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Aurantioporthe corni gen. et comb. nov., an endophyte and pathogen of *Cornus alternifolia*

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Abstract: *Cryptodiaporthe corni* is the causal agent of a destructive disease called golden canker, which affects *Cornus alternifolia*, known as the pagoda or alternate-leaved dogwood. Due to the association between *Cr. corni* and pagoda dogwood, we sought to determine whether this fungus was capable of living as an endophyte in pagoda dogwood and causing this disease. Forty asymptomatic stems of plants growing in nature were sampled from five sites across Minnesota. *Cryptodiaporthe corni* was present in more than half (62.5%) of the stems. Asymptomatic nursery material also was sampled, and the fungus was isolated from a small percentage (20%) of them. Inoculations carried out in the field and greenhouse suggested the endophytic isolates of *Cr. corni* were capable of causing disease. Asymptomatic stems of trees in the field inoculated with non-colonized (control) grain seed developed golden canker as frequently as those inoculated with grain seed colonized by *Cr. corni*, suggesting that the fungus was already present in these plants. In greenhouse pathogenicity trials an isolate of *Cr. corni* obtained from an asymptomatic stem was capable of causing golden canker disease, thus demonstrating causality, fulfilling Koch's postulates. The taxonomic placement of *Cr. corni* within Cryphonectriaceae was determined. Phylogenetic analysis of the ITS rDNA and β-tubulin gene regions, along with morphological characteristics, suggested *Cr. corni* is distinct from other genera within this family. Therefore, we propose a new genus, *Aurantioporthe*, as well as the new combination, *A. corni*, to accommodate this species within Cryphonectriaceae.

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Key words: Cryphonectriaceae, Diaporthales, golden canker, pagoda dogwood

Introduction

Pagoda dogwood or alternate-leaved dogwood (*Cornus alternifolia* L.) is native to the eastern half of North America (USDA 2013), and is most frequently found in rich woodlands and swampy thickets growing as a small understory tree, 3.0–7.5 m tall (Cullina 2002). The tree can be found in managed landscapes, with the major limitation to the use of pagoda dogwood in landscaping being the occurrence of golden canker caused by *Cryptodiaporthe corni* (Wehm.) Petrak. *Cornus alternifolia* is considered the only host of this widespread disease, which occurs throughout most of the host's range (Redlin and Rossman 1991). The disease is easily recognized by the small, bright orange pycnidia that appear on the cankered wood as raised protrusions on the surface of the dead bark (Redlin and Rossman 1991). The infection typically moves down the stem until it reaches a branch attachment or node. On some stems the fungus can progress past branch nodes and move into the main stem, killing the entire stem (Beier 2013). Infected plants can persist for years, sending new shoots that result in a cycle of regrowth and dieback.

Cornus alternifolia is currently grown in many states where it is not native, and golden canker disease is widespread and prevalent in many of these locations. For example, *Cr. corni* has been collected in eastern South Dakota, nearly 200 miles from native stands of pagoda dogwood in central Minnesota and Iowa, while landscape plantings infected with this fungus also have been found as far north as Fargo, North Dakota (Redlin and Stack 2001). The common association between *Cr. corni* and *C. alternifolia* led Sinclair and Lyon (2005) to hypothesize that the fungus was likely an endophyte of pagoda dogwood. Therefore, distribution patterns for this pathogen might be the result of the presence of *Cr. corni* within plants transported from nurseries, in addition to more typical means for disease transmission, such as wind dispersal of spores.

Endophytes typically are defined as fungi that live asymptomatically within plants; however, a wide variety of definitions exist for the term (Hyde and Soytong 2008). Whether latent pathogens should be

considered endophytes is still a matter of debate (Wennstrom 1994, Wilson 1995, Mostert et al. 2000, reviewed by Hyde and Soytong 2008, Porras-Alfaro and Bayman 2011). Herein we use the definition of Petrini (1991): ''All organisms inhabiting plant organs that, at some time in their life, can colonize internal plant tissues without causing apparent harm to the host.'' This definition includes fungi, such as *Cr. corni* that may live within plants but function as latent pathogens.

Although currently placed in *Cryptodiaporthe* Petrak within Gnomoniaceae (Petrak 1941), phylogenetic studies suggest *Cr. corni* belongs in Cryphonectriaceae (Castlebury et al. 2002; Gryzenhout et al. 2006, 2010). This placement is also supported by the staining reaction of *Cr. corni*, in which the orange pigmentation of its stromatic tissue turns purple in the presence of KOH (Redlin and Rossman 1991). This reaction occurs in all members of Cryphonectriaceae and has been used to differentiate this family from others in the Diaporthales that do not react in this manner (Gryzenhout et al. 2006). Gryzenhout et al. (2006) further suggested the need for a new generic placement of *Cr. corni* in Cryphonectriaceae due its phylogenetic position. Because Gryzenhout et al. (2006) had access to only two isolates, they recommended study involving more isolates before establishing a new genus to accommodate this species. Subsequent phylogenetic studies also have suggested a distinct position for *Cr. corni* within Cryphonectriaceae (Begoude et al. 2010, Gryzenhout et al. 2010, Chen et al. 2013a, Crane and Burgess 2013); however, formal taxonomic revisions have not been undertaken.

The objectives for this study were to (i) investigate whether *Cr. corni* exists as an endophyte asymptomatically within its host, *Cornus alternifolia*, (ii) verify *Cr. corni* as the cause of golden canker on pagoda dogwood by fulfilling Koch's postulates and (iii) carry out an assessment of the phylogenetic position of *Cr. corni* within Cryphonectriaceae.

MATERIALS AND METHODS

Endophytic assessment.—Plant samples were collected from five sites across Minnesota. Three sites had natural stands of *C. alternifolia*: Itasca State Park (47°11′48.36″N, 95° 10′15.56″W), Great River Bluffs State Park (43°56′16.48″N, 91°24′56.64″W), and William O'Brien State Park (45°13′25.43″N, 92°45′37.85″W). The other two sites had both natural and managed landscapes: University of Minnesota at St Paul (44°59′13.78″N, 93°11′1.81″W) and the Minnesota Landscape Arboretum (44°51′37.99″N, 93°37′5.30″W). All trees were categorized as naturally occurring or transplants at each site. Within each site stems were collected at two different times, first in Apr and later in Jul. Sixteen plants were labeled at each site. For each collection at all locations, four plants with at least one stem showing no symptoms of the disease were randomly selected. An asymptomatic stem from each plant was cut. Ten 3 y old *Cornus alternifolia*, 1.1–1.8 m tall, also were cut at ground level from a production nursery field. The nursery plants had been treated with fungicides approximately every 2 wk during the six mo before collection (SUPPLEMENTARY TABLE I). After stems were cut, all leaves and branches were removed from the main stem (SUPPLEMENTARY FIG. 1). The bottom 8 cm of the stem were discarded, and the remaining stem was cut into 15 cm long segments. The first segment was saved and labeled as segment 1, the next 15 cm segment was discarded, the third was labeled segment 2 etc. until reaching the apical meristem. Segments were sterilized as follows: 70% ethanol (EtOH) for 1 min, 20% bleach solution (5.25% available Cl) for 3 min and 70% EtOH for 30 s. The stem segments then were allowed to air dry in a laminar flow hood. After drying, the 15 cm stem segments were cut around the circumference down to the xylem at 5 and 10 cm to make a ring approximately 3 mm wide. Each ring was removed and cut into five smaller pieces and plated onto acidified 1.5% malt extract agar (MEA) containing 0.20% lactic acid (SUPPLEMENTARY FIG. 1). Samples were placed in a transparent container in the lab and monitored for fungal growth for 1 mo. Fungal colonies growing out of the wood pieces were subcultured and plated onto 1.5% MEA. Once the fungus was isolated it was identified by morphological characteristics including orange stromatic tissue, the lack of aerial mycelium and the characteristic purple staining reaction in the orange stromatic tissue with application of 3% KOH (Redlin and Rossman 1991).

