GU320793	USA, NJ	*Fragaria sp.*	L. Struwe, 2008
Gnomoniopsis guttulata	na	MS 0312	BPI 877452A	EU254812	na	na	Bulgaria	*Agrimonia eupatoria*	D. Stoykov, 2005
Gnomoniopsis idaeicola	CBS 125672	DMW 147.1	BPI 879164	GU320823	GU320781	GU320797	USA, CA	*Rubus sp.*	D.M. Walker, 2008
Gnomoniopsis idaeicola	CBS 125673	DMW 167.2	BPI 879176	GU320824	GU320782	GU320798	USA, OR	*Rubus pedatus*	D.M. Walker, 2008
Gnomoniopsis idaeicola	CBS 125674	DMW 103.5	BPI 879157	GU320820	GU320780	GU320796	France	*Rubus sp.*	L.C. Mejia, 2008
Gnomoniopsis idaeicola	CBS 125675	DMW 190.1	BPI 879170	GU320822	GU320783	GU320799	USA, OR	*Rubus procerus*	D.M. Walker, 2008
Gnomoniopsis idaeicola	CBS 125676	DMW 98.2	BPI 879180	GU320821	GU320784	GU320811	USA, WA	*Rubus procerus*	D.M. Walker, 2008
Gnomoniopsis macounii	CBS121468	AR 3866	BPI 871008	EU254762	EU219126	GU320804	USA, NY	*Spiraea sp.*	L. Vasilyeva, 2002
Gnomoniopsis occulta	CBS 125677	DMW 113.1	BPI 879189	GU320828	GU320785	GU320812	USA, OR	*Potentilla sp.*	D.M. Walker, 2008
Gnomoniopsis occulta	CBS 125678	DMW 206.1	BPI 879191	GU320829	GU320786	GU320800	USA, OR	*Potentilla sp.*	D.M. Walker, 2008
Gnomoniopsis occulta	na	MS 0292	BPI 877455	EU254811	na	na	Russia	*Potentilla anserina*	M.V. Sogonov, 2005
Gnomoniopsis paraclavulata	CBS 123202	AR 4127	CohenW645	GU320830	GU320775	GU320815	USA, MD	*Quercus alba*	S. Cohen, 2005
Gnomoniopsis racemula	CBS 121469	AR 3892	BPI 871003	EU254841	EU219125	GU320803	USA, MN	*Chamerion angustifolium*	L. Vasilyeva, 2002
Gnomoniopsis sanguisorbae	CBS 858.79	CBS 858.79	na	GU320818	GU320790	GU320805	Switzerland	*Sanguisorba minor*	M. Monod, 1979
Gnomoniopsis sp.	CBS 125299	DMW 187.1	BPI 879154	GU320819	GU320791	GU320806	USA, OR	*Rubus parviflorus*	D.M. Walker, 2008

Gnomoniopsis tormentillae	CBS 904.79	CBS 904.79	na	EU254856	EU219165	GU320795	Switzerland	*Potentilla sp.*	M. Monod, 1977
Plagiostoma euphorbiae	CBS 340.78	CBS 340.78	na	EU199198	GU367034	GU354016	Netherlands	*Euphorbia palustris*	W. Gams, 2001
Sirococcus conigenus	CBS 113.75	BBA 62043	BPI 871251	EF512482	EU219129	EF512544	Germany	*Picea pungens*	R. Schneider, 2002
Sirococcus piceicola	CBS 119620	AR 4038	BPI 871252	EF512480	EU219130	EF512542	Canada, BC	*Picea sitchensis*	B. Callan, 2004
Sirococcus tsugae	CBS 119627	AR 4010	BPI 871172	EF512478	EU219143	EF512540	USA, OR	*Cedrus deodara*	M. Putnam, 2003

Table 2.1. AR = Dr. Amy Rossman, third author; BPI = U.S. National Fungus Collections, USDA ARS, Beltsville, MD; CBS = Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; DMW = Donald M. Walker, first author; na = not available.

LITERATURE CITED

Anagnostakis, S.L., 1988. *Cryphonectria parasitica* cause of chestnut blight. Adv. Plant Pathol. 6, 123–136.

Arnaud, G., Arnaud, M., 1931. Traité de pathologie végétale. Tome 1, Vol.2. Paris: Paul Lechevalier et Fils. p 1558–1562.

Barr, M.E., 1978. The Diaporthales in North America with emphasis on *Gnomonia* and its segregates. Mycol. Mem. 7, 1–232.

Bolay, A., 1971. Contribution a la connaissance de *Gnomonia comari* Karsten (syn. *G. fructicola* [Arnaud] Fall). Etude taxonomique, phytopathologiqueet recherches sur sa croissance in vitro. Berichte der Schweizerischen Botanischen Gesellschaft 81, 398–482.

Bolton, A.T., 1954. *Gnomonia fructicola* on strawberry. Can. J. Bot. 32, 172–181.

Carbone, I., Kohn, L.M., 1999. A method for designing primer sets for speciation studies in filamentous ascomycetes. Mycologia 91, 553–556.

Cohen, S.D., 2004. Endophytic–host selectivity of *Discula umbrinella* on *Quercus alba* and *Quercus rubra* characterized by infection, pathogenicity and mycelial compatibility. Eur. J. Plant Pathol. 110, 713–721.

Danti, R., Sieber, T.N., Sanguineti, G., 2002. Endophytic mycobiota in twigs of healthy and declining *Fagus sylvatica* in the Apennines. Mycol. Res. 106, 1343–1348.

Edgar, R.C., 2004. MUSCLE: a multiple sequence alignment method with reduced time and space complexity. BMC Bioinf. 5, 113.

Fall, J., 1951. Studies on fungus parasites of strawberry leaves in Ontario. Can. J. Bot. 29, 299–315.

Farris, J.C., Källersjö, M., Kluge, A., Bult, B., 1995. Testing significance of incongruence. Cladistics 10, 315–319.

Felsenstein, J., 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 6, 227–242.

Huelsenbeck, J.P., Ronquist, F., 2001. MRBAYES: Bayesian inference of phylogeny. Bioinformatics 17, 754–755.

Johnson, L.A., Soltis, D.E., 1998. Assessing congruence: empirical examples from molecular data. In: Soltis, D.E., Soltis, P.S., Doyle, J.J., eds. Molecular Systematics of Plants 2. Boston: Kluwer. p 287–348.

Kellogg, E.A., Appels, R., Mason–Gamer, R.J., 1996. When genes tell different stories: the diploid genera of Triticeae (Graminae). Syst. Bot. 21, 321–347.

Kjer, K.M., 1995. Use of rRNA secondary structure in phylogenetic studies to identify homologous positions: an example of alignment and data presentation from the frogs. Mol. Phyl. Evol. 4, 314–330.

Maas, J.L., 1998. Compendium of Strawberry Diseases. 2nd edn. St. Paul, Minnesota: APS Press. p 1–98.

Mason–Gamer, R.J., Kellogg, E.A., 1996. Testing for phylogenetic conflict among molecular data sets in the tribe Triticeae. Syst. Biol. 45, 524–545.

Monod, M., 1983. Monographie taxonomique des Gnomoniaceae (Ascomycétes de l'ordre des Diaporthales I). Beihefte zur Sydowia 9, 1–315.

Moročko, I., Fatehi, J., 2007. Molecular characterization of strawberry pathogen *Gnomonia fragariae* and its genetic relatedness to other *Gnomonia* species and members of Diaporthales. Mycol. Res. 111, 603–614.

Nixon, K.C., Carpenter, J.M., 1993. On outgroups. Cladistics 9, 413–426.

O'Donnell, K., Cigelnik, E., 1997. Two divergent intragenomic rDNA ITS2 types within a monophyletic lineage of the fungus *Fusarium* are nonorthologous. Mol. Phyl. Evol. 7, 103–116.

Posada, D., Crandall, K.A., 1998. Modeltest: testing the model of DNA substitution. Bioinformatics 14, 817–818.

Rehner, S.A., 2001. EF1 alpha primers. Document available online at: http//ocid.nacse.org/research/deephyphae/EF1primer.pdf.

Ronquist, F., Huelsenbeck, J.P., 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19, 1572–1574.

Rossman, A.Y., Farr, D.F., Castlebury, L.A., 2007a. A review of the phylogeny and biology of the Diaporthales. Mycoscience 48, 135–144.

Rossman, A.Y., Castlebury, L.A., Farr, D.F., Stanosz, G.R., 2007b. *Sirococcus conigenus, Sirococcus piceicola* sp. nov. on conifers: anamorphic fungi in the Gnomoniaceae. For. Path. 38, 47–60.

Sanderson, P.G., Worf, G.L., 1986. *Phomopsis* and *Sirococcus* shoot blights of Colorado blue spruce in Wisconsin. Plant Dis. 70, 1159.

Shahin, E.A., Claflin, L.E., 1978. The occurrence and distribution of *Sirococcus* shoot blight of spruce in Kansas. Plant Dis. Rep. 62, 648–650.

Sieber, T., Hugentobler, C., 1987. Endophytische Pilze in Blättern und Ästen gesunder und geschädigter Buchen (*Fagus sylvatica* L.). Eur. J. Forest Pathol. 17, 411–425.

Smith, R.S., 1973. *Sirococcus* tip dieback of *Pinus* spp. in California forest nurseries. Plant Dis. Rep. 57, 69–73.

Sogonov, M.V., Castlebury, L.A., Rossman, A.Y., Mejía, L.C., White, J.F., 2008. Leaf–inhabiting genera of the Gnomoniaceae, Diaporthales. Stud. Mycol. 62, 1–77.

Sogonov, M.V., Castlebury, L.A., Rossman, A.Y., Mejía, L.C., White JF. 2007. The type species of *Apiognomonia, A. veneta,* with its *Discula* anamorph is distinct from *A. errabunda*. Mycol. Res. 111, 693–709.

Stoykov, D.Y., 2005. New records of Diaporthales in Bulgaria. I. Mycol. Balcanica 2, 69–74.

Swofford, D.L., 2002. PAUP*: phylogenetic analysis using parsimony (and other methods). Version 4.0b10. Sunderland, Massachusetts: Sinauer Associates Inc.

Torres, M.S., White, J.F., Bischoff, J.F., 2005. *Cordyceps spegazzinii* sp. nov. a new species of the *C. militaris* group. Mycotaxon 94, 253–263.

van Adrichem, M.C.J., Bosher, J.E., 1958. Leaf blotch and petiole blight of strawberry caused by *Gnomonia fructicola*. Plant Dis. Rep. 42, 772–775.

Watling, R., Turnbull, E., 1998. Cantharellaceae, Gomphaceae and amyloid–spored and xeruloid members of Tricholomataceae (excl. *Mycena*). In: Henderson, D.M., Orton, P.D., Watling, R., Gregory, N.M., Howell, K.M.R., eds. British Fungus Flora 8. United Kingdom: Alden. p 1–189.

White, T.J., Bruns, T., Lee, S., Taylor, J., 1990. Amplification and direct sequencing of fungal ribosomal DNA for phylogenetics. In: Innis, M.A., Gelfand, D.H., Sninsky, J.J., White, T.J., eds. PCR Protocols: a guide to methods and applications. San Diego: Academic Press. p 315–322.

Zuker, M., 2003. Mfold web server for nucleic acid folding and hybridization prediction. Nucleic Acids Res. 31, 3406–3415.

Zwickl, D.J., 2006. Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion. Doctoral dissertation. The University of Texas at Austin. 115 p.

Chapter 3

New molecular markers for fungal phylogenetics: Two genes for species-level systematics in the Sordariomycetes (Ascomycota)[2]

ABSTRACT

Although significant progress has been made resolving deep branches of the fungal tree of life, many fungal systematists are interested in species-level questions to both define species and assess fungal biodiversity. Fungal genome sequences are a useful resource to systematic biologists for developing new phylogenetic markers that better represent the whole genome. Here we report primers for two newly identified single-copy protein-coding genes, FG1093 and MS204, for use with ascomycetes. Although fungi were the focus of this study, this methodological approach could be easily applied to marker development for studies of other organisms. The tests used here to assess phylogenetic informativeness are computationally rapid, require only rudimentary datasets to evaluate existing or newly developed markers, and can be applied to other non-model organisms to assist in experimental design of phylogenetic studies. Phylogenetic utility of the markers was tested in two genera, *Gnomoniopsis* and *Ophiognomonia* (Gnomoniaceae, Diaporthales). The phylogenetic performance of *β-tubulin*, ITS, and *tef-lα* was compared with FG1093 and MS204. Phylogenies inferred from FG1093 and MS204 were largely in agreement with *β-tubulin*, ITS, and *tef-1α* although some topological conflict was observed. Resolution and support for branches differed based on the combination of markers used for each genus.

Based on two independent tests of phylogenetic performance, FG1093 and MS204 were

determined to be equal to or better than *β-tubulin*, ITS, and *tef-1α* in resolving species

relationships. Differences were found in site-specific rate of evolution in all five markers.

In addition, isolates from 15 orders and 22 families of Ascomycota were screened using

primers for FG1093 and MS204 to demonstrate primer utility across a wide diversity of

ascomycetes. The primer sets for the newly identified genes FG1093 and MS204 and

methods used to develop them are useful additions to the ascomycete systematists'

toolbox.

INTRODUCTION

      The field of fungal phylogenetics has been revolutionized with advances in

molecular biology leading to expanded multigene datasets and phylogenetic algorithms.

Multigene and phylogenomic studies have resolved many higher-level relationships (*e.g.*

Aguileta et al. 2008, Fitzpatrick et al. 2006, James et. al. 2006, Kuramae et al. 2006,

McLaughlin et al. 2009, Robbertse et al. 2006, Spatafora et al. 2006), leaving the tips of

the branches in question. The nuclear ribosomal large and small subunits and the internal

transcribed spacer (ITS) regions 1 and 2 including 5.8S ribosomal DNA make up the bulk

of available fungal DNA sequences and were the first genes to be used for DNA

sequence-based phylogenies. These ribosomal genes were followed by subunits 1 and 2

of RNA polymerase II, elongation factor 1α (*tef-lα*), and mitochondrial ATP synthase and

have predominated in fungal phylogenetic studies to date (*e.g.,* Damm et al. 2007,

Letcher et al. 2008, Mejía et al. 2011, Raja et al. 2008, Schoch et al. 2009, Spatafora et al.

2006, Walker et al. 2010). Although these genes have shown great utility, there are

problematic issues with some. For example, paralogs have been documented in *β-tubulin* and *tef-1α* (Corradi et al. 2004a, 2004b, James et al. 2006, Landvik et al. 2001). In addition, the ITS, which is informally accepted as an identification marker for many fungi, has undergone nonconcerted patterns of evolution leading to paralogous ITS types (Ko and Jung 2002, O'Donnell and Cigelnik 1997, O'Donnell et al. 1998) in some species and may be uninformative or even misleading in some situations (Crouch et al. 2009).

Recent progress in genome sequencing efforts among the fungi has aided in the identification of phylogenetically informative genes and expanded the suite of available markers for inferring evolutionary histories (Aguileta et al. 2008, Schmitt et al. 2009). Utilizing 21 fungal genomes, Aguileta et al. (2008) identified 246 single-copy orthologous gene clusters in an optimally performing gene set. Protein-coding genes such as *β-tubulin, tef-lα, γ-actin,* chitinases, chitin synthases, RNA polymerases and others were not among the best performing genes found by Aguileta et al. (2008). In addition, *β-tubulin, tef-lα,* chitinases, and dehydrogenases were not in the cross-genome single-copy ortholog set, most likely due to the presence of paralogs. Schmitt et al. (2009) developed primers sets for two single-copy genes *Mcm7* and *Tsr1,* which Aguileta et al. (2008) determined as the most phylogenetically informative markers in their optimally performing gene set. Although these genes show promise for taxonomic resolution from species to class levels, species amplified by Schmitt et al. (2009) belonged exclusively to the lichen-forming classes Eurotiomycetes, Lecanoromycetes, and Lichinomycetes. Primer sequences, however, were compared with published *Mcm7* and *Tsr1* sequences for representative taxa suggesting that the primers would work outside the tested groups

(Schmitt et al. 2009, Zhang et al. 2011). Raja et al. (2011) confirmed phylogenetic utility of *Mcm7* from species to class levels in the Dothideomycetes, Eurotiomycetes, Geoglossomycetes, Lecanoromycetes, Leotiomycetes, and Sordariomycetes.

In this study, the FUNgal phYlogenomic dataBASE (FUNYBASE, Marthey et al. 2008) was utilized in an effort to identify genes appropriate for resolving species-level relationships in the Ascomycota with focus on the diaporthalean family Gnomoniaceae (Sordariomycetes). FUNYBASE is a collection of single-copy orthologous genes extracted from 96 publicly available genomes and constitutes an effective resource to find conserved orthologs within user-defined taxon sets (Aguileta et al. 2008, Marthey et al. 2008). Two genera in the Gnomoniaceae, *Gnomoniopsis* and *Ophiognomonia,* were chosen as models for this study. As neither genus has a genome sequence to serve as a reference for marker design, these two genera were considered ideal candidates for testing the utility of the database for developing new primer sets. Both are widespread and easily collected in northern temperate regions on a diverse range of plant hosts including horticultural and agronomic crop plants. These genera include, plant pathogenic, non-pathogenic, endophytic, and saprobic species, and are amenable to filamentous growth in culture. According to Walker et al. (2010), the genus *Gnomoniopsis* includes 13 species, many of which are economically significant pathogens of rosaceous crop plants such as strawberry, raspberry, and blackberry (Bolay 1971, Maas 1998, Monod 1983). The genus *Ophiognomonia* includes 17 species, (Sogonov et al. 2008) which are known to cause numerous diseases of hardwood trees, such as the devastating butternut canker in North America (*e.g.,* Green 2004, Green and Castlebury 2007, Juhasova et al. 2006, Neely and Black 1976, Pennycook 2007). By

identifying appropriate species-level markers, it will be possible to address the evolutionary history of host associations, lifestyle transitions between pathogenic and endophytic or saprobic species, and the impact of agricultural systems on pathogens in this group as well as biogeographical history in a manner that has not previously been possible.

Our objectives in the present study are to: (1) identify additional or alternative markers that increase resolution and support in species-level relationships in the genera *Gnomoniopsis* and *Ophiognomonia*; (2) evaluate substitution rates and performance of the newly identified genes in comparison to *β-tubulin*, ITS, and *tef-1α*; (3) identify combination(s) of genes that produce fully resolved and robustly supported phylogenies in *Gnomoniopsis* and *Ophiognomonia*; and (4) demonstrate primer utility within a broad sampling of the Ascomycota.

METHODS

Gene screening process

Twenty-five single-copy protein-coding orthologous genes were extracted from FUNYBASE (Marthey et al. 2008) for the following ascomycete taxa: *Aspergillus nidulans, Fusarium graminearum, Magnaporthe grisea, Neurospora crassa, Sclerotinia sclerotiorum, Stagonospora nodorum,* and *Trichoderma reesei.* Predicted protein sequences were downloaded from FUNYBASE and used for BlastP searches (Altschul et al. 1997) of databases maintained by the Broad Institute (http://www.broadinstitute.org) or the Joint Genome Initiative (http://genome.jgi-psf.org/) to recover corresponding amino acid data. MAFFT v6 web server (http://mafft.cbrc.jp/alignment/server/) was

utilized to align translated amino acids with corresponding genomic nucleotide sequences. Sequences were examined for 400–1200 bp regions characterized by the presence of conserved exon and intron regions. When single or multiple conserved introns were identified with a flanking exon, greater than 10 amino acids in length, minimally degenerate primers were designed for the flanking exon regions with Oligo v6.9 (http://oligo.net/), using sequences from *Magnaporthe grisea,* the closest available relative to *Gnomoniopsis* and *Ophiognomonia*, as the primary guide. Primer sets were tested in 11 *Gnomoniopsis* species, 13 *Ophiognomonia* species, and 30 additional ascomycete taxa (Table 3.1). Additionally, primers were refined to reflect sequences determined for gnomoniaceous taxa after obtaining sequence data (Table 3.2).

DNA extraction, amplification, and sequencing

Fungal cultures were incubated and genomic DNA extracted as in Walker et al. (2010). Genomic DNA for each isolate was quantified at 10 ng/µL using a NanoDrop ND-1000 spectrophotometer (Nanodrop Products, Wilmington, DE). PCR amplification conditions and primers for *β-tubulin,* ITS, and *tef-1α* were as detailed in Walker et al. (2010), with the exception of the primers for *tef-1α* designed for this study based on existing gnomoniaceous sequences (Table 3.2). For FG1093 and MS204, reaction conditions were the same as Walker et al. (2010) with the following thermal cycling parameters: 2 m at 95 $^{\circ}$C, then 10 cycles of 1 m at 95 $^{\circ}$C, 30 s at 65–55 $^{\circ}$C decreasing by 1 degree each cycle, 1 m at 72 $^{\circ}$C, followed by 35 cycles of 1 m at 95 $^{\circ}$C, 30 s at 59 $^{\circ}$C or 62 $^{\circ}$C (FG1093 or MS204, respectively), 1 m at 72 $^{\circ}$C, with primer pairs FG1093 E1F1/E3R1 and MS204 E1F1/E5R1 or MS204 E1F1/E5R1a (Table 3.2). PCR products

were purified and directly sequenced as in Walker et al. (2010) using the PCR primers

and sequencing primers summarized in Table 3.2.

For optimization of annealing temperatures when non-specific amplification was

observed, ranges of theoretical optimal annealing temperature for 12 sequences from

isolates in eight orders of ascomycetes corresponding to the primer regions and the

FG1093 and MS204 primers were determined (BioMath Calculators,

http://www.promega.com/biomath/calc11.htm#melt_results; Rychlik and Rhoads 1989).

These ranges were used as median starting point temperatures for subsequent gradient

PCRs. Reaction conditions were as described above. Four isolates from different

taxonomic orders were evaluated, two of which previously amplified well and two

lacking previous amplification.

Sequence data analyses

Raw sequences were edited and assembled with Sequencher version 4.9 for

Windows (Gene Codes Corporation, Ann Arbor, MI). Individual alignments were

performed in MAFFT v6 (http://mafft.cbrc.jp/alignment/server/) according to the

algorithm in Table 3.3. Potential conflict among datasets was assessed with a conditional

comparison test (Johnson and Soltis 1998, Kellogg et al. 1996, Mason-Gamer and

Kellogg 1996) as in Walker et al. (2010). Due to minor conflict observed between

individual *Ophiognomonia* datasets, three additional methods were employed: (1)

evaluation of semi-strict combinable component trees (Collins et al. 2005) in PAUP* v.

4.0b10 (Swofford 2002) from single dataset GARLI BS replicates; (2) exclusion of all

alignment positions containing gaps (*β-tubulin* 84 bp, FG1093 77 bp, ITS 22 bp, MS204

263 bp, *tef-1α* 285 bp) and analysis of resulting trees; and (3) addition of all available *Ophiognomonia* isolates to the alignment to test for incomplete taxon sampling issues (Hedtke et al. 2006).

Phylogenetic trees were inferred through Bayesian, maximum likelihood (ML), and maximum parsimony (MP) phylogenetic analyses. Trees were rooted using the outgroup method (Nixon and Carpenter 1993), using *Plagiostoma* sp. and *Ophiognomonia setacea* for *Gnomoniopsis* and *Gnomoniopsis sanguisorbae* and *Plagiostoma* sp. for *Ophiognomonia* . Each individual alignment was analyzed and concatenated in all possible permutations with a minimum of three markers included, consisting of thirty-two separate data matrices (Table 3.4, Table S3.1). MP analysis was performed as described in Walker et al. (2010). Bayesian analysis was performed using the Bioportal website (www.bioportal.uio.no) as described in Walker et al. (2010) with the exception of a sampling frequency of every 500 generations. Each marker was independently partitioned according to ModelTest v. 3.7 results (Posada and Crandall 1998, Table S3.2). Convergence of each run was verified in three ways. The first 1,250,000 generations (25%) were eliminated as the "burn in" period and each parameter visualized for convergence using the graphic output from the Bioportal website. The standard deviation of split frequencies was determined to fall below 0.01 for each run and potential scale reduction factor verified as close to 1.0 for all parameters (Ronquist and Huelsenbeck 2003). The program GARLI v1.0 was used to calculate maximum likelihood trees and bootstrap (ML BS) support values (Zwickl 2006). GARLI was run through The Lattice Project (http://boinc.umiacs.umd.edu) utilizing grid computing (Bazinet et al. 2007, Bazinet and Cummings 2008, Cummings and Huskamp 2005, Myers

et al. 2008). Partitioned GARLI analyses were not available through The Lattice Project, therefore, branch support was estimated using the optimal models for combined datasets selected by ModelTest v. 3.7 (Posada and Crandall 1998, Table S3.2) under the default settings with 1000 ML BS replications. Majority rule consensus trees from the bootstrap analyses were calculated in PAUP*, with a threshold of ≥70 % used as the cutoff for supported nodes.

Assessment of combined and individual phylogenetic performance

The following two criteria were used to identify three- and four-marker combinations that accurately represent the topology of the five-marker datasets with equivalent or higher support for branches: (1) the total number of robustly supported (≥70% ML BS, MP BS or ≥95% PP) branches from each analysis and (2) the number of unique topologies in the 95% posterior credibility interval estimated by the Bayesian analyses (Buckley et al. 2002, Willows-Munro et al. 2005).

The individual markers were each compared to the five-marker dataset to test for topological congruence and phylogenetic performance in *Gnomoniopsis* and *Ophiognomonia*. The three- and four-marker combinations (Table 3.4) that fully recover the five-marker topology with equivalent or higher support for branches were also used to measure performance of the individual markers included in the respective datasets. These three- and four-marker combinations (Table 3.4) were also compared to the five-marker topology to confirm topological congruence between combined marker sets. For each analysis, congruence between the five individual ML trees and the ML tree from each of the three-, four-, and five-marker datasets was evaluated using the pairwise comparison

of phylogenies algorithm developed by Nye et al. (2005,

http://www.mas.ncl.ac.uk/~ntmwn/phylo_comparison/pairwise.html). Performance

scores (0–100) represent comparisons from an optimum 1-to-1 correspondence map of

branches and terminal groups (Nye et al. 2005). Results for independent marker

performance were interpreted as individual marker contribution to a given dataset.

Phylogenetic informativeness profiles (PI profiles) were calculated for each

marker using PhyDesign (Lopez-Giraldez and Townsend 2011, Townsend 2007,

http://phydesign.townsend.yale.edu/). The rate profiles of single-marker datasets

excluding gapped regions were calculated in HyPhy (Pond et al. 2005) for PhyDesign

analyses. The model parameters were altered in HyPhy according to ModelTest results

(Table S3.2). The most likely five-marker tree from the GARLI analysis was converted to

an ultrametric tree using nonparametric rate smoothing (Sanderson 1997) in TreeEdit

v1.0a10 for both genera (Rambaut and Charleston 2002). A relative timescale was used

to date tree nodes in phylogenies of *Gnomoniopsis* and *Ophiognomonia.*

Site specific rate evolution

Evaluation of the site specific rate of evolution for each marker in *Gnomoniopsis*

and *Ophiognomonia* was performed in the baseml application of PAML 4: Phylogenetic

Analysis by Maximum Likelihood (Yang 2007) on the Bioportal website

(www.bioportal.uio.no) using the default settings for all parameters except for the model

of evolution and input tree. The most likely ML trees from single marker GARLI

analyses were used as input trees in PAML 4. All alignment positions with gaps were

excluded from the analyses. The number of included sites/total sites can be determined by

comparing the alignment lengths in Table 3.3. The PAML alignment files excluding gapped sites were realigned with complete data matrices to determine exon and intron boundaries. The exons and introns were then mapped onto site-specific rate files for each gene region using Microsoft Excel. The substitution rate mean and variance of exons and introns for both genera were calculated in Microsoft Excel (2007).

Substitution saturation

The third codon position was evaluated for substitution saturation in FG1093 and MS204 for *Gnomoniopsis* and *Ophiognomonia* in DAMBE: Data Analysis in Molecular Biology and Evolution (Xia et al. 2003). An alignment file including exon regions was imported into DAMBE, and third codon positions tested for the index of substitution saturation (*Iss*) and critical index of substitution saturation (*Iss.c*). If the *Iss* was significantly ($P \leq 0.05$) less than the *Iss.c,* substitution saturation was determined as absent from the alignment (Xia et al. 2003).

RESULTS

Ortholog screen

Thirteen of the 25 single-copy orthologous genes extracted from FUNYBASE did not meet our standards due to either lack of variability or length constraints (Table S3.3). Primer design was not feasible for seven genes due to lack of conserved primer binding sites (Table S3.3). Primer sets for FG1093, FG1565, and MS204 produced successful amplification in all ten test species of *Gnomoniopsis* and *Ophiognomonia*. Approximately 400–600 bp of FG1093 and 1000–1500 bp of MS204 were amplified (Fig. 3.1).

However, due to extensive amplification failures in additional species of *Gnomoniopsis*, *Ophiognomonia,* and selected representatives of Ascomycota, further development of FG1565 was abandoned. Primers developed for FG1077 and FG1380 failed to amplify the test species and were also abandoned (Table S3.3).

PCR optimization

Gradient PCR and reagent optimizations were used to develop standard protocols for amplification of FG1093 and MS204 (Table 3.2). Nine reactions across an annealing temperature gradient of 66–58 ^{o}C for each of four isolates were performed, followed by a 62–70 ^{o}C gradient for MS204 and a 56–64 ^{o}C gradient for FG1093. Optimal annealing temperatures were determined as 59 ^{o}C (FG1093 E1F1/E3R1) and 62 ^{o}C (MS204 E1F1/E5R1) for the four isolates tested. An optimal annealing temperature of 65.5 ^{o}C was determined for amplification of MS204 for species in the order Hypocreales. Several additional steps were followed when background amplification was observed. Decreasing primer concentrations from 10 μM to 5 μM and DNA concentration from 10 ng/μL to 5 ng/μL decreased background amplification and increased sequence quality. Also, addition of MgCl$_2$ (final concentration: 2.5 mM) and 5x (0.5μL) combinatorial enhancer solution (Ralser et al. 2006) to PCR reactions increased amplification success for hypocrealean taxa.

Primer utility in Ascomycota

Thirteen primers were developed to amplify and sequence the two optimized gene regions from a diverse array of Ascomycota (Table 3.2) from five classes including 15

orders and 22 families. Two FG1093 primers and three MS204 primers were specific to

Gnomoniaceae (Table 3.2). Positive amplification results were obtained for 97% of the

54 isolates using FG1093 primers E1F1/E3R1 and 91% of the isolates using MS204

primers E1F1/E5R1 or E1F1/E5R1a (Table 3.1). Amplification of FG1093 failed for

Lasiosphaeria lanuginosa (Sordariomycetes) and *Saccharomyces cerevisiae*

(Saccharomycetes). Amplification of MS204 failed for *Mollisia cinerea* (Leotiomycetes),

Saccharomyces cerevisiae (Saccharomycetes), and *Lasiosphaeria lanuginosa,*

Sphaeronaemella fragariae, Xylaria enteroleuca (Sordariomycetes). For all isolates with

positive amplification, sequences were generated to confirm amplicon identity (Table

3.1).

Nucleotide alignments

Individual alignment lengths and characteristics for *Gnomoniopsis* and

Ophiognomonia are reported in Table 3.3. Alignments for *Gnomoniopsis* each consisted

of 15 sequences representing 11 species of *Gnomoniopsis,* two species of *Sirococcus,* and

two outgroup taxa, namely *Ophiognomonia setacea* and *Plagiostoma* sp. For *tef-1α,* only

the 404 bp intron region was available for the *Sirococcus* isolate CBS 113.75. All

Ophiognomonia alignments consisted of 15 isolates, representing 13 species in

Ophiognomonia and two outgroup taxa namely *Gnomoniopsis sanguisorbae* and

Plagiostoma sp. The numbers of positions excluded due to ambiguous alignment for

Gnomoniopsis are as follows: *β-tubulin* 41 bp, FG1093 9 bp, ITS 5 bp, MS204 42 bp, and

tef-1α 23 bp. The numbers of positions excluded due to ambiguous alignment for

Ophiognomonia are as follows: *β-tubulin* 46 bp, FG1093 15 bp, ITS 29 bp, MS204 6 bp,

and *tef-1α* 253 bp. A contiguous 235 bp region was excluded from *tef-1α* in *Ophiognomonia* that included numerous insertions and deletions rendering its alignment uncertain.

Phylogenetic analyses

No conflict was observed among trees generated from individual alignments for *Gnomoniopsis* as determined by conditional comparison tests; however, minor conflict was observed in *Ophiognomonia*. The conditional comparison test between the semi-conservative vs. conservative alignments showed no differences in number of conflicting branches (n=4). Conflict was observed independently between FG1093 vs. MS204 and *β-tubulin* and also *β-tubulin* vs. FG1093, ITS, MS204, and *tef-1α.* The topologies of ITS, MS204, and *tef-1α* were fully compatible in *Ophiognomonia*. Conflict was nearly absent in comparisons of semi-strict combinable consensus trees. Only a single conflicting branch was observed independently between FG1093 vs. MS204 and *β-tubulin.* Addition of isolates representing taxa already present in the analyses and representatives of potentially undescribed taxa decreased conflict from four to two supported branches. Since conflict decreased in alignments with additional taxa and was nearly absent from combinable consensus trees, single-marker data matrices of *Ophiognomonia* were concatenated for further analyses.

Although ML trees of the five-marker dataset are accepted as the most likely phylogeny for both *Gnomoniopsis* and *Ophiognomonia,* three- and four-marker combinations were identified as minimal marker sets that accurately represent the five-marker phylogenies of these genera. Branch support values from each analyzed

combination of markers for *Gnomoniopsis* are listed in Tables S3.1 and 3.4. For

Gnomoniopsis, the combination of three-markers that fully recovers the five-marker

topology with equivalent or higher support for branches was *β-tubulin*/FG1093/MS204

(Table 3.4). This three-marker phylogeny has support for 11 MP BS, 10 ML BS and 11

PP (n=12) branches and the lowest number of tree topologies (n=3) in the 95% credibility

interval (Fig. 3.2). In addition, from a total of 36 possible support values (MP BS, ML

BS, PP) across 12 nodes of this tree, 27 were greater than 90% (Fig. 3.2). Addition of *tef-*

1α to any combined dataset for *Gnomoniopsis* decreased support and increased the

number of tree topologies in the 95% credibility interval (Table 3.4). For *Gnomoniopsis,*

the four-marker combination that fully recovers the five-marker topology with equivalent

or higher support for branches was ITS/*β-tubulin*/FG1093/MS204 (Table 3.4). The

number of topologies in the 95% credibility interval increased (n=5) by a single tree and

MP/PP branch support decreased by one (Table 3.4); however, the topology was identical

to the best three- and five-marker trees.

The three-marker combination for *Ophiognomonia* that fully recovers the five-

marker topology with equivalent or higher support for branches was ITS/*tef-1α*/MS204

with 10 MP BS, 10 ML BS, 10 PP (n=12) and the lowest number of trees (n=4) in the

95% credibility interval (Table 3.4). This phylogeny shared an identical topology with

phylogenies produced from ITS/*β-tubulin*/*tef-1α*/MS204 and ITS/*tef-1α*/FG1093/MS204,

with the latter two datasets producing one or two additional trees in the 95% credibility

interval respectively. The ITS/*β-tubulin*/*tef-1α*/FG1093 phylogeny, which differed by a

single supported branch from the phylogenies above, was considered as a credible

alternative phylogeny due to high Bayesian support and low number (n=2) of unique

topologies in the 95% credibility interval (Table 3.4). The five-marker combined data matrix was accepted as a consensus phylogeny as it collapsed the conflicting branch in the three and four-marker topologies (Fig. 3.3).

Phylogenetic performance of individual gene regions

The three- and four-marker combinations that accurately represent the topology of the five-marker combinations (section 3.5) were used to evaluate individual sequence marker performance using the method of Nye et al. (2005), which tests the ability of a given dataset to recover a given reference topology. The range of topological scores for all individual datasets generated from these analyses were 77.8–92.5 and 69.3–85.4 for *Gnomoniopsis* and *Ophiognomonia*, respectively, out of a possible score of 100. For *Gnomoniopsis,* the ITS recovered the highest topological scores (92.5). MS204, FG1093, and *β-tubulin* were roughly equivalent and only fell slightly below the ITS dataset in their ability to recover the reference topology (84.4–91.1), while *tef-1α* performed at the lowest level (Table 3.5). Topological performance values were consistent for all independent *Gnomoniopsis* datasets regardless of whether they were tested using the three-, four-, or five-marker datasets. Topological comparison of the three- and four-marker datasets that accurately represent the five-marker dataset showed completely identical topologies (100%) for *Gnomoniopsis* (Table 3.5).

For *Ophiognomonia,* ITS, *β-tubulin,* FG1093, *tef-1α* and MS204 recovered the three-, four-, and five-marker datasets consistently (Table 3.5; 69.3–85.4). For *Ophiognomonia, β-tubulin* and MS204 recovered two of the four-marker datasets (76.6, 77.0/85.4, 80.3) better than the five-marker dataset (73.3/78.3) respectively. For *tef-1α,*

the three-marker dataset (75.8) was recovered with a higher score than the five-marker

dataset (73.8; Table 3.5). The MS204 dataset recovered the overall highest topological

scores (78.3–85.4; Table 3.5). Phylogenetic performance of FG1093 and MS204 did not

appear dependent on taxon sampling, as greater or nearly equal topological scores were

observed when comparing FG1093 or MS204 to *β-tubulin/*ITS/*tef-1α* independent of the

genus under evaluation (Table 3.5). Relatively high scores (87.4–94.2) were recorded for

topological comparison of the three-, four-, and five-marker datasets to one another

(Table 3.5). The four-marker *β-tubulin/*ITS/MS204/*tef-1α* combination recovered the

five-marker dataset with the lowest score (87.4). The other three combinations recovered

the five-marker dataset with high scores (Table 3.5; 94.2, 92, 94.2).

Phylogenetic informativeness profiles developed in Townsend (2007) were used

to determine a quantitative measure of signal and utility that individual sequence markers

contribute to phylogenetic inference. The sequence markers *β-tubulin*, MS204, and *tef-1α*

in *Gnomoniopsis* and *β-tubulin/*MS204 in *Ophiognomonia* show the highest net

informativeness profiles (Fig. 3.4A, C). When informativeness per site is considered,

FG1093 shifts to second in rank behind *β-tubulin* for both genera (Fig. 3.4B, D). The ITS

region has the lowest net and per site informativeness among all sequence markers for

both genera. In net PI profiles for both genera, ITS and FG1093 have relatively gentle

slopes, suggesting low but consistent phylogenetic signal (Fig. 3.4A, C). The steep

sloping PI profiles of *β-tubulin* and *tef-1α* indicate rapid accumulation of noise past the

profile peaks (indicated by colored arrows) for *Gnomoniopsis* (Fig. 3.4A–B). For both

genera, MS204 displayed the highest signal for resolution during the time of interest. The

tef-1α marker showed the highest level of informativeness for the most recent divergences in both genera.

Site specific rate of evolution

Site specific rates of nucleotide evolution were evaluated for each marker, including partitions of exons and introns. The highest average substitution rates were observed for introns and lowest in exons for all five sequence markers across both genera, as was expected (Fig. 3.5). The average nucleotide substitution rate of FG1093 was lower in *Gnomoniopsis* than *Ophiognomonia* (Fig. 3.5). The substitution rates of ITS, *tef-1α,* and MS204 were similar between *Gnomoniopsis* and *Ophiognomonia*. Overall, ITS had the lowest substitution rate among all gene regions for both genera (Fig. 3.5). The introns in *β-tubulin, tef-1α,* and MS204 had the highest average and variance in substitution rates for both genera (Fig. 3.5). Site rate variation was minimal to absent in the 5.8S region of ITS and exons of FG1093. Also, substitution saturation was completely absent from third codon positions in FG1093 and MS204 for both *Gnomoniopsis* and *Ophiognomonia* (Table 3.3).

DISCUSSION

Although significant progress has been made in resolving deep branches of the fungal tree of life and an increasing number of markers for defining supraspecific fungal groups is available, additional markers are needed to more fully resolve the tips of the branches for a more comprehensive understanding of genealogical relationships among species. Expanding the available number of markers also gives insight into the

evolutionary history of a species by exploring alternative genealogies and facilitates genome-wide molecular fingerprinting of organisms. A stumbling block in the development of species-level markers for many groups of fungi has traditionally been the lack of sequence resources necessary to develop such markers. For the majority of fungi, the implementation of phylogenetic marker systems continues to be driven by factors such as available informatic and financial resources, rather than the suitability of the markers to address a particular research question. In the present study, using published fungal genomes, we demonstrate an efficient approach for the identification, development, and testing of robust phylogenetic markers in non-model fungi.

The genera *Gnomoniopsis* and *Ophiognomonia* each lack a complete genome sequence and were chosen to implement this method. This approach could be applied to other non-model organisms using databases such as Inparanoid (Eukaryotes; O'Brien et al. 2005), OrthoDB (vertebrates, arthropods, fungi; Waterhouse et al. 2011), and TreeFam (Animals; Li et al. 2006). The topological test of Nye et al. (2005) and PI profiles developed in Townsend (2007) were used in this study to quantify phylogenetic informativeness and resolution of currently used or new marker candidates. These tests are computationally rapid, require only rudimentary datasets to evaluate existing or newly developed markers, and can be applied to other non-model organisms to assist in experimental design of phylogenetic studies.

Phylogenetic performance of the markers

The utility of the two newly developed sequence markers was tested in the genera *Gnomoniopsis* and *Ophiognomonia;* however, the primer sets developed will amplify a

wide diversity of ascomycetes and could easily be adopted for groups outside the

sampled taxa (Table 3.1). MS204, which codes for a guanine nucleotide-binding protein

subunit beta-like protein

(http://www.broadinstitute.org/annotation/genome/neurospora/GeneDetails.html?sp=S70

00006085100444), is among a category of binding proteins determined by Aguileta et al.

(2008) as overrepresented in their set of best performing genes, although MS204 itself

was not included. In comparison to the other gene regions used in this study, MS204

performs equally well or better in recovering the five-marker dataset for *Ophiognomonia*

and ranks second for *Gnomoniopsis* (Table 3.5). Although MS204 is not solely capable of

fully resolving and supporting the accepted phylogenies for *Gnomoniopsis* and

Ophiognomonia, it provides highly informative signal in combined dataset analyses (Fig.

3.4, Table 3.5). PI profiles were used to quantify marker signal and were interpreted

based on three criteria. (1) The height of the profile peak indicates the maximum amount

of signal a marker contributed to a phylogenetic analysis. (2) The location in a phylogeny

of a PI profile peak indicates the time epoch when characters are evolving at an optimal

rate and contributing meaningful phylogenetic signal to an analysis (colored arrows Fig.

3.4). A profile that peaks deeper in a phylogeny than the time of interest should contain

less characters that may have frequently evolved to convergent states (Townsend 2007,

Townsend and Leuenberger 2011). For example, in the net PI profiles for both genera,

MS204 displays high signal at the appropriate time for species delimitation. (3) The slope

of a PI profile determines how quickly homoplasy accumulates in a given marker from

character convergence (Townsend 2007, Townsend and Leuenberger 2011). Sequence

markers that display PI profiles with gentle slopes provide consistent signal over

historical time and are less subject to convergence, whereas steep sloping profiles suggest

rapid accumulation of noise past the optimal rate of character evolution, which is

indicated by the profile peak (Townsend 2007, Townsend and Leuenberger 2011). In

comparing net PI profiles for *Gnomoniopsis,* MS204 displays high signal and a relatively

gentle slope (Fig. 3.4A). The decrease in signal represented by per site PI profiles for

both genera indicate a limitation in MS204 (Fig. 3.4B, D). Independently MS204 is

useful for lower-level resolution, but most powerful when combined with other markers

in a phylogenetic analysis.

The second newly developed marker FG1093, which codes for the 60S

ribosomal protein L37, is homologous across Eukaryota (HomoloGene:68110

http://www.ncbi.nlm.nih.gov/sites/entrez?db=homologene&cmd=retrieve&list_uids=681

10). FG1093, at approximately 500 bp, required only two primers to sequence both

strands completely. For both genera, FG1093 displayed high per site PI profiles (Fig.

3.4B, D) indicating a high level of informativeness. The low signal in net PI profiles is a

limitation of FG1093 for both genera; however, this marker is less subject to homoplasy

given the gentle slope profile. The short length and ease of amplification and sequencing

of FG1093 makes this marker attractive for species-level phylogenetic studies.

The ITS regions have been useful for grouping isolates representing species in

genera in Gnomoniaceae, but well-supported phylogenies using ITS alone have not been

possible (Mejía et al. 2011, Walker et al. 2010). In this study, the low but consistent

signal of ITS was sufficient to recover the topology of the five-marker combinations

(Table 3.5), although it was determined to be less useful due to low signal in PI profiles

and the lowest substitution rates relative to the other markers (Fig. 3.4–3.5). However, the

gentle sloping profile of ITS predicted the least amount of susceptibility to phylogenetic noise among all markers in both genera (Townsend and Leuenberger 2011, Fig. 3.4).

Phylogenetic informativeness tests were useful to aid in development of markers and also assessment or comparison of currently used markers. Application of PI tests prior to experimental design of a phylogenetic study should aid in marker choice and allow more accurate phylogenetic reconstruction. For example, the average substitution rates for *β-tubulin* and MS204 are similar within and between genera (Fig. 3.5). The PI profile peak in the *Ophiognomonia* phylogeny shows that *β-tubulin* possesses utility mostly past the epoch of interest (Fig. 3.4). The MS204 profile peaks deeper in the epoch of interest and should, therefore, be more informative than *β-tubulin* for phylogenetic inference of species (Fig. 3.4, Table 3.5). Also, the ability of MS204 to recover the five-marker dataset better than *β-tubulin* (Table 3.5; 78.3, 73.3 respectively), indicates that MS204 is a superior marker for species-level relationships. Considering *Gnomoniopsis*, *tef-1α* has moderate to high net/per site PI profiles, but steep slopes and profile peaks mostly past the epoch of interest (Fig. 3.4). High intron substitution rates were observed in *tef-1α* (Fig. 3.5), suggesting that the introns are evolving too rapidly for resolving species relationships in *Gnomoniopsis*. The fast rate of evolution in *tef-1α* introns makes it ideal for species delimitation instead of inter-species relationships. Comparison of markers in this study using PI tests provides insight into a quantitative ranking system useful for experimental design of phylogenetic studies in Gnomoniaceae with easy application to other organisms.

Combined marker analyses

With the number of ever increasing genome scale datasets across the fungal and other kingdoms, the question of how many markers are needed for phylogenetic studies persists. To determine the minimal number of markers needed for a fully resolved and highly supported species tree in this study, all possible three-, four-, and five-marker combinations were analyzed. The identification of a minimal marker set that accurately represents the five-marker topology was intended for use as a method for design of phylogenetic studies in the Gnomoniaceae with application to other organism groups. For *Gnomoniopsis,* the topologies of the three- and four-marker datasets were determined as 100% identical to the five-marker dataset (Table 3.5) and considered an accurate representation of the five-marker topology using the test in Nye et al. (2005). The *β-tubulin*/FG1093/MS204 dataset fully recovered the five-marker topology with equivalent or higher support for branches. All but two branches (#6/12; Fig. 3.2; Table S3.1) were robustly supported in all analyses. The topology of this dataset was not in conflict with the topology produced from *β-tubulin*/ITS/*tef-1α* dataset (Table 3.4) used in Walker et al. (2010), although less resolution and support were achieved in the latter study. We attribute higher support in the three- vs. five-marker phylogeny of *Gnomoniopsis* to addition of the *tef-1α* marker to the five-marker dataset. As previously mentioned (Section 4.1) this marker is evolving at a rate too high to accurately assess species-level relationships in *Gnomoniopsis* (Fig. 3.4–3.5). The high substitution rates observed in noncoding regions of *tef-1α* for *Gnomoniopsis* demonstrate that neutral changes are occurring relatively freely within introns (Fig 3.5). The dynamically changing sites in the *tef-1α* marker could be useful for recent speciation events unless they introduce homoplasy (Townsend 2007). Characters that evolve near the optimal rate will be more

useful to resolve a polytomy than characters evolving at a different rate (Townsend 2007). Therefore, it is imperative to choose a gene that evolves at an appropriate rate to resolve the ancestral branching pattern among taxa of interest (Townsend 2007).

Increasing the number of phylogenetically informative markers in an analysis should reduce stochastic error and better represent the whole genome (Chen et al. 2008, Cummings et al. 2008). When increasing the number of markers in combined analyses, similar results to Wild and Maddison's (2008) study of beetles (Coleoptera) were expected; the addition of multiple nuclear loci increased both resolution and support. However, in this study, branch support decreased and the number of trees in the 95% credibility interval increased in the five-marker combined analyses for both genera. This does not necessarily indicate that there are different evolutionary histories in the genes; however, one cannot entirely rule this out (Buckley et al. 2002). The degree of gene tree heterogeneity can differ depending on details of gene divergence history (Knowles 2009). As in this study, the underlying species tree is dependent on gene tree congruence. As time since speciation increases, gene tree discord becomes less likely (Knowles 2009). Perhaps insufficient time for fixation of gene lineages is an alternative explanation for decreased branch support in the five-marker combined analyses for both genera. Hedtke et al. (2006) found with strategic taxon sampling, only 2–3 genes are needed to achieve a robustly supported phylogeny. Although the five-marker phylogenies are accepted as the most accurate interpretation of species relationships in *Gnomoniopsis* and *Ophiognomonia,* this study empirically supports a three-marker scenario as sufficient to obtain a highly resolved and supported phylogeny in topological agreement with the five-marker combinations. However, choosing the three markers with the highest PI profiles

did not result in the most resolved and supported phylogeny. For example, in *Gnomoniopsis* net PI profiles for *β-tubulin/*MS204*/tef-1α* display the highest peaks (Fig. 3.4A), but when combined they produce a poorly resolved and supported phylogeny (Table 3.4). High signal is important, but selection of markers with PI profile peaks distributed across the entire phylogeny with gentle sloping profiles will limit phylogenetic noise in a dataset and ensure meaningful marker selection.

CONCLUSION

Two novel phylogenetic markers were identified and developed for use in phylogenetic studies of Ascomycota. The systematic approach used to select, evaluate, and quantify phylogenetic informativeness of the markers can be easily applied to any organismal group. Application of this method should help to improve experimental design and increase confidence in hypothesized phylogenetic relationships. Use of PI profiles to choose a combined marker set that produced high resolution and support proved to be a superior method for experimental design. The markers FG1093 and MS204 contributed both resolution and support in combined dataset analyses of *Gnomoniopsis* and *Ophiognomonia* and should be useful for addressing species-level questions for a broad diversity of ascomycetes.

Mejía, Andrew Miller, and Gary Samuels for providing cultures; Stephen Rehner for

helpful discussions on primer design; Jo Anne Crouch, Conrad Schoch, and Lena Struwe

for presubmission comments on the manuscript; and Sasha Allen and Tunesha Phipps for

technical support.
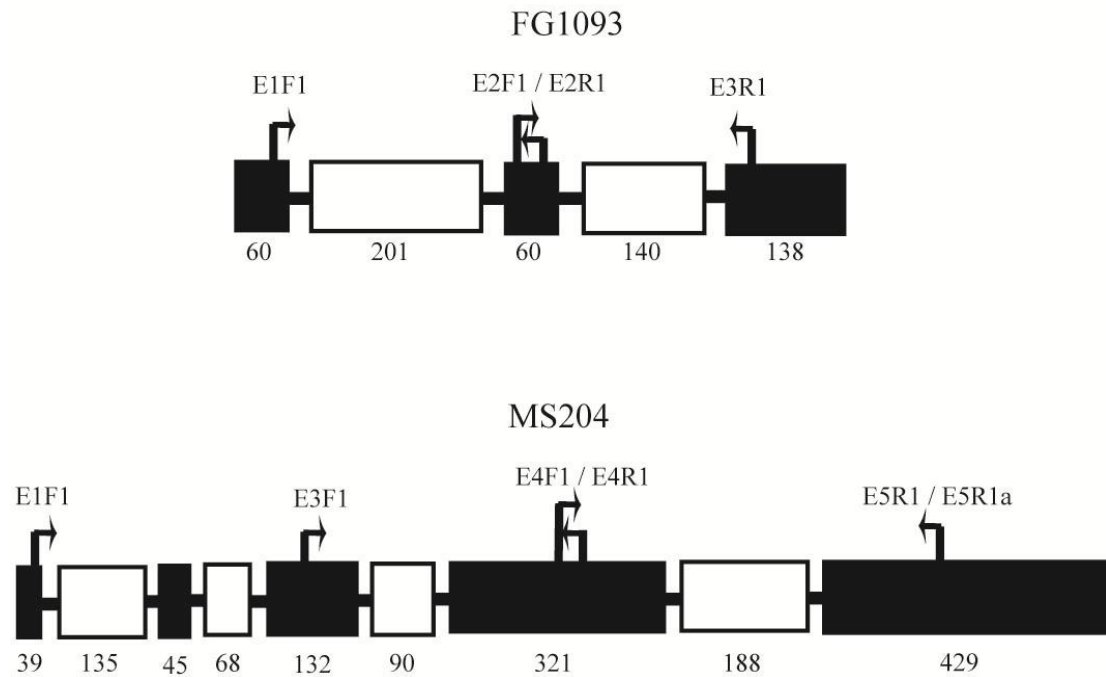
Fig. 3.1. Location of FG1093 and MS204 primers according to *Magnaporthe grisea* guide sequences. Exons are shaded black and introns white; Location and direction of primers are noted with arrows. Fragment size is noted beneath the respective exon and intron in base pairs.
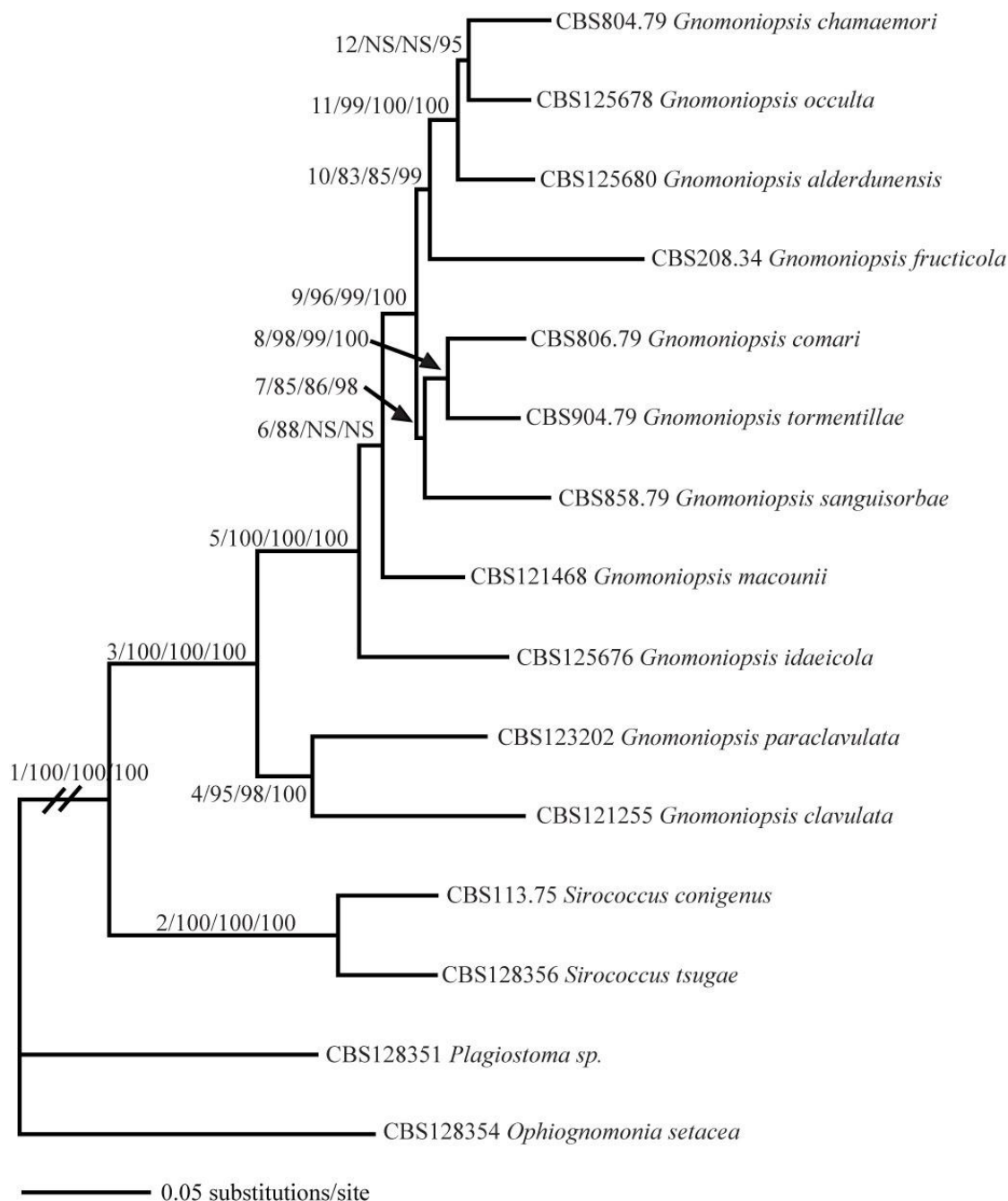
Fig. 3.2. ML phylogenetic analysis in GARLI (ML score = -lnL 13829.2864) of *β-tubulin*/FG1093/MS204 sequences from 11 species of *Gnomoniopsis,* two species of *Sirococcus,* and two outgroup taxa all within the Gnomoniaceae. Support values are displayed next to the corresponding branch in the following order: branch #

corresponding to Supplementary Table 3.1; MP BS values ≥ 70 %; GARLI ML BS

values ≥70 %; Bayesian PP ≥ 95 %.
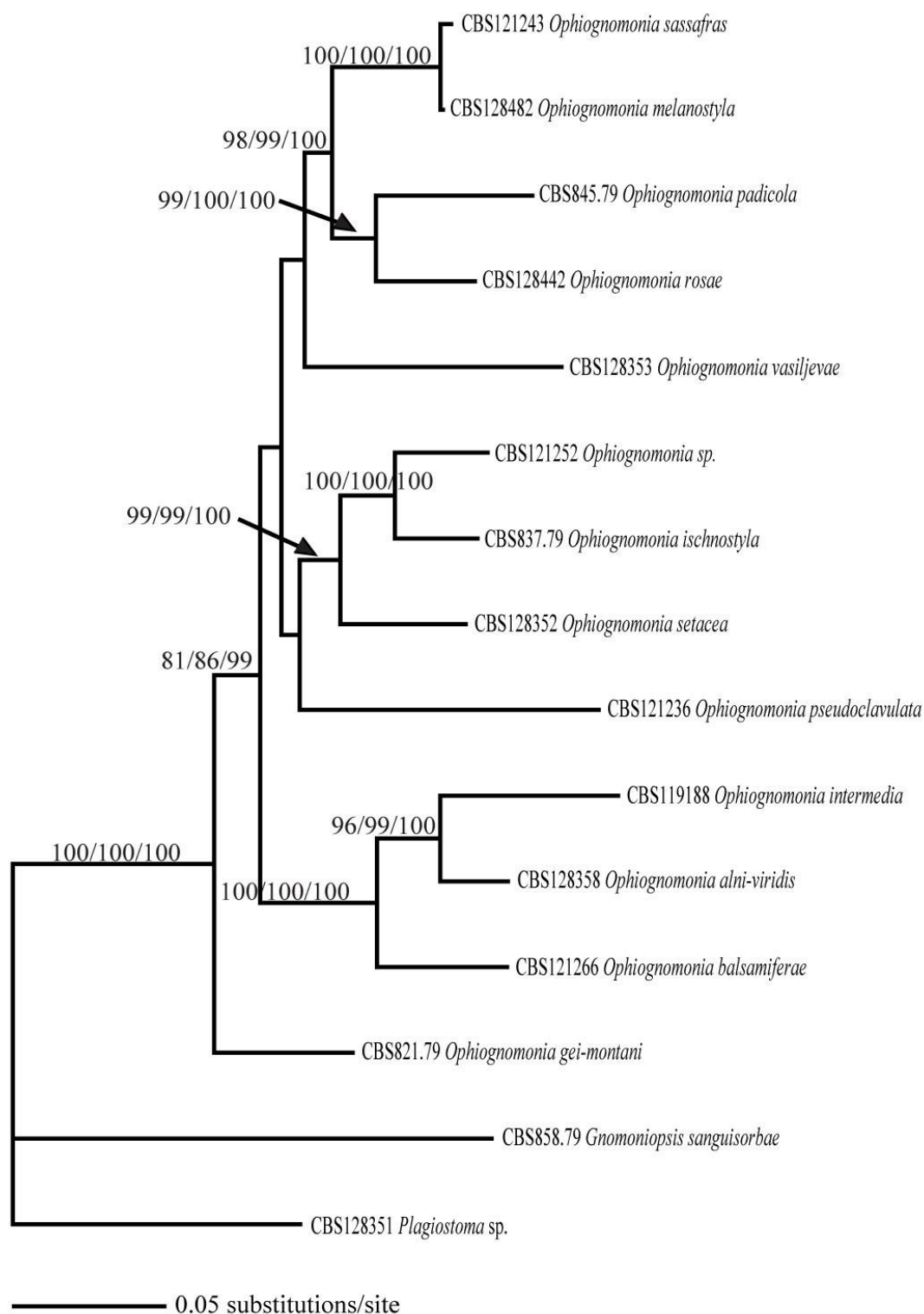
Fig. 3.3. ML phylogenetic analysis in GARLI (ML score = -lnL 20161.3299) of ITS/*β-tubulin*/*tef-1α*/FG1093/MS204 sequences from 13 species of *Ophiognomonia* and two

outgroup taxa all within the Gnomoniaceae. Support values are displayed next to the

corresponding branch in the following order: MP BS values ≥ 70 %; GARLI ML BS

values ≥70 %; Bayesian PP ≥ 95 %.
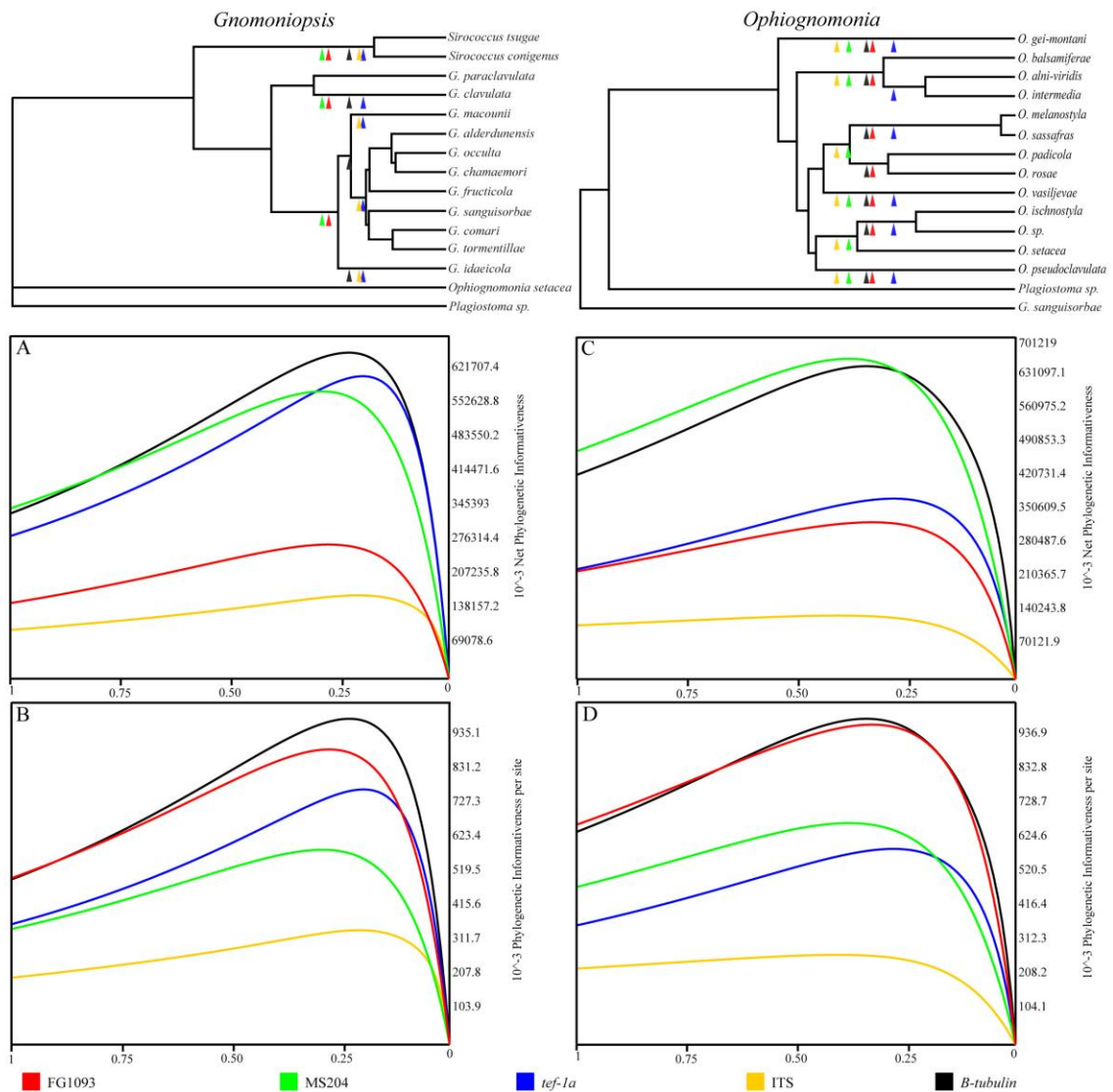
Fig. 3.4. Individual PI profiles of ITS/*β-tubulin*/ FG1093/*tef-1α*/MS204 in reference to

the GARLI ML five-marker tree which was converted to an ultrametric tree using

nonparametric rate smoothing. A, C. Net PI profile. B, D. Per site PI profile. Colored

arrows correspond to the region in the phylogeny where individual markers reach the

peak informativeness level. The relative timescale corresponds to the root-to-tip distance of *Gnomoniopsis* and *Ophiognomonia* phylogenies.
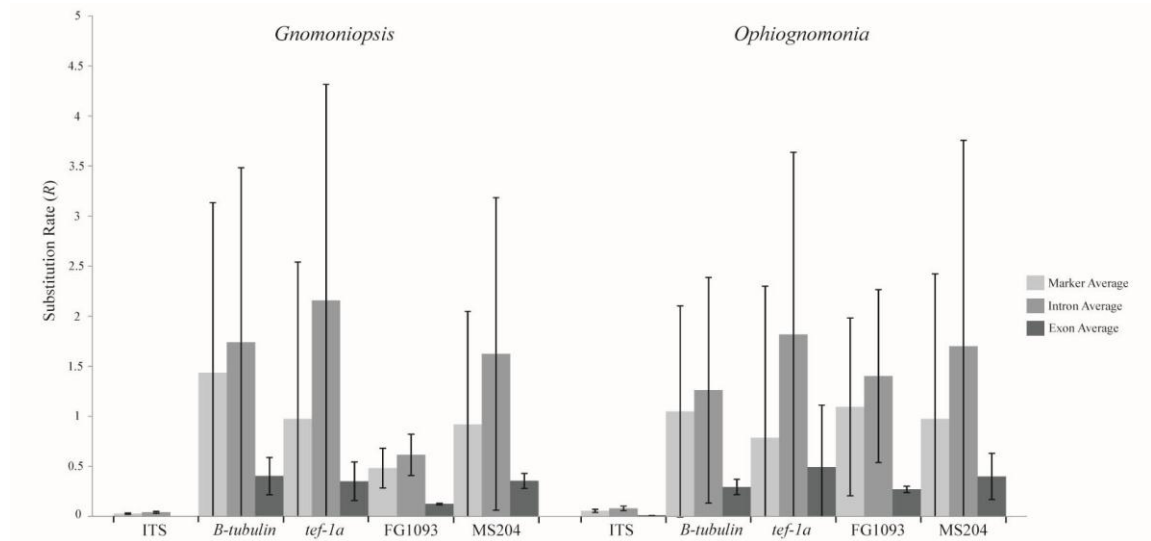
Fig. 3.5. Site specific substitution rate (*R*) of three partitions for each marker: 1) average rate; 2) intron average; 3) exon average. Each partition is represented by a shaded bar. Error bars represent the variance in substitution rate across each partition. The y-axis represents substitution rate *(R),* the ratio of expected number of transitions to transversions if one observes the substitution process over time.

Table 3.1. Isolates sequenced for this study

Class	Order	Family	Species	CBS number	Isolate	ITS	*β-tubulin*	*tef1-α*	FG1093	MS204
Dothideomycetes										
	Capnodiales	Davidiellaceae	*Cladosporium sphaerospermum*	CBS 109788	CBS 109788	NS	NS	NS	JF319012	JF319039
	Dothideales	Dothioraceae	*Aureobasidium pullulans var. pullulans*	CBS 584.75	CBS 584.75	FJ150906*	FJ157869.1*	FJ157895.1*	JF319016	JF319057
	Pleosporales	Pleosporaceae	*Alternaria alternata*	CBS 916.96	CBS 916.96	FJ196306*	NS	NS	JF319017	JF319058
Eurotiomycetes	Eurotiales									
		Trichocomaceae	*Byssochlamys spectabilis*	CBS 102.74	CBS 102.74	EU037055.1*	EU037073.1*	NS	JF319011	JF319055
		Trichocomaceae	*Penicillium chrysogenum*	CBS 306.48	CBS 306.48	AY213669.1*	AY495981.1*	NS	JF319014	JF319056
Leotiomycetes	Helotiales	Dermateaceae	*Mollisia cinerea*	CBS 128349	DMW 102.2	JF514855	NS	NS	JF319018	PF
Saccharomycetes	Saccharomycetales	Saccharomycetaceae	*Saccharomyces cerevisiae var cerevisiae*	CBS 8803	CBS 8803	NS	NS	NS	PF	PF
Sordariomycetes	Boliniales	Boliniaceae	*Camarops pugillus*	CBS 128346	AR 4686	NS	NS	NS	JF319008	JF319048
	Chaetosphaerales	Chaetosphaeriaceae	*Chaetosphaeria innumera*	NA	GJS 94-119	NS	NS	NS	JF319022	JF319059
		Chaetosphaeriaceae	*Melanochaeta hemipsila*	NA	AR 4690	NS	NS	NS	JF319010	JF319050
	Diaporthales	Cryphonectriaceae	*Cryphonectria nitschkei*	CBS 109776	AR 3433	DQ120761.1*	DQ120768*	NS	JF319002	JF319042
		Diaporthaceae	*Diaporthe eres*	CBS 109767	AR 3538	DQ491514*	NS	DQ479931*	JF319006	JF319046
		Gnomoniaceae	*Gnomoniopsis alderdunensis*	CBS 125680	DMW 72.1	GU320825*	GU320787*	GU320801*	JF274653	JF319036
			Gnomoniopsis chamaemori	CBS 804.79	CBS 804.79	GU320817*	GU320777*	GU320809*	JF274646	JF319029
			Gnomoniopsis clavulata	CBS 121255	MS 0397	EU254818*	EU219211*	GU320807*	JF274644	JF319027
			Gnomoniopsis comari	CBS 806.79	CBS 806.79	EU254821*	EU219156*	GU320810*	JF274647	JF319030
			Gnomoniopsis fructicola	CBS 208.34	CBS 208.34	EU254826*	EU219149*	GU320808*	JF274645	JF319028
			Gnomoniopsis idaeicola	CBS 125676	DMW 98.2	GU320821*	GU320784*	GU320811*	JF274654	JF319037
			Gnomoniopsis macounii	CBS 121468	AR 3866	EU254762*	EU219126*	GU320804*	JF274641	JF319024
			Gnomoniopsis occulta	CBS 125678	DMW 206.1	GU320829*	GU320786*	GU320800*	JF274650	JF319033
			Gnomoniopsis paraclavulata	CBS 123202	AR 4127	GU320830*	GU320775*	GU320815*	JF274642	JF319025
			Gnomoniopsis sanguisorbae	CBS 858.79	CBS 858.79	GU320818*	GU320790*	GU320805*	JF274648	JF319031

Order	Family	Species							
		Gnomoniopsis tormentillae	CBS 904.79	CBS 904.79	EU254856*	EU219165*	GU320795*	JF274649	JF319032
		Ophiognomonia alni-viridis	CBS 128358	LCM 494	JF514848	JF514841	JF514826	JF319072	JF319085
		Ophiognomonia balsamiferae	CBS 121266	AR 4320	EU254870.1*	EU219221.1*	JF514827	JF319064	JF319077
		Ophiognomonia gei-montani	CBS 821.79	CBS 821.79	EU254871*	JF514837	JF514828	JF319065	JF319078
		Ophiognomonia intermedia	CBS 119188	AR 4147	EU254875.1*	JF514835	JF514825	JF319061	JF319074
		Ophiognomonia ischnostyla	CBS 837.79	CBS 837.79	EU254890.1*	EU219161.1*	JF514821	JF319066	JF319079
		Ophiognomonia melanostyla	CBS 128482	LCM 389.01	JF514849	JF514840	JF514830	JF319071	JF319084
		Ophiognomonia sp.	CBS 121252	AR 4295	EU254901.1*	EU219205.1*	JF514820	JF319063	JF319076
		Ophiognomonia padicola	CBS 845.79	CBS 845.79	JF514845	EU219179.1*	JF514832	JF319067	JF319080
		Ophiognomonia pseudoclavulata	CBS 121236	AR 4059	EU254923.1*	EU219183.1*	JF514819	JF319060	JF319073
		Ophiognomonia rosae	CBS 128442	DMW 108.2	JF514851	JF514842	JF514824	JF319068	JF319081
		Ophiognomonia sassafras	CBS 121243	AR 4284	EU254941.1*	EU219197.1*	JF514829	JF319062	JF319075
		Ophiognomonia setacea	CBS 128352	DMW 291.1	JF514846	JF514838	JF514822	JF319069	JF319082
		Ophiognomonia setacea	CBS 128354	DMW 310.1	JF514847	JF514839	JF514823	JF274652	JF319035
		Ophiognomonia vasiljevae	CBS 128353	DMW 303.3	JF514850	JF514843	JF514831	JF319070	JF319083
		Plagiostoma sp.	CBS 128351	DMW 286.1	JF514852	JF514836	JF514833	JF274651	JF319034
		Sirococcus conigenus	CBS 113.75	CBS 113.75	EF512482*	EU219129*	EF512544*	JF274643	JF319026
		Sirococcus tsugae	CBS 128356	JB11.10.09.06	JF514853	JF514844	JF514834	JF274655	JF319038
	Melanconidaceae	*Melanconis stilbostoma*	CBS 109778	AR 3501	NG013198.1*	EU219104.1*	DQ862039*	JF319004	JF319044
	Schizoparmaceae	*Pilideiella castaneicola*	CBS 143.97	CBS 143.97	NS	NS	NS	JF319013	JF319051
	Valsaceae	*Valsa rubi*	CBS 109756	AR 3416	NS	NS	NS	JF319001	JF319041
		Valsa salicis	CBS 109754	AR 3514	NS	NS	DQ862042*	JF319005	JF319045
Hypocreales	Hypocreaceae	*Hypomyces australis*	NA	GJS 01-245	NS	NS	NS	JF319021	JF319054
		Trichoderma reesei	CBS 128355	GJS 00-23	NS	NS	NS	JF319020	JF319053
Melanosporales	Ceratostomataceae	*Melanospora fallax*	CBS 478.75	CBS 478.75	NS	EF469148.1*	EF469068.1*	JF319015	JF319052
		Melanospora zamiae	CBS 128345	AR 4202	NS	NS	NS	JF319007	JF319047

									JF319040
Microascales	Ceratocystidaceae	*Ambrosiella xylebori*	CBS 128348	AR 4689	NS	NS	NS	JF319000	
		Ceratocystis fimbriata	CBS 128347	AR 4688	NS	NS	NS	JF319009	JF319049
Microascales	Incertae sedis	*Sphaeronaemella fragariae*	CBS 128350	DMW 223.1	JF514854	NS	NS	JF319019	PF
Sordariales	Lasiosphaeriaceae	*Lasiosphaeria lanuginosa*	CBS 128441	AR 4683	NS	NS	NS	PF	PF
Xylariales	Diatrypaceae	*Diatrype virescens*	CBS 128344	AR 3482	NS	NS	NS	JF319003	JF319043
	Xylariaceae	*Xylaria enteroleuca*	CBS 128357	LCM 204	NS	NS	NS	JF319023	PF

Table 3.1. Isolates sequenced for this study. CBS = Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; NS = sequence not necessary for this study; * = GenBank number from an alternate study; PF = PCR failure.

