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The new family Septorioideaceae, within the Botryosphaeriales and *Septorioides strobi* as a new species associated with needle defoliation of *Pinus strobus* in the United States

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ABSTRACT

Recent sampling of eastern white pine (*Pinus strobus*) affected by white pine needle defoliation (WPND) within the northeastern U.S. has found that a putative new species, closely related to *Septorioides pini-thunbergii*, was the most frequently isolated species. *Septorioides pini-thunbergii* is currently the only known species of its genus in the family *Botryosphaeriaceae* and is associated with needle cast of *Pinus thunbergii* in Japan. This study aims to complete a morphological description of the putative new species and use DNA sequence data of six gene loci (SSU, LSU, ITS, β-tubulin, EF1, and RPB2) to accurately place the putative new species within the *Botryosphaeriales*. Morphological comparisons have shown that this putative new species is distinct from *S. pini-thunbergii*. Comparison of DNA sequence data has further confirmed our morphological findings, indicating the classification of a new species which we describe as *Septorioides strobi* sp. nov., marking the first report of the genus *Septorioides* within the U.S. Subsequently, our phylogenetic analysis has further revealed that *S. pini-thunbergii* and *S. strobi* do not reside within the *Botryosphaereace*, but comprise a new family within the order *Botryosphaeriales* we recognize as *Septorioideaceae* fam. nov.

Taxonomic novelties: **New family** – *Septorioideaceae* S. Wyka & K. Broders fam. nov. **New species** – *Septorioides strobi* S. Wyka & K. Broders sp. nov.

Introduction

The *Botryosphaeriales* is a highly varied order, containing species that range from endophytes to pathogens (Slippers & Wingfield 2007). This variety of lifestyles coupled with that fact that species are known to occur on a wide range of monocotyledonous, dicotyledonous, and gymnosperm hosts, along with sightings on grasses (Crous *et al.* 2015) and lichens (Barr 1987; Von Arx 1987), has allowed species of *Botryosphaeriales* to be globally distributed (Slippers & Wingfield 2007). While most of the taxa in the *Botryosphaeriales* exist as endophytes, living mostly in healthy tissue of woody plants for

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extended periods of time (Slippers & Wingfield 2007), many are characterized as pathogens that cause disease on a wide variety of economically and ecologically important plants (Slippers *et al.* 2013). However, these two ecological functions are not always exclusive. Pathogenic species of the *Botryosphaeriales* cause disease after the onset of an initial stress factor, such as drought or infection by another weak pathogen (Schoeneweiss 1981; Swart & Wingfield 1991; Blodgett & Stanosz 1995). Once the disease is initiated, symptoms can develop rapidly and cause severe damage over large areas if the stress factor is widespread (Slippers & Wingfield 2007). In addition to the expanding knowledge of the complexity of these ecological functions associated with species of the *Botryosphaeriales*, this order has undergone significant reconstruction over the past decade with the addition of several new genera (Crous *et al.* 2004; Damm *et al.* 2007; Phillips *et al.* 2008; Hyde *et al.* 2011; Liu *et al.* 2012; Crous *et al.* 2015) as well as the creation of four new families; *Phyllostictaceae, Aplosporellaceae*, *Melanopsaceae*, and *Saccharataceae* (Minnes *et al.* 2012; Slippers *et al.* 2013; Wikee *et al.* 2013).

Septorioides pini-thunbergii was first reported to be associated with needle blight and the cause of sooty mold of *Pinus thunbergii* in Japan (Kaneko *et al.* 1989; Suto 2000), but has since been isolated as an endophyte from *Pinus densiflora* in South Korea (Yoo & Eom 2012), confounding the true ecological role of this species. The genus *Septorioides* is characterized as resembling the genus *Septoria* by sharing hyaline filiform conidia with transverse eusepta along with an acervular conidiomata possessing conidophores reduced to conidiogenous cells. However, *Septorioides* is morphologically distinguished by having an acervulus, conidiomata that open by means of an irregular rupture, and paraphyses that are intermingled among conidiogenous cells (Quaedvlieg *et al.* 2013). Although recent complications in the *Septoria* complex addressed by Quaedvlieg *et al.* (2013), reveal that many *Septoria* morphological characters are conserved within many septoria-like genera which mostly reside in different phylogenetic clades rather than the tight cluster of *Septoria* within the family *Mycosphaerellaceae*. Their recent phylogenetic study further suggested that DNA sequence data was paramount when dealing with members of this complex. *Septorioides pini-thunbergii* is currently placed in the family *Botryosphaeriaceae* within the *Botryosphaeriales* (Quaedvlieg *et al.* 2013).