Field inoculations.—Twenty pagoda dogwood plants with asymptomatic branches were randomly selected at the Great River Bluffs State Park in Minnesota. The branches selected were 1.5–2.5 m from the ground. The cultures of *Cr. corni* used for field inoculations were isolated from two different plants exhibiting cankers; one from the Great River Bluffs State Park in Minnesota (MNS1005) and the other from the University of Minnesota at St Paul campus (MNS1007) (TABLE I). For inoculations, isolates were grown on wheat grain for 18 d (Beier 2013). Control grain was made by sterilizing wheat grain and inoculating with a plug of non-colonized 1.5% MEA.

All trees were inoculated with the control grain (mock-inoculated treatment) and either the MNS1005 or the MNS1007 inoculated grain (inoculated treatment) in Mar 2012. Twenty trees were numbered and odd-numbered trees were inoculated with MNS1005 and the even-numbered trees were inoculated with MNS1007. From each tree two branches were selected for treatment by wounding followed by placement of inoculated or control grain. Wounding consisted of cutting off the branch directly below a node (within 1 cm) creating a stub of internodal stem. Branches and tools were surface-sterilized before wounding with a 70% EtOH solution. Three of the inoculated or control grain seeds were placed on each of the wounds. The wound was wrapped with Parafilm to retain moisture and reduce the likelihood of contamination.

TABLE I. Geographical origin of isolates and samples of *Aurantioporthe corni* collected from *Cornus alternifolia* used in this study

Isolate No.[a]	Origin	Collector	GenBank accession No.		Herbarium accession No.	Culture accession No.[b]
			ITS	*TUB*		
CTS1001[cd]	Redding, CT	Kitka, K.	KF495033	KF495063	MIN 936355	CTS1001
DES1001[c]	Greenville, DE	Highland, A.	KF495034	KF495064	NA	DES1001
DES1002[cd]	Newark, DE	Mulrooney, R.	KF495035	KF495065	MIN 936354	DES1002
IAS1001[c]	Ames, IA	Rummery, S.	KF495037	KF495067	NA	IAS1001
IAS1002[c]	Dallas Center, IA	Schmitz, A.	KF495038	KF495068	NA	IAS1002
ILS1001[c]	Chicago, IL	Beier, G.	KF495036	KF495066	NA	ILS1001
MES1001[c]	Boothbay, ME	Cullina, W.	KF495039	KF495069	NA	MES1001
MIS1001[c]	Ontonagon, MI	Beier, G.	KF495040	KF495070	NA	MIS1001
MNS1001[c]	Carlos Avery WMA, MN	Beier, G.	KF495041	KF495071	NA	MNS1001
MNS1002[c]	Great River Bluffs State Park, MN	Beier, G.	KF495042	KF495072	NA	MNS1002
MNA1003[ce]	William O' Brien State Park, MN	Beier, G.	KF495043	KF495073	NA	MNA1003
MNS1005[e]	Great River Bluffs State Park, MN	Beier, G.	NA	NA	NA	MNS1005
MNS1007[e]	University of Minnesota, St. Paul, MN	Beier, G.	NA	NA	NA	MNS1007
MNS1008[e]	Pillsbury State Forest, MN	Beier, G.	NA	NA	NA	MNS1008
MNA1009[e]	Minnesota Landscape Arboretum, MN	Beier, G.	NA	NA	NA	MNA1009
MNS1010[d]	Frontenac State Park, MN	Beier, G.	NA	NA	MIN 936359	NA
MNS1011[d]	Interstate State Park, MN	Beier, G.	NA	NA	MIN 936356	NA
MNS1012[d]	Minneapolis, MN	Beier, G.	NA	NA	MIN 936361	NA
MNS1013[d]	Minnesota Landscape Arboretum, MN	Beier, G.	NA	NA	MIN 936360	NA
MNS1014[d]	Whitewater State Park, MN	Beier, G.	NA	NA	MIN 936357	NA
MNS1015[d]	William O'Brien State Park, MN	Beier, G.	NA	NA	MIN 936358	NA
NCS1001[c]	Ashville, NC	Blue, L.	KF495044	KF495074	NA	NCS1001
PAS1001[c]	Philadelphia, PA	Beerley, T.	KF495045	KF495075	NA	PAS1001
SDS1001[c]	Freeman, SD	Beier, G.	KF495046	KF495076	NA	SDS1001
WIS1001[c]	Burlington, WI	Beier, G.	KF495047	KF495077	NA	WIS1001

[a] The first two letters in the isolate number are abbreviations for the state collected from and the third letter represents whether the host plant was symptomatic or asymptomatic (S = symptomatic, A = asymptomatic).

[b] Forest Pathology Culture Collection, University of Minnesota, St Paul, Minnesota.

[c] Isolates used for phylogenetic analysis.

[d] Samples used for morphological study.

[e] Isolates used for pathogenicity study.

Branches were collected and observed 3 mo post inoculation (MPI). Observations were taken on lesion length, percent internode necrosis measured vertically from the wound to the first node down from the wound, lesion advance past the nearest node and presence of pycnidia. Pycnidia were confirmed to be *Cr. corni* by microscopic observations and use of KOH as described above. Branch segments for each treatment were sampled to determine if the fungus was present. Bark segments were taken from areas exhibiting pycnidia, and the segments were taken from necrotic regions. Segments were plated onto acidified 1.5% MEA with 0.20% lactic acid. The fungus was subcultured and identified as described above.

Greenhouse inoculations.—Thirty 30–45 cm tall bareroot *Cornus alternifolia,* 1 y old liners, were grown in size 3 containers with Sun Gro's Metro-Mix 950 (Sun Gro, Agawam, Massachusetts) media. Plants were grown under natural light conditions for 9 mo in a greenhouse at the University of Minnesota at St Paul and were watered weekly. Five isolates (TABLE I) and a mock-inoculated control were used. For each of the five different isolates and the mock-inoculated treatment five plants were used. The inoculated and control grain seed were prepared as described above.

For inoculations, a wound was made by cutting off the apical portion of the main stem, approximately 46 cm above the soil. The area to be inoculated was surface-sterilized with a paper towel soaked in 95% EtOH. A hand pruner was used to cut just below the node that was closest to 46 cm resulting in a stub of internodal stem. Three pieces of grain were placed onto the end of the cut stem and held in place with Parafilm. After inoculation the plants were randomly arranged in a cooler maintained at 10 C with no light. After 1 mo the plants were taken out of the cooler and

placed in a random arrangement in the greenhouse. Plants were watered when a majority of the plants showed slight wilting in their leaves.

Observations and measurements were made at seven MPI for percent internode necrosis measured vertically from the wound to the first node down from the wound, presence of pycnidia and lesion progression beyond the node immediately below the wound. All plants were sampled to determine whether the fungus was present. Cuts were made 15 cm below the initial cut, and the segments were surface-sterilized as described above. Bark rings were made 1.25 cm above and below the advancing front of the lesion, and the fungus was isolated and identified as described above.