Table 3.2. Primers designed for current study

Locus	Specificity	Protocol	Primer	Sequence 5 - 3'	Location in *Neurospora crassa* genome according to BROAD institute	
FG1093	Universal primers for the Ascomycota	PCR Rxn: E1F1–E3R1. Thermocycler conditions: Touchdown PCR 65–55 C with 35 additional rounds with annealing temperature of 59 C. Sequencing rxn with E1F1– E3R1. E2F1 and E2R1 are usually not required.	FG1093 E1F1	GCGCCACAMCAAGWCSCACRC	OR74A (NC10): NCU01966	(1838001-1838022)
			FG1093 E2F1	YCGHTCBCTCCACRWCCAGAA		(1837805-1837826)
			FG1093 E2R1	TTCTGGWYGTGGAGVGARCGR		(1837805-1837826)
			FG1093 E3R1	TTCTBCGCTTGGCCTTCTCRS		(1837055-1837076)
FG1093	Gnomoniaceae specific primers	Identical protocol to universal primers.	FG1093 E1F1a	GCGCCACMACAAGTCGCACGC		(1838001-1838022)
			FG1093 E3R1a	TTCTBCGCTTGGCCTTCTCGG		(1837055-1837076)
MS204	Universal primers for the Ascomycota	PCR Rxn: E1F1–E5R1. Thermocycler conditions: Touchdown PCR 65–55 C with 35 additional rounds with annealing temperature of 62 C. Sequencing rxn with all 4 primers.	MS204 E1F1	AAGGGCACCCTGGAGGGCCAC	OR74A (NC10): NCU05810	(346718-346739)
			MS204 E4F1	CGTYTCYTTCTCYGCCGAYAA		(345752-345773)
			MS204 E4R1	TTRTCGGCRGAGAARGARACG		(345752-345773)
			MS204 E5R1	GATGGTGACGGYGTTGATGTA		(344684-344705)
MS204	Gnomoniaceae specific primers	Identical protocol to universal primers.	MS204 E3F1	GAAGTCTCTGCACGGCCACTC		(346112-346133)
			MS204 E4R1a	CRAARCGGCGGGTGGTGGTGC		(345707-345728)
			MS204 E5R1a	GATGGTGACGGTGTTGATGTA		(344684-344705)
tef-1α	Gnomoniaceae specific primers	Standard conditions according to Walker et al. 2010	EF1 10F	CAGTGCGGAGGTATYGACAA	OR74A (NC10): NCU02003	(1711918-1711938)
			EF1 740F	TGYGCYGTTCTCATCATYGC		(1712461-1712481)
			EF1 740R	GCRATGATGAGAACRGCRCA		(1712461-1712481)
			EF1 1050R	TTCTTCCAGCCCTTGTACCA		(1712751-1712771)

Table 3.2. Primers designed for current study and respective PCR protocols for each gene. Primers with "E#F1" are used in forward reactions; Primers with "E#R1" or "E#R1a" are used in reverse reactions. Location in *Neurospora* genome according to Strain (#): Locus (Location).

Table 3.3. Alignment properties

Genus	Locus	Mafft Algorithm	No. Characters	No. Characters in PAML Analysis	No. variable sites (in %)	No. informative sites (in %)	Locus CI	Locus RI	Substitution saturation at third codon position. *Iss (Iss.c)* Saturation?
Gnomoniopsis	*β-tubulin*	E-INS-i + manual	771	631	350 (45)	218 (28)	0.736	0.62	NA
	tef-1α	FFT-NS-i	1002	749	400 (40)	238 (24)	0.716	0.487	NA
	ITS	FFT-NS-i	524	484	100 (19)	57 (11)	0.762	0.701	NA
	FG1093	FFT-NS-i	434	222	214 (49)	109 (25)	0.78	0.613	0.13 (1.14) No Saturation
	MS204	FFT-NS-i	1197	978	475 (40)	286 (24)	0.766	0.583	0.21 (0.64) No Saturation
Ophiognomonia	*β-tubulin*	FFT-NS-i + manual	741	657	324 (44)	168 (23)	0.748	0.5	NA
	tef-1α	FFT-NS-i + manual	805	631	247 (31)	143 (18)	0.691	0.531	NA
	ITS	FFT-NS-i + manual	507	485	83 (16)	32 (6)	0.792	0.658	NA
	FG1093	FFT-NS-i	397	320	181 (46)	85 (21)	0.779	0.622	0.04 (0.70) No Saturation
	MS204	FFT-NS-i	1223	960	438 (36)	241 (20)	0.744	0.612	0.04 (0.72) No Saturation

Table 3.3. Alignment properties from analyses of individual markers for *Gnomoniopsis* and *Ophiognomonia*. In the Mafft Algorithm column "+ manual" = with manual alignment adjustments. For the test of substitution saturation, if the *Iss* is significantly ($P \leq 0.05$) less than the *Iss.c,* substitution saturation is absent from the alignment (Xia et al. 2003). "NA" = Not Available.

Table 3.4. Summary of support values

Genus	Support value	ITS *β-tubulin tef-1α*	ITS *β-tubulin FG1093*	ITS *β-tubulin MS204*	ITS *tef-1α FG1093*	ITS *tef-1α MS204*	ITS FG1093 MS204	*β-tubulin tef-1α FG1093*	*β-tubulin tef-1α MS204*
Gnomoniopsis	# Branches MP BS ≥ 70%	9	10	9	7	7	9	7	8
	# Branches ML BS ≥ 70%	7	9	9	9	8	9	8	8
	# Branches PP ≥ 95%	9	9	9	8	8	9	8	8
	# trees Bayesian 95% credibility interval	22	6	11	24	28	23	28	24
Ophiognomonia	# Branches MP BS ≥ 70%	9	9	8	6	10	8	7	10
	# Branches ML BS ≥ 70%	6	6	9	7	10	8	8	7
	# Branches PP ≥ 95%	9	9	10	6	10	8	10	8
	# trees Bayesian 95% credibility interval	6	17	9	66	4	67	5	15

Table 3.4. Summary of support values continued

Genus	*β-tubulin* **FG1093 MS204**	*tef-1α* **FG1093 MS204**	**ITS** *β-tubulin tef-1α* **FG1093**	**ITS** *β-tubulin tef-1α* **MS204**	**ITS** *β-tubulin* **FG1093 MS204**	**ITS** *tef-1α* **FG1093 MS204**	*β-tubulin tef-1α* **FG1093 MS204**	**ITS** *β-tubulin tef-1α* **FG1093 MS204**
Gnomoniopsis								
	11	7	8	9	10	8	8	9
	10	9	8	8	10	9	8	8
	11	9	8	9	10	8	8	8
	3	22	31	19	5	31	19	27
Ophiognomonia								
	9	9	8	10	10	10	10	10
	8	9	9	10	9	10	8	9
	8	9	11	10	9	9	9	9
	28	11	2	5	8	6	10	5

Table 3.4. Summary of support values from Maximum Parsimony Boostrap (MP BS), Maximum Likelihood (ML BS), and Bayesian

(PP) analyses. All possible three-, four-, and five-marker combinations were analyzed for *Gnomoniopsis* and *Ophiognomonia.* Shaded

areas represent the three- and four-marker combinations that fully recover the five-marker topology with equivalent or higher support

for branches; ML BS = maximum likelihood bootstrap; MP BS = maximum parsimony bootstrap; PP = Bayesian posterior

probabilities.

Table 3.5. Phylogenetic performance of individual markers

	Gnomoniopsis			*Ophiognomonia*				
	B-tubulin, FG1093, MS204	ITS, *B-tubulin*, FG1093, MS204	Five-marker phylogeny	ITS, *tef-1a*, MS204	ITS, *B-tubulin*, *tef-1a*, FG1093	ITS, *B-tubulin*, *tef-1a*, MS204	ITS, *tef-1a*, FG1093, MS204	Five-marker phylogeny
FG1093	84.4	84.4	84.4	NA	74.7	NA	73	69.3
ITS	NA	92.5	92.5	72.9	71.4	72.2	72.9	74
tef-1α	NA	NA	77.8	75.8	71.2	71.2	75.8	73.8
β-tubulin	85.1	85.1	85.1	NA	76.6	77	NA	73.3
MS204	91.1	91.1	91.1	80.3	NA	85.4	80.3	78.3
Five-marker phylogeny	100	100	100	94.2	87.4	92	94.2	100

Table 3.5. Test of phylogenetic performance of GARLI ML trees from individual markers compared to the three- and four-marker

combinations that fully recover the five-marker topology with equivalent or higher support for branches in *Gnomoniopsis* and

Ophiognomonia. Topological scores (0–100 scale) represent individual marker ability to recover the reference phylogeny from an

optimum 1-to-1 correspondence map of branches and terminal groups (Nye et al., 2005). NA = individual marker was not analyzed since it was missing from the combined marker dataset.

Supplementary Table 3.1. Support values for *Gnomoniopsis* analyses

Genus	Support value	Branch #	ITS *β-tubulin* *tef-1α*	ITS *β-tubulin* FG1093	ITS *β-tubulin* MS204	ITS *tef-1α* FG1093	ITS *tef-1α* MS204	ITS FG1093 MS204	*β-tubulin* *tef-1α* FG1093	*β-tubulin* *tef-1α* MS204
Gnomoniopsis	MP BS		100	100	100	99	100	100	100	100
	ML BS	1	100	100	100	100	100	100	100	100
	PP		100	100	100	100	100	100	100	100
	MP BS		100	100	100	100	100	100	100	100
	ML BS	2	100	100	100	100	100	100	100	100
	PP		100	100	100	100	100	100	100	100
	MP BS		99	99	100	99	100	100	100	100
	ML BS	3	97	98	99	98	99	99	100	100
	PP		100	100	100	100	100	100	100	100
	MP BS		98	79	97	93	97	90	97	99
	ML BS	4	99	98	99	98	99	97	99	99
	PP		100	99	100	99	100	100	100	100
	MP BS		100	100	100	98	100	99	100	100
	ML BS	5	100	100	100	100	99	99	100	100
	PP		100	100	100	100	100	100	100	100
	MP BS		84	93	95	NS	NS	NS	NS	NS
	ML BS	6	NS	77	90	70	NS	NS	NS	NS
	PP		95	99	99	NS	NS	NS	NS	NS
	MP BS		NS	NS	NS	NS	NS	NS	NS	NS
	ML BS	7	NS	NS	NS	NS	NS	NS	NS	NS
	PP		NS	NS	NS	NS	NS	NS	NS	NS
	MP BS		99	98	99	99	98	90	99	99
	ML BS	8	100	99	100	100	99	99	100	100
	PP		100	100	100	100	100	100	100	100

	Branch #								
MP BS		NS	87	83	NS	NS	97	NS	NS
ML BS	9	NS	97	96	91	83	99	93	84
PP		98	100	100	100	99	100	100	99
MP BS		NS	70	NS	NS	NS	NS	NS	NS
ML BS	10	NS	NS	NS	NS	NS	NS	NS	NS
PP		NS	NS	NS	NS	NS	NS	NS	NS
MP BS		100	100	100	100	99	98	99	99
ML BS	11	100	100	100	99	99	99	100	100
PP		100	100	100	100	100	100	100	100
MP BS		INC (81)	NS	NS	NS	NS	76	NS	INC (77)
ML BS	12	NS	NS	NS	NS	NS	86	NS	NS
PP		NS	NS	NS	NS	NS	97	NS	NS
# Branches MP BS ≥ 70%		9	10	9	7	7	9	7	8
# Branches ML BS ≥ 70%		7	9	9	9	8	9	8	8
# Branches PP ≥ 95%		9	9	9	8	8	9	8	8
95% credibility interval		22	6	11	24	28	23	28	24

Supplementary Table 3.1. Support values for *Gnomoniopsis* analyses. Continued

Genus	Support value	Branch #	*β-tubulin* FG1093 MS204	*tef-1α* FG1093 MS204	ITS *β-tubulin tef-1α* FG1093	ITS *β-tubulin tef-1α* MS204	ITS *β-tubulin* FG1093 MS204	ITS *tef-1α* FG1093 MS204	*β-tubulin tef-1α* FG1093 MS204	ITS *β-tubulin tef-1α* FG1093 MS204
Gnomoniopsis	MP BS		100	100	100	100	100	100	100	100
	ML BS	1	100	100	100	100	100	100	100	100
	PP		100	100	100	100	100	100	100	100
	MP BS		100	100	100	100	100	100	100	100
	ML BS	2	100	100	100	100	100	100	100	100
	PP		100	100	100	100	100	100	100	100
	MP BS		100	100	99	100	100	99	100	100
	ML BS	3	100	100	99	99	100	100	100	100
	PP		100	100	100	100	100	100	100	100
	MP BS		95	97	98	99	96	97	99	99
	ML BS	4	98	99	100	99	99	99	99	100

PP		100	100	100	100	100	100	100	100
MP BS		100	99	100	100	100	100	100	100
ML BS	5	100	99	100	100	100	100	100	100
PP		100	100	100	100	100	100	100	100
MP BS		88	NS	76	80	93	NS	NS	75
ML BS	6	NS	92	NS	NS	82	71	NS	NS
PP		NS	99	NS	97	99	NS	NS	NS
MP BS		85	NS	NS	NS	NS	NS	NS	NS
ML BS	7	86	NS	NS	NS	NS	NS	NS	NS
PP		98	NS	NS	NS	NS	NS	NS	NS
MP BS		98	98	99	100	99	99	99	99
ML BS	8	99	99	100	100	100	100	100	100
PP		100	100	100	100	100	100	100	100
MP BS		96	NS	NS	NS	97	80	73	85
ML BS	9	99	97	94	91	99	98	99	99
PP		100	100	100	100	100	100	100	100
MP BS		83	NS	NS	NS	71	NS	NS	NS
ML BS	10	85	NS	NS	NS	NS	NS	NS	NS
PP		99	NS	NS	NS	NS	NS	NS	NS
MP BS		99	99	100	100	100	100	99	100
ML BS	11	100	99	100	100	100	99	100	100
PP		100	100	100	100	100	100	100	100
MP BS		NS	NS	NS	INC (71)	NS	NS	NS	NS
ML BS	12	NS	NS	NS	NS	74	NS	NS	NS
PP		95	NS	NS	NS	97	NS	NS	NS
# Branches MP BS ≥ 70%		11	7	8	9	10	8	8	9
# Branches ML BS ≥ 70%		10	9	8	8	10	9	8	8
# Branches PP ≥ 95%		11	9	8	9	10	8	8	8
95% credibility interval		3	22	31	19	5	31	19	27

Supplementary Table 3.1. Support values from Maximum Parsimony Boostrap (MP BS), Maximum Likelihood (ML BS), and Bayesian (PP) analyses of *Gnomoniopsis*. INC = incongruence relative to other two analyses for respective branch and dataset; NS = no support; ML BS = maximum likelihood bootstrap; MP BS = maximum parsimony bootstrap; PP = Bayesian posterior probabilities; Branch # corresponds to values in Fig. 3.2.

Supplementary Table 2. Model Test Results

Genus	Align	Model	*base	**rmat	shape	pinvar
Gnomoniopsis	ITS	TrN+I+G	0.2589, 0.2300, 0.2111, 0.3000	1.0000, 1.6883, 1.0000, 1.0000, 4.3444, 1.0000	0.6871	0.5854
	β-tubulin	HKY+I+G	0.2015, 0.3097, 0.2383, 0.2505	Ti/tv ratio = 1.6207	4.7717	0.3807
	tef-1α	GTR+I+G	0.2106, 0.3037, 0.2294, 0.2563	1.4855, 3.5604, 1.8655, 0.9981, 5.0659, 1.0000	1.2999	0.3904
	FG1093	GTR+I+G	0.2816, 0.3018, 0.2534, 0.1632	0.7777, 2.2169, 1.4174, 0.5573, 3.4136, 1.0000	2.8389	0.2753
	MS204	GTR+I+G	0.2147, 0.3234, 0.2342, 0.2277	0.7936, 3.4787, 1.3505, 0.5892, 4.4206, 1.0000	1.8665	0.4666
	ITS *β-tubulin* *tef-1α*	GTR+I+G	0.2129, 0.2942, 0.2308, 0.2621	1.2457, 3.0912, 1.3455, 0.9173, 3.8924, 1.0000	1.6296	0.4328
	ITS *β-tubulin* FG1093	TrN+I+G	0.2376, 0.2865, 0.2321, 0.2439	1.0000, 2.7966, 1.0000, 1.0000, 3.2810, 1.0000	2.3605	0.423
	ITS *β-tubulin* MS204	GTR+I+G	0.2164, 0.3020, 0.2329, 0.2488	0.9430, 3.0072, 1.1457, 0.7323, 3.6266, 1.0000	1.9161	0.4624
	ITS *tef-1α* FG1093	GTR+I+G	0.2384, 0.2832, 0.2320, 0.2464	1.3088, 2.9723, 1.4759, 0.9254, 4.4876, 1.0000	1.2994	0.4112

ITS *tef-1α* MS204	GTR+I+G	0.2219, 0.2969, 0.2294, 0.2519	1.1519, 3.1821, 1.4731, 0.8198, 4.7760, 1.0000	1.1372	0.4377
ITS FG1093 MS204	GTR+I+G	0.2379, 0.2969, 0.2338, 0.2314	0.9622, 2.9982, 1.3088, 0.6832, 4.0744, 1.0000	1.6612	0.4556
β-tubulin *tef-1α* FG1093	GTR+I+G	0.2182, 0.3086, 0.2374, 0.2358	1.1342, 3.2065, 1.4326, 0.8413, 3.7761, 1.0000	2.4085	0.3726
β-tubulin *tef-1α* MS204	GTR+I+G	0.2078, 0.3145, 0.2346, 0.2431	1.0514, 3.3904, 1.4257, 0.7799, 4.0268, 1.0000	1.9966	0.4201
β-tubulin FG1093 MS204	TVM+I+G	0.2163, 0.3194, 0.2363, 0.2280	0.9076, 3.3469, 1.2558, 0.6863, 3.3469, 1.0000	3.2128	0.4152
tef-1α FG1093 MS204	GTR+I+G	0.2248, 0.3101, 0.2374, 0.2277	1.0475, 3.3263, 1.5788, 0.7383, 4.5502, 1.0000	1.6603	0.4027
ITS *β-tubulin* *tef-1α* FG1093	GTR+I+G	0.2245, 0.2950, 0.2345, 0.2460	1.1644, 2.9878, 1.3235, 0.8672, 3.8021, 1.0000	1.7892	0.4093
ITS *β-tubulin* *tef-1α* MS204	GTR+I+G	0.2146, 0.3026, 0.2322, 0.2506	1.0907, 3.1333, 1.3511, 0.8103, 4.0637, 1.0000	1.6223	0.4391
ITS *β-tubulin* FG1093 MS204	GTR+I+G	0.2253, 0.3027, 0.2357, 0.2362	0.9536, 2.9899, 1.2025, 0.7243, 3.5811, 1.0000	2.213	0.4423

	ITS *tef-1α* FG1093 MS204	GTR+I+G	0.2295, 0.2983, 0.2329, 0.2393	1.1166, 3.1554, 1.4826, 0.7920, 4.5067, 1.0000	1.388	0.4265
	β-tubulin *tef-1α* FG1093 MS204	GTR+I+G	0.2171, 0.3128, 0.2370, 0.2331	1.0326, 3.2326, 1.4199, 0.7624, 3.9640, 1.0000	2.1283	0.4032
	ITS *β-tubulin* *tef-1α* FG1093 MS204	GTR+I+G	0.2219, 0.3025, 0.2345, 0.2411	1.0731, 3.1175, 1.3648, 0.7947, 3.9846, 1.0000	1.771	0.4236
Ophiognomonia	ITS	SYM+I+G	equal frequency	1.8291, 1.8074, 1.3373, 0.3107, 3.8807, 1.0000	0.6389	0.5104
	β-tubulin	HKY+I+G	0.2166, 0.3201, 0.2265, 0.2368	Ti/tv ratio = 1.8493	2.7815	0.3624
	tef-1α	TIM+G	0.2140, 0.3011, 0.2404, 0.2444	1.000, 2.4303, 1.3931, 1.3931, 5.0802, 1.0000	0.2638	0
	FG1093	GTR+G	0.2891, 0.2873, 0.2629, 0.1607	1.1175, 2.7865, 1.6622, 0.6304, 7.3582, 1.0000	0.6252	0
	MS204	TVM+G	0.2252, 0.3215, 0.2294, 0.2239	0.8941, 3.4523, 1.3953, 0.6116, 3.4523, 1.0000	0.3492	0
	ITS *β-tubulin* *tef-1α*	TrN+I+G	0.2282, 0.2931, 0.2304, 0.2483	1.0000, 2.5982, 1.0000, 1.0000, 3.9467, 1.0000	0.6587	0.3091

ITS *β-tubulin* FG1093	GTR+I+G	0.2417, 0.2886, 0.2399, 0.2299	1.4968, 3.7246, 1.4918, 0.9519, 5.1085, 1.0000	0.6292	0.2157
ITS *β-tubulin* MS204	GTR+G	0.2290, 0.3009, 0.2309, 0.2392	1.1228, 3.4578, 1.3403, 0.7799, 3.9573, 1.0000	0.3418	0
ITS *tef-1α* FG1093	GTR+I+G	0.2426, 0.2821, 0.2441, 0.2312	1.4040, 2.9708, 1.6443, 1.1248, 6.0364, 1.0000	0.4409	0.2189
ITS *tef-1α* MS204	GTR+G	0.2287, 0.2986, 0.2352, 0.2375	1.0517, 2.7779, 1.4073, 0.8104, 4.1665, 1.0000	0.2846	0
ITS FG1093 MS204	GTR+I+G	0.2464, 0.2933, 0.2369, 0.2234	1.2068, 3.2329, 1.4414, 0.7374, 4.5890, 1.0000	0.4567	0.1559
β-tubulin *tef-1α* FG1093	GTR+I+G	0.2255, 0.3107, 0.2454, 0.2184	1.1667, 3.3612, 1.5730, 0.9905, 5.2419, 1.0000	1.0317	0.3161
β-tubulin *tef-1α* MS204	GTR+I+G	0.2176, 0.3160, 0.2372, 0.2291	1.0116, 3.2913, 1.4868, 0.8384, 4.2279, 1.0000	1.1201	0.3649
β-tubulin FG1093 MS204	GTR+G	0.2325, 0.3135, 0.2386, 0.2154	1.0813, 3.5770, 1.4559, 0.7786, 4.5470, 1.0000	0.4296	0
tef-1α FG1093 MS204	GTR+G	0.2336, 0.3102, 0.2418, 0.2145	1.0249, 3.0538, 1.5722, 0.8173, 4.8599, 1.0000	0.354	0

		*base	**rmat	shape	pinvar
ITS *β-tubulin* *tef-1α* FG1093	GTR+G	0.2325, 0.2950, 0.2400, 0.2325	1.2853, 3.2885, 1.4612, 1.0529, 5.1093, 1.0000	0.3377	0
ITS *β-tubulin* *tef-1α* MS204	GTR+G	0.2247, 0.3033, 0.2342, 0.2378	1.0815, 3.1349, 1.3778, 0.8687, 4.2034, 1.0000	0.3228	0
ITS *β-tubulin* FG1093 MS204	GTR+G	0.2368, 0.3002, 0.2358, 0.2271	1.1828, 3.4943, 1.4035, 0.8046, 4.4667, 1.0000	0.3684	0
ITS *tef-1α* FG1093 MS204	GTR+G	0.2376, 0.2979, 0.2388, 0.2258	1.1304, 2.9685, 1.4726, 0.8374, 4.6950, 1.0000	0.3143	0
β-tubulin *tef-1α* FG1093 MS204	GTR+G	0.2276, 0.3123, 0.2398, 0.2203	1.0641, 3.2983, 1.4851, 0.8568, 4.6550, 1.0000	0.3862	0
ITS *β-tubulin* *tef-1α* FG1093 MS204	GTR+G	0.2317, 0.3023, 0.2373, 0.2287	1.1356, 3.2181, 1.4222, 0.8779, 4.5714, 1.0000	0.3441	0

Supplementary Table 3.2. ModelTest Results. *base = A,C,G,T; **rmat = [A-C], [A-G], [A,T], [C-G], [C-T], [G-T]; shape = gamma distribution shape parameter; pinvar = proportion of invariant sites.

Supplementary Table 3.3. Summary of evaluated gene regions

Gene ID according to FUNYBASE	Locus ID from *Neurospora crassa* genome according to BROAD institute	Gene name	Comment	Primer	Sequence 5-3'
FG1073	NCU 09896.2	Adenylyl-sulfate kinase.	Rejected, one short intron.	NA	NA
FG1084	NCU 08963.2	60S ribosomal protein L30.	Rejected, lack of intron synteny.	NA	NA
FG1145	NCU 10498.5	60S ribosomal protein L35.	Rejected, one short intron.	NA	NA
FG1211	NCU 08627.2	40S ribosomal protein S21.	Rejected, one short intron.	NA	NA
FG1213	NCU 00706.2	60S ribosomal protein L44.	Rejected, lack of intron synteny.	NA	NA
FG1222	NCU 02998.2	Nicotinate-nucleotide pyrophosphorylase.	Rejected, lacks introns entirely.	NA	NA
FG1304	NCU 08333.2	Bleomycin hydrolase.	Rejected, one short intron.	NA	NA
FG1406	NCU 06702.2	yop-1.	Rejected, lack of intron synteny.	NA	NA
FG1841	NCU 06429.2	Alpha-actinin	Rejected, lack of intron synteny.	NA	NA
FG1850	NCU 09864.2	2-oxoisovalerate dehydrogenase alpha subunit.	Rejected, one short intron.	NA	NA
MS230	NCU 02546.2	Nucleolar GTP-binding protein 2.	Rejected, due to intron length.	NA	NA
MS255	NCU 03806.5	Ribosomal protein L27a.e.	Rejected, lack of intron synteny.	NA	NA
MS264	NCU 00566.5	Synaptobrevin.	Rejected, due to intron length.	NA	NA

FG379	NCU 03302.5	60S ribosomal protein L36.	Rejected, lack of primer binding sites.	NA	NA
FG1077	NCU 09880.5	Small nuclear ribonucleoprotein SmG.	Primer faliure, no template amplification.	FG1077 F1	TCCTCARCCNGARCTNAARAA
				FG1077 R1	CTTCTCWCCRCCGNCYTTCTC
FG1093	NCU 01966	60S ribosomal protein L37.	See manuscript.	FG1093 E1F1	GCGCCACAMCAAGWCSCACRC
				FG1093 E2F1	YCGHTCBCTCCACRWCCAGAA
				FG1093 E2R1	TTCTGGWYGTGGAGVGARCGR
				FG1093 E3R1	TTCTBCGCTTGGCCTTCTCRS
				FG1093 E1F1a	GCGCCACMACAAGTCGCACGC
				FG1093 E3R1a	TTCTBCGCTTGGCCTTCTCGG
FG1281	NCU 03610.2	NEDD8-conjugating enzyme UBC12.	Rejected, lack of primer binding sites.	NA	NA
FG1380	NCU 06201.5	Cell wall biogenesis protein Ecm15.	Primer faliure, no template amplification.	FG1380 E2F1	VGHCTCMGTVKCVAADGARGT
				FG1380 E3F1	AGARGGHWVCTGGGAYGADGT
				FG1380 E3R1	TGACHTCRTCCCAGBWDCCYT
				FG1380 E4R1	TGTCCTCWYKKGTRTGCTTCT
FG1552	NCU 02472.2	NADH-ubiquinone oxidoreductase 20.8 kd subunit.	Rejected, lack of primer binding sites.	NA	NA
FG1565	NCU 11173.5	Thiamine-phosphate pyrophosphorylase.	Some template amplification, but excessive primer failure in test group.	FG1565 E1F1	YMTTGGDCAGGATGAYATGRG
				FG1565 E3F1	TGCARAACTTCGCVGCCAAYG
				FG1565 E3R1	CRTTGGCBGCGAAGTTYTGCA
				FG1565 E4R1	CDCCYTCATTGCCYTTGATBA
				FG1565 E4R1a	CGCCTTCATTGCCTTTGATGA
				FG1565 E3F1a	TTCAAAACTTYGCDGCYAACG
				FG1565 E3R1a	CGTTRGCHGCRAAGTTTTGAA
				FG1565 E1F1a	YMTTGGGCAGGATGAYATGGG
				FG1565 E3R1b	CGTTRGCDGCRAARTTTTGMA
				FG1565 E1F1b	YMTTGGGCAGGATGACATGGG
MS204	NCU 05810.5	Cross pathway control-2, guanine nucleotide-binding protein subunit beta-like protein.	See manuscript.	MS204 E1F1	AAGGGCACCCTGGAGGGCCAC
				MS204 E4F1	CGTYTCYTTCTCYGCCGAYAA
				MS204 E4R1	TTRTCGGCRGAGAARGARACG
				MS204 E5R1	GATGGTGACGGYGTTGATGTA

				MS204 E3F1	GAAGTCTCTGCACGGCCACTC
				MS204 E4R1a	CRAARCGGCGGGTGGTGGTGC
				MS204 E5R1a	GATGGTGACGGTGTTGATGTA
				MS204 E5R1b	TGGTGACGGTGTTGATGTAGC
MS270	NCU 06482.5	Pyruvate dehydrogenase E1 component alpha subunit.	Rejected, lack of primer binding sites.	NA	NA
MS272	NCU 00081.5	DNA topoisomerase 3-beta.	Rejected, lack of primer binding sites.	NA	NA
MS2701	NCU 04807.5	Universal stress protein family domain-containing protein.	Rejected, lack of primer binding sites.	NA	NA
MS2705	NCU 01760.5	Pumilio-family RNA binding repeat protein.	Rejected, lack of primer binding sites.	NA	NA

Supplementary Table 3.3. Summary of all evaluated genes regions for molecular marker development. NA = Not Available;

"Comment" column indicates the reason why each gene region was pursued/rejected. The shaded region indicates gene regions where

primer design was attempted or completed.

LITERATURE CITED

Aguileta, G., Marthey, S., Chiapello, H., Lebrun, M.-H., Rodolphe, F., Fournier, E., Gendrault-Jacquemard, A., Giraud, T., 2008. Assessing the performance of single-copy genes for recovering robust phylogenies. Syst. Biol. 57, 613–627.

Altschul, S.F., Madden, T.L., Schaffer, A.A., Zhang, J., Zhang, Z., Miller, W., Lipman, D.J., 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. 25, 3389–3402.

Bazinet, A.L., Myers, D.S., Fuetsch, J., Cummings, M.P., 2007. Grid Services Base Library: A high-level, procedural application programming interface for writing Globus-based grid services. Future Gener. Comp. Sy. 23, 517–522.

Bazinet, A.L., Cummings, M.P., 2008. The Lattice Project: a grid research and production environment combining multiple grid computing models. In: Distributed & Grid Computing - Science Made Transparent for Everyone. Principles, Applications, and Supporting Communities. Rechenkraft.net, Marburg.

Bolay, A., 1971. Contribution a la connaissance de *Gnomonia comari* Karsten (syn. *G. fructicola* [Arnaud] Fall). Etude taxonomique, phytopathologiqueet recherches sur sa croissance in vitro. Berichte der Schweizerischen Botanischen Gesellschaft 81, 398–482.

Buckley, T.R., Arensburger, P., Simon, C., Chambers, G.K., 2002. Combined data, Bayesian phylogenetics, and the origin of the New Zealand cicada genera. Syst. Biol. 51, 4–18.

Chen, W.-J., Miya, M., Saitoh, K., Mayden, R.L., 2008. Phylogenetic utility of two existing and four novel nuclear gene loci in reconstructing Tree of Life of ray-finned fishes: The order Cypriniformes (Ostariophysi) as a case study. Gene 423, 125–134.

Collins, T.M., Fedrigo, O., Naylor, G.J.P., 2005. Choosing the best genes for the job: The case for stationary genes in genome-scale phylogenetics. Syst. Biol. 54, 493–500.

Corradi, N., Hijri, M., Fumagalli, L., Sanders, I.R., 2004a. Arbuscular mycorrhizal fungi (Glomeromycota) harbour ancient fungal tubulin genes that resemble those of the chytrids (Chytridiomycota). Fungal Genet. Biol. 41, 1037–1045.

Corradi, N., Kuhn, G., Sanders, I.R., 2004b. Monophyly of [beta]-tubulin and H+-ATPase gene variants in *Glomus intraradices*: consequences for molecular evolutionary studies of AM fungal genes. Fungal Genet. Biol. 41, 262–273.

Crouch, J.A., Clarke, B.B., Hillman, B.I., 2009. What is the value of ITS sequence data in *Colletotrichum* systematics and species diagnosis? A case study using the falcate-spored, graminicolous *Colletotrichum* group. Mycologia 101, 648–656.

Cummings, M.P., Huskamp, J.C., 2005. Grid computing. Educause Review 40, 116–117.

Cummings, M.P., Neel, M.C., Shaw, K.L., 2008. A Genealogical Approach to Quantifying Lineage Divergenece. Evolution 62, 2411–2422.

Damm, U., Crous, P.W., Fourie, P.H., 2007. Botryosphaeriaceae as potential pathogens of *Prunus* species in South Africa, with descriptions of *Diplodia africana* and *Lasiodiplodia plurivora* sp. nov. Mycologia 99, 664–680.

Fitzpatrick, D.A., Logue, M.E., Stajich, J.E., Butler, G., 2006. A fungal phylogeny based on 42 complete genomes derived from supertree and combined gene analysis. BMC Evol. Biol. 6, 99.

Green, S., 2004. Fungi associated with shoots of silver birch (*Betula pendula*) in Scotland. Mycol. Res. 108, 1327–1336.

Green, S., Castlebury, L.A., 2007. Connection of *Gnomonia intermedia* to *Discula betulina* and its relationship to other taxa in Gnomoniaceae. Mycol. Res. 111, 62–69.

Hedtke, S.M., Townsend, T.M., Hillis, D.M., 2006. Resolution of phylogenetic conflict in large data sets by increased taxon sampling. Syst. Biol. 55, 522–529.

James, T.Y., Kauff, F., Schoch, C.L., Matheny, P.B., Hofstetter, V., Cox, C.J., Celio, G., Gueidan, C., Fraker, E., Miadlikowska, J., Lumbsch, H.T., Rauhut, A., Reeb, V., Arnold, A.E., Amtoft, A., Stajich, J.E., Hosaka, K., Sung, G.-H., Johnson, D., O'Rourke, B., Crockett, M., Binder, M., Curtis, J.M., Slot, J.C., Wang, Z., Wilson, A.W., Schuszler, A., Longcore, J.E., O'Donnell, K., Mozley-Standridge, S., Porter, D., Letcher, P.M., Powell, M.J., Taylor, J.W., White, M.M., Griffith, G.W., Davies, D.R., Humber, R.A., Morton, J.B., Sugiyama, J., Rossman, A.Y., Rogers, J.D., Pfister, D.H., Hewitt, D., Hansen, K., Hambleton, S., Shoemaker, R.A., Kohlmeyer, J., Volkmann-Kohlmeyer, B., Spotts, R.A., Serdani, M., Crous, P.W., Hughes, K.W., Matsuura, K., Langer, E., Langer, G., Untereiner, W.A., Lucking, R., Budel, B., Geiser, D.M., Aptroot, A., Diederich, P., Schmitt, I., Schultz, M., Yahr, R., Hibbett, D.S., Lutzoni, F., McLaughlin, D.J., Spatafora, J.W., Vilgalys, R., 2006. Reconstructing the early evolution of Fungi using a six-gene phylogeny. Nature 443, 818–822.

James, T.Y., Kauff, F., Schoch, C.L., Matheny, P.B., Hofstetter, V., Cox, C.J., Celio, G., Gueidan, C., Fraker, E., Miadlikowska, J., Lumbsch, H.T., Rauhut, A., Reeb, V., Arnold, A.E., Amtoft, A., Stajich, J.E., Hosaka, K., Sung, G.-H., Johnson, D., O'Rourke, B., Crockett, M., Binder, M., Curtis, J.M., Slot, J.C., Wang, Z., Wilson, A.W., Schuszler, A., Longcore, J.E., O'Donnell, K., Mozley-Standridge, S., Porter, D., Letcher, P.M., Powell, M.J., Taylor, J.W., White, M.M., Griffith, G.W., Davies, D.R., Humber, R.A., Morton, J.B., Sugiyama, J., Rossman, A.Y., Rogers, J.D., Pfister, D.H., Hewitt, D., Hansen, K., Hambleton, S., Shoemaker, R.A., Kohlmeyer, J., Volkmann-Kohlmeyer, B., Spotts, R.A., Serdani, M., Crous, P.W., Hughes, K.W., Matsuura, K., Langer, E., Langer, G., Untereiner, W.A., Lucking, R., Budel, B., Geiser, D.M., Aptroot, A., Diederich, P.,

Schmitt, I., Schultz, M., Yahr, R., Hibbett, D.S., Lutzoni, F., McLaughlin, D.J., Spatafora, J.W., Vilgalys, R., 2006. Reconstructing the early evolution of Fungi using a six-gene phylogeny. Nature 443, 818-822.

Johnson, L.A., Soltis, D.E., 1998. Assessing congruence: empirical examples from molecular data. In: Soltis, D.E., Soltis, P.S., Doyle, J.J. (Eds.), Molecular Systematics of Plants 2. Kluwer, U.S.A., pp. 287–348.

Juhasova, G., Ivanova, H., Spisak, J., 2006. Biology of fungus *Gnomonia leptostyla* in agro-ecological environments of Slovakia. Mikologica Fitopatologie 40, 538–547.

Kellogg, E.A., Appels, R., Mason-Gamer, R.J., 1996. When genes tell different stories: the diploid genera of Triticeae (Graminae). Syst. Bot. 21, 321–347.

Ko, K.S., Jung, H.S., 2002. Three nonorthologous ITS1 types are present in a polypore fungus *Trichaptum abietinum*. Mol. Phylogenet. Evol. 23, 112–122.

Kuramae, E.E., Robert, V., Snel, B., Weiß, M., Boekhout, T., 2006. Phylogenomics reveal a robust fungal tree of life. FEMS Yeast Res. 6, 1213–1220.

Landvik, S., Eriksson, O.E., Berbee, M.L., 2001. *Neolecta* - a fungal dinosaur? Evidence from beta-tubulin amino acid sequences. Mycologia 93, 1151–1163.

Letcher, P.M., Powell, M.J., Viusent, M.C., 2008. Rediscovery of an unusual chytridiaceous fungus new to the order Rhizophydiales. Mycologia 100, 325–334.

Li, H., Coghlan, A., Ruan, J., Coin, L.J., Heriche, J.-K., Osmotherly, L., Li, R., Liu, T., Zhang, Z., Bolund, L., Wong, G.K.-S., Zheng, W., Dehal, P., Wang, J., Durbin, R., 2006. TreeFam: a curated database of phylogenetic trees of animal gene families. Nucleic Acids Res. 34, 572–580.

Lopez-Giraldez, F., Townsend, J.P., 2011. PhyDesign: a webapp for profiling phylogenetic informativeness. BMC Evol. Biol. 11, 152.

Maas, J.L., 1998. Compendium of strawberry diseases. 2nd edn. The American Phytopathological Society. U.S.A.

Marthey, S., Aguileta, G., Rodolphe, F., Gendrault, A., Giraud, T., Fournier, E., Lopez-Villavicencio, M., Gautier, A., Lebrun, M.-H., Chiapello, H., 2008. FUNYBASE: a FUNgal phYlogenomic dataBASE. BMC Bioinformatics 9, 456.

Mason-Gamer, R.J., Kellogg, E.A., 1996. Testing for phylogenetic conflict among molecular data sets in the tribe Triticeae. Syst. Biol. 45, 524–545.

McLaughlin, D.J., Hibbett, D.S., Lutzoni, F., Spatafora, J.W., Vilgalys, R., 2009. The search for the fungal tree of life. Trends Microbiol. 17, 488–497.

Mejía, L.C., Rossman, A.Y., Castlebury, L.A., White, J.F., Jr., 2011. New species, phylogeny, host-associations, and geographic distribution of the genus *Cryptosporella* (Gnomoniaceae, Diaporthales). Mycologia 103, 379–399.

Monod, M., 1983. Monographie taxonomique des Gnomoniaceae (Ascomycétes de l'ordre des Diaporthales I). Beihefte zur Sydowia 9, 1–315.

Myers, D.S., Bazinet, A.L., Cummings, M.P., 2008. Expanding the reach of grid computing: combining Globus- and BOINC-based systems. In Grids for Bioinformatics and Computational Biology. John Wiley & Sons, New York.

Neely, D., Black, W.M., 1976. Anthracnose of black walnuts in the Midwest. Plant Dis. Rep. 60, 519–521.

Nixon, K.C., Carpenter, J.M., 1993. On outgroups. Cladistics 9, 413–426.

Nye, T.M.W., Liò, P., Gilks, W.R., 2005. A novel algorithm and web-based tool for comparing two alternative phylogenetic trees. Bioinformatics 22, 117–119.

O'Brien, K.P., Remm, M., Sonnhammer, E.L.L., 2005. Inparanoid: a comprehensive database of eukaryotic orthologs. Nucleic Acids Res. 33, 476–480.

O'Donnell, K., Cigelnik, E., 1997. Two divergent intragenomic rDNA ITS2 types within a monophyletic lineage of the fungus *Fusarium* are nonorthologous. Mol. Phylogenet. Evol. 7, 103–116.

O'Donnell, K., Cigelnik, E., Nirenber, H.I., 1998. Molecular systematics and phylogeography of the *Gibberella fujikuroi* species complex. Mycologia 90, 465–473.

Pennycook, S.R., 2007. *Discula betulae* comb. nov., correct name for the anamorph of *Gnomonia intermedia*. Mycotaxon 101, 361–364.

Pond, S.L.K., Frost, S.D.W., Muse, S.V., 2005. HyPhy: hypothesis testing using phylogenies. Bioinformatics 21, 676–679.

Posada, D., Crandall, K.A., 1998. Modeltest: testing the model of DNA substitution. Bioinformatics 14, 817–818.

Raja, H.A., Miller, A.N., Shearer, C.A., 2008. Freshwater ascomycetes: *Aquapoterium pinicola*, a new genus and species of Helotiales (Leotiomycetes) from Florida. Mycologia 100, 141–148.

Raja, H., Schoch, C., Hustad, V., Shearer, C., Miller, A., 2011. Testing the phylogenetic utility of MCM7 in the Ascomycota. MycoKeys 1, 63–94.

Ralser, M., Querfurth, R., Warnatz, H.J., Lehrach, H., Yaspo, M.L., Krobitsch, S., 2006. An efficient and economic enhancer mix for PCR. Biochem. Bioph. Research Communications 347, 747–751.

Rambaut., A., Charleston., M.A., 2002. TreeEdit: phylogenetic tree editor v1.0 alpha 10.

Robbertse, B., Reeves, J.B., Schoch, C.L., Spatafora, J.W., 2006. A phylogenomic analysis of the Ascomycota. Fungal Genet. Biol. 43, 715–725.

Ronquist, F., Huelsenbeck, J.P., 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19, 1572–1574.

Rychlik, W., Rhoads, R.E., 1989. A computer program for choosing optimal oligonudeotides for filter hybridization, sequencing and in vitro amplification of DNA. Nucleic Acids Res. 17, 8543–8551.

Sanderson, M.J., 1997. A nonparametric approach to estimating divergence times in the absence of rate constancy. Mol. Biol. Evol. 14, 1218–1231.

Schmitt, I., Crespo, A., Divakar, P.K., Fankhauser, J.D., Herman-Sackett, E., Kalb, K., Nelsen, M.P., Nelson, N.A., Rivas-Plata, E., Shimp, A.D., Widhelm, T., Lumbsch, H.T., 2009. New primers for promising single-copy genes in fungal phylogenetics and systematics. Persoonia 23, 35–40.

Schoch, C.L., Sung, G.-H., López-Giráldez, F., Townsend, J.P., Miadlikowska, J., Hofstetter, V., Robbertse, B., Matheny, P.B., Kauff, F., Wang, Z., Gueidan, C., Andrie, R.M., Trippe, K., Ciufetti, L.M., Wynns, A., Fraker, E., Hodkinson, B.P., Bonito, G., Groenewald, J.Z., Arzanlou, M., Sybren de Hoog, G., Crous, P.W., Hewitt, D., Pfister, D.H., Peterson, K., Gryzenhout, M., Wingfield, M.J., Aptroot, A., Suh, S.-O., Blackwell, M., Hillis, D.M., Griffith, G.W., Castlebury, L.A., Rossman, A.Y., Lumbsch, H.T., Lücking, R., Büdel, B., Rauhut, A., Diederich, P., Ertz, D., Geiser, D.M., Hosaka, K., Inderbitzin, P., Kohlmeyer, J., Volkmann-Kohlmeyer, B., Mostert, L., O'Donnell, K., Sipman, H., Rogers, J.D., Shoemaker, R.A., Sugiyama, J., Summerbell, R.C., Untereiner, W., Johnston, P.R., Stenroos, S., Zuccaro, A., Dyer, P.S., Crittenden, P.D., Cole, M.S., Hansen, K., Trappe, J.M., Yahr, R., Lutzoni, F., Spatafora, J.W., 2009. The Ascomycota tree of life: A phylum-wide phylogeny clarifies the origin and evolution of fundamental reproductive and ecological traits. Syst. Biol. 58, 224–239.

Sogonov, M.V., Castlebury, L.A., Rossman, A.Y., Mejía, L.C., White, J.F., 2008. Leaf-inhabiting genera of the Gnomoniaceae, Diaporthales. Stud. Mycol. 62, 1–77.

Spatafora, J.W., Sung, G.-H., Johnson, D., Hesse, C., O'Rourke, B., Serdani, M., Spotts, R., Lutzoni, F., Hofstetter, V., Miadlikowska, J., Reeb, V., Gueidan, C., Fraker, E., Lumbsch, T., Lucking, R., Schmitt, I., Hosaka, K., Aptroot, A., Roux, C., Miller, A.N., Geiser, D.M., Hafellner, J., Hestmark, G., Arnold, A.E., Budel, B., Rauhut, A., Hewitt,

D., Untereiner, W.A., Cole, M.S., Scheidegger, C., Schultz, M., Sipman, H., Schoch, C.L., 2006. A five-gene phylogeny of Pezizomycotina. Mycologia 98, 1018–1028.

Swofford, D.L., 2002. PAUP 4.*0b10: Phylogenetic Analysis Using Parsimony*. Sinauer Associates, U.S.A.

Townsend, J.P., 2007. Profiling phylogenetic informativeness. Syst. Biol. 56, 222–231.

Townsend, J.P., Leuenberger, C., 2011. Taxon sampling and the optimal rates of evolution for phylogenetic inference. Syst. Biol. 60, 358–365.

Walker, D.M., Castlebury, L.A., Rossman, A.Y., Sogonov, M.V., White, J.F., Jr., 2010. Systematics of the genus *Gnomoniopsis* (Gnomoniaceae, Diaporthales) based on a three-gene phylogeny, host associations, and morphology. Mycologia 102, 1479–1496.

Waterhouse, R.M., Zdobnov, E.M., Tegenfeldt, F., Li, J., Kriventseva, E.V., 2011. OrthoDB: the hierarchical catalog of eukaryotic orthologs in 2011. Nucleic Acids Res. 39, 283–288.

Wild, A.L., Maddison, D.R., 2008. Evaluating nuclear protein-coding genes for phylogenetic utility in beetles. Mol. Phylogenet. Evol. 48, 877–891.

Willows-Munro, S., Robinson, T.J., Matthee, C.A., 2005. Utility of nuclear DNA intron markers at lower taxonomic levels: Phylogenetic resolution among nine *Tragelaphus* spp. Mol. Phylogenet. Evol. 35, 624–636.

Xia, X., Xie, Z., Salemi, M., Chen, L., Wang, Y., 2003. An index of substitution saturation and its application. Mol. Phylogenet. Evol. 26, 1–7.

Yang, Z., 2007. PAML 4: Phylogenetic analysis by maximum likelihood. Mol. Biol. Evol. 24, 1586–1591.

Zhang, N., Zhao, S., Shen, Q., 2011. A six-gene phylogeny reveals the evolution of mode of infection in the rice blast fungus and allied species. Mycologia. doi: 10.3852/11-022.

Zwickl, D.J., 2006. Genetic algorithm approaches for the phylogenetic analysis of large biological sequence data sets under the maximum likelihood criterion. Ph.D. dissertation. The University of Texas at Austin. 115 p.

Chapter 4

Phylogeny of the genus *Ophiognomonia* (Gnomoniaceae, Diaporthales) based on analyses of three molecular markers, host associations, and morphology[3]

ABSTRACT

Species of *Ophiognomonia* are leaf-inhabiting endophytes, pathogens, and saprobes that infect plants in the families Betulaceae, Fagaceae, Juglandaceae, Lauraceae, Malvaceae, Platanaceae, Rosaceae, Salicaceae, and Sapindaceae. Based on extensive collecting, this species-rich genus is now known to have a world wide distribution in primarily temperate areas, although some species are known from the subtropics. Analyses of DNA sequences from three markers including guanine nucleotide-binding protein subunit beta-like protein (MS204), translation elongation factor 1α (*tef-1α*), and the ITS region including ITS1, 5.8S rDNA and ITS2 regions (ITS) were used to define phylogenetic species in *Ophiognomonia*. Host plant association correlated with these species. Twenty-five new species of *Ophiognomonia* and two new combinations are proposed with descriptions and illustrations. In addition, descriptions and illustrations are provided for 12 other species of *Ophiognomonia*. A key is provided to the 45 currently accepted species of *Ophiognomonia*. The disposition of additional names in *Ophiognomonia* is also discussed.

INTRODUCTION

Fungi in the family Gnomoniaceae (Diaporthales, Sordariomycetes, Ascomycota) are associated with a diverse range of herbaceous plants, shrubs, and trees from over 330 host genera in North America and Europe (Farr, D.F. and Rossman, A.Y. Fungal Databases, Systematic Mycology and Microbiology Laboratory, ARS, USDA. Retrieved September 19, 2011, from http://nt.ars-grin.gov/fungaldatabases/) and function in the environment as endophytes, pathogens, and saprobes. Recently nine genera were recognized in a comprehensive monograph of the Gnomoniaceae (Sogonov et al. 2008). These nine genera were identified on the basis of a three-marker phylogeny based on the 5' region of the large ribosomal subunit (nrLSU) and exons from the translation elongation factor 1-alpha (*tef-1α)* and RNA polymerase II (*rpb2*) genes. Mejía et al. (2011a) increased the number of gnomoniaceous genera to ten by describing the monotypic genus *Occultocarpon,* which occurs on *Alnus nepalensis* in China. Although the modern genera of Gnomoniaceae have been defined by DNA sequence data, other characters such as host association, presence/absence of stroma, and perithecial habit are also important (Sogonov et al. 2008)*.* For example, the genus *Cryptosporella* produces perithecia aggregated in stromata on twigs, whereas the perithecia of *Gnomonia* are solitary and erumpent on overwintered leaves.

The purpose of this study is to document species diversity in the genus *Ophiognomonia* using multiple molecular markers. *Ophiognomonia* has a worldwide distribution, primarily in temperate forests, but with a few species that occur in subtropical regions, and is based on the type species *O. melanostyla* (DC. : Fr.) Berl. found on *Tilia* spp. in temperate forests in USA and Europe. Sogonov et al. (2008)

recognized 17 species in the genus *Ophiognomonia* on host plants in the Betulaceae, Fagaceae, Juglandaceae, Lauraceae, Malvaceae, Platanaceae, Rosaceae, Salicaceae, and Sapindaceae. Historically, knowledge of geographic distribution and host association of species in this genus was limited, especially in Asia and South America. Kobayashi (1970) collected a single species, *O. setacea* as *Gnomonia setacea* on *Quercus* in Japan. Otani (1995) observed *O. leptostyla* on *Juglans* sp. and *O. setacea* on *Castanea* sp. and *Quercus* sp. from Japan. Reports and collections of *Ophiognomonia* from Europe and North America are more common than in Asia, but are still somewhat limited. For example, Barr (1978) accepted a single species, *O. melanostyla* on *Tilia* sp. from Europe and the United States. Monod (1983) described eight additional species distributed throughout Europe and North America.

Species of *Ophiognomonia* cause diseases of economically important hardwood trees, including *O. intermedia* (Rehm) Sogonov with the asexual state *Discula betulae* (Westend.) Pennycook, which causes a foliar disease of birch and dieback of young shoots (Green 2004, Green and Castlebury 2007, Pennycook 2007). Walnut anthracnose and leaf blotch are caused by virulent strains of *Ophiognomonia leptostyla* in the eastern half of the United States, South America, Europe, and Asia (Belisario 2008, Berry 1981, Juhasova et al. 2006, Neely and Black 1976). Disease epidemics caused by *O. leptostyla* are particularly destructive during the rainy and cool seasons in Iran, which is the third highest walnut producer in the world (Behdad 1991, Belisario 2008, Salahi et al. 2009). Perhaps the most devastating member of the genus in North America is the asexually reproducing *O. clavigignenti-juglandacearum* (Nair, Kostichka, & Kuntz) Broders & Boland, which causes butternut canker (*Juglans cinerea* L.) with past reports

documenting 70–90% tree decline in some areas (Anderson and LaMadeleine 1978, Broders and Boland 2011).

Prior to Sogonov et al. (2008) considerable confusion existed about the generic concept of *Ophiognomonia*. *Ophiognomonia melanostyla* was originally described in the genus *Sphaeria* and then transferred to *Cryptoderis*, *Gnomonia,* and *Gnomoniella* before being designated as the type species of *Ophiognomonia* in 1899 (see Sogonov et al. 2008). Many species now in *Ophiognomonia* were scattered amongst various gnomoniaceous genera due to emphasis of differing morphological characters by different authors. For many years considerable importance was placed on the shape and septation of ascospores. For example, Monod (1983) included both *O. rubi-idaei* (M. Monod) Sogonov and *O. trientensis* (M. Monod) Sogonov in *Gnomonia* based on the short, ellipsoidal, one-septate ascospores. Barr (1978) emphasized placement of the perithecial neck thus recognizing *Plagiostoma micromegala* (Ellis & Everh.) M.E. Barr and *Pleuroceras sassafras* (Ellis & Everh.) M.E. Barr, now both included in *Ophiognomonia* (Sogonov et al. 2008).

Within the Gnomoniaceae species are based on the phylogenetic analyses of molecular markers. Host association and morphological characters such as ascospore size and septation can also be useful for species identification. Recent phylogenetic studies have shown that species of Gnomoniaceae often have a narrow host range associating with a single host genus or species (Mejía et al. 2008, 2011a–c, Sogonov et al. 2008, Walker et al. 2010). For example, in the genus *Cryptosporella* nine species are associated with a single host species or subspecies and seven fungal species occur on a single host genus (Mejía et al. 2011b). Mejía et al. (2011b) suggest that the genus *Cryptosporella* has

undergone speciation within the geographic host ranges of Betulaceae, Fagaceae, and

Salicaceae. Walker et al. (2010) used ascospore size, septation, and host association to

supplement phylogenetic recognition of species in the genus *Gnomoniopsis.* Four species

of *Gnomoniopsis* are specific to the host genus *Rubus* and ten additional species associate

with nine other host genera in the Fagaceae, Onagraceae, and Rosaceae.

Based on theory from Avise and Ball (1990), Taylor et al. (2000) coined

genealogical concordance phylogenetic species recognition (GCPSR) as an approach for

defining fungal species based on congruent gene trees. Seven genes in various

combinations have been commonly used for GCPSR of fungi, specifically nuclear large

and small ribosomal subunits, 5.8S ribosomal RNA gene, subunits 1 and 2 of RNA

polymerase II, *tef-1α*, and mitochondrial ATP synthase as well as the nuclear ribosomal

internal transcribed spacer (ITS) regions 1 and 2 (*e.g.,* Damm et al. 2007, Letcher et al.

2008, Mejía et al. 2011b, Raja et al. 2008, Spatafora et al. 2006, Walker et al. 2010). As

fungal genomic data became available, additional molecular markers were added to the

mycologist's toolbox (Aguileta et al. 2008, Schmitt et al. 2009, Walker et al. accepted)

but it can be difficult to evaluate species limits and the contribution and usefulness of the

individual genes in a phylogenetic analysis. More recently the genealogical sorting index

(*gsi*; Cummings et al. 2008) has been used to quantify exclusivity of ancestry of

monophyletic groups. Phylogenetic informativeness profiles incorporate nucleotide

substitution rates over evolutionary time and can assist in marker selection for

phylogenetic questions (Townsend 2007). Walker et al. (accepted) applied phylogenetic

informativeness (Townsend 2007, Townsend and Leuenberger 2011) to assess the

usefulness of five molecular markers including *β-tubulin*, FG1093 (60S ribosomal protein

L37), ITS, MS204 (guanine nucleotide-binding protein subunit beta-like protein), and *tef-1α,* in resolving lower-level relationships in *Ophiognomonia* and determined that concatenation of ITS, MS204, and *tef-1α* accurately represent the topology of the combined five-marker dataset.

DNA sequences from three ribosomal DNA and protein coding molecular markers, namely MS204, *tef-1α*, and ITS are used in this study to determine the species diversity of *Ophiognomonia*. Monophyletic species are evaluated using GCPSR and *gsi* in single and combined-marker genealogies. Twenty-five new species of *Ophiognomonia* and two new combinations are proposed with descriptions and illustrations. In addition, descriptions and illustrations are provided for the 12 combinations included without description in Sogonov et al. (2008). A key is provided to the 45 currently accepted species of *Ophiognomonia.*

METHODS

Morphological observations

Macroscopic and microscopic characters were observed and digital images captured as in Walker et al. (2010). Freshly collected specimens were isolated and grown in culture according to Walker et al. (2010). Freshly collected specimens determined as immature due to lack of ascospore germination in culture were placed in moist chambers. The moist chambers were airtight plastic boxes/bags with moist paper towels lining the bottom surface. They were placed at 4 °C in complete darkness and observed weekly for ascospore maturation and germination in culture. Cultures were deposited at the Centraalbureau voor Schimmelcultures (CBS) in the Netherlands (Table 4.1).

DNA extraction, amplification, and sequencing

Cultures were grown and genomic DNA extracted using the QIAGEN Puregene Core Kit A (QIAGEN Inc., Chatsworth, California) as in Walker et al. (2010). The markers ITS, MS204, and *tef-1α* were selected for analysis based on phylogenetic informativeness test results from Walker et al. (accepted). ITS and *tef-1α* were amplified and sequenced according to Walker et al. (2010) with the addition of four gnomoniaceae-specific *tef-1α* primers designed in Walker et al. (accepted). The marker MS204 was amplified and sequenced as in Walker et al. (accepted).

Sequence data analyses

Raw sequences were edited and assembled into contigs with Sequencher 4.9 for Windows (Gene Codes Corp., Ann Arbor, Michigan). Eight alignments were prepared using the MAFFT v.6 web server (http://mafft.cbrc.jp/alignment/server/) and curated with the Gblocks (Castresana 2000, Talavera and Castresana 2007) web server (http://molevol.cmima.csic.es/castresana/Gblocks_server.html). The alignment strategy for each marker was set at L-INS-i for nucleotide sequences in MAFFT v.6. Manual alignment modifications were performed before running Gblocks with the default parameters. Alignments one, two, and three correspond to the markers ITS, *tef-1α,* and MS204, respectively. Each alignment was composed of DNA sequences for 45 isolates, representing 43 species in *Ophiognomonia* and the outgroup taxa *Ambarignomonia petiolorum* and *Discula destructiva* in the Gnomoniaceae. The three individually aligned sequence markers were concatenated into a single file to form alignment four.

Alignments 5–7 correspond to combined three-marker alignments for three independently

supported clades of species within *Ophiognomonia.* Each marker was aligned

individually as previously mentioned, then concatenated to form a single file for each of

the three clades. Alignment five (clade one) consisted of 39 isolates, representing 15

species in *Ophiognomonia,* and the outgroup taxon *O. longispora*. Alignment six (clade

two) consisted of 25 isolates, representing 11 species in *Ophiognomonia,* and the

outgroup *O. monticola*. Alignment seven (clade three) consisted of 35 isolates,

representing 15 species in *Ophiognomonia,* and the outgroup taxa *O. gei-montani* and *O.*

leptostyla. Alignment eight consisted of ITS sequences from the same 25 isolates in

alignment six, plus four additional ITS sequences representing two species of

Ophiognomonia lacking a culture, for a total of 29 ITS sequences representing 13 species

of *Ophiognomonia* and the outgroup *O. monticola.* All alignments have been deposited in

TreeBase.

Potential conflict among datasets was assessed by comparing the three individual

gene trees across all alignments with a conditional comparison test using maximum

parsimony bootstrap (MPBS) analyses with a cutoff value of ≥70% for a supported clade

(Johnson and Soltis 1998, Kellogg et al. 1996, Mason-Gamer and Kellogg 1996).

Phylogenetic trees were inferred with maximum parsimony (MP), maximum likelihood

(ML), and Bayesian analyses. In all analyses rooting was accomplished with the outgroup

method (Nixon and Carpenter 1993) using results from this study and from Sogonov et

al. (2008). For MP analyses each gene was analyzed individually and then together in a

three-marker combined alignment using PAUP 4.0b10 (Swofford 2002) according to

Walker et al. (2010). The University of Oslo Bioportal (http://www.bioportal.uio.no/) and

The Lattice Project (http://boinc.umiacs.umd.edu) web servers were used for performing

partitioned ML and Bayesian analyses with the programs GARLI v2.0 (Zwickl, 2006)

and MrBayes 3.1.2 (Huelsenbeck and Ronquist 2001, Ronquist and Huelsenbeck 2003)

respectively, with implementation of parameters from Walker et al. (accepted). In order

to reach convergence, the Bayesian analyses of alignments four and eight were run for

20,000,000 generations.

Phylogenetic species recognition was based on three methods, specifically the

genealogical sorting index (gsi; Cummings et al. 2008), genealogical concordance

phylogenetic species recognition (GCPSR; Talyor et al. 2000), and genealogical

nondiscordance (Dettman et al. 2003). The gsi is a standardized method to determine

exclusive ancestry of predefined groups in a tree. It is based on a 0 to 1 continuum with 0

= lack of genealogical divergence from other groups and 1 = monophyly. The gsi statistic

can be used to test hypothesized species lineages measured by coalescent patterns in gene

trees against the null hypothesis of no divergence (Cummings et al. 2008). Alignments 5–

7 (clades 1–3) were independently tested with gsi using 100 trees randomly selected from

the GARLI maximum likelihood bootstrap (MLBS) tree distribution with 10,000

permutations to determine statistical significance (P-value ≤ 0.05) using the gsi web

server (http://www.genealogicalsorting.org/index.php). All 100 gsi measurements from

the MLBS tree distribution were pooled to calculate an ensemble gsi_T statistic for each

marker. The gsi_T statistic is a summary measurement of genealogical exclusivity for a

species lineage across the MLBS tree distribution for a given marker. The results from

the conditional comparison tests were used for GCPSR and genealogical nondiscordance.

Twenty-nine species were evaluated. Since these methods use comparisons of clades

consisting of multiple isolates to represent a phylogenetic species, 16 species represented by a single isolate were excluded from the analyses.

RESULTS

After manual adjustment and curation in Gblocks, alignment one (ITS) consisted of 410 (73%) of the original 556 position dataset with 321 constant, 38 non-parsimony informative, and 51 parsimony informative sites; alignment two (*tef-1α*) consisted of 531 (38%) of the original 1364 position dataset with 392 constant, 39 non-parsimony informative, and 100 parsimony informative sites; and alignment three (MS204) consisted of 810 (65%) of the original 1244 position dataset with 494 constant, 85 non-parsimony informative, and 231 parsimony informative. The combined three-marker alignment consisted of 1751 of the available 3164 positions (55%) with 1207 constant, 162 non-parsimony informative, and 382 parsimony informative sites. A maximum parsimony (MP) heuristic search of the three marker alignment produced 12 equally parsimonious trees with a length of 1596 steps (CI = 0.490, RI = 0.617, RC = 0.302, HI = 0.510). Only ITS sequences were available for *Ophiognomonia gei* and *O. rubi-idaei*. Therefore these species were not included in the combined alignment but were included in alignment eight. Alignment eight (ITS) consisted of 455 (83%) of the original 545 position dataset with 385 constant, 17 non-parsimony informative, and 53 parsimony informative sites. A maximum parsimony (MP) heuristic search produced a single most parsimonious tree with a length of 107 steps (CI = 0.832, RI = 0.933, RC = 0.776, HI = 0.168). Hereafter, alignments four and eight will be referred to as the "combined alignment" and "ITS alignment" respectively.

Based on the results of the combined alignment, three additional datasets containing taxa corresponding to the three identified major clades were prepared to more fully investigate variation in these clades because many potentially informative sites were discarded due to ambiguous alignment in the all-taxa combined alignment. Alignment five (clade one) consisted of 2189 (79%) of the original 2783 position dataset with 1684 constant, 125 non-parsimony informative, and 380 parsimony informative sites. A maximum parsimony (MP) heuristic search produced eight equally parsimonious trees with a length of 829 steps (CI = 0.765, RI = 0.898, RC = 0.687, HI = 0.235). Alignment six (clade two) consisted of 2126 (72%) of the original 2940 position dataset with 1617 constant, 85 non-parsimony informative, and 424 parsimony informative sites. A maximum parsimony (MP) heuristic search produced two equally parsimonious trees with a length of 855 steps (CI = 0.756, RI = 0.879, RC = 0.664, HI = 0.244). Alignment seven (clade three) consisted of 2096 (72%) of the original 2925 position dataset with 1538 constant, 166 non-parsimony informative, and 392 parsimony informative sites. A maximum parsimony (MP) heuristic search produced 30 equally parsimonious trees with a length of 1104 steps (CI = 0.655, RI = 0.770, RC = 0.504, HI = 0.345). Hereafter, alignments 5–7 will be referred to as clades one, two, and three, respectively.

The conditional comparison test showed conflict independently between *tef-1α* vs. ITS and MS204 single-marker trees for placement of a single species, *Ophiognomonia lenticulispora*, which was represented by the single isolate CBS 131363. The single-marker alignments were reduced to one isolate representing each species and analyzed to eliminate taxon sampling as a possible reason for any observed incongruence. The same minor conflict remained independently between *tef-1α* vs. ITS and MS204.

Nucleotide substitution models were determined individually for each marker in all eight alignments (Supplementary Table 4.1). The ML analysis in GARLI v2.0 for the combined analysis resulted in one tree with a -lnL 10931.08 (Fig. 4.1); clade one resulted in one tree with a -lnL 7963.63 (Fig. 4.2); clade two resulted in one tree with a -lnL 7605.96 (Fig. 4.3); clade three resulted in one tree with a -lnL 8916.31 (Fig. 4.4); alignment of ITS sequences corresponding to clade two resulted in one tree with a -lnL 1888.25 (Fig. 4.5).

The ML analyses of the combined alignment and clades 1–3 resolves all included species of *Ophiognomonia* (Figs. 4.1–4.4). Three major clades (100% PP, ML, MP) were supported. Clade one consists of 15 species that occur on the host families Betulaceae, Fagaceae, and Rosaceae (Fig. 4.2). Within clade one, a group of closely related species including *O. asiatica, O. kobayashii, O. otanii, O. setacea,* and *O. sogonovii* occur on *Quercus* spp. and *Castanea* spp. within the Fagaceae (100% PP, 91% ML, < 70% MP). Clade two consists of 11 species of *Ophiognomonia* occurring on the host families Juglandaceae, Lauraceae, Rosaceae, and Malvaceae (Fig. 4.3). One group within clade two (100% PP, ML, 99% MP) containing *O. cordicarpa, O. longispora, O. melanostyla,* and *O. sassafras* shares elongated filiform ascospores (Figs. 4.11, 4.24, 4.40, 4.44), a character not observed among the remaining species of *Ophiognomonia.* Another group (99% PP, 94% ML, 86% MP) within clade two consisting of *O. nipponicae, O. padicola,* and *O. rosae* occurs only on hosts in the Rosaceae. Also within clade two, the species *O. micromegala, O. pseudoclavulata,* and *O. vasiljevae* form a supported group (100% PP, 99% ML, 97% MP) that occurs on hosts in the Juglandaceae, except for *O. lenticulispora,* which was collected on *Prunus* sp. (Fig. 4.3). Clade three contains 15 species of

Ophiognomonia on the host families Betulaceae, Juglandaceae, and Salicaceae. One group of eight species is supported (100% PP, 98% ML, 92% MP) within clade three, including *O. alni-viridis, O. bugabensis, O. ibarakiensis, O. intermedia, O. maximowiczianae, O. multirostrata, O. nana,* and *O. tucumanensis,* which occur on *Alnus* spp. and *Betula* spp.

Twenty-nine of the 45 species of *Ophiognomonia* were tested using the three criteria for GCPSR defined in the methods and were confirmed as distinct evolutionary lineages. The remaining 16 species were represented by a single isolate and could not be subjected to these analyses. Using GCPSR, MS204 supported all 29 species tested (Table 4.2). Analysis of *tef-1α* resulted in strong support for 27 species, excluding *O. hiawathae* and *O. michiganensis.* Only 18 of 29 species were supported in the ITS gene tree using GCPSR. Genealogical nondiscordance was not observed in any of the 29 species of *Ophiognomonia.* In addition, all species were strongly supported in 2/3 or 3/3 marker genealogies, except for *O. hiawathae* and *O. michiganensis,* which were strongly supported by MS204. The *gsi* results for each marker differed, but were in general agreement with GCPSR of species (Table 4.2). The gsi_T range of values for MS204 was 0.5727–1.0 with 27 of 29 species $\geq$ 0.7504. This marker exhibits the highest degree of exclusive ancestry among species for the combination of MLBS trees tested. The gsi_T range of values for *tef-1α* was 0.4782–1.0, with 26 of 29 species $\geq$ 0.7346 (Table 4.2). The gsi_T for *O. hiawathae* was not significant indicating incomplete lineage sorting in the *tef-1α* marker for this species. The tree distribution representing the genealogical history of the ITS region indicated high exclusive ancestry for most but not all species of *Ophiognomonia.* The ITS region showed a diverse range of gsi_T values (Table 4.2;

0.1551–1.0). The *gsi* results for the ITS region were as follows: five species had statistically significant $gsi_T < 0.5$, 21 species with $gsi_T > 0.5$ and three species with non-statistically significant gsi_T values. ITS sequences representing *Ophiognomonia rubi-idaei* show a high statistically significant gsi_T value (0.8194) suggesting that this species is a distinct evolutionary lineage.

DISCUSSION

The genus *Ophiognomonia* is a highly diverse group of fungi with economically significant pathogens of shade, lumber, and nut-producing trees (Anderson and LaMadeleine 1978, Behdad 1991, Belisario 2008, Berry 1981, Broders and Boland 2011, Green 2004, Green and Castlebury 2007, Juhasova et al. 2006, Neely and Black 1976, Pennycook 2007, Salahi et al. 2009). In this study, descriptions and illustrations for 27 new combinations and species and 12 previously recognized species are provided as well as a key to all species of *Ophiognomonia*.

Monod (1983) characterized the genus *Ophiognomonia* as having elongated filiform ascospores with 1–3 septations. Of the eight species recognized by Monod (1983) in *Ophiognomonia, O. padicola,* and *O. sassafras* are confirmed in this genus with molecular data by Sogonov et al. (2008). Many of the species recognized here as members of *Ophiognomonia* were placed in the genus *Gnomonia* by Monod (1983). He characterized species in the genus *Gnomonia* as having asci with 8, rarely 2, 4, or 20–30, ascospores each with a median to slightly submedian septum and appendages. Although included in the genus *Gnomonia* by Monod (1983), the following species were accepted by Sogonov et al. (2008) and confirmed herein as members of the genus *Ophiognomonia:*

O. alni-viridis, O. gei-montani, O. intermedia, O. leptostyla, O. rosae, O. rubi-idaei, O. setacea, and *O. trientensis.* In addition *O. micromegala* was placed in the genus *Plagiostoma* based on the presence of lateral perithecial necks and *O. nana* in the genus *Gnomoniella* based on aseptate ascospores by Monod (1983). A culture (BRIP 29308a) of *O. elasticae* (Koords.) M. Monod was obtained, sequenced, and determined to fall outside of the Gnomoniaceae, in the Basidiomycota. The remaining species of *Ophiognomonia* recognized by Monod (1983), specifically *O. capillaris, O. langii,* and *O. lapponica,* could not be obtained for this study. Barr's (1978) generic concepts of *Gnomonia, Gnomoniella, Ophiognomonia,* and *Plagiostoma* were accepted by Monod (1983), however, the species in each genus differ. She recognized only the type species of *Ophiognomonia*, *O. melanostyla*.

Within the genus *Ophiognomonia* most morphological characters such as shape and size of perithecia and perithecial necks and ascospore length, width, and septation have limited use for identification of species of *Ophiognomonia.* The most common morphological characteristic in *Ophiognomonia* occurring in 28 of 45 species is fusiform ascospores that are approximately $10–20 \times 2–4$ µm with a median septum. A distinct submedian septum was observed in ascospores of *O. alni-cordatae, O. apiospora, O. gei-montani,* and *O. otanii* (Figs. 4.6, 4.8, 4.14, 4.35). Aseptate ascospores were documented only in *O. nana* (Fig. 4.32). *Ophiognomonia cordicarpa, O. longispora, O. melanostyla,* and *O. sassafras* forming a phylogenetically distinct group were the only species with filiform ascospores (Figs. 4.11, 4.24, 4.26, 4.42). Ascospore appendages were observed in *O. balsamiferae, O. gei, O. hiawathae, O. intermedia, O. ischnostyla, O. longispora, O. melanostyla, O. michiganensis, O. nipponicae, O. pseudoclavulata, O.*

pseudoischnostyla, and *O. setacea.* Uncommonly large ascospores (40 × 7 μm) for

Ophiognomonia were observed in *O. micromegala* (Fig. 4.28). Among the species of

Ophiognomonia, only *O. lenticulispora* and *O. pseudoclavulata* have oval to ellipsoidal

ascospores (Fig. 4.22, 4.36). Multiple-necked perithecia were occasionally observed in *O.*

michiganensis and *O. multirostrata,* a phenomenon often occurring in culture, but rarely

in nature for species of *Gnomoniopsis* and *Ophiognomonia* (Fig. 4.27, 4.30; Sogonov et

al. 2008, Walker et al. 2010). *Ophiognomonia apiospora* has an unusually thick

perithecial cell wall for this genus that becomes distinctly concave upon drying (Fig. 4.8).

