1 **Five new *Pseudophialophora* species from grass roots in the oligotrophic pine**
2 **barrens ecosystem**

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14 **ABSTRACT**

15 During our recent survey of fungi in the oligotrophic pine barrens ecosystem, five new
16 *Pseudophialophora* species, *P. angusta*, *P. dichanthii*, *P. magnispora*, *P. tarda*, and *P.*
17 *whartonensis*, were uncovered from the roots of switchgrass (*Panicum virgatum*) and
18 tapered rosette grass (*Dichantheium acuminatum*). The five new fungal species are
19 described based on morphological characteristics and DNA sequences of SSU, ITS,
20 LSU, *MCM7*, *RPB1* and *TEF1* genes. The 6-locus phylogeny indicates that
21 *Pseudophialophora* species form a monophyletic clade in Magnaporthaceae of
22 Magnaporthales. A key for all described species in *Pseudophialophora* is provided,

including these five and three previously published species. Distinctions among the new species and other related species are discussed. The plant-fungal interaction experiment indicates that *P. angusta*, *P. eragrostis*, *P. magnispora*, *P. schzachyrii*, *P. tarda* and *P. whartonensis* have negative effects on the growth of switchgrass. Runner hyphae were observed from the inoculated switchgrass roots, which are typical structures of root-infecting pathogens.

Keywords:

Magnaporthaceae; Magnaporthales; Multi-gene phylogeny; *Pseudophialophora*; Systematics; Taxonomy

1 Introduction

Magnaporthales is an order in *Sordariomycetes*, *Ascomycota* (Thongkantha et al. 2009; Zhang et al. 2011; Luo et al. 2015). It contains over 200 species, about 50% of which are pathogens of monocot plants, e.g., rice, maize and wheat (Kirk et al. 2008; Luo and Zhang 2013). The typical morphological characteristics of *Magnaporthales* include absence of stromata, black perithecia with a long beak, well-developed paraphyses, cylindrical to clavate asci with a refractive apical ring, and fusoid to filiform ascospores with septa and smooth surface (Huhndorf et al. 2008; Thongkantha et al. 2009).

Recently, three major lineages were recognized in *Magnaporthales* based on phylogenomic analysis (Luo et al. 2015) and multi-locus phylogenetic analyses (Zhang et al. 2011; Klaubauf et al. 2014; Luo et al. 2014b), and three families were named accordingly: *Magnaporthaceae*, *Pyriculariaceae* and *Ophioceraceae* (Klaubauf et al. 2014). *Ophioceraceae*, the early diverging lineage in *Magnaporthales* are saprobes on submerged wood substrates (Shearer 1989, 1999; Luo et al. 2004). *Pyriculariaceae* species have *Pyricularia* asexual morphs and are pathogenic to *Poaceae* or other monocot plants. The rice blast fungus *Pyricularia oryzae*, a model system for plant-fungal interaction studies belongs to this family (Sesma and Osbourn 2004, Dean et al 2005). Most species in *Magnaporthaceae* have phialophora-like or *Nakataea* asexual morphs, and often are root pathogens of grasses or cereals, e.g., the take-all fungus *Gaeumannomyces graminis* and the summer patch pathogen *Magnaporthiopsis poae* (Klaubauf et al. 2014; Luo et al. 2015).

Pseudophialophora J. Luo & N. Zhang is a recently established genus in

1 *Magnaporthaceae* of *Magnaporthales* (Luo et al. 2014b). Three species have been
2 accepted in *Pseudophialophora* including *P. panicorum* (type species), *P. eragrostis*
3 and *P. schizachyrii*. They are characterized by slow growth rates on potato dextrose
4 agar (PDA), thin and compact colonies, simple and short conidiophores, curved and
5 phialidic conidiogenous cells, oblong ellipsoid to ellipsoid and unicellular conidia
6 usually forming slimy heads, and inhabiting *Poaceae* grass roots. These species were
7 all collected from apparently healthy grass roots in New Jersey Pine Barrens. Their
8 ecological functions are unknown.

