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Pinibarreniales, a new order of Sordariomycetes from pine barrens ecosystem

Jing Luo[a], Emily Walsh[a], Alexis Faulborn[a], Kevin Gao[a], James White[a], and Ning Zhang[a,b]

[a]Department of Plant Biology, Rutgers University, 59 Dudley Road, New Brunswick, New Jersey 08901; [b]Department of Biochemistry and Microbiology, Rutgers University, 76 Lipman Drive, New Brunswick, New Jersey 08901

ABSTRACT

Pinibarrenia chlamydospora, sp. nov. isolated from the roots of highbush blueberry in the New Jersey Pine Barrens, is described and illustrated. Based on multigene phylogenetic analysis, as well as morphological and ecological characteristics, Pinibarreniales and Pinibarreniaceae are established to accommodate this novel lineage in Sordariomycetidae, Sordariomycetes. Pinibarreniales, Tracyllalales, and Vermiculariopsiellales are proposed to be included in the subclass Sordariomycetidae. Pinibarreniales likely have a wide distribution and forms association with Ericaceae plants that live in acidic and oligotrophic environments because its DNA barcode matches with environmental sequences from other independent ecological studies. The plant-fungal interaction experiment revealed negative impacts on *Arabidopsis*, indicating its pathogenicity. This uncovered new fungal lineage will contribute to a better understanding of the diversity and systematics of Sordariomycetes.

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Multigene phylogeny; *Pinibarrenia*; Pinibarreniaceae; root fungi; taxonomy; 4 new taxa

INTRODUCTION

Sordariomycetes is a large fungal class in Ascomycota that contains many economically and ecologically important species. Members of Sordariomycetes are ubiquitous and known as pathogenetic, endophytic, mycoparasitic, and saprobic but non-lichenized. They have perithecial to cleistothecial ascomata, paraphysate and periphysate hamathecia, unitunicate, inoperculate, and pseudoprototunicate to fissitunicate asci, and hyphomycetous to coelomycetous asexual states (Wijayawardene et al. 2022). Eriksson and Winka (1997) first introduced the class to accommodate three subclasses, Hypocreomycetidae, Sordariomycetidae, and Xylariomycetidae, and seven orders based on the nuclear 18S rDNA (18S) phylogeny and morphology. Lumbsch and Huhndorf (2007) reported three subclasses, 16 orders, 54 families, and 912 genera. Kirk (2008) included three subclasses, 15 orders, 64 families, and 1119 genera. Based on extensive molecular phylogenetic studies, the taxonomy of Sordariomycetes has undergone significant changes in recent years (Kirk et al. 2008). Based on morphology and multigene analyses of 18S, nuclear 28S rDNA (28S), the translation elongation factor 1-α (*TEF1*), and the second largest subunit of RNA polymerase II (*RPB2*), Maharachchikumbura et al. (2015) proposed three more subclasses: Diaporthomycetidae, Lulworthiomycetidae, and Meliomycetidae. Diaporthomycetidae includes Diaporthales and its relatives, which were classified in the subclass Sordariomycetidae. Then they (Maharachchikumbura et al. 2016) outlined the class and included six subclasses, 32 orders, 105 families, and 1331 genera. Based on the phylogenetic and molecular clock evidence using the same genes, Hongsanan et al. (2017) treated the subclass Meliolomycetidae as a synonym of Sordariomycetidae and erected the sixth subclass in Sordariomycetes, Savoryellomycetidae, for Conioscyphales, which was assigned to Diaporthomycetidae, as well as Fuscosporellales, Pleurotheciales, and Savoryellales, which were assigned to Hypocreomycetidae. Hyde et al. (2020) raised the seventh subclass, Pisorisporiomycetidae, for Pisorisporiales, which was classified as Lulworthiomycetidae, and accepted seven subclasses, 45 orders, 167 families, and 1499 genera. Wijayawardene et al. (2022) further expanded the class to seven subclasses, 46 orders, 184 families, and 1595 genera.