No single, distinct, morphological characteristic allows recognition of individual species

in the phylogenetically diverse genus *Ophiognomonia.*

Barr (1978) documented the North American distribution of gnomoniaceous

species as far north as British Columbia, Canada. The northernmost range of

Ophiognomonia is expanded here to Finland where *O.rosae* was collected; the

southernmost distribution is extended to Central America (Panama) where *O. bugabensis*

was collected and to South America (Argentina) for *O. tucumanensis.* Sogonov et al.

(2008) documented several genera in the Gnomoniaceae including *Ophiognomonia*

occurring in Russia. Mejía et al. (2011a) expanded the biogeographic range of the

Gnomoniaceae by describing the monotypic genus *Occultocarpon* and several new

species of *Plagiostoma* from the Yunnan province of China. This study presents the first

report of the genus *Ophiognomonia* from China. Kobayashi (1970) documented a single

species of *Ophiognomonia, O. setacea,* in Japan, and that report is confirmed here. On a

two-week trip to Japan, 16 new species were collected and are described here. These

results suggest that gnomoniaceous fungi are plentiful throughout temperate regions.

The biogeographic structure represented in the phylogeny of *Ophiognomonia* indicates allopatric speciation as a driving force for several endemic species in this group. Multiple species have limited geographic distribution to regions such as Japan/China, Europe/North America, or Central/South America. *Ophiognomonia setacea* is the only exception, exhibiting a global distribution without geographic constraints. For example, 14 species are endemic in Japan, two in Central and South America, 13 in North America, and four in Europe suggesting that these species are genetically and, in many cases, geographically isolated from other species of *Ophiognomonia.* It is unclear to what extent these taxa are truly endemic or are present but undocumented in other locations.

Phylogenetic analyses of variable molecular markers are the primary means of species delimitation in *Ophiognomonia.* This genus has a diverse host range occurring on plants in the families Betulaceae, Fagaceae, Juglandaceae, Lauraceae, Malvaceae, Platanaceae, Rosaceae, Salicaceae, and Sapindaceae. Most species of *Ophiognomonia* show preference to a single host genus or several genera from the same host family. For example, *O. monticola* was collected on *Carpinus* sp. (Betulaceae) from Japan and *O. rosae* on *Fragaria vesca, Rosa* sp., and *Rubus* sp. (Rosaceae) from Europe and the U.S. However, one species, *O. michiganensis,* was associated with genera in the Betulaceae and Rosaceae similar to host/fungus associations for *Apiognomonia errabunda,* which causes anthracnose disease of shade trees in 10 different plant families (Sogonov et al. 2007). The genus *Alnus* is the most common host plant for species of *Ophiognomonia.* Thirteen species in clades one and three (Figs. 4.2, 4.4) are associated with *Alnus* spp. *Ophiognomonia balsamiferae* on *Populus* spp. is the only species of *Ophiognomonia* that occurs on the Salicaceae and thus may represent a host jump to a novel host family.

Despite extensive collecting on salicaceous hosts, no additional species of

Ophiognomonia were discovered in this family. Multiple species including *O.*

clavigignenti-juglandacearum, O. leptostyla, O. micromegala, O. pseudoclavulata, and

O. vasiljevae occur on plants in the Juglandaceae in addition to *O. cordicarpa* and *O.*

pterocaryae, the first records of the Gnomoniaceae on the host genus *Pterocarya* in the

Juglandaceae. Several patterns of host plant association at the family rank were observed

throughout the phylogeny of *Ophiognomonia.* A group of closely related species

including *O. asiatica, O. kobayashii, O. otanii,* and *O. sogonovii* are specific to *Quercus*

spp. and *Castanea* spp. within the Fagaceae (Fig. 4.2). In addition, a group including *O.*

nipponicae, O. padicola, and *O. rosae* occur only on hosts in the Rosaceae (Fig. 4.3).

Similarly a distribution of fungal species on only one host family was observed for other

genera in the Gnomoniaceae. Mejía et al. (2011c) discovered 11 species of *Plagiostoma*

associated with the Salicaceae while Walker et al. (2010) found similar host/fungus

relationships in the genus *Gnomoniopsis*. Sogonov et al. (2008) observed similar

relationships for species of *Gnomonia* associating with a single host genus or species

within the Coryloideae. A clade consisting of eight species of *Ophiognomonia,* including

O. alni-viridis, O. bugabensis, O. ibarakiensis, O. intermedia, O. maximowiczianae, O.

multirostrata, O. nana, and *O. tucumanensis* are host specific to *Alnus* spp. and *Betula*

spp. (Fig. 4.4). The genus *Cryptosporella* exhibits similar host/fungus associations on

Alnus/*Betula* (Mejía et al. 2008, 2011b). These examples suggest close host/fungus

associations and possible host specificity or coevolution within the Gnomoniaceae.

The criteria used here for GCPSR proved useful for species recognition in the

Gnomoniaceae. Similar criteria for GCPSR have been used in the model organisms

Aspergillus spp. (Pringle et al. 2005), *Neurospora* (Dettman et al. 2003), and *Fusarium* spp. (O'Donnell et al. 2004, Sarver et al. 2011). For example, in Pringle et al. (2005) a distinct evolutionary lineage was recognized if the majority of single-marker genealogies were congruent. Two criteria were considered important for GCPSR in Dettman et al. (2003). A clade must be present in (1) the majority of single-marker genealogies or (2) strongly supported with a single-marker and lack genealogical nondiscordance in any other locus genealogy. These GCPSR concepts were expanded here by including the genealogical sorting index (*gsi*) to determine exclusive genetic ancestry (Cummings et al. 2008).

The *gsi* provided a tree-based measure for identification of reciprocally monophyletic species clades (Cummings et al. 2008). For example, the ITS genealogies of *O. multirostrata, O. setacea,* and *O. tucumanensis* lack support under GCPSR. However, *gsi* of the ITS tree distribution indicate near exclusive ancestry (Table 4.2; gsi_T = 0.6435*/0.8835*/0.6406* respectively). The *gsi* measure can also be used to confirm and quantify the lack of genealogical structure indicated by GCPSR for a species clade at a given locus. For example, in the ITS region, *O. alni-viridis, O. asiatica, O. kobayashii,* and *O. intermedia* are not supported under GCPSR, which is confirmed and quantified by low gsi_T values (Table 4.2). An explanation for the previously mentioned example is that time to evolve reciprocal monophyly is longer than time since initial genetic isolation of these species for this locus (Tajima 1983, Hudson and Coyne 2002, Rosenberg 2003). *Ophiognomonia hiawathae* and *O. michiganensis* were the least supported species in single-marker genealogies under the GCPSR criteria used here (Table 4.2). However, both the gsi_T and GCPSR analyses for the combined dataset detected distinct genealogical

structure for these species. Cummings et al. (2008) observed similar results using the gsi_T statistic to evaluate single-marker genealogies of field crickets (*Grylllus* spp.; dataset from Broughton and Harrison 2003). When analyzing the combined marker dataset for field crickets, genealogical structure at the species level became apparent (Cummings et al. 2008).

Resolving various taxonomic ranks of phylogenetic relationships requires markers with signal at different levels of divergence or different rates of evolution (Hillis and Dixon 1991, Townsend 2007). The markers ITS, MS204, and *tef-1α* were selected for this study based on evidence from Walker et al. (accepted), which assessed various combinations of five markers using phylogenetic informativeness tests (Townsend 2007) and determined this combination of three markers to fully recover the five-marker topology with equivalent or higher support for branches. The markers MS204 and *tef-1α* performed exceptionally well in nearly all cases under the criteria for GCPSR (Table 4.2). The ITS region (ITS1, 5.8S rDNA and ITS2) performed poorly, most likely due to the low rate of evolution in this marker (Walker et al. accepted).

Three clade-specific alignments (Figs. 4.2–4.4) were necessary to make accurate decisions of homologous intron regions in ITS, MS204, and *tef-1α.* Exclusion of unnecessary positions after alignment across the entire genus caused a great loss of phylogenetic signal and did not support the true molecular diversity in each species clade. In addition, hidden phylogenetic signal in single-marker analyses often becomes apparent in concatenated analyses (Sullivan 1996). Several species not supported in individual marker analyses were strongly supported by combined three-marker analysis under the criteria proposed here for GCPSR (Table 4.2). Similar results were indicated by Wild and

Maddison (2008), who determined the necessity of multiple-marker concatenation for reconstructing the beetle tree of life.

This study is an account of the 45 currently known species of *Ophiognomonia* including 25 species new to science. Developing phylogenetic concepts for species recognition in an economically significant group of fungi that lack distinct morphological characters provide the basis for future studies of Gnomoniaceae and other non-model organisms. Knowledge of the species of *Ophiognomonia* has interesting ecological implications given their association and pathogenic potential on important shade, lumber, and nut-producing trees. Accurate species definition is essential for developing effective measures and quarantine policies to control the diseases they cause and spread of these plant pathogens. Additional collection of this highly diverse group will likely lead to the discovery of many new species in diverse habitats worldwide and associations with known and novel host plants.

TAXONOMY

Ophiognomonia (Sacc.) Sacc., Syll. Fung. 14: 613. 1899.

Lectotype designated by Höhnel (1919): *Ophiognomonia melanostyla* (DC.: Fr.) Berl.

≡ *Gnomoniella* subgenus *Ophiognomonia* Sacc., Syll. Fung. 1: 419. 1882.

Perithecia solitary, aggregated up to three, or in loose clusters, without stroma, epiphyllous and hypophyllous on overwintered leaf blades or on overwintered petioles, rachises, stems, or fruits of woody or herbaceous plants. Perithecia dark brown to glossy black, rarely cream, globose to subglobose, immersed or partially erumpent, occasionally

causing host tissue to swell and break. Neck central, lateral, or marginal, straight, curved, or sinuous, long to short. Asci fusiform to oval or filiform, apical ring often conspicuous, eight ascospores per ascus arranged uni-, bi-, and multiseriate or parallel, occasionally intertwined. Ascospores two-celled, rarely one-celled, oval, fusiform, or filiform, ends blunt to rounded, with or without appendages.

Hosts: On Betulaceae, Fagaceae, Juglandaceae, Lauraceae, Malvaceae, Platanaceae, Rosaceae, Salicaceae, and Sapindaceae.

Ophiognomonia alni-cordatae D.M. Walker, sp. nov.   Fig. 4.6a–f.

MycoBank: MB 564079

Etymology: alni-cordatae refers to the host on which the holotype was collected.

Holotypus: JAPAN, NAGANO: Ueda-shi, Sugadaira, Kakuma River Trail, on overwintered leaves of *Alnus cordata,* 14 April 2010, D.M. Walker (BPI 882233, culture DMW 384.1 = CBS 131353).

Perithecia immersed, occasionally causing host tissue to swell, on leaf petioles and veins, epiphyllous or hypophyllous, solitary or aggregated up to two, glossy black, globose to subglobose, (134–)177–234 μm high × 228–294 μm diam (mean = 182 × 261, S.D. 49.7, 46.7, n1 = 3, n2 = 2). Necks central to marginal, mostly straight or curved to sinuous, occasionally swollen at tip, (180–)189–394(–438) μm long (mean = 263, S.D. 69.8, n = 18). Asci fusiform with rounded or papillate apex and acute or long tapering stipe, apical ring conspicuous, (43–)45–50(–52) × (13–)16–21(–22) μm (mean = 48 × 18, S.D. 3.6, 2.6, n1 = 8, n2 = 11), with ascospores arranged irregularly uni- to multiseriate. Ascospores fusiform, ends rounded, straight to slightly curved, one-septate, distinct

submedian septum, slight constriction at septum, $(21–)22–24(–25) \times (4–)5–6(–7)$ µm (mean = 22.6×5.5, S.D. 1.2, 0.7, n1 = 27, n2 = 25).

Habitat: On dead leaves of *Alnus cordata* (Loisel.) Duby (Betulaceae).

Distribution: Japan (Nagano prefecture).

Notes: O. alni-cordatae is one of 17 species known from Japan, and one of four occuring on *Alnus* from this country. A distinct submedian septum was only observed in ascospores of four species including, *O. alni-cordatae, O. apiospora, O. gei-montani,* and *O. otanii.*

Ophiognomonia alni-viridis (Podlahova & Svrček) Sogonov, Stud. Mycol. 62: 55. 2008. Fig. 4.7a–j.

Basionym: *Gnomonia alni-viridis* Podlahova & Svrček, Česká Mycol. 24: 129. 1970.

MycoBank: MB 512215

Perithecia immersed, occasionally causing host tissue to swell, on leaf blades, veins, and petioles, hypophyllous and epiphyllous, solitary or aggregated up to two, glossy black, subglobose, $(135–)136–301(–311)$ µm high $\times$ $(187–)197–363(–432)$ µm diam (mean = 235×296, S.D. 59.5, 65.6, n1 = 15, n2 = 15). Necks central, straight, curved, or slightly sinuous, $(331–)641–1620(–1653)$ µm long (mean = 1069, S.D. 371.1, n = 20). Asci ellipsoid to fusiform with papillate or rounded apex, stipe acute or long tapering, apical ring conspicuous, $(28–)29–43(–50) \times (8–)9–18(–19)$ µm (mean = 34×15, S.D. 4.6, 2.5, n1 = 30, n2 = 28), ascospores arranged parallel or irregularly uniseriate to multiseriate. Ascospores fusiform with rounded ends, straight to slightly curved, one-septate, median to indistinctly sub- or supramedian, slightly constricted at septum, each cell with 0–2

distinct and several small guttules, (11–)12–16(–17) × 2–3 µm (mean = 14 × 3, S.D. 1.6, 0.5, n1 = 30, n2 = 30).

Habitat: On overwintered leaves of *Alnus rhombifolia* Nutt., *A. serrulata* (Aiton) Willd., *A. sinuata* Rydb., *A. viridis* (Chaix) D.C.*, Betula papyrifera* Marshall, and overwintered fruits of *A. viridis* (Betulaceae).

Distribution: Canada (British Columbia), Europe (Czech Republic, Switzerland), and United States (CA, MI, NY, WA).

Materials examined: CANADA, BRITISH COLUMBIA: 15 km south of Princeton, near Indian Reserve #3, on overwintered leaves of *Betula papyrifera,* 13 May 2006, M.V. Sogonov (BPI 877600, GenBank EU 254869); CZECH REPUBLIC: on overwintered fruits of *Alnus viridis,* 14 July 1969, coll. R. Podlahová, det. Svrček (PRM 685743, HOLOTYPE of *Gnomonia alni-viridis,* PRM); SWITZERLAND: Valais, vicinity of Martigny, on overwintered leaves of *Alnus viridis,* 21 May 2005, M. Monod (BPI 877585A, GenBank EU 254866). UNITED STATES, CALIFORNIA: Shasta County, Shasta, Trinity National Park, Ellery Creek, on *Alnus rhombifolia,* 19 May 2008, L.C. Mejía, det. D.M. Walker (BPI 879529, culture LCM 459.01); MICHIGAN: Houghton County, boat dock near FJ McClain Campground, on overwintered leaves of *Betula* sp., 31 May 2010, D.M. Walker (BPI 882251, culture DMW 439.3 = CBS 131408); NEW YORK: Franklin County, Adirondack high peaks region, Adirondack Loj, trail head, on overwintered leaves of *Betula papyrifera,* 9 June 2007, L.C. Mejía, det. D.M. Walker (BPI 881497, cultures LCM 158.01, LCM 158.02); NEW YORK: White Face Mountain, 4000 ft elevation, on *Alnus serrulata,* 12 June 2007, L.C. Mejía, det. D.M. Walker (BPI 881512, cultures LCM 164.01, LCM 164.02); WASHINGTON: King County, Mount

Baker-Snoqualmie National Forest, Snoqualmie ranger district, near exit 42 on highway US 90, on overwintered leaves of *Alnus viridis,* 16 May 2006, M.V. Sogonov (BPI 877595, GenBank EU 254867); WASHINGTON: Clallam County, Olympic National Park, Heart O' the Hills Campground, on *Alnus sinuata,* May 2008, L.C. Mejía, det. D.M. Walker (BPI 879541, culture LCM 494 = CBS 128358).

Notes: Ophiognomonia alni-viridis is one of four species that occur on both *Alnus* spp. and *Betula* spp. in the Betulaceae. This species has relatively long perithecial necks compared to many other species in *Ophiognomonia.*

Ophiognomonia apiospora L.C. Mejía & D.M. Walker, sp. nov.   Fig. 4.8a–g.

MycoBank: MB 564080

Etymology: apiospora refers to the distinct submedian location of the ascospore septum.

Holotypus: CHINA, YUNNAN PROVINCE: Kunming, Kunming Institute of Botany, botanical garden, on overwintered leaves of *Alnus nepalensis,* 12 July 2008, L.C. Mejía, det. D.M. Walker (BPI 879601, ex-type cultures LCM 503.05 = CBS 131425, LCM 503.06 = CBS 131426).

Perithecia immersed, occasionally causing host tissue to swell, concave from base when dry, thick cell walls, on leaf petioles and veins, hypophyllous and epiphyllous, solitary or aggregated up to three, glossy black, subglobose, (289–)336–423(–482) μm high × (671–)677–724(–840) μm diam (mean = 375 × 717, S.D. 76.6, 72.5, n1 = 5, n2 = 5). Necks central, elongated, straight to curved, (1478–)1525–2671(–3074) μm long (mean = 2208, S.D. 579.2, n = 8). Asci ellipsoid to fusiform, apex papillate or rounded, stipe acute, apical ring conspicuous, (42–)45–50(–60) × 18–20 μm (mean = 49 × 20, S.D. 6.9, 2.8, n1

= 5, n2 = 2), ascospores arranged uniseriate to irregularly multiseriate. Ascospores fusiform, rounded ends, straight to slightly curved, one-septate, submedian, distinctly constricted at septum, each cell with 0–5 large guttules, $(24–)25–28(–29) \times 4–5$ µm (mean = 26 × 4, S.D. 1.3, 0.2, n1 = 30, n2 = 28).

Habitat: On overwintered leaf blades, petioles, and veins of *Alnus nepalensis* D. Don (Betulaceae).

Distribution: China (Yunnan Province).

Notes: This is the only species of *Ophiognomonia* with an unusually thick perithecial cell wall. In addition, *O. apiospora* has the longest perithecial necks in the genus *Ophiognomonia.* This species has a distinct submedian septum that was also observed in ascospores of *O. alni-cordatae, O. gei-montani,* and *O. otanii.* This is only species of *Ophiognomonia* known to occur in China on the genus *Alnus.*

Ophiognomonia asiatica D.M. Walker & L.C. Mejía, sp. nov.   Fig. 4.9a–g.

MycoBank: MB 564081

Etymology: asiatica refers to the location where the holotype was collected.

Holotypus: JAPAN, IBARAKI: Tsukuba City, National Museum, on overwintered leaves of *Quercus serrata,* 2 April 2010, D.M. Walker (BPI 882231, ex-type culture DMW378.2 = CBS 131351).

Perithecia immersed, occasionally causing host tissue to swell and rupture, on leaf petioles, veins, and blades, solitary or aggregated up to three, glossy black, subglobose, $(143–)154–263(–292)$ µm high $\times (239–)256–413(–514)$ µm diam (mean = 212 × 345, S.D. 58, 91, n1 = 8, n2 = 8). Necks central, straight, curved, or sometimes sinuous, (438–

)518–1176(–1225) μm long (mean = 738, S.D. 178.7, n = 23). Asci fusiform to ellipsoid, apex papillate, stipe long tapering, apical ring large, 3 μm diam, conspicuous, (24–)25–40(–41) × (10–)11–16(–17) μm (mean = 31 × 14, S.D. 5.6, 1.8, n1 = 30, n2 = 25), ascospores arranged irregularly uniseriate, multiseriate, or parallel. Ascospores fusiform with rounded ends, straight to slightly curved, one-septate, median or indistinctly supramedian, not constricted or slightly constricted at septum, each cell with 0–2 distinct guttules, (11–)12–15(–16) × 2–3 μm (mean = 14 × 2, S.D. 1.6, 0.4, n1 = 30, n2 = 30).

Habitat: On overwintered leaves of *Quercus aliena* Blume, *Quercus dentata* Thunb.*,* and *Q. serrata* Murray (Fagaceae).

Distribution: China (Kunming) and Japan (Ibaraki prefecture).

Materials examined: CHINA, KUNMING: Kunming Botanical Garden, on overwintered leaves of *Quercus dentata*, 11 July 2008, L.C. Mejía (BPI 879600, LCM 500.01 = CBS 131424). JAPAN, IBARAKI: Ushiku, Ushiku Nature Reserve, on overwintered leaves of *Quercus serrata,* 9 April 2010, D.M. Walker (BPI 882220, cultures DMW 351.3 = CBS 131345, DMW 351.2); IBARAKI: Ushiku, Ushiku Nature Reserve, on overwintered leaves of *Quercus aliena,* 9 April 2010, D.M. Walker (BPI 882225, culture DMW361.1 = CBS 131347).

Notes: This is the only species of *Ophiognomonia* known from both China and Japan on the genus *Quercus*. It is one of four species of *Ophiognomonia* known to occur exclusively on *Quercus.* A group of closely related species including *O. asiatica, O. kobayashii, O. otanii,* and *O. sogonovii* are specific to *Quercus* spp. and *Castanea* spp. within the Fagaceae (Fig. 4.2).

Ophiognomonia balsamiferae Sogonov, Stud. Mycol. 62: 51. 2008.

MycoBank: MB 512180

Habitat: On overwintered petioles of *Populus balsamifera* L. (Salicaceae).

Distribution: Canada (British Columbia).

Notes: This is the only species of *Ophiognomonia* known to occur on *Populus* in the Salicaceae. Ascospore appendages were observed in *O. balsamiferae, O. gei, O. hiawathae, O. intermedia, O. ischnostyla, O. longispora, O. melanostyla, O. michiganensis, O. nipponicae, O. pseudoclavulata, O. pseudoischnostyla,* and *O. setacea.* For a detailed description of this species, see Sogonov et al. (2008).

Ophiognomonia bugabensis L.C. Mejía & D.M. Walker, sp. nov.   Fig. 4.10a–g.

MycoBank: MB 564082

Etymology: bugabaensis refers to the district of Bugaba in Panama where the holotype was collected.

Holotypus: PANAMA, CHIRIQUI: District of Bugaba, Las Nubes, Parque Internacional La Amistad, main trail close to the gamewarden house in the entrance of the park, at 2225 masl on dead leaves of *Alnus acuminata,* 27 December 2006, L.C. Mejía, det. D.M. Walker (BPI 879256).

Perithecia immersed, on leaf blades and veins, hypophyllous, solitary to aggregated up to two, glossy black, subglobose, (178–)247–282(–303) μm high × (252–)275–474(–497) μm diam (mean = 255 × 387, S.D. 48, 102, n1 = 5, n2 = 6). Necks central or marginal, straight, curved, or sinuous, (340–)349–559(–667) μm long (mean = 461, S.D. 110, n = 11). Asci obovoid to oval, apex rounded, stipe acute to rounded, (40–)43–55(–57) × (23–

)25–26(–27) µm (mean = 48 × 25, S.D. 7.7, 1.8, n1 = 5, n2 = 5), ascospores arranged irregularly uniseriate to multiseriate. Ascospores broadly fusiform, ends rounded, straight to slightly curved, one-septate, supramedian, slightly constricted at septum, (17–)18–19(–20) × (4–)5–6 µm (mean = 18 × 5, S.D. 0.9, 0.6, n1 = 30, n2 = 17).

Habitat: On dead leaves or as an endophyte of *Alnus acuminata* Kunth (Betulaceae).

Distribution: Panama (Chiriqui).

Materials examined: PANAMA, CHIRIQUI: District of Bugaba, Las Nubes, Parque Internacional La Amistad, isolated as an endophyte from a twig of of *Alnus acuminata,* 22 December 2004, L.C. Mejía, det. D.M. Walker (culture LCM 362); CHIRIQUI: District of Bugaba, Las Nubes, Parque Internacional La Amistad, isolated as an endophyte from leaf of *Alnus acuminata,* 22 December 2004, L.C. Mejía, det. D.M. Walker (LCM 368 = CBS 131399).

Notes: When compared to other species, *O. bugabensis* was isolated in high frequency as an endophyte of leaves and twigs of *Alnus acuminata* in Panama. This species was also collected on dead leaves of *Alnus acuminata* in Panama. This host plant occurs in montane cloud forest from Mexico to the Andes. Only *O. bugabensis* and *O. tucumanensis* are known to occur on *Alnus acuminata.* These two species can be distinguished by geographic location; also *O. bugabensis* has larger ascospores and shorter perithecial necks than *O. tucumanensis.* Interestingly, *O. bugabensis* was found at the same time of year when perithecia of another species of Gnomoniaceae, *Cryptosporella amistadensis*, is commonly found in the same geographic area.

Ophiognomonia clavigignenti-juglandacearum (Nair, Kostichka, & Kuntz) Broders & Boland, Fung. Biol. 115: 5. 2010.

Basionym: *Sirococcus clavigignenti-juglandacearum* Nair, Kostichka, & Kuntz, Mycologia 71: 643. 1979.

Habitat: Causing butternut canker of *Juglans ailantifolia* Carriére var. *cordiformis* (Makino) Rehder*, J. cinerea* L.*,* and *J. nigra* L.

Distribution: Canada (New Brunswick, Ontario, Quebec) and United States (AK, CT, IN, MI, MN, MO, NC, NH, NY, OH, TN, VT, WI).

Notes: This species causes the devastating butternut canker disease in North America. It is known to occur only in the asexual state. For a detailed description of this species, see Broders and Boland (2010).

Ophiognomonia cordicarpa D.M. Walker, sp. nov.   Fig. 4.11a–h.

MycoBank: MB 564083

Etymology: cordicarpa refers to the heart-shaped perithecia of this species.

Holotypus: JAPAN, NAGANO: Ueda-shi, Sugadaira, waterfall at the Sugadaira Montane Research Center, on overwintered leaves of *Pterocarya rhoifolia,* 13 April 2010, D.M. Walker (BPI 882217, ex-type culture DMW 344.2 = CBS 131342).

Perithecia immersed, occasionally causing host tissue to swell, on leaf blades and veins, solitary, glossy black, cordate to subglobose, 223–268 μm high × 357–474 μm diam (mean = 252 × 400, S.D. 25.4, 64.1, n1 = 3, n2 = 3). Necks central, lateral, or marginal, straight, curved, or sinuous, (672–)1093–1111(–1117) μm long (mean = 998, S.D. 217.7, n = 4). Asci narrowly fusiform, apex bluntly rounded, stipe acute or bluntly rounded,

apical ring conspicuous, (69–)77–85(–92) × (7–)9–11(–13) μm (mean = 82 × 10, S.D. 7.2, 1.9, n1 = 7, n2 = 7), ascospores arranged parallel to intertwined. Ascospores filiform with bluntly rounded ends, curved to sinuous, one-septate, supramedian, not constricted at septum, with many small guttules, (55–)56–77(–78) × 1–2 μm (mean = 64 × 1, S.D. 7.8, 0.4, n1 = 26, n2 = 20).

Habitat: On overwintered leaves of *Pterocarya rhoifolia* Siebold & Zucc. (Juglandaceae).

Distribution: Japan (Nagano prefecture).

Notes: *Ophiognomonia cordicarpa* is one of 17 species known from Japan, and one of two known to occur on *Pterocarya* (Juglandaceae). Several other species are known to occur on *Carya* and *Juglans* (Juglandaceae) including the pathogens *O. leptostyla* and *O. clavigignenti-juglandacearum. Ophiognomonia cordicarpa* has long filiform ascospores, whereas *O. pterocaryae* has much shorter fusiform ascospores. The distinctive heart-shaped ascomata of this species is unusual for perithecia in *Ophiognomonia.* The species *O. cordicarpa, O. longispora, O. melanostyla,* and *O. sassafras* share elongated filiform ascospores and form a clade of closely related species (Fig. 3).

Ophiognomonia gardiennetii D.M. Walker, sp. nov.   Fig. 4.12a–g.

MycoBank: MB 564084

Etymology: gardiennetii refers to Alain Gardiennet to honor his contribution as a collector of many specimens of the Gnomoniaceae.

Holotypus: UNITED STATES, MICHIGAN: Mackinac County, Brevort campground, on overwintered leaves of *Alnus serrulata,* 27 May 2010, D.M. Walker (BPI 882262, ex-type culture DMW 469.3 = CBS 131417).

Perithecia immersed to partially erumpent, occasionally causing host tissue to swell, on leaf blades, petioles, and veins, hypophyllous and epiphyllous, solitary, glossy black, subglobose, (178–)180–243(–253) μm high × (238–)248–309(–351) μm diam (mean = 214 × 283, S.D. 29.9, 34, n1 = 9, n2 = 9). Necks central, marginal, or lateral, straight to curved, (356–)364–686(–697) μm long (mean = 487, S.D. 131, n = 15). Asci ellipsoid to fusiform, apex rounded to papillate, stipe acute to short tapering, (21–)24–34(–37) × (11–)12–15(–16) μm (mean = 28 × 13, S.D. 3.4, 1.5, n1 = 21, n2 = 21), ascospores arranged uniseriate to irregularly multiseriate. Ascospores fusiform with rounded ends, straight to slightly curved, one-septate, median to indistinctly sub- or supramedian, slightly to not constricted at septum, each cell with 0–2 distinct and several small guttules, (9–)10–12(–13) × 2–3 μm (mean = 11 × 3, S.D. 0.9, 0.3, n1 = 30, n2 = 30).

Habitat: On overwintered leaves of *Alnus serrulata* Willd. (Betulaceae).

Distribution: United States (MI).

Materials examined: UNITED STATES, MICHIGAN: Houghton County, FJ McClain State Park, on overwintered leaves of *Alnus serrulata,* 30 May 2010, D.M. Walker (BPI 882252, culture DMW 442.1 = CBS 131409); MICHIGAN: Marquette County, hiking trail along Peshekee river, on overwintered leaves of *Alnus serrulata,* 30 May 2010, D.M. Walker (BPI 882276, culture DMW 513.1 = CBS 131429).

Notes: Only *O. gardiennetii* and *O. trientensis* are known to occur exclusively on *Alnus* from the U.S. Morphologically these species are very similar and can only be

distinguished by DNA sequence data. In addition, they form a clade of closely related species with the butternut pathogen *O. clavigignenti-juglandacearum* (Fig. 4.4).

Ophiognomonia gei (Pat. & Doass.) D.M. Walker, comb. nov.   Fig. 4.13a–g.

Basionym: *Gnomonia gei* Pat. & Doass., in Patouillard, Tabl. analyt. Fung. France (Paris) 5: 214. 1886.

MycoBank: MB 564085

Perithecia immersed, causing host tissue to swell, bases visible under thin layer of host tissue, on herbaceous stems, leaves, or petioles, hypophyllous, solitary, glossy black, subglobose, 196–244 μm high × 325–400 μm diam (mean = 220 × 363, S.D. 34, 53, n1 = 2, n2 = 2). Necks central, long, straight to curved, (1248–)1451–1784 μm long (mean = 1494, S.D. 270, n = 3). Asci pyriform to clavate, apex rounded, stipe curved tapering, 24–36 × 4–6 μm (mean = 35 × 5, S.D. 8.5, 1.4, n1 = 2, n2 = 2), ascospores arranged uniseriate. Ascospores fusiform, ends rounded, straight to slightly curved, one-septate, median to submedian, not constricted or slightly constricted at septum, each cell with several small guttules, with appendages at each end subulate to whip-shaped or absent, (15–)16–18(–19) × 2 μm (mean = 17 × 2, S.D. 1.1, 0.0, n1 = 11, n2 = 10).

Habitat: On overwintered leaves *Fragaria vesca* L. and *Geum pyrenaicum* Mill. (Rosaceae).

Distribution: Europe (France).

Materials examined: FRANCE: *Geum pyrenaicum,* 26 June 1885, J.E. Doassans & N. Patouillard 5304 (LECTOTYPE of *Gnomonia gei* designated here, FH).

Notes: Ophiognomonia gei based on *Gnomonia gei* was originally collected in France on *Geum pyrenaicum.* Monod (1983) collected and isolated what he considered to be *Gnomonia gei* from Switzerland on *Fragaria vesca.* His description is in agreement with measurements taken from original material collected by Doassans and Patouillard in 1885 (FH 5304). Monod's specimen (Monod 301 = culture CBS 818.79) was not available from LAU, however, the isolate was used here as a molecular representative of *O. gei.* This species is one of two that occur on *Geum,* and one of nine that occur on the host family Rosaceae. Of these species *O. gei, O. nipponicae, O. padicola, O. rosae, O. rubi-idaei* form a clade according to ITS sequence data (Fig. 4.5). Ascospore appendages were only observed in *O. balsamiferae, O. gei, O. hiawathae, O. intermedia, O. ischnostyla, O. longispora, O. melanostyla, O. michiganensis, O. nipponicae, O. pseudoclavulata, O. pseudoischnostyla,* and *O. setacea.*

Ophiognomonia gei-montani (Ranoj.) Sogonov, Stud. Mycol. 62: 58. 2008. Fig. 4.14a–f.
Basionym: *Gnomonia gei-montani* Ranoj., Ann. Mycol. 8: 362. 1910.
MycoBank: MB 512183
Perithecia immersed, on leaf blades, petioles, and veins, causing swelling and rupture of host tissue, hypophyllous, solitary, glossy black, subglobose, (245–)315–331(–345) μm high × (300–)341–363(–383) μm diam (mean = 309 × 347, S.D. 44.4, 35.6, n1 = 4, n2 = 4). Necks marginal, straight to curved, (289–)301–472(–530) μm long (mean = 368, S.D. 84, n = 9). Asci ellipsoid to fusiform, apex rounded, stipe tapering, apical ring not conspicuous, (39–)48–50(–56) × 12–17 μm (mean = 51 × 15, S.D. 4.2, 3.5, n1 = 3, n2 = 2), ascospores arranged irregularly uni- or biseriate. Ascospores fusiform, rounded ends,

straight to slightly curved, one-septate, distinctly submedian, slightly to not constricted at septum, lacking guttules, $(11–)13–14(–15) \times (2–)3–4$ μm (mean $= 14 \times 3$, S.D. 0.6, 0.3, n1 = 21, n2 = 18).

Habitat: On overwintered leaves of *Geum bulgaricum* Panc., *G. coccineum* Sm., *G. montanum* L., and *G. rhodopeum* Stoj. & Stef. (Rosaceae).

Distribution: Europe (Serbia, Switzerland).

Materials examined: SERBIA: on dead leaves of *Geum montanum,* 1910, N. Ranojević (S-F190027 HOLOTYPE of *Gnomonia gei-montani*); SWITZERLAND: Salvan, La Tendraz, 1600 m, on dead leaves of *Geum montanum,* 28 May 2005, M. Monod (BPI 877589, GenBank EU 254872).

Notes: This species is one of two that occur on *Geum,* and one of nine that occur on the host family Rosaceae. A distinct submedian septum was only observed in ascospores of four species including *O. alni-cordatae, O. apiospora, O. gei-montani,* and *O. otanii.*

Ophiognomonia gunmensis D.M. Walker, sp. nov.   Fig. 4.15a–g.

MycoBank: MB 564086

Etymology: gunmensis refers to the Japanese prefecture where the holotype of this species was collected.

Holotypus: JAPAN, GUNMA: Azuma, Azuma Nature Park, on overwintered leaves of *Quercus serrata,* 12 April 2010, D.M. Walker (BPI 882236, ex-type culture DMW 388.1 = CBS 131401).

Perithecia immersed, on leaf blades and veins, epiphyllous or hypophyllous, solitary or up to two, glossy black, globose to subglobose, $(108–)146–191(–220)$ μm high $\times$ (143–

)146–244(–246) µm diam (mean = 167 × 205, S.D. 37.1, 44.4, n1 = 7, n2 = 8). Necks central, short, straight, (230–)363–370(–390) µm long (mean = 365, S.D. 52.1, n = 8). Asci fusiform to ellipsoid, apex rounded, stipe acute, apical ring conspicuous, (26–)27–32(–42) × (8–)9–14(–15) µm (mean = 30 × 12, S.D. 4.1, 2.2, n1 = 13, n2 = 13), ascospores arranged parallel or irregularly uniseriate to multiseriate. Ascospores fusiform, ends rounded, straight to slightly curved, one-septate, median to indistinctly supramedian, (14–)15–17(–18) × 2 µm (mean = 17 × 2, S.D. 0.9, 0.6, n1 = 30, n2 = 12).

Habitat: On overwintered leaves of *Quercus serrata* Murray (Fagaceae).

Distribution: Japan (Gunma prefecture).

Notes: Ophiognomonia gunmensis is one of 17 species from Japan, and one of four species known to occur specifically on *Quercus.* The perithecial necks are short relative to other species on *Quercus.*

Ophiognomonia hiawathae D.M. Walker, sp. nov.   Fig. 4.16a–g.

MycoBank: MB 564087

Etymology: hiawathae refers to the national park where this species was collected, which was named to honor the Native American leader of the Onondaga tribe, Hiawatha.

Holotypus: UNITED STATES, MICHIGAN: Mackinac County, Brevort campground, on overwintered leaves of *Betula lutea,* 27 May 2010, D.M. Walker (BPI 882261, ex-type culture DMW 466.1 = CBS 131416).

Perithecia immersed, occasionally causing host tissue to swell and rupture, on leaf blades and veins, solitary, glossy black, subglobose (183–)190–255(–261) µm high × (196–)200–261(–321) µm diam (mean = 218 × 246, S.D. 33, 46, n1 = 6, n2 = 6). Necks central,

straight to curved, (332–)368–696(–961) µm long (mean = 569, S.D. 179, n = 11). Asci fusiform to ellipsoid, apex papillate or rounded, stipe acute or tapering, apical ring conspicuous, (23–)24–33(–34) × (15–)16–19(–20) µm (mean = 28 × 18, S.D. 2.7, 1.4, n1 = 22, n2 = 26), ascospores arranged parallel or irregularly uniseriate. Ascospores fusiform, ends rounded, straight to slightly curved, one-septate, median to indistinctly sub- or supramedian, slightly to not constricted at septum with appendages subulate, whip-shaped, or absent, (12–)13–15(–16) × 2–3(–4) µm (mean = 14 × 3, S.D. 0.9, 0.6, n1 = 30, n2 = 28).

Habitat: On overwintered leaves of *Betula lutea* Michx. (Betulaceae).

Distribution: United States (MI).

Materials examined: UNITED STATES, MICHIGAN: Schoolcraft County, Manistique, Hiawatha National Forest, Indian lake campground, on overwintered leaves of *Betula lutea,* 28 May 2010, D.M. Walker (BPI 882256, culture DMW 458.3 = CBS 131413).

Notes: This species is similar to *O. michiganensis,* however, *O. hiawathae* has larger ascospores. *Ophiognomonia hiawathae* is one of four species of *Ophiognomonia* known to occur on *Betula* in the U.S. Ascospore appendages were only observed in *O. balsamiferae, O. gei, O. hiawathae, O. intermedia, O. ischnostyla, O. longispora, O. melanostyla, O. michiganensis, O. nipponicae, O. pseudoclavulata, O. pseudoischnostyla,* and *O. setacea.*

Ophiognomonia ibarakiensis D.M. Walker, sp. nov.   Fig. 4.17a–h.

MycoBank: MB 564088

Etymology: ibarakiensis refers to the Japanese prefecture where the holotype was collected.

Holotypus: JAPAN, IBARAKI: Hirasawa, rice fields at the foot of Mt. Tsukuba, on overwintered leaves of *Alnus* sp., 8 April 2010, D.M. Walker (BPI 882247, culture DMW 419.3 = CBS 131405).

Perithecia immersed, occasionally causing host tissue to swell and rupture, on leaf blades, petioles, and veins, solitary or aggregated up to three, glossy black, globose, (154–)171–186(–187) µm high × (161–)178–186(–187) µm diam (mean = 176 × 178, S.D. 13.7, 12, n1 = 5, n2 = 4). Necks central to marginal, mostly straight or curved, tips occasionally hamate, (71–)153–545(–546) µm long (mean = 335, S.D. 118.7, n = 24). Asci fusiform to ellipsoid, apex rounded, stipe acute to short tapering, (23–)25–44(–50) × (10–)11–17(–19) µm (mean = 32 × 14, S.D. 8.7, 2.6, n1 = 12, n2 = 12), ascospores arranged irregularly bi- to multiseriate. Ascospores ellipsoidal to oval, rounded ends, straight to slightly curved, one-septate, median to indistinctly sub- or supramedian, not constricted at septum, (10–)11–12 × 3–4 µm (mean = 11 × 4, S.D. 0.6, 0.6, n1 = 30, n2 = 30).

Habitat: On overwintered leaves of *Alnus* sp. Mill. (Betulaceae).

Distribution: Japan (Ibaraki prefecture).

Materials examined: JAPAN, IBARAKI: Hirasawa, rice fields at the foot of Mt. Tsukuba, on overwintered leaves of *Alnus* sp., 8 April 2010, D.M. Walker (BPI 882227, culture DMW 371.1 = CBS 131349).

Notes: *Ophiognomonia ibarakiensis* is one of 17 species known from Japan, and one of four occuring on *Alnus* from this country. This species has slightly smaller ascospores

than *O. naganoensis* and the ascospores overlap in size with *O. multirostrata,* which both occur on *Alnus* from Japan.

Ophiognomonia intermedia (Rehm) Sogonov, Stud. Mycol. 62: 58. 2008. Fig. 4.18a–g.

Basionym: *Gnomonia intermedia* Rehm, Ann. Mycol. 6: 489. 1908.

=*Discula betulina* (Westend.) Arx, Verh. K. Akad. Wet., tweede sect. 51(3): 64. 1957.

=*Gloeosporidium betulinum* (Westend.) Höhn., Sber. Akad. Wiss. Wien, Math.-naturw. Kl., Abt. 1 125(1–2): 95. 1916.

=*Gloeosporium betulinum* Westend., Pl. crypt. exsicc. 19–20(nos 901-1000): no. 978. 1857.

MycoBank: MB 512185

Perithecia immersed, occasionally causing host tissue to swell and rupture, on leaf blades and veins, epiphyllous or hypophyllous, solitary or in loose clusters, glossy black, globose to subglobose, (191–)207–250(–268) μm high × (195–)217–279(–331) μm diam (mean = 228 × 261, S.D. 25.9, 46, n1 = 8, n2 = 8). Necks central, mostly straight, sometimes curved, (408–)464–1047(–1050) μm long (mean = 678, S.D. 191, n = 18). Asci fusiform to ellipsoid, apex papillate or rounded, apical ring not conspicuous, stipe acute to long tapering, (19–)20–41(–48) × (10–)11–16(–17) μm (mean = 26.2 × 13.1, S.D. 8.7, 2.4, n1 = 18, n2 = 18), ascospores arranged parallel or irregularly uniseriate. Ascospores ellipsoid to fusiform with rounded ends, straight to slightly curved, one-septate, median to indistinctly sub- or supramedian, slightly to not constricted at septum

with appendages at each end short, blunt, subulate or absent, (11–)12–14(–15) × 2–3 μm

(mean = 13 × 2, S.D. 0.8, 0.6, n1 = 30, n2 = 30).

Habitat: On overwintered leaves of *Alnus serrulata* Willd., *Betula lutea* Michx., *B. nana*

L., *B. nigra* L., *B. papyrifera* Marshall, *B. pedula* Roth, and *B. pubescens* Ehrh.

(Betulaceae).

Distribution: Canada (British Columbia), Europe (Germany, Scotland), Russia (Tver' and

Novgorod provinces), and United States (MD, MI).

Materials examined: CANADA, BRITISH COLUMBIA: Agassiz, 15 km NE from

Agassiz, route 7, on overwintered leaves of *Betula papyrifera,* 13 May 2005, M.V.

Sogonov (BPI 877599, GenBank EU 254884); BRITISH COLUMBIA: Burnaby,

Burnaby Lake Regional Park, on overwintered leaves of *Betula papyrifera,* 12 May 2006,

M.V. Sogonov (BPI 877602, GenBank EU 254886). GERMANY: *Betula* sp., 1908,

Rehm (Rehm Ascomyceten 1794, BPI-bound, LECTOTYPE of *Gnomonia intermedia*

designated here); SCOTLAND: Blair Atholl Estates, *Betula pendula,* 23 March 2005, S.

Green (BPI 880534, EPITYPE of *Gnomonia intermedia* designated here, ex-epitype

culture AR 4147 = CBS 119188). RUSSIA, NOVGOROD PROVINCE: Kholm district,

Rdeysky Natural Reserve, vicinity of the village Fryunino, on overwintered leaves of

Betula nana, 11 June 2005, M.V. Sogonov (BPI 877496, GenBank EU 254881);

NOVGOROD PROVINCE: Naberezhnaya reki Lovat' str., on overwintered leaves of

Betula pendula, 23 August 2004, M.V. Sogonov (BPI 877498, GenBank EU 254878);

TVER' PROVINCE: Toropets district, v. Kosilovo, on overwintered leaves of *Betula*

pendula, 5 June 2005, M.V. Sogonov (BPI 877488B, GenBank EU 254887). UNITED

STATES, MARYLAND: Prince George's County, Beltsville, Little Paint Branch Park,

on overwintered leaves of *Betula nigra,* 17 March 2005, M.V. Sogonov (BPI 877597,

GenBank EU 254879); MARYLAND: Prince George's County, Beltsville, Little Paint

Branch Park, on overwintered leaves of *Betula nigra,* 11 April 2005, M.V. Sogonov (BPI

877598, GenBank EU 254880); MICHIGAN: Mackinac County, Cut River Bridge, on

overwintered leaves of *Alnus serrulata,* 25 May 2010, D.M. Walker (BPI 882263, culture

DMW 470.1 = CBS 131418); MICHIGAN: Sanilac County, roadside south of

Forestville, on overwintered leaves of *Betula papyrifera,* 27 May 2010, D.M. Walker

(BPI 882266, culture DMW 482.2); MICHIGAN: Mackinac County, Brevort

campground, on overwintered leaves of *Betula lutea,* 28 May 2010, D.M. Walker (BPI

882267, culture DMW 486.1 = CBS 131421).

Notes: Ophiognomonia intermedia causes a foliar disease and dieback of young birch

shoots (Green 2004). The anamorph/teleomorph connection between *Discula betulae*

(Westend.) Pennycook and *O. intermedia* was documented by Green and Castlebury

(2007). Ascospore appendages were observed in this species and *O. balsamiferae, O. gei,*

O. hiawathae, O. ischnostyla, O. longispora, O. melanostyla, O. michiganensis, O.

nipponicae, O. pseudoclavulata, O. pseudoischnostyla, and *O. setacea.*

Ophiognomonia ischnostyla (Desm.) Sogonov, Stud. Mycol. 62: 59. 2008. Fig. 4.19a–j.

Basionym: *Sphaeria ischnostyla* Desm., Annls Sci. nat., Bot., sér. 3 11: 357. 1849.

≡ *Gnomonia ischnostyla* (Desm.) Auersw. in Gonn. & Rabenh., Mycol. Europ.

5/6: 2. 1869.

MycoBank: MB 512185

Perithecia immersed, occasionally causing host tissue to swell, on leaf petioles and veins, hypophyllous to epiphyllous, solitary or aggregated up to two, glossy black, globose to subglobose (137–)139–162(–166) μm high × (179–)200–212(–257) μm diam (mean = 150 × 210, S.D. 13.2, 28.8, n1 = 5, n2 = 5). Necks central to marginal, mostly straight or curved to sinuous, occasionally swollen at the tip (350–)351–583(–590) μm long (mean = 480, S.D. 83.7, n = 11). Asci fusiform, apex rounded, acute or long tapering stipe, apical ring conspicuous (30–)34–42(–46) × 10–17 μm (mean = 38 × 14, S.D. 7.3, 4.9, n1 = 4, n2 = 2), ascospores arranged parallel or irregularly uniseriate, fusiform, ends rounded, straight to slightly curved, one-septate, median to indistinctly sub- or supramedian, slight constriction at septum, appendages subulate to whip-shaped or absent (14–)15–17(–18) × 2–3 μm (mean = 16 × 2, S.D. 1.4, 0.3, n1 = 30, n2 = 17).

Habitat: On overwintered leaves of *Carpinus betulus* L. and *Corylus avellana* L. (Betulaceae).

Distribution: Europe (France, Switzerland) and Russia (Novogorod Province).

Materials examined: FRANCE: *Carpinus betulus,* 1849, Desmazieres (Pl. Crypt. France 2084, BPI-bound, LECTOTYPE of *Sphaeria ischnostyla* designated here); RUSSIA, NOVOGOROD PROVINCE: Kholm district, Arboretum (Dendropark), near tree #560, on overwintered leaves of *Corylus avellana,* June 2005, M.V. Sogonov (BPI 877514B, EU 254899); SWITZERLAND: Ticino, Monte San Salvatore, on leaves of *Corylus avellana,* 28 May 2005, M.V. Sogonov (BPI 871054B, culture CBS 121234).

Notes: This species is morphologically similar to *O. pseudoischnostyla,* however, *O. ischnostyla* occurs on *Carpinus* spp. and *Corylus* spp., whereas *O. pseudoischnostyla* occurs on *Alnus* spp. and *Betula* spp. These two species both occur in Europe. Ascospore

appendages were observed in *O. ischnostyla* and *O. balsamiferae, O. gei, O. hiawathae, O. intermedia, O. longispora, O. melanostyla, O. michiganensis, O. nipponicae, O. pseudoclavulata, O. pseudoischnostyla,* and *O. setacea.* For a more detailed discussion on the taxonomy of this species, see Sogonov et al. (2008).

Ophiognomonia japonica D.M. Walker, sp. nov.   Fig. 4.20a–f.

MycoBank: MB 564089

Etymology: japonica refers to the host plant from which the holotype was collected.

Holotypus: JAPAN, GUNMA: Kawarayu, Kawarayu Trail, on overwintered leaves of *Prunus japonica,* 12 April 2010, D.M. Walker (BPI 882235, ex-type culture DMW 387.2 = CBS 131355).

Perithecia immersed, occasionally causing host tissue to swell and rupture, on leaf petioles and veins, solitary, glossy black, subglobose 175–222 μm high × (275–)325–340(–369) μm diam (mean = 206 × 327, S.D. 27, 39.3, n1 = 3, n2 = 4). Necks central to marginal, mostly straight or slightly curved, (437–)462–613(–619) μm long (mean = 540, S.D. 74.9, n = 10). Asci fusiform to ellipsoid, apex papillate or rounded, stipe acute, tapering, or rarely whip-shaped, apical ring conspicuous, (20–)22–23(–25) × 14–15 μm (mean = 23 × 14, S.D. 2, 0.5, n1 = 11, n2 = 10), ascospores arranged parallel or uniseriate. Ascospores fusiform with rounded ends, straight to slightly curved, one-septate, median to indistinctly supramedian, not constricted at septum, (12–)13–16(–17) × 2–3 μm (mean = 14 × 2, S.D. 0.9, 0.2, n1 = 30, n2 = 19).

Habitat: On overwintered leaves of *Prunus japonica* Thunb. (Rosaceae).

Distribution: Japan (Gunma prefecture).

Notes: Ophiognomonia japonica is one of 17 species known from Japan and one of two occuring on *Prunus* from that country. This species has shorter perithecial necks and smaller ascospores than *O. nipponicae,* which also occurs on *Prunus* in Japan.

Ophiognomonia kobayashii D.M. Walker, sp. nov.   Fig. 4.21a–i.

MycoBank: MB 564090

Etymology: kobayashii was named after Takao Kobayashi to honor his contributions to the taxonomy of the Diaporthales of Japan.

Holotypus: JAPAN, IBARAKI: Tsukuba City, Natural Forest, on overwintered leaves of *Castanea crenata,* 4 April 2010, D.M. Walker (BPI 882232, ex-type culture DMW 379.3 = CBS 131352).

Perithecia immersed, on leaf blades and veins, solitary, glossy black, globose to subglobose, (122–)127–169(–228) μm high × (124–)127–212(–217) μm diam (mean = 151 × 170, S.D. 31.1, 36.9, n1 = 10, n2 = 10). Necks central, mostly straight, sometimes curved, (329–)400–645(–699) μm long (mean = 493, S.D. 114.4, n = 15). Asci fusiform to ellipsoid, apex papillate or rounded, stipe tapering, apical ring conspicuous, (20–)21–30(–31) × (10–)11–16(–17) μm (mean = 26 × 14, S.D. 3.3, 1.8, n1 = 30, n2 = 30), ascospores arranged uniseriate or parallel, rarely multiseriate. Ascospores fusiform with rounded ends, straight to slightly curved, one-septate, median to indistinctly supramedian, slightly constricted at septum, each cell with one large and one small guttule, (11–)12–13(–14) × 2–3 μm (mean = 13 × 2, S.D. 0.9, 0.3, n1 = 30, n2 = 30).

Habitat: On overwintered leaves of *Castanea crenata* Siebold & Zucc. (Fagaceae).

Distribution: Japan (Ibaraki prefecture).

Materials examined: JAPAN, IBARAKI: Tsukuba City, Mt. Tsukuba, shrine trail, on

overwintered leaves of *Castanea crenata,* 8 April 2010, D.M. Walker (BPI 882245,

culture DMW 416.1 = CBS 131403); IBARAKI: Tsukuba City, Natural Forest, on

overwintered leaves of *Castanea crenata,* 4 April 2010, D.M. Walker (BPI 882229,

culture DMW374.2 = CBS 131350); IBARAKI: Ushiku, Ushiku Nature Reserve, on

overwintered leaves of *Castanea crenata,* 9 April 2010, D.M. Walker (BPI 882218,

culture DMW347.2 = CBS 131343).

Notes: Ophiognomonia kobayashii is one of 17 species known from Japan and one of

three occuring on *Castanea* in that country. A group of closely related species including

O. asiatica, O. kobayashii, O. otanii, and *O. sogonovii* are specific to *Quercus* spp. and

Castanea spp. within the Fagaceae (Fig. 4.2).

Ophiognomonia lenticulispora D.M. Walker, sp. nov.   Fig. 4.22a–f.

MycoBank: MB 564091

Etymology: lenticulispora refers to the lens shaped ascospores of this species.

Holotypus: UNITED STATES, MARYLAND: Prince George's County, Beltsville

Agricultural Research Center, on overwintered leaves of *Prunus* sp., 25 April 2011, D.M.

Walker (BPI 882287, ex-type culture DMW 544 = CBS 131363).

Perithecia immersed, on leaf blades and veins, hypophyllous, solitary or loosely

aggregated, glossy black, globose to subglobose, (189–)190–197(–204) µm high × (231–

)235–263(–271) µm diam (mean = 195 × 250, S.D. 7, 20, n1 = 4, n2 = 4). Necks central

to marginal, straight to slightly curved, (317–)323–327(–372) µm long (mean = 335, S.D.

25.2, n = 4). Asci ellipsoid to fusiform, apex rounded, stipe tapering, apical ring

conspicuous, (28–)30–37(–39) × (12–)13–15(–17) μm (mean = 35 × 14, S.D. 3.5, 1.3, n1
= 11, n2 = 10), ascospores arranged irregularly uniseriate to biseriate. Ascospores oval to
ellipsoid, rounded ends, straight, one-septate, median to indistinctly sub- or supramedian,
slightly to not constricted at septum, one cell slightly larger than the other, each cell with
several small guttules, (7–)8–9 × 3 μm (mean = 8 × 3, S.D. 0.6, 0.0, n1 = 30, n2 = 22).

Habitat: On overwintered leaves of *Prunus* sp. (Rosaceae).

Distribution: United States (MD).

Notes: Perithecia for this species only appeared on overwintered leaves after two weeks
of incubation in a moist chamber at 4 °C in complete darkness. Only *O. lenticulispora*
and *O. pseudoclavulata* have oval to ellipsoid ascospores in *Ophiognomonia*. These
species can be distinguished from each other by ascospore shape, size, and
presence/absence of appendages. *Ophignomonia lenticulispora* is one of two species of
Ophiognomonia known to occur on *Prunus* in the U.S.

Ophiognomonia leptostyla (Fr.) Sogonov, Stud. Mycol. 62: 62. 2008.　　　Fig. 4.23a–n.

Basionym: *Sphaeria leptostyla* Fr., Syst. Mycol. 2: 517. 1823.

　　　　≡ *Gnomonia leptostyla* (Fr.) Ces & De Not., Comment. Soc. Crittog. Ital. 1(4):

　　　　232. 1863.

MycoBank: MB 512187

Teleomorph: Perithecia immersed, on leaf blades, petioles, and veins, causing host tissue
to swell and rupture, hypophyllous, solitary or aggregated up to three, glossy black,
subglobose, 175–252(–302) μm high × 247–295(–309) μm diam (mean = 243 × 284,
S.D. 64, 32.5, n1 = 3, n2 = 3). Necks central, straight to curved, (240–)254–551(–601)

μm long (mean = 406, S.D. 150.3, n = 7). Asci ellipsoid to fusiform, apex rounded, stipe short, tapering, apical ring conspicuous, (28–)29–30(–33) × (12–)15–16(–17) μm (mean = 30 × 15, S.D. 2, 1.8, n1 = 8, n2 = 8), ascospores arranged irregularly uniseriate or parallel. Ascospores fusiform with rounded ends, straight to slightly curved, one-septate, median to indistinctly supramedian, not constricted at septum, each cell with several small guttules, (13–)14–15 × 2 μm (mean = 14 × 2, S.D. 0.6, 0.0, n1 = 27, n2 = 23).

Anamorph: Macroconidia lunate, reniform, or straight, basal cell bluntly rounded, apical cell with acute end, one-septate, median to indistinctly sub- or supramedian, distinctly constricted at septum, basal cell equal or larger than distal cell, hilum sometimes conspicuous, (22–)23–32(–35) × (6–)7–8(–9) μm (mean = 28 × 7, S.D. 3.6, 1, n1 = 14, n2 = 16). Microconidia fusiform, ends rounded, aseptate, hilum sometimes conspicuous, (6–)9–12(–13) × 2–3(–4) μm (mean = 11 × 3, S.D. 2.7, 0.7, n1 = 7, n2 = 7).

Habitat: On living and overwintered leaves of *Juglans nigra* L., *Juglans regia* L., and *Juglans* sp. L. (Juglandaceae) causing leaf blotch.

Distribution: Canada (Ontario), Europe (Austria, Bulgaria, Germany, Poland, Russia, Switzerland), Iran, and United States (AL, DE, IA, IL, MA, MD, NY, PA, VA, WV).

Materials examined: BULGARIA: Sofia region, Zapaden Park, on overwintered leaves of *Juglans regia*, 5 June 2005, D. Stoykov (BPI 878231). UNITED STATES, PENNSYLVANIA: Centre County, State College, on symptomatic leaves of *Juglans regia,* 29 September 1919, L.O. Overholts (BPI 870007); WEST VIRGINIA: Monongalia County, Morgantown, on symptomatic leaves of *Juglans nigra,* 12 September 1928, W.A. Archer (BPI 611485).

Notes: Ophiognomonia leptostyla is the cause of the virulent disease called walnut anthracnose or walnut leaf blotch, which is prevalent in the Midwestern and Eastern United States (Neely and Black 1976, Berry 1981, Juhasova et al. 2006). This species has a broad geographic distribution in Europe, the Middle East, and North America. This is one of three species that occur on *Juglans*. Several other species are known to occur on *Carya* and *Juglans* in the Juglandaceae including the pathogen *O. clavigignenti-juglandacearum.*

Ophiognomonia longispora D.M. Walker, sp. nov.   Fig. 4.24a–j.

MycoBank: MB 564093

Etymology: longispora refers to the long ascospores of this species.

Holotypus: : JAPAN, NAGANO: Ueda-shi, Sugadaira, arboretum at the Sugadaira Montane Research Center, on overwintered leaves of *Tilia maximowicziana,* 13 April 2010, D.M. Walker (BPI 882239, ex-type culture DMW 394.3 = CBS 131358).

Perithecia immersed, occasionally causing host tissue to swell and rupture, on leaf blades and veins, epiphyllous or hypophyllous, solitary or aggregated up to two, glossy black, subglobose, (175–)177–256(–261) μm high × (218–)262–378(–380) μm diam (mean = 216 × 308, S.D. 40.6, 71.3, n1 = 6, n2 = 5). Necks central to marginal, straight, curved, or slightly sinuous, (305–)399–1058(–1090) μm long (mean = 795, S.D. 235, n = 17). Asci narrowly fusiform, apex acute to rounded, stipe acute, (49–)51–60(–62) × (5–)6–9(–10) μm (mean = 55 × 7, S.D. 4, 1.5, n1 = 11, n2 = 10), ascospores arranged parallel. Ascospores narrowly clavate, filiform or sinuous, rounded ends, straight to curved, one-septate, supramedian, basal cell narrower than distal cell, with several small guttules,

(33–)34–43(–44) × 1–2 µm (mean = 38 × 1, S.D. 3.2, 0.4, n1 = 30, n2 = 28) and appendages at each end subulate to whip-shaped.

Habitat: On overwintered leaves of *Tilia maximowicziana* Shiras. (Malvaceae).

Distribution: Japan (Nagano prefecture).

Materials examined: JAPAN, NAGANO: Ueda-shi, Sugadaira, Arboretum at the Sugadaira Montane Research Center, on overwintered leaves of *Tilia maximowicziana,* 13 April 2010, D.M. Walker (BPI 882210, culture DMW 325.4 = CBS 131337).

Notes: *Ophiognomonia longispora* is one of 17 species known from Japan. In addition, this species is one of two that occur on *Tilia* and the only species on this host genus from Japan. The species *O. cordicarpa, O. longispora, O. melanostyla,* and *O. sassafras* share elongated filiform ascospores. These species form a closely related clade (Fig. 4.3). Ascospore appendages were only observed in *O. balsamiferae, O. gei, O. hiawathae, O. intermedia, O. ischnostyla, O. longispora, O. melanostyla, O. michiganensis, O. nipponicae, O. pseudoclavulata, O. pseudoischnostyla,* and *O. setacea.*

Ophiognomonia maximowiczianae D.M. Walker, sp. nov.   Fig. 4.25a–e.

MycoBank: MB 564094

Etymology: maximowiczianae refers to the plant host epithet from which the holotype was collected.

Holotypus: JAPAN, NAGANO: Ueda-shi, Sugadaira, Arboretum at Sugadaira Montane Research Center, on overwintered leaves of *Betula maximowicziana,* 13 April 2010, D.M. Walker (BPI 882238, ex-type culture DMW 392.1 = CBS 131357).

Perithecia immersed, occasionally causing host tissue to swell and rupture, on leaf blades and veins, solitary, glossy black, subglobose, 188–253 µm high × 207–287 µm diam (mean = 221 × 247, S.D. 46, 56.6, n1 = 2, n2 = 2). Necks central, straight to curved, (517–)658–868(–1010) µm long (mean = 763, S.D. 218.8, n = 4). Asci fusiform to ellipsoid, apex papillate or rounded, stipe acute or tapering, (23–)24–31(–33) × (11–)12–14(–15) µm (mean = 28 × 13, S.D. 2.9, 1.1, n1 = 19, n2 = 15), ascospores arranged irregularly uniseriate to multiseriate. Ascospores fusiform, rounded ends, straight to slightly curved, one-septate, median to indistinctly sub- or supramedian, not constricted at septum, (9–)10–11(–12) × 2 µm (mean = 11 × 2, S.D. 0.6, 0.0, n1 = 30, n2 = 30).

Habitat: On overwintered leaves of *Betula maximowicziana* Regel (Betulaceae).

Distribution: Japan (Nagano prefecture).

Notes: Ophiognomonia maximowiczianae is one of 17 species known from Japan, and the only species known to occur on *Betula* from that country.

Ophiognomonia melanostyla (DC.: Fr.) Berl., Icon. Fung. 2: 146. 1899.   Fig. 4.26a–h.

Basionym: *Sphaeria melanostyla* DC.: Fr., Fl. Franç. 5/6: 129. 1815: Syst. Mycol. 2: 517. 1823.

≡ *Gnomonia melanostyla* (DC.: Fr.) Auersw. in Gonn. & Rabenh., Mycol. Europ. 5/6: 28. 1869.

≡ *Gnomoniella melanostyla* (DC.: Fr.) Sacc., Syll. Fung. 1: 419. 1882.

≡ *Cryptoderis melanostyla* (DC.: Fr.) G. Winter, Rabenhorst's Kryptogamen Flora I, Abt. 2: 592. 1887.

Habitat: On overwintered leaves of *Tilia americana* L., *T. cordata* Mill., *T. heterophylla* Vent., and *Tilia* sp. L. (Malvaceae).

Distribution: Europe (Austria, Bulgaria, Czech Republic, France, Germany, Switzerland, Ukraine), Canada (Ontario), and United States (NY, PA).

Materials examined: FRANCE: Veronnes, on leaves of *Tilia* sp., 18 March 2011, A. Gardiennet (BPI 882278, culture DMW 522 = CBS 131430); FRANCE: Le Mazeldan, Barre des Cevenes, on leaves of *Tilia* sp., Y. Mourgues & M. Chovillon (BPI 882279, EPITYPE designated here, ex-epitype culture DMW 533 = CBS 131431); GERMANY: Frankfurt, Langen, on leaves of *Tilia heterophylla,* 2008, L.C. Mejía (BPI 879257, culture LCM 389.01 = CBS 128482); SWITZERLAND: Vaud, Lausanne, Parc Bourge, on *Tilia cordata,* 28 May 2005, M.V. Sogonov (BPI 877611, GenBank EU 254913); SWITZERLAND: Vaud, St. Cergue, on *Tilia cordata,* 20 May 2005, M.V. Sogonov (BPI 877610, GenBank EU 254911). UNITED STATES, NEW YORK: Sullivan County, Roscoe vicinity, area around Campbell Inn, on *Tilia americana,* July 2005, M.V. Sogonov (BPI 877608, GenBank EU 254912).

Notes: This is the type species of *Ophiognomonia.* For a detailed description of this species, see Sogonov et al. (2008). The species *O. cordicarpa, O. longispora, O. melanostyla,* and *O. sassafras* share elongated filiform ascospores and form a clade of closely related species (Fig. 4.3). Ascospore appendages were observed for this species as well as in *O. balsamiferae, O. gei, O. hiawathae, O. intermedia, O. ischnostyla, O. longispora, O. michiganensis, O. nipponicae, O. pseudoclavulata, O. pseudoischnostyla,* and *O. setacea.*

Ophiognomonia michiganensis D.M. Walker, sp. nov.   Fig. 4.27a–l.

MycoBank: MB 564095

Etymology: michiganensis refers to the state where the holotype was collected.

Holotypus: UNITED STATES, MICHIGAN: Houghton County, FJ McClain State Park, on overwintered leaves of *Betula papyrifera,* 30 April 2010, D.M. Walker (BPI 882255, ex-type culture DMW 454.3 = CBS 131412).

Perithecia immersed, occasionally causing host tissue to swell and rupture, on leaf blades and veins, solitary, glossy black, globose to subglobose (141–)188–265(–287) μm high × (178–)214–341(–405) μm diam (mean = 227 × 269, S.D. 34.5, 56.4, n1 = 16, n2 = 16). Occasionally two necks per base, necks central, straight or slightly curved, (228–)285–771(–879) μm long (mean = 501, S.D. 191, n = 20). Asci fusiform to ellipsoid, apex papillate or rounded, stipe tapering or occasionally acute to papillate, apical ring conspicuous (20–)23–34(–38) × (8–)11–17(–18) μm (mean = 27 × 14, S.D. 4, 2, n1 = 30, n2 = 30), ascospores arranged parallel, irregularly uniseriate, or multiseriate. Ascospores fusiform with rounded ends, straight to slightly curved, one-septate, median to indistinctly submedian or distinctly submedian when on *Prunus* sp., slightly to not constricted at septum, (9–)10–14(–15) × 2–3 μm (mean = 12 × 2, S.D. 1.8, 0.4, n1 = 30, n2 = 30). Appendages subulate to whip-shaped or absent.

Habitat: On overwintered leaves of *Alnus serrulata* Willd.*, Alnus* sp. Mill., *Betula alleghaniensis* Britton*, B. lutea* Michx.*, B. papyrifera* Marshall*, Betula* sp., *Carpinus americana* Michx (Betulaceae)*,* and *Prunus* sp. L. (Rosaceae).

Distribution: United States (MI, NC, NY).

Materials examined: UNITED STATES, MICHIGAN: Mackinac County, Brevort campground, on overwintered leaves of *Betula* sp.*,* 28 May 2010, D.M. Walker (BPI 882273, culture DMW 508.3 = CBS 131428); MICHIGAN: Mackinac County, Brevort campground, on overwintered leaves of *Betula papyrifera,* 27 May 2010, D.M. Walker (BPI 882254, culture DMW 451.2 = CBS 131411); MICHIGAN: Mackinac County, Brevort campground, on overwintered leaves of *Prunus* sp.*,* 27 May 2010, D.M. Walker (BPI 882271, culture DMW 505.3 = CBS 131427); MICHIGAN: Mackinac County, Cut River Bridge, on overwintered leaves of *Betula papyrifera,* 28 May 2010, D.M. Walker (BPI 882259, culture DMW 464.1); MICHIGAN: Schoolcraft County, Manistique, Indian campground, on overwintered leaves of *Alnus serrulata,* 28 May 2010, D.M. Walker (BPI 882269, culture DMW 494.2 = CBS 131423); MICHIGAN: Schoolcraft County, Manistique, Indian campground, on overwintered leaves of *Betula lutea,* 28 May 2010, D.M. Walker (BPI 882260, culture DMW 465.2 = CBS 131415); MICHIGAN: Roscommon County, Marl Lake, on overwintered leaves of *Betula papyrifera,* 27 May 2010, D.M. Walker (BPI 882258, culture DMW 461.2 = CBS 131414); MICHIGAN: Alger County, Miners Falls, on overwintered leaves of *Betula lutea,* 31 May 2010, D.M. Walker (BPI 882253, culture DMW447.1 = CBS 131410); MICHIGAN: Sanilac County, roadside park south of Forestville, on overwintered leaves of *Alnus* sp., 27 May 2010, D.M. Walker (BPI 882264, culture DMW 475.1 = CBS 131419); MICHIGAN: Alger County, Sable Falls, on overwintered leaves of *Alnus serrulata,* 29 May 2010, D.M. Walker (BPI 882268, culture DMW492.1 = CBS 131422); MICHIGAN: Alger County, Sable Falls, on overwintered leaves of *Betula papyrifera,* 29 May 2010, D.M. Walker (BPI 882265, culture DMW478.1 = CBS 131420); NEW YORK: Franklin County,

Adirondack High Peaks Region, Marcy Dam, on leaves of *Betula alleghaniensis,* 9 June

2007, L.C. Mejía (BPI 881487, culture LCM 161); NORTH CAROLINA: Haywood

County, Great Smoky Mountains National Park, Cataloochee, beginning of the trail, on

overwintered leaves of *Betula lenta,* 23 May 2006, M.V. Sogonov (BPI 877624);

NORTH CAROLINA: Haywood County, Great Smoky Mountains National Park,

Cataloochee, beginning of the trail, on overwintered leaves of *Carpinus americana,* 23

May 2006, M.V. Sogonov (BPI 877467B, culture CBS 121908).

Notes: This species is very common in the Eastern and Midwestern U.S. on several

genera in the Betulaceae. *Ophiognomonia setacea* and *O. michiganensis* are the only

species of *Ophiognomonia* that occur on more than one plant family or order. Ascospore

appendages were observed in *O. michiganensis* as well as *O. balsamiferae, O. gei, O.*

hiawathae, O. intermedia, O. ischnostyla, O. longispora, O. melanostyla, O. nipponicae,

O. pseudoclavulata, O. pseudoischnostyla, and *O. setacea.* Multiple-necked perithecia

were occasionally observed in *O. michiganensis* and *O. multirostrata,* a phenomenon

often occurring in culture, but rarely in nature for species of *Gnomoniopsis* (Walker et al.

2010) and *Ophiognomonia.*

Ophiognomonia micromegala (Ellis & Everh.) Sogonov, Stud. Mycol. 62: 63. 2008.

Fig. 4.28a–j.

Basionym: *Diaporthe micromegala* Ellis & Everh., Proc. Acad. nat. Sci. Philad. 45: 449.

1894.

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

1978.

MycoBank: MB 512188

Perithecia immersed, occasionally causing host tissue to swell, on leaf rachises and veins, solitary or aggregated 2–3, glossy black, globose to subglobose, (209–)227–379(–399) μm high × (351–)388–478(–491) μm diam (mean = 311 × 440, S.D. 59.1, 57.4, n1 = 13, n2 = 13). Necks central to marginal, straight to curved, (223–)227–537(–624) μm long (mean = 384, S.D. 121.8, n = 17). Asci fusiform, apex rounded, stipe short tapered or rounded, apical ring conspicuous, (51–)61–70 × (16–)18–20 μm (mean = 61 × 18, S.D. 9.5, 2, n1 = 3, n2 = 3), ascospores arranged irregularly parallel or multiseriate. Ascospores fusiform to broadly fusiform with bluntly rounded ends, straight to slightly curved, one-septate, median to indistinctly sub- or supramedian, slightly to distinctly constricted at septum, each cell with many large and small distinct guttules, (26–)27–50(–53) × (3–)4–11(–12) μm (mean = 40 × 7, S.D. 8.8, 3.5, n1 = 30, n2 = 30).

Habitat: On overwintered leaves and rachises of *Carya* sp. Nutt. (Juglandaceae).

Distribution: United States (MD).

Materials examined: UNITED STATES: *Carya* sp., 21 August 1893, A. Commons (Commons 2309, ISOTYPE of *Diaporthe micromegala,* NY); MARYLAND: Prince George's County, Beltsville Agricultural Reseach Center, on overwintered leaves and rachises of *Carya* sp., 21 April 2011, D.M. Walker (BPI 882280, EPITYPE designated here, ex-epitype culture DMW 535 = CBS 131432); MARYLAND: Prince George's County, Beltsville Agricultural Research Center, on overwintered leaves and rachises of *Carya* sp., 21 April 2011, D.M. Walker (BPI 882281, culture DMW 536 = CBS 131433).

Notes: Ophiognomonia micromegala has large fusiform ascospores unlike the narrowly fusiform ascospores of *O. melanostyla. Ophiognomonia micromegala* is one of seven

species that occur on plants in the Juglandaceae, and one of two that occur on *Carya* in the U.S.

Ophiognomonia monticola D.M. Walker, sp. nov.   Fig. 4.29a–g.

MycoBank: MB 564096

Etymology: monticola refers to Mt. Tsukuba where the holotype was collected.

Holotypus: JAPAN, IBARAKI: Tsukuba City, west side of Mt. Tsukuba, on overwintered leaves of *Carpinus* sp., 5 April 2010, D.M. Walker (BPI 882222, ex-type culture DMW 357.3 = CBS 131346).

Perithecia immersed, occasionally causing host tissue to swell, on leaf blades, petioles, and veins, solitary or aggregated up to two, glossy black, globose, subglobose, or ellipsoidal, (88–)109–123(–146) μm high × (110–)154–232(–247) μm diam (mean = 121 × 184, S.D. 23.4, 56.3, n1 = 5, n2 = 5). Necks central to marginal, straight, curved, or sinuate, (385–)390–595(–665) μm long (mean = 481, S.D. 81.5, n = 16). Asci fusiform to ellipsoid, apex rounded, stipe acute or long tapering, apical ring conspicuous, (19–)20–22(–25) × (15–)16–17 μm (mean = 22 × 16, S.D. 2.4, 0.9, n1 = 12, n2 = 12), ascospores arranged parallel or irregularly uniseriate. Ascospores fusiform with rounded ends, straight to slightly curved, one-septate, median to indistinctly sub- or supramedian, slightly constricted at septum, (12–)13–14 × 2–3 μm (mean = 13 × 2, S.D. 0.6, 0.4, n1 = 30, n2 = 16).