A recent disease epidemic affecting the forests of Northeastern North America, known as white pine needle defoliation (WPND), is suspected to be caused by multiple fungal pathogens. This disease was first documented in the spring of 2010 affecting 24 328 ha in the state of Maine, and has since become an increasing concern to both the public and private landowners (Frament *et al.* 2011). *Pinus strobus* is not only an essential economic lumber resource but a crucial ecological component to the forest of northern USA and eastern Canada. Mature and regeneration *P. strobus* are similarly affected with symptoms including thin crowns, discolouration and premature defoliation of one-year-old needles, leaving behind only the new emergent needles of the current growing season (Munck *et al.* 2012). Symptoms along with current research point to three foliar pathogens causing the disease epidemic: (1) *Lecanosticta acicola* (Synonym *Mycosphaerella dearnessii*) the cause of brown spot needle blight (Quaedvlieg *et al.* 2012); (2) *Lophophacidium dooksii* (Synonym *Canavirgella banfieldii*) (Laflamme *et al.* 2015) the cause of dooks needle cast; and (3) *Bifusella linearis*, another needle cast pathogen (Minter & Millar 1984; Munck *et al.* 2012; Broders *et al.* 2015).

However, preliminary studies of diseased needles from 70 sites across New England revealed a putative new species, closely related to *S. pini-thunbergii*, as the most frequently isolated species from symptomatic needles (Wyka *et al.* unpublished). While *L. acicola*, *L. dooksii*, and *B. linearis* are speculated to be the primary causal agents due to historical research and their ubiquitous presence across the Northeastern United States, they have been previously documented to cause only limited disease of *P. strobus* (Skilling & Nicholls 1974; Minter & Millar 1984; Merril *et al.*1996; Wenner & Merril 1998; Munck *et al.* 2012; Broders *et al.* 2014, 2015). Previous research and preliminary data suggests that this severe epidemic arose after several consecutive years of warmer and wetter weather during the spring, creating a more favourable environment for these foliar pathogens (Munck *et al.* 2012; Broders *et al.* 2014, 2015; Wyka *et al. unpublished*). While this might be true, these three foliar pathogens could instead be acting as a widespread stress factor, rather than the primary cause, to which the frequently isolated putative new species is exploiting, as has been the case for many species within the order *Botryosphaeriales* (Schoeneweiss 1981; Swart & Wingfield 1991; Blodgett & Stanosz 1995).

It is therefore crucial to correctly categorize the ecological function and phylogenetic placement of this putative new species. Aside from morphological characteristics, the phylogenetic relationship of this putative new species to all known genera of the *Botryosphaeriales* is essential for understanding the evolution of the genus *Septorioides*. Based on this information the objectives of this study were to: (1) complete a taxonomic description, including morphological features and optimal growth rates, of the putative new species and document its association with the WPND epidemic; (2) use DNA sequence data of five gene loci to accurately place this putative new species within the *Botryosphaeriales*. The taxonomic changes for the establishment of a new family and a new species are considered in this study.

Materials and methods

Isolates

Pinus strobus needle samples were collected from 70 locations throughout Maine, New Hampshire, Vermont, and Massachusetts from April to June 2011–2014. At each location, three to five branch tips, from three trees exhibiting WPND symptoms, thin crowns with yellowing and browning of 2nd and 3rd year needles (Munck *et al.* 2012), were randomly sampled and shipped to the USFS Durham field office in a quart-size (1L) paper bag. Branch samples were first visually inspected for symptomatic needles containing fruiting structures, tip necrosis, and discolouration. Twenty to thirty symptomatic needles per tree were surfaced sterilized in a 10 % bleach solution for 1 min, rinsed three times with de-ionized water, and allowed to dry in a fume hood. Dry needles were incubated