9 Pine barrens is a general name for a unique type of ecosystem with acidic and
10 nutrient-poor soils (Forman 1998). Pines and oaks are the dominant trees in pine
11 barrens, whereas the grasses (*Poaceae*), sedges (*Cyperaceae*), blueberries and other
12 heath family members (*Ericaceae*) are dominant on disturbed open and dry areas. The
13 largest and most uniform area of pine barrens in the United States is the 1.4 million acre
14 (57,000 km²) pine barrens of New Jersey, which has a temperate climate with average
15 monthly temperatures from 0.3 to 24.3 °C and average annual precipitation of 116.5 cm
16 (1981–2010, NJ State Climatologist). The podzolic soil in this region is highly acidic
17 (pH ~4.0 with very low cation exchange capacity), sandy, dry (low moisture holding
18 capacity) and nutrient poor (low in P, K, etc) (Joffe and Watson 1933, Tedrow 1952,
19 Turner et al. 1985, Forman 1998). The New Jersey Pine Barrens represents one of a
20 series of barrens ecosystems along the eastern seaboard of the United States and similar
21 ecosystems around the world. Scarce attention has been received on studies of fungi
22 in the pine barrens, and much remains unknown about fungal diversity and functions in

this ecosystem (Forman 1998, Tuininga and Dighton 2004).

Since 2011, we started sampling grass root-associated fungi in the New Jersey Pine Barrens and uncovered many new fungal lineages. We recently have described two new genera including *Pseudophialophora* from these collections (Luo et al. 2014a, b; Walsh et al. 2014). In this study we aim to 1) describe five new *Pseudophialophora* species from the pine barrens grass roots based on morphology, biology, ecology and multi-gene phylogenetic analysis; 2) investigate the phylogenetic position of *Pseudophialophora* in Magnaporthales; and 3) understand the ecological functions of *Pseudophialophora* species by the plant-fungal interaction experiment.

2 Material and methods

2.1 Fungal isolation

Grass roots were sampled from Colliers Mills and Wharton State Forest in New Jersey Pine Barrens in June 2014. Within 24 h after collection, the roots were rinsed in tap water to remove soil particles on the surface and cut into about 5 mm long segments. The segments were then surface sterilized with 95% ethanol for 30 sec, followed by 2 min in 0.6% sodium hypochlorite (NaOCl), 2 min in 70 % ethanol and two rinses in sterile distilled water. The disinfested segments were placed on malt extract agar (MEA, BD) with 0.07% lactic acid and incubated at room temperature. Fungal cultures were isolated and purified by subculturing from emergent hyphal tips.

2.2 Morphological study

Cultural characteristics were recorded from potato dextrose agar (PDA, BD) and corn meal agar (CMA, BD), and the colour names of colonies followed Ridgway's nomenclature (Ridgway 1912). Microscopic examinations, measurements and photographs were taken from slides of fungi mounted in distilled water. The specimens examined were deposit in the Rutgers Mycological Herbarium, New Brunswick, New Jersey, USA (RUTPP), and cultures were kept in the Centraalbureau voor Schimmelcultures Fungal Biodiversity Centre, the Netherlands (CBS).

2.3 DNA extraction, amplification and sequencing

The protocols used in (Zhang et al. 2011) and (Luo and Zhang 2013) were followed for DNA extraction, PCR amplification and sequencing of small subunit (SSU), internal transcribed spacer (ITS), and large subunit (LSU) of ribosomal RNA genes, DNA replication licensing factor (*MCM7*), the largest subunit of RNA polymerase II (*RPB1*) and translation elongation factor 1- α (*TEF1*) genes. A total of 53 fungal isolates, representing 31 species and three varieties in Magnaporthales, were included in our phylogenetic analyses. Forty-eight newly generated sequences in this study were deposited in GenBank, and their taxon names, isolate numbers, sources, hosts, and GenBank accession numbers are provided in Table 1. The other reference taxa were

listed in Luo and Zhang (2013) and Luo et al. (2014b).

2.4 Sequence alignment and phylogenetic analyses

Sequences of each gene were aligned with Clustal X 1.8 (Thompson et al. 1997) and revised manually with BioEdit 7.0.5 (Hall 1999). A six-gene dataset was generated by concatenating the individual sequence alignments. *Cryphonectria parasitica* was chosen as the outgroup taxon. MrModeltest (Nylander 2004) was used to specify the best-fit nucleotide substitution model under hierarchical likelihood ratio tests (hLRT) and Akaike information criterion (AIC). Maximum likelihood (ML) analysis with the selected model was carried out in RAxML 8.1.1 (Stamatakis 2014). Branch support was assessed with 1000 bootstraps replicates. Maximum parsimony (MP) analysis was performed with heuristic search in PAUP* 4.0b10 (Swofford 2002). All characters were given equal weight. Gaps were treated as missing characters. Starting trees were obtained via random sequence stepwise addition with 1000 replicates. The branch swapping algorithm was tree-bisection-reconnection (TBR). Steepest descent and Multitree opinions were not in effect. To calculate all branch support values, a bootstrap analysis was performed with 1000 replicates using heuristic searches with simple sequence stepwise addition for each replicate. Bayesian inference (BI) was conducted with the Markov Chain Monte Carlo method in MrBayes 3.2.1 (Ronquist et al. 2012) under the selected nucleotide substitution model. Trees were sampled every 100 generations from 5 000 000 generations resulting in 100 000 trees. The first 12 500

trees were discarded as the burn-in and the remaining 37 500 trees were chosen to calculate posterior probability values of clades in a consensus tree.