Habitat: On overwintered leaves of *Carpinus* sp. L. (Betulaceae).

Distribution: Japan (Ibaraki prefecture).

Materials examined: JAPAN, IBARAKI: Tsukuba City, west side of Mt. Tsukuba, on overwintered leaves of *Carpinus* sp., 5 April 2010, D.M. Walker (BPI 882243, culture DMW 405.3 = CBS 131361).

Notes: Ophiognomonia monticola is one of 17 species known from Japan. It is one of three species worldwide known to occur on *Carpinus*, and the only species to occur on this genus in Japan.

Ophiognomonia multirostrata D.M. Walker, sp. nov.   Fig. 4.30a–g.

MycoBank: MB 564097

Etymology: multirostrata refers to the multiple necks on perithecia of this species.

Holotypus: JAPAN, IBARAKI: Tsukuba City, Tsukuba Botanical Garden, on overwintered leaves of *Alnus firma,* 6 April 2010, D.M. Walker (BPI 882226, ex-type culture DMW 364.3 = CBS 131348).

Perithecia immersed, occasionally causing host tissue to swell and rupture, on leaf blades, petioles, and veins, hypophyllous and epiphyllous, solitary, glossy black, subglobose, (143–)228–260(–285) μm high × (195–)299–408(–501) μm diam (mean = 232 × 358, S.D. 54, 103, n1 = 5, n2 = 6). Necks central, straight, curved, sinuous or up to six necks per base, (752–)789–1066(–1203) μm long (mean = 920, S.D. 114, n = 18). Asci ellipsoid or fusiform, apex rounded, stipe acute to long tapering, (25–)26–39(–44) × (14–)15–17(–18) μm (mean = 32 × 16, S.D. 3.9, 1.3, n1 = 30, n2 = 26), ascospores arranged uniseriate to irregularly multiseriate. Ascospores fusiform, ends rounded, straight to slightly curved, one-septate, median to indistinctly sub- or supramedian, not constricted

at septum, each cell with 0–2 distinct and several small guttules, (11–)12–14(–15) × 2–3 μm (mean = 13 × 3, S.D. 1.1, 0.5, n1 = 30, n2 = 15).

Habitat: On overwintered leaves of *Alnus firma* Siebold & Zucc. (Betulaceae).

Distribution: Japan (Ibaraki prefecture).

Materials examined: JAPAN, IBARAKI: Ushiku, Ushiku Nature Reserve, on overwintered leaves of *Alnus firma,* 9 April 2010, D.M. Walker (BPI 882228, culture DMW 373.1 = CBS 131400); IBARAKI: Ushiku, Ushiku Nature Reserve, on overwintered leaves of *Alnus firma,* 9 April 2010, D.M. Walker (BPI 882248, culture DMW423.1 = CBS 131406).

Notes: *Ophiognomonia multirostrata* is one of 17 species known from Japan, and one of four occurring on *Alnus* from that country. Multiple-necked perithecia were occasionally observed in *O. michiganensis* and *O. multirostrata,* a phenomenon often occurring in culture, but rarely in nature for species of *Ophiognomonia. Ophiognomonia multirostrata* has slightly smaller ascospores than *O. naganoensis* and ascospores that overlap in size with *O. ibarakiensis,* which also occurs on *Alnus* from Japan.

Ophiognomonia naganoensis D.M. Walker, sp. nov.   Fig. 4.31a–f.

MycoBank: MB 564098

Etymology: naganoensis refers to the Japanese prefecture where the holotype was collected.

Holotypus: JAPAN, NAGANO: Ueda-shi, Sugadaira, waterfall at the Sugadaira Montane Research Center, on overwintered leaves of *Alnus hirsuta,* 13 April 2010, D.M. Walker (BPI 882246, ex-type culture DMW 418.3 = CBS 131404).

Perithecia immersed, occasionally causing host tissue to swell and rupture, on leaf blades and veins, hypophyllous and epiphyllous, solitary to aggregated up to two, glossy black, subglobose, (351–)372–386(–391) μm high × (432–)456–523(–565) μm diam (mean = 376 × 494, S.D. 18.8, 61, n1 = 4, n2 = 4). Necks central, straight, curved, or sinuous, (434–)491–913(–917) μm long (mean = 683, S.D. 127.5, n = 17). Asci ellipsoid to fusiform, apex rounded, stipe acute, rounded, or long tapering, apical ring conspicuous, (32–)33–47(–48) × (8–)9–20(–21) μm (mean = 38 × 16, S.D. 5.5, 4.5, n1 = 30, n2 = 30), ascospores arranged uniseriate to irregularly multiseriate. Ascospores fusiform, rounded ends, straight to slightly curved, one-septate, median to indistinctly sub- or supramedian, slightly constricted at septum, each cell with 0–2 distinct guttules and several small guttules, (18–)19–20(–21) × 3–4 μm (mean = 19 × 4, S.D. 0.8, 0.5, n1 = 30, n2 = 15).

Habitat: On overwintered leaves of *Alnus hirsuta* Turcz. and *A. hirsuta* Turcz. *f. sibirica* (Spach) H. Ohba (Betulaceae).

Distribution: Japan (Nagano prefecture).

Materials examined: JAPAN, NAGANO: Ueda-shi, Sugadaira, waterfall at the Sugadaira Montane Research Center, on overwintered leaves of *Alnus hirsuta* var. *sibirica,* 6 April 2010, D.M. Walker (BPI 882244, culture DMW 410.1 = CBS 131362); NAGANO: Ueda-shi, Sugadaira, waterfall at the Sugadaira Montane Research Center, on overwintered leaves of *Alnus hirsuta* var. *sibirica,* 13 April 2010, D.M. Walker (BPI 882211, culture DMW 331.2 = CBS 131338).

Notes: *Ophiognomonia naganoensis* is one of 17 species known from Japan, and one of four occurring on *Alnus* from that country. This species has slightly larger ascospores than *O. multirostrata* and *O. ibarakiensis,* which also occur on *Alnus* in Japan.

Ophiognomonia nana (Rehm) Sogonov, Stud. Mycol. 62: 63. 2008.　　　Fig. 4.32a–f.

Basionym: *Gnomoniella nana* Rehm, Hedwigia 42: 349. 1903.

MycoBank: MB 512189

Perithecia immersed to partially erumpent, causing host tissue to swell, on leaf blades and veins, epiphyllous and hypophyllous, solitary, glossy black, subglobose, 287 μm high × 347 μm diam (n1 = 1, n2 = 1). Necks central, straight to curved, 808–841 μm long (mean = 824, S.D. 23, n = 2). Asci obovoid to pyriform, apex papillate, stipe acute to long tapering, apical ring conspicuous, (42–)45–49(–60) × (21–)25–26(–27) μm (mean = 48 × 25, S.D. 7, 2.5, n1 = 5, n2 = 5), ascospores arranged irregularly multiseriate. Ascospores lenticular with acute to rounded ends, single celled, non-septate, lacking guttules, (12–)13–15(–16) × 6–7 μm (mean = 14 × 6, S.D. 1, 0.5, n1 = 30, n2 = 23).

Habitat: On leaves of *Betula nana* L. (Betulaceae).

Distribution: Europe (Germany).

Materials examined: GERMANY: Oberbayern, Bernried, on leaves of *Betula nana,* May 1903, Rehm (Rehm Ascomyceten 1522, LECTOTYPE of *Gnomoniella nana* designated here, FH).

Notes: This is the only species of *Ophiognomonia* with single celled, non-septate ascospores. Seven species of *Ophiognomonia* including *O. nana* occur on the genus *Betula* having a global temperate distribution.

Ophiognomonia nipponicae D.M. Walker, sp. nov.　　　　　　　Fig. 4.33a–i.

MycoBank: MB 564099

Etymology: nipponicae refers to the host plant epithet on which the holotype was collected.

Holotypus: JAPAN, IBARAKI: Tsukuba City, hiking trail around Mt. Tsukuba shrine, on overwintered leaves of *Prunus nipponica,* 6 April 2010, D.M. Walker (BPI 882249, ex-type culture DMW 424.1 = CBS 131407).

Perithecia immersed, on leaf blades and veins, solitary or in dense clusters, glossy black, globose to subglobose, (201–)244–298(–316) μm high × (–227)261–306(–422) μm diam (mean = 265 × 302, S.D. 45.4, 73.8, n1 = 5, n2 = 5). Necks central to marginal, curved, sinuous, or straight, (965–)968–1385(–1403) μm long (mean = 1153, S.D. 126.7, n = 15). Asci fusiform to ellipsoid, apex papillate or rounded, stipe tapering, (34–)35–46(–48) × (13–)14–15(–16) μm (mean = 38 × 15, S.D. 3.8, 0.8, n1 = 22, n2 = 21), ascospores arranged parallel or irregularly uniseriate. Ascospores fusiform with rounded ends, straight to slightly curved, one-septate, median to indistinctly sub- or supramedian, not constricted at septum, with appendages short, corniform to subulate or absent, (15–)16–17(–18) × 2 μm (mean = 17 × 2, S.D. 3.2, 0.0, n1 = 30, n2 = 23).

Habitat: On overwinterd leaves of *Prunus nipponica* Matsum. (Rosaceae).

Distribution: Japan (Ibaraki prefecture).

Notes: Ophiognomonia nipponicae is one of 17 species known from Japan, and one of two species occuring on *Prunus* from that country. This species has longer perithecial necks and larger ascospores than *O. japonica,* which also occurs on *Prunus* in Japan. Ascospore appendages were observed in *O. balsamiferae, O. gei, O. hiawathae, O. intermedia, O. ischnostyla, O. longispora, O. melanostyla, O. michiganensis, O. nipponicae, O. pseudoclavulata, O. pseudoischnostyla,* and *O. setacea.*

Ophiognomonia ostryae-virginianae D.M. Walker & L.C. Mejía, sp. nov. Fig. 4.34a–f.

MycoBank: MB 564100

Etymology: ostryae-virginianae refers to the host *Ostrya virginiana* from which the holotype was collected.

Holotypus: UNITED STATES, NEW YORK: Tompkins County, Ithaca, Buttermilk Falls State Park, on overwintered leaves of *Ostrya virginiana,* 7 June 2007, L.C. Mejía, det. D.M. Walker (BPI 879596, ex-type culture LCM 155.01 = CBS 131398).

Perithecia immersed, occasionally causing host tissue to swell, on leaf blades and veins, hypophyllous and epiphyllous, solitary, glossy black, globose to subglobose, (136–)146–166(–179) µm high × (164–)166–168(–200) µm diam (mean = 157 × 175, S.D. 19, 17, n1 = 4, n2 = 4). Necks central, straight to curved, (236–)325–432(–438) µm long (mean = 361, S.D. 74, n = 7). Asci ellipsoid to fusiform, apex papillate or rounded, stipe acute, rounded or tapering, apical ring conspicuous, (26–)27–40(–43) × (13–)14–16(–17) µm (mean = 32 × 15, S.D. 5.3, 1, n1 = 17, n2 = 17), ascospores arranged parallel to irregularly uniseriate. Ascospores fusiform with rounded ends, straight to slightly curved, one-septate, median, slightly to not constricted at septum, each cell with 0–2 distinct and several small guttules, (13–)14–15(–16) × 2–3 µm (mean = 14 × 2, S.D. 0.8, 0.5, n1 = 29, n2 = 21).

Habitat: On overwintered leaves of *Ostrya virginiana* K. Koch (Betulaceae).

Distribution: United States (NY).

Notes: This is the only species known to occur on *Ostrya* in the Betulaceae and may represent a novel host shift to this genus.

Ophiognomonia otanii D.M. Walker, sp. nov. Fig. 4.35a–h.

MycoBank: MB 564101

Etymology: otanii was named after Yoshio Otani to honor his contribution to the taxonomy of the Diaporthales of Japan.

Holotypus: JAPAN, NAGANO: Ueda-shi, Sugadaira, Kakuma River Trail, on overwintered leaves of *Castanea crenata,* 14 April 2010, D.M. Walker (BPI 882234, ex-type culture DMW385.1 = CBS 131354).

Perithecia immersed, occasionally causing host tissue to swell and rupture, on leaf petioles, veins, and blades, solitary or aggregated up to two, glossy black, subglobose, (165–)175–323(–330) high × (220–)226–387(–406) µm diam (mean = 242 × 310, S.D. 54, 62, n1 = 11, n2 = 11). Necks central to marginal, straight to curved, (482–)508–1032(–1174) µm long (mean = 746, S.D. 171.6, n = 30). Asci fusiform to ellipsoid, apex papillate or rounded, stipe tapering or occasionally papillate to rounded, apical ring conspicuous (24–)25–33(–34) × (13–)14–16(–17) µm (mean = 28 × 16, S.D. 2.6, 1.1, n1 = 26, n2 = 27), ascospores arranged parallel or irregularly uniseriate. Ascospores fusiform with rounded ends, mostly straight, rarely slightly curved, one-septate, indistinctly submedian, not constricted at septum, each cell with 0–2 distinct and several small guttules, 14–15(–16) × 2–3 µm (mean = 15 × 2, S.D. 0.7, 0.4, n1 = 30, n2 = 30).

Habitat: On overwintered leaves of *Castanea crenata* Siebold & Zucc. (Fagaceae).

Distribution: Japan (Gunma, Ibaraki, and Nagano prefectures).

Materials examined: JAPAN, GUNMA: Azuma, Azuma Forest Park, on overwintered leaves of *Castanea crenata,* 12 April 2010, D.M. Walker (BPI 882237, culture DMW

390.1 = CBS 131356); IBARAKI: Ushiku Nature Reserve, on overwintered leaves of *Castanea crenata,* 9 April 2010, D.M. Walker (BPI 882242, culture DMW 401.3 = CBS 131402); NAGANO: Ueda-shi, Sugadaira, Kakuma River Trail, on overwintered leaves of *Castanea crenata,* 14 April 2010, D.M. Walker (BPI 882241, culture DMW 397.1 = CBS 131360).

Notes: Ophiognomonia otanii is one of 17 species known from Japan and one of three occuring on *Castanea* in that country. A distinct submedian septum was observed in ascospores of four species including *O. alni-cordatae, O. apiospora, O. gei-montani,* and *O. otanii.* A group of closely related species including *O. asiatica, O. kobayashii, O. otanii,* and *O. sogonovii* are specific to *Quercus* spp. and *Castanea* spp. within the Fagaceae (Fig. 4.2).

Ophiognomonia padicola (Lib.) M. Monod, Beih. Sydowia 9: 158. 1983.

Basionym: *Sphaeria padicola* Lib., Plant. Cryptog. Arduenn. Cent. 2: 149. 1832.

≡ *Gnomonia padicola* (Lib.) Kleb., Z. Pflkrankh. 18: 137. 1908.

=*Ophiognomonia padi* Jaap, Verh. bot. Ver. Prov. Brandenburg 47: 87. 1905 *fide* Monod 1983.

Habitat: On overwintered leaves of *Prunus padus* L. (Rosaceae).

Distribution: Europe (Germany, Switzerland).

Notes: This is the only species of *Ophiognomonia* known to occur on *Prunus* from Europe. For a detailed description of this species, see Monod (1983).

Ophiognomonia pseudoclavulata Sogonov, Stud. Mycol. 62: 51. 2008.   Fig. 4.36a–g.

Habitat: On overwintered leaves of *Carya* sp. Nutt. *Carya tomentosa* (Lam.) Nutt. (Juglandaceae).

Distribution: United States (DC, IL, IN, MD, NC, NJ, PA, TN, VA).

Materials examined: UNITED STATES, MARYLAND: Frederick and Carroll Counties, Patapsco State Park, on overwintered leaves of *Carya* sp., 11 April 2011, D.M. Walker (BPI 882283, culture DMW 538 = CBS 131434); MARYLAND: Prince George's County, Beltsville Agricultural Research Center, on overwintered leaves of *Carya* sp., 28 April 2011, D.M. Walker (BPI 882290, culture DMW 551 = CBS 131367); PENNSYLVANIA: Kennett Square County, vicinity of Philadelphia, near Phillips mushroom farm, *Carya tomentosa,* 17 April 2004, M.V. Sogonov (HOLOTYPE, BPI 844280, ex-type culture AR4059 = CBS 121236).

Notes: Ophiognomonia lenticulispora and *O. pseudoclavulata* are the only species of *Ophiognomonia* with oval to ellipsoid ascospores. In addition, ascospore appendages were observed in *O. balsamiferae, O. gei, O. hiawathae, O. intermedia, O. ischnostyla, O. longispora, O. melanostyla, O. michiganensis, O. nipponicae, O. pseudoclavulata, O. pseudoischnostyla,* and *O. setacea.* For a detailed description of this species, see Sogonov et al. (2008).

Ophiognomonia pseudoischnostyla, D.M. Walker, sp. nov.　　　　　　Fig. 4.37a–f.

Mycobank: MB 564102

Etymology: pseudoischnostyla refers to the resemblance to *O. ischnostyla.*

Holotypus: RUSSIA, TVER' PROVINCE: Toropets district, vicinity of v. Bubonitsy, biological research station Chisty Les, on leaves of *Betula verrucosa,* 31 August 2004, M.V. Sogonov (BPI 877616, ex-type culture CBS 121228).

Perithecia immersed, occasionally causing host tissue to swell, on leaf petioles and veins, hypophyllous to epiphyllous, solitary or aggregated up to two, glossy black, globose to subglobose, (205–)222–272(–316) µm high $\times$ (227–)280– 397(–537) µm diam (mean = 248 $\times$ 335, S.D. 38, 96, n1 = 8, n2 = 8). Necks central to marginal, mostly straight or curved to sinuous, occasionally swollen at the tip, (509–)557–890(–902) µm long (mean = 684, S.D. 117.8, n = 15). Asci fusiform, apex rounded, acute stipe, apical ring conspicuous, (33–)34–47(–48) $\times$ (14–)16–17 µm (mean = 40 $\times$ 16, S.D. 6.3, 1.1, n1 = 8, n2 = 8), ascospores arranged parallel or irregularly uniseriate, fusiform, ends rounded, straight to slightly curved, one-septate, median to indistinctly sub- or supramedian, slight constriction at septum, appendages subulate to whip-shaped or absent, (13–)14–19(–20) $\times$ 2–3 µm (mean = 17 $\times$ 2, S.D. 2.1, 0.5, n1 = 30, n2 = 28).

Habitat: On overwintered leaves of *Alnus glutinosa* (L.) Gaertn.*, A. incana* (L.) Moench*,* and *Betula pubescens* Ehrh. (Betulaceae).

Distribution: Europe (Switzerland) and Russia (Novogorod and Tver' provinces).

Materials examined: RUSSIA, NOVOGOROD PROVINCE: Kholm district, Rdeysky Natural Reserve, vicinity of village Fryunino, on overwintered leaves of *Alnus glutinosa,* 11 June 2005, M.V. Sogonov (BPI 877619, GenBank EU 294900); TVER' PROVINCE: Toropets district, v. Kosilovo, on overwintered leaves of *Alnus glutinosa,* 5 June 2005, M.V. Sogonov (BPI 877617, EU 254907); TVER' PROVINCE: Toropets district, vicinity of v. Bubonitsy, biological research station Chisty Les, leaves of *Alnus glutinosa,* 14 June

2005, M.V. Sogonov (BPI 877618, GenBank EU 254908). SWITZERLAND: Wallis, Mörel, on overwintered leaves of *Alnus incana,* 28 May 2005, M.V. Sogonov (BPI 877620, GenBank EU 254898).

Notes: This species is morphologically similar to *O. ischnostyla,* however, *O. ischnostyla* occurs on *Carpinus* spp. and *Corylus* spp., whereas *O. pseudoischnostyla* occurs on *Alnus* spp. and *Betula* spp. Ascospore appendages were observed in this species and *O. balsamiferae, O. gei, O. hiawathae, O. intermedia, O. ischnostyla, O. longispora, O. melanostyla, O. michiganensis, O. nipponicae, O. pseudoclavulata,* and *O. setacea.*

Ophiognomonia pterocaryae D.M. Walker, sp. nov.   Fig. 4.38a–f.

MycoBank: MB 564103

Etymology: pterocaryae refers to the host genus on which the holotype was collected.

Holotypus: JAPAN, NAGANO: Ueda-shi, Sugadaira, Kakuma River Trail, on overwintered leaves of *Pterocarya rhoifolia,* 14 April 2010, D.M. Walker (BPI 882240, ex-type culture DMW 396.3 = CBS 131359).

Perithecia immersed to erumpent, occasionally causing host tissue to swell, on leaf blades, veins, petioles, and rachises, hypophyllous, solitary, loosely aggregated, or clusters up to three, glossy black, subglobose, (206–)212–312(–313) μm high × (287–)307–423(–424) μm diam (mean = 274 × 353, S.D. 41.6, 49.7, n1 = 9, n2 = 9). Necks central, marginal, or lateral, straight to curved, (351–)400–646(–726) μm long (mean = 533, S.D. 107.5, n = 13). Asci clavate to fusiform, apex rounded to papillate, stipe acute to long tapering, (38–)39–59(–68) × (15–)16–17(–18) μm (mean = 47 × 17, S.D. 8.5, 1.1, n1 = 16, n2 = 16), ascospores arranged uniseriate to irregularly multiseriate. Ascospores

fusiform with rounded ends, straight to slightly curved, one-septate, median to indistinctly sub- or supramedian, not constricted at septum, (14–)15–18(–19) × 3–4 µm (mean = 17 × 3, S.D. 1.1, 0.3, n1 = 30, n2 = 27).

Habitat: On overwintered leaves of *Pterocarya rhoifolia* Siebold & Zucc. (Juglandaceae).

Distribution: Japan (Nagano prefecture).

Materials examined: JAPAN, NAGANO: Ueda-shi, Sugadaira, Kakuma River Trail, on overwintered leaves of *Pterocarya rhoifolia,* 14 April 2010, D.M. Walker (BPI 882219, culture DMW 350.2 = CBS 131344).

Notes: *Ophiognomonia pterocaryae* is one of 17 species known from Japan, and one of two known to occur on *Pterocarya* from that country. Of the species on *Pterocarya, O. cordicarpa* has long filiform ascospores, whereas *O. pterocaryae* has much shorter fusiform ascospores. Several other species are known to occur on *Carya* and *Juglans* in the Juglandaceae, including the pathogens *O. leptostyla* and *O. clavigignenti-juglandacearum.*

Ophiognomonia quercus-gambellii (M. Monod) D.M. Walker, comb. nov. Fig. 4.39a–h.

Basionym: *Gnomonia quercus-gambellii* M. Monod, Beih. Sydowia 9: 98. 1983.

MycoBank: MB 564104

Perithecia immersed, causing host tissue to swell, rupture, and expose bases, on leaf blades and veins, hypophyllous, solitary, glossy black, globose to subglobose, (142–)163–209(–229) µm high × (157–)178–255(–268) µm diam (mean = 192 × 221, S.D. 29, 36, n1 = 10, n2 = 10). Necks central, rarely two necks per base, upright, straight to curved

or sinuous, tips often swollen, (229–)331–439(–480) μm long (mean = 310, S.D. 85, n = 13). Asci fusiform to obovoid with rounded apex and stipe, apical ring sometimes conspicuous, (29–)30–44(–46) × (10–)11–15(–16) μm (mean = 38 × 12, S.D. 4.7, 1.8, n1 = 21, n2 = 21), ascospores arranged obliquely uniseriate to irregularly multiseriate. Ascospores fusiform with rounded ends, straight to slightly curved, one-septate, median to submedian or supramedian, not constricted or slightly constricted at septum, each cell with several small guttules, (11–)12–14(–15) × (2–)3–4 μm (mean = 13 × 3, S.D. 0.9, 0.6, n1 = 30, n2 = 30).

Habitat: On overwintered leaves of *Quercus gambellii* Liebm. and *Q. kelloggii* Newberry (Fagaceae).

Distribution: United States (AZ, OR).

Materials examined: UNITED STATES, ARIZONA: Coconino County, North Rim of the Grand Canyon, leaves of *Quercus gambellii,* 18 August 1973, M.E. Barr (Barr 6095 collected as *Gnomonia fasciculata*, HOLOTYPE of *Gnomonia quercus-gambellii,* NY); OREGON: Jackson County, McGregor and Casey Park, on overwintered leaves of *Quercus kelloggii,* 20 May 2010, D.M. Walker (BPI 882202, EPITYPE designated here, ex-epitype culture DMW 117.1 = CBS 131397).

Notes: Ophiognomonia quercus-gambellii, based on *Gnomonia quercus-gambellii*, was originally collected by M.E. Barr in Arizona, U.S.A. who identified this specimen as *Gnomonia fasciculata* Fuckel (Barr, 1978). A specimen was collected and culture obtained (BPI 882202 = CBS 131397) on *Quercus kellogii* from Oregon, U.S.A. that is morphologically identical to the type specimen of *G. quercus-gambellii* (Barr 6095). The Oregon specimen is designated as the epitype. Both *O. quercus-gambellii* and *G.*

fasciculata occur on *Quercus* spp. *Ophiognomonia quercus-gambellii* is one of four species of *Ophiognomonia* known to occur exclusively on *Quercus.*

Ophiognomonia rosae (Fuckel) Kirschst., Annls mycol. 37(1/2): 129. 1939. Fig. 4.40a–h.

Basionym: *Gnomonia rosae* Fuckel, Jb. nassau. Ver. Naturk. 23–24: 122. 1870.

≡ *Gnomoniella rosae* (Fuckel) Sacc., Syll. Fung. 1: 416. 1882.

MycoBank: MB 276702

Perithecia immersed, occasionally causing host tissue to swell, on leaf blades and veins, hypophyllous, solitary, glossy black, subglobose, (249–)296–312(–336) μm high × (247–)300–389(–442) μm diam (mean = 298 × 338, S.D. 32, 77, n1 = 5, n2 = 5). Necks central, straight to curved, (245–)430–1451(–1784) μm long (mean = 611, S.D. 223.1, n = 10). Asci fusiform, apex papillate or rounded, stipe long tapering, apical ring conspicuous, (26–)29–38(–40) × (11–)12–15(–16) μm (mean = 34 × 13, S.D. 3.3, 1.3, n1 = 25, n2 = 29), ascospores arranged irregularly multiseriate or parallel. Ascospores narrowly fusiform to fusiform, straight to slightly curved, one-septate, median to indistinctly sub- or supramedian, slightly constricted at septum, each cell with 0–2 distinct guttules, (13–)14–20(–21) × (1–)2–3 μm (mean = 16 × 2, S.D. 2.4, 0.6, n1 = 30, n2 = 30).

Habitat: On overwintered leaves of *Fragaria vesca* L., *Rosa* sp. L., and *Rubus* sp. L. (Rosaceae).

Distribution: Europe (France, Switzerland) and United States (OR).

Materials examined: FRANCE: Veronnes, leaves of *Rubus* sp., April 2011, A. Gardiennet (BPI 882286, EPITYPE designated here, ex-epitype culture DMW 543 = CBS 131365); SWITZERLAND: *Rosa* sp., 1870, Fuckel, (Fuckel Fungi Rhenani 1790,

LECTOTYPE of *Sphaeria rosae* designated here, FH). UNITED STATES, OREGON: Jackson County, Prospect, River Bridge campground, Upper Rouge River trailhead, on overwintered leaves of *Fragaria vesca,* D.M. Walker (BPI 882201, culture DMW 108.2 = CBS 128442).

Notes: This species is one of nine that occur on hosts in the Rosaceae, and one of eight that occur on multiple genera in this host family. *Ophiognomonia rosae* has long perithecial necks relative to many other species of *Ophiognomonia.*

Ophiognomonia rubi-idaei (M. Monod) Sogonov, Stud. Mycol. 62: 64. 2008. Fig. 4.41a–g.

Basionym: *Gnomonia rubi-idaei* M. Monod, Beih. Sydowia 9: 106. 1983.

MycoBank: MB 512190

Perithecia immersed, occasionally causing host tissue to swell, on leaf blades and veins, hypophyllous, solitary, glossy black, subglobose, (325–)373–520(–521) μm high × (447–)483–686(–719) μm diam (mean = 430 × 588, S.D. 77, 105, n1 = 7, n2 = 7). Necks central to lateral, straight to curved, (835–)883–1973(–2054) μm long (mean = 1460, S.D. 521, n = 6). Asci fusiform, narrow, apex rounded or papillate, stipe long tapering, apical ring conspicuous, (27–)28–48(–49) × (7–)8–14(–17) μm (mean = 38 × 10, S.D. 7.1, 2.6, n1 = 24, n2 = 21), ascospores arranged regularly to irregularly parallel or multiseriate. Ascospores narrowly fusiform with rounded ends, straight to slightly curved, one-septate, median to submedian, not constricted at septum, (12–)13–16(–17) × 2 μm (mean = 15 × 2, S.D. 1.1, 0, n1 = 30, n2 = 25).

Habitat: On overwintered leaves of *Rubus idaeus* L., *Rubus* sp. L., and *R. spectabilis* Pursh. (Rosaceae).

Distribution: Canada (British Columbia) and Europe (Switzerland).

Materials examined: CANADA, BRITISH COLUMBIA: Manning Provincial Park, on overwintered leaves of *Rubus* sp., 13 May 2006, M.V. Sogonov (BPI 877559B, GenBank EU 254939); BRITISH COLUMBIA: Victoria Island, Route 14, on overwintered leaves of *Rubus spectabilis,* 10 May 2006, M.V. Sogonov (BPI 877638, GenBank EU 254938). SWITZERLAND: on overwintered leaves of *Rubus idaeus,* 21 May 2005, M.V. Sogonov (BPI 877637, GenBank EU 254937).

Notes: This species is one of nine that occur on hosts in the Rosaceae, and the only species of *Ophiognomonia* known to occur exclusively on *Rubus.* This species has the second longest perithecial neck length. Only *O. apiospora* has a longer perithecial neck in the genus *Ophiognomonia.*

Ophiognomonia sassafras (Ellis & Everh.) M. Monod, Beih. Sydowia 9: 157. 1983. Fig. 4.42a–j.

Basionym: *Gnomonia sassafras* Ellis & Everh., Bull. Torrey bot. Club 10(7): 98. 1883.

    ≡ *Pleuroceras sassafras* (Ellis & Everh.) M.E. Barr, Mycol. Mem. 7: 122. 1978.

MycoBank: MB 108295

Perithecia immersed, occasionally causing host tissue to swell and rupture, on leaf blades and veins, hypophyllous or epiphyllous, solitary or loosely grouped, glossy black, globose to subglobose, (216–)217–278(–290) µm high × (279–)287–333(–345) µm diam (mean = 249 × 279, S.D. 26, 90.8, n1 = 11, n2 = 12). Necks central to marginal, straight

to slightly sinuous, (520–)543–950(–1058) µm long (mean = 776, S.D. 147, n = 22). Asci
narrowly fusiform, apex rounded, stipe rounded or tapering, apical ring conspicuous,
(59–)62–68(–70) × (4–)5–7 µm (mean = 66 × 6, S.D. 3.2, 1.2, n1 = 11, n2 = 11),
ascospores arranged obliquely parallel. Ascospores clavately filiform to sinuous, rounded
ends, one-septate, supramedian, not constricted at septum, basal cell narrower than distal
cell, several small guttules, (42–)43–48(–52) × 1– 2 µm (mean = 44 × 2, S.D. 8.6, 0.5, n1
= 30, n2 = 30).

Habitat: On overwintered leaves of *Sassafras albidum* (Nutt.) Nees and *S. officinale*
Siebold (Lauraceae).

Distribution: United States (MD, OH, WV).

Materials examined: UNITED STATES, MARYLAND: Prince George's County,
Beltsville Agricultural Research Center, on overwintered leaves of *Sassafras albidum,* 25
April 2010, D.M. Walker (BPI 882282, culture DMW 537); MARYLAND: Prince
George's County, Beltsville Agricultural Research Center, on overwintered leaves of
Sassafras albidum, 25 April 2010, D.M. Walker (BPI 882285, EPITYPE designated here,
ex-epitype culture DMW 542 = CBS 131366); OHIO: Fairfield County, fallen leaves of
Sassafras officinale, May 1883, Kellerman (NY 00921946, HOLOTYPE of *Gnomonia
sassafras*); WEST VIRGINIA: Pendleton County, Franklin, on overwintered leaves of
Sassafras albidum, 2 April 2010, coll. C.M. Milensky, det. D.M. Walker (BPI 882284,
culture DMW 541 = CBS 131435).

Notes: Ophiognomonia sassafras is the only species of Gnomoniaceae known to occur
on *Sassafras* in the Lauraceae and may represent a shift to a novel host family. The

species *O. cordicarpa, O. longispora, O. melanostyla,* and *O. sassafras* share elongated filiform ascospores and form a clade of closely related species (Fig. 4.3).

Ophiognomonia setacea (Pers.: Fr.) Sogonov, Stud. Mycol. 62: 64. 2008. Fig. 4.43a–f. Basionym: *Sphaeria setacea* Pers.: Fr., Syn. Method. Fung. 62. 1801 : Syst. Mycol. 2: 517. 1823.

≡ *Gnomonia setacea* (Pers.: Fr.) Ces. & De Not., Comment. Soc. Crittog. Ital. 1: 232. 1863.

Habitat: On overwintered leaves of *Acer* sp., *Castanea dentata* L., *Castanea* sp., *Corylus* sp., *Fagus* sp., *Platanus* sp., *Quercus acutissima* Carruth., *Q. alba* L., *Q. bicolor* Willd., *Q. cerris* L., *Q. macrocarpa* Michx, *Q. montana* Willd., *Q. palustris* Münchh., *Q. phellos* L., *Q. pubescens* Willd., *Q. robur* L., and *Quercus* sp. (Betulaceae, Fagaceae, Platanaceae, Sapindaceae).

Distribution: Canada (Ontario), Europe (Austria, Bulgaria, Germany, Italy, Montenegro, Sweden, Switzerland), Japan (Ibaraki prefecture), and United States (LA, MD, MI, NJ, NY, OH, PA, TN, VA, WV).

Materials examined: JAPAN, IBARAKI: Tsukuba City, Botanical Garden, on overwintered leaves of *Quercus acutissima,* 5 April 2010, D.M. Walker (BPI 882212, culture DMW 333.2 = CBS 131339); IBARAKI: Ushiku, Ushiku nature reserve, on overwintered leaves of *Quercus acutissima,* 9 April 2010, D.M. Walker (BPI 882223, culture DMW 358.4). UNITED STATES, MICHIGAN: Sanilac County, Lakeport campground, on overwintered leaves of *Quercus* sp., 27 May 2010, D.M. Walker (BPI 882275, culture DMW 510.1); NEW JERSEY: Middlesex County, New Brunswick,

Kilmer reserve, on overwintered leaves of *Quercus palustris,* 24 April 2009, D.M.

Walker (BPI 882204, culture DMW 289.1);

Notes: This is the only globally distributed species found in most temperate parts of the

world. *Ophiognomonia setacea* and *O. michiganensis* are the only species of

Ophiognomonia that occur on several different host plant families or orders. For a

detailed description of this species, see Sogonov et al. (2008).

Ophiognomonia sogonovii D.M. Walker, sp. nov.   Fig. 4.44a–i.

MycoBank: MB 564105

Etymology: sogonovii was named after Mikhail Sogonov to honor his contribution to the

taxonomy of the Gnomoniaceae.

Holotypus: JAPAN, IBARAKI: Tsukuba City, West side of Mt. Tsukuba, on

overwintered leaves of *Quercus serrata,* 5 April 2010, D.M. Walker (BPI 882214, ex-

type culture DMW 337.1 = CBS 131341).

Perithecia immersed, on leaf petioles, veins, and blades, solitary or aggregated up to two,

glossy black, subglobose, (166–)188–294(–335) μm high × (204–)243–397(–498) μm

diam (mean = 224 × 322, S.D. 50.7, 81.2, n1 = 12, n2 = 11). Necks central to marginal,

mostly straight or slightly curved, (513–)543–949(–1172) μm long (mean = 724, S.D.

184.5, n = 13). Asci fusiform to ellipsoid, apex papillate or rounded, stipe tapering, apical

ring large, conspicuous (22–)23–38(–39) × (11–)12–19(–20) μm (mean = 32 × 15, S.D.

4.4, 2.4, n1 = 28, n2 = 28), ascospores arranged irregularly uniseriate or multiseriate.

Ascospores fusiform with rounded ends, straight to slightly curved, one-septate, distinctly

submedian or supramedian, not constricted or slightly constricted at septum, each cell

with 0–2 distinct or several small guttules, (12–)13–16(–17) × (2–)3–4 µm (mean = 14 × 3, S.D. 1.4, 0.6, n1 = 30, n2 = 30).

Habitat: On overwintered leaves of *Quercus mongolica* Fisch. ex Turcz.*, Q. mongolica* Fisch. ex Turcz.*,* var. *grosseserrata* (Blume) Rehder & E.H. Wilson*,* and *Q. serrata* Murray (Fagaceae).

Distribution: Japan (Ibaraki and Nagano prefectures) and Russia (Primorsky Territory).

Materials examined: JAPAN, IBARAKI: Tsukuba City, West side of Mt. Tsukuba, on overwintered leaves of *Quercus mongolica,* 5 April 2010, D.M. Walker (BPI 882213, cultures DMW 336.1, DMW336.3 = CBS 131340); NAGANO: Ueda-shi, Sugadaira, Arboretum at Sugadaira Montane Research Center, on overwintered leaves of *Quercus mongolica* var. *grosseserrata,* 13 April 2010, D.M. Walker (BPI 882221, culture DMW 353.1 = CBS 131661). RUSSIA, PRIMORSKY TERRITORY: Russky Island, on dead leaves of *Quercus mongolica,* 25 May 2003, L.N. Vasilyeva (BPI 872323, culture CBS 121914).

Notes: This is one of four species of *Ophiognomonia* known to occur exclusively on *Quercus.* A group of closely related species including *O. asiatica, O. kobayashii, O. otanii,* and *O. sogonovii* are specific to *Quercus* spp. and *Castanea* spp. within the Fagaceae (Fig. 4.2).

Ophiognomonia trientensis (M. Monod) Sogonov, Stud. Mycol. 62: 64. 2008.

Fig. 4.45a–g.

Basionym: *Gnomonia trientensis* M. Monod, Beih. Sydowia 9: 90. 1983.

MycoBank: MB 512192

Perithecia immersed, occasionally causing host tissue to swell, on leaf blades and veins, hypophyllous and epiphyllous, solitary or aggregated up to two, glossy black to cream, subglobose, (134–)136–255(–264) μm high × (203–)213–364(–386) μm diam (mean = 198 × 288, S.D. 62, 71.3, n1 = 6, n2 = 8). Necks central, straight, curved, or contorted, (302–)326–1019(–1073) μm long (mean = 597, S.D. 236, n = 21). Asci ellipsoid to fusiform, apex rounded, stipe rounded to acute, (33–)35–41(–44) × (7–)8–10(–11) μm (mean = 37 × 9, S.D. 3.9, 1.4, n1 = 8, n2 = 8), ascospores irregularly uniseriate, biseriate, overlapping. Ascospores oval to broadly fusiform with rounded ends, straight, one-septate, median to sub- or supramedian, not constricted at septum, each cell with two large and several small guttules, 9–10 × 3–4 μm (mean = 10 × 3, S.D. 0.5, 0.3, n1 = 25, n2 = 20).

Habitat: On overwintered leaves of *Alnus tenuifolia* Nutt. and *A. viridis* (Chaix) DC. (Betulaceae).

Distribution: Canada (British Columbia), Europe (Switzerland), and United States (WA).

Materials examined: CANADA, BRITISH COLUMBIA: Hope, on overwintered leaves of *Alnus tenuifolia,* 13 May 2006, M.V. Sogonov (BPI 877672, GenBank EU 254986); BRITISH COLUMBIA: Manning Provincial Park, Engineers Trail, on overwintered leaves of *Alnus viridis,* 13 May 2006, M.V. Sogonov (BPI 877673, GenBank EU 254987). UNITED STATES, ALASKA: Kenai Peninsula County, In between Augustine Island, Shaw Island, and Kamishak Bay, on overwintered leaves of *Alnus* sp., 21 June 2011, D.M. Walker (BPI 882638, DMW 554 = CBS 131604); WASHINGTON: King County, Mount Baker-Snoqualmie National Forest, Snoqualmie Ranger District, near exit

42 on highway US 90, on overwintered but still attached leaves of *Alnus viridis,* 16 May 2006, M.V. Sogonov (BPI 877674, GenBank EU 254985).

Notes: Only *O. gardiennetii* and *O. trientensis* are known to occur exclusively on *Alnus* from the U.S. Morphologically these species are very similar and can only be distinguished by DNA sequence data. In addition, they form a clade of closely related species with the butternut pathogen *O. clavigignenti-juglandacearum* (Fig 4.4)*.*

 Ophiognomonia tucumanensis L.C. Mejía & D.M. Walker, sp. nov.   Fig. 4.46a–g.

MycoBank: MB 564106

Etymology: tucumanensis refers to the province of Tucuman where the holotype was collected.

Holotypus: ARGENTINA, TUCUMAN: on dead leaves of *Alnus acuminata,* 20 April 2011, A.Y. Rossman, det. D.M. Walker (BPI 882288, ex-type culture DMW 549 = CBS 131364).

Perithecia immersed to erumpent, causing host tissue to swell, on leaf blades, veins, and petioles, hypophyllous and epiphyllous, solitary or loosely aggregated up to four, glossy black, globose to subglobose, (198–)203–277(–285) µm high × (191–)202–296(–320) µm diam (mean = 238 × 257, S.D. 28.2, 40.3, n1 = 10, n2 = 10). Necks central to marginal, straight, curved, or slightly sinuous, neck base occasionally disc shaped, (298–)436–1056(–1059) µm long (mean = 756, S.D. 213, n = 24). Asci ellipsoid to fusiform with rounded apex, stipe acute or short tapering, apical ring sometimes conspicuous, (21–)22–29(–32) × (11–)12–16(–17) µm (mean = 25 × 14, S.D. 2.6, 1.4, n1 = 26, n2 = 25), ascospores arranged irregularly uniseriate to multiseriate. Ascospores fusiform with

rounded ends, straight to slightly curved, one-septate, median to indistinctly sub- or supramedian, slightly to not constricted at septum, each cell with 0–2 large and several small guttules, (9–)10–12(–13) × 2–3(–4) μm (mean = 12 × 3, S.D. 1.2, 0.4, n1 = 30, n2 = 30).

Habitat: On dead leaves of *Alnus acuminata* Kunth (Betulaceae).

Distribution: Argentina (Tucuman).

Materials examined: ARGENTINA, TUCUMAN: Villa Nougues, dead leaves of *Alnus acuminata,* 16 November 2008, L.C. Mejía, det. D.M. Walker (BPI 879565, culture LCM 622.01 = CBS 131368).

Notes: Ophiognomonia tucumanensis is the only species of Gnomoniaceae known from South America on *Alnus acuminata.* This plant host occurs in montane cloud forests from Mexico to the Andes. *Ophiognomonia tucumanensis* represents the southernmost distribution of the Gnomoniaceae. Only *O. bugabensis* and *O. tucumanensis* are known to occur on *Alnus acuminata.* These species can be distinguished by geographic location. In addition, *O. bugabensis* has larger ascospores and shorter perithecial necks than *O. tucumanensis.*

Ophiognomonia vasiljevae Sogonov, Stud. Mycol. 62: 53. 2008.

Habitat: On overwintered leaves of *Juglans nigra* L. and *Juglans* sp. L. (Juglandaceae).

Distribution: United States (MD, TN, VA).

Materials examined: UNITED STATES, MARYLAND: Frederick and Carroll Counties, Patapsco State Park, on overwintered leaves of *Juglans* sp., 11 April 2011, D.M. Walker (BPI 882289, culture DMW 550 = CBS 131436); TENNESSEE: Blount County, Great

Smoky Mountains National Park, along loop near Methodist Church, on leaves of

Juglans nigra, 24 May 2006, M.V. Sogonov (HOLOTYPE, BPI 877671, ex-type culture

CBS 121253); VIRGINIA: Fairfax County, Burke, Zion Rd. and Guinea Rd., on leaves of

Juglans nigra, 1 June 2009, M.V. Sogonov (BPI 882206, culture DMW 303.3 = CBS

128353).

Notes: This is one of three species that occur on *Juglans.* Several other species are known

to occur on *Carya* and *Juglans* in the Juglandaceae including the pathogens *O. leptostyla*

and *O. clavigignenti-juglandacearum.* For a detailed description of this species, see

Sogonov et al. (2008).

Synoptic Key to Species in *Ophiognomonia*

Perithecia

1. Average Height

100–200 µm..1, 12, 14, 16, 18, 19, 26, 31, 34, 37, 43

200–300 µm..............2, 4, 6, 8, 9, 10, 13, 15, 17, 20, 21, 22, 23, 24, 27, 29, 30, 32,

35, 36, 38, 40, 42, 44, 41

300–400 µm...3, 5, 11, 25, 28, 45, 33

400–500 µm..39

2. Average Diameter

100–200 µm...14, 18, 26, 31

200–300 µm..........................1, 2, 9, 12, 13, 15, 16, 19, 20, 22, 24, 34, 37, 40, 43, 44

300–400 µm........................4, 6, 10, 11, 17, 21, 23, 27, 29, 30, 32, 35, 36, 38, 42, 41

400–500 µm..5, 8, 25, 28

500–600 µm..39, 33

600–700 µm...45

700–800 µm...3

Perithecial Neck

1. Average Length

100–200 µm...34

200–300

µm...1

300–400 µm..11, 12, 13, 19, 25, 31, 37

400–500 µm..6, 9, 16, 18, 20, 26

500–600 µm...13, 17, 24, 36, 43

600–700 µm..15, 28, 35, 38, 45, 41

700–800 µm...4, 21, 22, 32, 40, 42, 44

800–900 µm...29

900–1000 µm..5, 8, 27, 33

1000–1100 µm...2, 23

1100–1200 µm..30

1400–1500 µm...10, 39

2200–2300 µm...3

Ascospores

1. Shape

    filiform..8, 21, 23, 40, 33

    fusiform.........1, 2, 3, 4, 5, 9, 10, 11, 12, 13, 15, 16, 17, 18, 20, 22, 24, 26, 27, 28, 30, 31, 32, 35, 36, 37, 38, 39, 42, 44, 45, 41

    broadly fusiform..6, 25, 43

    broadly ellipsoid...34

    oval...14, 19

    lenticular..29

2. Septation

    aseptate...29

    one-septate.........1, 2, 3, 4, 5, 6, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 30, 31, 32, 34, 35, 36, 37, 38, 39, 40, 42, 43, 44, 45, 33, 41

3. Location of Septation

    submedian...1, 3, 10, 11, 24, 32, 42

    median...........2, 4, 5, 14, 15, 16, 17, 18, 19, 20, 22, 25, 26, 27, 28, 30, 31, 34, 35, 36, 37, 38, 39, 43, 44, 45, 33, 41

    supramedian...6, 8, 9, 12, 13, 21, 23, 40, 42

6. Appendages

    present.....................................5, 10, 13, 15, 16, 21, 23, 24, 30, 34, 35, 41

absent.........1, 2, 3, 4, 6, 8, 9, 11, 12, 14, 17, 18, 19, 20, 22, 25, 26, 27, 28, 29, 31, 32, 36, 37, 38, 39, 40, 42, 43, 44, 45, 33

4. Average Length

5–10 µm..19, 34, 43

10–15 µm.............2, 4, 9, 11, 13, 14, 15, 17, 18, 20, 22, 24, 26, 27, 29, 31, 37, 44, 41

15–20 µm..5, 6, 10, 12, 16, 30, 32, 35, 36, 38, 39, 42, 45

20–25 µm..1, 28

25–30 µm..3

35–40 µm...21, 23, 25, 40

45–50 µm...33

60–65 µm...8

5. Average Width

0–1 µm...8, 21, 23, 33

1–2 µm...10, 30, 39, 40, 41

2–3 µm..............................2, 4, 5, 9, 11, 12, 13, 15, 16, 17, 18, 19, 20, 22, 24, 26, 27, 31, 32, 34, 35, 38, 44, 45

3–4 µm..3, 14, 28, 36, 37, 42, 43

4–5 µm...6

5–6 µm...1, 29

6–7 µm..25

Host Plant

Only Known In Anamorphic State... 7

Geographic Distribution

Iran…………………………………………………………………………….20

Japan………………....1, 4, 8, 12, 14, 17, 18, 21, 22, 26, 27, 28, 30, 32, 36, 41, 42

Panama…………………………………………..………………………......…..6

Russia…………………………………………………………....15, 16, 20, 35, 42

United States……….2, 7, 9, 13, 15, 19, 20, 23, 24, 25, 31, 34, 37, 38, 40, 41, 43, 45

1. *Ophiognomonia alni-cordatae*

2. *Ophiognomonia alni-viridis*

3. *Ophiognomonia apiospora*

4. *Ophiognomonia asiatica*

5. *Ophiognomonia balsamiferae*

6. *Ophiognomonia bugabensis*

7. *Ophiognomonia clavigignenti-juglandacearum*

8. *Ophiognomonia cordicarpa*

9. *Ophiognomonia gardiennetii*

10. *Ophiognomonia gei*

11. *Ophiognomonia gei-montani*

12. *Ophiognomonia gunmensis*

13. *Ophiognomonia hiawathae*

14. *Ophiognomonia ibarakiensis*

15. *Ophiognomonia intermedia*

16. *Ophiognomonia ischnostyla*

17. *Ophiognomonia japonica*

18. *Ophiognomonia kobayashii*

19. *Ophiognomonia lenticulispora*

20. *Ophiognomonia leptostyla*

21. *Ophiognomonia longispora*

22. *Ophiognomonia maximowiczianae*

23. *Ophiognomonia melanostyla*

24. *Ophiognomonia michiganensis*

25. *Ophiognomonia micromegala*

26. *Ophiognomonia monticola*

27. *Ophiognomonia multirostrata*

28. *Ophiognomonia naganoensis*

29. *Ophiognomonia nana*

30. *Ophiognomonia nipponicae*

31. *Ophiognomonia ostryae-virginianae*

32. *Ophiognomonia otanii*

33. *Ophiognomonia padicola*

34. *Ophiognomonia pseudoclavulata*

35. *Ophiognomonia pseudoischnostyla*

36. *Ophiognomonia pterocaryae*

37. *Ophiognomonia quercus-gambellii*

38. *Ophiognomonia rosae*

39. *Ophiognomonia rubi-idaei*

40. *Ophiognomonia sassafras*

41. *Ophiognomonia setacea*

42. *Ophiognomonia sogonovii*

43. *Ophiognomonia trientensis*

44. *Ophiognomonia tucumanensis*

45. *Ophiognomonia vasiljevae*

Excluded or doubtful names in *Ophiognomonia*:

Ophiognomonia capillaris (Penz. & Sacc.) M. Monod, Beih. Sydowia 9: 160. 1983.

Basionym: *Linospora capillaris* Penz. & Sacc., Malpighia 11: 409. 1904.

   = *Linospora liquidambaris* Teng, Sinensia 4: 384. 1934.

Holotypus: JAVA: Tjibodas, on dead leaves of undetermined host (PAD-not examined).

Also, reported from China on *Liquidambar formosana* by Teng (1934).

Notes: Monod (1983) examined the type and second specimen of this taxon. Based on his description of ascomata with very long perithecial necks, 1100–1600 μm long, and elongated ascospores, 49–58 × 1–1.2 μm, it is possible that this species belongs in *Ophiognomonia*, however, it was not encountered during this study. The most well-known species of the Gnomoniaceae on *Liquidambar* is *Ambarignomonia petiolorum,* which has ascomata each with a thin, elongated perithecial neck surrounded by a white collar at the base and 9–15 × 1.5–2 μm, one-septate ascospores, quite unlike the description of *O. capillaris* (Sogonov et al. 2008).

Ophiognomonia caulicola Hohn., Sber. Akad. Wiss. Wien, Math.-naturw. Kl., Abt. 1 117: 1213. 1908.

Holotypus: AUSTRIA: bei Ybbsitz, on dead branch of *Salvia glutinosa*, April 1909, Strasser (FH-not examined).

Notes: Monod (1983) examined the depauperate type specimen and provided a partial description of this species. This host is unusual for a member of the Gnomoniaceae and it seems doubtful that this species belongs in that family. It is known only from the type specimen.

Ophiognomonia cryptica D. Wilson & M.E. Barr in Wilson, Barr & Faeth, Mycologia 89: 539. 1997.

Holotypus: UNITED STATES, ARIZONA: Pinal Co., 100 km E of Phoenix, alt. 1292 ft, isolated from leaves of *Quercus emoryi,* December 1994, D. Wilson (BPI 749237).

Notes: This species was isolated as an endophyte of *Quercus emoryi* and produced ascomata in culture. The holotype specimen that consists of five dried cultures labeled with differing numbers was examined superficially. Based on the description, the ascomata have long beaks 400–1400 μm and filiform ascospores 38–48 x 2–2.5 μm. These characteristics suggest that this species belongs in *Ophiognomonia*; however, no living material was encountered during this study. Attempts to obtain DNA and sequence data from the dried culture of the holotype specimen produced only that of contaminants.

Ophiognomonia elasticae (Koord.) M. Monod, Beih. Sydowia 9: 157. 1983.

Basionym: *Linospora elasticae* Koord., Botan. Untersuch. 193. 1907.

Holotypus: JAVA: on leaves of *Ficus elastica* (not examined).

Materials examined: PHILIPPINES: Province Laguna, near Los Banos, Mount Maquiling, on dead leaves of *Ficus* sp., June 1914, Baker, (Rehm Fungi Malayan 151, BPI 626855).

Notes: This species was described from Java, later reported from the Philippines (Teodoro 1937), and recently noted as the dominant fungus isolated from fallen leaves of *Ficus pleurocarpa* in Australia (Paulus et al. 2007). Monod (1983) examined a non-type specimen and retained it in *Ophiognomonia*; however, we examined that same specimen and concluded that this species should be placed in the genus *Ophiobolous* (Walker 1980) because of the lack of an apical ring in the ascus and the multiseptate, filiform ascospores. Cultures of this species from Australia were sequenced but proved to be basidiomycetes.

Ophiognomonia helvetica Rehm, Annls mycol. 5(6): 543. 1907.

≡ *Pleuroceras helvetica* (Rehm) Barr, Mycologia Memoir 7:121. 1978.

Holotypus: SWITZERLAND: on dead leaves of *Salix herbacea* (S-not examined).

Notes: Based on a specimen from northern Quebec, Barr (1978) placed this name in *Pleuroceras* stating that this is a "subarctic-subalpine species". Monod (1983) examined the type specimen from a high elevation in Switzerland and agreed with this placement, thus we accept this species as *P. helvetica*.

Ophiognomonia langii M. Monod, Sydowia Beih. 9: 156. 1983.

Holotypus: NORWAY: Tromso, on dead leaves of *Salix reticulate*, 19 July 1977, M. Monod (Monod 373 LAU-not examined).

Notes: Based on the description in Monod (1983), it seems likely that this arctic-alpine species belongs in *Pleuroceras*, related to *P. helvetica* mentioned above. This species has also been reported from Sweden (Eriksson 1992).

Ophiognomonia lapponica Vestergr., Bot. Notiser: 125. 1902.

Holotypus: SWEDEN: Lapponia, Lulensis, Lulleketje, Randijaure, on leaves of *Betula odorata*, 19 June 1900, C. Skottsberg and T. Vestergren (Vestergren Micromycetes Rariores Selecti 408, BPI 626912).

Notes: Based on an examination of the type specimen, this species could be accepted in the Gnomoniaceae in either *Ophiognomonia* or *Pleuroceras*. The basally immersed ascomata are relatively thick-walled, collapsing from the bottom when dry, each with a beak 200–300 x 60–120 μm. The ascospores are very thin, 65–75 x 1.5–2 μm, one-septate. No living material of this species was encountered.

Ophiognomonia procumbens (Fuckel) Berl., Icon. Fung. 2: 146. 1900.

Basionym: *Linospora procumbens* Fuckel, Jb. nassau. Ver. Naturk. 23–24: 124. 1870.

Notes: Monod (1983) examined type material of *L. procumbens* and suggested that this name is a synonym of *Pleuroceras pleurostylum* (Auersw.) Barr. This species is known to occur only on *Salix* in Europe, thus the reports of *O. procumbens* in California on dead leaves of *Quercus agrifolia* (French, 1980) are erroneous.

Ophiognomonia pseudoplatani (Tubeuf) D.K. Barrett & R.B. Pearce, Trans. Br. mycol. Soc. 76(2): 317. 1981.

Basionym: *Gnomonia pseudoplatani* Tubeuf, Z. PflKrankh. 40: 364. 1930.

≡ *Pleuroceras pseudoplatani* (Tubeuf) M. Monod, Beih. Sydowia 9: 171. 1983.

= *Asteroma pseudoplatani* Butin & Wulf, Sydowia 40: 39. 1987.

Holotypus: GERMANY: on fallen leaves of *Acer pseudoplatanus* (not examined).

Notes: Based on the description in Barrett & Pearce (1981), *Pleuroceras pseudoplatani* occurs in Europe and has ascospores 45–65 x 0.5 x 1.5 μm that resemble in shape but are longer than those of *P. tenellum* in North America having ascospores 20–36 x 1–2 μm. Ascospores of both species are elongate, slightly narrowing toward one septum with long appendages at each end (Barr 1978), characteristic of many species of *Pleuroceras* (Monod 1983). This species should be referred to as *Pleuroceras pseudoplatani* and causes a disease called giant leaf blotch of sycamore as described and illustrated by Barrett and Pearce (1981) and Butin and Wulf (1987).

Ophiognomonia sacchari Speg., Revta Fac. Agron. Vet. Univ. nac. La Plata 2(19): 231. 1896.

Holotypus: Argentina, Tucuman, on weakened leaves and sheath of *Saccharum officinalis* (LPS-not examined).

Notes: Nothing except the type description and specimen is known about this name but it seems unlikely as a member of the Gnomoniaceae.

Ophiognomonia umbelliferarum (M.E. Barr) Lar. N. Vassiljeva, Pyrenomycetes of the Russian Far East, 1. Gnomoniaceae (Vladivostok): 39 (1993).

Basionym: *Linocarpon umbelliferarum* M.E. Barr, Can. J. Bot. 39: 320. 1961.

   ≡ *Plagiosphaera umbelliferarum* (M.E. Barr) M.E. Barr, Mycol. Mem. 7: 123. 1978.

Holotypus: CANDA: Quebec, on dead stems of *Heracleum lanatum,* M.E. Barr (Barr 2198A-not examined).

Notes: This species is known only from the type specimen. The description and illustration of this species by Barr (1961) show a refractive, globular cluster in the ascal apex characteristic of genera in the Sordariales such as *Lasiosphaeria* and *Neolinocarpon*, thus it is unrelated to *Ophiognomonia*. Walker (1980) considered this species to be similar to *Plagiosphaera immersa* (≡ *Ophiobolus immersa*), but could not distinguish them. A GenBank BLAST search of the ITS region (ITS1, 5.8S rDNA and ITS2) of *P. immersa*, type of *Plagiosphaera*, suggests that this genus belongs outside of the Gnomoniaceae, rather it is distantly related to *Gaeumannomyces* in the Magnaporthales (on *Urtica dioica* from Veronnes, France, culture BPI 883014, culture DMW 571).

ACKNOWLEDGEMENTS

Fig. 4.1. ML phylogenetic analysis (ML score = -lnL 10931.08) of ITS, MS204, and *tef-*

1α sequences of 43 species in *Ophiognomonia* and two outgroup taxa within the

Gnomoniaceae. Bayesian posterior probabilities ≥ 95 % are displayed above each branch. GARLI ML bootstrap values ≥70 % are displayed to the bottom left and MP bootstrap values ≥ 70 % to the bottom right of each branch. Taxa in bold are new combinations or new species.
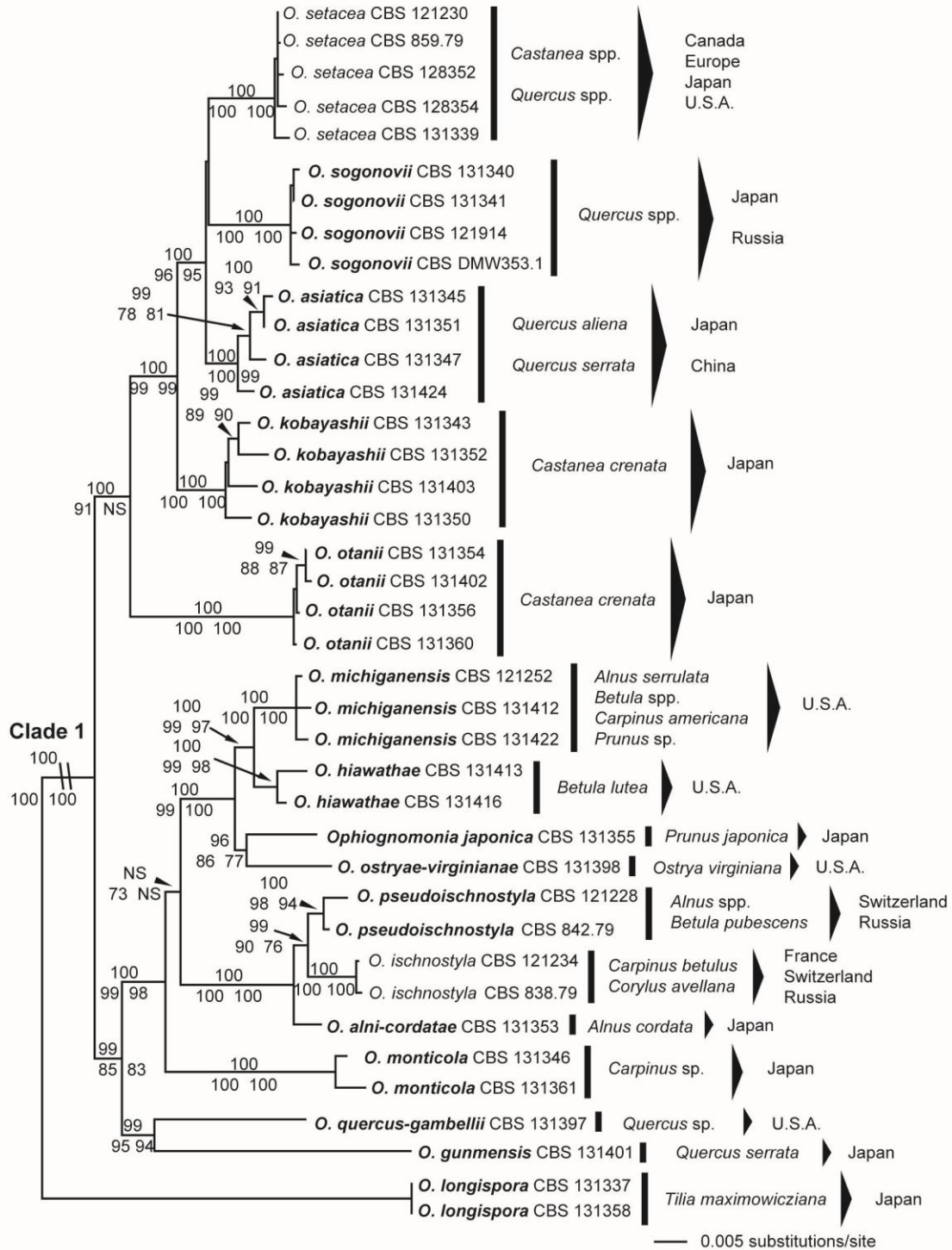
Fig. 4.2. ML phylogenetic analysis (ML score = -lnL 7963.63) of ITS, MS204, and *tef-1α*

sequences of 15 species in *Ophiognomonia* (Clade one) and one outgroup taxon within

Ophiognomonia. Bayesian posterior probabilities ≥ 95 % are displayed above each

branch. GARLI ML bootstrap values ≥70 % are displayed to the bottom left and MP

bootstrap values ≥ 70 % are displayed to the bottom right of each branch. Taxa in bold

are new combinations or new species.
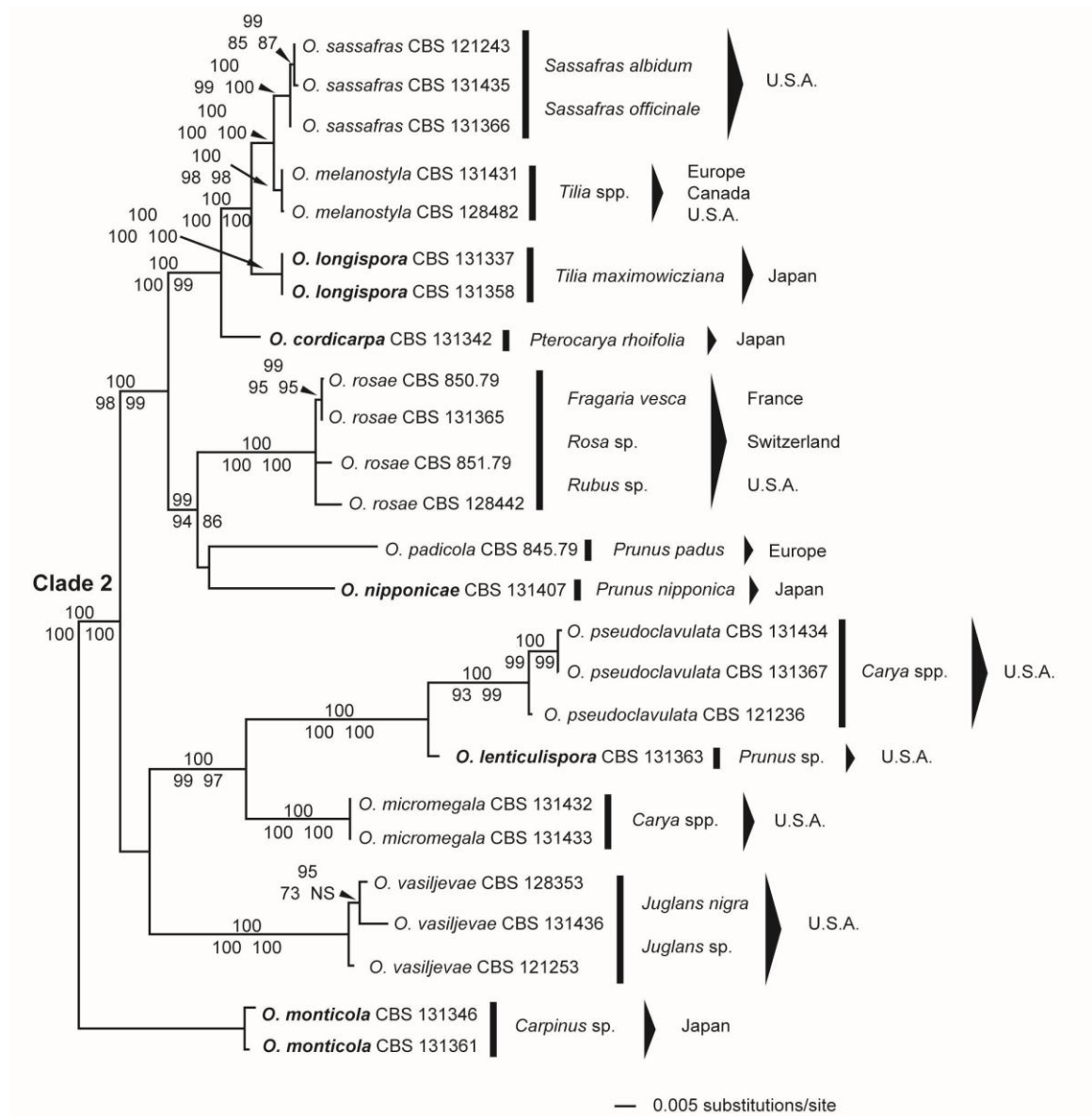
Fig. 4.3. ML phylogenetic analysis (ML score = -lnL 7605.96) of ITS, MS204, and *tef-1α*

sequences of 11 species in *Ophiognomonia* (Clade two) and one outgroup taxon all

within *Ophiognomonia*. Bayesian posterior probabilities ≥ 95 % are displayed above each

branch. GARLI ML bootstrap values ≥70 % are displayed to the bottom left and MP bootstrap values ≥ 70 % are displayed to the bottom right of each branch. Taxa in bold are new combinations or new species.
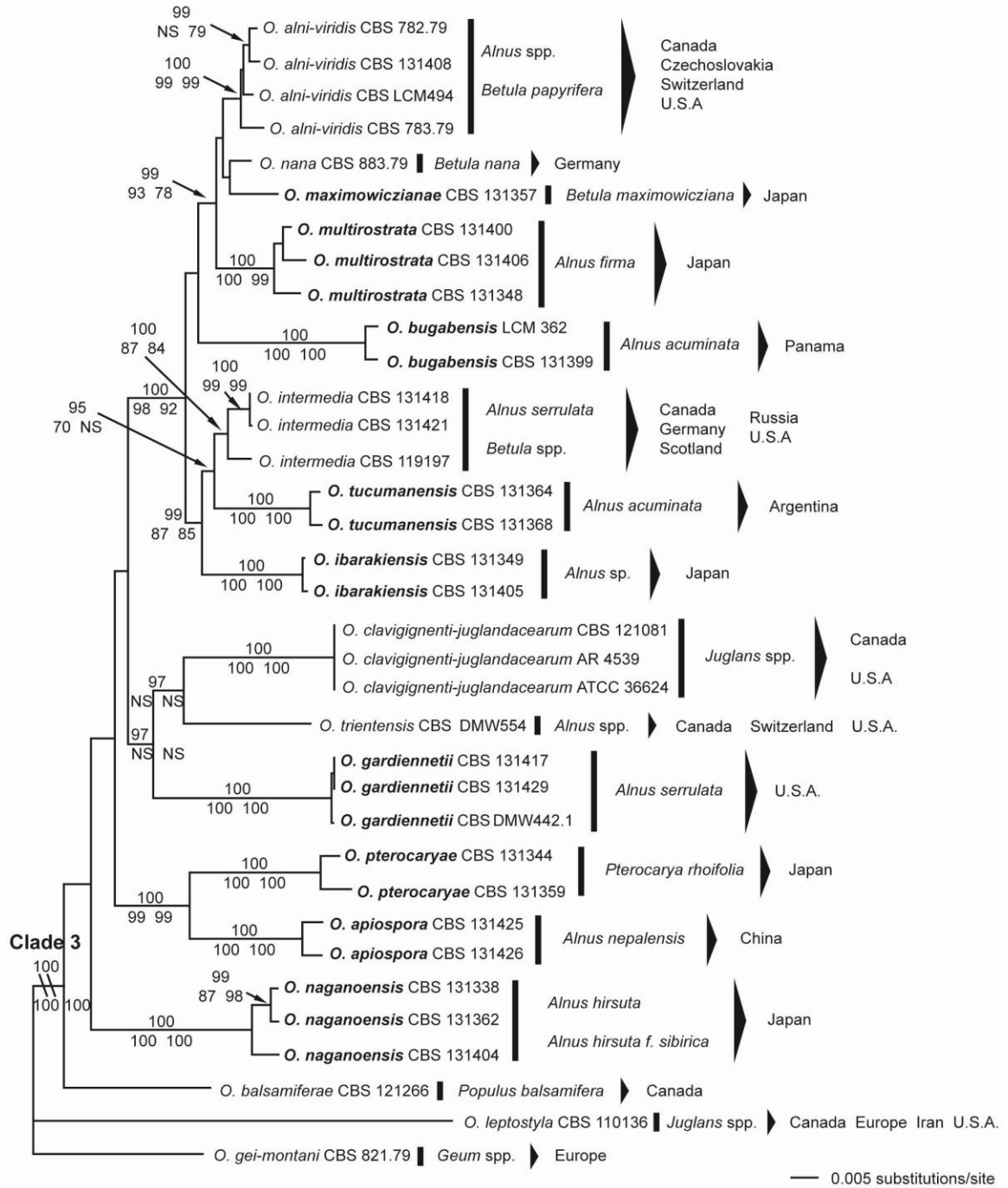
Fig. 4.4. ML phylogenetic analysis (ML score = -lnL 8916.31) of ITS, MS204, and *tef-1α*

sequences of 15 species in *Ophiognomonia* (Clade three) and two outgroup taxa all

within *Ophiognomonia*. Bayesian posterior probabilities ≥ 95 % are displayed above each

branch. GARLI ML bootstrap values ≥70 % are displayed to the bottom left and MP

bootstrap values ≥ 70 % are displayed to the bottom right of each branch. Taxa in bold

are new combinations or new species.
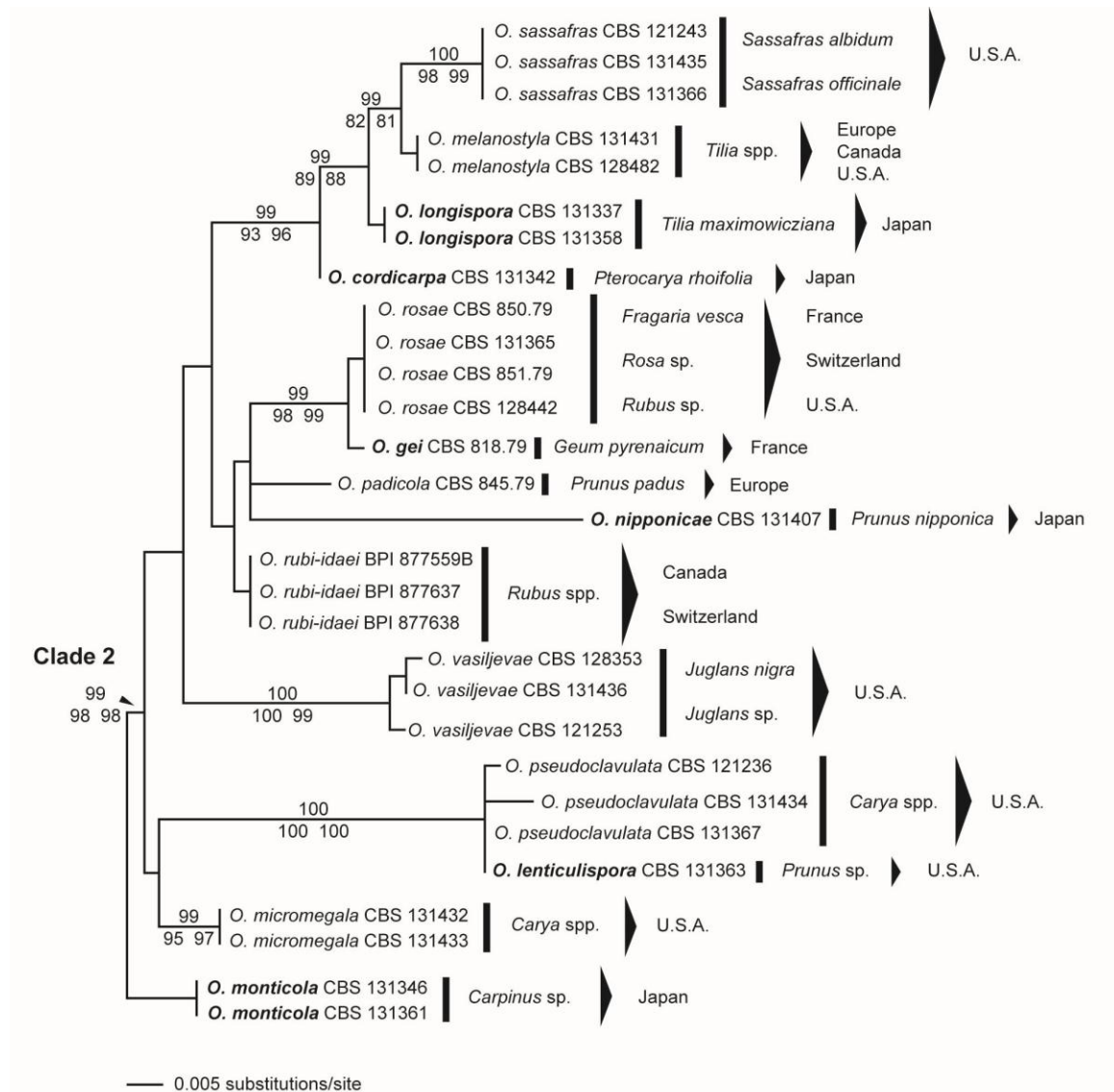
Fig. 4.5. ML phylogenetic analysis (ML score = -lnL 1888.25) of ITS sequences of 11

species in *Ophiognomonia* (Clade two) and one outgroup taxon all within

Ophiognomonia. Bayesian posterior probabilities ≥ 95 % are displayed above each

branch. GARLI ML bootstrap values ≥70 % are displayed to the bottom left and MP

bootstrap values ≥ 70 % are displayed to the bottom right of each branch. Taxa in bold

are new combinations or new species.