Analysis.—All categorical data were analyzed using a 2 × 2 contingency table. Graph Pad Software (Graph Pad Software Inc., La Jolla, California) was used to perform Fisher's exact test on all contingency tables. Continuous data was analyzed using a Student's *t*-test in R 2.9.2 (R Development Core Team, Vienna, Austria). A chi-squared test was used to analyze the difference between the presence of fungi on the top half of the plant versus the bottom half, and a linear mixed model (by REML) was used to examine relationships between stem diameter and the increased probability of finding the fungus.

Isolates and DNA extraction.—Samples of *Cr. corni* were obtained from across the eastern United States and have been deposited in the Forest Pathology Culture Collection, University of Minnesota (TABLE I). Isolates were obtained from samples of *Cornus alternifolia* with visible pycnidia of *Cr. corni* except for isolate MNA1003, which was taken from an asymptomatic stem. Bark segments from the advancing canker or pycnidia on the surface of the canker were plated onto Petri dishes containing acidified 1.5% MEA containing 0.20% lactic acid. The fungus was subcultured to 1.5% MEA containing 0.05% yeast extract to promote the growth of aerial mycelium. A cetyl trimethylammonium bromide (CTAB) procedure was used to extract DNA from each isolate as described in Beier (2013).

PCR and sequencing.—Sequences of the nuc rDNA ITS1-5.8S-ITS2 regions (ITS) and exons 6 and 7 of the β-tubulin gene (*TUB*) were examined in the phylogenetic analysis. The ITS region was amplified with the ITS1/ITS4 primer pair (White et al. 1990). The Bt1a and Bt1b primers were used for *TUB* (Glass and Donaldson 1995). PCR were carried out in a PTC-200 thermal-cycler (MJ Research, Watertown, Massachusetts). The amplification reaction mixtures were based on that of Blanchette et al. (2010). The PCR protocol followed that of Arenz and Blanchette (2009) for amplification of the ITS gene region. For *TUB* the following protocol was used: 94 C for 1 min, 30 cycles of 94 C for 1 min, 60 C for 1 min, and 72 C for 1 min. For sequencing 4.9 µL water, 0.75 µL PCR product and 0.42 µL 10 µM primer was used. Samples were sent for sequencing to the University of Minnesota Genomics Center. Forward and reverse sequencing for all amplified products was done with an ABI 3730xl DNA sequencer (Applied Biosystems, Foster City, California). Consensus sequences for individual contigs were assembled and edited with ChromasPro 1.42 (Technelysium Ltd., Helensvale, Australia). Sequences were aligned with ClustalW in MEGA 5.05 (Tamura et al. 2011). The alignments were manually assessed, and ambiguous regions were excluded. Alignments contained sequences from 65 isolates: 16 *Cr. corni*, 47 Cryphonectriaceae and two outgroups (*Diaporthe ambigua*). For each genus in Cryphonectriaceae two sequences from the type species were used. Also, a single representative for each taxon in Cryphonectriaceae with both ITS and *TUB* sequences available in GenBank were included. Alignments are available at TreeBASE (http://purl.org/phylo/treebase/phylows/study/TB2:S15115?x-access-code=fc40370b5a9a01359a8e3d2a0d45b5ea&format=html).

Phylogenetic analysis.—Maximum likelihood (ML) and Bayesian (BI) analyses were performed. To determine whether the gene regions could be combined for analysis, a model comparison was carried out with Bayes factors in MrBayes 3.2.1 (Ronquist et al. 2012). To determine the appropriate model, jModelTest 2.1.4 (Darriba et al. 2012, Guindon and Gascuel 2003) was used. For both the ITS and *TUB* regions the selected models were not implemented in MrBayes 3.2.1, therefore the next most complex model (GTR + I + G) was used. Four chains were run for 10 000 000 Markov chain Monte Carlo (MCMC) generations with a sampling frequency every 1000 generations. The first 25% of sampled trees were eliminated as burn in. To calculate Bayes factors Tracer 1.5 (Drummond and Rambaut 2007) was used. Maximum likelihood analysis was conducted with GARLI (genetic algorithm for rapid likelihood inference; Zwickl 2006) and grid computing (Cummings and Huskamp 2005) through the Lattice Project (Bazinet and Cummings 2008). A fast ML stepwise-addition algorithm was used to generate a starting tree. To assess the confidence levels of branches, 1000 bootstrap replicates were used. MrBayes 3.2.1 was used for BI. The same parameters as described above were used. A consensus tree was generated from two individual runs for both gene regions.

Morphology.—Morphological characteristics were examined on eight canker samples containing both sexual and asexual fruiting structures. Samples were collected from six sites in Minnesota, one site in Connecticut and one site in Delaware (TABLE I). Asci, ascospores and conidia were examined under light microscopy at 1000× by preparing squash mounts and staining with 0.25% cotton blue. Twenty measurements were made for asci and spore types for each sample. Perithecia and pycnidia were placed in 3% KOH to determine color change. Microscopic sections were made to obtain measurements for perithecia, pycnidia and conidiophores. Sections were made by infiltrating bark samples with 25% tissue freezing medium (Electron Microscopy Sciences, Hatfield, Pennsylvania). Sections, 10–20 µm, were cut with a freezing microtome at −20 C. Sections were stained with 0.25% cotton blue and measured at 400× for fruiting bodies and 1000× for conidiophores. For each tissue sample 10 measurements were taken on each type of fruiting body and for conidiophores. Color descriptions follow the system of Rayner (1970). Microscopic measurements were made with a Nikon Labophot (Nikon Instruments Inc., Melville, New York). All images were taken with

a Nikon Digital Camera DXM1200F (Nikon Instruments Inc., Melville, New York).

RESULTS

Endophytic assessment.—Plants at field sites were categorized as either native (naturally occurring) or landscaped (planted). A higher percentage of the native plants (68%) were found to have *Cr. corni* compared with the landscape plants (44.5%), but the difference was not statistically significant ($P = 0.26$). *Cryptodiaporthe corni* was isolated from asymptomatic stems at Minnesota sites, with the April collection having a slightly higher rate of occurrence (65% on average) than that of July (60% on average), although it was not found to be statistically significant ($P = 1$). There was considerable variation in the number of plants with the fungus present at the different sites, but when April and July collections were combined the difference between locations was not found to be statistically significant ($P = 0.13$). The percentage of segments within a plant with the fungus present varied widely 0–100% with a mean of 33%.

A pattern was observed within individual stems, where a majority of the plants had the fungus present in more segments from the bottom half of the stem than the top. For stems with the fungus present, 52.5% of the plants had more segments containing *Cr. corni* in the bottom half of the plant compared with the top and only 5% had more segments containing *Cr. corni* found in the top half of the plant compared with the bottom ($P < 0.001$). The remaining plants with the fungus present had equal amounts of infection in the bottom and top halves. Diameters for the 15 cm segments were 2.7–21.0 mm. Statistical analysis under a linear mixed model (by REML) was used to determine the potential relationship between segment diameter and presence or absence of the fungus. It was found that within a tree the probability of finding the fungus increased 1.15 times for each unit increase (mm) in segment diameter.