Sordariomycetidae is a subclass of Sordariomycetes. Members of this subclass are mainly characterized by dark and perithecial ascomata with inoperculate and unitunicate asci. They can be found from both terrestrial and aquatic ecosystems as pathogens, endophytes, and saprotrophs worldwide (Hyde et al. 2020). Eriksson and Winka (1997) established Sordariomycetidae for three orders, Diaporthales, Ophiostomatales, and Sordariales. Huhndorf et al. (2004) added Boliniales, Chaetosphaeriales, and

CONTACT Ning Zhang ningz@rutgers.edu
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Coniochaetales in based on 28S data, but the support for the Sordariomycetidae clade was low. However, Zhang et al. (2006) revealed a monophyletic and well-supported Sordariomycetidae containing Boliniales, Chaetosphaeriales, Coniochaetales, Diaporthales, Ophiostomatales, and Sordariales based on 18S, 28S, *TEF1*, and *RPB2* data. After removing Coniochaetales, Diaporthales, and Ophiostomatales but adding Phyllachorales, Maharachchikumbura et al. (2015) narrowed the subclass with four orders based on 18S, 28S, *TEF1*, and *RPB2* data. Hernández-Restrepo et al. (2015) proposed the order Cordanales based on 28S data. However, Hongsanan et al. (2017) combined it under Coniochaetales and recognized six orders, Boliniales, Chaetosphaeriales, Coniochaetales, Meliolales, Phyllachorales, and Sordariales, based on 18S, 28S, *TEF1*, and *RPB2* data. With the inclusion of Cephalothecales and Pseododactylariales, Hyde et al. (2020) accepted eight orders in Sordariomycetidae using the same genes.

Pine barrens is an understudied fire-prone ecosystem that has acidic, sandy, and oligotrophic soil, with pine, oak, and Ericaceae as dominant plants (Forman 1998). Since 2010, we have been studying fungi in the pine barrens ecosystem and described a number of novel fungal taxa, including a family and several genera (Luo et al. 2015, 2014; Walsh et al. 2021, 2015, 2014). The decade-long data collection demonstrated that plant roots in the pine barrens contain high fungal diversity and are rich in fungi new to science. In this paper, we aim to (i) identify and describe a new fungal lineage uncovered from the highbush blueberry roots in the New Jersey Pine Barrens based on morphology, biology, ecology, and multigene phylogenetic analyses; (ii) determine its taxonomic level within Sordariomycetidae; and (iii) understand its ecological function by plant-fungal interaction experiments.

MATERIALS AND METHODS

Fungal isolation.—On 30 January 2023, the highbush blueberry (*Vaccinium corymbosum*) were collected from the pine barrens ecosystem in New Jersey: New Jersey Pine Plains (39.70570, −74.37407, altitude of 40 m) and the surrounding New Jersey Pine Barrens (39.69727, −74.36865, altitude of 28 m). Apparently healthy root samples, comprising coarse roots and fibrous fine roots, were obtained from the plant root system by excavating it up to a depth of 50 cm in the soil. 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 30s, followed by 2 min in 0.6% sodium hypochlorite (NaOCl; Clorox, Oakland, California), 2 min in 70% ethanol, three consecutive rinses with sterile distilled water, and dried with sterile paper towel. The disinfected segments were transferred to malt extract agar (MEA; BD, Sparks, Maryland) containing antibiotics (25 mg/L chloramphenicol, streptomycin, and ampicillin) and cultured at room temperature. Fungal cultures were isolated and purified by subculturing from emergent hyphal tips.