Fig. 4.6. *Ophiognomonia alni-cordatae.* a–f. Holotype BPI 882233. Scale bars of

perithecia = 100 μm. Scale bars of asci and ascospores = 10 μm.
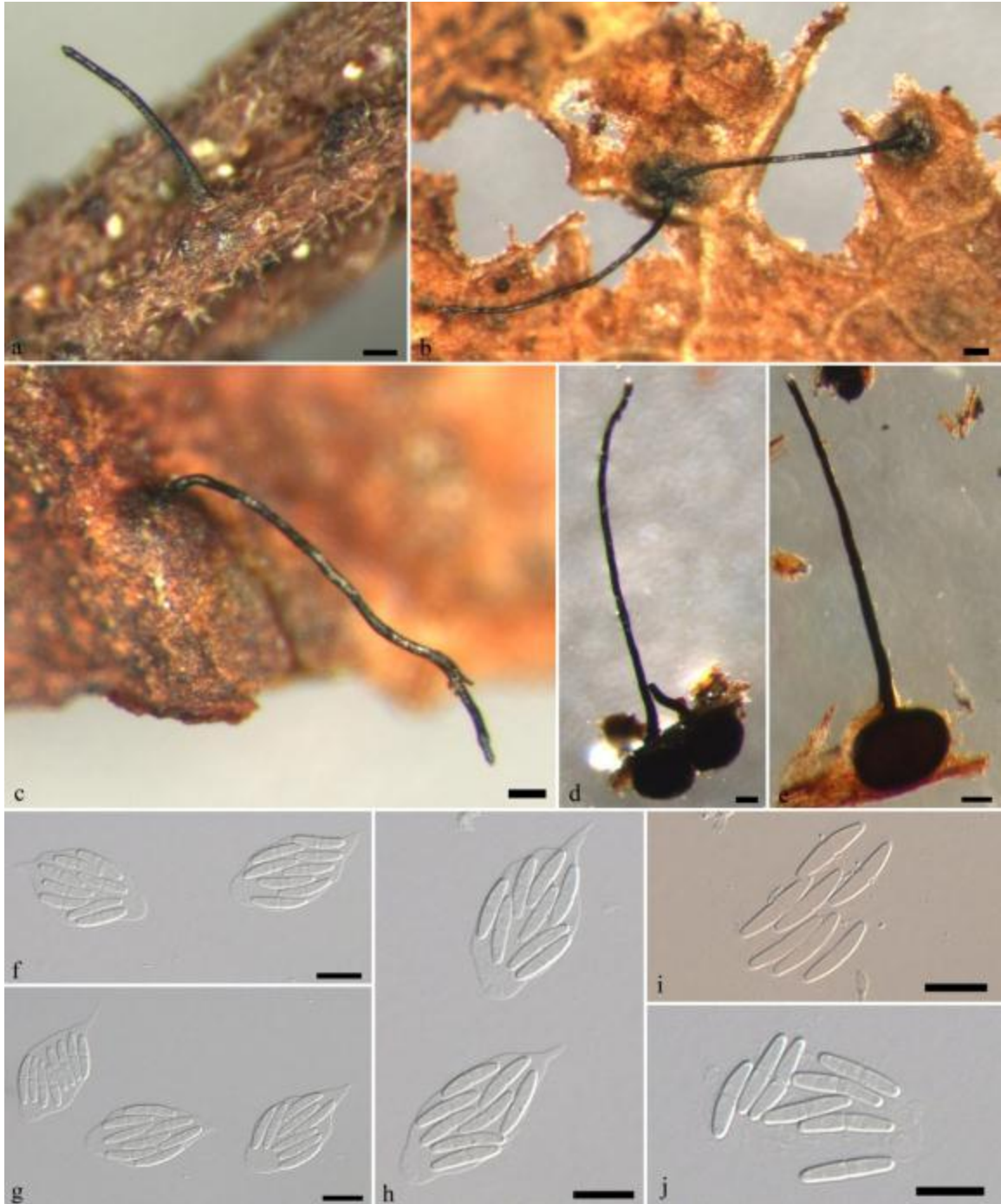
Fig. 4.7. *Opiognomonia alni-viridis*. a, e, i. BPI 879541; b–d, f–h, j. BPI 879541. Scale

bars of perithecia = 100 μm. Scale bars of asci and ascospores = 10 μm.

Fig. 4.8. *Ophiognomonia apiospora*. a–g. Holotype BPI 879601. Scale bars of perithecia = 100 μm. Scale bars of asci and ascospores = 10 μm.

Fig. 4.9. *Ophiognomonia asiatica*. a–c. BPI 882225; d–g. Holotype BPI 882231. Scale bars of perithecia = 100 μm. Scale bars of asci and ascospores = 10 μm.

Fig. 4.10. *Ophiognomonia bugabensis*. a–g. Holotype BPI 879256. Scale bars of perithecia = 100 μm. Scale bars of asci and ascospores = 10 μm.

Fig. 4.11. *Ophiognomonia cordicarpa.* a–h. Holotype BPI 882217. Scale bars of perithecia = 100 μm. Scale bars of asci and ascospores = 10 μm.
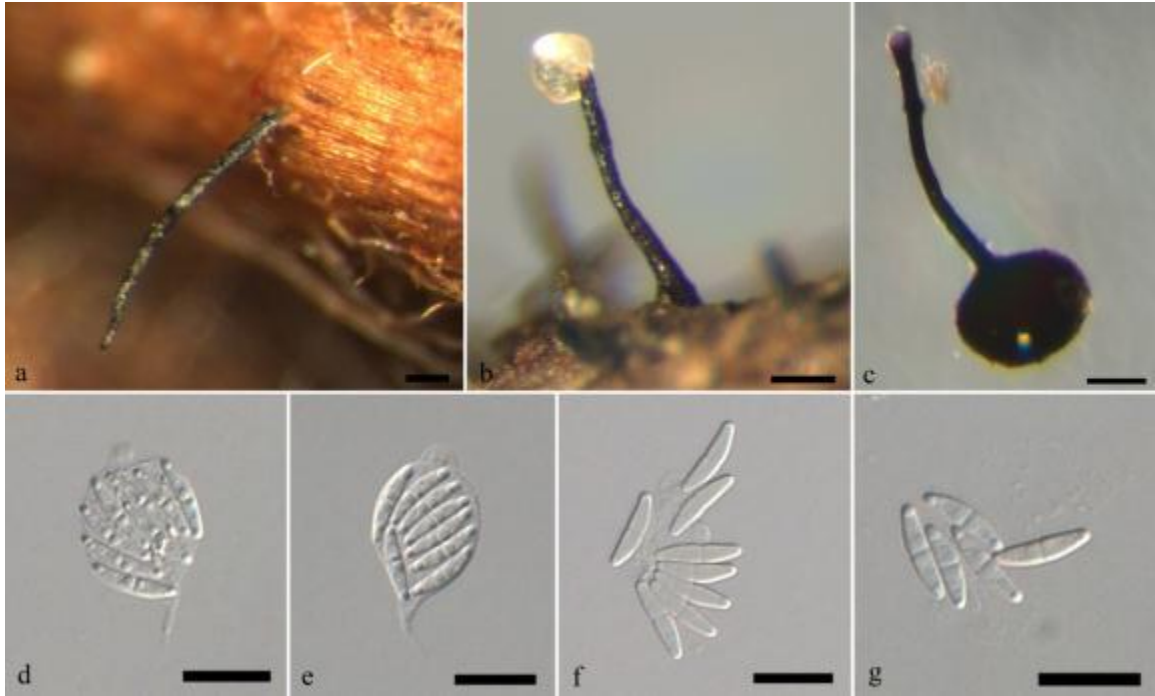
Fig. 4.12. *Ophiognomonia gardiennetii*. a. BPI 882252; b–g. BPI 882276. Scale bars of

perithecia = 100 μm. Scale bars of asci and ascospores = 10 μm.
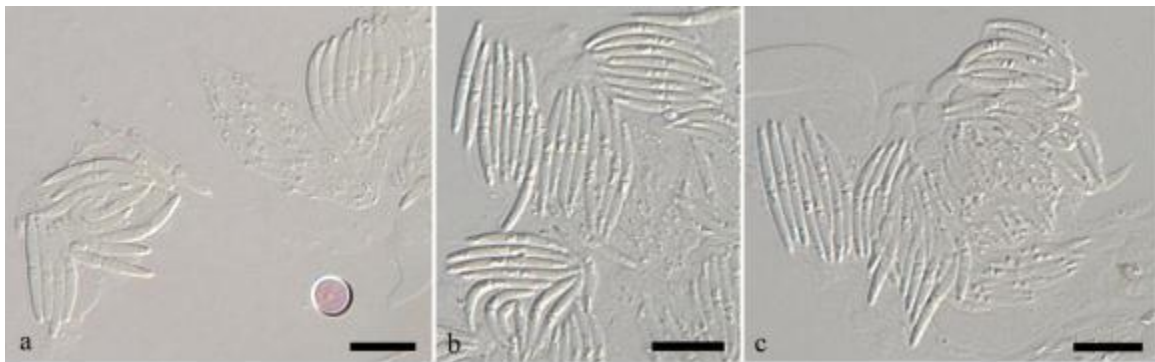
Fig. 4.13. *Ophiognomonia gei*. a–c. Lectotype Patouillard 5304. Scale bars of asci and
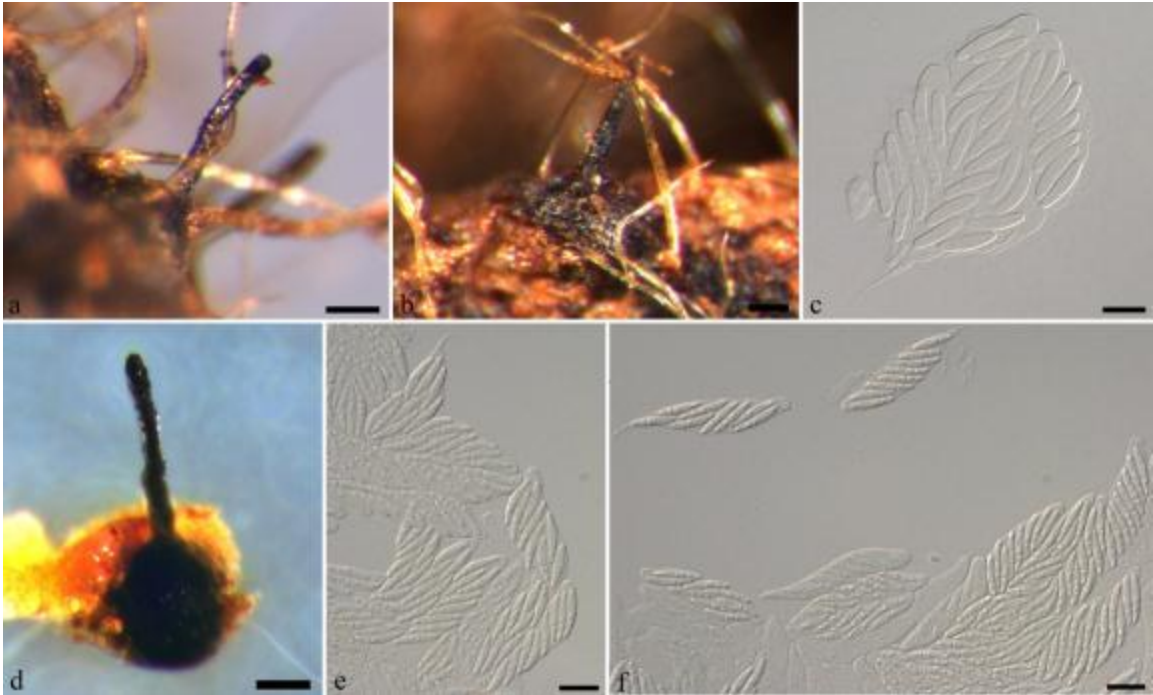
ascospores = 10 μm.

Fig. 4.14. *Ophiognomonia gei-montani*. a–c. BPI 877589; d–f. Holotype F 190027. Scale bars of perithecia = 100 μm. Scale bars of asci and ascospores = 10 μm.
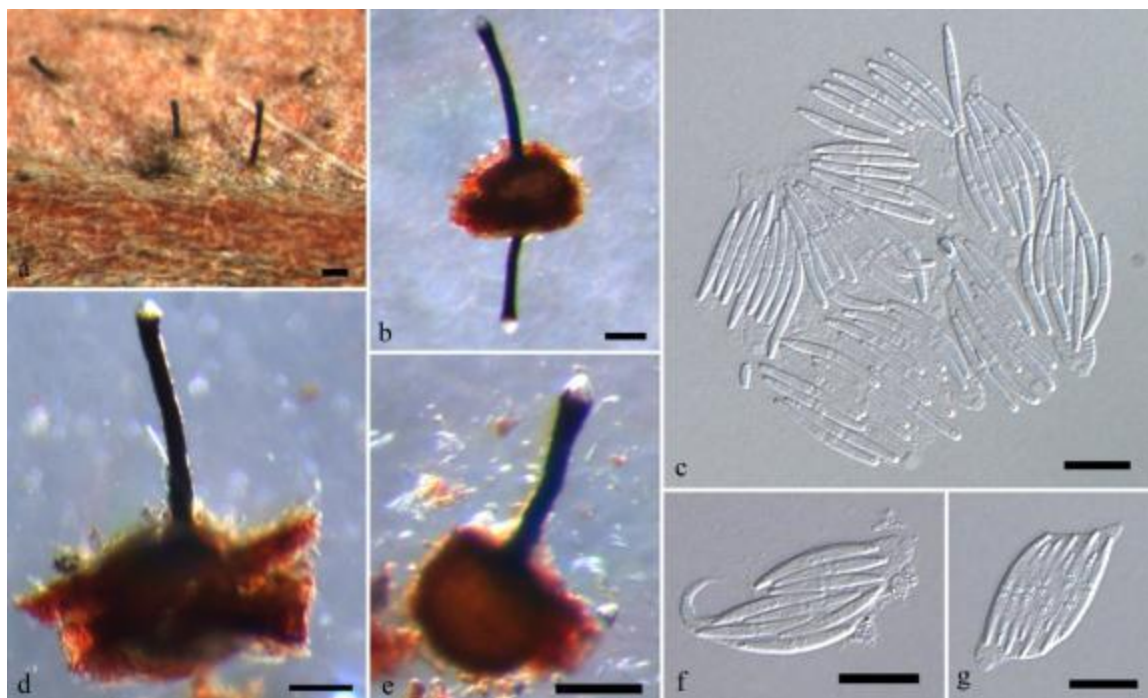
Fig. 4.15. *Ophiognomonia gunmensis*. a–g. Holotype BPI 882236. Scale bars of

perithecia = 100 μm. Scale bars of asci and ascospores = 10 μm.
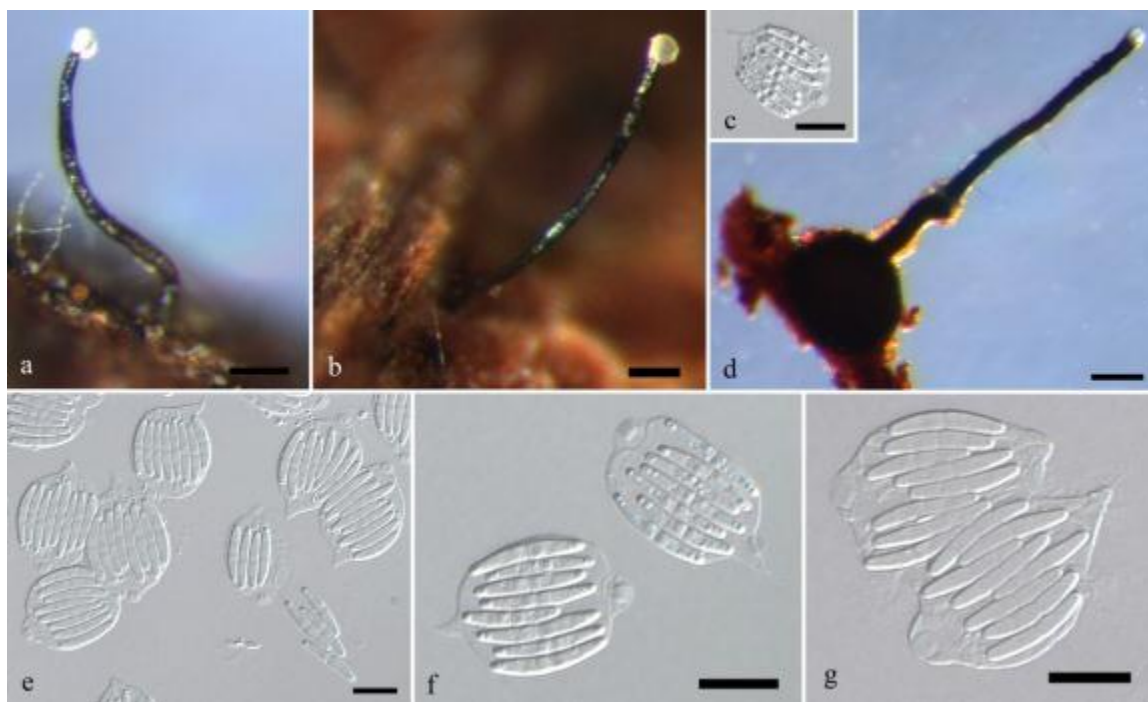
Fig. 4.16. *Ophiognomonia hiawathae*. a, g. BPI 882256; b–f. Holotype BPI 882261.

Scale bars of perithecia = 100 μm. Scale bars of asci and ascospores = 10 μm.

Fig. 4.17. *Ophiognomonia ibarakiensis*. a–d, f, h. Holotype BPI 882247; e, g. BPI

882227. Scale bars of perithecia = 100 μm. Scale bars of asci and ascospores = 10 μm.

Fig. 4.18. *Ophiognomonia intermedia*. a–b, g–i. Lectotype Rehm 1794; c–d. BPI 882266;

e–f. BPI 882267. Scale bars of perithecia = 100 μm. Scale bars of asci and ascospores = 10 μm.
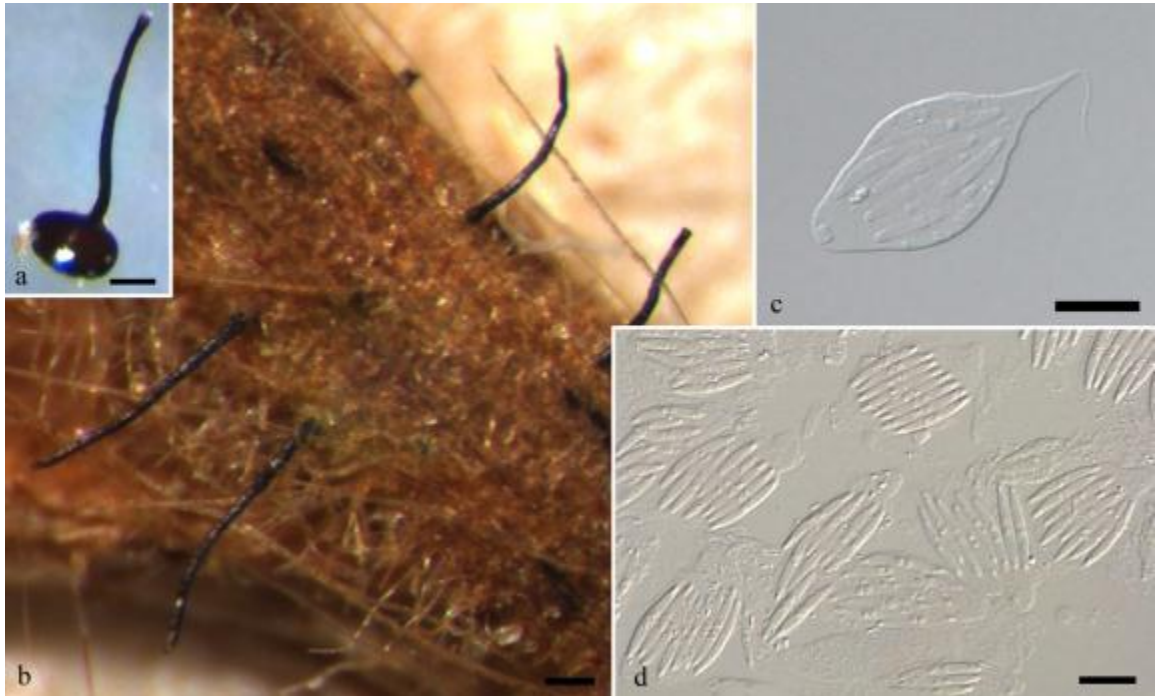
Fig. 4.19. *Ophiognomonia ischnostyla*. a–b, d. Lectotype Desmazieres, Pl. crypt. France 2084; c. BPI 871054B. Scale bars of perithecia = 100 μm. Scale bars of asci and ascospores = 10 μm.
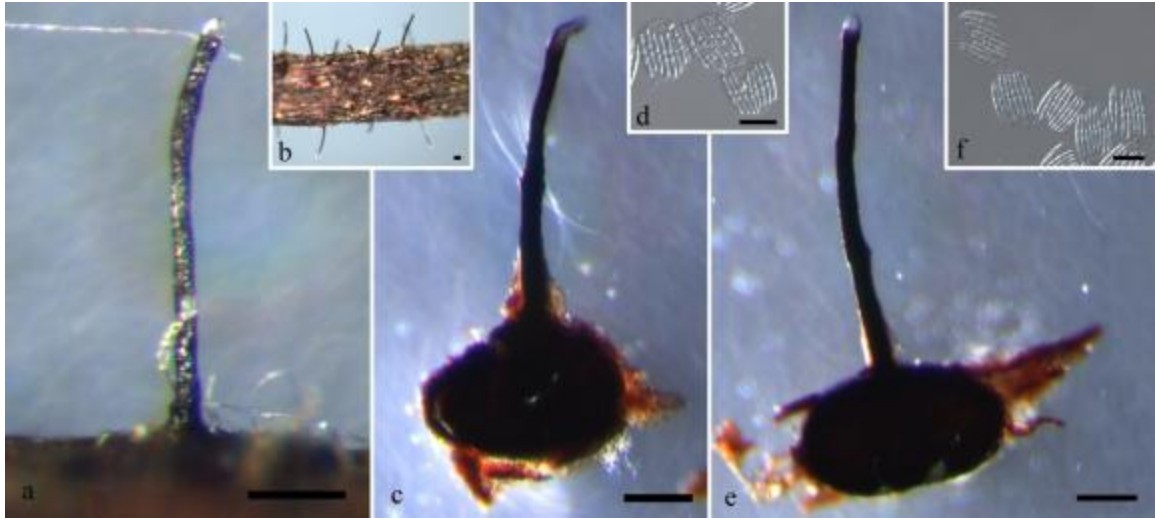
Fig. 4.20. *Ophiognomonia japonica*. a–f. Holotype BPI 882235. Scale bars of perithecia = 100 μm. Scale bars of asci and ascospores = 10 μm.
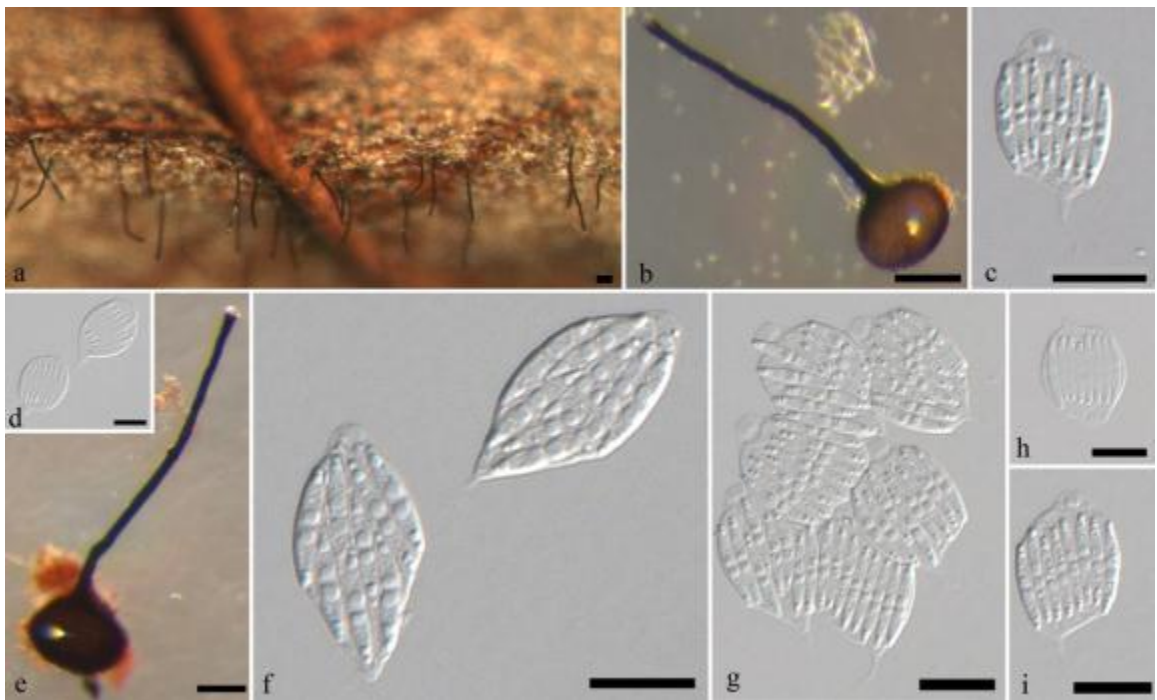
Fig. 4.21. *Ophiognomonia kobayashii*. a, c, i. BPI 882245; b, g–h. BPI 882229; d, e. Holotype BPI 882232; f. BPI 882218. Scale bars of perithecia = 100 μm. Scale bars of asci and ascospores = 10 μm.
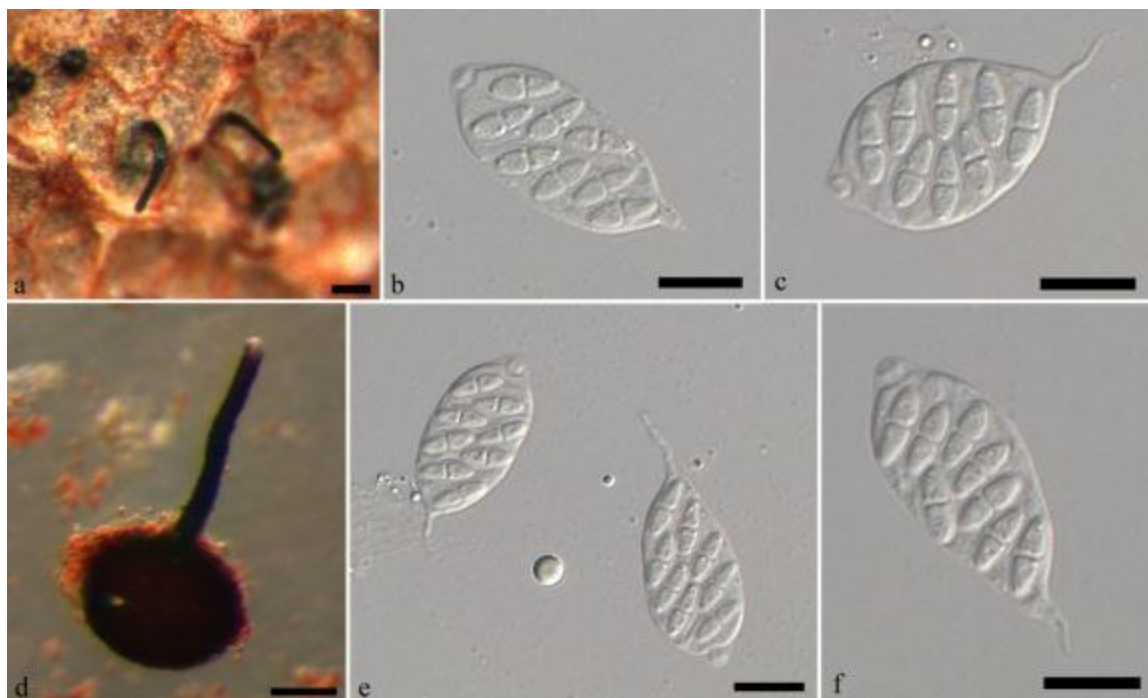
Fig. 4.22. *Ophiognomonia lenticulispora*. a–f. Holotype BPI 882287. Scale bars of perithecia = 100 μm. Scale bars of asci and ascospores = 10 μm.
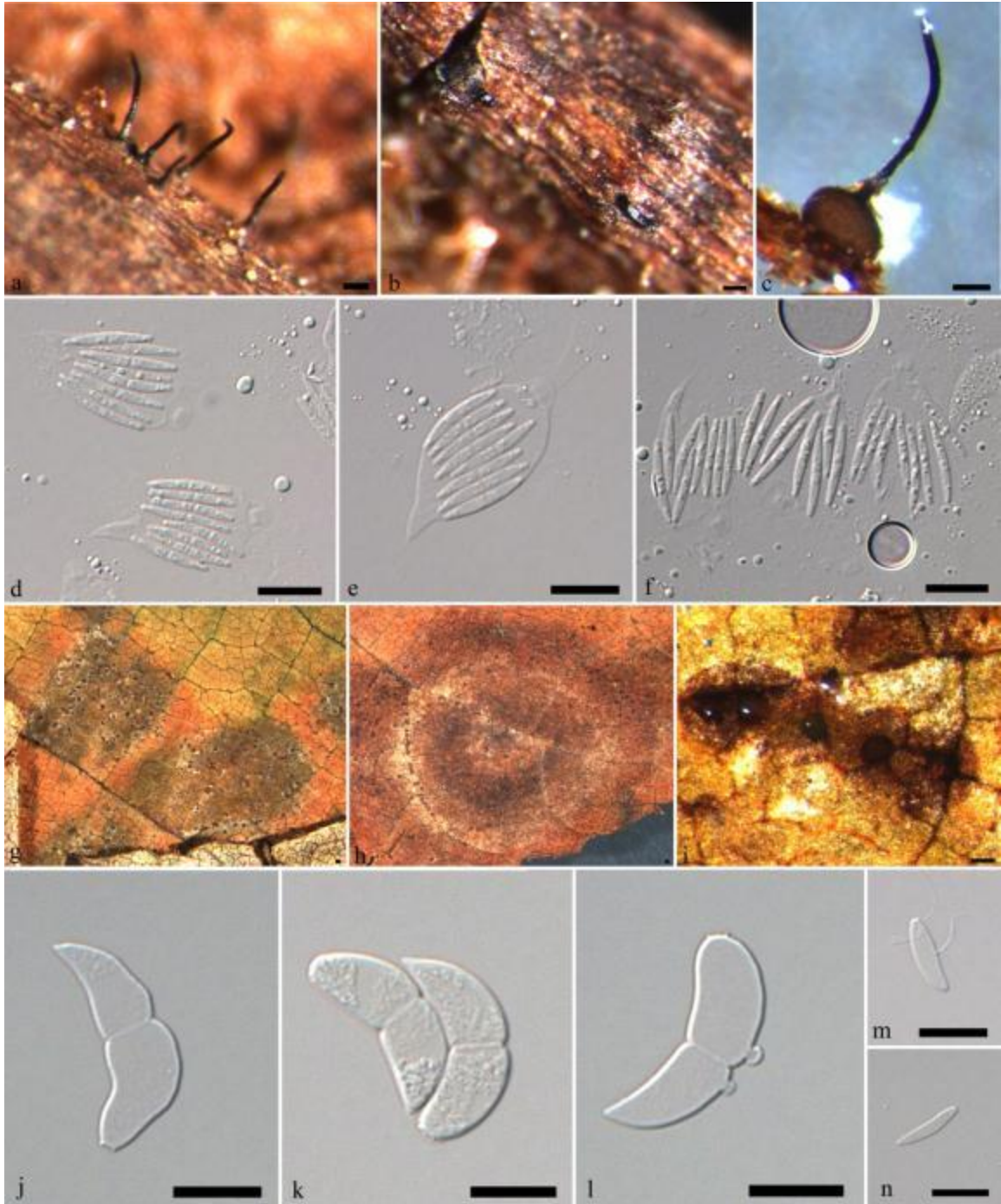
Fig. 4.23. *Ophiognomonia leptostyla*. a–f. BPI 878231; g, i–j, l, n. BPI 611485; h, k, m.

BPI 870007. Scale bars of perithecia and disease leasions= 100 μm. Scale bars of all asci,

ascospores, macro, and micro conidia = 10 μm.
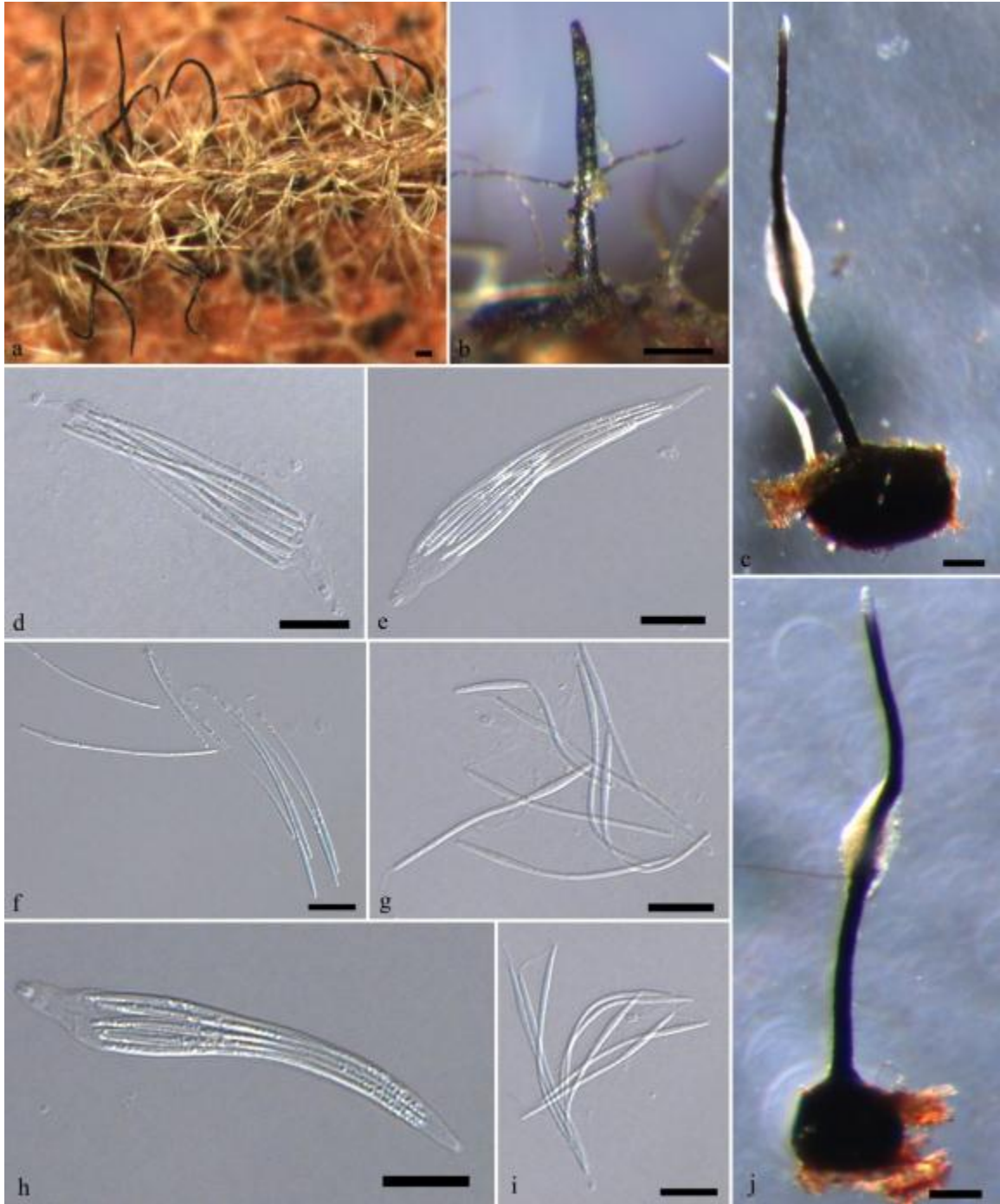
Fig. 4.24. *Ophiognomonia longispora*. b, d, g, i. BPI 882210; a, c, e–f, h, j. Holotype BPI 882239. Scale bars of perithecia = 100 μm. Scale bars of asci and ascospores = 10 μm.
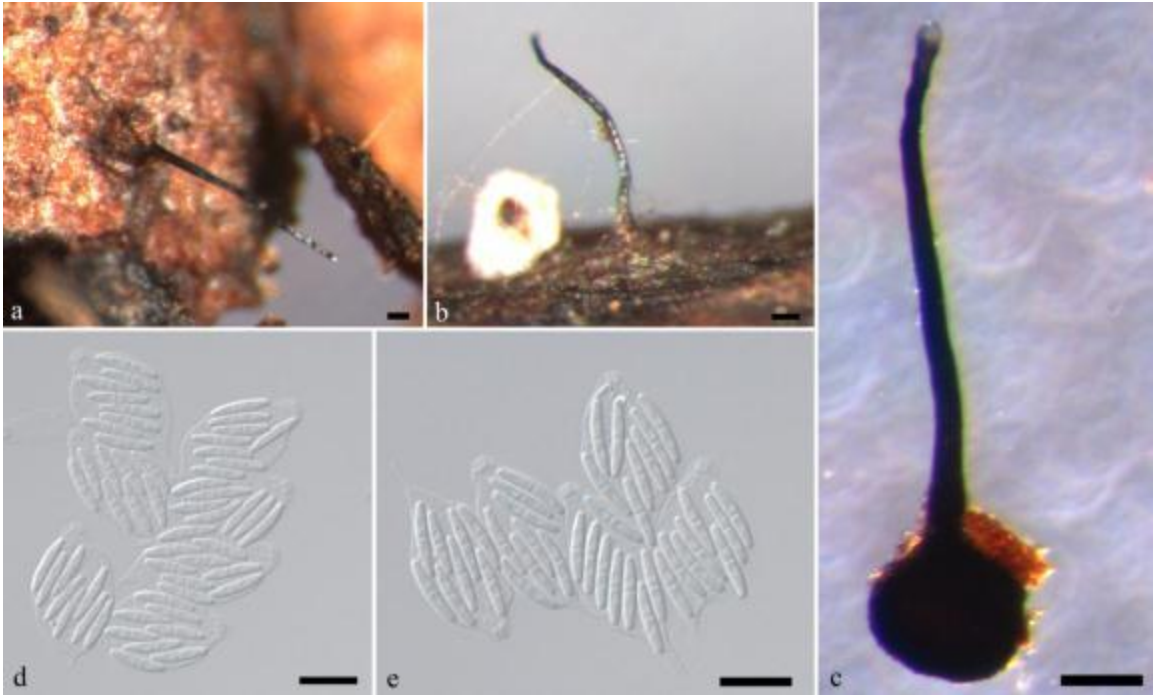
Fig. 4.25. *Ophiognomonia maximowiczianae*. a–e. Holotype BPI 882238. Scale bars of

perithecia = 100 μm. Scale bars of asci and ascospores = 10 μm.
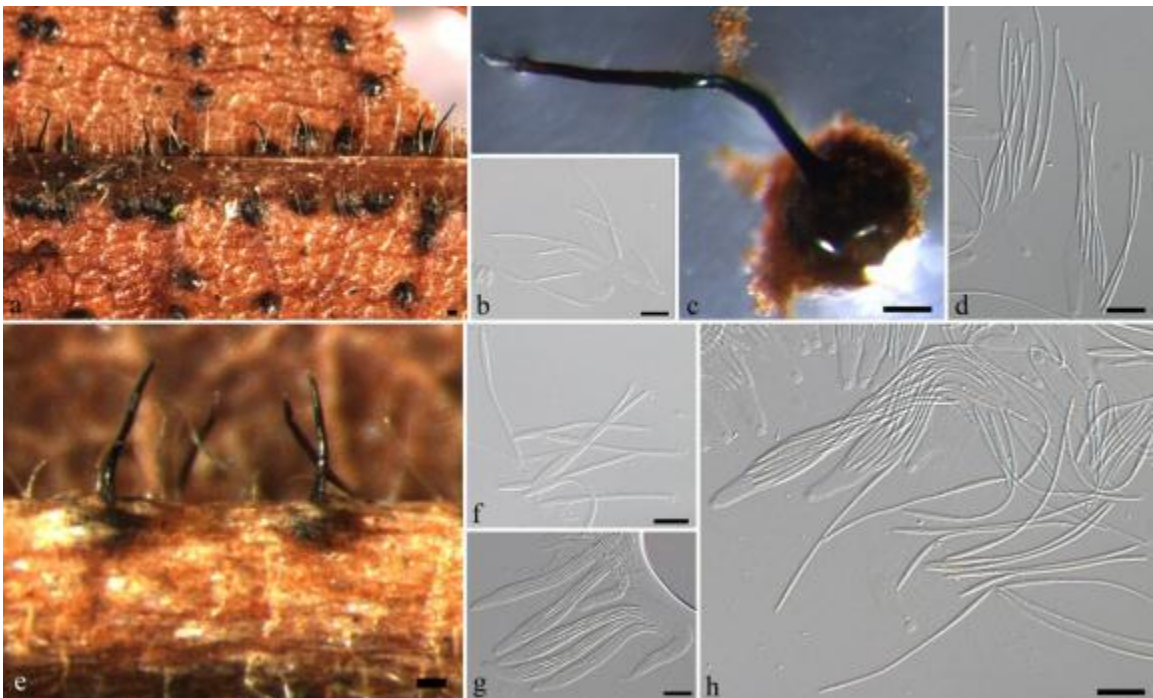
Fig. 4.26. *Ophiognomonia melanostyla*. a–c, f. Epitype BPI 882279; d, g–h. BPI 879257;

e. BPI 882278. Scale bars of perithecia = 100 μm. Scale bars of asci and ascospores = 10

μm.
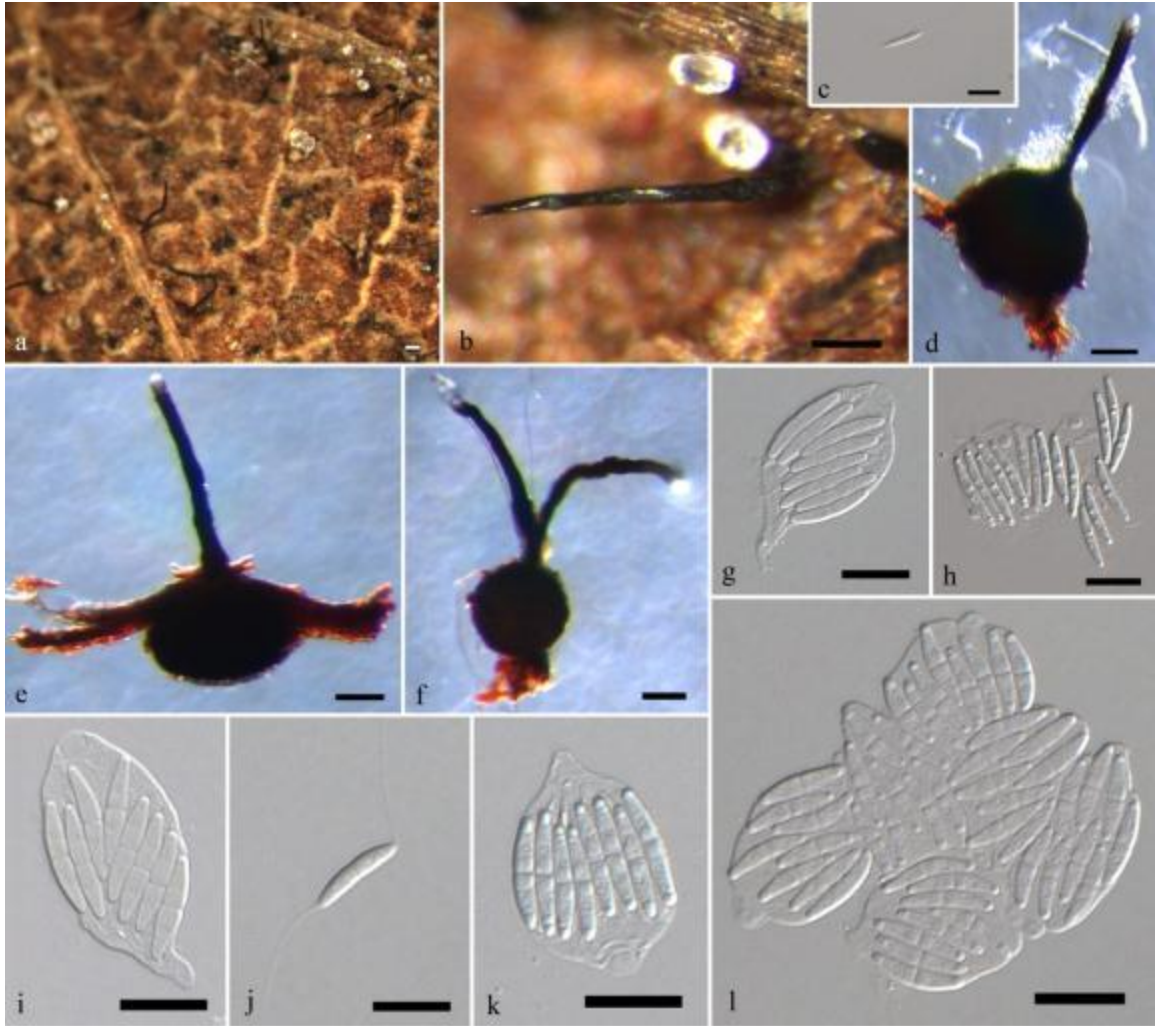
Fig. 4.27. *Ophiognomonia michiganensis.* a–b. BPI 882268; c–d, f, h. BPI 882273; e, l. BPI 882271; i, k, g. BPI 882268; j. BPI 882259. Scale bars of perithecia = 100 μm. Scale bars of asci and ascospores = 10 μm.
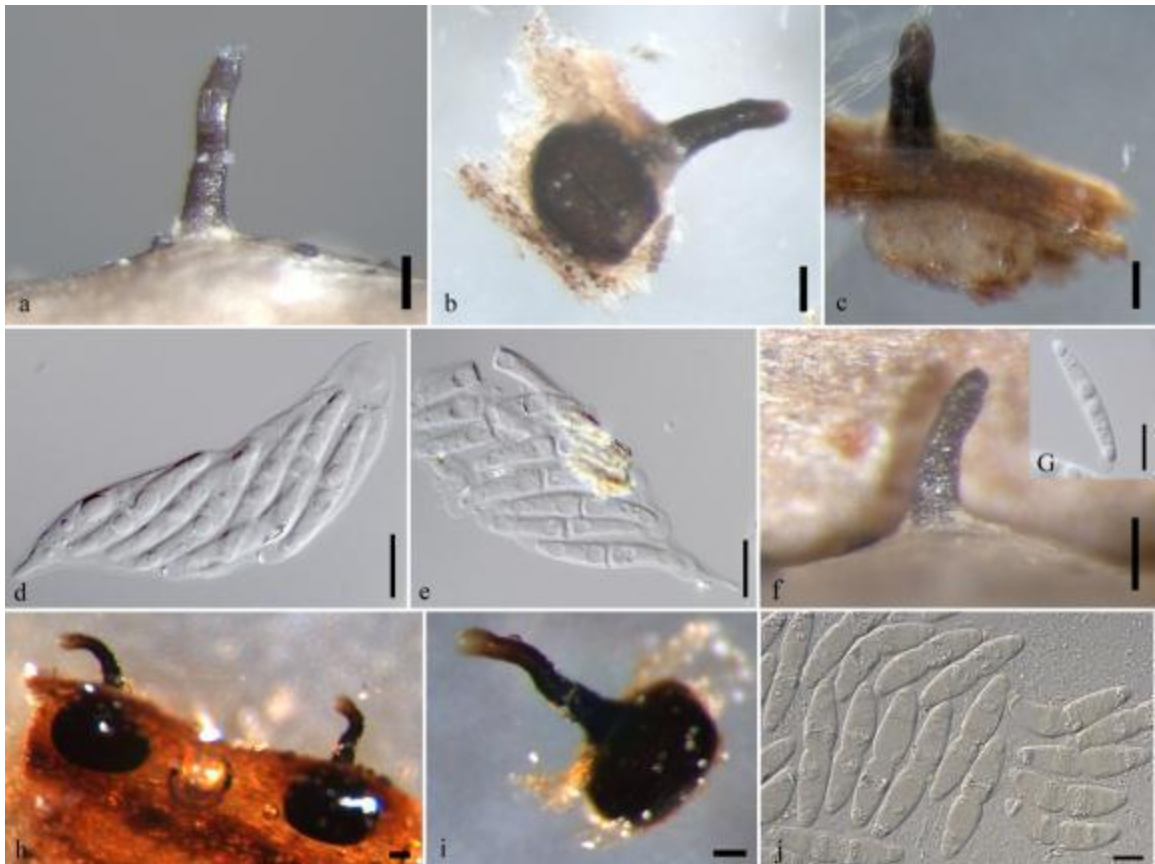
Fig. 4.28. *Ophiognomonia micromegala*. a–b, d–g. BPI 877612; c. BPI 877614; h– j.

Isotype Ellis and Everhart 2309. Scale bars of perithecia = 100 µm. Scale bars of asci and

ascospores = 10 µm.

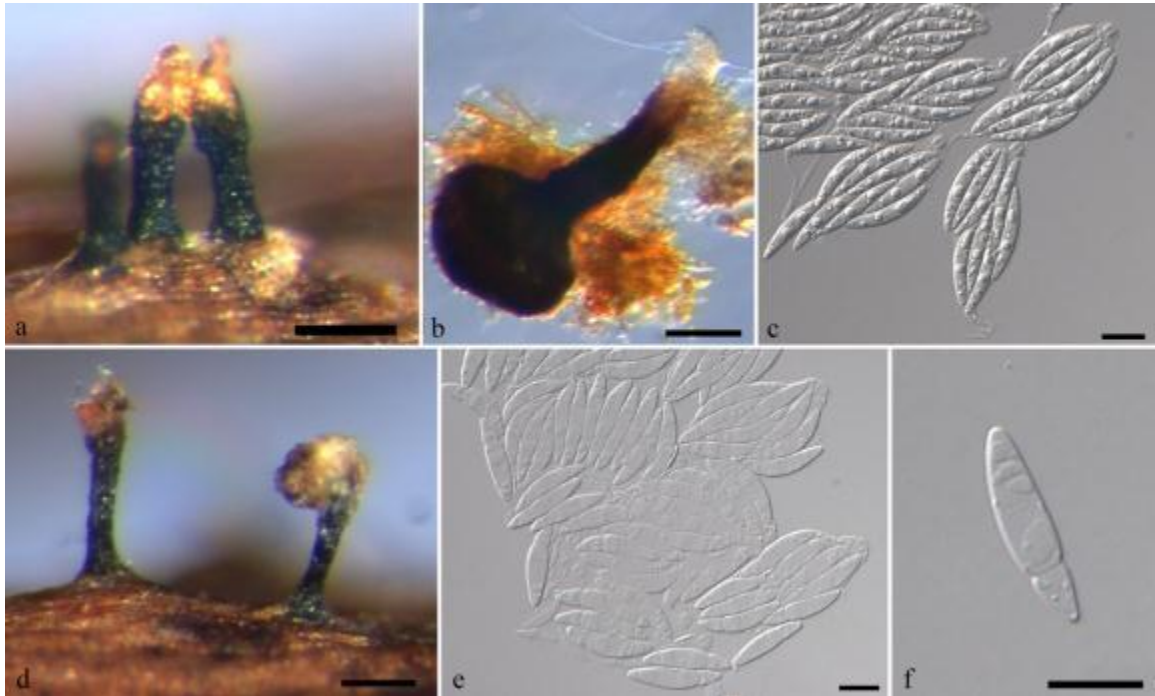**Fig. 4.29**. *Ophiognomonia monticola*. a–g. BPI 882243. Scale bars of perithecia = 100 μm. Scale bars of asci and ascospores = 10 μm.

Fig. 4.30. *Ophiognomonia multirostrata*. d–f. BPI 882248; b–c. Holotype BPI 882226; a, g. BPI 882228. Scale bars of perithecia = 100 μm. Scale bars of asci and ascospores = 10 μm.
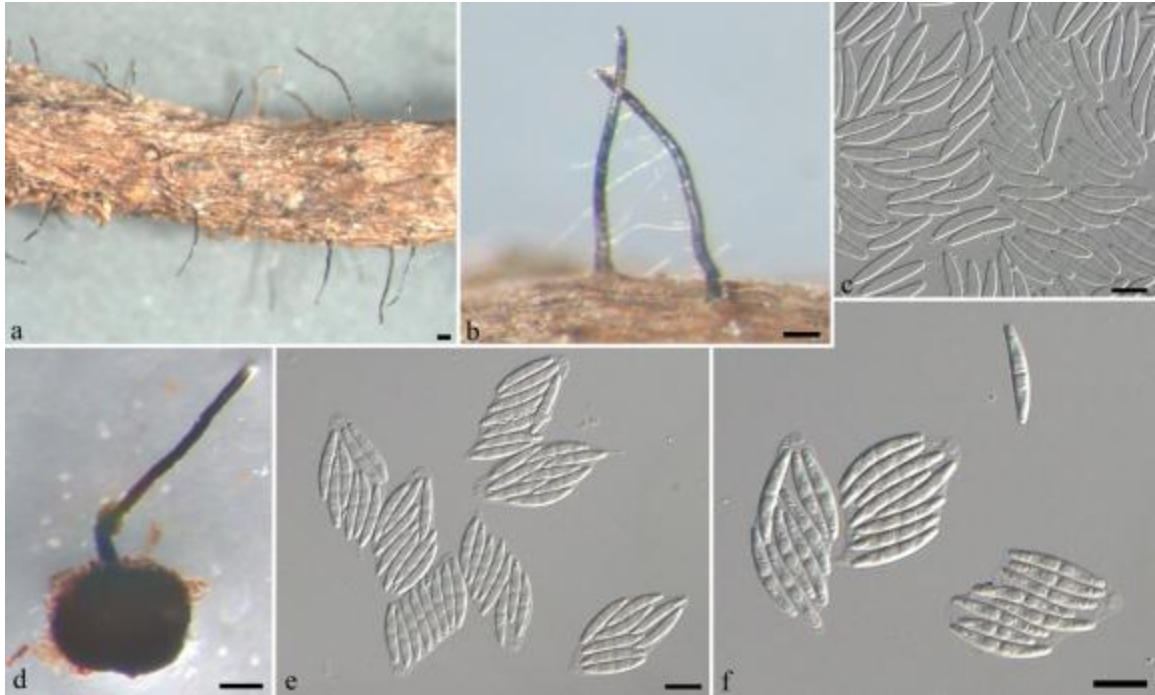
Fig. 4.31. *Ophiognomonia naganoensis*. a–b. Holotype BPI 882246; c, e–f. BPI 882244;

d. BPI 882211. Scale bars of perithecia = 100 μm. Scale bars of asci and ascospores = 10

μm.
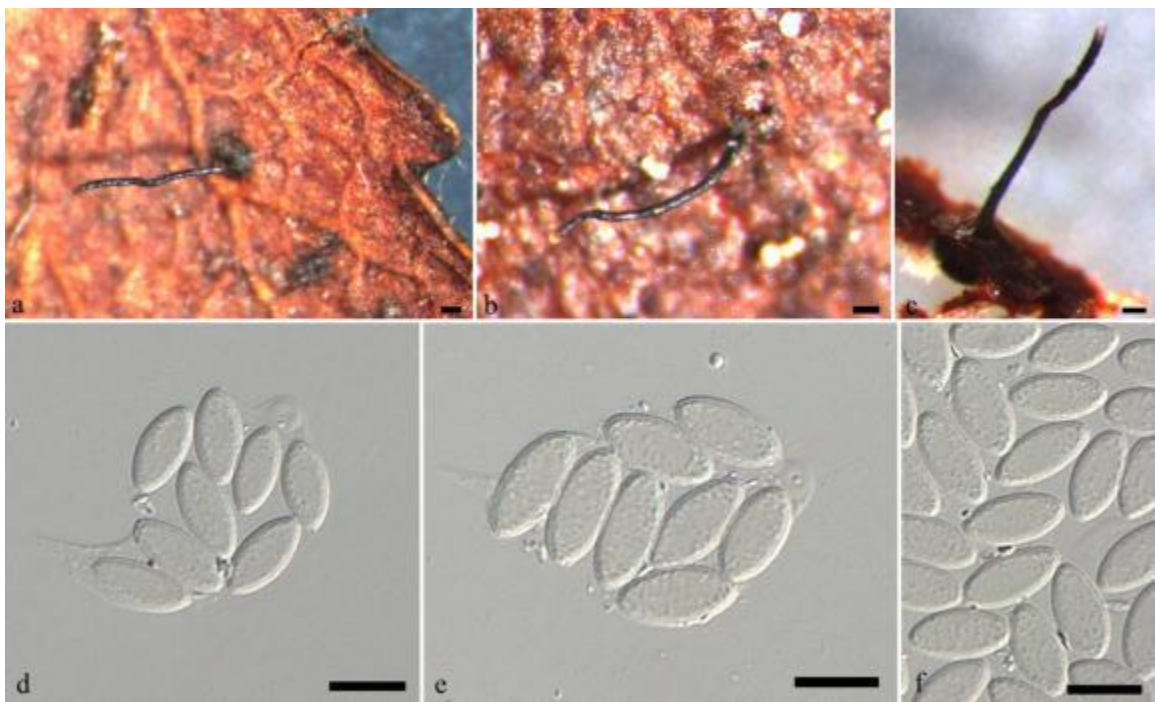
Fig. 4.32. *Ophiognomonia nana*. a–f. Lectotype Rehm Ascomyceten 1522. Scale bars of

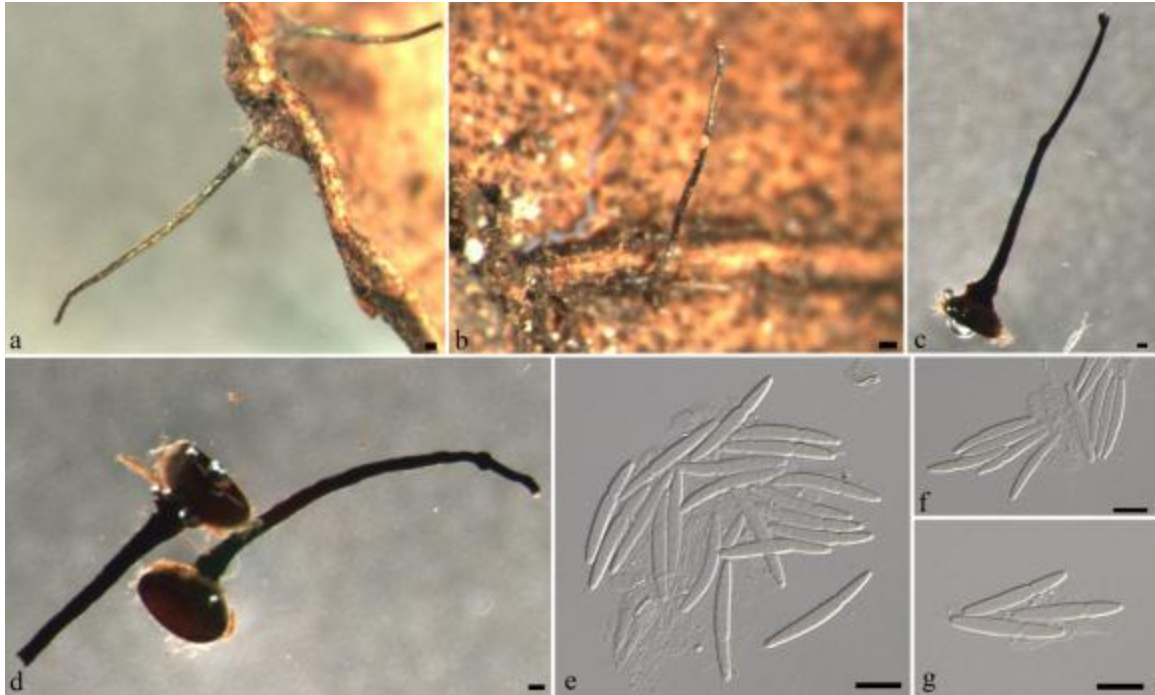perithecia = 100 μm. Scale bars of asci and ascospores = 10 μm.
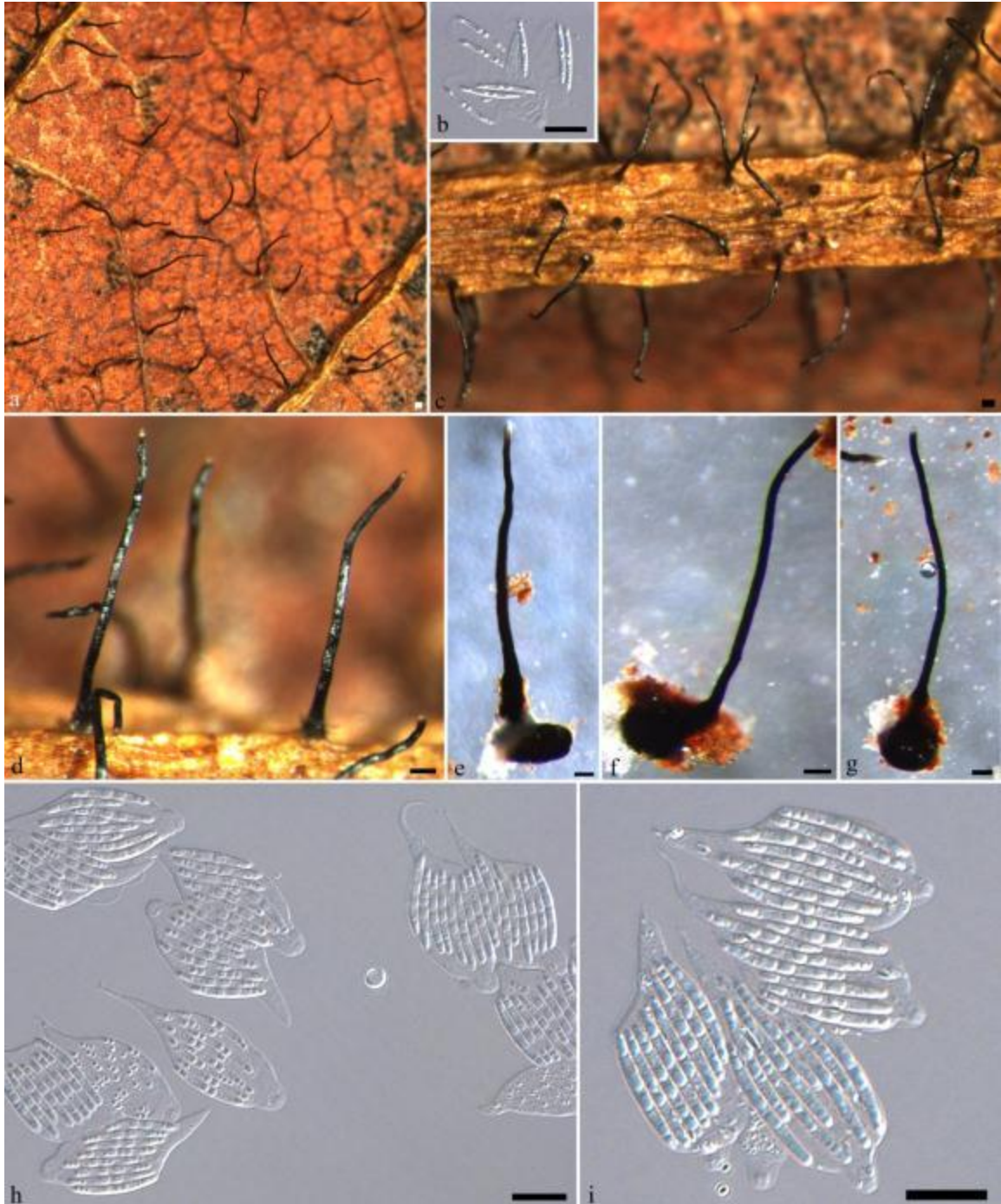
Fig. 4.33. *Ophiognomonia nipponicae*. a–i. Holotype BPI 882249. Scale bars of

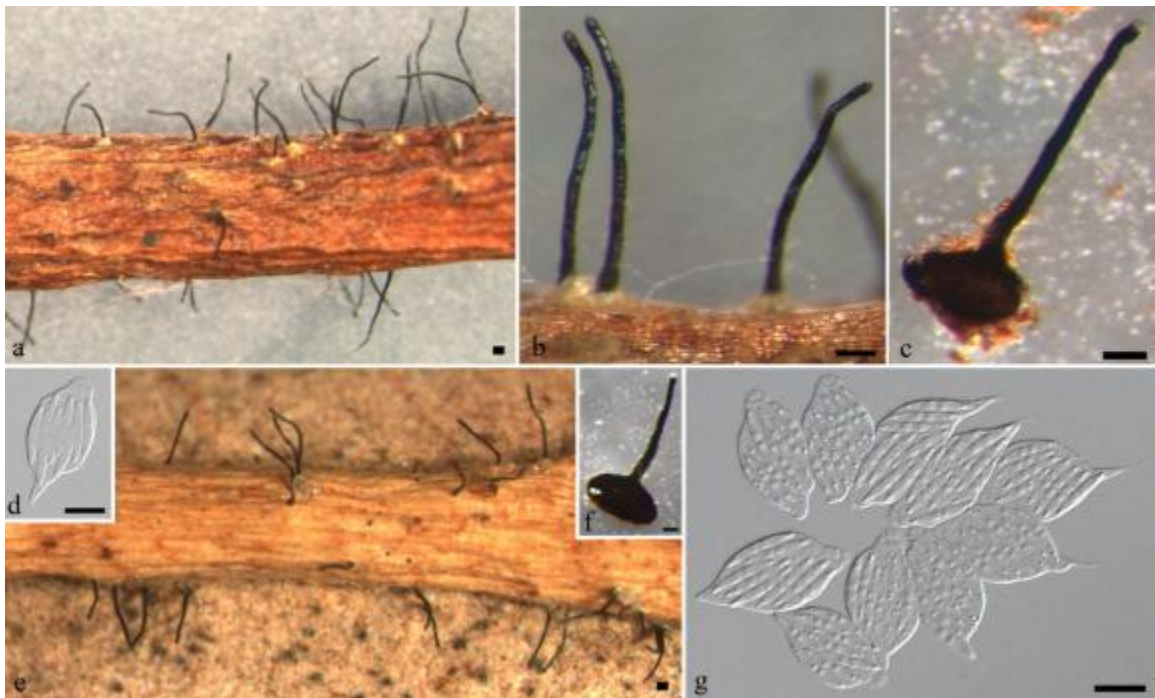perithecia = 100 μm. Scale bars of asci and ascospores = 10 μm.
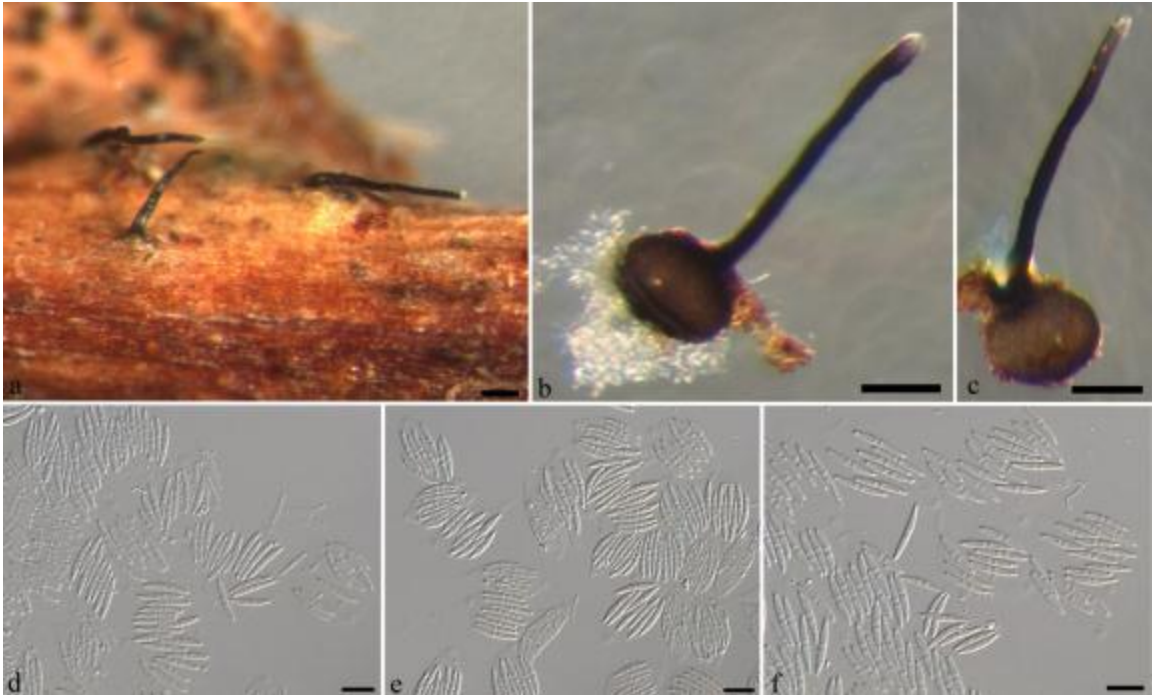
Fig. 4.34. *Ophiognomonia ostryae-virginianae*. a–f. Holotype BPI 879596. Scale bars of perithecia = 100 μm. Scale bars of asci and ascospores = 10 μm.
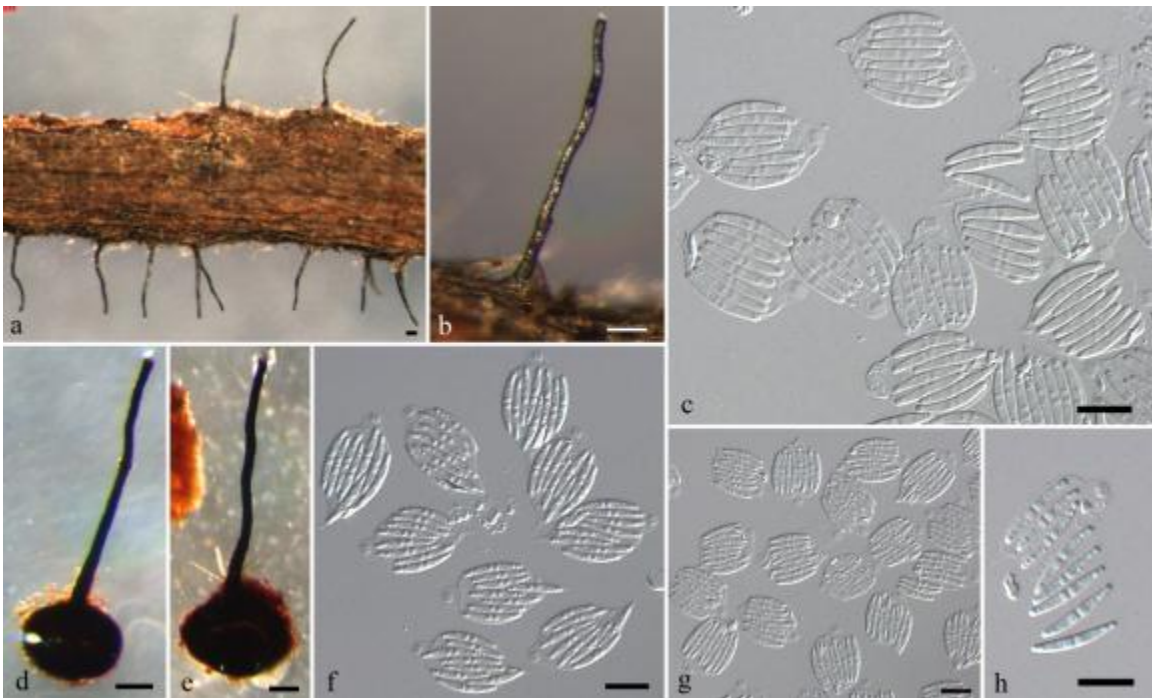
Fig. 4.35. *Ophiognomonia otanii*. a–b. Holotype BPI 882234; e–f, h. BPI 882237; c–d, g. BPI 882241. Scale bars of perithecia = 100 μm. Scale bars of asci and ascospores = 10 μm.
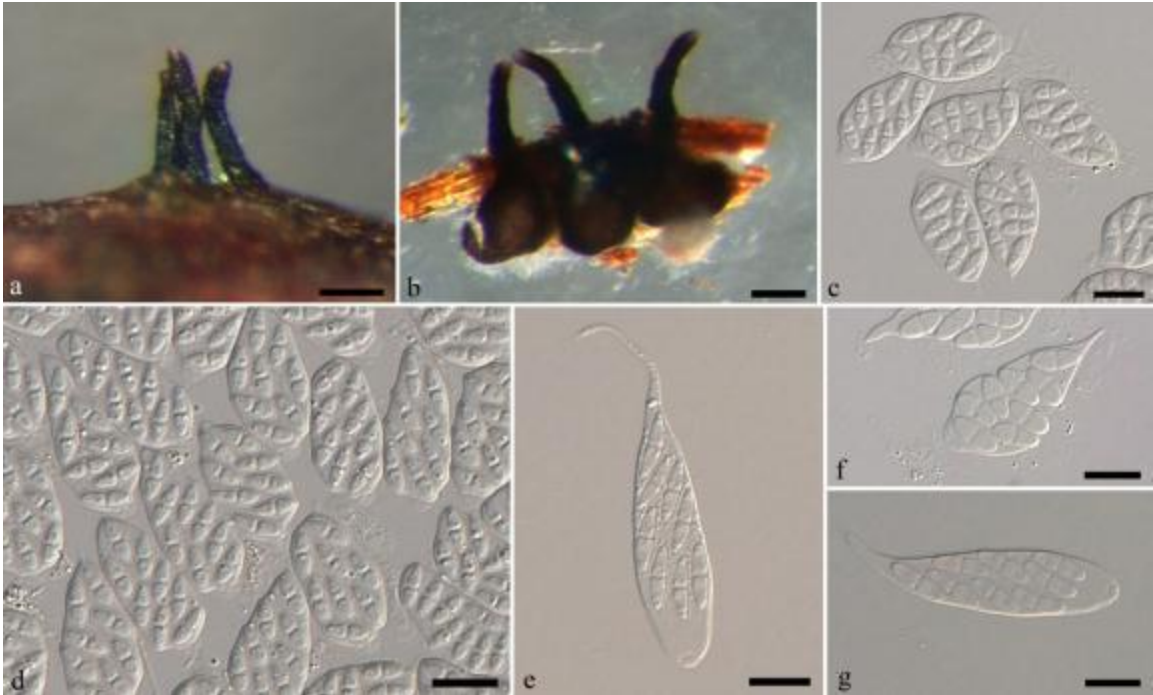
Fig. 4.36. *Ophiognomonia pseudoclavulata*. a–b, e, g. BPI 882283; c–d, f. BPI 882290.