Cryptodiaporthe corni was obtained from two of the trees (20%) from the asymptomatic nursery plants. When the fungus was isolated, it was found near the base of the plant. In the two plants where the fungus was found it was located in the lowest and second lowest 15 cm segment in one plant and the lowest 15 cm segment for the other.

Field inoculations.—No statistically significant differences existed between the mock-inoculated and inoculated treatments for any of the observations or measurements. When the two isolates (MNS1005, MNS1007) were compared there were also no statistical differences between any of the observations or measurements. The mock-inoculated treatment had a slightly higher percentage of branches with pycnidia present compared with the inoculated treatment, 94% compared to 83%, respectively, ($P > 0.05$). Also the mock-inoculated treatment had a higher average percentage of internode necrosis than the inoculated treatment, 97% and 83%, respectively ($P > 0.05$). Overall the fungus was isolated from 89% of samples from both the mock-inoculated and inoculated treatments. Two of the branch samples died due to golden canker so they were not included in analysis because the differences between treatments could not be assessed.

Greenhouse inoculations.—Differences were found between the mock-inoculated and inoculated groups. Only 20% of the stems in the mock-inoculated group were completely dead from the wound to the first node down, while 96% of the inoculated group was dead to the node ($P < 0.05$). Also the inoculated group had a higher proportion of lesions, which had advanced past the first node compared with the mock-inoculated group (16% versus 0%, respectively), but the difference was not found to be significant ($P > 0.05$). None of the mock-inoculated plants had any pycnidia present, and we were not able to reisolate the fungus from these plants, while in the inoculated group, 80% of plants treated had pycnidia present and 60% had *Cr. corni* reisolated from them ($P < 0.05$).

Variability existed in observations and measurements among the five isolates used in the experiment. By seven MPI all inoculation sources resulted in $\geq 80\%$ of plants being dead to the node ($P = 1.0$). The greatest variation among the isolates was for the presence/absence of pycnidia on the plant tissue. At seven MPI, 20% of plants inoculated with MNA1003 (asymptomatic isolate source) had pycnidia, which was significantly different than three of the other isolates, which resulted in 100% of the plants with pycnidia ($P < 0.05$). However, the fungus was isolated from above the advancing front of the lesion in 60% of the plants treated with MNA1003, which was equal to the average of the other four isolates.

Phylogenetic analyses.—The model comparison using Bayes factors strongly suggested that the gene regions were not congruent (log10 Bayes factor = 24.144); therefore they were not combined for analysis. For the ITS gene region alignment there were 550 characters, including 332 constant characters, 199 parsimony-informative characters and 19 uninformative variable characters. For the *TUB* alignment there were 464 characters: 283 constant characters, 171 parsimony-informative characters and 10 uninforma-

tive variable characters. The best trees from the maximum likelihood analyses of ITS and *TUB* are presented (FIGS. 1, 2, respectively).

Results from the phylogenetic analysis suggested that regardless of which gene region was used in the analysis *Cr. corni* forms a monophyletic clade, distinct from clades representing other genera within Cryphonectriaceae. These results were strongly supported in both the ML and BI analyses. The confidence level and posterior probability at the node separating *Cr. corni* from other genera was 95% and 1.0, respectively, in the ITS tree (FIG. 1) and 92% and 1.0, respectively, in the *TUB* tree (FIG. 2).

TAXONOMY

Aurantioporthe G. Beier & Blanchette, gen. nov.
MycoBank MB807278

Type species: Aurantioporthe corni (Wehm.) G. Beier & Blanchette

Etymology: Latin, *aurantiacus*, orange, referring to the color of the pycnidia covering the bark of infected plants, and Greek, *porthe*, destroyer, describing the destructive capabilities of the fungus.

Sexual morph: Ascigerous stromata immersed to semi-immersed, orange, upper region eustromatic to pseudostromatic, lower region pseudostromatic. Perithecia singular or in groups of 2–4, valsoid to diatrypoid, surrounded by host tissue, globose to subglobose, fuscous black, mature ostiolar necks emerge at stomatal surface having black clypei. Asci fusiform to ellipsoidal to clavate, containing eight ascospores, refractile ring present. Ascospores hyaline, fusiform to ellipsoidal to pyriform, with a median to sub-median septum, occasionally with additional septa.

Asexual morph: Conidiomata separate from ascigerous stromata, orange, immersed to semi-immersed, irregularly subspherical to flattened, unilocular, single ostiole, cells near ostiole pseudoparenchymatous. Conidiophores obclavate, narrowing to a point, branched, conidiogenous cells phialidic. Conidia minute, hyaline, fusiform to ellipsoidal, aseptate, exuded as orange droplets.

Habitat: On/in *Cornus alternifolia* bark and phloem.

Distribution: USA, Canada

Notes: On the basis of morphological differences and the phylogenetic analysis presented herein, the genus *Aurantioporthe* is established to accommodate *A. corni*, the only known species in the genus. The morphological features that distinguish this genus from other genera in the family include a combination of single to multiple septate ascospores and ascigerous stromata and conidiomata immersed to semi-immersed in the bark.

Aurantioporthe corni (Wehm.) G. Beier & Blanchette, comb. nov. FIG. 3a–f
MycoBank MB807279

Basionym: Apioporthe corni Wehm., Univ. Mich. Stud. Sci. Ser. 9: 220. 1933, nom. cons. prop.

≡ *Cryptodiaporthe corni* (Wehm.) Petrak, Ann. Mycol. 39:302. 1941.

≡ *Hapalocystis corni* (Wehm.) M.E. Barr, Mycotaxon 10:214. 1979.

= *Myxosporium nitidum* Berk. and Curtis in Berk., Grevillea 3:13. 1874, nom. rej. prop.

≡ *Phoma nitidum* (Berk. and Curtis) Höhn., Z. Gärungsphysiol. 5:208. 1915.

= *Sphaeronema aurantiacum* Peck, Rep. N.Y. St. Mus. Bot. 30:51. 1878, nom. rej. prop.

≡ *Zythia aurantiaca* (Peck) Sacc., Syll. Fung. 3:614. 1884.

≡ *Phoma aurantiacum* (Peck) Höhn., Z. Gärungsphysiol. 5:208. 1915.

Sexual morph: Ascigerous stromata immersed to semi-immersed, orange, upper region eustromatic to pseudostromatic, lower region pseudostromatic. Perithecia singular or in groups of 2–4, valsoid to diatrypoid, surrounded by host tissue, globose to subglobose, 128–288 high × 200–348 µm wide (mean = 235 × 277; SD 28, 37; n = 70), beak 88–348 high × 88–200 µm wide at base (mean = 217 × 127; SD 51, 25; n = 70), 28–200 µm wide at beak opening (mean = 101; SD 34; n = 68), fuscous black, mature ostiolar necks emerge at stomatal surface having black clypei, clypeus 95–240 µm wide (mean = 166; SD 33; n = 58). Asci fusiform to ellipsoidal to clavate, with eight ascospores, refractile ring present, 38–67 × 6–13 µm (mean = 54 × 8; SD 6, 1; n = 160), 6–14 µm wide at widest point (mean = 9; SD 2; n = 160). Ascospores 10–18 µm (mean = 13; SD 2; n = 160) hyaline, fusiform to ellipsoidal to pyriform, with a median to sub-median, slightly constricted septum, occasionally with additional septa, upper cell 5–10 × 3–7 µm (mean = 7 × 5; SD 1, 0.8; n = 160), lower cell 3–9 × 3–5 µm (mean = 6 × 4, SD 1, 0.7; n = 160).