2.5 *Plant-fungal interaction experiment*

The type strains of the eight *Pseudophialophora* species, including the five newly described and three previously published species were used in the plant-fungal interaction experiment. Switchgrass seeds ('Kanlow' variety) were surface disinfested as follows: 95% ethanol for 30s, 0.5% NaOCl for 1 min, 70% ethanol for 1 min, rinsed with sterile distilled water and allowed to germinate in dark at 25 C for 3 days.

Agargel (Sigma-Aldrich, USA) Petri plates (0.5%) were made following the manufacturer's instructions, and were cut in half, with one side removed. On the cut surface of an Agargel plate, three 10 mm × 10 mm × 5 mm plugs from a one-week old fungal culture grown on MEA were placed equidistance from one another. Germinated switchgrass seeds were then placed on the Agargel plugs with fungal mycelium. Sterile MEA plugs were used as the negative control. The switchgrass-fungal cultures were incubated at 25 C under 12 hr light and dark cycle. The shoot height of the switchgrass seedlings was recorded at 7 and 14 days after inoculation. Root length and root hair density were also measured following the methods in Sasan and Bidochka (2012).

There were fifteen replicates for each switchgrass-fungal interaction experiment.

3 Results

3.1 Sequence data and phylogeny

There were 408 nucleotide characters including gaps in the SSU alignment, 633 in ITS, 882 in LSU, 499 in *MCM7*, 623 in *RPB1*, and 865 in *TEF1*. The 6-gene dataset comprised 3910 characters, among which 1207 were parsimony informative, 367 were variable and parsimony uninformative, and 2336 were constant. It was deposited in TreeBASE as S17151. The general time reversible + proportion of invariable sites + gamma distributed for rate variation among sites (GTR+I+G) was selected as the best-fit model for ML and BI analyses. The phylogenetic tree was reconstructed by using ML, MP and BI methods, and each method yielded one tree. These trees were similar in topology and only the ML tree was showed in Fig 1. The branches with significant support were noted with thickened lines. Strong branch support was defined when ML bootstrap proportion (MLBP) was $\geq 75\%$, MP bootstrap proportion (MPBP) $\geq 75\%$, and BI posterior probability (BIPP) ≥ 0.95 .

Three major clades are recognized in our phylogenetic tree (Fig 1). Three *Ophioceras* and one *Pseudohalonectria* species constitute Clade A. Clade B includes seven species in *Pyricularia*, *Xenopyricularia*, *Neopyricularia*, *Macgarvieomyces* and *Pseudopyricularia*. Clade C contains 18 species and three varieties belonging to *Magnaporthiopsis*, *Gaeumannomyces*, *Buergenerula*, *Nakataea*, *Omnidemptus*, *Slopeiomyces* and *Pseudophialophora*.

All our collections, including fourteen fungal isolates, fell into the subclade of

Clade C, *Pseudophialophora*, from which five new species are recognized and each species is distinct from other species in the genus. Based on the molecular phylogeny together with morphological, biological and ecological characteristics, five new species are proposed.

3.2 Taxonomy

Pseudophialophora angusta J. Luo & N. Zhang, **sp. nov.** Figs. 2E–H

MycoBank no.: MB811707.

Etymology: The specific epithet refers to the narrow conidia (1.5–2.5 μm wide) produced by this fungus.

Morphological description: Colonies on PDA 2.3 cm diam after 7 d in the dark at 25 C, Sulphur Yellow, surface velvety, aerial mycelium yellowish, reverse Pale Green-Yellow. Colonies on CMA reaching 1.9 cm after 7 d in the dark at 25 C, Sulphur Yellow, aerial mycelium sparse, reverse Light Green-Yellow. Conidiophores single or branched. Conidiogenous cells phialidic, curved, yellowish, 7–25 $\times$ 2–3 μm , 1–2 μm wide at the base, 0.5–1.2 μm wide near the apex (n = 50). Conidia aggregated in slimy heads, oblong ellipsoidal, straight or slightly curved, aseptate, hyaline, smooth, 7.5–10 $\times$ 1.5–2.5 μm (n = 50).