Scale bars of perithecia = 100 μm. Scale bars of asci and ascospores = 10 μm.

Fig. 4.37. *Ophiognomonia pseudoischnostyla.* a, c–d, f. BPI 877617; b, e. BPI 877619.

Scale bars of perithecia = 100 μm. Scale bars of asci and ascospores = 10 μm.
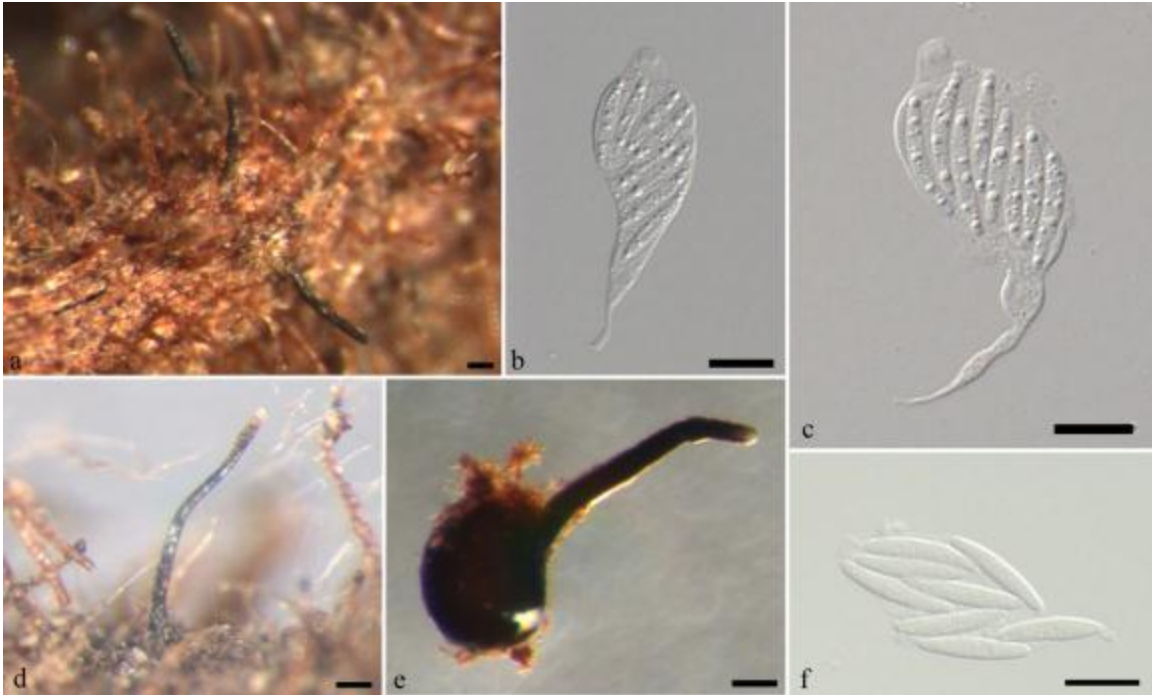
Fig. 4.38. *Ophiognomonia pterocaryae*. a–c, e. BPI 882219; d, f. Holotype BPI 882240.

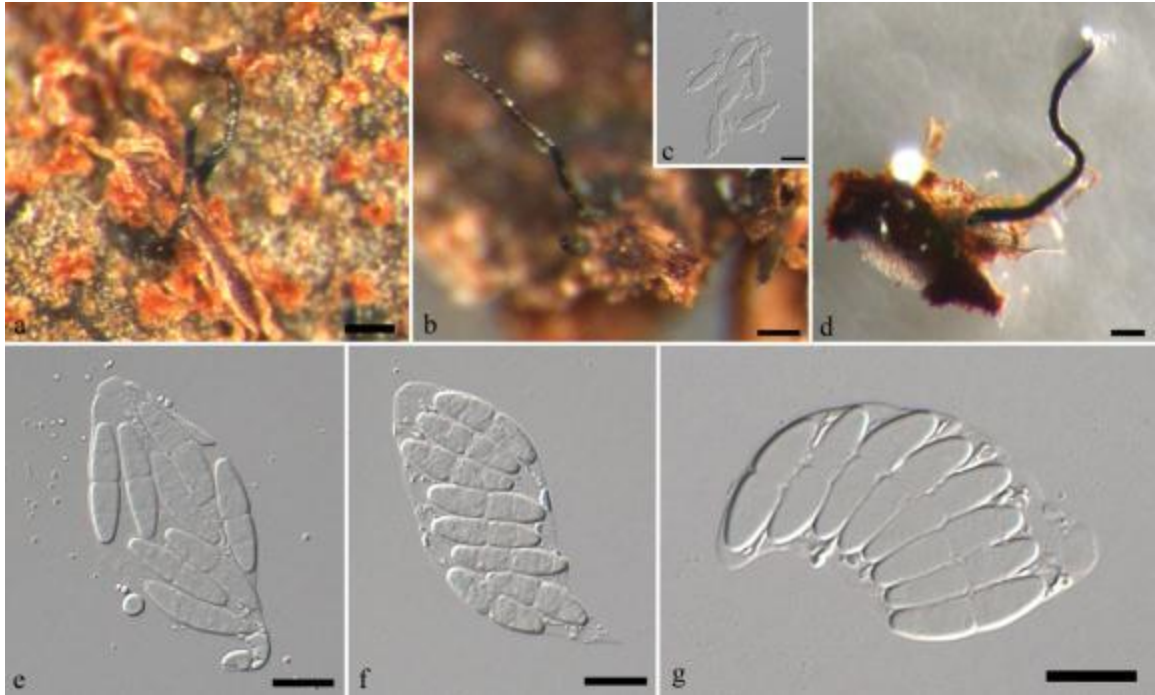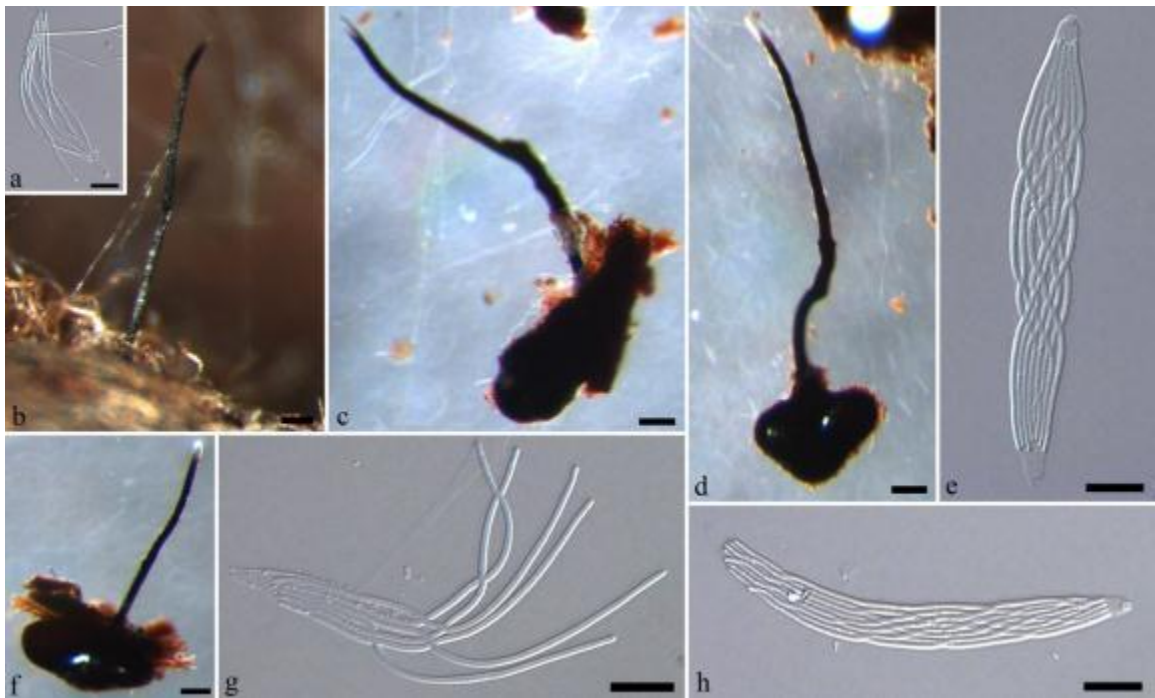Scale bars of perithecia = 100 μm. Scale bars of asci and ascospores = 10 μm.
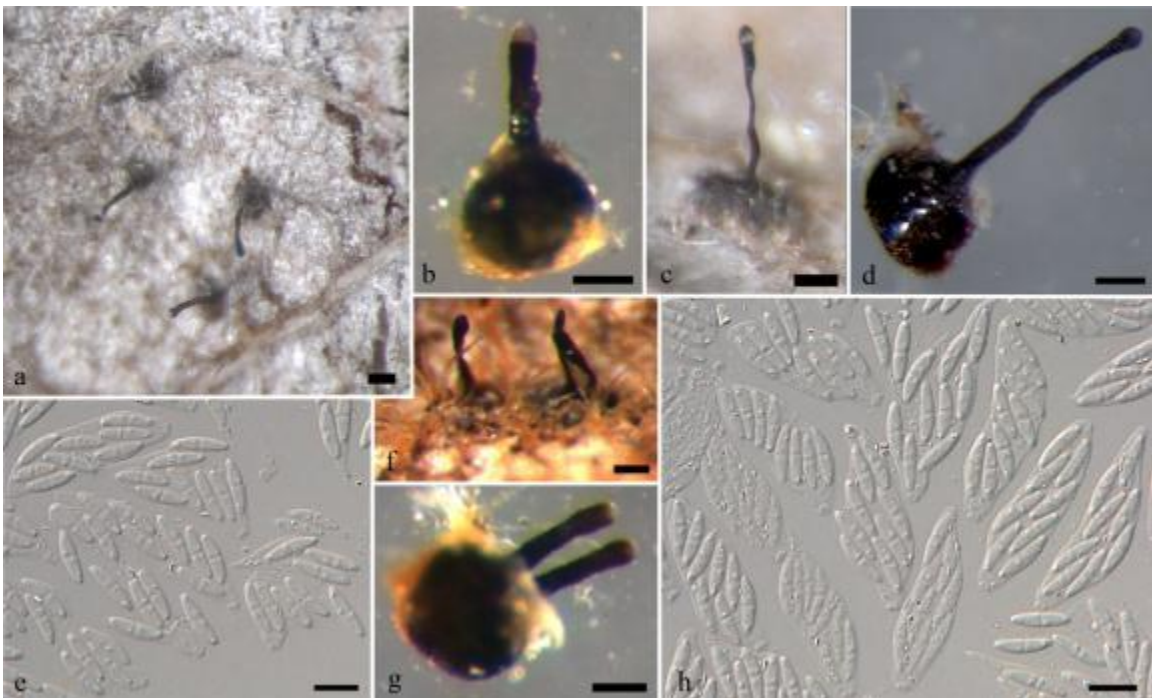
Fig. 4.39. *Ophiognomonia quercus-gambellii*. a, c, d. Epitype BPI 882202; b, e–h.

Holotype Barr 6095. Scale bars of perithecia = 100 μm. Scale bars of asci and ascospores

= 10 μm.
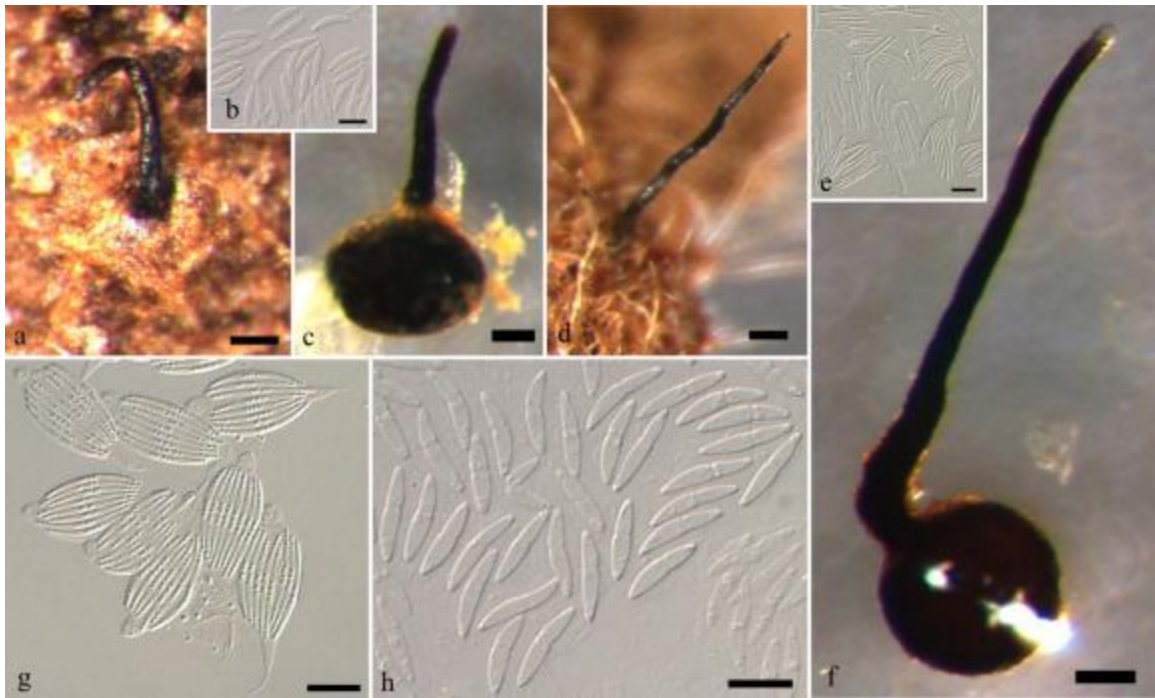
Fig. 4.40. *Ophiognomonia rosae*. a–c. h. Holotype Fuckel Fungi Rehnani 1790; d–g. Epitype BPI 882286. Scale bars of perithecia = 100 μm. Scale bars of asci and ascospores = 10 μm.

Fig. 4.41. *Ophiognomonia rubi-idaei*. a, f. BPI 877559B; b–c, e, g. BPI 877638; d. BPI 877637;. Scale bars of perithecia = 100 μm. Scale bars of asci and ascospores = 10 μm.
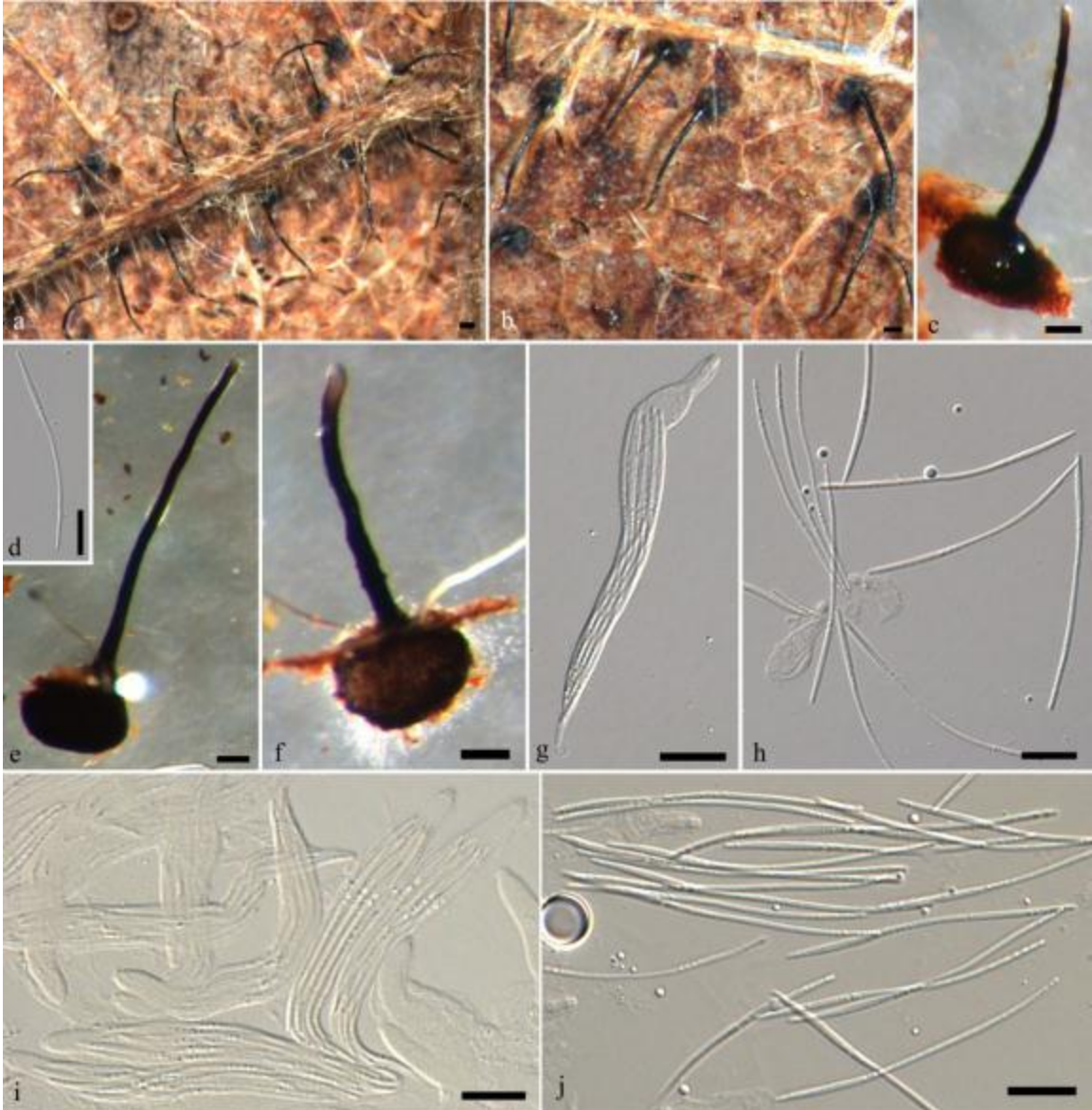
Fig. 4.42. *Ophiognomonia sassafras*. a–c, i. Holotype Ellis and Everhart 1684; d–e, g–h.

BPI 882282; f, j. Epitype BPI 882285. Scale bars of perithecia = 100 μm. Scale bars of

asci and ascospores = 10 μm.

Fig. 4.43. *Ophiognomonia setacea*. a, d. BPI 882275; b. BPI 882204; c, e, f. BPI 882223. Scale bars of perithecia = 100 μm. Scale bars of asci and ascospores = 10 μm.
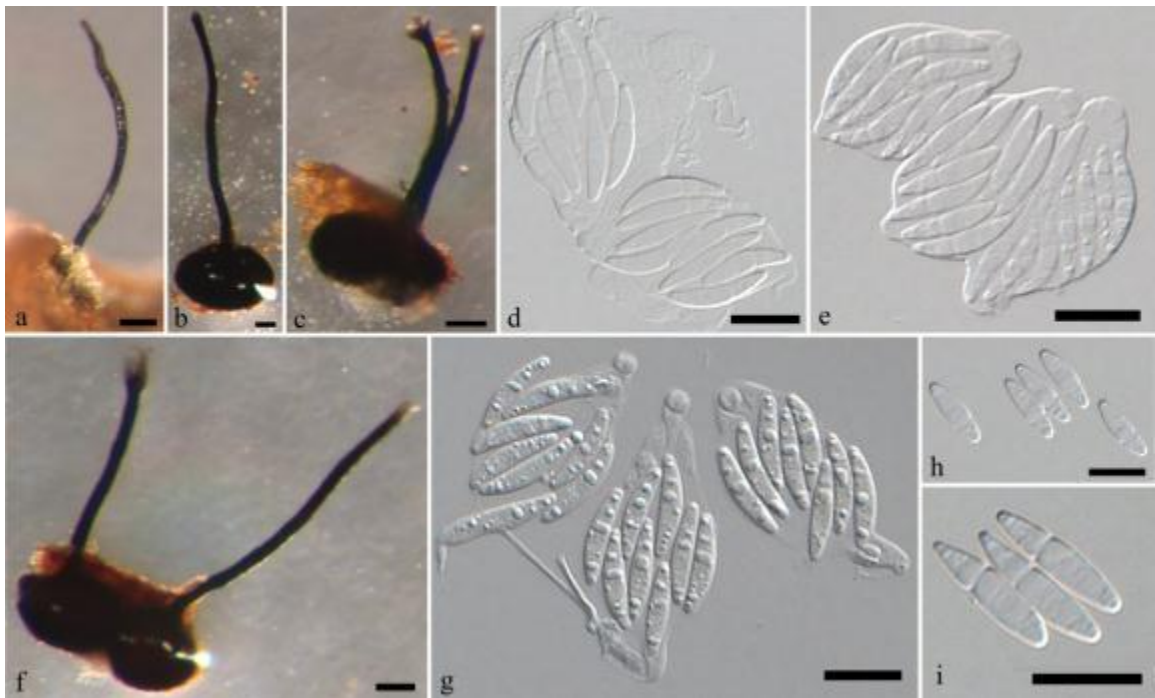
Fig. 4.44. *Ophiognomonia sogonovii*. a–e. BPI 882213; f–i. BPI 882221. Scale bars of perithecia = 100 μm. Scale bars of asci and ascospores = 10 μm.

Fig. 4.45. *Ophiognomonia trientensis*. a, c. BPI 877673; b, d–g. BPI 877672. Scale bars

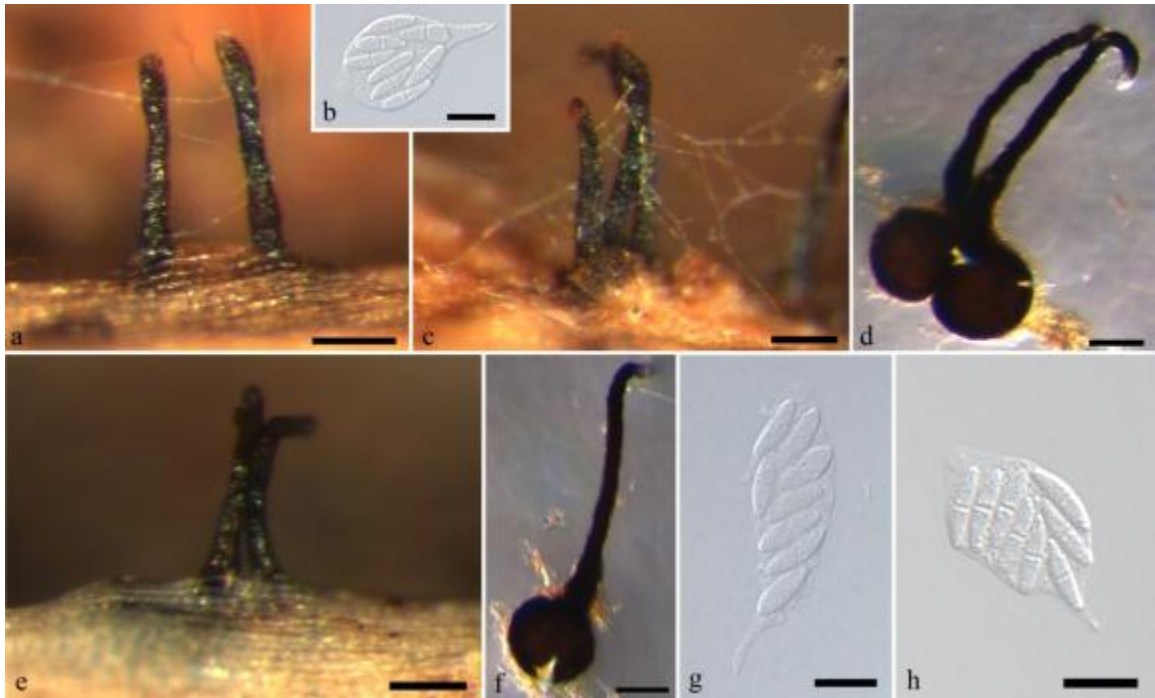of perithecia = 100 μm. Scale bars of asci and ascospores = 10 μm.
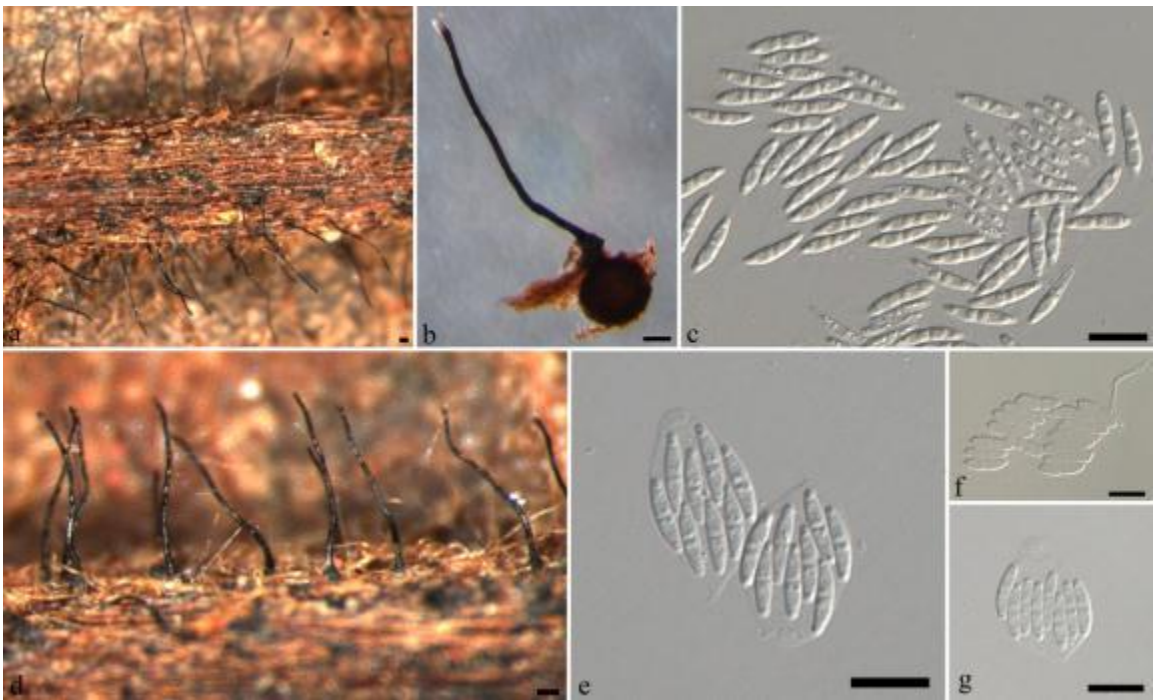
Fig. 4.46. *Ophiognomonia tucumanensis*. a–d, f. Holotype BPI 882288; e, g. BPI 879565.

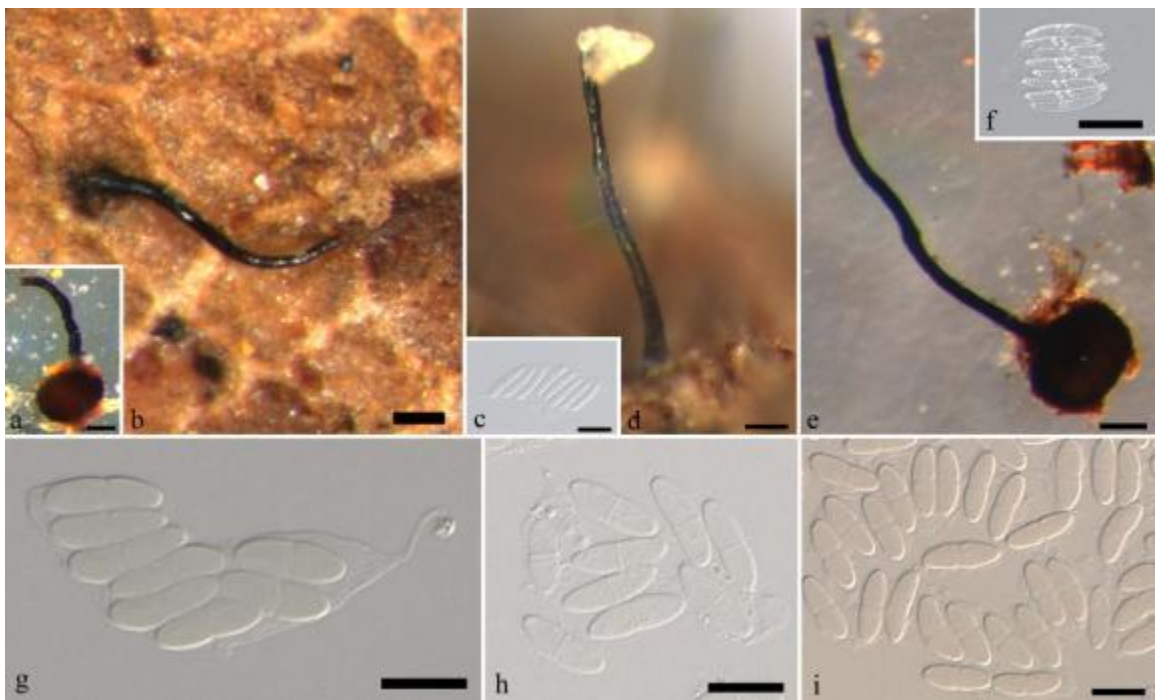Scale bars of perithecia = 100 μm. Scale bars of asci and ascospores = 10 μm.

Table 4.1. Specimens and cultures of Gnomoniaceae sequenced for this study.

Species	CBS #	Isolate	Specimen	ITS	MS204	*tef1-α*	Country	Host	Collector
Ambarignomonia petiolorum	CBS 121227	AR 4082	BPI 844274	EU254748.1*	JQ414056	JQ414140	USA	*Liquidambar styraciflua*	M.V. Sogonov
Discula destructiva	CBS 109771	AR 2596	BPI 1107757	JQ414221	JQ414053	JQ414137	USA	*Cornus nuttallii*	S. Redlin
Ophiognomonia alni-cordatae	CBS 131353	DMW 384.1	BPI 882233	JQ414243	JQ414091	JQ414175	Japan	*Alnus cordata*	D.M. Walker
Ophiognomonia alni-viridis	CBS 782.79	CBS 782.79	NA	EU254864.1*	JQ414064	JQ414148	Switzerland	*Alnus viridis*	M. Monod
Ophiognomonia alni-viridis	CBS 783.79	CBS 783.79	NA	EU254865.1*	JQ414065	JQ414149	Switzerland	*Betula* sp.	M. Monod
Ophiognomonia alni-viridis	CBS 131408	DMW 439.3	BPI 882251	JQ414260	JQ414108	JQ414192	USA	*Betula* sp.	D.M. Walker
Ophiognomonia alni-viridis	CBS 128358	LCM 494	BPI 879541	JF514848*	JF319085*	JF514826*	USA	*Alnus sinuata*	L.C. Mejía
Ophiognomonia apiospora	CBS 131425	LCM 503.05	BPI 879601	JQ414286	JQ414134	JQ414218	China	*Alnus nepalensis*	L.C. Mejía
Ophiognomonia apiospora	CBS 131426	LCM 503.06	BPI 879601	JQ414287	JQ414135	JQ414219	China	*Alnus nepalensis*	L.C. Mejía
Ophiognomonia asiatica	CBS 131351	DMW 378.2	BPI 882231	JQ414241	JQ414089	JQ414173	Japan	*Quercus serrata*	D.M. Walker
Ophiognomonia asiatica	CBS 131345	DMW 351.3	BPI 882220	JQ414233	JQ414081	JQ414165	Japan	*Quercus serrata*	D.M. Walker
Ophiognomonia asiatica	CBS 131347	DMW 361.1	BPI 882225	JQ414236	JQ414084	JQ414168	Japan	*Quercus aliena*	D.M. Walker
Ophiognomonia asiatica	CBS 131424	LCM 500.01	BPI 879600	JQ414285	JQ414133	JQ414217	China	*Quercus* sp.	L.C. Mejía
Ophiognomonia balsamiferae	CBS 121266	AR 4320	BPI 877606	EU254870.1*	JF319077*	JF514827*	Canada	*Populus balsamifera*	M.V. Sogonov
Ophiognomonia bugabensis	NA	LCM 362	NA	JQ414283	JQ414131	JQ414215	Panama	*Alnus acuminata*	L.C. Mejía
Ophiognomonia bugabensis	CBS 131399	LCM 368	NA	JQ414284	JQ414132	JQ414216	Panama	*Alnus acuminata*	L.C. Mejía
Ophiognomonia clavigignenti-juglandacearum	CBS 121081	AR 3791	NA	DQ323533.1*	JQ414054	JQ414138	USA	*Juglans cinerea*	M. Ostry
Ophiognomonia clavigignenti-juglandacearum	NA	AR 4539	NA	JQ414222	JQ414061	JQ414145	USA	*Juglans cinerea*	S. Anagnostakis
Ophiognomonia clavigignenti-juglandacearum	NA	ATCC 36624	BPI 880702	EU255069.1*	JQ414062	JQ414146	USA	*Juglans cinerea*	V.M.G. Nair
Ophiognomonia cordicarpa	CBS 131342	DMW 344.2	BPI 882217	JQ414230	JQ414078	JQ414162	Japan	*Pterocarya rhoifolia*	D.M. Walker

Species							Country	Host	Collector
Ophiognomonia gardiennetii	CBS 131409	DMW 442.1	BPI 882252	JQ414261	JQ414109	JQ414193	USA	*Alnus serrulata*	D.M. Walker
Ophiognomonia gardiennetii	CBS 131417	DMW 469.3	BPI 882262	JQ414265	JQ414113	JQ414197	USA	*Alnus serrulata*	D.M. Walker
Ophiognomonia gardiennetii	CBS 131429	DMW 513.1	BPI 882276	JQ414269	JQ414117	JQ414201	USA	*Alnus serrulata*	D.M. Walker
Ophiognomonia gei	CBS 818.79	CBS 818.79	NA	EU254928.1*	NA	NA	Switzerland	*Fragaria vesca*	M. Monod
Ophiognomonia gei-montani	CBS 821.79	CBS 821.79	NA	EU254871*	JF319078*	JF514828*	Switzerland	*Geum montanum*	M. Monod
Ophiognomonia gunmensis	CBS 131401	DMW 388.1	BPI 882236	JQ414246	JQ414094	JQ414178	Japan	*Quercus serrata*	D.M. Walker
Ophiognomonia hiawathae	CBS 131413	DMW 458.3	BPI 882256	JQ414263	JQ414111	JQ414195	USA	*Betula lutea*	D.M. Walker
Ophiognomonia hiawathae	CBS 131416	DMW 466.1	BPI 882261	JQ414264	JQ414112	JQ414196	USA	*Betula lutea*	D.M. Walker
Ophiognomonia ibarakiensis	CBS 131405	DMW 419.3	BPI 882247	JQ414257	JQ414105	JQ414189	Japan	*Alnus* sp.	D.M. Walker
Ophiognomonia ibarakiensis	CBS 131349	DMW 371.1	BPI 882227	JQ414238	JQ414086	JQ414170	Japan	*Alnus* sp.	D.M. Walker
Ophiognomonia intermedia	CBS 119197	AR 4147	BPI 880534	EU254875.1*	JF319074*	JF514825*	United Kingdom	*Betula alba*	S. Green
Ophiognomonia intermedia	CBS 131421	DMW 486.1	BPI 882267	JQ414267	JQ414115	JQ414199	USA	*Betula lutea*	D.M. Walker
Ophiognomonia intermedia	CBS 131418	DMW 470.1	BPI 882263	JQ414266	JQ414114	JQ414198	USA	*Alnus serrulata*	D.M. Walker
Ophiognomonia ischnostyla	CBS 121234	AR 4190	BPI 871054B	EU254897.1*	JQ414058	JQ414142	Switzerland	*Corylus avellana*	M.V. Sogonov
Ophiognomonia ischnostyla	CBS 838.79	CBS 838.79	NA	EU254891.1*	JQ414066	JQ414150	Switzerland	*Carpinus betulus*	M. Monod
Ophiognomonia japonica	CBS 131355	DMW 387.2	BPI 882235	JQ414245	JQ414093	JQ414177	Japan	*Prunus japonica*	D.M. Walker
Ophiognomonia kobayashii	CBS 131343	DMW 347.2	BPI 882218	JQ414231	JQ414079	JQ414163	Japan	*Castanea crenata*	D.M. Walker
Ophiognomonia kobayashii	CBS 131352	DMW 379.3	BPI 882232	JQ414242	JQ414090	JQ414174	Japan	*Castanea crenata*	D.M. Walker
Ophiognomonia kobayashii	CBS 131350	DMW 374.2	BPI 882229	JQ414240	JQ414088	JQ414172	Japan	*Castanea crenata*	D.M. Walker
Ophiognomonia kobayashii	CBS 131403	DMW 416.1	BPI 882245	JQ414255	JQ414103	JQ414187	Japan	*Castanea crenata*	D.M. Walker
Ophiognomonia lenticulispora	CBS 131363	DMW 544	BPI 882287	JQ414277	JQ414125	JQ414209	USA	*Prunus* sp.	D.M. Walker
Ophiognomonia leptostyla	CBS 110136	CBS 110136	NA	DQ323535.1*	JQ414063	JQ414147	USA	*Juglans* sp.	D. Farr
Ophiognomonia longispora	CBS 131337	DMW 325.4	BPI 882210	JQ414225	JQ414073	JQ414157	Japan	*Tilia maximowicziana*	D.M. Walker

Ophiognomonia longispora	CBS 131358	DMW 394.3	BPI 882239	JQ414249	JQ414097	JQ414181	Japan	*Tilia maximowicziana*	D.M. Walker
Ophiognomonia maximowiczianae	CBS 131357	DMW 392.1	BPI 882238	JQ414248	JQ414096	JQ414180	Japan	*Betula maximowicziana*	D.M. Walker
Ophiognomonia melanostyla	CBS 131431	DMW 533	BPI 882279	JQ414270	JQ414118	JQ414202	France	*Tilia* sp.	Y. Mourgues, M. Chovillon
Ophiognomonia melanostyla	CBS 128482	LCM 389.01	BPI 879257	JF514849*	JF319084*	JF514830*	Germany	*Tilia heterophylla*	L.C. Mejía
Ophiognomonia michiganensis	CBS 131412	DMW 454.3	BPI 882255	JQ414262	JQ414110	JQ414194	USA	*Betula papyrifera*	D.M. Walker
Ophiognomonia michiganensis	CBS 131422	DMW 492.1	BPI 882268	JQ414268	JQ414116	JQ414200	USA	*Alnus serrulata*	D.M. Walker
Ophiognomonia michiganensis	CBS 121252	AR 4295	BPI 877624	EU254901.1*	JF319076*	JF514820*	USA	*Betula lenta*	M.V. Sogonov
Ophiognomonia micromegala	CBS 131432	DMW 535	BPI 882280	JQ414271	JQ414119	JQ414203	USA	*Carya* sp.	D.M. Walker
Ophiognomonia micromegala	CBS 131433	DMW 536	BPI 882281	JQ414272	JQ414120	JQ414204	USA	*Carya* sp.	D.M. Walker
Ophiognomonia monticola	CBS 131346	DMW 357.3	BPI 882222	JQ414235	JQ414083	JQ414167	Japan	*Carpinus* sp.	D.M. Walker
Ophiognomonia monticola	CBS 131361	DMW 405.3	BPI 882243	JQ414253	JQ414101	JQ414185	Japan	*Carpinus* sp.	D.M. Walker
Ophiognomonia multirostrata	CBS 131348	DMW 364.3	BPI 882226	JQ414237	JQ414085	JQ414169	Japan	*Alnus firma*	D.M. Walker
Ophiognomonia multirostrata	CBS 131400	DMW 373.1	BPI 882228	JQ414239	JQ414087	JQ414171	Japan	*Alnus firma*	D.M. Walker
Ophiognomonia multirostrata	CBS 131406	DMW 423.1	BPI 882248	JQ414258	JQ414106	JQ414190	Japan	*Alnus firma*	D.M. Walker
Ophiognomonia naganoensis	CBS 131338	DMW 331.2	BPI 882211	JQ414226	JQ414074	JQ414158	Japan	*Alnus hirsuta var. sibirica*	D.M. Walker
Ophiognomonia naganoensis	CBS 131362	DMW 410.1	BPI 882244	JQ414254	JQ414102	JQ414186	Japan	*Alnus hirsuta var. sibirica*	D.M. Walker
Ophiognomonia naganoensis	CBS 131404	DMW 418.3	BPI 882246	JQ414256	JQ414104	JQ414188	Japan	*Alnus hirsuta*	D.M. Walker
Ophiognomonia nana	CBS 883.79	CBS 883.79	NA	JQ414223	JQ414071	JQ414155	Finland	*Betula nana*	M. Monod
Ophiognomonia nipponicae	CBS 131407	DMW 424.1	BPI 882249	JQ414259	JQ414107	JQ414191	Japan	*Prunus nipponica*	D.M. Walker
Ophiognomonia ostryae-virginianae	CBS 131398	LCM 155.01	BPI 879596	JQ414282	JQ414130	JQ414214	USA	*Ostrya virginiana*	L.C. Mejía
Ophiognomonia otanii	CBS 131402	DMW 401.3	BPI 882242	JQ414252	JQ414100	JQ414184	Japan	*Castanea crenata*	D.M. Walker
Ophiognomonia otanii	CBS 131354	DMW 385.1	BPI 882234	JQ414244	JQ414092	JQ414176	Japan	*Castanea crenata*	D.M. Walker

Ophiognomonia otanii	CBS 131356	DMW 390.1	BPI 882237	JQ414247	JQ414095	JQ414179	Japan	*Castanea crenata*	D.M. Walker
Ophiognomonia otanii	CBS 131360	DMW 397.1	BPI 882241	JQ414251	JQ414099	JQ414183	Japan	*Castanea crenata*	D.M. Walker
Ophiognomonia padicola	CBS 845.79	CBS 845.79	NA	JF514845*	JF319080*	JF514832*	Switzerland	*Prunus padus*	M. Monod
Ophiognomonia pseudoclavulata	CBS 121236	AR 4059	BPI 844280	EU254923.1*	JF319073*	JF514819*	USA	*Carya tomentosa*	M.V. Sogonov
Ophiognomonia pseudoclavulata	CBS 131434	DMW 538	BPI 882283	JQ414273	JQ414121	JQ414205	USA	*Carya* sp.	D.M. Walker
Ophiognomonia pseudoclavulata	CBS 131367	DMW 551	BPI 882290	JQ414280	JQ414128	JQ414212	USA	*Carya* sp.	D.M. Walker
Ophiognomonia pseudoischnostyla	CBS 121228	AR 4120	BPI 877616	EU254919.1*	JQ414057	JQ414141	Russia	*Betula pubescens*	M.V. Sogonov
Ophiognomonia pseudoischnostyla	CBS 842.79	CBS 842.79	NA	EU254892.1*	JQ414067	JQ414151	Switzerland	*Alnus incana*	M. Monod
Ophiognomonia pterocaryae	CBS 131359	DMW 396.3	BPI 882240	JQ414250	JQ414098	JQ414182	Japan	*Pterocarya rhoifolia*	D.M. Walker
Ophiognomonia pterocaryae	CBS 131344	DMW 350.2	BPI 882219	JQ414232	JQ414080	JQ414164	Japan	*Pterocarya rhoifolia*	D.M. Walker
Ophiognomonia quercus-gambellii	CBS 131397	DMW 117.1	BPI 882202	JQ414224	JQ414072	JQ414156	USA	*Quercus kellogii*	D.M. Walker
Ophiognomonia rosae	CBS 850.79	CBS 850.79	NA	EU254929.1*	JQ414068	JQ414152	Switzerland	*Rubus* sp.	M. Monod
Ophiognomonia rosae	CBS 851.79	CBS 851.79	NA	EU254930.1*	JQ414069	JQ414153	Finland	*Comarum palustre*	M. Monod
Ophiognomonia rosae	CBS 128442	DMW 108.2	BPI 882201	JF514851*	JF319081*	JF514824*	USA	*Fragaria vesca*	D.M. Walker
Ophiognomonia rosae	CBS 131365	DMW 543	BPI 882286	JQ414276	JQ414124	JQ414208	France	*Rubus* sp.	A. Gardiennet
Ophiognomonia rubi-idaei	NA	NA	BPI 877559B	EU254939.1*	NA	NA	Canada	*Rubus* sp.	M.V. Sogonov
Ophiognomonia rubi-idaei	NA	NA	BPI 877637	EU254937.1*	NA	NA	Switzerland	*Rubus ideaus*	M.V. Sogonov
Ophiognomonia rubi-idaei	NA	NA	BPI 877638	EU254938.1*	NA	NA	Canada	*Rubus spectabilis*	M.V. Sogonov
Ophiognomonia sassafras	CBS 121243	AR 4284	BPI 877639	EU254941.1*	JF319075*	JF514829*	USA	*Sassafras albidum*	M.V. Sogonov
Ophiognomonia sassafras	CBS 131435	DMW 541	BPI 882284	JQ414274	JQ414122	JQ414206	USA	*Sassafras albidum*	C.M. Milensky
Ophiognomonia sassafras	CBS 131366	DMW 542	BPI 882285	JQ414275	JQ414123	JQ414207	USA	*Sassafras albidum*	D.M. Walker
Ophiognomonia setacea	CBS 121230	AR 4193	BPI 877646	EU254955.1*	JQ414059	JQ414143	USA	*Castanea dentata*	M.V. Sogonov
Ophiognomonia setacea	CBS 859.79	CBS 859.79	NA	AY818958.1*	JQ414070	JQ414154	Switzerland	*Quercus* sp.	M. Monod

Ophiognomonia setacea	CBS 128352	DMW 291.1	BPI 882205	JF514846*	JF319082*	JF514822*	USA	*Quercus palustris*	D.M. Walker
Ophiognomonia setacea	CBS 128354	DMW 310.1	BPI 882208	JF514847*	JF319035*	JF514823*	USA	*Quercus* sp.	D.M. Walker
Ophiognomonia setacea	CBS 131339	DMW 333.2	BPI 882212	JQ414227	JQ414075	JQ414159	Japan	*Quercus acutissima*	D.M. Walker
Ophiognomonia sogonovii	CBS 121914	AR 4000	BPI 872323	EU199190.1*	JQ414055	JQ414139	Russia	*Quercus mongolica*	L. Vasilyeva
Ophiognomonia sogonovii	CBS 131340	DMW 336.3	BPI 882213	JQ414228	JQ414076	JQ414160	Japan	*Quercus mongolica*	D.M. Walker
Ophiognomonia sogonovii	CBS 131341	DMW 337.1	BPI 882214	JQ414229	JQ414077	JQ414161	Japan	*Quercus serrata*	D.M. Walker
Ophiognomonia sogonovii	CBS 131661	DMW 353.1	BPI 882221	JQ414234	JQ414082	JQ414166	Japan	*Quercus mongolica var. grosseserrata*	D.M. Walker
Ophiognomonia trientensis	CBS 131604	DMW 554	BPI 882638	JQ414281	JQ414129	JQ414213	USA	*Alnus* sp.	D.M. Walker
Ophiognomonia tucumanensis	CBS 131364	DMW 549	BPI 882288	JQ414278	JQ414126	JQ414210	Argentina	*Alnus acuminata*	A.Y. Rossman
Ophiognomonia tucumanensis	CBS 131368	LCM 622.01	BPI 879565	JQ414288	JQ414136	JQ414220	Argentina	*Alnus acuminata*	L.C. Mejía
Ophiognomonia vasiljevae	CBS 121253	AR 4298	BPI 877671	EU254977.1*	JQ414060	JQ414144	USA	*Juglans nigra*	M.V. Sogonov
Ophiognomonia vasiljevae	CBS 128353	DMW 303.3	BPI 882206	JF514850*	JF319083*	JF514831*	USA	*Juglans nigra*	M.V. Sogonov
Ophiognomonia vasiljevae	CBS 131436	DMW 550	BPI 882289	JQ414279	JQ414127	JQ414211	USA	*Juglans* sp.	D.M. Walker

Table 4.1. Specimens and cultures of Gnomoniaceae sequenced for this study. AR = Dr. Amy Rossman, third author; BPI = U.S. National Fungus Collections, USDA, ARS, Beltsville, MD; CBS = Centraalbureau voor Schimmelcultures, Utrecht, the Netherlands; DMW = Donald M. Walker, first author; NA= not available; * = DNA sequence from an alternative study.

Table 4.2. Phylogenetic Species Recognition

Clade #	Species	Genealogical Sorting Index GSI				GCPSR			
		ITS	MS204	*tef-1a*	Combined	ITS n = ≥ 70%	MS204 n = ≥ 70%	*tef-1a* n = ≥ 70%	Combined n = ≥ 70%
		gsiT	*gsiT*	*gsiT*	*gsiT*				
1	O. setacea	0.8835*	1.0*	0.9911*	1.0*	NS	x	x	x
	O. sogonovii	0.9338*	0.9972*	0.9972*	1.0*	x	x	x	x
	O. asiatica	0.4692*	0.9972*	0.9848*	1.0*	NS	x	x	x
	O. kobayashii	0.3853*	0.9837*	1.0*	0.9972*	NS	x	x	x
	O. otanii	0.9237*	0.9945*	1.0*	0.9918*	x	x	x	x
	O. michiganensis	0.2717*	1.0*	0.6815*	1.0*	NS	x	NS	x
	O. hiawathae	0.1321	1.0*	0.2174	1.0*	NS	x	NS	x
	O. pseudoischnostyla	0.1411	1.0*	0.7346*	1.0*	NS	x	x	x
	O. ischnostyla	0.1207	1.0*	1.0*	1.0*	NS	x	x	x
	O. monticola	0.917*	1.0*	1.0*	1.0*	x	x	x	x
2	O. sassafras	0.9934*	0.5727*	1.0*	0.9963*	x	x	x	x
	O. melanostyla	0.8323*	0.7504*	0.9191*	0.9739*	x	x	x	x
	O. longispora	0.9547*	0.9895*	0.9226*	1.0*	x	x	x	x
	O. rosae	1.0*	1.0*	0.9914*	0.9942*	x	x	x	x
	O. pseudoclavulata	0.6309*	0.640*	1.0*	1.0*	x	x	x	x
	O. micromegala	0.9321*	1.0*	0.4782*	0.9947*	x	x	x	x
	O. vasiljevae	0.9927*	0.9963*	0.9818*	1.0*	x	x	x	x
	O. rubi-idaei	0.8194*	NA	NA	NA	x	NA	NA	NA
3	O. alni-viridis	0.2083*	0.9846*	0.9890*	0.9972*	NS	x	x	x
	O. multirostrata	0.6435*	0.9893*	0.9893*	0.9858*	NS	x	x	x
	O. bugabensis	0.8609*	0.9845*	0.9742*	0.9793*	x	x	x	x
	O. intermedia	0.1551*	0.8186*	0.9415*	0.9858*	NS	x	x	x
	O. tucumanensis	0.6406*	0.9948*	1.0*	1.0*	NS	x	x	x
	O. ibarakiensis	0.8449*	0.9896*	0.9948*	1.0*	x	x	x	x

O. clavigignenti-juglandacearum	0.9079*	1.0*	1.0*	1.0*	x	x	x	x
O. gardiennetii	0.9929*	1.0*	1.0*	0.9964*	x	x	x	x
O. pterocaryae	0.9244*	1.0*	1.0*	1.0*	x	x	x	x
O. apiospora	0.9587*	0.9948*	0.9845*	0.9948*	x	x	x	x
O naganoensis	0.9858*	0.9964*	1.0*	0.9964*	x	x	x	x

Table 4.2. Phylogenetic Species Recognition. The Clade # correlates with Figs. 2–4; The GSI statistic is based on a 0 to 1 continuum, with 0 = lack of genealogical divergence from other groups and 1 = monophyly; * = statistically significant; X = MPBS (maximum parsimony bootstrap) support ≥ 70%; NS = MPBS ≤ 70%; NA = not available.

Supplementary Table 4.1. jModelTest results

	Molecular marker		
Alignment	**ITS**	***tef-1α***	**MS204**
Complete dataset	SYM+I+G	TIM1+I+G	GTR+I+G
Clade 1	TVMef+I	TIM1+G	TrN+G
Clade 2	TPM+I+G	TIM1+G	TPM1uf+G
Clade 3	SYM+I+G	TIM1+I+G	GTR+I+G

Supplementary Table 4.1. Model selected using jModelTest for Bayesian and ML analyses.

LITERATURE CITED

Aguileta, G., Marthey, S., Chiapello, H., Lebrun, M-H., Rodolphe, F., Fournier, E., Gendrault-Jacquemard, A., Giraud, T., 2008. Assessing the performance of single-copy genes for recovering robust phylogenies. Syst. Biol. 57 (4), 613–627. doi:10.1080/10635150802306527

Anderson, R.L., LaMadeleine, L.A., 1978. The distribution of butternut decline in the eastern United States. Forest Survey Report USDA Forest Service, 5.

Avise, J.C., Ball, R.M., Jr., 1990. Principles of genealogical concordance in species concepts and biological taxonomy. In Oxford Surveys in Evolutionary Biology, vol 7. Oxford Univ. Press, Oxford.

Barr, M.E., 1978. The Diaporthales in North America with emphasis on *Gnomonia* and its segregates. Mycologia Memoir 7, 1–232.

Barrett, D.K., Pearce, R.B., 1981. Giant leaf blotch disease of sycamore (*Acer pseudoplatanus*) in Britain. Trans. Brit. Mycol. Soc. 76, 317–345.

Behdad, E., 1991. Plant protection encyclopedia of Iran: pests, diseases, and weeds. Isfahan, Yad-boud Publisher.

Belisario, A., Scotton, M., Santori, A., Onofri, S., 2008. Variability in the Italian population of *Gnomonia leptostyla*, homothallism and resistance of *Juglans* species to anthracnose. Forest Pathol. 38 (2), 129–145. doi:10.1111/j.1439-0329.2007.00540.x

Berry, F.H., 1981. Walnut anthracnose forest insect & disease leaflet 85. US Department of Agriculture, Forest Service, Northern Area State & Private Forestry, Broomall, PA.

Broders, K.D., Boland, G.J., 2011. Reclassification of the butternut canker fungus, *Sirococcus clavigignenti-juglandacearum*, into the genus *Ophiognomonia*. Fungal. Biol. 115 (1), 70–79.

Broughton, R.E., Harrison, R.G., 2003. Nuclear gene genealogies reveal historical, demographic and selective factors associated with speciation in field crickets. Genetics 163 (4), 1389–1401.

Butin, H., Wulf, A., 1987. *Asteroma pseudoplatani* sp. nov., anamorph of *Pleuroceras pseudoplatani* (v. Tubeuf) Monod. Sydowia 40, 38–41.

Castresana, J., 2000. Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. Mol. Biol. Evol. 17 (4), 540–552.

Cummings, M.P., Neel, M.C., Shaw, K.L., 2008. A genealogical approach to quantifying lineage divergenece. Evolution 62 (9), 2411–2422. doi:10.1111/j.1558-5646.2008.00442.x

Damm, U., Crous, P.W., Fourie, P.H., 2007. Botryosphaeriaceae as potential pathogens of *Prunus* species in South Africa, with descriptions of *Diplodia africana* and *Lasiodiplodia plurivora* sp. nov. Mycologia 99 (5), 664–680. doi:10.3852/mycologia.99.5.664

Dettman, J.R., Jacobson, D.J., Taylor, J.W., 2003. A multilocus genealogical approach to phylogenetic species recognition in the model eukaryote *Neurospora*. Evolution 57 (12), 2703–2720. doi:10.1111/j.0014-3820.2003.tb01514.x

Eriksson, O.E., 1992. The non-lichenized pyrenomycetes of Sweden. Btjtryck, Lund, Sweden, 208 pgs.

Green, S., 2004. Fungi associated with shoots of silver birch (*Betula pendula*) in Scotland. Mycol. Res. 108, 1327–1336.

Green, S., Castlebury, L.A., 2007. Connection of *Gnomonia intermedia* to *Discula betulina* and its relationship to other taxa in Gnomoniaceae. Mycol. Res. 111, 62–69.

Hillis, D.M., Dixon, M.T., 1991. Ribosomal DNA: molecular evolution and phylogenetic inference. Q. Rev. Biol. 66 (4), 411–453.

Hudson, R.R., Coyne, J.A., 2002. Mathematical consequences of the genealogical species concept. Evolution 56 (8), 1557–1565. doi:10.1111/j.0014-3820.2002.tb01467.x

Huelsenbeck, J.P., Ronquist, F., 2001. MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17, 754–755.

Johnson, L.A., Soltis, D.E., 1998. Assessing congruence: empirical examples from molecular data. In: Soltis, D.E., Soltis, P.S., Doyle, J.J., eds. Molecular Systematics of Plants 2. Kluwer, U.S.A., pp 287–348.

Juhasova, G., Ivanova, H., Spisak, J., 2006. Biology of fungus *Gnomonia leptostyla* in agro-ecological environments of Slovakia. Mikologica Fitopatologie 40, 538–547.

Höhnel. F., von., 1919. Mycologische Fragmenta. Ann. Mycol. 17, 114–133.

Kellogg, E.A., Appels, R., Mason-Gamer, R.J., 1996. When genes tell different stories: the diploid genera of Triticeae (Graminae). Syst. Bot. 21, 321–347.

Kobayashi, T., 1970. Taxonomic studies of Japanese Diaporthaceae with special reference to their life-histories. Bulletin of the Government Forest Experiment Station No. 226.

Letcher, P.M., Powell, M.J., Viusent, M.C., 2008. Rediscovery of an unusual chytridiaceous fungus new to the order Rhizophydiales. Mycologia 100 (2), 325–334. doi:10.3852/mycologia.100.2.325

Mason-Gamer, R.J., Kellogg, E.A., 1996. Testing for phylogenetic conflict among molecular data sets in the tribe Triticeae. Syst. Biol. 45, 524–545.

Mejía, L.C., Castlebury, L.A., Rossman, A.Y., Sogonov, M.V., White, J.F., 2008. Phylogenetic placement and taxonomic review of the genus *Cryptosporella* and its synonyms *Ophiovalsa* and *Winterella* (Gnomoniaceae, Diaporthales). Mycol. Res. 112 (1), 23–35.

Mejía, L.C., Rossman, A.Y., Castlebury, L.C., Yang, Z., White, J.F., 2011a. *Occultocarpon*, a new monotypic genus of Gnomoniaceae on *Alnus nepalensis* from China. Fungal Divers. 52 (1), 99–105. doi:10.1007/s13225-011-0108-y

Mejía, L.C., Rossman, A.Y., Castlebury, L.A., White, J.F., Jr., 2011b. New species, phylogeny, host-associations, and geographic distribution of the genus *Cryptosporella* (Gnomoniaceae, Diaporthales). Mycologia 103, 379–399. doi:10.3852/10-134

Mejía, L.C., Castlebury, L.A., Rossman, A.Y., Sogonov, M.V., White, J.F., 2011c. A systematic account of the genus *Plagiostoma* (Gnomoniaceae, Diaporthales) based on morphology, host-associations, and a four-gene phylogeny. Stud. Mycol. 68 (1), 211–235. doi:10.3114/sim.2011.68.10

Monod, M., 1983. Monographie taxonomique des Gnomoniaceae (Ascomycétes de l'ordre des Diaporthales I). Beihefte zur Sydowia 9, 1–315.

Neely, D., Black, W.M., 1976. Anthracnose of black walnuts in the Midwest. Plant Dis. Reporter 60, 519–521.

Nixon, K.C., Carpenter, J.M., 1993. On outgroups. Cladistics 9, 413–426.

O'Donnell, K., Ward, T.J., Geiser, D.M., Kistler, H.C., Aoki, T., 2004. Genealogical concordance between the mating type locus and seven other nuclear genes supports formal recognition of nine phylogenetically distinct species within the *Fusarium graminearum* clade. Fungal Genet. Biol. 41 (6), 600–623.

Otani, Y., 1995. Mycological Flora of Japan. Vol III Ascomycotina No 3 Sordariales, Diaporthales Tokyo, Yokendo LTD.

Paulus, B., Gadek, P., Hyde, K., 2007. Successional patterns of microfungi in fallen leaves of *Ficus pleurocarpa* (Moraceae) in an Australian tropical rain forest. Biotropica 38, 42–51.

Pennycook, S.R., 2007. *Discula betulae* comb. nov., correct name for the anamorph of *Gnomonia intermedia*. Mycotaxon 101, 361–364.

Pringle, A., Baker, D.M., Platt, J.L., Wares, J.P., Latgé, J.P., Taylor, J.W., 2005. Cryptic Speciation in the Cosmopolitan and Clonal Human Pathogenic Fungus *Aspergillus Fumigatus*. Evolution 59 (9), 1886–1899. doi:10.1111/j.0014-3820.2005.tb01059.x

Raja, H.A., Miller, A.N., Shearer, C.A., 2008. Freshwater ascomycetes: *Aquapoterium pinicola*, a new genus and species of Helotiales (Leotiomycetes) from Florida. Mycologia 100 (1), 141–148. doi:10.3852/mycologia.100.1.141

Ronquist, F., Huelsenbeck, J.P., 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19 (12), 1572–1574. doi:10.1093/bioinformatics/btg180

Rosenberg, N.A., Harrison, R., 2003. The shapes of neutral gene genealogies in two species: probabilities of monophyly, paraphyly, and polyphyly in a coalescent model. Evolution 57 (7), 1465–1477. doi:10.1554/03-012

Salahi, S., Javan, N.M., Jamshidi, S., 2009. Study on population structure of *Gnomonia leptostyla*, causal agent of walnut anthracnose in East Azarbaijan province, Iran. New Agricultural Science Journal 3, 53–68.

Sarver, B.A.J., Ward, T.J., Gale, L.R., Broz, K., Kistler, C.H., Aoki, T., Nicholson, P., Carter, J., O'Donnell, K., 2011. Novel *Fusarium* head blight pathogens from Nepal and Louisiana revealed by multilocus genealogical concordance. Fungal Genet. Biol. 48 (12), 1096–1107.

Schmitt, I., Crespo, A., Divakar, P.K., Fankhauser, J.D., Herman-Sackett, E., Kalb, K., Nelsen, M.P., Nelson, N.A., Rivas-Plata, E., Shimp, A.D., Widhelm, T., Lumbsch, H.T., 2009. New primers for promising single-copy genes in fungal phylogenetics and systematics. Persoonia 23, 35–40.

Sogonov, M.V., Castlebury, L.A., Rossman, A.Y., Mejía, L.C., White, J.F., 2008. Leaf-inhabiting genera of the Gnomoniaceae, Diaporthales. Stud. Mycol. 62, 1–77.

Spatafora, J.W., Sung, G-H., Johnson, D., Hesse, C., O'Rourke, B., Serdani, M., Spotts, R., Lutzoni, F., Hofstetter, V., Miadlikowska, J., Reeb, V., Gueidan, C., Fraker, E., Lumbsch, T., Lucking, R., Schmitt, I., Hosaka, K., Aptroot, A., Roux, C., Miller, A.N., Geiser, D.M., Hafellner, J., Hestmark, G., Arnold, A.E., Budel, B., Rauhut, A., Hewitt, D., Untereiner, W.A., Cole, M.S., Scheidegger, C., Schultz, M., Sipman, H., Schoch, C.L., 2006. A five-gene phylogeny of Pezizomycotina. Mycologia 98 (6), 1018–1028. doi:10.3852/mycologia.98.6.1018

Sullivan, J., 1996. Combining data with different distributions of among-site rate variation. Syst. Biol. 45 (3), 375–380. doi:10.1093/sysbio/45.3.375

Swofford, D.L., 2002. PAUP 4.0b10: Phylogenetic analysis using parsimony. Sinauer Associates, USA.

Tajima, F., 1983. Evolutionary relationship of DNA sequences in finite populations. Genetics 105, 437–460.

Talavera, G., Castresana, J., 2007. Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. Syst. Biol. 56 (4), 564–577. doi:10.1080/10635150701472164

Taylor, J.W., Jacobson, D.J., Kroken, S., Kasuga, T., Geiser, D.M., Hibbett, D.S., Fisher, M.C., 2000. Phylogenetic species recognition and species concepts in fungi. Fungal Genet. Biol. 31 (1), 21–32.

Teodoro, N.G., 1937. An enumeration of Philippine Fungi. Techn. Bull. Dept. Agric. Comm. Manila 4, 1–585.

Townsend, J.P., 2007. Profiling phylogenetic informativeness. Syst. Biol. 56 (2), 222–231. doi:10.1080/10635150701311362

Townsend, J.P., Leuenberger, C., 2011. Taxon sampling and the optimal rates of evolution for phylogenetic inference. Syst. Biol. doi: 10.1093/sysbio/syq097. doi:10.1093/sysbio/syq097

Walker, J., 1980. *Gaeumannomyces*, *Linocarpon*, *Ophiobolus* and several other genera of scolecospored ascomycetes and *Phialophora* conidial states, with a note on hyphopodia. Mycotaxon 11, 1–129.

Walker, D.M., Castlebury, L.A., Rossman, A.Y., Sogonov, M.V., White, J.F., Jr., 2010. Systematics of the genus *Gnomoniopsis* (Gnomoniaceae, Diaporthales) based on a three gene phylogeny, host associations, and morphology. Mycologia 102 (6), 1479–1496. doi:10.3852/10-002

Wild, A.L., Maddison, D.R., 2008. Evaluating nuclear protein-coding genes for phylogenetic utility in beetles. Mol Phylogenet and Evol 48 (3), 877–891.

Zwickl, D.J., 2006. Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion. Ph.D. dissertation, The University of Texas at Austin, USA. 115 p.

Chapter 5

Host conservatism or host specialization? Patterns of fungal diversification are influenced by host specificity in *Ophiognomonia* (Gnomoniaceae, Diaporthales)

ABSTRACT

Species of *Ophiognomonia* (Gnomoniaceae) are perithecial fungi that occur as endophytes, pathogens, and latent saprobes on leaf and stem tissue of plants in the Betulaceae, Fagaceae, Juglandaceae, Lauraceae, Malvaceae, Platanaceae, Rosaceae, Salicaceae, and Sapindaceae. In this study host plant patterns at the order, family, genus, and species level were analyzed using Spatial Evolutionary and Ecological Vicariance Analysis (SEEVA) using a multi-gene phylogeny of 45 species of *Ophiognomonia.* The objective of this study was to better understand speciation events and host associations in *Ophiognomonia*. Also to determine if fungal speciation events are influenced by host conservatism, specialization, or switching at different taxonomic host ranks. Host taxonomic data were coded with the four variables order, family, genus, and species. Host plant differences between sister clades were interpreted using the divergence index (D), ranging from 0 for no divergence, to 1 for maximum possible divergence. Several subclades showed strong patterns of order/family conservatism ($D = 1.00$) for hosts in the Betulaceae, Fagaceae, Juglandaceae, and Rosaceae. Trends of host specialization at the genus and species ranks ($D = 1.00$) were suggested within these same host families. Two independent host switches at the family and three at the order rank were observed for the species *O. clavigignenti-juglandacearum, O. pterocaryae, O. japonica, O. lenticulispora,* and *O. sassafras,* respectively. In summary, host specificity was

hypothesized as a mechanism strongly contributing to speciation patterns in the genus

Ophiognomonia.

INTRODUCTION

Fungi in the family Gnomoniaceae (Diaporthales, Sordariomycetes, Ascomycota) are integral members in temperate and tropical ecosystems functioning as endophytes, pathogens, and saprobes. The Diaporthales have been documented in North America and Europe on a diverse range of herbaceous plants, shrubs, and trees from over 330 host plant genera (Farr and Rossman 2011). DNA sequence data, host association, and morphology have been used to classify ten genera in the Gnomoniaceae (Sogonov et al. 2008, Mejía et al. 2008, 2011 a–c, Walker et al. 2010). Many species of Gnomoniaceae cause significant damage as plant pathogens to ornamental, nut, and lumber producing trees including *Discula destructiva* (dogwood anthracnose), *Ophiognomonia clavigignenti-juglandacearum* (butternut canker), and *Apiognomonia erythrostoma* (cherry leaf scorch) [Anderson and LaMadeleine 1978, Broders and Boland 2011, Redlin 1991, Rossman et al. 2007].

Species of Gnomoniaceae have been documented in recent phylogenetic studies to have generally a very narrow host range, commonly associating with a single host genus or species (Mejía et al. 2008, 2011a–c, Sogonov et al. 2008, Walker et al. 2010, Chapter 4). Within the highly diverse genus *Cryptosporella,* Mejía et al. (2011c) documented seven fungal species each occurring only on a single host genus and nine only on a single host species or subspecies, representing the majority of species in the genus *Cryptosporella* (19 total species). Furthermore, the authors suggested the genus

Cryptosporella is constricted to, and have undergone speciation within, the host plant

families of Betulaceae, Fagaceae, and Salicaceae (Mejía et al. 2011c). Similarly, in a

study of the genus *Gnomoniopsis,* Walker et al. (2010) found four species specific to the

host genus *Rubus* in the Rosaceae. Tight host associations within the Gnomoniaceae are

not always the case, for example *Apiognomonia errabunda* causes anthracnose disease on

10 different host families (Sogonov et al. 2007).

Species within the genus *Ophiognomonia* associate with a diverse host range

including the plant the families Betulaceae, Fagaceae, Juglandaceae, Lauraceae,

Malvaceae, Platanaceae, Rosaceae, Salicaceae, and Sapindaceae (mostly rosids or more

basally placed angiosperms). Within the genus *Ophiognomonia,* Walker et al. (in review,

Chapter 4) observed patterns of host conservatism and diverse speciation within the

Betulaceae, as well as host jumps from Betulaceae to the Lauraceae, Platanaceae, and

Sapindaceae. Most species of *Ophiognomonia* are restricted to a single host genus or

several genera from the same host family (Walker et al. in review, Chapter 4). For

example, *O. rosae* has been documented on the genera *Fragaria, Rosa,* and *Rubus*

(Rosaceae), whereas, *O. longispora* occurs only on *Tilia* (Malvaceae).

Thirteen species of *Ophiognomonia* were documented in Walker et al. (in review,

Chapter 4) from a worldwide distribution on the genus *Alnus* (Betulaceae), indicating

diverse speciation within this genus in the past. Inversely, only two species *O.*

michiganensis and *O. setacea* are accepted generalists and are known to occur on plant

hosts from several different taxonomic orders. Understanding the tradeoffs between

generalist and specialist life strategies is important to interpret the coevolution of

host/fungus systems over geological space and time (Kawecki 1998, Whitlock 1996). In

addition understanding phylogenetic niche conservatism in an evolutionary context within monophyletic clades of fungi/hosts and how they behave over time is critical to explain coevolutionary patterns among species of *Ophiognomonia* and their host plants.

Host plant specificity is an important concept to recognize when contemplating the interaction of agriculturally and horticulturally relevant fungi with their hosts. A switch within a pathogen to a novel host are of great concern due to a pathogens possible massive impact on world trade, plant quarantine decisions, food availability, and agricultural health. Understanding evolutionary mechanisms that contribute to complex host/fungus interactions is important for elucidating plant pathogen host range. For example, we hypothesize that in species of the Gnomoniaceae, sex occurs on still growing or overwintered leaves and twigs of the host plant when homo- or heterothallic hyphae fuse and enter the sexual cycle, with the end product of ascospore (sexual spore) production. During wet spring conditions, the Gnomoniaceae disperse large amounts of aerial ascospores and conidia (asexual spores). Spores often land on both host and non-host plants, and must germinate and infect the host as an endophyte or pathogen to survive and complete the seasonal lifecycle. Due to these dispersal and life cycles strategies, opportunities for new host plant associations must often occur for species of the Gnomoniaceae (Giraud et al. 2008).

Undoubtedly, a diverse suite of factors not limited to, but including host/fungus chemistry, abiotic conditions, and coevolution contribute to the host range of a given fungus. However, when focusing at the molecular level, fungi utilize a suite of pathogenicity factors that determine host association and the potential to cause disease in their respective host plants (Kirzinger and Stavrinides 2011). It is the product of these

pathogenicity factors that partially define the host range and degree of specificity of a given fungus (Kirzinger and Stavrinides 2011). Since fungal pathogenicity factors often interact directly with host substrates and components of the host defense response, they are subject to strong selective pressures (Kirzinger and Stavrinides 2011). Host specificity can be affected by a mutation in pathogenicity factors offering a selective advantage that expands host range or leads to host jumps (Kirzinger and Stavrinides 2011). Farrell (2001) showed that closely related host plants are more susceptible to a given plant pathogen than distantly related host relatives due to phylogenetic conservation of host morphology and chemistry.

The niche was defined by Hutchinson (1957) as a set of abiotic and biotic conditions within an environment where a species is able to exist over time. The host plant defines the abiotic environment in which species of the Gnomoniaceae can persist. In this study, the host plant and its taxonomic neighborhood was explored as a key component of the fungal niche since it determines some of the abiotic environmental conditions that species of *Ophiognomonia* have encountered over evolutionary space and time. Several authors suggest that evolutionary divergence among fungi is often driven by host factors (Barrett et al. 2009, Eshed and Dinoor, 1980, Fournier and Giraud 2008, Shykoff et al., 1999). Niche-related traits have been documented to evolve from rapidly (e.g. Schluter 2000) to very slowly (e.g. Peterson et al. 1999, Wiens & Graham 2005). Over time, slow and gradual evolution leads to niche divergence, and may offer a novel habitat for occupation by parapatrically diverging populations. Species often exhibit phylogenetic niche conservatism, or persistent occupation of a niche constrained by ancestral preferences over evolutionary time (Martínez-Meyer et al. 2004, Prinzing et al.

2001, Struwe et al. 2011, Wiens and Graham, 2005). Wiens et al. (2010) considers both patterns of niche conservatism and specialization as theories to explain observed similarities and also processes driving evolutionary divergence. Niche conservatism can occur at different spatial, temporal, and phylogenetic scales and can be used to explain patterns of host plant conservatism or specialization within many fungi, including *Ophiognomonia*. The purpose of this study was to 1) map host plant preference on a phylogenetic tree of *Ophiognomonia,* 2) determine how patterns of fungal diversification are influenced by host specificity, and 3) determine if speciation events in the phylogeny of *Ophiognomonia* are associated with host switching or host conservation at certain taxonomic host ranks.

The method called Spatial Evolutionary and Ecological Vicariance Analysis (SEEVA, Struwe et al. 2011) integrates phylogenetic (temporal), geographical (spatial), and host plant data (in this case) in a single analysis to evaluate the patterns of host associated niche separation on clade differences within a phylogeny. This method of analyzing host and phylogenetic data provides both a visual and statistically solid understanding of evolutionary mechanisms that might have strongly influenced speciation events, host switches, and niche evolution. In this study, the SEEVA methodology of analyzing host plant data was utilized to provide a better understanding of evolutionary mechanisms influencing speciation events in the genus *Ophiognomonia*. A comprehensive picture of host/fungus evolution in *Ophiognomonia* should also provide a framework for evolutionary hypotheses about other genera in the Gnomoniaceae. This methodology could be useful for plant pathologists, mycologists, and plant quarantine officials for making predictions about host specificity of plant pathogens, potential host

jumps of known pathogens, as well as evolutionary histories of host-parasite relationships.