Asexual morph: Conidiomata separate from ascigerous stromata, orange, immersed to semi-immersed, irregularly subspherical to flattened, unilocular, single ostiole, cells near ostiole pseudoparenchymatous, 143–385 high × 180–510 µm wide at base (mean = 241 × 282; SD 48, 62; n = 80), neck 58–183 high × 80–265 µm wide at widest point (mean = 113 × 155; SD 28, 33; n = 80), 28–223 µm wide at neck opening (mean = 114; SD 45; n = 80). Conidiophores obclavate narrowing to a point, branched, conidiogenous cells phialidic, 10–15 × 1.5–3.0 µm (mean = 12 × 2; SD 1.2, 0.4; n = 80). Conidia minute, hyaline, fusiform to ellipsoidal, aseptate, exuded as orange droplets, 6–10 × 2–4 µm (mean = 7 × 3; SD 1, 0.3; n = 160).

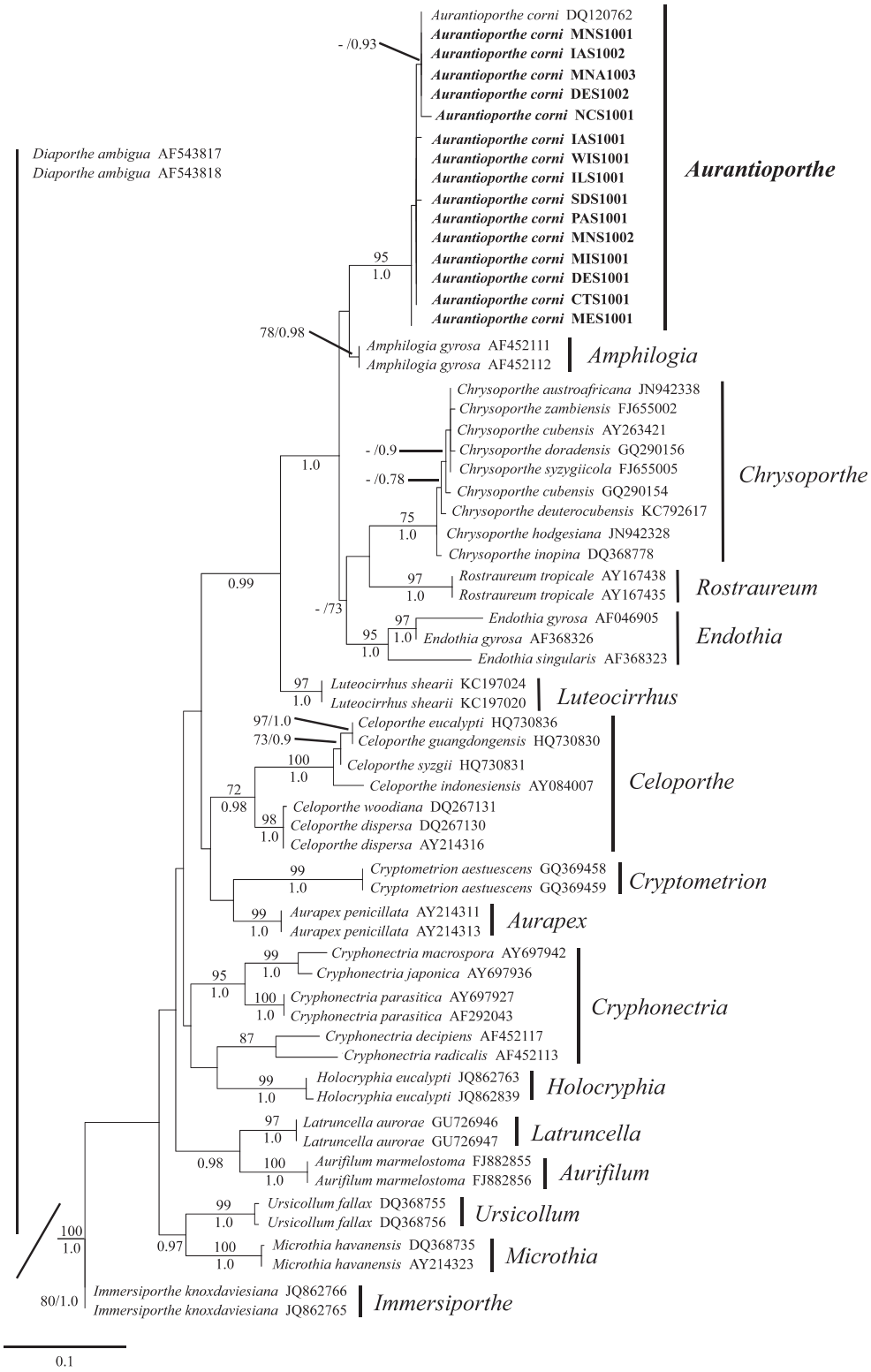

FIG. 1. Best tree from the maximum likelihood phylogenetic analysis of ITS sequences, which shows the relationship between *Aurantioporthe corni* (≡ *Cryptodiaporthe corni*) and other members of Cryphonectriaceae. Included in the analysis are 16 *A. corni* isolates, 47 isolates of other Cryphonectriaceae and two isolates serving as outgroup (*Diaporthe ambigua*). ML bootstrap values ≥ 70% (1000 replicates) are displayed above each branch node, and Bayesian posterior probabilities ≥ 0.70 are displayed below each branch node. Isolates of *A. corni* in boldface were sequenced. The newly proposed genus, *Aurantioporthe*, is in boldface. Sequences obtained from GenBank are followed by their accession numbers.