Morphological study.—Cultural characteristics were recorded from corn meal agar (CMA; BD), MEA, and potato dextrose agar (PDA; BD) at 25 C under 12 h light and dark cycle for 14 days, and the colony colors were named based on Ridgway's color charts (Ridgway 1912). Microscopic examinations, measurements, and photographs were taken from slides of fungi mounted in distilled water by using an Eclipse 80i compound microscope (Nikon, Tokyo, Japan) and a SMZ800 dissecting microscope (Nikon). The specimens examined were deposited in the Rutgers Mycological Herbarium, New Brunswick, New Jersey, USA (RUTPP), and cultures were submitted to the Westerdijk Fungal Biodiversity Institute, the Netherlands (CBS).

DNA extraction, amplification, and sequencing.—The protocols used in Zhang et al. (2011) and Luo and Zhang (2013) were followed for DNA extraction, polymerase chain reaction (PCR) amplification, and sequencing of nuc rDNA internal transcribed spacer region ITS1-5.8S-ITS2 (ITS), partial 18S and 28S regions, and *RPB2*. Sixty-four Sordariomycetidae taxa were included in our phylogenetic analyses. Four taxa of Tracyllalales and Vermiculariopsiellales that have uncertain status but are closely related to Sordariomycetidae were also included. *Cryptosporella hypodermia* and *Plagiostoma aesculi*, two Diaporthales taxa of Diaporthomycetidae, were chosen as outgroup. Taxon information including names, isolate numbers, substrates, locations, and GenBank accession numbers is given in TABLE 1.

Sequence alignment and phylogenetic analyses.—A preliminary phylogeny based on 18S and 28S was generated to infer phylogenetic placement of our collections at the subclass level within the class Sordariomycetes. Another phylogeny based on ITS, 28S, and *RPB2* was used to infer their relationships to taxa within the subclass Sordariomycetidae.

The data set for each gene were aligned with MAFFT 7 (Katoh et al. 2019) and edited manually with MEGA11 (Tamura et al. 2021), if needed. Phylogenetic analyses of

Table 1. Taxonomic names, isolate numbers, substrates, locations, and GenBank accession numbers of the fungi in this study.