The purpose of Walker et al. (in review, Chapter 4) was to document and classify the species of *Ophiognomonia* using multiple molecular markers, host associations, and morphology. According to phylogenetic informativeness tests (Townsend 2007, Townsend and Leuenberger 2011), the markers ITS (ITS1, 5.8S rDNA and ITS2), MS204 (guanine nucleotide-binding protein subunit beta-like protein), and *tef-1α* (translation elongation factor 1α) were used to reconstruct the phylogeny of *Ophiognomonia* (Walker et al., in review, accepted, Chapter 3–4). Several phylogenetic trees from Walker et al. (accepted, Chapter 4) were used in this study to statistically evaluate host/fungus associations between species of *Ophiognomonia* using the software program SEEVA v. 1.0 (Struwe et al. 2011).

METHODS

DNA extraction, amplification, and sequencing

Fungal isolates were grown in culture and genomic DNA extracted after 5–7 days using the QIAGEN Puregene Core Kit A (QIAGEN Inc., Chatsworth, California) following Walker et al. (2010). Three molecular markers, ITS, MS204, and *tef-1α*, were selected for analysis based on phylogenetic informativeness test results from Walker et al. (accepted, Chapter 3). The markers ITS and *tef-1α* were amplified and sequenced according to Walker et al. (2010), except for the Gnomoniaceae-specific *tef-1α* primers designed in Walker et al. (accepted, Chapter 3). MS204 was amplified and sequenced as

in Walker et al. (accepted, Chapter 3). DNA sequence data used in this study were published by Sogonov et al. (2008) and Walker et al. (accepted, chapter 3).

Sequence data analyses

Raw nucleotide sequences were edited and assembled into contigs with the program Sequencher 4.9 for Windows (Gene Codes Corp., Ann Arbor, Michigan). The three-marker alignments (alignments 5–7 = Clades 1–3) from Walker et al. (in review, Chapter 4) including the ITS region, *tef-1α,* and MS204 were used for phylogenetic analyses in this study. The Gblocks (http://molevol.cmima.csic.es/castresana/Gblocks_server.html) and MAFFT v.6 (http://mafft.cbrc.jp/alignment/server/) web servers were used to prepare three alignments for analyses. The alignments were stripped down to one isolate representing each species of *Ophiognomonia.* Alignment five (Clade 1) consisted of 16 isolates, representing 15 species in *Ophiognomonia*, and the outgroup taxon *O. longispora*. Alignment six (Clade 2) consisted of 14 isolates, representing 13 species in *Ophiognomonia*, and the outgroup *O. monticola*. Alignment seven (Clade 3) consisted of 17 isolates, representing 15 species in *Ophiognomonia*, and the outgroup taxa *O. gei-montani* and *O. leptostyla*. Only the ITS region was available for analysis in the taxa *O. gei* and *O. rubi-idaei* (Clade 2). Alignments 5−7 will be referred to hereafter as Clades 1−3 respectively.

Phylogenetic trees were inferred with maximum likelihood (ML) analyses. In all analyses rooting was accomplished with the outgroup method (Nixon and Carpenter 1993) using results from Walker et al. (in review, Chapter 4). The Lattice Project (http://boinc.umiacs.umd.edu) web server was used for performing partitioned ML

analyses with the program GARLI v2.0 (Zwickl, 2006), with implementation of parameters from Walker et al. (accepted, Chapter 3). The three ML phylogenetic trees representing Clades 1–3 were used for Spatial Evolutionary and Ecological Vicariance Analysis (SEEVA, Struwe et al. 2011).

Identification of unknown herbarium specimens

An ITS nucleotide sequence from single isolates representing each of the 45 species of *Ophiognomonia* from Walker et al. (accepted, Chapter 4) was used to create a local BLAST database in Bioedit v.7.0.9.0 (http://www.mbio.ncsu.edu/bioedit/bioedit.html). Local BLAST searches were performed to identify 136 herbarium specimens. The remaining 98 specimens were included in Walker et al.'s (in review, Chapter 4) multi-gene analyses, where they were identified to species. Two criteria were used to verify an ITS sequence for a given herbarium specimen, 1) 99 % sequence identity to a verified species of *Ophiognomonia,* and 2) a BLAST query sequence coverage of approximately 500 bp.

Host plant data

All 234 herbarium specimens representing 45 species of *Ophiognomonia* were assembled in a Microsoft Excel spreadsheet. Four host taxonomic ranks (order, family, genus, and species) were recorded for each herbarium specimen. The Angiosperm Phylogeny Group III system (http://en.wikipedia.org/wiki/APG_III_system) was used to verify the current host plant taxonomic ranks above genus and Mabberley (1997) for genus and species ranks.

SEEVA analysis

The program Spatial Evolutionary and Ecological Vicariance Analysis v. 1.0 (SEEVA, Struwe et al. 2011) was used under the default parameters to analyze patterns and differences in host plant association at any particular phylogenetic split within the phylogeny of *Ophiognomonia.* The GARLI ML trees for Clades 1–3 were used as input trees for three independent SEEVA analyses, one per clade. The total number of collection records included in each clade was as follows, 1) Clade 1 = 105 specimens (15 ingroup species), 2) Clade 2 = 48 specimens (13 ingroup species), and 3) Clade 3 = 81 specimens (15 ingroup species). Three separate SEEVA analyses were conducted based on results from Chapter 4 (Walker et al. in review), which indicate sequence variation in Clades 1−3 that would otherwise be discarded in an all-taxa combined alignment. In addition, the three clades were analyzed individually since they lack bootstrap support indicating each clades relationship to one another.

The host taxonomic ranks (4 variables: order, family, genus, species) were used as qualitative non-ordered categorical data in the SEEVA analyses. The program SEEVA combines all specimen records for comparison between two daughter clades using a contingency table (Struwe et al. 2011). For the 45 species of *Ophiognomonia* in this study, 43 contingency tables are produced, which are statistically independent from one another. Each contingency table can be viewed as a hypothesis, with the null hypothesis indicating no divergence between sister clades and host plant (Struwe et al. 2011). However, significant divergence indicates a fungus/host association within the phylogeny of *Ophiognomonia* (Struwe et al. 2011). Due to small sample sizes a sample-permuted

version of Fisher's (1958) exact test was used to compute the test criteria and *P*-values

(Struwe et al. 2011). In addition, to reduce the rate of false positives, a Bonferroni

correction (Rice, 1989) was used to declare a significant result at a given node as follows,

1) Clade 1 ($P \leq 0.0034$), 2) Clade 2 ($P \leq 0.0039$), and 3) Clade 3 ($P \leq 0.0034$). The

divergence index (*D*) was used to measure the strength of association between

phylogenetic-host plant patterns of sister clades in *Ophiognomonia* (Struwe et al. 2011).

A *D*-value of 0.0 indicates no difference between sister clades, whereas, a *D*-value of 1.0

indicates the maximum possible difference (Struwe et al. 2011).

RESULTS

Phylogenetic analyses

The alignment statistics for Clades 1–3 are reported in Walker et al. (in review,

Chapter 4, alignments 5-7). The GARLI v2.0 ML analysis for Clade 1 resulted in one tree

with a -lnL 7156.46 (Fig. 5.1); Clade 2 resulted in one tree with a -lnL 7136.87 (Fig. 5.2);

Clade 3 resulted in one tree with a -lnL 8106.78 (Fig. 5.3).

Summary of host associations

Clade 1 consists of 15 species that occur on hosts in the families Betulaceae,

Fagaceae, Platanaceae, Rosaceae, and Sapindaceae (Fig. 5.1, Table 5.1). Within Clade 1,

two separate subclades (Node 1:12) of species including *O. asiatica, O. gunmensis, O.*

kobayashii, O. otanii, O. setacea, O. sogonovii and *O. gunmensis/O. quercus-gambellii*

(Node 1:11) occur on *Quercus* spp. and *Castanea* spp. within the Fagaceae (Fig. 5.1). A

separate subclade consisting of the species *O. alni-cordatae, O. hiawathae, O.*

ischnostyla, O. japonica, O. michiganensis, O. monticola, O. ostryae-virginianae, O. pseudoischnostyla occur on several genera in the Betulaceae and Rosaceae (Fig 5.1, Table 5.1)

Clade 2 consists of 11 species of *Ophiognomonia* occurring on the host families Juglandaceae, Lauraceae, Rosaceae, and Malvaceae (Fig. 5.2. Table 5.1). Within Clade 2, the subclade (Node 3:7) consisting of *O. gei, O. nipponicae, O. padicola, O. rosae,* and *O. rubi-idaei* occur on multiple host genera in the Rosaceae (Fig. 5.2, Table 5.1). Also within this Clade 2, the species *O. micromegala, O. pseudoclavulata,* and *O. vasiljevae* form a subclade (2:3) that occurs on *Carya* and *Juglans* in the Juglandaceae. The species *O. lenticulispora* is also found in the subclade (Node 2:3) of *O. micromegala, O. pseudoclavulata,* and *O. vasiljevae* but was collected on *Prunus* sp. in the Rosaceae (Fig. 5.2).

Clade 3 contains 15 species of *Ophiognomonia* that occur on the host families Betulaceae, Juglandaceae, and Salicaceae. The subclade (Node 3:6, Fig. 5.3) of species including *O. alni-viridis, O. bugabensis, O. ibarakiensis, O. intermedia, O. maximowiczianae, O. multirostrata, O. nana,* and *O. tucumanensis* occur only on *Alnus* spp. and *Betula* spp. in the Betulaceae. Two independent subclades (Nodes 3:14, 3:15, Fig. 5.3) that include *O. clavigignenti-juglandacearum* and *O. pterocaryae* associate with *Juglans* spp. and *Pterocarya rhoifolia* (respectively) in the Juglandaceae. The species *O. balsamiferae* is positioned at the basal Node 3:2 and occurs on *Populus balsamiferae* in the Salicaceae.

SEEVA analyses

The program SEEVA v. 1.0 was used to analyze four different host taxonomic ranks across 43 phylogenetic nodes. Of the 172 total comparisons, 43 were significant using the Bonferonni correction mentioned in the methods (Figs. 5.1–5.3, * significant p-value). The degree of host plant divergence was indicated with the divergence index (D). The following paragraphs report the divergence index values in Clades 1–3 for each node as Clade # : Node # (e.g. 2:3, corresponds to Clade 2 : Node 3). Nodes 1:1, 2:1, and 3:1 are comparisons between the outgroup and ingroup and will not be presented or discussed. Overall, a broad range of host divergence was observed within and between each clade, and will be reported in the following sections on a clade-by-clade basis.

SEEVA, Clade 1 of Ophiognomonia

Divergence values for host order, family, and genus varied widely (Nodes 1:2−1:15, D = 0.00–1.00, Fig. 5.1). Higher divergence values and a more narrow range were observed for the host species rank (Nodes 1:2−1:15, D = 0.65–1.00, Fig. 5.1) when compared to the other taxonomic ranks. Host divergence at the family rank showed significantly higher D-values at basally placed nodes (Node 1:2, D = 0.88*, Node 1:3, D = 0.97*, Fig. 5.1) when compared to more terminally placed nodes. The only exception is the clear host split between *O. japonica* and *O. ostryae-virginianae* (Node 1:8, D = 1.00, Fig. 5.1). A strong directional trend showing host genus and species specialization and order/family conservation was observed at Nodes 1:12→1:15 in the species *O. asiatica, O. kobayashii, O. otanii, O. setacea,* and *O. sogonovii.*

SEEVA, Clade 2 of Ophiognomonia

A wide range (Nodes 2:2−2:13, D = 0.00–1.00, Fig. 5.2) of divergence values were observed in Clade 2 in all four variables, host order to species. High and statistically significant differences were observed at basal Nodes 2:2 and 2:6 for all host ranks (0.58–0.99, 0.79−1.00, respectively, Fig. 5.2). A strong directional trend in host divergence at the genus and species rank was observed in the subclade of species including *O. gei, O. nipponicae, O. padicola, O. rosae,* and *O. rubi-idaei* (Nodes 2:7–2:10, Fig. 5.2). The sister species clades *O. lenticulispora/O. pseudoclavulata* (Node 2:5) and *O. melanostyla/O. sassafras* (Node 2:13) independently show strong host divergence (D = 1.00, 1.00* respectively) at all four host ranks (Fig. 5.2).

SEEVA, Clade 3 of Ophiognomonia

A wide range of divergence indices was observed in this clade for host order, family, and genus (Nodes 3:2−3:15, D = 0.00–1.00, Fig. 5.3). Divergence indices for host species were higher when compared to host order→genus (Nodes 3:2−3:15, D = 0.67–1.00, Fig. 5.3). High divergence values were observed for several more terminally positioned nodes at the host family, genus, and species ranks (Nodes 3:13, 3:14, 3:15). A strong directional trend for host species divergence was documented in the subclade consisting of *O. alni-viridis, O. bugabensis, O. maximowiczianae, O. multirostrata,* and *O. nana* (Nodes 3:9→3:12, Fig. 5.3). A similar trend showing host genus and species divergence was present in the subclade including *O. ibarakiensis, O. intermedia,* and *O. tucumanensis* (Nodes 3:6→3:8, Fig. 5.3).

To summarize, host plant preference was mapped onto three clade specific phylogenetic trees representing the genus *Ophiognomonia* (Figs. 5.1−5.3). Several trends

throughout the phylogeny of *Ophiognomonia* suggest that patterns of fungal diversification are influenced by host specificity. For example, the subclade of species (Node 2:7→2:10) show patterns of strong host specialization at the genus and species rank within the family Rosaceae ($D = 0.55−1.00$, Fig. 5.2). In addition, multiple speciation events in the phylogeny of *Ophiognomonia* are associated with host switching or host conservation at different taxonomic host ranks. For example, host jumps were observed at Nodes 1:9, 2:5, 3:14, 3:15 at different taxonomic host ranks (Figs. 5.1−5.3).

DISCUSSION

General trends in Ophiognomonia

Several trends indicating host conservation, divergence, specialization and switching were noted in each clade of the phylogeny of *Ophiognomonia.* When comparing broad trends, a major host splitting event was suggested in more basally positioned nodes for all three clades of *Ophiognomonia* (Nodes 1:2, 2:2, 2:6, 3:5). Patterns of host specialization differed from family→species for Nodes 1:2 and 3:5 when compared to Nodes 2:2 and 2:6, which show specialization from order→species (Figs. 5.1−5.3). A general trend (with exceptions) of host genus and species specialization was observed at more terminally positioned nodes in all three clades of extant species of *Ophiognomonia* (Figs. 5.1−5.3). For example, host genus and species specialization was observed in the more terminally positioned nodes 1:6, 1:7, 1:12→1:15 in Clade 1, 2:7→2:10 in Clade 2, and 3:7, 3:8, 3:9→3:12 in Clade 3 (Figs. 5.1−5.3). Although the nodes in this phylogeny were not dated, perhaps this suggests major host splits (order,

family) early in the evolution of *Ophiognomonia* and a trend of host genus and species specialization more recently. The remaining sections will discuss defined patterns of host conservation, divergence, specialization and switching.

Host plant conservatism

The time-for-speciation effect (Stephens and Wiens 2003) states that species richness will be greater in an area that has been evolutionarily conserved for dramatically longer when compared to richness in a recently occupied niche. We hypothesize that this may explain the rich diversity of species occurring on hosts in the Juglandaceae (Node 2:3) and Rosaceae (Node 2:7) in Clade 2 and Fagaceae in Clade 1 (Node 1:12, Fig 5.1). For example, the subclade (Node 1:12) including *O. asiatica, O. kobayashii, O. otanii, O. setacea,* and *O. sogonovii* show strong host conservatism at the family rank for plants in the Fagaceae (Fagales; Fig. 5.1). The subclade of species (Nodes 2:7→2:10) including *O. gei, O. nipponicae, O. padicola, O. rosae,* and *O. rubi-idaei* suggest a pattern of host conservatism at the order and family rank and specialization within host genus and species (Fig. 5.2). This subclade (Node 2:7) only associates with hosts in the Rosaceae, therefore, we hypothesize that these species will be collected on other undocumented genera in the Rosaceae and continue to speciate in this family. Mejía et al. (2011b) observed a similar pattern within a clade of 11 species in the genus *Plagiostoma* that only associate with hosts in the Salicaceae. If analyzed using SEEVA, we hypothesize that the genus *Plagiostoma* would show a pattern that resembles this lineage (Node 2:7→2:10) of *Ophiognomonia.*

Species in Clade 3 have the narrowest host range when compared to the other two clades of *Ophiognomonia* (Table 5.1. Fig. 5.3). New species have been documented to diverge gradually and occupy niches similar to their most recent ancestors, and therefore, associate with phylogenetically related hosts (Martinez-Meyer et al. 2004, Prinzing et al. 2001, Wiens and Graham 2005). For Clade 3, much of the phylogenetic structure in host/fungus range can be considered niche conservatism for the host family Betulaceae (Nodes 3:3, 3:4, 3:6, Fig. 5.3). When simply listing host/fungus relationships, 12 of 15 species in Clade 3 associate with *Alnus* spp. and *Betula* spp. (Betulaceae), only *O. balsamiferae* occurs on *Populus balsamiferae* in the Salicaceae, and *O. clavigignenti-juglandacearum*/*O. pterocaryae* associate with *Juglans* spp./*Pterocarya rhoifolia* (respectively, Juglandaceae) [Table 5.1, Fig. 5.3]. Within a phylogenetic context, a strong pattern of host order conservatism was observed from Nodes 3:3→3:15, and host family conservatism from Nodes 3:6→3:12 (Fig. 5.3). Within the Betulaceae, the species *O. bugabensis, O. ibarakiensis, O. maximowiczianae, O. multirostrata, O. nana,* and *O. tucumanensis* (Node 3:6) occur only on *Alnus* spp. or *Betula* spp. (Betulaceae, Fagales), whereas the species *O. alni-viridis* and *O. intermedia* have an expanded host range occurring on both *Alnus* spp. and *Betula* spp. Sogonov et al. (2008) found that the genus *Gnomonia* (Gnomoniaceae) has tight host association patterns with the family Betulaceae. In fact, species from this genus are not known to occur on plants outside of the Betulaceae. We hypothesize that most of the species in Clade 3 (Node 3:3, excluding *O. clavigignenti-juglandacearum/O. pterocaryae*) have associated and possibly coevolved throughout evolutionary time with *Alnus* and *Betula*.

Host plant specialization

The lifecycle of *Ophiognomonia* consists of dispersing ascospores and conidia (sexual and asexual spores, respectively) into the environment via rain and wind. The spores land on both target and non-suitable hosts, and must germinate and infect the plant as an endophyte, pathogen, or latent saprobe to survive. Therefore, the opportunity for species of *Ophiognomonia* to associate with new hosts at the community level in mixed forests should be frequent (Giraud et al. 2008). Giraud et al. (2008) consider a host shift event as one factor contributing to evolution of a new species, but not the sole mechanism of speciation, rather allopatry or reproductive isolation must occur to solidify the event. For example, we hypothesize that the host switch (Node 1:9, Fig. 5.1) in the sister species *O. ostryae-virginianae* and *O. japonica* (*Ostrya virginiana,* Betulaceae→*Prunus* sp., Rosaceae) represents a speciation event influenced by host association but solidified by spatial allopatry (*O. ostryae-virginianae,* North America; *O. japonica,* Japan).

Host specificity has been hypothesized to contribute to a reproductive barrier essential for sympatric speciation events (Giraud et al. 2006, Peever et al. 2007). Two interesting patterns representing host-based specialization among parapatric/sympatric species were observed in Clade 1. The sister species *O. ischnostyla* and *O. pseudoischnostyla* (Node 1:7) occur on *Carpinus/Corylus* and *Alnus/Betula* (Betulaceae) in Europe and Western Asia from a broadly overlapping geographic range [Latitude: (45.98) 46.32−57.08 (57.14); Longitude: (6.58) 6.92−31.52 (35.32)]. Similarly, the species *O. asiatica* and *O. sogonovii* (Node 1:15) associate with *Quercus aliena/Q. crenata* and *Q. mongolica/Q. serrata* (respectively) and occur in closely overlapping spatial ranges in East Asia [Latitude: (25.14) 35.98−36.23 (36.31); Longitude: (102.75)

138.21−140.11 (140.20)]. *Ophiognomonia ischnostyla* and *O. pseudoischnostyla* show strong patterns of host genus and species specialization (*Carpinus/Corylus, Alnus/Betula,* respectively, *D*=1.00, Node 1:7) and *O. asiatica/O. sogonovii* show specialization at the species rank (*Quercus aliena/Q. crenata, Q. mongolica/Q. serrata,* respectively, *D*=0.85, Node 1:15). The later four species of *Ophiognomonia* were confirmed as genetically distinct lineages based on genealogical congruence of multiple molecular markers and the genealogical sorting index (Walker et al. in review, Chapter 4). We hypothesize that these four species of *Ophiognomonia* evolved partially due to parapatric/sympatric divergence by host usage, which triggered strong reproductive isolation (Giraud et al. 2006, 2008).

The subclade of species (Node 2:11) including *O. cordicarpa, O. longispora, O. melanostyla,* and *O. sassafras* are associated with hosts in the Juglandaceae, Malvaceae, and Lauraceae (Table 5.1, Fig. 5.2). After the strong divergence event at Node 2:6 (*D* = 0.79−1.00), these species suggest patterns typical of host specialization from order→species (Nodes 2:11→2:13, Fig. 5.2). Within this subclade, *O. longispora* and *O. melanostyla* (Nodes 2:12→2:13) occur on *Tilia* spp. in the Malvaceae, *O. cordicarpa* on *Pterocarya rhoifolia* (Juglandaceae), and *O. sassafras* on *Sassafras albidum* (Lauraceae). Interestingly, these four species share very long filiform ascospores ($\approx 40 \times 1$ µm), a character not documented in any other species of *Ophiognomonia* (Walker et al. in review, Chapter 4). Perhaps these patterns suggest a recent host jump to the Lauraceae and Malvaceae, and evolutionary conservation throughout geological history in the more common host families of *Ophiognomonia* including, Betulaceae, Juglandaceae, Fagaceae, and Rosaceae (Clades 1−3, Figs. 5.1−5.3). In addition, *O. sassafras* is the only species of Gnomoniaceae known to occur on hosts in the Laurales.

Host switching

Two separate host jumps from the Fagales to the Rosales (order rank) were observed in the phylogeny of *Ophiognomonia* (Nodes 1:9, 2:5). The subclade of species including *O. micromegala, O. pseudoclavulata,* and *O. vasiljevae* (Nodes 2:3→2:5) are associated with *Carya* and *Juglans* (Juglandaceae), except for *O. lenticulispora* which occurs on *Prunus* in the Rosaceae (Node 2:5, Fig. 5.2). A pattern of host conservatism in this subclade was observed at the order (Fagales) and family (Juglandaceae) ranks (Nodes 2:3→2:4). *Ophiognomonia lenticulispora* represents a host switch from *Carya tomentosa*→*Prunus* sp., which occurred at the order rank (Fagales→Rosales) in this subclade (Node 2:5, Fig. 5.2). The host genera *Carya* and *Prunus* often co-occur in forests where *O. lenticulispora, O. micromegala,* and *O. pseudoclavulata* were collected, therefore, we hypothesize that the ancestor of *O. lenticulispora* originated on *Carya* (or another host in the Juglandaceae) and has evolved to specialize on *Prunus.* We also speculate that *O. lenticulispora* has the ability to switch within and between hosts in the Rosaceae and Juglandaceae. Host shifting events that resemble this example have been documented in *Puccinia/Crucifer* (Roy 2001) and *Microbotryum/*Caryophyllaceae (Refrégier et al. 2008) species complexes.

The other host jump from Fagales→Rosales occurred at Node 1:9 (Fig. 5.1). The strong divergence event at node 1:9 is representative of a host shift from Betulaceae (*Ostrya*)→Rosaceae (*Prunus*) for *O. japonica* ($D = 1.00$, Fig. 5.1). The complete subclade (Node 1:4) of species is associated with several genera in the Betulaceae. Only *O. japonica* and *O. michiganensis* occur on *Prunus* in the Rosaceae. We hypothesize that

the shift to *Prunus* in *O. japonica* is a host jump (Node 1:9), whereas the shift in *O. michiganensis* is an expansion of the host range (Node 1:10, Fig. 5.1).

Several authors hypothesized that pathogen association with a narrow host range of species provides an advantage over a generalist species with respect to the endless coevolution of host/fungus competition (Kawecki 1998, Whitlock 1996). The subclade of species indicated at Nodes 3:4→3:15 associate primarily with *Alnus* and *Betula* (Betulaceae) and generally show patterns of strong host conservatism at the order/family ranks (Fig. 5.3). Two host switching events were observed in the subclade consisting of Nodes 3:4→3:15 from hosts in the Betulaceae→Juglandaceae. The species *O. clavigignenti-juglandacearum* showed a host switch from *Alnus*→*Juglans* and *O. pterocaryae* from *Alnus*→*Pterocarya* (Nodes 3:14, 3:15, Fig. 5.3). A similar pattern was observed in the genus *Cryptosporella* (Gnomoniaceae); all species occur on hosts in the Betulaceae, except for *C. hypodermia* (Ulmaceae) and *C. wehmeyeriana* (Malvaceae, Mejía et al. 2011c). Based on this pattern, Mejía et al. (2011c) hypothesize that species of *Cryptosporella* share a close association with hosts in the Betulaceae. In this study we hypothesize that species of *Ophiognomonia* have coevolved in close association with several host families in the Fagales, yet show several host shifts to other plant orders.

Host generalists vs. specialists

Host generalist and specialist strategies have most likely contributed to host associations in extant species of *Ophiognomonia.* The species *O. michiganensis* is associated with *Alnus, Betula,* and *Carpinus* in the Betulaceae (Fagales), and *Prunus* in the Rosaceae (Rosales) [Node 1:10, Fig. 5.1, Table 5.1]. Another species, *O. setacea*

occurs on plants from several host orders including the Fagales, Proteales, and Sapindales (Node 1:14, Fig. 5.1, Table 5.1). Both *O. michiganensis* and *O. setacea* are the only species of *Ophiognomonia* known to occur on several host orders and considered as generalists with respect to host association. This is not uncommon among the Gnomoniaceae, in fact, Sogonov et al. (2007) documented the species *Apiognomonia errabunda* occurring on a diverse range of host orders including Fagales, Malpighiales, Malvales, Rosales, and Sapindales. The remaining 43 species of *Ophiognomonia* associate with a single host genus or several genera from the same family indicating tight host/fungus evolution for the majority of species in this genus over time.

Sample size effect

Inevitably, only a subset of the total geographic and host range of *Ophiognomonia* was sampled in this study. For example, 11 species of *Ophiognomonia* are represented by a single herbarium specimen. Struwe et al. (2011) suggest inclusion of all detailed specimen records even if they represent a singleton species to ensure a comprehensive analysis in SEEVA. It is probable that host records for new collections of *Ophiognomonia* will closely coordinate with host associations from past species records (Martinez-Meyer et al. 2004, Wiens and Graham 2005). We do not discount the possibility of new host records for any species of *Ophiognomonia*, however, we hypothesize that this is less likely for some than others. For example, in Clade 2 the subclade of species including *O. gei, O. nipponicae, O. padicola, O. rosae,* and *O. rubi-idaei* is restricted to the family Rosaceae. We hypothesize that this is a tight host/fungus association and these species are unlikely to occur on plants outside of the Rosaceae. In

Clade 3 (Node 3:3→3:15) nearly all species are associated with *Alnus* and *Betula* (Betulaceae), except for *O. clavigignenti-juglandacearum* and *O. pterocaryae,* which occur on hosts in the Juglandaceae. We hypothesize that this indicates the potential for other species in this subclade (Nodes 3:3→3:15) to shift to hosts in the Juglandaceae, but still maintaining a strong association within the Betulaceae.

CONCLUSION

Additional sampling is likely to expand the host range and geographical distribution for species of *Ophiognomonia.* Just as Gilbert and Webb (2007) provide a quantitative measure for risk assessment of potential host ranges for pathogenic fungi, this study statistically supports host/fungus relationships and provides a means for hypothesizing host switches within phylogenetic lineages. Host conservatism, specialization, and switching were observed in the phylogeny of *Ophiognomonia* and hypothesized to contribute to speciation patterns in this genus. This methodology could be useful to plant quarantine officials, pathologists, or mycologists for making predictions about host specificity of plant pathogens.

ACKNOWLEDGEMENTS
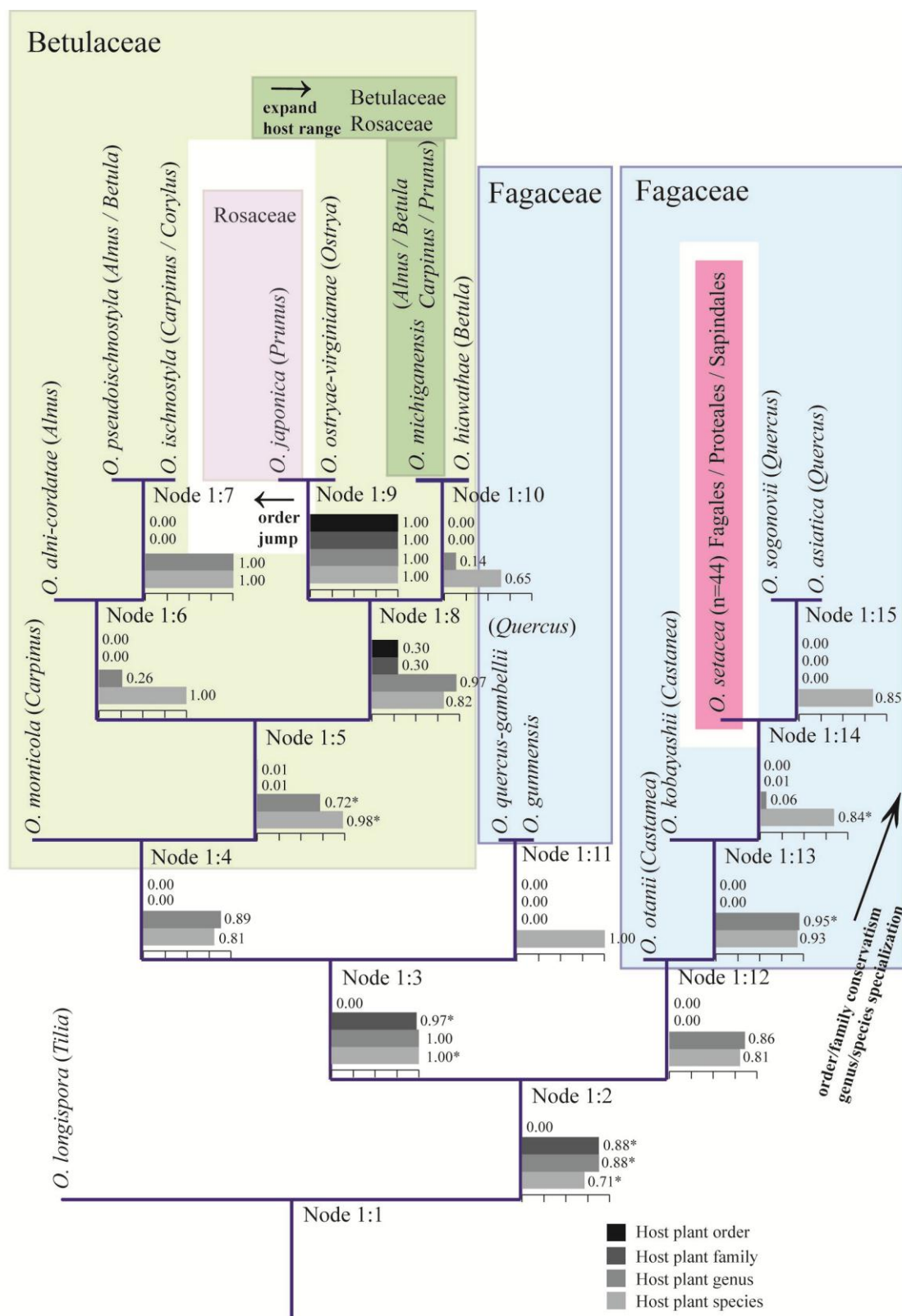
Fig. 5.1. Clade 1 of the genus *Ophiognomonia*. Spatial Evolutionary and Ecological

Vicariance Analysis (SEEVA) results. Divergence indices of four host plant variables:

Host plant order, family, genus, and species. Histograms representing divergence indices

(0–1) for each variable are mapped onto the ML tree from the GARLI analysis for 16

species of *Ophiognomonia.* After applying a Bonferroni correction ($P \leq 0.0034$), the

statistically significant divergence indices are indicated by "*" at each node. Nodes are

labeled as Clade # : Node #. The host plant families are represented as shaded boxes. The

complete host/fungus associations are listed in Table 5.1. Patterns of host conservatism,

specialization, or switching are indicated by bold arrows.
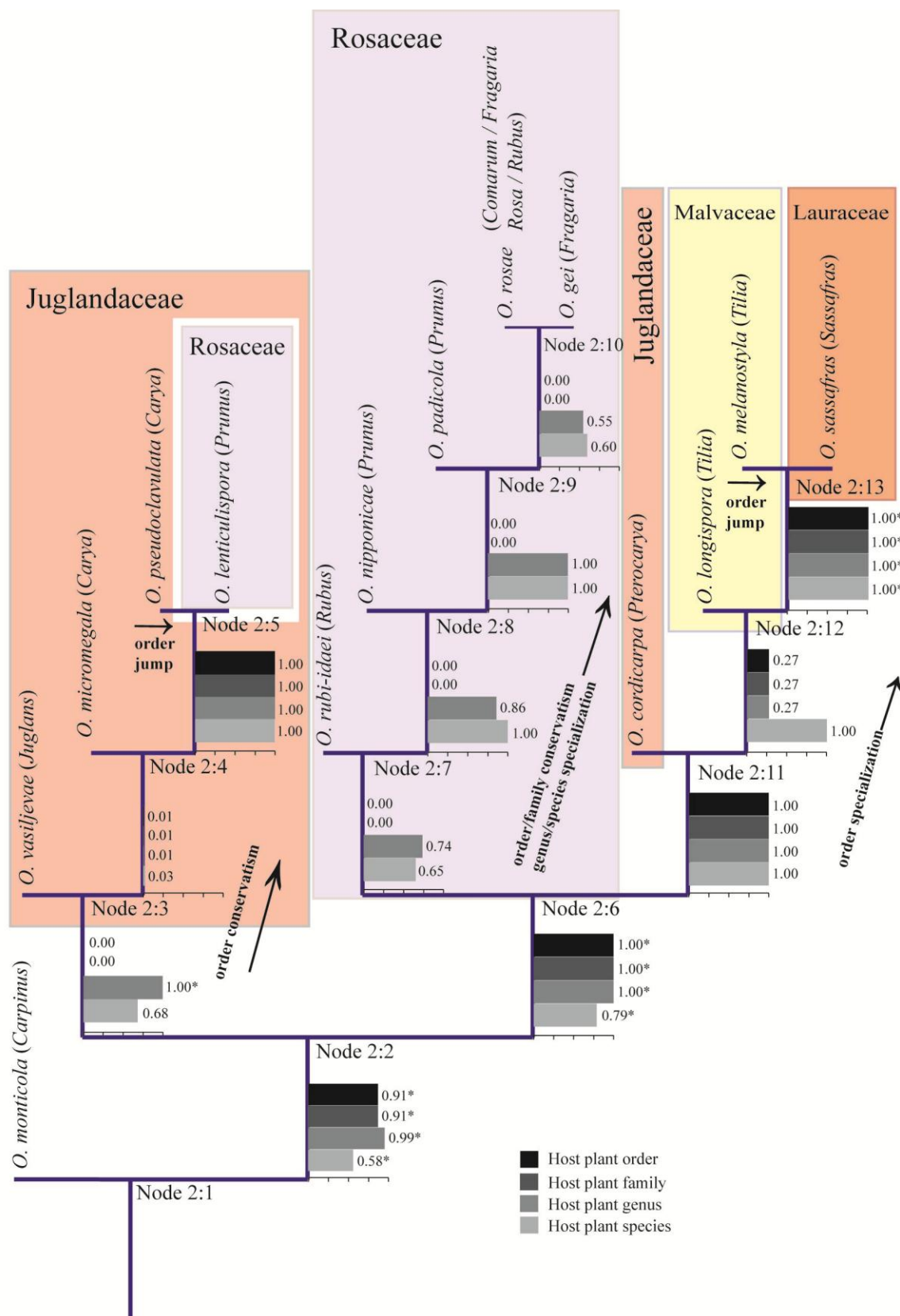
Fig. 5.2. Clade 2 of the genus *Ophiognomonia*. Spatial Evolutionary and Ecological

Vicariance Analysis (SEEVA) results. Divergence indices of four host plant variables:

Host plant order, family, genus, and species. Histograms of divergence indices (0–1) for

each variable are mapped onto the ML tree from the GARLI analysis for 14 species of

Ophiognomonia. After applying a Bonferroni correction ($P \leq 0.0039$), the statistically

significant divergence indices are indicated by "*" at each node. Nodes are labeled as

Clade # : Node #. The host plant families are represented as shaded boxes. The complete

host/fungus associations are listed in Table 5.1. Patterns of host conservatism,

specialization, or switching are indicated by bold arrows.

Fig. 5.3. Clade 3 of the genus *Ophiognomonia*. Spatial Evolutionary and Ecological

Vicariance Analysis (SEEVA) results. Divergence indices of four host plant variables:

Host plant order, family, genus, and species. Histograms of divergence indices (0–1) for each variable are mapped onto the ML tree from the GARLI analysis for 17 species of *Ophiognomonia.* After applying a Bonferroni correction ($P \leq 0.0034$), the statistically significant divergence indices are indicated by "*" at each node. Nodes are labeled as Clade # : Node #. The host plant families are represented as shaded boxes. The complete host/fungus associations are listed in Table 5.1. Patterns of host conservatism, specialization, or switching are indicated by bold arrows.

Table 5.1. Host plant relationships and geographic distribution of *Ophiognomonia*

Fungal Species	Host Order	Host Family	Host Genus	Host Species	Distribution	Number of Collections
Ophiognomonia alni-cordatae	Fagales	Betulaceae	*Alnus*	*cordata*	East Asia	1
Ophiognomonia alni-viridis	Fagales	Betulaceae	*Alnus*	*glutinosa*	West Asia, Europe, North America	17
				rhombifolia *rubra* *serrulata* *sinuata* *viridis*		
			Betula	*papyrifera* *nana*		
Ophiognomonia apiospora	Fagales	Betulaceae	*Alnus*	*nepalensis*	East Asia	2
Ophiognomonia asiatica	Fagales	Fagaceae	*Quercus*	*aliena* *crenata*	East Asia	5
Ophiognomonia balsamiferae	Malpighiales	Salicaceae	*Populus*	*balsamifera*	West North America	2
Ophiognomonia bugabensis	Fagales	Betulaceae	*Alnus*	*acuminata*	Central America	3
Ophiognomonia clavigignenti-juglandacearum	Fagales	Juglandaceae	*Juglans*	*cinerea*	East North America	14
Ophiognomonia cordicarpa	Fagales	Juglandaceae	*Pterocarya*	*rhoifolia*	East Asia	1
Ophiognomonia gardiennetii	Fagales	Betulaceae	*Alnus*	*serrulata*	East North America	3

Ophiognomonia gei	Rosales	Rosaceae	*Fragaria*	*vesca*	West Asia, Europe	3
Ophiognomonia gei-montani	Rosales	Rosaceae	*Geum*	*montana*	Europe	1
Ophiognomonia gunmensis	Fagales	Fagaceae	*Quercus*	*serrata*	East Asia	1
Ophiognomonia hiawathae	Fagales	Betulaceae	*Betula*	*lenta* *lutea* *papyrifera* *pubescens* *verrucosa*	West Asia, East North America	10
Ophiognomonia ibarakiensis	Fagales	Betulaceae	*Alnus*	sp.	East Asia	2
Ophiognomonia intermedia	Fagales	Betulaceae	*Alnus* *Betula*	*serrulata* *lutea* *papyrifera* *pendula* *verrucosa* *nigra*	West Asia, Europe, West North America	18
Ophiognomonia ischnostyla	Fagales	Betulaceae	*Carpinus* *Corylus*	*betulus* *avellana*	West Asia, Europe	4
Ophiognomonia japonica	Rosales	Rosaceae	*Prunus*	sp.	East Asia	1
Ophiognomonia kobayashii	Fagales	Fagaceae	*Castanea*	*crenata*	East Asia	4
Ophiognomonia lenticulispora	Rosales	Rosaceae	*Prunus*	sp.	East North America	1

Ophiognomonia leptostyla	Fagales	Juglandaceae	*Juglans*	*nigra*	Southwest Asia, Europe, East and Southeast North America	3
Ophiognomonia longispora	Malvales	Malvaceae	*Tilia*	*maximowicziana*	East Asia	2
Ophiognomonia maximowiczianae	Fagales	Betulaceae	*Betula*	*maximowicziana*	East Asia	1
Ophiognomonia melanostyla	Malvales	Malvaceae	*Tilia*	*americana* *cordata* *heterophyla*	East North America, Europe	6
Ophiognomonia michiganensis	Fagales	Betulaceae	*Alnus* *Betula* *Carpinus* *Prunus*	*serrulata* *alleghaniensis* *lutea* *papyrifera* *americana* sp.	East North America	16
	Rosales	Rosaceae				
Ophiognomonia micromegala	Fagales	Juglandaceae	*Carya*	*tomentosa*	East North America	7
Ophiognomonia monticola	Fagales	Betulaceae	*Carpinus*	sp.	East Asia	2
Ophiognomonia multirostrata	Fagales	Betulaceae	*Alnus*	*firma*	East Asia	4
Ophiognomonia naganoensis	Fagales	Betulaceae	*Alnus*	*hirsuta* *hirsuta* var. *sibitica*	East Asia	3
Ophiognomonia nana	Fagales	Betulaceae	*Betula*	*nana*	Europe	1
Ophiognomonia nipponicae	Rosales	Rosaceae	*Prunus*	*nipponica*	East Asia	1
Ophiognomonia ostryae-virginianae	Fagales	Betulaceae	*Ostrya*	*virginiana*	East North America	1

Ophiognomonia otanii	Fagales	Fagaceae	*Castanea*	*crenata*	East Asia	4
Ophiognomonia padicola	Rosales	Rosaceae	*Prunus*	*padus*	Europe	1
Ophiognomonia pseudoclavulata	Fagales	Juglandaceae	*Carya*	*tomentosa*	East North America	8
Ophiognomonia pseudoischnostyla	Fagales	Betulaceae	*Alnus* *Betula*	*glutinosa incana verrucosa*	West Asia, Europe	6
Ophiognomonia pterocaryae	Fagales	Juglandaceae	*Pterocarya*	*rhoifolia*	East Asia	2
Ophiognomonia quercus-gambellii	Fagales	Fagaceae	*Quercus*	*garryana* *kellogii*	West and Southwest North America	2
Ophiognomonia rosae	Rosales	Rosaceae	*Comarum* *Fragaria* *Rosa* *Rubus*	*palustre* *vesca* sp. sp.	West Asia, Europe, East and West North America	7
Ophiognomonia rubi-idaei	Rosales	Rosaceae	*Rubus*	*idaeus* *spectabilis*	Europe, West North America	3
Ophiognomonia sassafras	Laurales	Lauraceae	*Sassafras*	*albidum*	East North America	5

Ophiognomonia setacea	Sapindales	Sapindaceae	*Acer*	*saccharum*	East and West Asia, Europe, East and Southeast North America	44
	Fagales	Betulaceae Fagaceae	*Corylus Castanea* *Fagus Quercus*	sp. *dentata sativa* *grandifolia acutissima alba bicolor coccinea palustris prinus robur rubra velutina*		
	Proteales	Platanaceae	*Platanus*	*occidentalis*		
Ophiognomonia sogonovii	Fagales	Fagaceae	*Quercus*	*mongolica mongolica var. grosseserrata serrata*	East Asia	4
Ophiognomonia trientensis	Fagales	Betulaceae	*Alnus*	*tenuifolia* *viridis*	Europe, West North America	3
Ophiognomonia tucumanensis	Fagales	Betulaceae	*Alnus*	*acuminata*	South America	2
Ophiognomonia vasiljevae	Fagales	Juglandaceae	*Juglans*	*nigra*	East North America	3

Table 5.1. Host plant relationships and geographic distribution of 45 species in *Ophiognomonia.*

LITERATURE CITED

Anderson, R.L., LaMadeleine, L.A., 1978. The Distribution of Butternut Decline in the Eastern United States. Forest Survey Report. USDA Forest Service, 5.

Barrett, L.G., Kniskern, J.M., Bodenhausen, N., Zhang, W., Bergelson, J., 2009. Continua of specificity and virulence in plant host–pathogen interactions: causes and consequences. New Phytol. 183, 513−529.

Broders, K.D., Boland, G.J., 2011. Reclassification of the butternut canker fungus, *Sirococcus clavigignenti-juglandacearum*, into the genus *Ophiognomonia*. Fungal Biology 115, 70−79.

Eshed, N., Dinoor, A., 1980. Genetics of pathogenicity in *Puccinia coronata* − pathogenic specialization at the host genus level. Phytopathology 70, 1042−1046.

Farr, D.F., Rossman, A.Y. Fungal Databases, Systematic Mycology and Microbiology Laboratory, ARS, USDA. Retrieved September 19, 2011, from http://nt.ars-grin.gov/fungaldatabases/.

Farrell, B.D., 2001. Evolutionary Assembly of the Milkweed Fauna: Cytochrome Oxidase I and the Age of *Tetraopes* Beetles. Mol. Phylogenet. Evol. 18, 467–478.

Fisher, R.A., 1958. Statistical methods for research workers. 13th edn. Hefner, New York.

Fournier, E., Giraud, T., 2008. Sympatric genetic differentiation of a generalist pathogenic fungus, *Botrytis cinerea*, on two different host plants, grapevine and bramble. J. Evolution. Biol. 21, 122−132.

Gilbert, G.S., Webb, C.O., 2007. Phylogenetic signal in plant pathogen-host range. Proc. Natl. Acad. Sci. 104, 4979−4983.

Giraud, T., Villaréal, L.M.M.A., Austerlitz, F., Le Gac, M., Lavigne, C., 2006. Importance of the Life Cycle in Sympatric Host Race Formation and Speciation of Pathogens. Phytopathology 96, 280−287.

Giraud, T., Refregier, G., Le Gac, M., de Vienne, D.M., Hood, M.E., 2008. Speciation in fungi. Fungal Genetics and Biology 45, 791−802.

Hutchinson, G.E., 1957. Concluding remarks. Cold Spring Harbor Symp. 22, 415−427.

Kawecki, T.J., 1998. Red Queen meets Santa Rosalia: arms races and the evolution of host specialization in organisms with parasitic lifestyles. Am. Nat. 152, 635–651.

Kirzinger, M.W.B., Stavrinides, J., 2012. Host specificity determinants as a genetic continuum. Trends Microbiol. 20, 88−93.

Mabberley, D.J., 1997. The plant-book: A portable dictionary of the vascular plants. Second edition. Cambridge University Press.

Martínez-Meyer, E., Townsend Peterson, A., Hargrove, W.W., 2004. Ecological niches as stable distributional constraints on mammal species, with implications for Pleistocene extinctions and climate change projections for biodiversity. Global Ecol. Biogeogr. 13, 305−314.

Mejía, L.C., Castlebury, L.A., Rossman, A.Y., Sogonov, M.V., White, J.F., 2008. Phylogenetic placement and taxonomic review of the genus *Cryptosporella* and its synonyms *Ophiovalsa* and *Winterella* (Gnomoniaceae, Diaporthales). Mycol. Res. 112, 23−35.

Mejía, L., Rossman, A., Castlebury, L., Yang, Z., White, J., 2011a. *Occultocarpon*, a new monotypic genus of Gnomoniaceae on *Alnus nepalensis* from China. Fungal Divers. 52, 99−105.

Mejía, L.C., Castlebury, L.A., Rossman, A.Y., Sogonov, M.V., White, J.F., 2011b. A systematic account of the genus *Plagiostoma* (Gnomoniaceae, Diaporthales) based on morphology, host-associations, and a four-gene phylogeny. Stud. Mycol. 68, 211−235.

Mejía, L.C., Rossman, A.Y., Castlebury, L.A., White, J.F., Jr., 2011c. New species, phylogeny, host-associations, and geographic distribution of the genus *Cryptosporella* (Gnomoniaceae, Diaporthales). Mycologia 103, 379−399.

Nixon, K.C., Carpenter, J.M., 1993. On outgroups. Cladistics 9, 413−426.

Peever, T., 2007. Role of host specificity in the speciation of *Ascochyta* pathogens of cool season food legumes. Eur. J. Plant Pathol. 119, 119−126.

Peterson, A.T., Soberon, J., Sanchez-Cordero, V., 1999. Conservatism of ecological niches in evolutionary time. Science 285, 1265−1267.

Prinzing, A., 2001. The niche of higher plants: evidence for phylogenetic conservatism. Proceedings of the Royal Society of London. Series B: Biological Sciences 268, 2383−2389.

Redlin, S.C., 1991. *Discula destructiva* sp. Nov., Cause of Dogwood Anthracnose. Mycologia 83, 633−642.

Refregier, G., Le Gac, M., Jabbour, F., Widmer, A., Hood, M., Yockteng, R., Shykoff, J., Giraud, T., 2008. Cophylogeny of the anther smut fungi and their Caryophyllaceous

hosts: prevalence of host shifts and importance of delimiting parasite species. BMC Evol. Biol. 100, doi:10.1186/1471-2148-1188-1100.

Rice, W.R., 1989. Analyzing tables of statistical tests. Evolution 43, 223−225.

Rossman, A.T., Farr, D.F., Castlebury, L.A., 2007. A review of the phylogeny and biology of the Diaporthales. Mycoscience 48, 135−144.

Roy, B.A., 2001. Patterns of association between crucifers and their flower-mimic pathogens: host-jumps are more common than coevolution or cospeciation. Evolution 55, 41−53.

Schluter, D., 2000. The Ecology of Adaptive Radiations. Oxford University Press, Oxford.

Shykoff, J.A., Meyhofer, A., Bucheli, E., 1999. Genetic isolation among host races of the anther smut fungus *Microbotryum violaceum* on three host plant species. Int. J. Plant Sci. 160, 907−916.

Sogonov, M.V., Castlebury, L.A., Rossman, A.Y., White, J.F., 2007. The type species of *Apiognomonia, A. veneta*, with its *Discula* anamorph is distinct from *A. errabunda*. Mycol. Res. 111, 693−709.

Sogonov, M.V., Castlebury, L.A., Rossman, A.Y., Mejía, L.C., White, J.F., 2008. Leaf-inhabiting genera of the Gnomoniaceae, Diaporthales. Stud. Mycol. 62, 1−77.

Stephens, P.R., Wiens, J.J., 2003. Explaining species richness from continents to communities: the time-for-speciation effect in emydid turtles. Am. Nat. 161, 112−128.

Struwe, L., Smouse, P.E., Heiberg, E., Haag, S., Lathrop, R.G., 2011. Spatial evolutionary and ecological vicariance analysis (SEEVA), a novel approach to biogeography and speciation research, with an example from Brazilian Gentianaceae. J. Biogeogr. 38, 1841−1854.

Townsend, J.P., 2007. Profiling Phylogenetic Informativeness. Syst. Biol. 56, 222−231.

Townsend, J.P., Leuenberger, C., 2011. Taxon Sampling and the Optimal Rates of Evolution for Phylogenetic Inference. Syst. Biol. 60, 358−365.

Walker, D.M., Castlebury, L.A., Rossman, A.Y., Sogonov, M.V., White, J.F., 2010. Systematics of genus *Gnomoniopsis* (Gnomoniaceae, Diaporthales) based on a three gene phylogeny, host associations and morphology. Mycologia 102, 1479−1496.

Walker, D.M., Castlebury, L.A., Rossman, A.Y., and White, J.F. 2012. New molecular markers for fungal phylogenetics: Two genes for species-level systematics in the

Sordariomycetes (Ascomycota). Mol. Phylogenet. Evol. Accepted, doi: 10.1016/j.ympev.2012.05.005.

Walker, D.M., Castlebury, L.A., Rossman, A.Y., Mejía, L.C., White, J.F. Phylogeny of the genus *Ophiognomonia* (Gnomoniaceae, Diaporthales) based on analyses of three molecular markers, host associations, and morphology. Fungal Divers. In review, manuscript # FUDI-D-12-00071.

Whitlock, M.C., 1996. The Red Queen beats the Jack-of-all-trades: the limitations of phenotypic plasticity and niche breadth. Am. Nat. 148, S65–S77.

Wiens, J.J., Graham, C.H., 2005. Niche conservatism: integrating evolution, ecology, and conservation biology. Ann. Rev. Ecol. Evol. Syst. 36, 519−539.

Wiens, J.J., Ackerly, D.D., Allen, A.P., Anacker, B.L., Buckley, L.B., Cornell, H.V., Damschen, E.I., Jonathan Davies, T., Grytnes, J.-A., Harrison, S.P., Hawkins, B.A., Holt, R.D., McCain, C.M., Stephens, P.R., 2010. Niche conservatism as an emerging principle in ecology and conservation biology. Ecol. Lett. 13, 1310−1324.

Zwickl, D.J., 2006. Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion. Ph.D. dissertation. The University of Texas at Austin, U.S.A.

Chapter 6

Environmental influence on speciation and niche evolution in the fungal genus

Ophiognomonia **(Gnomoniaceae, Diaporthales)**

ABSTRACT

Species of *Ophiognomonia* (Gnomoniaceae) are leaf- and stem-inhabiting

perithecial fungi occurring on host tree species of the Betulaceae, Fagaceae,

Juglandaceae, Lauraceae, Malvaceae, Platanaceae, Rosaceae, Salicaceae, and

Sapindaceae. In this study environmental data was analyzed with SEEVA (Spatial

Evolutionary and Ecological Vicariance Analysis) using a multi-gene phylogeny of 45

species of *Ophiognomonia* to better understand speciation events in this genus. Eight

temperature and precipitation variables were analyzed in order to integrate evolutionary

hypotheses from phylogenetic data and detailed specimen records with environmental

(climate) information. Our objectives were to determine: 1) if patterns of fungal

diversification in this genus are influenced by climate constraints, 2) if species of

Ophiognomonia exhibit patterns of niche conservatism or specialization, and 3) if niche

conservatism or specialization influences speciation events in *Ophiognomonia*. The

differences in climate variables between sister clades were interpreted using the

divergence index (D), ranging from 0 for no divergence, to 1 for maximum possible

divergence. Patterns suggesting both niche conservatism (n=5, $D < 0.32$) and

specialization (n=8, $D = 0.48–0.72$) were observed at basal nodes in the phylogeny of

Ophiognomonia. Two species of *Ophiognomonia* with syngeneric hosts and sympatric

geographic ranges show patterns of ecological vicariance in three different temperature

and precipitation variables ($D > 0.72$), indicating climate-based niche specialization without spatial or host changes. We hypothesize that a host shift in sister species from *Carya tomentosa* → *Prunus* sp. was facilitated within an overlapping and ecologically similar host environment (n=6, $D < 0.47$). These and several other patterns are presented and discussed in this comprehensive study of the genus *Ophiognomonia.*

INTRODUCTION

Fungi in the family Gnomoniaceae (Diaporthales, Sordariomycetes, Ascomycota) are commonly found in temperate forests and at high elevations in tropical biomes. These species have been documented as endophytes, pathogens, and saprobes on a diverse range of trees used commercially to produce nuts, lumber, and shade, but are also common on wild tree species. Species of the Diaporthales have been documented on over 330 host genera in North America and Europe (Farr and Rossman 2011). The Gnomoniaceae, a family within this order consisting of ten genera, were characterized based on phylogenetic analyses of DNA sequence data, host association, and morphology (Sogonov et al. 2008, Mejía et al. 2008, 2011 a–c, Walker et al. 2010). Many species of Gnomoniaceae cause significant economic and ecological damage as plant pathogens, including *Discula destructiva* (dogwood anthracnose), *Ophiognomonia leptostyla* (walnut anthracnose), and *Apiognomonia errabunda* (Belisario 2008, Berry 1981, Juhasova et al. 2006, Neely and Black 1976, Redlin 1991, Rossman et al. 2007).

A narrow host range for most species has been documented in recent phylogenetic studies of the Gnomoniaceae, with most species associating with a single host genus or species (Mejía et al. 2008, 2011a-c, Sogonov et al. 2008, Walker et al. 2010). For

example, Mejía et al. (2011c) documented seven species of *Cryptosporella* occurring only on the host genus *Alnus* (Betulaceae). In addition, Walker et al. (2010) found four species of *Gnomoniopsis* that were specific to the host genus *Rubus* (Rosaceae), and Sogonov et al. (2008) documented the genus *Gnomonia* associating only with plants in the family Betulaceae. Sogonov et al. (2007) documented an exception to such tight host associations in the species *Apiognomonia errabunda*, which occurs on ten different plant families.

The genus *Ophiognomonia* contains 45 known species and has a diverse host range including plants in the families Betulaceae, Fagaceae, Juglandaceae, Lauraceae, Malvaceae, Platanaceae, Rosaceae, Salicaceae, and Sapindaceae. Most species in this genus are each restricted to a single host genus or several genera classified in the same host family (Sogonov et al. 2008, Walker et al. in review, Chapter 4). For example, *O. pseudoischnostyla* is documented on the host genera *Alnus* and *Betula* (Betulaceae), whereas, *O. quercus-gambellii* occurs only on *Quercus*. Only two species of *Ophiognomonia, O. michiganensis* and *O. setacea*, are known to occur on hosts from several different taxonomic orders/families (Walker et al. in review, Chapter 4).

Species of the Gnomoniaceae have been documented primarily in temperate regions, but also at high elevations in tropical ecosystems that often include the same host plant families as temperate areas. The northernmost reach of the Gnomoniaceae (*Ophiognomonia*) has been documented in Finland (latitude: 66.2695) for the species *O. rosae* (Walker et al. in review, Chapter 4). *Ophiognomonia tucumanensis* was recently discovered in South America (Argentina; latitude: -26.8500) and represents the southernmost distribution of the Gnomoniaceae (Walker et al. in review, Chapter 4).

Most accounts of the Gnomoniaceae are from North America and Europe; however, several genera have been documented from Argentina, China, Japan, and Panama (Mejía et al. 2011, Sogonov et al. 2008, Walker et al. in review, Chapter 4). Many species of *Ophiognomonia* have been collected in temperate areas in The Americas, Europe, and Asia (Walker et al. in review, Chapter 4) representing high species richness in these regions.

The ecological niche was defined by Hutchinson (1957) as the range of abiotic and biotic conditions in which a species can survive over time. The actual niche depends on ancestral adaptations to both ancient and recent habitats (Prinzing et al. 2001). The tendency for a species to occupy similar ecological niches that resemble that of its ancestors is referred to as phylogenetic niche conservatism (Harvey and Pagel 1991, Wiens et al. 2010). Some studies utilizing phylogenetics, ecophysiology, or Quaternary ecology have concluded that niche conservatism is of minor importance on speciation (Bennett 1997, Cronquist 1988, Stebbins 1975) whereas, other studies have found it to be substantial (Hodgson 1986) We conducted this study to evaluate the role of niche conservatism on speciation in the genus *Ophiognomonia*.

Biological reasons underlying a shared trait among several closely related species cannot be explained exclusively with phylogenetic analyses. Rather, integration of population-level processes, evolutionary biology, and ecological data allow for elucidation of patterns that resemble niche conservatism or specialization (Wiens et al. 2010). Studies that simultaneously link and evaluate these types of variables are rare. For example, Struwe et al. (2009, 2011) suggested that niche-based constraints have influenced speciation within three angiosperm genera from the Gentianaceae

(*Macrocarpaea, Prepusa, Senaea*). In this study we link local ecological patterns inferred from georeferenced herbarium specimens of host species to a phylogenetic analysis of the genus *Ophiognomonia* to better understand how the environment may have influenced speciation in this genus.

Within the Gnomoniaceae, host plant presence in a given habitat is an important factor determining the geographic range of these fungi. Documentation of the actual distribution of gnomoniaceous species is undoubtedly incomplete. We hypothesize that many of these species occur only in part of the total host distribution. If so, then the presence of the plant is only one factor determining fungal distribution, with other criteria such as ancestral biogeography and niche restrictions on the fungus/host that limit the actual distribution of the fungus. Abiotic and biotic factors such as temperature, precipitation, and the local plant community effect host distributions. Both the local plant community and microhabitat in which a fungus persists must exhibit selective pressures on the evolution of these species. In Chapter 5, we empirically demonstrate that host association is contributing to speciation in the genus *Ophiognomonia*. In this study we were interested to determine if the microhabitat affects fungal speciation and spatial distribution in this genus.

The program Spatial Evolutionary and Ecological Vicariance Analysis (SEEVA, Struwe et al. 2011) has the ability to integrate phylogenetic (temporal), geographical (spatial), and ecological data extracted from georeferenced herbarium specimens into a single analysis to evaluate niche divergence associated with speciation. Using SEEVA, patterns of ecological conservatism or specialization can be elucidated using a statistically solid method to quantify ecological shifts in closely related species. In this

study, the SEEVA methodology was utilized to better understand patterns of ecological divergence and possible effects of abiotic conditions on speciation events in the genus *Ophiognomonia*. Examining the interaction between the host, fungus, and environment should provide a framework for evolutionary hypotheses about future projects on plant pathogenic fungi.

The currently known species of *Ophiognomonia* were recently described (Walker et al. in review, Chapter 4) using multiple molecular markers, host associations, and morphology. Three molecular markers, ITS (ITS1, 5.8S rDNA and ITS2), MS204 (guanine nucleotide-binding protein subunit beta-like protein), and *tef-1α* (translation elongation factor 1α), were selected according to phylogenetic informativeness tests (Townsend 2007, Townsend and Leuenberger 2011) from data provided in Walker et al. (accepted, in review, Chapter 3–4) to reconstruct the phylogeny of *Ophiognomonia.* Three supported clades were recovered in the latter study (Walker et al. in review, Chapter 4) that represents the 45 sampled species of *Ophiognomonia.* The phylogenies from Walker et al. (in review, Chapter 4) were used in this study in the SEEVA analysis to evaluate niche separation associated with lineage divergence. The purpose of this study was to 1) determine which ecological variables show high/low levels of divergence throughout the phylogeny of *Ophiognomonia,* 2) distinguish between niche conservatism or specialization at basal and terminal phylogenetic nodes, and 3) determine if ecological niche conservatism or specialization may have influenced speciation in *Ophiognomonia.*

METHODS

A brief account of the methods is provided here; see chapter 5 for more details since the methodological approach for that chapter is similar to the one used here.

DNA extraction, amplification, and sequencing

Fungal isolates were grown in culture and genomic DNA extracted as in Walker et al. (2010). Three molecular markers, ITS, MS204, and *tef-1α*, were selected for analysis based on results from Walker et al. (accepted, Chapter 3). The markers were amplified and sequenced following Walker et al. (2010, accepted, Chapter 3).

Sequence data analyses

Based on results from the all-taxa combined alignment in Chapter 4 (Fig. 4.1), three additional datasets were formed corresponding to the three separate clades of *Ophiognomonia.* Sequence variation was detected and investigated in the three clades that would otherwise be discarded due to ambiguous alignment in the all-taxa combined alignment (Walker et al. in review, Chapter 4). SEEVA analyses (explained below) were performed on all three clades independently since support is lacking to determine the relationships of the clades to one another, hence inhibiting an all-taxa combined analysis in SEEVA.

Phylogenetic analyses for the three clades were completed using the three-marker alignments (alignments 5–7 = Clades 1–3) from Walker et al. (in review, Chapter 4, Chapter 5). The programs and parameters used to edit sequence data and prepare the alignments are identical to the methods used in Chapters 4–5. The complete DNA sequence dataset was stripped down to one isolate representing each of the 45 species of

Ophiognomonia. Each isolate was selected at random from the available isolates representing each species. The three alignments are hereafter referred to as Clades 1–3 (alignments 5–7 in Chapter 4, respectively), and explained in detail in Chapter 5. Phylogenetic trees were inferred with maximum likelihood (ML) analyses using the program GARLI v2.0 (Zwickl 2006), with implementation of parameters from Walker et al. (accepted, Chapter 4). The three resulting phylogenetic trees, each representing Clades 1, 2, and 3, were used as phylogenetic raw data for the Spatial Evolutionary and Ecological Vicariance Analysis (SEEVA, Struwe et al. 2011), as in Chapter 5.

Identification of unknown herbarium specimens

Herbarium specimens with ITS nucleotide sequence data verified in Walker et al. (in review, Chapter 4) to the species rank were used to create a local BLAST database in Bioedit v.7.0.9.0 (http://www.mbio.ncsu.edu/bioedit/bioedit.html), see Chapter 5. Local BLAST searches were used to identify 136 unknown herbarium samples with ITS sequence data using the same criteria as in Chapter 5.

Geolocation of taxonomic specimens

All herbarium specimens (n=234; Clade 1, n=105; Clade 2, n=48; Clade 3, n=81) with nucleotide sequence data were georeferenced using as precise as possible locality descriptions or GPS coordinates. The criteria in Struwe et al. (2011) were used for quality control of georeferenced collections. All 234 collections were georeferenced to the nearest second in decimal degrees. Specimens were discarded if the locality data was too vague. Google Maps (http://maps.google.com) was used to georeference specimens, and

custom Java script (javascript:void(prompt('',gApplication.getMap().getCenter())));
[http://www.tech-recipes.com/rx/2403/google_maps_get_latitude_longitude_values/])
was used to extract GPS coordinates. Latitude and longitude decimal degrees for each
collection were assembled in Microsoft Excel.

Environmental data

Eight sets of environmental variables representing the current climate (≈1950–
2000) were acquired from the website WorldClim v1.4 (Hijmans et al. 2005). The climate
dataset was downloaded in 2.5 arc-minute format. The following eight BIOCLIM
variables were used for analysis in the program SEEVA, 1) annual temperature range, 2)
minimum temperature of the coldest month, 3) maximum temperature of the warmest
month, 4) mean temperature of the wettest quarter (three month period), 5) annual
precipitation, 6) precipitation of the warmest quarter, 7) precipitation of the wettest
month, 8) precipitation of the driest month. The program DIVA-GIS (http://www.diva-
gis.org) was used to extract data for the eight previously mentioned BIOCLIM variables
for each herbarium specimen point location and data stored in Microsoft Excel.

SEEVA analyses

The program Spatial Evolutionary and Ecological Vicariance Analysis (SEEVA,
Struwe et al. 2011) was used to investigate ecological vicariance and divergence patterns
associated with each node of the phylogeny of *Ophiognomonia.* For an explanation of the
SEEVA rational see Chapter 5 and Struwe et al. (2011). The GARLI ML trees for Clades
1–3 were converted into cladograms and used for the SEEVA analyses. In the SEEVA

analysis, DNA sequences are one per species, but all specimens representing a species were included for georeferencing purposes. The eight BIOCLIM variables were labeled as quantitative, ordered, continuous data in four quartile classes for the SEEVA analyses. Due to small sample sizes a sample-permuted version of Fisher's (1958) exact test and Bonferroni correction (Rice 1989) were used to compute the test criteria and p-values and reduce the rate of false positives (Chapter 5, Struwe et al. 2011). The following p-values were used to declare a statistically significant result at a given node, 1) Clade 1 ($P \leq$ 0.0034), 2) Clade 2 ($P \leq 0.0039$), and 3) Clade 3 ($P \leq 0.0034$). Sister clades in *Ophiognomonia* were compared using the divergence index (D) to measure the strength of association between phylogenetic and ecological vicariance patterns (Struwe et al. 2011). A D-value of 0.0 indicates no difference when comparing sister clades, whereas, 1.0 indicates the maximum possible difference (Struwe et al. 2011).

RESULTS

Sample sizes and divergence indices (D) for the eight BIOCLIM variables for each of the three clades of *Ophiognomonia* are listed in Tables 6.1–6.6 and Figures 6.1–6.6 (select data). The following paragraphs report the divergence values in Clades 1–3 for each node as Clade # : Node # (e.g. 1:2, corresponds to Clade 1 : Node 2). A summary of host plant association and geographic location for all herbarium specimens of *Ophiognomonia* is listed in Table 6.7.

Ophiognomonia, Clade 1

For Clade 1, species divergence for all eight environmental variables showed a wide range of diversity (0.00–1.00 Tables 6.1–6.2, Figs. 6.1−6.2). Species divergence for temperature (0.00–1.00, Table 6.1) and precipitation (0.00–1.00, Table 6.2) variables were also highly diverse. Patterns of strong divergence in environmental characteristics of sister clades was observed at Node 1:4 (0.61–0.96) when comparing *O. monticola* vs. the clade composed of *O. alni-cordatae, O. hiawathae, O. ischnostyla, O. japonica, O. ostryae-virginianae,* and *O. pseudoischnostyla* (Fig. 6.1, Tables 6.1–6.2). Strong divergence patterns (0.85−1.00) for the split between *O. alni-cordatae* and *O. ischnostyla/O. pseudoischnostyla* suggest niche specialization at Node 1:6, and also between *O. gunmensis* and *O. quercus-gambellii* at Node 1:11 (Fig. 6.1, Tables 6.1–6.2). Trends of climate based-niche specialization without changes in host or geographic range was suggested independently for the sister species pairs of *O. ischnostyla/O. pseudoischnostyla* and *O. asiatica/O. sogonovii.* Strong divergence between these sister taxa was documented in minimum temperature during the coldest month, maximum temperature during the warmest month, and precipitation during the driest month (Tables 6.1−6.2). The sister species *O. hiawathae* and *O. michiganensis* (Node 1:10) show a pattern of weak ecological divergence across all environmental variables, which is representative of niche conservatism between these species (0.01–0.48, Table 6.1–6.2).

At Node 1:2, seven of eight environmental variables showed statistically significant divergence between two larger groups (Group A, B) of species (0.19–0.62, Fig. 6.2, Tables 6.1–6.2). The annual temperature range was the only variable without statistical significance. The species in Group A were found in colder and drier climates, whereas, Group B persisted in warmer and wetter climates (Fig. 6.2).

When examining all eight environmental variables across Clade 1, the strongest patterns of species divergence were observed for minimum temperature during the coldest month and precipitation during the wettest month. For minimum temperature during the coldest month six D-values were > 0.75 and seven > 0.50 (Table 6.1–6.2) indicating niche-based divergence among species in cold temperatures. The trend of progressively cold-tolerant taxa was observed from Node 1:4→Node 1:10 (Fig. 6.1). For precipitation during the wettest month, five D-values were > 0.75, and eight > 0.50 (Table 6.1–6.2), indicating precipitation-based niche specialization.

Ophiognomonia, Clade 2

A wide range of divergence index values (0.03–1.00, Tables 6.3–6.4) was indicated for all environmental variables in Clade 2. Clade divergence between temperature (0.03–1.00, Tables 6.3–6.4) and precipitation (0.05–1.00, Tables 6.3–6.4) varied greatly within Clade 2.

Trends of ecological specialization between clades at Nodes 2:2, 2:8, and 2:12 was indicated by strong divergence values for all environmental variables tested. The split at Node 2:2 (0.48–0.72) represents a pattern suggesting a basal ecological divergence event in Clade 2, followed by ecological conservatism in species Groups A and B (Fig. 6.3–6.4, Tables 6.3–6.4). For Node 2:2, all eight environmental variables were statistically significant (Fig. 6.4). After the divergence event at Node 2:2, ecological conservation is apparent at Node 2:6 within species Group B (0.03–0.53). A similar pattern was observed for species Group A, consisting of ecological divergence at Node 2:2, followed by conservation at Nodes 2:3→2:5 (Tables 6.3–6.4). Strong ecological

divergence was indicated for species at Nodes 2:8 (0.44–1.00) and 2:12 (0.57–0.87), followed by the trend toward drier habitats (Nodes 2:8→2:10, 2:12→2:13, Fig. 6.3) in these species lineages.

When comparing species divergence across all eight environmental variables, precipitation during the wettest month showed strong patterns suggesting niche-based evolution (Fig. 6.3, Table 6.4). Five nodes exhibited D-values > 0.75, nine ≥ 0.50, and three < 0.50, indicating strong niche specialization for this variable (Table 6.4).

Ophiognomonia, Clade 3

A wide range of divergence between clades was exhibited for all environmental variables tested in the SEEVA analysis for Clade 3 (0.00–1.00, Tables 6.5–6.6). Both temperature (0.02–1.00) and precipitation (0.00–1.00) variables shared similar patterns of divergence between clades (Tables 6.5–6.6).

Strong patterns suggesting niche specialization at Nodes 3:2, 3:3, 3:7, and 3:14 were observed for all environmental variables tested (Tables 6.5–6.6). At Nodes 3:2 and 3:3, moderate divergences were observed between the sister group and *O. balsamiferae* (0.52–0.66) and the sister group and *O. naganoensis* (0.47–0.67), respectively, when compared to the other species of Clade 3 (Table 6.5–6.6). A strong ecological split occurred at Node 3:7 between *O. ibarakiensis* and the sister clade *O.intermedia/O. tucumanensis* (0.60–0.93). Patterns suggesting strong niche-based divergence was also observed between the sister species *O. clavigignenti-juglandacearum* and *O. trientensis* (Node 3:14, 0.48–1.00). Inversely, trends of ecological conservatism were indicated at Nodes 3:5 and 3:6 (Node 3:5 = 0.02–0.45, Node 3:6 = 0.10–0.28) within species Groups

A and B, except for the annual temperature range, minimum temperature during the coldest month, and precipitation during the warmest quarter (D = 0.41*, 0.38*, 0.45*, respectively, Fig. 6.6), which show low to moderate divergence.

Strong ecological vicariance was suggested among the spatially allopatric sister species *O. maximowiczianae* and *O. nana* (Node 3:12, Tables 6.5−6.6). All D-values were high (1.00) except for precipitation during the warmest quarter (0.00), indicating both ecological specialization and conservatism among these species. Similarly, the sister species *O. apiospora* and *O. pterocaryae* show patterns of ecological vicariance. Strong divergence was documented in all four temperature variables, annual precipitation, and precipitation during the driest month (D = 1.00).

When comparing all eight environmental variables, the mean temperature during the wettest quarter showed the strongest ecological divergence between species of *Ophiognomonia* in Clade 3 (six D-values > 0.75, nine > 0.50, and five < 0.50). For example, the trend toward warmer/wetter climates was observed independently for *O. tucumanensis/O. clavigignenti-juglandacearum,* and colder/wetter climates for *O. intermedia/O. trientensis* (Node 3:8, 3:14, Fig. 6.5). The inverse was observed across all nodes for precipitation during the wettest month (three D-values > 0.75, six > 0.50, and eight < 0.50) indicating a pattern of moderate niche conservatism for this environmental variable in Clade 3.

DISCUSSION

Climatic conditions including temperature and precipitation are just a subset of factors influencing the current distribution of fungal and plant species. To completely

understand species richness patterns we must understand if and how climatic factors

influence speciation, extinction, and species dispersal (Wiens et al. 2006). Species of the

Gnomoniaceae rely on their host plants for survival and completion of their lifecycle.

Integrating broad climatic patterns with host/fungus relationship data will contribute to a

comprehensive study on the evolutionary history and biology of these fungi. In this study,

eight BIOCLIM (http://www.andra.fr/bioclim/, Tables 6.1–6.6) variables were linked to

234 georeferenced fungal herbarium collections in order to map environmental data on a

phylogenetic tree to assess divergence patterns and infer niche conservatism or

differentiation as a contributing factor in speciation. Our objective was to integrate

evolutionary hypotheses from phylogenetic analyses with spatial, temporal,

environmental, host, and geographical information.