Holotype: **United States:** *New Jersey:* Wharton State Forest, N 39 46.136, W 074 40.885, alt. 40 m, from roots of *Dichanthelium acuminatum*, 26 Jun. 2014, J. Luo & N. Zhang WSF14RG40 (RUTPP-WSF14RG40).

Notes: This species has the narrowest conidia, which made it distinct from other species in *Pseudophialophora*. It showed affinity to *P. schizachyrii* phylogenetically. However they were differed in some molecular characters, including 26 nucleotide positions in ITS, 9 in LSU, 11 in *MCM7*, 10 in *RPB1* and 19 in *TEF1*.

Pseudophialophora dichanthii J. Luo & N. Zhang, **sp. nov.** Figs. 2I–L

MycoBank no.: MB811708.

Etymology: The specific epithet refers to the host generic name *Dichantheium*.

Morphological description: Colonies on PDA 2.5 cm diam after 7 d in the dark at 25 C, Light Yellow-Green, surface velvety, aerial mycelium yellowish, reverse Calliste Green. Colonies on CMA reaching 2.3 cm after 7 d in the dark at 25 C, Light Green-Yellow, aerial mycelium sparse, reverse Pale Green-Yellow. Conidiophores single or branched. Conidiogenous cells phialidic, hyaline, curved, $6\text{--}20 \times 2\text{--}3.5 \mu\text{m}$, $1\text{--}2.2 \mu\text{m}$ wide at the base, $0.5\text{--}1.2 \mu\text{m}$ wide near the apex ($n = 50$). Conidia aggregated in slimy heads, oblong ellipsoidal to ellipsoidal, aseptate, hyaline, smooth, $4\text{--}8 \times 2.5\text{--}3.5 \mu\text{m}$ ($n = 50$).

Holotype: **United States:** *New Jersey:* Wharton State Forest, N 39 46.136, W 074 40.885, alt. 40 m, from roots of *Dichantheium acuminatum*, 26 Jun. 2014, J. Luo & N. Zhang, WSF14RG82 (RUTPP-WSF14RG82).

Other materials examined: **United States:** *New Jersey:* Wharton State Forest, N 39 46.136, W 074 40.885, alt. 40 m, from roots of *Dichantheium acuminatum*, 26 Jun. 2014, J. Luo & N. Zhang, WSF14RG12; Wharton State Forest, N 39 46.136, W 074

40.885, alt. 40 m, from roots of *Dichantheium acuminatum*, 26 Jun. 2014, J. Luo & N. Zhang WSF14RG41; Wharton State Forest, N 39 46.136, W 074 40.885, alt. 40 m, from roots of *Dichantheium acuminatum*, 26 Jun. 2014, J. Luo & N. Zhang WSF14RG70; Wharton State Forest, N 39 46.136, W 074 40.885, alt. 40 m, from roots of *Dichantheium acuminatum*, 26 Jun. 2014, J. Luo & N. Zhang WSF14RG72.

Notes: Two collections of this species were grouped together and showed affinity to *Pseudophialophora schizachyrii*. The latter, however, differed in lower growth rates on PDA (2.2 cm/7 days), sulphine yellow coloured colonies on PDA, and *Schizachyrium* host (Luo et al. 2014b). Molecularly, it differed from *P. schizachyrii* by 1 nucleotide position in SSU, 32 in ITS, 7 in LSU, 11 in *MCM7*, 8 in *RPB1* and 10 in *TEF1*.

***Pseudophialophora magnispora* J. Luo & N. Zhang, sp. nov.** Figs. 2M–P

MycoBank no.: MB811709.

Etymology: The specific epithet refers to the large conidia ($8\text{--}12 \times 3\text{--}5 \mu\text{m}$) of this fungus.

Morphological description: Colonies on PDA 2.6 cm diam after 7 d in the dark at 25 C, Yellow Green, surface velvety to floccus, aerial mycelium yellowish, reverse Peacock Green. Colonies on CMA reaching 2.4 cm after 7 d in the dark at 25 C, Light Green-Yellow, aerial mycelium sparse, reverse Pale Green-Yellow. Conidiophores single or branched. Conidiogenous cells phialidic, hyaline to yellowish, curved, $13\text{--}30 \times 2.5\text{--}4 \mu\text{m}$, $1.5\text{--}2.5 \mu\text{m}$ wide at the base, $0.5\text{--}1.5 \mu\text{m}$ wide near the apex ($n = 50$).

1 Conidia aggregated in slimy heads, ellipsoidal, aseptate, hyaline, smooth, $8\text{--}12 \times 3\text{--}5$
 2 μm ($n = 50$).