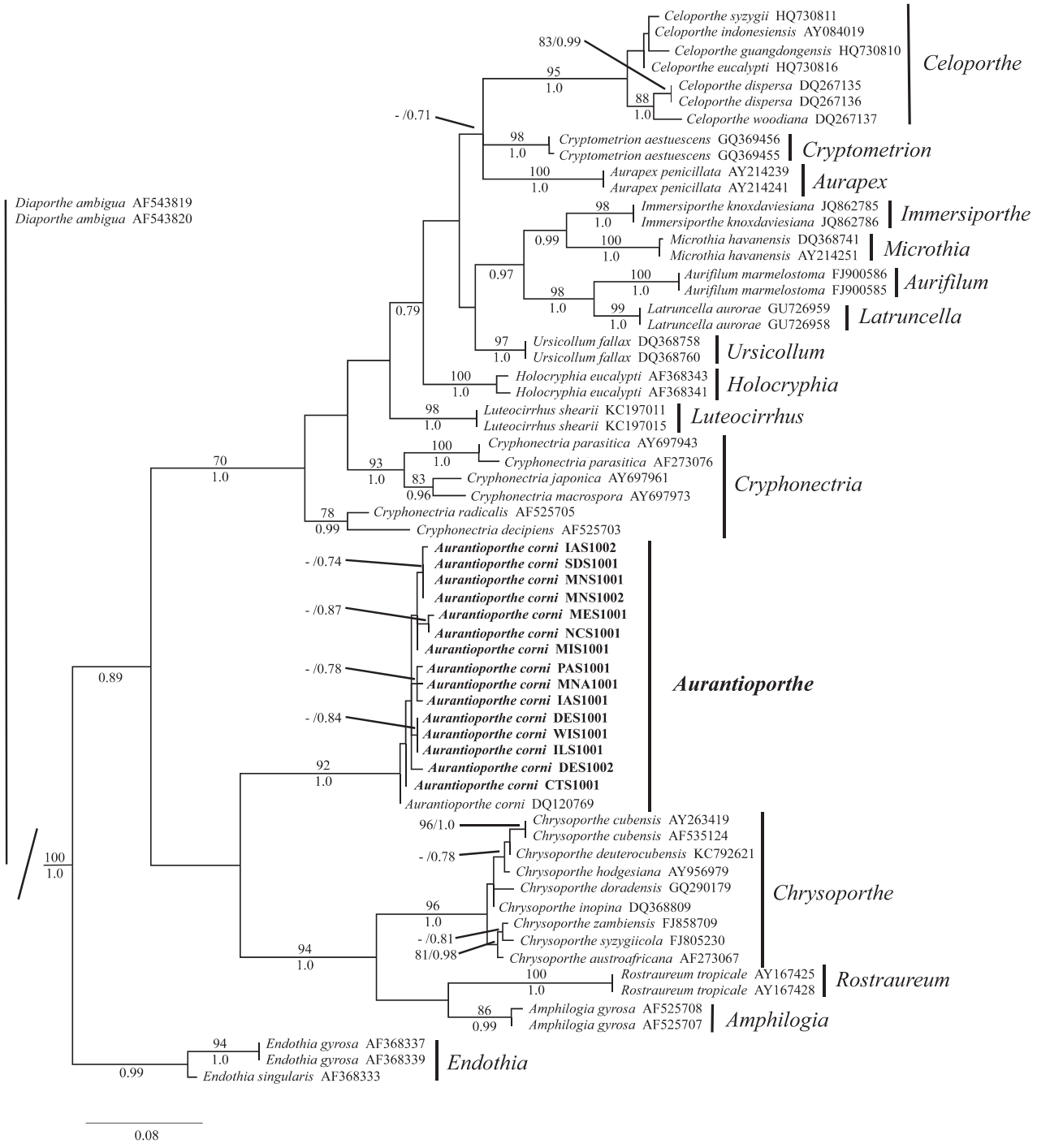

FIG. 2. Best tree from the maximum likelihood phylogenetic analysis of *TUB* sequences, which shows the relationship between *Aurantioporthe corni* (≡ *Cryptodiaporthe corni*) and other members of Cryphonectriaceae. Included in the analysis are 16 *A. corni* isolates, 47 isolates of other Cryphonectriaceae and two isolates serving as outgroup (*Diaporthe ambigua*). ML bootstrap values ≥ 70% (1000 replicates) are displayed above each branch node, and Bayesian posterior probabilities ≥ 0.70 are displayed below each branch node. Isolates of *A. corni* in boldface were sequenced. The newly proposed genus, *Aurantioporthe*, is in boldface. Sequences obtained from GenBank are followed by their accession numbers.

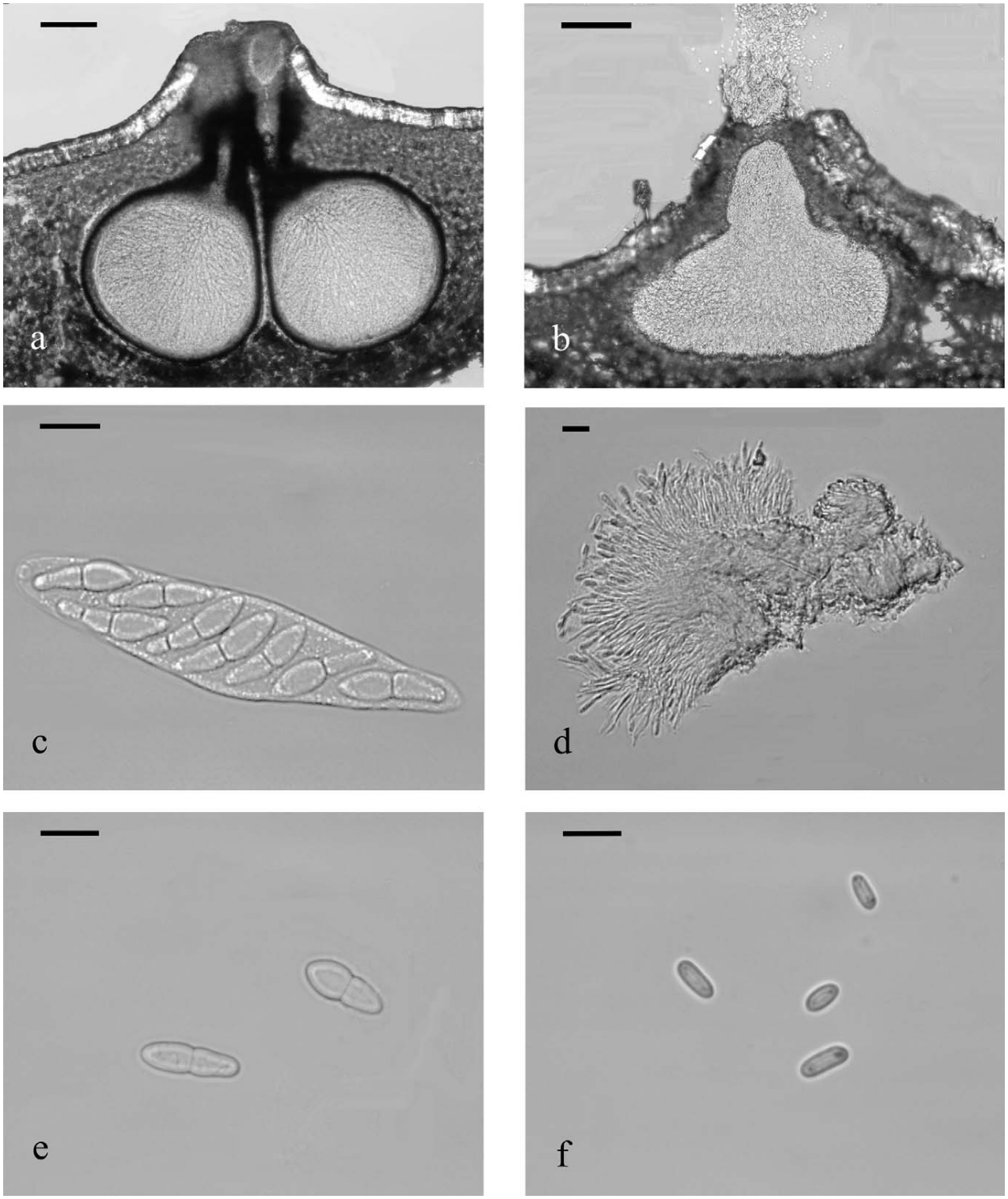

FIG. 3. Micrographs of the sexual morph (a, c, e) and asexual morph (b, d, f) of *Aurantioporthe corni* on *Cornus alternifolia*. a. Longitudinal section through the ascigerous stroma. b. Longitudinal section through the conidioma. c. Asci. d. Conidiophores. e. Ascospores. f. Conidia. Scale bars: a, b = 100 μm; c–f = 10 μm.

Habitat: On/in *Cornus alternifolia* bark and phloem.