Taxon	Isolate number	Substrate	Location	GenBank accession numbers			
				18S	ITS	28S	*RPB2*
Achroceratosphaeria potamia	JF08139	NA	NA	GQ996541	NA	GQ996538	NA
Albertiniella polyporicola	CBS457.88	*Ganoderma applanatum*	Germany	AF096170	LT633939	LT633939	LT634061
Amesia atrobrunnea	CBS379.66	Moldy mattress	Solomon Islands	NA	MH858833	MH870470	KX976798
Amphisphaeria umbrina	AFTOL-ID1229	*Quercus petraea*	Austria	FJ176809	NA	FJ176863	NA
Annulatascus velatisporus	MFLUCC16-1441	NA	Australia	KY244032	NA	KY244031	NA
Apiorhynchostoma curreyi	UAMH11088	NA	NA	NG_065685	NR_120207	NG_042715	KY931926
Ascotaiwania lignicola	NIL00005	NA	NA	HQ446284	NA	HQ446364	NA
Ascovaginospora stellipala	P5-13A	NA	NA	U85087	NA	U85088	NA
Asteridiella obesa	VIC31239	*Balfourodendron riedelianum*	Brazil	NG_065009	NR_120256	NG_057014	NA
Barrina polyspora	AWR9560A	NA	NA	NA	NA	AY346261	NA
Bionectria ochroleuca	AFTOL-ID187	NA	USA	DQ862044	NA	DQ862027	NA
Bombardia bombarda	AFTOL-ID967	NA	NA	DQ471021	NA	DQ470970	DQ470923
Botryotinia fuckeliana	AFTOL-ID59	NA	NA	AY544695	NA	AY544651	NA
Botryotrichum verrucosum	CBS116.64	Salt-marsh soil, mature dunes	England	NA	NR_168165	LT993567	LT993486
Brunneodinemasporium jonesii	GZCC16-0050	Decaying wood	China	NA	NR_166384	NG_068242	NA
Camarops tubulina	SMH4614	NA	Denmark	NA	NA	AY346266	AY780157
Caudatispora biapiculata	SMH1873	Decaying petioles of *Prestoea* sp.	USA	NA	NA	AY346269	NA
Cercophora ambigua	CBS215.60	Decorticated twig	Canada	NA	AY999137	AY999114	NA
Chaetomium globosum	CBS160.62	Compost	Germany	NA	KT214565	MH869713	KT214666
Chaetomium tenue	CBS139.38	NA	NA	NA	MH855933	MH867433	KT214669
Chaetosphaeria aquatica	MFLUCC18-1618	Submerged wood	China	MK834748	MK828639	MK835841	MN156524
Cladorrhinum foecundissimum	CBS180.66	Soil	Netherlands	NA	MH858767	NG_073585	MK876818
Clohiesia corticola	HKUCC3712	Submerged wood	Australia	NA	NA	AF132329	NA
Clypeosphaeria uniseptata	HKUCC6349	NA	NA	DQ810255	NA	DQ810219	NA
Coccodiella miconiae	ppMP1342	*Miconia* sp.	Panama	KX451871	MF460365	KX430506	NA
Cocoicola californica	F59034	*Washingtonia robusta*	USA	KX451866	KX451918	KX430468	KX451958
Coniochaeta luteoviridis	CBS206.38	Butter	NA	NA	NR_154769	NG_067348	NA
Coniochaeta navarrae	LTA3/CBS141016	*Ulmus* sp.	Spain	NA	KU762326	KU762326	KU762329
Conioscyphascus varius	CBS113653	NA	NA	AY484511	NA	AY484512	NA
Cordana aquatica	MFLUCC18-1625	Submerged wood	China	NA	MK828602	MK835799	MN156529
Cordana inaequalis	CBS508.83	Decaying wood of *Carya ovata*	Canada	NA	NR_145363	NG_067424	NA
Cordana lignicola	MFLUCC18-1624	Submerged wood	China	MK834761	MK828600	MK835797	MN156527
Corynascus sepedonium	CBS111.69	Soil	India	NA	MH859271	MH871003	HQ871827
Cryphonectria parasitica	CMW7048/ATCC48198	NA	NA	JN938760	NA	JN940858	NA
Cryptendoxyla hypophloia	CBS508.70	*Acer saccharum*, wood, under bark	Canada	NA	MH859822	MH871593	NA
Cryptosporella hypodermia	CBS171.69	Dead twigs of *Ulmus campestris*	Netherlands	DQ862049	MH859289	MH871020	DQ862018
Delonicicola siamense	MFLUCC15-0670	NA	NA	NG_061278	NA	NG_059172	NA
Diaporthe maritima	DAOM695742	NA	NA	NG_065078	NA	NG_069330	NA
Diatrype disciformis	AFTOL-ID927	NA	NA	DQ471012	NA	DQ470964	NA
Doratomyces stemonitis	AFTOL-ID1380	NA	NA	DQ836901	NA	DQ836907	NA
Endomeliola dingleyae	PDD98304	*Coprosma robusta*	New Zealand	NA	GU138865	GU138866	NA
Endoxyla operculata	UAMH11085	NA	NA	KY931895	JX460987	JX460992	KY931927
Falcocladium thailandicum	CBS121717	Leaves of *Eucalyptus camaldulensis*	Thailand	JF831930	NA	JF831934	NA
Fragosphaeria purpurea	CBS133.34	Wood of *Fagus sylvatica*	United Kingdom	AF096176	NA	AF096191	NA
Gondwanamyces capensis	AFTOL-ID1907	NA	NA	FJ176834	NA	FJ176888	NA
Graphostroma platystoma	AFTOL-ID1249	NA	NA	DQ836900	NA	DQ836906	NA
Helminthosphaeria hyphodermae	SMH4192	NA	NA	NA	NA	KF765608	NA
Humicola cuyabenoensis	CBS398.97	NA	Ecuador	NA	NR_160060	MH874258	LT993492
Humicola fuscoatra	CBS118.14	Soil	Norway	AY706333	LT993579	LT993579	KX976882
Hypocrea rufa	DAOMJBT1003	NA	NA	JN939042	NA	JN938865	NA
Lasiosphaeria ovina	SMH1538	NA	USA	NA	AY587926	AF064643	AY600287
Leptosporella arengae	MFLUCC15-0330	Dead rachis of *Arenga pinnata*	Thailand	MG366594	MG272255	MG272246	MG272260