3 *Holotype*: **United States**: *New Jersey*: Colliers Mills, N40 04.093, W74 26.598,
 4 alt. 42 m, from roots of *Dichanthelium acuminatum*, 4 Jun. 2014, *J. Luo & N. Zhang*
 5 CM14RG38 (RUTPP-CM14RG38).

6 *Other materials examined*: **United States**: *New Jersey*: Colliers Mills, N40
 7 04.093, W74 26.598, alt. 42 m, from roots of *Dichanthelium acuminatum*, 4 Jun. 2014,
 8 *J. Luo & N. Zhang* CM14RG50.

9 *Notes*: Compared to other species in the genus, this species has the largest
 10 conidia and longest conidiogenous cells. It was closely related to *Pseudophialophora*
 11 *whartonensis* phylogenetically. However they were differed in 17 nucleotide positions
 12 in ITS, 5 in LSU, 3 in *MCM7*, 5 in *RPB1* and 3 in *TEF1*.

13

14 ***Pseudophialophora tarda* J. Luo & N. Zhang, sp. nov.** Figs. 2A–D

15 MycoBank no.: MB811710.

16 *Etymology*: The specific epithet refers to its slow growth rate on PDA.

17 *Morphological description*: Colonies on PDA 2.0 cm diam after 7 d in the dark at
 18 25 C, Sulphur Yellow, surface velvety, aerial mycelium yellowish, reverse Pale
 19 Green-Yellow. Colonies on CMA reaching 1.5 cm after 7 d in the dark at 25 C,
 20 Sulphur Yellow, aerial mycelium sparse, reverse Light Green-Yellow. Conidiophores
 21 single or branched. Conidiogenous cells phialidic, hyaline, curved, $8\text{--}20 \times 2.5\text{--}4.5 \mu\text{m}$,
 22 $1.5\text{--}2.5 \mu\text{m}$ wide at the base, $0.5\text{--}1.2 \mu\text{m}$ wide near the apex ($n = 50$). Conidia

1 aggregated in slimy heads, oblong ellipsoidal to ellipsoidal, aseptate, hyaline, smooth,
2 7.5–9.5 × 2.5–3.5 µm (n = 50).

3 *Holotype: United States: New Jersey: Wharton State Forest, N 39 46.136, W 074*
4 *40.885, alt. 40 m, from roots of *Panicum virgatum*, 26 Jun. 2014, J. Luo & N. Zhang*
5 *WSF14SW13 (RUTPP-WSF14SW13).*

6 *Other materials examined: United States: New Jersey: Wharton State Forest, N*
7 *39 46.136, W 074 40.885, alt. 40 m, from roots of *Dichanthelium acuminatum*, 26 Jun*
8 *2014, J. Luo & N. Zhang WSF14RG22; Wharton State Forest, N 39 46.136, W 074*
9 *40.885, 40 m alt., from roots of *Dichanthelium acuminatum*, 26 Jun. 2014, J. Luo & N.*
10 *Zhang WSF14RG45; Wharton State Forest, N 39 46.136, W 074 40.885, alt. 40 m,*
11 *from roots of *Dichanthelium acuminatum*, 26 Jun. 2014, J. Luo & N. Zhang*
12 *WSF14RG48; Wharton State Forest, N 39 46.136, W 074 40.885, alt. 40 m, from*
13 *roots of *Dichanthelium acuminatum*, 26 Jun. 2014, J. Luo & N. Zhang*
14 *WSF14RG64-2.*

15 *Notes:* Two collections of this species were phylogenetically situated at the base
16 of subclade *Pseudophialophora* and closely related to *P. panicorum*. Morphologically,
17 *P. panicorum* differed from *P. tarda* in narrower conidiogenous cells (1.5–2.7 µm wide
18 in *P. panicorum*), and higher growth rates on PDA (2.6 cm/7 days in *P. panicorum*)
19 (Luo et al. 2014b). Molecularly, *P. panicorum* differed from this species by 14
20 nucleotide position in ITS, 5 in LSU, 3 in *MCM7*, 3 in *RPB1* and 6 in *TEF1*.

21

22 *Pseudophialophora whartonensis* J. Luo & N. Zhang, **sp. nov.**

Figs. 2Q–T

MycoBank no.: MB811711.

Etymology: The specific epithet refers to the location (Wharton State Forest) where this fungus was collected.