Distribution: USA, Canada

Specimens examined (all Aurantioporthe corni were collected from Cornus alternifolia): CANADA. ONTARIO: London, *J. Dearness*, (LECTOTYPE of *Apioporthe corni* NY), middle left packet on sheet, designated by Redlin and Rossman (1991); London, on dead limbs of *Cornus*, *Ellis and Everhart*, North American Fungi 2820 *Diaporthe albocarnis*, May 1891, *J. Dearness* (NY 00921764); London, Apr–Jun 1892, *J. Dearness*, (PARATYPE of *Apioporthe corni* NY), top right packet on sheet; London, Aug 1895, *J. Dearness*, (PARATYPE of *Apioporthe corni* NY), bottom right packet on sheet; London, May 1891, *J. Dearness* (ISOTYPE of *Diaporthe albocarnis* Ellis and Everh. NY 00921764); London, on bark of *Cornus alternifolia*, *E. Bartholomew*, Fungi Columbiani 3809 *Diaporthe corni*, Apr, May 1912, *J. Dearness* (NY). USA. CONNECTICUT: Redding, Highstead Arboretum, 13 Aug 2010, *K. Kitka*, (MIN 936355); DELAWARE: Newark, University of Delaware, 10 Jun 2011, *R. Mulrooney* (MIN 936354); MASSACHUSETTS: Sunderland, Mount Toby, 6 Jul 1967, *H.E. Bigelow, M.E. Bigelow and J. Grow* (NY fun. coll.); MINNESOTA: Chaska Landscape Arboretum, 24 May 2013, *G. Beier* (MIN 936360); Chisago County, Interstate State Park, 21 May 2013, *G. Beier* (MIN 936356); Goodhue County, Frontenac State Park, 26 May 2013, *G. Beier* (MIN 936359); Hennepin County, 11 Apr 1927, *H. Johnson* (MIN 431094); Hennepin County, Minneapolis, Minnehaha Park, 28 May 2013, *G. Beier* (MIN 936361); Washington County, William O'Brien State Park, 21 May 2013, *G. Beier* (MIN 936358); Winona County, Whitewater State Park, 18 May 2013, *G. Beier* (MIN 936357); NEW YORK: Ithaca, 1918, *G.H. Smith* (NY fun coll.); Mclean, 14 May 1923 (NY); Syracuse, on twigs of *Cornus*, Underwood and Cooke, *Illustrative Fungi 73 Myxosporium nitidum*, Apr 1889 (NY); PENNSYLVANIA: West Chester, on dead branches of *Cornus*, *Ellis*, North American Fungi 351 *Myxosporium nitidum*, *Haines, Everhart, and Wood* (NY).

Additional specimens examined: Diaporthe corni: GERMANY. Parc de Reichartshausen, on the dead branches of *Cornus alba*, *Fuckel* (NY). *Massarina albocarnis*: CANADA. ONTARIO: London, on *Ostrya*, 1877 (PARATYPE NY 00921768 as *Diaporthe albocarnis*); ONTARIO: London, on dead *Ostrya virginica*, Jun 1892 (PARATYPE NY 00921767 as *Diaporthe albocarnis*); USA. NEW YORK: Mclean, on *Cornus stolonifera*, 14 May 1923 (NY as *Diaporthe albocarnis*).

Notes: Wehmeyer (1933) described *Apioporthe corni* Wehm., stating in the protolog, ''Specimen typicum in Herb. New York Bot. Gard. Legit Dearness, prope London, Ontario.'' A lectotype for *Cr. corni* (Wehm.) Petrak was designated by Redlin and Rossman (1991) among three packets affixed to an herbarium sheet that matched Wehmeyer's description but labeled *Diaporthe corni* Fuckel. The protolog for *Apioporthe corni* (Wehmeyer 1933) also cites authentic material representing this taxon among the exsicatti *Fungi Columbiani* (Barthol. *Diaporthe corni* No. 3809). Specimens of Fungi Columbiani No. 3809 that we examined from the New York Botanical Garden (NY Fun. Col. 3809) were *Aurantioporthe corni* and exhibited septate ascospores. This material is distinct from *D. corni* (Fuckel 1870), which the protolog suggests is a different fungus with aseptate ascospores and explains Wehmeyer's (1933) mistaken inclusion of this taxon in the synonym of *Apioporthe corni*.

Wehmeyer (1933) also cites a specimen of *Diaporthe albocarnis* Ellis and Everh. as authentic material representing *Apioporthe corni* Wehm. Our examination of type collections on *Cornus* among *North American Fungi* (Ellis and Everh. No. 2820; NY 00921764), however, proved to be mixed collections that included *Aurantioporthe corni*, a fact that Redlin and Rossman (1991) also recognized. After examining the type material of *D. albocarnis* on *Ostrya* (NY 00921767, NY 00921768), it was confirmed not to be *Aurantioporthe corni*. Barr (1992) considers *Massarina albocarnis* (Ellis and Everh) M.E. Barr as the current name for *D. albocarni*.

Redlin and Rossman (1991) cited an authentic specimen of *Cr. corni* from Maine that was sequenced by Castlebury et al. (2002) to resolve the phylogenetic placement of taxa within the Diaporthales. That sequence (GenBank AF408343, as ''BPI 747916''), which has been included in the phylogenetic analysis here, actually corresponds to BPI 1107157 (Castlebury pers comm).

An asexual morph of *Cryptodiaporthe corni* (Wehm.) Petrak has long been recognized in *Myxosporium nitidum* Berk. and Curtis (Berkeley 1874). Peck (1878) described *Sphaeronema aurantiacum* (≡ *Zythia aurantiaca* [Peck] Sacc. Syll. Fung. 3:614, 1884), a heterotypic synonym of *M. nitidum* (Redlin and Rossman 1991). Each of the anamorph-typified species names cited here reference the distinct orange coloration of the conidiomata and list *Cornus alternifolia* L. as the host. Under Article 59 of the International Code of Nomenclature for Algae, Fungi and Plants (Melbourne Code) pleomorphic fungi now must have only one name, and each of the legitimate names cited above (both sexual and asexual morphs) now compete for priority, leaving *Myxosporium nitidum* as the earliest name (McNeill et al. 2012). Article 57.2 of the Melbourne Code, however, recommends that anamorph-typified species names should not displace the teleomorph-typified name in cases where these names were widely used. Considering that the teleomorph-typified name has been used commonly in recent studies, especially when referring to ''golden canker'' disease, and the specific epithet ''corni'' refers specifically to the host, *Cornus* L., on which the disease occurs, we propose the conservation of *Apioporthe corni* Wehm. following the provisions under Article 57.2 of the Melbourne Code. Likewise, and to avoid undue confusion under

the new ''one fungus, one name'' nomenclatural rules, the anamorph-typified species names, *Myxosporium nitidum* Berk. And Curtis and *Sphaeronema aurantiacum* Peck, are proposed to be rejected. Thus, *Aurantioporthe corni* (Wehm.) G. Beier & Blanchette comb. nov. should be used for both asexual and sexual states of the fungus described here.