(*Continued*)

Table 1. (Continued).

Taxon	Isolate number	Substrate	Location	GenBank accession numbers			
				18S	ITS	28S	*RPB2*
Leptosporella elaeidis	SRWD14b	Dead rachis and petioles of *Elaeis guineensis*	Thailand	MK659774	MK659767	MK659772	NA
Lindra thalassiae	AFTOL-ID413	NA	NA	DQ470994	NA	DQ470947	NA
Linocarpon arengae	MFLUCC15-0331	Dead rachis of *Arenga pinnata*	Thailand	MG272252	MG272257	MG272247	NA
Lulworthia fucicola	ATCC64288	NA	NA	AY879007	NA	AY878965	NA
Magnaporthiopsis poae	M47	*Poa pratensis*	USA	JF414860	NA	JF414885	NA
Meliola centellae	VIC31244	*Centella asiatica*	Brazil	NA	NR_137799	JQ734545	NA
Monilochaetes infuscans	CBS869.96	*Ipomoea batatas*	South Africa	GU180620	NA	GU180639	NA
Neolinocarpon rachidis	MFLUCC15-0814a	Dead petioles of *Cocos nucifera*	Thailand	MK106367	MK106342	MK106353	NA
Neonawawia malaysiana	CBS125544	Leaves of *Eucalayptus urophylla*	Malaysia	NA	NR_168754	NG_068512	NA
Neurospora sitophila	CBS112.19	Bread	Europe	NA	MH854676	MH866192	NA
Ophioceras commune	M91	Rotten wood	China	JX134661	NA	JX134687	NA
Ophiostoma piliferum	AFTOL-ID910	NA	NA	DQ471003	NA	DQ470955	NA
Pararamichloridium verrucosum	CBS128.86	Old fungi on leaf of *Bambusa* sp.	Andhra Pradesh, India	NG_065540	NA	NG_057768	NA
Phialemonium atrogriseum	CBS604.67	Noodles	Ukraine	NG_065691	MH859062	NG_057883	LT633959
Phialemonium inflatum	CBS259.39	*Apis mellifera* (worker bee), abdomen, adult	USA	NG_065530	NR_165996	HE610464	LT633974
Phialemonium obovatum	CBS279.76	Man, systemic mycotic infection in a 6-month-old burned child	USA	NG_065692	NR_165935	NG_057631	LT634006
Phyllachora graminis	TH-544	*Dichanthelium viscidellum*	Panama	KX451873	NA	KX430508	NA
Pinibarrenia chlamydospora	PB23.R23	Roots of *Vaccinium corymbosum*	USA	**PP340160**	**PP192098**	**PP192108**	**PP198911**
Pinibarrenia chlamydospora	PB23.R32	Roots of *Vaccinium corymbosum*	USA	**PP340161**	**PP192099**	**PP192107**	**PP198912**
Pinibarrenia chlamydospora	PB23.R41	Roots of *Vaccinium corymbosum*	USA	**PP340162**	**PP192101**	**PP192109**	**PP198914**
Pinibarrenia chlamydospora	RB23.R23	Roots of *Vaccinium corymbosum*	USA	**PP340163**	**PP192102**	**PP192105**	**PP198915**
Pinibarrenia chlamydospora	RB23.R34	Roots of *Vaccinium corymbosum*	USA	**PP340164**	**PP192100**	**PP192106**	**PP198913**
Pinibarrenia chlamydospora	RB23.R45	Roots of *Vaccinium corymbosum*	USA	**PP340165**	**PP192103**	**PP192104**	**PP198916**