Morphological description: Colonies on PDA 2.8 cm diam after 7 d in the dark at 25 C, Calliste Green, surface velvety to floccus, aerial mycelium yellowish, reverse Parrot Green. Colonies on CMA reaching 2.6 cm after 7 d in the dark at 25 C, Pale Green-Yellow, aerial mycelium sparse, reverse Pale Greenish Yellow. Conidiophores single or branched. Conidiogenous cells phialidic, hyaline to yellowish, curved, 8–25 $\times$ 2.5–3.5 μ m, 1.2–2.2 μ m wide at the base, 0.5–1.5 μ m wide near the apex (n = 50). Conidia aggregated in slimy heads, oblong ellipsoidal to ellipsoidal, aseptate, hyaline, smooth, 4–8 $\times$ 2.5–4 μ m (n = 50).

Holotype: **United States:** *New Jersey:* Wharton State Forest, N 39 46.136, W 074 40.885, alt. 40 m, from roots of *Dichantherium acuminatum*, 26 Jun. 2014, J. Luo & N. Zhang WSF14RG66 (RUTPP-WSF14RG66).

Notes: This species appeared to be closely related to *Pseudophialophora eragrostis* in the phylogenetic tree. However, *P. eragrostis* differed from it in longer conidia (7.5–10.5 long in *P. eragrostis*) and *Eragrostis* host (Luo et al. 2014b). Molecularly, *P. eragrostis* differed from our collection by 13 nucleotide position in ITS, 5 in LSU, 7 in *MCM7*, 7 in *RPB1* and 4 in *TEF1*.

A key to the species accepted in *Pseudophialophora*

1	1 Colony diameter ≥ 2.5 cm on PDA in the dark after 7 days at 25 C	2
2	1 Colony diameter < 2.5 cm on PDA in the dark after 7 days at 25 C	6
3	2(1) Conidia averaging ≥ 7.5 μm long	3
4	2(1) Conidia averaging < 7.5 μm long	5
5	3(2) Colony dark green on PDA, associated with <i>Eragrostis</i> sp.	<i>P. eragrostis</i>
6	3(2) Colony greenish on PDA, associated with other hosts	4
7	4(3) Conidia 7.5–10.5 x 2.5–3.5 μm , associated with <i>Panicum</i> sp.	<i>P. panicorum</i>
8	4(3) Conidia 8–12 x 3–5 μm , associated with <i>Dichanthelium acuminatum</i>	
9		<i>P. magnispora</i>
10	5(2) Colony dark green on PDA	<i>P. wharttonensis</i>
11	5(2) Colony greenish on PDA	<i>P. dichanthii</i>
12	6(1) Conidia averaging ≥ 2.5 μm wide	7
13	6(1) Conidia averaging < 2.5 μm wide	<i>P. angusta</i>
14	7(6) Colony sulphine yellow on PDA, conidia 5.5–8 x 2.5–3.5 μm	<i>P. schizachyrii</i>
15	7(6) Colony yellowish on PDA, conidia 7.5–9.5 x 2.5–3.5 μm	<i>P. tarda</i>
16		

17 3.3 Plant-fungal interaction experiment

18 Our inoculation experiments showed that the shoot heights of switchgrass treated with
19 *Pseudophialophora tarda* and *P. schzachyrii* were significantly shorter than the
20 control at day 7 and day 14 after inoculation ($p < 0.05$), while no significant
21 difference was detected for the other *Pseudophialophora* species ($p \geq 0.05$). The
22 roots of switchgrass co-cultured with *P. angusta*, *P. eragrostis*, *P. magnispora*, *P.*

1 *schzachyrii*, *P. tarda* and *P. whartonensis* were significantly shorter than the control at
 2 day 7 and day 14 after inoculation ($p < 0.05$) (Figs 3 and 4). The root lengths for *P.*
 3 *dichanthii* and *P. panicorum* were not significantly shorter than the control ($p \geq 0.05$).
 4 For the root hair density, significant differences were observed for *P. angusta*, *P.*
 5 *magnispora*, *P. schzachyrii* and *P. tarda* ($p < 0.05$), while not significant for *P.*
 6 *dichanthii*, *P. eragrostis*, *P. panicorum* and *P. whartonensis* ($p \geq 0.05$) (Figs 3 and 4).
 7 Based on these data, *Pseudophialophora tarda* and *P. schzachyrii* decreased both
 8 shoot and root growth of switchgrass, *P. angusta*, *P. eragrostis*, *P. magnispora* and *P.*
 9 *whartonensis* decreased root growth only, while *P. dichanthii* and *P. panicorum*
 10 showed no effects on switchgrass growth.

11 After 35 days of inoculation, brown runner hyphae and intracellular coiled
 12 hyphae were observed from the roots of all *Pseudophialophora* treated switchgrass
 13 plants (Fig 5). No fungal structures were observed from the control roots. In addition,
 14 all *Pseudophialophora* species used for inoculation were recovered from the roots of
 15 treated switchgrass after surface sterilization. These results confirmed the success of
 16 fungal inoculation to switchgrass plants.