　　　We acknowledge that the limited sample sizes in this study do not represent the

total geographical distribution of *Ophiognomonia.* However, the sampled distribution

here likely represents the habitat of unknown areas in which these species occur. Future

collections in new geographic areas will likely represent the habitats and host plants

sampled in this study. We do not rule out the discovery of novel habitats or host plants

for species of *Ophiognomonia.* In addition, based on results from Walker et al. (in

review, Chapter 4) we hypothesize that more new species of *Ophiognomonia* will be

discovered in novel temperate and tropical areas.

　　　　The tendency for a species to occupy a niche that closely resembles that of its

most recent ancestor is termed niche conservatism (Wiens and Graham 2005, Wiens et al.

2010). The time-for-speciation effect predicts that species which occur in a given region

or habitat for a longer time period will accumulate higher species richness relative to

younger species in same area (Stephens and Wiens 2003). Stephens and Wiens (2003) predict that the combination of niche conservatism and time-for-speciation effect are capable of explaining patterns of species richness and distributions along latitudinal and elevational gradients, as well as, at the community level. We hypothesize that niche conservatism, niche specialization, and the time-for-speciation effect are viable theories capable of explaining several patterns observed in the phylogeny of *Ophiognomonia*. The remaining sections discuss these and other patterns in *Ophiognomonia*.

Phylogenetic niche conservatism and species richness in temperate regions

The trend of consistently low divergence index values (n=5, $D < 0.32$) was observed across the majority of environmental variables at Node 3:5, however, moderate divergence in annual temperature range ($D = 0.41*$), minimum temperature during the coldest month ($D = 0.38*$), and precipitation during the warmest quarter ($D = 0.45*$) was evident in species Groups A and B (Node 3:5, Fig. 6.6). The clade delimited at Node 3:5 represents species from a worldwide distribution in both temperate and tropical regions, which associate with the host plants *Alnus* spp. and *Betula* spp. (Betulaceae), except for *O. clavigignenti-juglandacearum* (*Juglans cinerea,* Juglandaceae). Although patterns of niche specialization were observed in several terminal groups (e.g. Nodes 3:12, 3:14), this clade of species (Node 3:5, $D = 0.02-0.45$), occurs in similar environments and under similar host constraints (*Alnus* spp. and *Betula* spp.). The 11 species delimited at Node 3:5 have been collected in both the Northern and Southern hemispheres at a diverse range of latitudes (-26.85−57.71). The spatial distribution of a particular host is partially determined by the environment (and many other complex factors). The host plant is the

primary factor limiting the distribution of species in *Ophiognomonia*. It is currently

unknown if the species of *Ophiognomonia* occur in all or are limited to part of their

respective host environment. Teasing apart the spatial distributions of host and fungus to

explore environmental constraints, either due to chance and/or history (incomplete

dispersal throughout the host range), will be explored in a later study.

A similar pattern suggesting niche conservatism is present at Node 3:6 (n=8, *D* <

0.29, Tables 6.5−6.6), which delimits the clade of species *O. alni-viridis, O. bugabensis,*

O. ibarakiensis, O. intermedia, O. maximowiczianae, O. multirostrata, O. nana, and *O.*

tucumanensis. The later species associate only with *Alnus* spp. and *Betula* spp.

(Betulaceae) from a worldwide distribution including Eastern/Western Asia, Europe and

The Americas (Table 6.7, Fig. 6.5). We hypothesize that a long standing relationship with

respect to host association and the environment has persisted throughout evolutionary

time for these species of *Ophiognomonia* (Node 3:6). Also, that both niche conservatism

and the time-for-speciation effect help explain species diversity in temperate regions

worldwide, and only a recent shift to the tropics (*O. bugabensis, O. tucumanensis,* Fig.

6.5). Higher species richness in temperate versus tropical regions has also been

documented in both frogs and snakes, suggesting an origin in temperate regions and

limited dispersal outside of these areas due to climatic constraints (Pyron and Burbrink

2009, Smith et al. 2005).

Trends of ecological specialization at basal nodes in Ophiognomonia

Our objective in this study was to understand the ecological mechanisms that

underlie phylogenetic-based conservatism or specialization for a specific climate (ex.

Clayton et al. 2004); host associations and host range evolution was explored in Chapter

5. The two major subclades of Clade 1 (Group A, B) are separated at Node 1:2, and show

several patterns that could be explained by ecological specialization. Species in Groups A

and B are from a worldwide distribution occurring on hosts in the families Betulaceae,

Fagaceae, Platanaceae, Rosaceae, and Sapindaceae (Table 6.7, Fig. 6.1). Although

divergence among the eight environmental variables for these two clades (Groups A, B)

is low to moderate (0.19−0.62), all are significant, except for annual temperature range.

When examining the patterns of divergence closer, the clade of species including *O. alni-cordatae, O. gunmensis, O. hiawathae, O. ischnostyla, O. japonica, O. michiganensis, O. monticola, O. ostryae-virginianae, O. pseudoischnostyla,* and *O. quercus-gambellii*

(Group A) tend to occur in warmer and wetter conditions when compared to the clade

including *O. asiatica, O. kobayashii, O. otanii, O. setacea,* and *O. sogonovii* (Group B),

which occur in colder and drier environments. These patterns suggest ecological

specialization in distinct environments for these two clades of *Ophiognomonia.*

Patterns suggesting strong ecological divergence were observed for basal node

2:2, which splits the phylogeny into subclades (Group A, B, Fig. 6.3). These two clades

of species (Group A, B) occur on hosts in the Juglandaceae, Lauraceae, Malvaceae, and

Rosaceae from a worldwide temperate distribution (Table 6.7, Fig. 6.3). When comparing

Group A to Group B at Node 2:2, a trend suggesting moderate to strong ecological

divergence was observed between these clades for all eight environmental variables ($D =$

0.48−0.72, Fig. 6.4). Species in Group A associate with hosts primarily in the

Juglandaceae, except for *O. lenticulispora* (*Prunus* sp., Rosaceae), and occur within

warmer and wetter environments when compared to species in Group B. Species in

Group B form two subclades (Node 2:6), one of which associates with hosts in the Rosaceae (Nodes 2:7→2:10), and the other with Lauraceae, Juglandaceae, and Malvaceae (Nodes 2:11→2:13, Table 6.7, Fig 6.3). Species in Group B are spatially distributed in colder and drier environments, and despite the diverse host and geographic range, show patterns suggesting niche conservatism at the split between host specific subclades (Node 2:6, $D = 0.03−0.53$). A general trend of niche conservatism across all environmental variables was observed for the species delimited by Nodes 2:3→2:5 (n = 20 of 24, $D < 0.50$, Tables 6.3−6.4). This clade of species (Group A) including *O. lenticulispora, O. micromegala, O. pseudoclavulata,* and *O. vasiljevae* is documented from an overlapping range in Eastern North America [Latitude: (35.61) 35.82-39.61 (39.83); Longitude: (-85.83) -83.82−-76.73 (-75.71)]. Perhaps, long term association with hosts in the Juglandaceae and Rosaceae accompanied by ecological niche conservatism may be an explanation for species richness on these plants in temperate forests along the east coast of North America. Similarly, Prinzing et al. (2001) quantified niche conservatism among extant European plant species, and found clear conservation representing the hypothesized habitat of their ancestors and opportunities these plants encountered during diversification.

Patterns of phylogenetic niche specialization without spatial or host changes

Ecological theory predicts that coexistence of identical competitors will not exist within a local community (Douhan et al. 2008). Different ecological roles may be present for closely related species that co-occur within the same niche. Therefore, environmental influences probably contribute to the speciation process of co-occurring organisms

(Douhan et al. 2008). At the molecular level, theoretical models have shown that sympatric speciation is possible if mating takes place within a specific habitat and the same gene(s) control both fitness and habitat preference (Rice 1984). Although this scenario was not tested in this study, it must be relatively common in *Ophiognomonia,* since both the host plant and environment are critical elements in the life cycle of these fungi.

The spatially sympatric species *O. asiatica* and *O. sogonovii* occur on the syngeneric host genera *Quercus aliena/Q. crenata* and *Q. mongolica/Q. serrata* from China/Japan, respectively (Node 1:15, Table 6.7, Fig. 6.1). Both species were collected from overlapping geographic ranges in East Asia [Latitude: (25.14) 35.98−36.23 (36.31); Longitude: (102.75) 138.21−140.11 (140.20)]. Patterns suggesting ecological specialization were observed between these species for maximum temperature during the warmest month ($D = 1.00$) and minimum temperature during the coldest month ($D = 1.00$). *Ophiognomonia sogonovii* was documented in a colder environment when compared with *O. asiatica*. Another strong split ($D = 0.72$) was observed for precipitation levels during the driest month between these sister species. *Ophiognomonia sogonovii* was collected in drier environments when compared to *O. asiatica.* We hypothesize that populations of these species regularly come into contact but are reproductively isolated due to host species specialization (Chapter 5) and strong ecological vicariance. This is an excellent example of a pattern suggesting climate-based niche specialization without spatial or host genus change. Douhan et al. (2008) found a similar example of ecological vicariance within three co-occurring populations of

Claviceps purpurea that specialize independently on salt-water tolerant, riparian, and terrestrial grasses in costal estuary habitats.

The sister species (Node 1:7, Fig. 6:1) *O. ischnostyla* and *O. pseudoischnostyla* occur on *Carpinus/Corylus* and *Alnus/Betula* in the Betulaceae (respectively), and show a trend resembling climate-based niche specialization without extensive spatial or host family changes (Table 6.7). *Ophiognomonia ischnostyla* and *O. pseudoischnostyla* are documented in Europe and Western Asia from a broadly overlapping geographic range [Latitude: (45.98) 46.32−57.08 (57.14); Longitude: (6.58) 6.92−31.52 (35.32)]. For minimum temperature during the coldest month, *O. pseudoischnostyla* showed patterns resembling ecological specialization ($D = 0.70$) in colder environments when compared to *O. ischnostyla,* which occurs in warmer climates. Patterns suggestive of climate-based niche specialization were also observed for precipitation during the driest month ($D = 0.72$), with *O. pseudoischnostyla* occurring in drier, and *O. ischnostyla* in wetter environments. As documented in Chapter 5, host genus specialization was most likely a driving factor in the speciation event at Node 1:7, but patterns of climate-based niche specialization are supported herein as contributing to reproductive isolation of these spatially overlapping species.

An additional trend of climate-based niche specialization without host family changes (Rosaceae) was observed when comparing the species *O. padicola* (*Prunus padus*) to the sister clade of *O. gei/O. rosae* (*Fragaria vesca/Comarum palustre, Fragaria vesca, Rosa* sp., *Rubus* sp., respectively, Node 2:9, Table 6.7, Fig. 6.3). *Ophiognomonia padicola* is known to occur in Europe, *O. gei* in Europe and Western Asia, and *O. rosae* in Europe and North America [Node 2:9 complete range = Latitude:

(42.76) 45.17−56.73 (66.27); Longitude: (-122.49) -70.42−29.38 (35.32)]. These species

share an overlapping geographic range in Europe [Latitude: (46.10) 46.26−47.55 (66.27);

Longitude (5.26) 6.37−7.11(29.38)]. Comparisons of these species suggest patterns of

climate-based niche specialization within an overlapping spatial range on hosts in the

Rosaceae. For example, for annual temperature range, *O. padicola* is present in colder

climates than *O. gei/O. rosae* ($D = 0.54$). Similarly, for the mean temperature during the

wettest quarter, *O. padicola* occurs within colder and wetter environments than *O. gei/O.*

rosae ($D = 0.62$). The SEEVA analysis suggests that *O. padicola* occurs in a distinctly

wetter environment than *O. gei/O. rosae*. For example, these species show strong trends

of divergence in three environmental variables including annual precipitation ($D = 0.87$),

precipitation during the wettest month ($D = 0.87$), and precipitation during the driest

month ($D = 0.86$). The patterns observed in this example support the hypothesis that

fungal speciation may be influenced in part by the environment.

Host shifts facilitated by overlapping host environments?

Similar host plant ecology may play an important role in facilitating host shifts in

similar or overlapping ecological host habitats (Refregier et al. 2008). We hypothesize

that the host shift from *Carya* spp. (Juglandaceae) → *Prunus* sp. (Rosaceae) in the

sympatric sister species *O. lenticulispora* and *O. pseudoclavulata* was facilitated by a

highly similar host environment (Node 2:5, Tables 6.3−6.4, Fig. 6.3). These species are

located in closely overlapping spatial ranges in Eastern North America and often

collected in the same forest [Latitude: (35.82) 38.55-39.23 (39.83); Longitude: (-85.83) -

78.72−-76.73 (-75.71)]. Trends suggesting strong to moderate ecological conservatism

were observed at Node 2:5 for six environmental variables ($D < 0.47$), excluding annual

precipitation and precipitation during the wettest month ($D = 0.69, 0.51$, respectively). As

shown in Chapter 5, the clade of species (Node 2:3) including *O. micromegala, O.*

pseudoclavulata, and *O. vasiljevae* are specific to host genera *Carya* and *Juglans*

(Juglandanceae), except for *O. lenticulispora* (*Prunus* sp., Rosaceae). The most recent

common ancestor shared by *O. lenticulispora* and *O. pseudoclavulata* (Node 2:4) was

most likely *Carya tomentosa* in the Juglandaceae (Figs. 5.2, Chapter 5). Since *O.*

lenticulispora and *O. pseudoclavulata* occur in the same forest on host trees within

meters of one another (personal observation), perhaps the environment facilitated a shift

in the most recent common ancestor from *Carya tomentosa* to *Prunus* sp. and subsequent

speciation.

Speculation on the lifecycle of Ophiognomonia

The species *O. apiospora* and *O. pterocaryae* represent a host shift from *Alnus*

nepalensis (Betulaceae) → *Pterocarya rhoifolia* (Juglandaceae; respectively)

accompanied by patterns of ecological divergence (Node 3:15, Table 6.5−6.6, Fig. 6.5).

Both of these species occur in Eastern Asia over a broad geographic area with *O.*

apiospora found in China and *O. pterocaryae* in Japan (Latitude: 25.14−36.31;

Longitude: 102.75−138.22). *Ophiognomonia pterocaryae* was documented in warmer

environments than *O. apiospora,* except for the minimum temperature during the coldest

month. Species of the Gnomoniaceae are known to overwinter in dead leaves and twigs

on the forest floor, producing perithecia (sexual structures) and dispersing ascospores into

the environment during cool/moist spring conditions (Walker et al. 2010). Perhaps cold

winter temperatures (-12−-7 °C) are critical for *O. pterocaryae* to trigger dormancy of immature perithecia allowing survival throughout the cold winter months, and warm conditions necessary during the remainder of the year for ascospore/mycelium maturation, growth, and survival. Patterns of phylogenetic niche conservatism were observed in *O. apiospora* and *O. pterocaryae* species for precipitation during the warmest quarter and wettest month (Table 6). High levels of precipitation are critical for *O. apiospora* and *O. pterocaryae* during hot (> 364 mm precipitation) and wet conditions (>166 mm precipitation). This illustrates the importance of high moisture levels for survival of these species regardless of host specificity.

Gradual niche evolution in Ophiognomonia

The niche of closely related species has been hypothesized to differentiate to some degree during a speciation event (Warren et al. 2008). To fully understand the evolutionary biology of an organism, it is critical to understand if the environment influenced the speciation event or if the species adapted to the new habitat following reproductive isolation. Warren et al. (2008) empirically demonstrated that niches of closely related Cuban anoles tend to be similar to one another but rarely identical. A similar trend was suggested in the SEEVA analysis for this study in all species of *Ophiognomonia.* Moderate niche divergence ($D \geq 0.45$) was suggested in at least one environmental variable at every node, except for nodes 2:4 and 3:6 (Tables 6.1−6.6). This supports our hypothesis that gradual niche change is occurring in the extant species of *Ophiognomonia.* For example, when examining precipitation during the wettest month a general trend toward more and more drier environments was observed for Nodes

2:6→2:13 in species Group B of Clade 2 (Fig. 6.3). This pattern suggests gradual niche evolution toward drier environments for the species *O. cordicarpa, O. gei, O. longispora, O. melanostyla, O. nipponicae, O. padicola, O. rosae, O. rubi-idaei,* and *O. sassafras.* Undoubtedly, both the diverse host range (Juglandaceae, Lauraceae, Malvaceae, Rosaceae) and spatial distribution (Asia, Europe, North America) in this clade also had a significant effect on speciation.

CONCLUSION

Patterns of niche-based conservatism and specialization without spatial or host changes were suggested within the phylogeny of *Ophiognomonia*. We hypothesize that host specificity and niche-based evolution are mechanisms strongly contributing to speciation patterns in this genus. This method of analyzing environmental data provides a visual and statistically solid understanding of niche evolution in the genus *Ophiognomonia.* This methodology could be used for predictions about environmental constraints of plant pathogens, as well as determining the evolutionary histories of host-pathogen-environment relationships.

ACKNOWLEDGEMENTS

Legend:

- > -4.7 °C (warmest)
- -6.2 – -4.7 °C
- -12 – -6.2 °C
- < -12 °C (coldest)

Fig. 6.1. Clade 1 of the genus *Ophiognomonia*. Spatial Evolutionary and Ecological Vicariance Analysis (SEEVA) results for the BIOCLIM variable minimum temperature during the coldest month for the current climate (1950−2000) mapped onto the ML tree from the GARLI analysis for 16 species of *Ophiognomonia.* The minimum temperature during the coldest month has been coded in four different quantitative states and displayed as histograms at each branch terminal. The total height of each histogram represents the number of observations in each category indicated in the figure legend. The total number of observations (n) is listed after each species. Divergence indices are listed at each node in the phylogeny with * indicating a statistically significant relationship. Arrows at selected nodes suggest patterns of niche-based specialization. Spatial data is indicated by shaded boxes corresponding to Table 6.7. The following abbreviations were used to represent each geographic location: e Asia = Eastern Asia; w Asia = Western Asia; c Am = Central America; e N Am = Eastern North America (East of Rocky Mountains); s Am = South America; se N Am = Southeastern North America; w N Am = Western North America (West of Rocky Mountains).

Fig. 6.2. Clade 1, Node 1:2 of the genus *Ophiognomonia*. Spatial Evolutionary and Ecological Vicariance Analysis (SEEVA) results for eight different BIOCLIM variables for the current climate (1950−2000) extracted from Node 1:2 of Figure 6.1. The letters "A" and "B" on the x-axis correspond to the two groups of species delimited at Node 1:2 on Figure 6.1. The total height of each histogram represents the number of observations in each temperature or precipitation category indicated in the figure legend. Divergence values are listed above the histograms for each environmental variable. A statistically significant divergence value is indicated by * after the Bonferroni correction ($P \leq$ 0.0034). The total number of observations (n) for Group A = 44 and B = 61. Variables with the word "Quarter" = three month period.

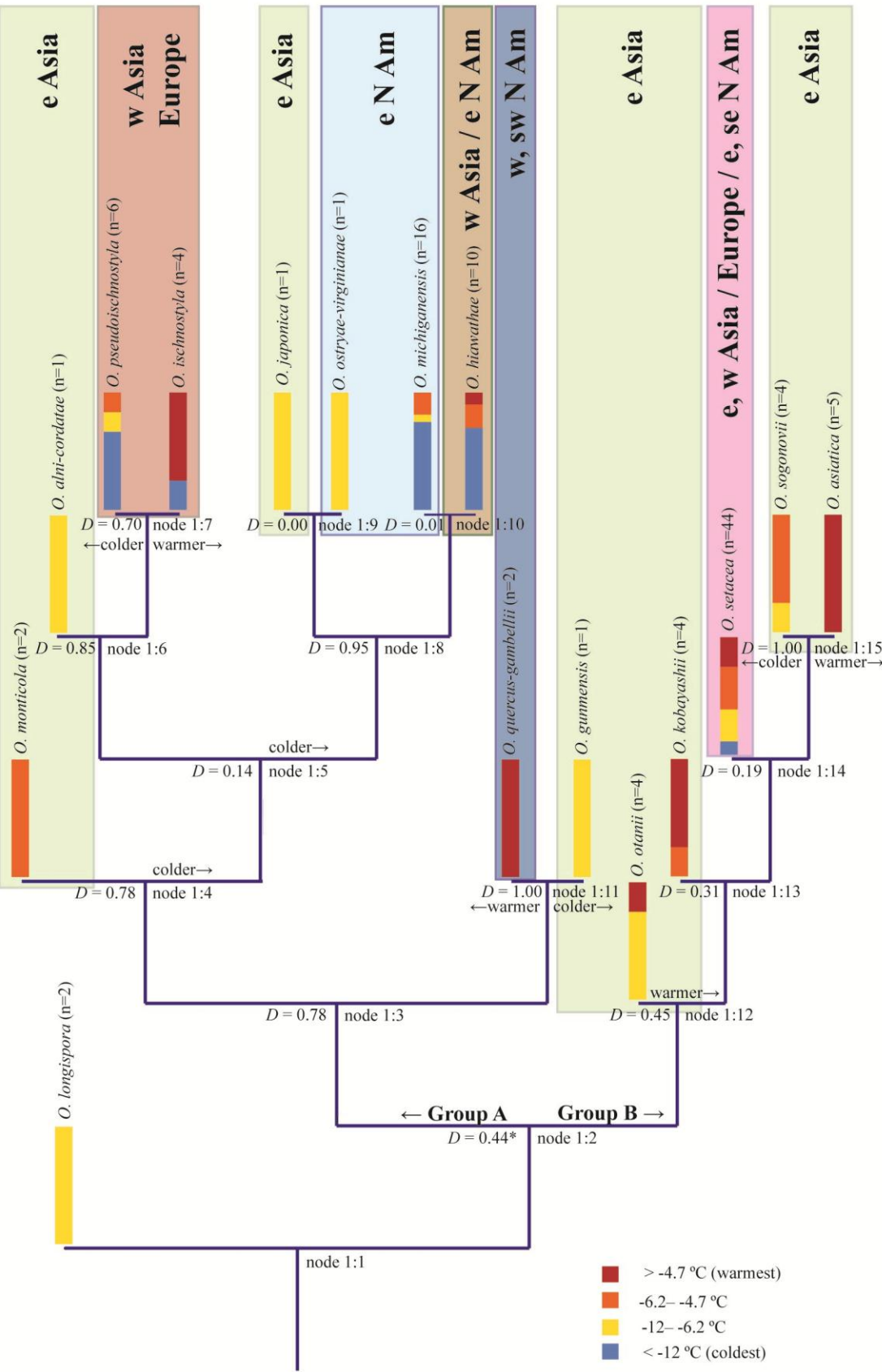> 129 mm (wettest)
105–129 mm
101–105 mm
< 101 mm (driest)

Fig. 6.3. Clade 2 of the genus *Ophiognomonia*. Spatial Evolutionary and Ecological Vicariance Analysis (SEEVA) results for the BIOCLIM variable precipitation during the wettest month for the current climate (1950−2000) mapped onto the ML tree from the GARLI analysis for 14 species of *Ophiognomonia*. The precipitation during the wettest month has been coded in four different quantitative states and displayed as histograms at each branch terminal. The total height of each histogram represents the number of observations in each category indicated in the figure legend. The total number of observations (n) is listed after each species. Divergence indices are listed at each node in the phylogeny with * indicating a statistically significant relationship. Arrows at selected nodes suggest patterns of niche-based specialization. Spatial data is indicated by shaded boxes corresponding to Table 6.7. The following abbreviations were used to represent each geographic location: e Asia = Eastern Asia; w Asia = Western Asia; c Am = Central America; e N Am = Eastern North America (East of Rocky Mountains); s Am = South America; se N Am = Southeastern North America; w N Am = Western North America (West of Rocky Mountains).

Fig. 6.4. Clade 2, Node 2:2 of the genus *Ophiognomonia*. Spatial Evolutionary and Ecological Vicariance Analysis (SEEVA) results for eight different BIOCLIM variables for the current climate (1950−2000) extracted from Node 2:2 of Figure 6.3. The letters "A" and "B" on the x-axis correspond to the two groups of species delimited at Node 2:2 on Figure 6.3. The total height of each histogram represents the number of observations in each temperature or precipitation category indicated in the figure legend. Divergence values are listed above the histograms for each environmental variable. A statistically significant divergence value is indicated by * after the Bonferroni correction ($P \leq$ 0.0039). The total number of observations (n) for Group A = 19 and B = 29. Variables with the word "Quarter" = three month period.
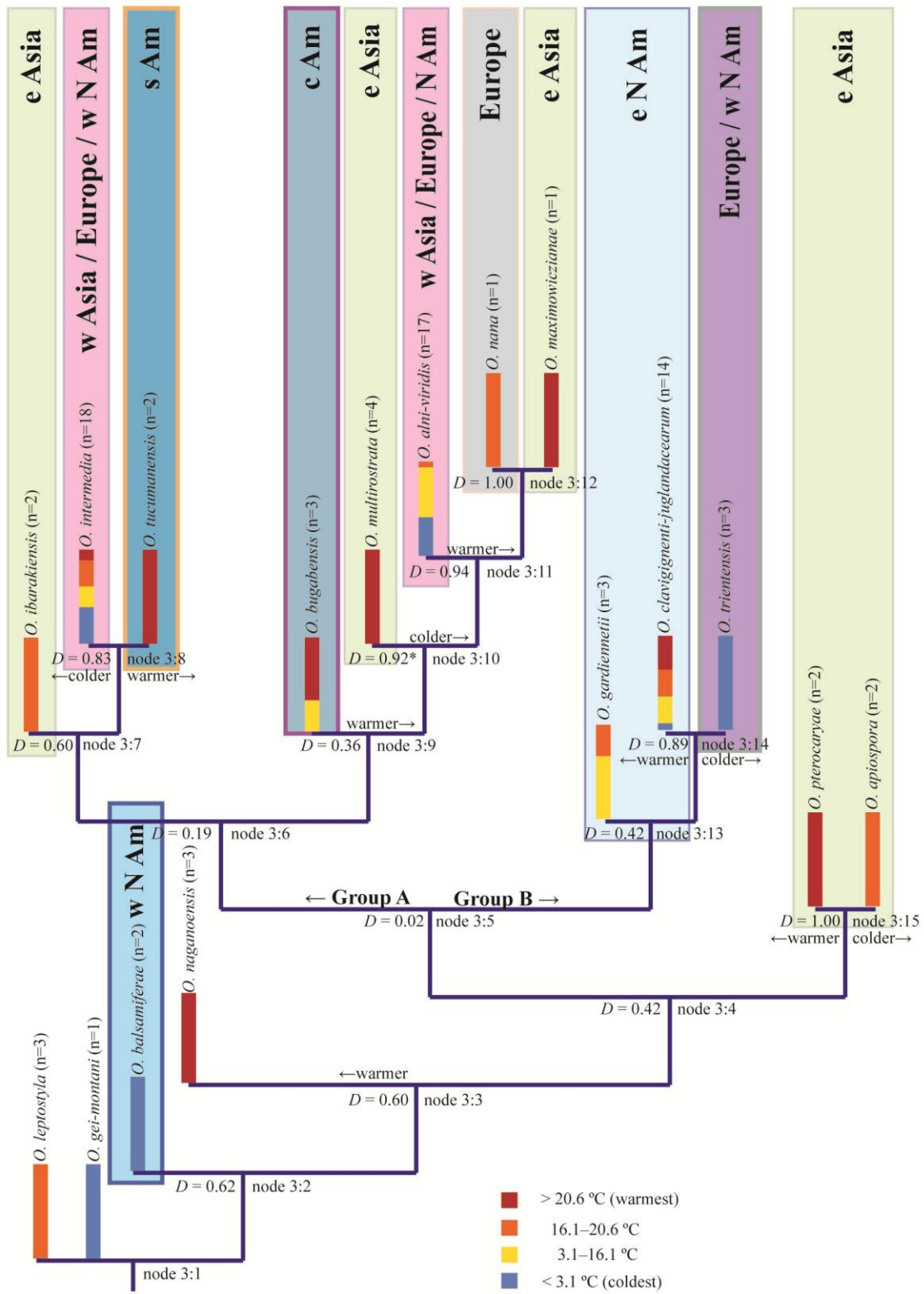
Fig. 6.5. Clade 3 of the genus *Ophiognomonia*. Spatial Evolutionary and Ecological

Vicariance Analysis (SEEVA) results for the BIOCLIM variable mean temperature

during the wettest quarter for the current climate (1950−2000) mapped onto the ML tree

from the GARLI analysis for 17 species of *Ophiognomonia.* The mean temperature

during the wettest quarter has been coded in four different quantitative states and

displayed as histograms at each branch terminal. The total height of each histogram

represents the number of observations in each category indicated in the figure legend.

The total number of observations (n) is listed after each species. Divergence indices are

listed at each node in the phylogeny with * indicating a statistically significant

relationship. Arrows at selected nodes suggest patterns of niche-based specialization.

Spatial data is indicated by shaded boxes corresponding to Table 6.7. The following

abbreviations were used to represent each geographic location: e Asia = Eastern Asia; w

Asia = Western Asia; c Am = Central America; e N Am = Eastern North America (East

of Rocky Mountains); s Am = South America; se N Am = Southeastern North America;

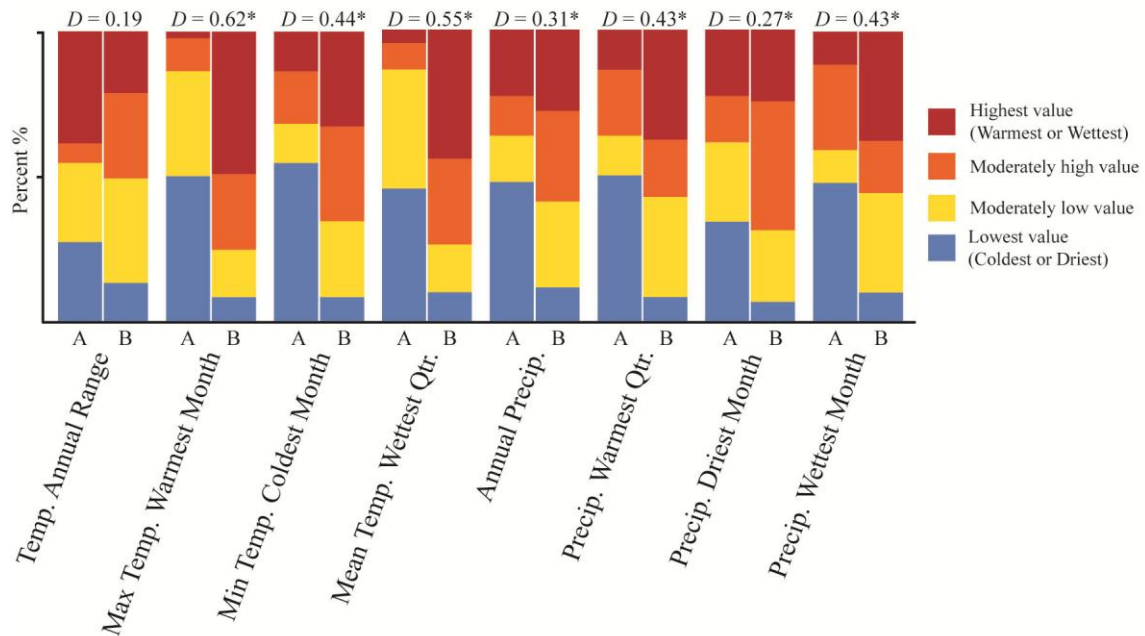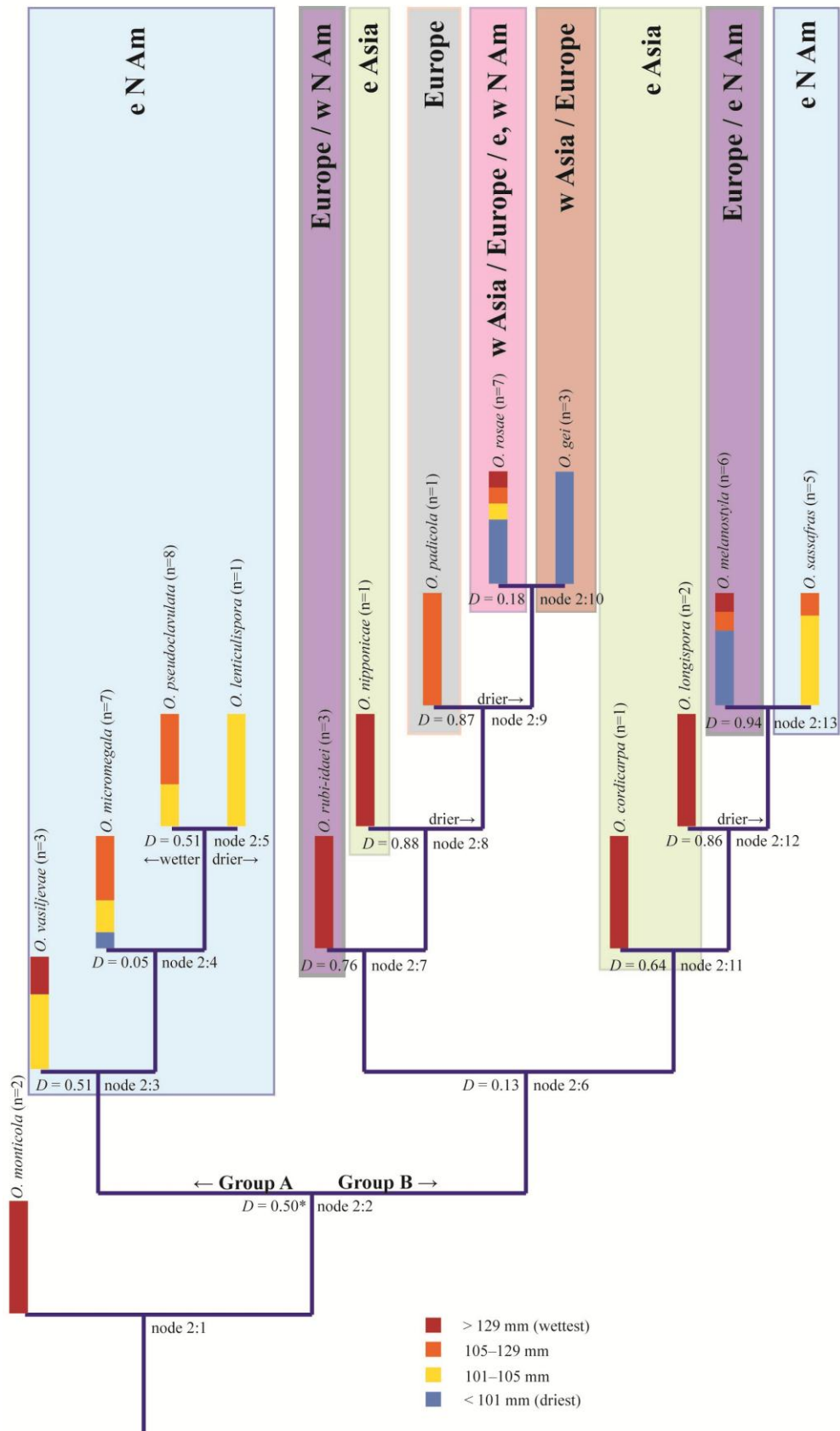w N Am = Western North America (West of Rocky Mountains).

Fig. 6.6. Clade 3, Node 3:5 of the genus *Ophiognomonia*. Spatial Evolutionary and Ecological Vicariance Analysis (SEEVA) results for eight different BIOCLIM variables for the current climate (1950−2000) extracted from Node 3:5 of Figure 6.5. The letters "A" and "B" on the x-axis correspond to the two groups of species delimited at Node 3:5 on Figure 6.5. The total height of each histogram represents the number of observations in each temperature or precipitation category indicated in the figure legend. Divergence values are listed above the histograms for each environmental variable. A statistically significant divergence value is indicated by * after the Bonferroni correction ($P \leq 0.0034$). The total number of observations (n) for Group A = 20 and B = 48. Variables with the word "Quarter" = three month period.

Table 6.1. Index of divergence (*D*) from the SEEVA analysis of Clade 1 in *Ophiognomonia* using four temperature-based variables.

|	Number of samples		Index of divergence (*D*)			
Phylogenetic node	nA	nB	Annual temperature range	Minimum temperature, coldest month	Maximum temperature, warmest month	Mean temperature, wettest quarter
1: 1 outgroup vs. ingroup	2	105	X	X	X	X
1: 2	44	61	0.19	0.44*	0.62*	0.55*
1: 3	3	41	0.59	**0.78**	0.21	0.33
1: 4	2	39	0.61	**0.78**	**0.89**	**0.96**
1: 5	11	28	0.61*	0.14	0.28	0.29
1.6	1	10	**1.00**	**0.85**	**1.00**	**1.00**
1: 7	4	6	0.29	0.70	0.08	0.14
1: 8	2	26	0.35	**0.95**	0.44	0.65
1: 9	1	1	**1.00**	0.00	**1.00**	**1.00**
1: 10	10	16	0.48	0.01	0.17	0.19
1: 11	1	2	**1.00**	**1.00**	**1.00**	**1.00**
1: 12	4	57	0.36	0.45	0.20	0.16
1: 13	4	53	0.49	0.31	0.11	0.34
1: 14	44	9	0.49	0.19	0.14	0.37
1: 15	4	5	0.08	**1.00**	**1.00**	0.47

Table 6.1. Index of divergence (*D*) from the SEEVA analysis of Clade 1 in *Ophiognomonia* using four temperature-based variables.

The phylogenetic nodes correspond to those in Fig. 6.1 as Clade # : Node #. Variables with *D*-values > 0.75 are listed in bold type

face. The columns nA / nB indicate the number of samples compared between the two sister clades. "X" indicates a *D*-value excluded

from the results. *Nodes show significant difference between sister groups using a Bonferroni criterion of $P \leq 0.0034$.

Table 6.2. Index of divergence (D) from the SEEVA analysis of Clade 1 in *Ophiognomonia* using four precipitation-based variables.

	Number of samples		Index of divergence (D)			
Phylogenetic node	nA	nB	Annual Precipitation	Precipitation, warmest quarter	Precipitation, wettest month	Precipitation, driest month
1: 1 outgroup vs. ingroup	2	105	X	X	X	X
1: 2	44	61	0.31*	0.43*	0.43*	0.27*
1: 3	3	41	0.73	0.18	0.54	0.26
1: 4	2	39	0.70	**0.89**	**0.93**	0.63
1: 5	11	28	0.04	0.06	0.06	0.16
1.6	1	10	**1.00**	**0.85**	**1.00**	**1.00**
1: 7	4	6	0.46	0.06	0.01	0.72
1: 8	2	26	**0.79**	**0.88**	**0.83**	0.50
1: 9	1	1	**1.00**	**1.00**	**1.00**	**1.00**
1: 10	10	16	0.04	0.04	0.04	0.33
1: 11	1	2	**1.00**	**1.00**	**1.00**	**1.00**
1: 12	4	57	0.37	0.49	0.49	0.43
1: 13	4	53	0.32	0.37	0.39	0.49
1: 14	44	9	0.43	0.73*	0.72*	0.45
1: 15	4	5	0.06	0.00	0.00	0.72

Table 6.2. Index of divergence (D) from the SEEVA analysis of Clade 1 in *Ophiognomonia* using four precipitation-based variables. The phylogenetic nodes correspond to those in Fig. 6.1 as Clade # : Node #. Variables with D-values > 0.75 are listed in bold type face. The columns nA / nB indicate the number of samples compared between the two sister clades. "X" indicates a D-value excluded from the results. *Nodes show significant difference between sister groups using a Bonferroni criterion of $P \leq 0.0034$.

Table 6.3. Index of divergence (*D*) from the SEEVA analysis of Clade 2 in *Ophiognomonia* using four temperature-based variables.

	Number of samples		Index of divergence (*D*)			
Phylogenetic node	nA	nB	Annual temperature range	Minimum temperature, coldest month	Maximum temperature, warmest month	Mean temperature, wettest quarter
2: 1 outgroup vs. ingroup	2	48	X	X	X	X
2: 2	19	29	0.48*	0.60*	0.70*	0.66*
2: 3	3	16	0.63	0.13	0.05	0.22
2: 4	7	9	0.04	0.05	0.03	0.19
2: 5	1	8	0.27	0.47	0.33	0.15
2: 6	14	15	0.30	0.03	0.53	0.20
2: 7	3	12	0.17	0.12	0.12	0.54
2: 8	1	11	0.44	**1.00**	**0.77**	**1.00**
2: 9	1	10	0.54	0.44	0.09	0.62
2: 10	3	7	0.58	0.21	0.20	0.24
2: 11	1	13	0.42	0.40	0.65	0.64
2: 12	2	11	0.59	0.57	**0.87**	**0.86**
2: 13	5	6	**0.88**	**0.77**	0.42	0.53

Table 6.3. Index of divergence (*D*) from the SEEVA analysis of Clade 2 in *Ophiognomonia* using four temperature-based variables. The phylogenetic nodes correspond to those in Fig. 6.3 as Clade # : Node #. Variables with *D*-values > 0.75 are listed in bold type face. The columns nA / nB indicate the number of samples compared between the two sister clades. "X" indicates a *D*-value excluded from the results. *Nodes show significant difference between sister groups using a Bonferroni criterion of $P \leq 0.0039$.

Table 6.4. Index of divergence (*D*) from the SEEVA analysis of Clade 2 in *Ophiognomonia* using four precipitation-based variables.

| Phylogenetic node | Number of samples | | Index of divergence (*D*) | | | |
	nA	nB	Annual Precipitation	Precipitation, warmest quarter	Precipitation, wettest month	Precipitation, driest month
2: 1 outgroup vs. ingroup	2	48	X	X	X	X
2: 2	19	29	0.59*	0.71*	0.50*	0.72*
2: 3	3	16	0.21	0.28	0.51	0.35
2: 4	7	9	0.09	0.11	0.05	0.13
2: 5	1	8	0.69	0.08	0.51	0.18
2: 6	14	15	0.14	0.20	0.13	0.08
2: 7	3	12	0.27	0.10	**0.76**	0.28
2: 8	1	11	**0.75**	**0.88**	**0.88**	0.47
2: 9	1	10	**0.87**	0.74	**0.87**	**0.86**
2: 10	3	7	0.20	0.20	0.18	0.09
2: 11	1	13	0.52	0.51	0.64	0.64
2: 12	2	11	0.72	0.71	**0.86**	**0.86**
2: 13	5	6	**1.00**	**1.00**	**0.94**	0.29

Table 6.4. Index of divergence (*D*) from the SEEVA analysis of Clade 2 in *Ophiognomonia* using four precipitation-based variables.

The phylogenetic nodes correspond to those in Fig. 6.3 as Clade # : Node #. Variables with *D*-values > 0.75 are listed in bold type

face. The columns nA / nB indicate the number of samples compared between the two sister clades. "X" indicates a *D*-value excluded

from the results. *Nodes show significant difference between sister groups using a Bonferroni criterion of P ≤ 0.0039.

Table 6.5. Index of divergence (*D*) from the SEEVA analysis of Clade 3 in *Ophiognomonia* using four temperature-based variables.

| | Number of samples | | Index of divergence (*D*) | | | |
| | | | Annual temperature range | Minimum temperature, coldest month | Maximum temperature, warmest month | Mean temperature, wettest quarter |
Phylogenetic node	nA	nB				
3: 1 outgroup vs. ingroup	4	77	X	X	X	X
3: 2	2	75	0.64	0.62	0.66	0.62
3: 3	3	72	0.65	0.67	0.65	0.60
3: 4	4	68	0.36	0.40	0.36	0.42
3: 5	20	48	0.41*	0.38*	0.32	0.02
3: 6	22	26	0.12	0.20	0.16	0.19
3: 7	2	20	**0.93**	**0.85**	0.69	0.60
3: 8	2	18	0.34	0.25	0.65	**0.83**
3: 9	3	23	0.66	**0.80**	0.49	0.36
3: 10	4	19	0.58	0.67	**0.93***	**0.92***
3: 11	2	17	0.37	0.25	0.40	**0.94**
3: 12	1	1	**1.00**	**1.00**	**1.00**	**1.00**
3: 13	3	17	0.30	0.31	0.54	0.42
3: 14	3	14	0.71	0.64	**1.00***	**0.89**
3: 15	2	2	**1.00**	**1.00**	**1.00**	**1.00**

Table 6.5. Index of divergence (*D*) from the SEEVA analysis of Clade 3 in *Ophiognomonia* using four temperature-based variables. The phylogenetic nodes correspond to those in Fig. 6.5 as Clade # : Node #. Variables with *D*-values > 0.75 are listed in bold type face. The columns nA / nB indicate the number of samples compared between the two sister clades. "X" indicates a *D*-value excluded from the results. *Nodes show significant difference between sister groups using a Bonferroni criterion of P ≤ 0.0034.

Table 6.6. Index of divergence (*D*) from the SEEVA analysis of Clade 3 in *Ophiognomonia* using four precipitation-based variables.

	Number of samples		Index of divergence (*D*)			
Phylogenetic node	nA	nB	Annual Precipitation	Precipitation, warmest quarter	Precipitation, wettest month	Precipitation, driest month
3: 1 outgroup vs. ingroup	4	77	X	X	X	X
3: 2	2	75	0.62	0.66	0.52	0.57
3: 3	3	72	0.67	0.58	0.47	0.62
3: 4	4	68	0.44	0.65	0.39	0.35
3: 5	20	48	0.32	0.45*	0.25	0.10
3: 6	22	26	0.11	0.28	0.23	0.10
3: 7	2	20	0.61	**0.86**	**0.76**	**0.76**
3: 8	2	18	0.29	**1.00**	0.51	0.65
3: 9	3	23	0.53	0.46	0.28	0.44
3: 10	4	19	0.67	0.67	0.43	0.24
3: 11	2	17	0.72	**0.82**	0.33	0.30
3: 12	1	1	**1.00**	0.00	**1.00**	**1.00**
3: 13	3	17	**0.83**	**0.76**	0.64	**0.78**
3: 14	3	14	0.48	**1.00***	**0.90**	**0.91**
3: 15	2	2	**1.00**	0.00	0.33	**1.00**

Table 6.6. Index of divergence (*D*) from the SEEVA analysis of Clade 3 in *Ophiognomonia* using four precipitation-based variables. The phylogenetic nodes correspond to those in Fig. 6.5 as Clade # : Node #. Variables with *D*-values > 0.75 are listed in bold type face. The columns nA / nB indicate the number of samples compared between the two sister clades. "X" indicates a *D*-value excluded from the results. *Nodes show significant difference between sister groups using a Bonferroni criterion of P ≤ 0.0034.

Table 6.7. Host plant relationships and geographic distribution

Fungal Species	Host Order	Host Family	Host Genus	Host Species	Distribution: Country (Locality)	Number of Georeferenced Collections
Ophiognomonia alni-cordatae	Fagales	Betulaceae	*Alnus*	*cordata*	Japan (Nagano prefecture)	1
Ophiognomonia alni-viridis	Fagales	Betulaceae	*Alnus* *Betula*	*glutinosa rhombifolia rubra serrulata sinuata viridis papyrifera nana*	Canada (British Columbia), Europe (Czech Republic, Switzerland), Russia (Tver' and Novgorod Province), United States (CA, MI, NY, WA)	17
Ophiognomonia apiospora	Fagales	Betulaceae	*Alnus*	*nepalensis*	China (Yunnan Province)	2
Ophiognomonia asiatica	Fagales	Fagaceae	*Quercus*	*aliena crenata*	China (Kunming), Japan (Ibaraki prefecture)	5
Ophiognomonia balsamiferae	Malpighiales	Salicaceae	*Populus*	*balsamifera*	Canada (British Columbia)	2
Ophiognomonia bugabensis	Fagales	Betulaceae	*Alnus*	*acuminata*	Panama (Chiriqui)	3

Ophiognomonia clavigignenti-juglandacearum	Fagales	Juglandaceae	*Juglans*	*cinerea*	Canada (New Brunswick, Ontario, Quebec), United States (AR, CT, IN, MI, MN, MO, NC, NH, NY, OH, TN, VT, WI)	14
Ophiognomonia cordicarpa	Fagales	Juglandaceae	*Pterocarya*	*rhoifolia*	Japan (Nagano prefecture)	1
Ophiognomonia gardiennetii	Fagales	Betulaceae	*Alnus*	*serrulata*	United States (MI)	3
Ophiognomonia gei	Rosales	Rosaceae	*Fragaria*	*vesca*	Europe (France), Russia (Tver' Province)	3
Ophiognomonia gei-montani	Rosales	Rosaceae	*Geum*	*montana*	Europe (Switzerland)	1
Ophiognomonia gunmensis	Fagales	Fagaceae	*Quercus*	*serrata*	Japan (Gunma prefecture)	1
Ophiognomonia hiawathae	Fagales	Betulaceae	*Betula*	*lenta lutea papyrifera pubescens verrucosa*	Russia (Tver' and Novgorod province), United States (MI)	10
Ophiognomonia ibarakiensis	Fagales	Betulaceae	*Alnus*	sp.	Japan (Ibaraki prefecture)	2
Ophiognomonia intermedia	Fagales	Betulaceae	*Alnus*	*serrulata*	Canada (British Columbia), Europe	18

			Betula	*lutea papyrifera pendula verrucosa nigra*	(Germany, Scotland), Russia (Tver' and Novgorod provinces), United States (MD, MI)	
Ophiognomonia ischnostyla	Fagales	Betulaceae	*Carpinus Corylus*	*betulus avellana*	Europe (France, Switzerland), Russia (Novogorod Province)	4
Ophiognomonia japonica	Rosales	Rosaceae	*Prunus*	sp.	Japan (Gunma prefecture)	1
Ophiognomonia kobayashii	Fagales	Fagaceae	*Castanea*	*crenata*	Japan (Ibaraki prefecture)	4
Ophiognomonia lenticulispora	Rosales	Rosaceae	*Prunus*	sp.	United States (MD)	1
Ophiognomonia leptostyla	Fagales	Juglandaceae	*Juglans*	*nigra*	Canada (Ontario), Europe (Austria, Bulgaria, Germany, Poland, Russia, Switzerland), Iran, United States (AL, DE, IA, IL, MA, MD, NY, PA, VA, WV)	3

Ophiognomonia longispora	Malvales	Malvaceae	*Tilia*	*maximowicziana*	Japan (Nagano prefecture)	2
Ophiognomonia maximowiczianae	Fagales	Betulaceae	*Betula*	*maximowicziana*	Japan (Nagano prefecture)	1
Ophiognomonia melanostyla	Malvales	Malvaceae	*Tilia*	*americana* *cordata* *heterophyla*	Europe (Austria, Bulgaria, Czech Republic, France, Germany, Switzerland, Ukraine), Canada (Ontario), United States (NY, PA)	6
Ophiognomonia michiganensis	Fagales	Betulaceae	*Alnus* *Betula* *Carpinus* *Prunus*	*serrulata* *alleghaniensis* *lutea* *papyrifera* *americana* sp.	United States (MI, NC, NY)	16
	Rosales	Rosaceae				
Ophiognomonia micromegala	Fagales	Juglandaceae	*Carya*	*tomentosa*	United States (MD)	7
Ophiognomonia monticola	Fagales	Betulaceae	*Carpinus*	sp.	Japan (Ibaraki prefecture)	2
Ophiognomonia multirostrata	Fagales	Betulaceae	*Alnus*	*firma*	Japan (Ibaraki prefecture)	4

Ophiognomonia naganoensis	Fagales	Betulaceae	*Alnus*	*hirsuta* *hirsuta* var. *sibitica*	Japan (Nagano prefecture)	3
Ophiognomonia nana	Fagales	Betulaceae	*Betula*	*nana*	Europe (Germany)	1
Ophiognomonia nipponicae	Rosales	Rosaceae	*Prunus*	*nipponica*	Japan (Ibaraki prefecture)	1
Ophiognomonia ostryae-virginianae	Fagales	Betulaceae	*Ostrya*	*virginiana*	United States (NY)	1
Ophiognomonia otanii	Fagales	Fagaceae	*Castanea*	*crenata*	Japan (Gunma, Ibaraki, Nagano prefectures)	4
Ophiognomonia padicola	Rosales	Rosaceae	*Prunus*	*padus*	Europe (Germany, Switzerland)	1
Ophiognomonia pseudoclavulata	Fagales	Juglandaceae	*Carya*	*tomentosa*	United States (DC, IL, IN, MD, NC, NJ, PA, TN, VA)	8
Ophiognomonia pseudoischnostyla	Fagales	Betulaceae	*Alnus* *Betula*	*glutinosa* *incana* *verrucosa*	Europe (Switzerland), Russia (Novogorod and Tver' provinces)	6
Ophiognomonia pterocaryae	Fagales	Juglandaceae	*Pterocarya*	*rhoifolia*	Japan (Nagano prefecture)	2
Ophiognomonia quercus-gambellii	Fagales	Fagaceae	*Quercus*	*garryana* *kellogii*	United States (AZ, OR)	2
Ophiognomonia rosae	Rosales	Rosaceae	*Comarum*	*palustre*	Europe (Finland,	7

					France, Switzerland), Russia (Tver' Province), United States (ME, OR)	
			Fragaria	*vesca*		
			Rosa	sp.		
			Rubus	sp.		
Ophiognomonia rubi-idaei	Rosales	Rosaceae	*Rubus*	*idaeus*	Canada (British Columbia), Europe (Switzerland)	3
				spectabilis		
Ophiognomonia sassafras	Laurales	Lauraceae	*Sassafras*	*albidum*	United States (MD, OH, WV)	5
Ophiognomonia setacea	Sapindales	Sapindaceae	*Acer*	*saccharum*	Canada (Ontario), Europe (Austria, Bulgaria, Germany, Italy, Montenegro, Sweden, Switzerland), Japan (Ibaraki prefecture), Russia (Tver' province), United States (GA, LA, MD, MI, NC, NJ, NY, OH, PA, TN, VA, WV)	44
	Fagales	Betulaceae	*Corylus*	sp.		
		Fagaceae	*Castanea*	*dentata*		
				sativa		
			Fagus	*grandifolia*		
			Quercus	*acutissima*		
				alba		
				bicolor		
				coccinea		
				palustris		
				prinus		
				robur		
				rubra		
				velutina		
	Proteales	Platanaceae	*Platanus*	*occidentalis*		

Ophiognomonia sogonovii	Fagales	Fagaceae	*Quercus*	*mongolica* *mongolica var. grosseserrata* *serrata*	Japan (Ibaraki and Nagano prefectures), Russia (Primorsky Territory)	4
Ophiognomonia trientensis	Fagales	Betulaceae	*Alnus*	*tenuifolia* *viridis*	Canada (British Columbia), Europe (Switzerland), United States (WA)	3
Ophiognomonia tucumanensis	Fagales	Betulaceae	*Alnus*	*acuminata*	Argentina (Tucuman)	2
Ophiognomonia vasiljevae	Fagales	Juglandaceae	*Juglans*	*nigra*	United States (MD, TN, VA)	3

Table 6.7. Host plant relationships and geographic distribution of 45 species in *Ophiognomonia.*

LITERATURE CITED

Belisario, A., Scotton, M., Santori, A., Onofri, S., 2008. Variability in the Italian population of *Gnomonia leptostyla*, homothallism and resistance of *Juglans* species to anthracnose. Forest Pathol. 38, 129−145.

Bennett, K.D., 1997. Evolution and ecology. Cambridge University Press.

Berry, F.H., 1981. Walnut Anthracnose Forest Insect & Disease Leaflet 85. U.S. Department of Agriculture, Forest Service, Northern Area State & Private Forestry, Broomall, PA.

Clayton, D.H., Bush, S.E., Johnson, K.P., 2004. Ecology of congruence: past meets present. Syst. Biol. 53.

Cronquist, A., 1988. The evolution and classification of fowering plants. New York: New York Botanical Garden.

Douhan, G.W., Smith, M.E., Huyrn, K.L., Westbrook, A., Beerli, P., Fisher, A.J., 2008. Multigene analysis suggests ecological speciation in the fungal pathogen *Claviceps purpurea*. Mol. Ecol. 17, 2276−2286.

Farr, D.F., Rossman, A.Y. Fungal Databases, Systematic Mycology and Microbiology Laboratory, ARS, USDA. Retrieved September 19, 2011, from http://nt.ars-grin.gov/fungaldatabases/.

Fisher, R.A., 1958. Statistical methods for research workers. 13th edn. Hefner, New York.

Harvey, P.H., Pagel, M.R., 1991. The comparative method in evolutionary biology. Oxford University Press.

Hijmans, R.J., Cameron, S.E., Parra, J.L., Jones, P.G., Jarvis, A., 2005. Very high resolution interpolated climate surfaces for global land areas. Int J Climatol 25, 1965−1978.

Hodgson, J.G., 1986. Commonness and rarity in plants with special reference to the Sheffield flora. III. Taxonomic and evolutionary aspects. Biol. Conserv. 36, 275−296.

Hutchinson, G.E., 1957. Concluding remarks. Cold Spring Harbor Symp. 22, 415−427.

Juhasova, G., Ivanova, H., Spisak, J., 2006. Biology of fungus *Gnomonia leptostyla* in agro-ecological environments of Slovakia. Mikologica Fitopatologie 40, 538−547.

Mejía, L.C., Castlebury, L.A., Rossman, A.Y., Sogonov, M.V., White, J.F., 2008. Phylogenetic placement and taxonomic review of the genus *Cryptosporella* and its synonyms *Ophiovalsa* and *Winterella* (Gnomoniaceae, Diaporthales). Mycol. Res. 112, 23−35.

Mejía, L., Rossman, A., Castlebury, L., Yang, Z., White, J., 2011a. *Occultocarpon*, a new monotypic genus of Gnomoniaceae on *Alnus nepalensis* from China. Fungal Divers. 52, 99−105.

Mejía, L.C., Castlebury, L.A., Rossman, A.Y., Sogonov, M.V., White, J.F., 2011b. A systematic account of the genus *Plagiostoma* (Gnomoniaceae, Diaporthales) based on morphology, host-associations, and a four-gene phylogeny. Stud. Mycol. 68, 211−235.

Mejía, L.C., Rossman, A.Y., Castlebury, L.A., White, J.F., Jr., 2011c. New species, phylogeny, host-associations, and geographic distribution of the genus *Cryptosporella* (Gnomoniaceae, Diaporthales). Mycologia 103, 379−399.

Neely, D., Black, W.M., 1976. Anthracnose of black walnuts in the Midwest. Plant Dis. Reporter 60, 519−521.

Prinzing, A., 2001. The niche of higher plants: evidence for phylogenetic conservatism. Proceedings of the Royal Society of London. Series B: Biological Sciences 268, 2383-2389.

Pyron, R.A., Burbrink, F.T., 2009. Can the tropical conservatism hypothesis explain temperate species richness patterns? An inverse latitudinal biodiversity gradient in the New World snake tribe Lampropeltini. Glob. Ecol. Biogeogr. 18, 406−415.

Redlin, S.C., 1991. *Discula destructiva* sp. Nov., Cause of Dogwood Anthracnose. Mycologia 83, 633−642.

Refregier, G., Le Gac, M., Jabbour, F., Widmer, A., Hood, M., Yockteng, R., Shykoff, J., Giraud, T., 2008. Cophylogeny of the anther smut fungi and their Caryophyllaceous hosts: prevalence of host shifts and importance of delimiting parasite species. BMC Evol. Biol. 100, doi:10.1186/1471-2148-1188-1100.

Rice, W.R., 1984. Disruptive selection on habitat preference and the evolution of reproductive isolation: a simulation study. Evolution 38, 1251−1260.

Rice, W.R., 1989. Analyzing tables of statistical tests. Evolution 43, 223−225.

Rossman, A.T., Farr, D.F., Castlebury, L.A., 2007. A review of the phylogeny and biology of the Diaporthales. Mycoscience 48, 135-144.

Smith, S.A., Stephens, P.R., Wiens, J.J., 2005. Replicate patterns of species richness, historical biogeography, and phylogeny in Holarctic treefrogs. Evolution 59, 2433−2450.

Sogonov, M.V., Castlebury, L.A., Rossman, A.Y., White, J.F., 2007. The type species of *Apiognomonia*, *A. veneta*, with its *Discula* anamorph is distinct from *A. errabunda*. Mycol. Res. 111, 693−709.

Sogonov, M.V., Castlebury, L.A., Rossman, A.Y., Mejía, L.C., White, J.F., 2008. Leaf-inhabiting genera of the Gnomoniaceae, Diaporthales. Stud. Mycol. 62, 1−77.

Stebbins, G.L., 1975. Flowering plants: evolution above species level. Cambridge, MA: Belknap Press of Harvard University Press.

Stephens, P.R., Wiens, J.J., 2003. Explaining species richness from continents to communities: the time-for-speciation effect in emydid turtles. Am. Nat. 161, 112−128.

Struwe, L., Haag, S., Heiberg, E., Grant, J.R., 2009. Andean Speciation and Vicariance in Neotropical *Macrocarpaea* (Gentianaceae-Helieae). Ann. Mo. Bot. Gard. 96, 450−469.

Struwe, L., Smouse, P.E., Heiberg, E., Haag, S., Lathrop, R.G., 2011. Spatial evolutionary and ecological vicariance analysis (SEEVA), a novel approach to biogeography and speciation research, with an example from Brazilian Gentianaceae. J. Biogeogr. 38, 1841−1854.

Townsend, J.P., 2007. Profiling Phylogenetic Informativeness. Syst. Biol. 56, 222−231.

Townsend, J.P., Leuenberger, C., 2011. Taxon Sampling and the Optimal Rates of Evolution for Phylogenetic Inference. Syst. Biol. 60, 358−365.

Walker, D.M., Castlebury, L.A., Rossman, A.Y., Sogonov, M.V., White, J.F., 2010. Systematics of genus *Gnomoniopsis* (Gnomoniaceae, Diaporthales) based on a three gene phylogeny, host associations and morphology. Mycologia 102, 1479−1496.

Walker, D.M., Castlebury, L.A., Rossman, A.Y., and White, J.F. 2012. New molecular markers for fungal phylogenetics: Two genes for species-level systematics in the Sordariomycetes (Ascomycota). Mol. Phylogenet. Evol. Accepted, doi: 10.1016/j.ympev.2012.05.005.

Walker, D.M., Castlebury, L.A., Rossman, A.Y., Mejía, L.C., White, J.F. Phylogeny of the genus *Ophiognomonia* (Gnomoniaceae, Diaporthales) based on analyses of three molecular markers, host associations, and morphology. Fungal Divers. In review, manuscript # FUDI-D-12-00071.

Warren, D.L., Glor, R.E., Turelli, M., 2008. Environmental niche equivalency versus conservatism: quantitative approaches to niche evolution. Evolution 62, 2868−2883.

Wiens, J.J., Graham, C.H., 2005. Niche conservatism: integrating evolution, ecology, and conservation biology. Ann. Rev. Ecol. Evol. Syst. 36, 519−539.

Wiens, J.J., Graham, C.H., Moen, D.S., Smith, S.A., Reeder, T.W., 2006. Evolutionary and Ecological Causes of the Latitudinal Diversity Gradient in Hylid Frogs: Treefrog Trees Unearth the Roots of High Tropical Diversity. The University of Chicago Press for The American Society of Naturalists, pp. 579−596.

Wiens, J.J., Ackerly, D.D., Allen, A.P., Anacker, B.L., Buckley, L.B., Cornell, H.V., Damschen, E.I., Jonathan Davies, T., Grytnes, J.-A., Harrison, S.P., Hawkins, B.A., Holt, R.D., McCain, C.M., Stephens, P.R., 2010. Niche conservatism as an emerging principle in ecology and conservation biology. Ecol. Lett. 13, 1310−1324.

Zwickl, D.J., 2006. Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion. Ph.D. dissertation. The University of Texas at Austin, U.S.A.

Donald M. Walker

Rutgers University   *Current Laboratory Location*:
Plant Biology and Pathology   Systematic Mycology and Microbiology
59 Dudley Road, Foran Hall   USDA-ARS
New Brunswick, NJ 08901   10300 Baltimore Avenue, Bldg. 010A
(716) 807-9830   Beltsville, MD 20705
dmwalker.dmw@gmail.com

EDUCATION

Ph.D. candidate **Rutgers University**, New Brunswick, NJ. Plant Biology and
Pathology.
- *Dissertation*: Ecological Vicariance, Host Associations, and Systematics of the Gnomoniaceae (Diaporthales, Fungi).
- *Committee members*: James F. White (chair), Lisa A. Castlebury, Amy Y. Rossman, Lena Struwe, Peter V. Oudemans.
- **2007–present.** GPA 3.9; anticipated filing date: June 7, 2012.

B.S. 2007   **State University of New York**, **College of Environmental Science and Forestry**, Syracuse, NY. Environmental and Forest Biology. GPA 3.6. Magna Cum Laude. Advisor: Alex Weir.

A.S. 2005   **Niagara County Community College**, Sanborn, NY. Environmental Science. GPA 3.7.

PROFESSIONAL EXPERIENCE

2011   **Teaching Assistant**, *Fungal diversity and ecology*. Cranberry Lake Biological Research Station, Cranberry Lake, NY.

2007–present **Graduate Research Assistant**, Rutgers University, New Brunswick, NJ.

2008–2009   **Graduate Teaching Assistant**, *General Biology I & II*. Rutgers University, New Brunswick, NJ.

2007   **Mycology Research Assistant**, *Dematiaceous Hyphomycetes of New York*. State University of New York, College of Environmental Science and Forestry, Syracuse, NY.

2007   **Mycology Research Assistant**, *Systematics of the Laboulbeniales*. State University of New York, College of Environmental Science and Forestry, Syracuse, NY.

2006   **Wetlands Research Leader**, Thousand Islands Biological Station, Clayton, NY.

2005   **Lab Assistant**, *Biogeochemistry*. State University of New York, Syracuse, College of Environmental Science and Forestry, NY.

AWARDS, HONORS AND GRANTS

2011   **Mycological Society of America Registration Award**, $250.

2011	**C. Reed Funk Student Award,** Rutgers University, $500.
2011	**Conference Travel Award**, Rutgers University, $250.
2010	**Mycological Society of America International Travel Award**, $250.
2010	**Mycological Society of America, Everett Lutrell Mentor Student Travel Award**, $250.
2010	**Mycological Society of America Backus Award**, $1000.
2010	**C. Reed Funk Student Award**, Rutgers University, $500.
2009	**Conference Travel Award**, Rutgers University, $250.
2008	**Organization for Tropical Studies Research Fellowship**, $2000.
2008	**C. Reed Funk Student Award**, Rutgers University, $500.
2008	**Graduate Research Prize for Best Student Poster**, Mycological Society of America, $100.
2007	**Presidents High Honors List**, four semesters, SUNY Environmental Science and Forestry.
2007	**Patricia & Jeffery Morrell '77 Distinguished Mycology Student Scholarship**, SUNY Environmental Science and Forestry, $800.
2005	**Transfer Student Scholarship**, SUNY Environmental Science and Forestry.
2003	**Distinguished Student Scholarship**, Niagara County Community College.
2003–5	**Dean's List**, four semesters, Niagara County Community College.

TEACHING

- **Teaching Assistant:** Cranberry Lake Biological Research Station, *Fungal diversity and ecology*, EFB342: 3 credits. Field and lab assistant. Summer 2011.
- **Graduate Teaching Assistant:** Rutgers University, *General Biology I & II,* BIO119:101/102: 4 credits/semester. Lecture and laboratory, lab preparation, lab practical design and proctor, exam proctor. Fall 2008 and Spring 2009.
- **Mycology Research Assistant**: SUNY College of Environmental Science and Forestry, *Dematiaceous Hyphomycetes of New York*. Provided microscopy and sterile technique training to B.S. student. Summer 2007.
- **Mycology Research Assistant**: SUNY College of Environmental Science and Forestry*, Systematics of the Laboulbeniales*, EFB 498: 1 credit. Provided microscopy training to B.S. student. Spring 2007.
- **Wetlands Research Leader:** Thousand Islands Biological Station, EFB 498: 3 credits. Aquatic and wetland vegetation surveys. Summer 2006 and Fall 2006.

STUDENT MENTORING

- **Ryan Vo, University of Maryland**. B.S. student, Cell Biology. Supervised and provided training in molecular biology and sterile technique. 2011–2012.
- **Dr**. **Yuuri Hirooka, University of Maryland.** Post doctoral research associate. Provided training in phylogenetic analyses during the project entitled, "A monograph of *Allantonectria, Nectria*, and *Pleonectria* (*Nectriaceae, Hypocreales, Ascomycota*) and their pycnidial, sporodochial, and synnematous

anamorphs". 2010–2011.

- **Demetra Skaltsas, University of Maryland.** Ph.D. student. Supervised and provided training in molecular biology technique during her thesis research. 2011–2012.
- **General Biology Students, Rutgers University**. B.S. students. **Cory Haluska**: reference for awarded Hamo Hachnasarian Scholarship and placement in Dr. Larry Katz's laboratory at Rutgers University; **Keya Thakkar**: placement in Dr. Tamar Barkay's laboratory at Rutgers University. 2008–2009.
- **Ryan Brown, SUNY College of Environmental Science and Forestry**. B.S. student, Environmental and Forest Biology. Supervised during wetland vegetation survey at the Thousand Islands Biological Station, NY. Summer 2006.
- **Eva Sztechmiler, SUNY College of Environmental Science and Forestry**. B.S. student, Environmental and Forest Biology. Supervised during project entitled, "Bryophyte community ecology of stream beds in the Adirondacks". Summer 2006.

SERVICE AND PROFESSIONAL MEMBERSHIPS

- **Beltsville Academy Science Fair Judge:** Beltsville Academy, MD. Participated as a science fair judge for grades 4–8. January 31, 2012 and February 1, 2012.
- **Member**: Mycological Society of America, Society of Systematic Biologists, American Association of University Professors.
- **Ad hoc reviewer, journals**: Mycologia, Studies in Mycology.
- **Professional meeting contributions:** Organized local arrangements at the Mycological Society of America meetings in Lexington, KY (2010) and Fairbanks, AK (2011), Seminar Chair at Mycological Society of America meeting in Fairbanks, AK (2011).

INVITED PRESENTATIONS

Walker, D.M. 2012. Biodiversity, ecology, and evolutionary biology of the fungal genus *Ophiognomonia.* The University of Findlay, Findlay, OH, April 11, 2012.

Walker, D.M. 2011. Diversity of microfungi in the Gnomoniaceae (Diaporthales). Cranberry Lake Biological Research Station, Cranberry Lake, NY, June 23, 2011.

Walker, D.M. 2011. Microfungi in Our Midst; Diversity in the family Gnomoniaceae. Mycological Association of Washington DC, MD, June 6, 2011.

Walker, D.M. 2011. Profiling phylogenetic informativeness. University of Maryland, College Park, MD, April 13, 2011.

Walker, D.M. 2010. Biodiversity and systematics of ascomycete fungi. Radio Tsukuba 84.2, Tsukuba, Japan, April 8, 2010.

PUBLISHED ABSTRACTS AND CONTRIBUTED PRESENTATIONS

Walker, D.M. 2012. Phylogenetic marker development and systematics of *Ophiognomonia,* with an emphasis on host association and ecological vicariance. Presentation via Skype to Dr. Young-Ki Jo's lab at Texas A&M, March 19, 2012.

Walker, D.M., Struwe, L., Castlebury, L.A., Rossman, A.Y., White, J.F. 2012. Coevolution or host jumping? Host-plant association may influence speciation in *Ophiognomonia* (Gnomoniaceae, Diaporthales). Poster presented at: American Phytopathological Society Potomac Division, Winchester, VA, March 14−16, 2012.

Walker, D.M., Castlebury, L.A., Rossman, A.Y., White, J.F. 2011. Environmental influences on speciation and role of the niche in the genus *Ophiognomonia* (Gnomoniaceae, Diaporthales). Inoculum 62, 47. Oral presentation at: Mycological Society of America meeting, Fairbanks, AK, August 2–5, 2011.

Mejía, L.C., **Walker, D.M.**, Rossman, A.Y., Castlebury, L.A., Sogonov, M.V., White, J.F., Gnomoniaceae of neotropical montane forests: are there more species to be found? VII Latin American Mycology Conference, San Jose, Costa Rica, July 18–21, 2011.

Walker, D.M., Castlebury, L.A., Rossman, A.Y., White, J.F. 2010. Use of three new single copy loci for systematics in the genus *Ophiognomonia* (Gnomoniaceae, Diaporthales). Inoculum 61, 82. Oral presentation at: Mycological Society of America meeting, Lexington, KY, June 28–July 1, 2010.

Walker, D.M., Castlebury, L.A., Rossman, A.Y., White, J.F. 2010. Identification of three new loci to resolve species in *Ophiognomonia* (Gnomoniaceae, Diaporthales). Poster presented at: the 9th International Mycological Congress: The Biology of Fungi, Edinburgh, UK, August 1–6, 2010.

Walker, D.M., Rossman, A.Y., Castlebury, L.A., Mejía, L.C., Sogonov, M.V., White, J.F. 2008. Assessing the monophyly of *Gnomoniopsis* (Gnomoniaceae). A phylogeny based on morphological, cultural, and ribosomal DNA sequences. Inoculum 59, 62. Poster presented at: Mycological Society of America meeting, State College, PA, August 10–13, 2008. Awarded the Graduate Research Prize for Best Student Poster.

PEER REVIEWED PUBLICATIONS

Walker, D.M., Castlebury, L.A., Rossman, A.Y., Mejía, L.C., and White, J.F. Phylogeny of the genus *Ophiognomonia* (Gnomoniaceae, Diaporthales) based on analyses of three molecular markers, host associations, and morphology. Fungal Diversity. In review, manuscript # FUDI-D-12-00071.

Walker, D.M., Castlebury, L.A., Rossman, A.Y., and White, J.F. 2012. New molecular markers for fungal phylogenetics: Two genes for species-level systematics in the Sordariomycetes (Ascomycota). Molecular Phylogenetics and Evolution. Accepted, doi: 10.1016/j.ympev.2012.05.005.

Rossman, A.Y., Melgar, J.C., **Walker, D.M.**, Gonzales, A., Ramirez, T., and Rivera, J.M. 2012. First report of *Dolabra nepheliae* causing stem canker of rambutan and pulasan in Honduras. Plant Disease 96, 765.

Walker, D.M., Castlebury, L.A., Rossman, A.Y., Sogonov, M.V., and White, J.F. 2010. Systematics of genus *Gnomoniopsis* (Gnomoniaceae, Diaporthales) based on a three-gene phylogeny, host associations and morphology. Mycologia 102, 1479–1496.

MANUSCRIPTS IN PREPARATION

Walker, D.M., Struwe, L., Castlebury, L.A., Rossman, A.Y., White, J.F. Host conservatism or specialization? Patterns of fungal diversification are influenced by host specificity in *Ophiognomonia* (Gnomoniaceae, Diaporthales). In preparation for *Evolution.*

Walker, D.M., Struwe, L., Castlebury, L.A., Rossman, A.Y., White, J.F. Environmental influences on speciation and role of the niche in the genus *Ophiognomonia* (Gnomoniaceae, Diaporthales). In preparation for *Journal of Ecology.*

Walker, D.M., Olsen, R., and Rossman, A.Y. First report of *Plagiostoma fraxini* on *Fraxinus mandshurica* from Maryland, USA. In preparation for *Plant Disease.*

Walker, D.M., Tredway, L.P., and Crouch, J.A. Epitypification of *Magnaporthe salvinii*, *Pyricularia grisea*, and the rice blast fungus *Pyricularia oryzae*. In preparation for *Mycologia.*