DISCUSSION

This study has shown that *Aurantioporthe corni* is capable of living as an endophyte in *Cornus alternifolia*, in that this fungus was found in a number of asymptomatic stems of *C. alternifolia* throughout Minnesota. The fungus was found not only in plants from native stands but also those in managed landscapes as well as field-grown nursery stock, although with less prevalence. This is the first study to report *A. corni* growing asymptomatically as an endophyte in *C. alternifolia*, as hypothesized by Sinclair and Lyon (2005). Our study demonstrated that golden canker developed after mock-inoculating asymptomatic branches in the field, suggesting the endophytic form of *A. corni* was already present within these plants and capable of causing disease. This was supported further by the greenhouse inoculation experiment, where *A. corni* isolated from asymptomatic plants was used as an inoculum source that caused the characteristic signs of golden canker in other plants. Other studies also have examined whether endophytes, which can become pathogenic, are capable of causing disease in newly inoculated plant material if they are isolated from asymptomatic plant material. Dakin et al. (2010) isolated *Neofusicoccum australe* (Slippers, Crous and M.J. Wingfield) Crous, Slippers and A.J.L. Phillips from asymptomatic *Agonis flexuosa* (Wild.) Sweet and used it in a pathogenicity trial and found no statistically significant difference between lesions on *A. flexuosa* when isolates collected from asymptomatic trees or diseased trees were used.

Aurantioporthe corni was present in asymptomatic stems of *C. alternifolia* in managed landscapes at a relatively high incidence (44.5%) and all plants studied in the managed landscapes had golden canker present on at least one of the multiple stems. Because only a small portion of the nursery material studied had the fungus present in asymptomatic tissue (20%), it appears that nursery plants are infected after being placed in the landscape, either from spores of infected plants growing in the area or by those already present on the plant in the nursery. The nursery material examined had been treated with a frequent fungicide regimen (SUPPLEMENTARY TABLE I). However, if a less stringent fungicide regimen had been used on the nursery material, the percentage of plants infected with the fungus might have been higher. Future opportunities exist in investigating whether preventative application of fungicides reduces colonization by *A. corni* in asymptomatic nursery stock. However, fungicides are not always effective at controlling endophytic fungi that can become pathogenic. Hartman et al. (2009) performed a study on *Sphaeropsis sapinea* (Fr.) Dyko and B. Sutton (now called *Diplodea pinea*) growing in *Pinus nigra* J.F.Arnold, Austrian pine, and found no statistically significant differences in the recovery of *D. pinea* in symptomless shoots after injection with debacarb, tebuconazole, oxycarboxin or water. The fact that the disease has been detected on *C. alternifolia* in landscape settings in North Dakota (Redlin and Stack 2001) and South Dakota, with no apparent susceptible hosts nearby, suggests that infected asymptomatic nursery material could account for the infections, although long distance dispersal of spores or spores already on the plant material is also plausible.

Redlin and Rossman (1991) considered *Aurantioporthe corni* as the causal agent of golden canker disease of *Cornus alternifolia*, yet the fulfillment of Koch's postulates has never been reported. In the greenhouse trial 80% of stems inoculated with *A. corni* developed pycnidia and characteristics of golden canker, while none of the controls developed pycnidia. At the conclusion of the experiment the fungus was isolated from a high number (60%) of inoculated plants, thus filling Koch's postulates for these replicates. The fact that we were not able to isolate *A. corni* from all replicates in this experiment could be due to environmental conditions in the greenhouse that were beyond our control.

Due to the increased use of *Cornus alternifolia* in managed landscapes, and the relatively high rate of *Aurantioporthe corni* living endophytically in asymptomatic stems, further studies are needed to determine what triggers this fungus to cause disease. Previous published studies suggest that the development of golden canker is induced by stress (Redlin and Rossman 1991, Grabowski 2009, Pataky 2010, Steiner 2011). For other pathosystems where endophytes considered to be latent pathogens have become pathogenic, the main factors suggested were wounding and drought stress (Bagga and Smalley 1974, Appel and Stipes 1984, Bachi and Peterson 1985, Blodgett et al. 1997, Swart et al. 1987). Stress caused by drought can be a factor in allowing an endophyte to colonize a tree more quickly (Chapela and Boddy 1988, Capretti and Battisti 2007). One of the common contributing factors to the outbreaks of the disease caused by the latent pathogen *Diplodea pinea* is hail damage after drought (Kotze 1935, Laughton 1937,

Luckhoff 1964, reviewed by Wingfield and Knox-Davis 1980). Determining what factors play a role in causing *A. corni* to transition to a parasitic phase will help in developing recommendations to control and minimize the impact of golden canker disease.

Recommendations for management and control of golden canker of pagoda dogwood can be inferred from our study. Although a plant with signs or symptoms of the disease may have the fungus in asymptomatic tissue, branches with signs of the disease should be removed to help reduce the amount of inoculum. This is especially important in the managed landscape, where a lower proportion of stems were found to have the fungus growing asymptomatically in stems. Removal of potential inoculum is also recommended for other diseases caused by endophytic fungi that can become pathogenic. Johnson et al. (1996) recommended pruning branches of *Corylus avelana* L with eastern filbert blight caused by *Anisogramma anomala* (Peck) E. Muller., to reduce the amount of inoculum and remove the fungus from the plant. However, they discuss the limitations of pruning in removing a latent pathogen from trees (Johnson et al. 1996). Determining whether an asymptomatic *Cornus alternifolia* plant is infected with *Aurantioporthe corni* is important when trying to minimize the spread of the disease, especially when moving nursery material to areas that are great distances from susceptible hosts. For those wanting to determine whether an asymptomatic plant has *A. corni* we recommend sampling from the bottom half of the stem, because the majority of stems that were found to be infected with *A. corni* had more segments colonized in that area.

We confirm *Aurantioporthe corni* belongs in Cryphonectriaceae and that it is both morphologically and phylogenetically distinct from any of the preexisting genera in this family. Although numerous phylogenetic studies have suggested the placement of *Cr. corni* within Cryphonectriaceae rather than Gnomoniaceae, these studies have relied on sequence data from only one or two isolates (Gryzenhout et al. 2006; Begoude et al. 2010; Chen et al. 2011, 2013a; Crane and Burgess 2013). When comparing the 28S nrDNA region (LSU) sequence information from the isolates collected in this study, all isolates had a 99–100% identity match in GenBank BLAST to the *Cr. corni* isolate used by Chen et al. (2013a) illustrating that *Cr. corni* was more appropriately placed in Cryphonectriaceae. In addition to these phylogenetic studies, a key morphological characteristic also supports the placement of *A. corni* outside Gnomoniaceae. *Aurantioporthe corni* exhibits orange stromatic tissue that turns purple in the presence of 3% KOH, a characteristic that unites all members of Cryphonectriaceae within Diaporthales (Gryzenhout et al. 2006). Within Cryphonectriaceae, *A. corni* is both morphologically and phylogenetically distinct from all other known genera. Ascospores of *A. corni* are most frequently single-septate, which is common in Cryphonectriaceae, but occasionally they can be multiseptate (FIG. 3c). *Amphilogia* Gryzenh., Glen and M.J. Wingf. is the only other genus in the family having single to multiseptate ascospores (Vermeulen et al. 2011; Chen et al. 2013a, b; Crane and Burgess 2013). *Aurantioporthe* can easily be distinguished from *Amphilogia* by observing the conidiomata as they are immersed to semi-immersed in *Aurantioporthe* and superficial in *Amphilogia* (Gryzenhout et al. 2005). Previous phylogenetic analysis also supports that *Aurantioporthe* is distinct from other genera in the family (Gryzenhout et al. 2006, 2010). The separation of *A. corni* from clades representing other genera in the family is strongly supported by our phylogenetic analysis (95% confidence level/1.0 posterior probability for ITS and 92% confidence level/1.0 posterior probability for *TUB*). Morphological characteristics and the phylogenetic position of *A. corni* both reinforce the need to establish a new genus within Cryphonectriaceae to accommodate *Aurantioporthe*.

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