17

18 **4 Discussion**

19

20 *Magnaporthaceae* was established by Cannon (1994) to accommodate *Magnaporthe*,
 21 *Gaeumannomyces* and related taxa, which had been placed in various orders in
 22 *Ascomycota* due to a lack of convincing phenotypic characters. Thongkantha et al.

(2009) later proposed a single-family order, *Magnaporthales* for these fungi based on phylogenetic analyses. Originally described with about 20 species, *Magnaporthales* currently contains over 200 species (Kirk et al. 2008; Huhndorf et al. 2008; Luo and Zhang 2013; Luo et al 2014a, 2014b). In addition, an increasing number of sequences of uncultured *Magnaporthales* have been deposited in GenBank by different researchers, suggesting cryptic diversity of *Magnaporthales*. Recently *Magnaporthales* was divided into three families (Klaubauf et al. 2014), representing three major lineages recognized by phylogenomic (Luo et al. 2015) and multi-gene phylogenetic analyses (Klaubauf et al. 2014; Luo et al. 2014a, 2014b). Each lineage has a unique set of phenotypic, biological and ecological characteristics (Luo et al. 2015).

The five new *Pseudophialophora* species together with the other three *Pseudophialophora* species described previously formed a monophyletic group. The 6-gene phylogeny indicated that the well-supported *Pseudophialophora* clade belongs to *Magnaporthaceae* in *Magnaporthales*. Many species in *Magnaporthaceae* are known pathogens of wild or domesticated grasses, such as the take-all pathogen of cereals and grasses *Gaeumannomyces graminis*, the summer patch pathogen of turfgrasses *Magnaporthiopsis poae*, the late wilt pathogen of maize *Magnaporthiopsis maydis*, and the root rot pathogen of maize *Gaeumannomyces radicola* (McKeen 1952; Deacon and Scott 1983; Cannon 1994; Besi et al. 2009; Garcia-Carneros et al. 2012). Within *Pseudophialophora*, *P. eragrostis*, *P. whartonensis*, *P. magnispora*, *P.*

1 *dichanthii* and *P. schizachyrii* formed a well-supported subclade. *Pseudophialophora*
 2 *angusta* is shown as a singleton in the genus, while *P. panicorum* and *P. tarda*
 3 clustered in another subclade.

4
 5 Morphologically, *Pseudophialophora* species are phialophora-like. They produce
 6 dark septate hyphae, simple and short conidiophores, typical phialides and unicellular
 7 conidia (Luo et al. 2014b). However, *Phialophora* species have flaring collarets at the
 8 tips of the phialides and belong to *Chaetothyriales*, *Eurotiomycetes* (Gams 2000).
 9 *Pseudophialophora* also resembles *Magnaporthiopsis*, *Gaeumannomyces*, and
 10 *Slopeiomyces* in phialidic asexual morphs and compact colonies on PDA. Compared
 11 to *Pseudophialophora*, *Magnaporthiopsis* differs in fast growing colonies, straight
 12 conidiogenous cells, ovoid conidia, and presence of simple to lobed hyphopodia (Luo
 13 and Zhang 2013). *Gaeumannomyces* differs in fast growing colonies, straight
 14 conidiogenous cells, densely branched conidiophores, sickle-shaped conidia, and
 15 presence of simple to lobed hyphopodia (Luo et al. 2014b). *Slopeiomyces* differs in
 16 straight conidiogenous cells, densely branched conidiophores, and sickle-shaped
 17 conidia, and presence of simple hyphopodia on host (Hornby et al. 1977; Klaubauf et
 18 al. 2014).

19
 20 Our inoculation results showed that several *Pseudophialophora* species
 21 decreased the shoot and/or root growth of switchgrass seedlings. Coupled with the
 22 production of runner hyphae, and the phylogenetic position, *Pseudophialophora*

1 species likely are root pathogens to switchgrass. Hyphopodia and runner hyphae are
2 typical infection and dispersal structures produced by the root-infecting fungi in
3 *Magnaporthaceae*. Observation of runner hyphae on *Pseudophialophora* inoculated
4 plants supports that these fungi are root pathogens. Further studies are needed to
5 search for hyphopodia and host symptoms caused by *Pseudophialophora*.
6 *Magnaporthales* contains many scientifically and economically important species,
7 and switchgrass is a promising biofuel crop. The report on new fungal lineages in
8 *Magnaporthales* and their interactions with switchgrass may provide useful
9 information for future fungal biodiversity studies and crop protection.

10

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Figure Legends:

Fig 1 – A maximum likelihood tree inferred from the combined SSU, ITS, LSU, *MCM7*, *RPB1* and *TEF1* sequence datasets. New species proposed in this study are in bold face. Branches with significant support are noted with thickened lines. Strong branch support is defined when ML bootstrap proportion (MLBP) is $\geq 75\%$, MP bootstrap proportion (MPBP) $\geq 75\%$, and BI posterior probability (BIPP) ≥ 0.95 .

Fig 2 – Asexual morphs with conidiophores and conidia. (A–D) *Pseudophialophora tarda* (WSF14SW13). (E–H) *P. angusta* (WSF14RG40). (I–L) *P. dichanthii* (WSF14RG82). (M–P) *P. magnispora* (CM14RG38). (Q–T) *P. whartonensis* (WSF14RG66).

Fig 3 – (A) Root lengths (mm) for control and *Pseudophialophora* treated switchgrass seedlings at day 7 after inoculation (n = 15). (B) Root lengths (mm) for control and *Pseudophialophora* treated switchgrass seedlings on day 14 after inoculation (n = 15). (C) Root hair densities (root hair/mm) for control and *Pseudophialophora* treated switchgrass seedlings at day 7 after inoculation (n = 15). (D) Root hair densities (root hair/mm) for control and *Pseudophialophora* treated switchgrass seedlings at day 14 after inoculation (n = 15). Each column indicates the mean $\pm$ SD of values.

Fig 4 – Switchgrass seedlings treated with control and eight *Pseudophialophora*

1 species at day 14 after inoculation. (A) Control. (B) *P. panicorum* (CM3m7). (C) *P.*
2 *dichanthii* (WSF14RG82). (D) *P. eragrostis* (CM12m9). (E) *P. whartonensis*
3 (WSF14RG66). (F) *P. angusta* (WSF14RG40). (G) *P. magnispora* (CM14RG38). (H)
4 *P. schzachyrii* (AL3s4). (I) *P. tarda* (WSF14SW13).

5

6 Fig 5 – The roots of switchgrass after 35 days of inoculation, stained with Rose
7 Bengal Stain (0.02%). (A–B) The runner hyphae growing on the surface of roots.

8 (C–D) The coiled hyphae formed within the root cells. A from *P. eragrostis*

9 (CM12m9), B from *P. angusta* (WSF14RG40), C from *P. tarda* (WSF14SW13), D

10 from *P. schzachyrii* (AL3s4).

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Table 1 – Species name, isolate number, source, host and GenBank accession numbers of the fungi in this study.

Species	Isolate number ^a	Source	Host	SSU	ITS	LSU	<i>MCM7</i>	<i>RPB1</i>	<i>TEF1</i>
<i>Pseudophialophora angusta</i>	WSF14RG40	New Jersey, USA	roots of <i>Dichantherium acuminatum</i>	KP769825	KP769841	KP769833	KP784816	KP784824	KP784832
<i>Pseudophialophora dichanthii</i>	WSF14RG82	New Jersey, USA	roots of <i>Dichantherium acuminatum</i>	KP769822	KP769838	KP769830	KP784813	KP784821	KP784829
<i>Pseudophialophora dichanthii</i>	WSF14RG72	New Jersey, USA	roots of <i>Dichantherium acuminatum</i>	KP769821	KP769837	KP769829	KP784812	KP784820	KP784828
<i>Pseudophialophora magnispora</i>	CM14RG38	New Jersey, USA	roots of <i>Dichantherium acuminatum</i>	KP769819	KP769835	KP769827	KP784810	KP784818	KP784826
<i>Pseudophialophora magnispora</i>	CM14RG50	New Jersey, USA	roots of <i>Dichantherium acuminatum</i>	KP769820	KP769836	KP769828	KP784811	KP784819	KP784827
<i>Pseudophialophora tarda</i>	WSF14SW13	New Jersey, USA	roots of <i>Dichantherium acuminatum</i>	KP769823	KP769839	KP769831	KP784814	KP784822	KP784830
<i>Pseudophialophora tarda</i>	WSF14RG48-2	New Jersey, USA	roots of <i>Panicum virgatum</i>	KP769824	KP769840	KP769832	KP784815	KP784823	KP784831
<i>Pseudophialophora wharttonensis</i>	WSF14RG66	New Jersey, USA	roots of <i>Dichantherium acuminatum</i>	KP769818	KP769834	KP769826	KP784809	KP784817	KP784825

Figure1

Figure2

Figure3

Figure4Color

Figure4Grey

Figure5