Pisorisporium cymbiforme	PRM924377	NA	NA	KM588901	NA	KM588904	NA
Plagiostoma aesculi	CBS109765	*Aesculus hippocastanum*	Austria	DQ836899	DQ323530	MH874424	DQ836892
Pleurostoma repens	CBSH7594	Pine lumber	USA	AY761067	NA	AY761078	NA
Pleurotheciella rivularia	CBS125238	Wood of *Hedera helix* submerged in a stream	France	NG_061124	NA	NG_057950	NA
Podospora fimicola	CBS482.64	Dung of cow	Switzerland	NA	MH858488	MH870122	KP981623
Pseudodactylaria fusiformis	MFLUCC20-0085	Submerged decaying bamboo culms	China	MT184897	MT184905	MT184906	MT188555
Pseudodactylaria xanthorrhoeae	CBS143414	*Xanthorrhoea* sp.	Australia	NA	NR_156669	NG_058521	NA
Pseudolachnella brevicoronata	HHUF30119	*Sasa* sp.	Japan	NA	NR_154277	NG_059408	NA
Pseudovalsaria ferruginea	UAMH11490	NA	NA	NA	JX460988	JX460993	NA
Pyxidiophora arvernensis	AFTOL-ID2197	NA	NA	FJ176839	NA	FJ176894	NA
Rubellisphaeria abscondita	CBS132078	NA	NA	NG_061221	NA	NG_058203	NA
Savoryella lignicola	NF00204	NA	NA	HQ446300	NA	HQ446378	NA
Sordaria fimicola	SMH4106	NA	USA	NA	NA	AY780079	AY780194
Spadicoides atra	CBS489.77	Branch of *Quercus petraea*	Czech Republic	EF204521	NA	EF204506	NA
Synaptospora plumbea	SMH3962	NA	NA	NA	NA	KF765621	NA
Tirisporella beccariana	BCC38312	NA	NA	JQ655457	NA	JQ655449	NA
Torpedospora radiata	AFTOL-ID751	NA	NA	DQ470999	NA	DQ470951	NA
Tracylla aristata	CBS141404	Leaves of *Eucalyptus regnans*	Australia	NA	OL654129	OL654186	NA
Tracylla eucalypti	CBS144429	Leaves of *Eucalyptus urophylla*	Colombia	NA	OL654130	OL654187	NA
Triangularia bambusae	CBS352.33	Shoots of *Bambusa* sp.	NA	NA	MH855456	MH866923	MK876830
Vermiculatiosiella australiensis	CBS141499	Leaves of *Grevillea* sp.	Australia	NA	KX306772	KX306797	ON399361
Vermiculatiosiella eucalypticola	CBS143442	Leaves of *Eucalyptus dalrympleana*	Australia	NA	MG386070	MG386123	ON399368
Xylaria acuta	AFTOL-ID63	NA	NA	AY544719	NA	AY544676	NA
Zopfiella tabulata	CBS230.78	Dung of porcupine	Canada	NA	MK926854	MK926854	MK876816

Note. AFTOL = Assembling the Fungal Tree of Life project; ATCC = American Type Culture Collection, Manassas, Virginia, USA; CBS = Westerdijk Fungal Biodiversity Institute, Utrecht, the Netherlands; DAOM = Canadian National Mycological Herbarium, Ottawa, Canada; F = Field Museum, Chicago, Illinois, USA; GZCC = Guizhou Culture Collection, Guiyang, China; HKUCC = University of Hong Kong Culture Collection, Hong Kong, China; MFLUCC = Mae Fah Luang University Culture Collection, Chiang Rai, Thailand; PDD = New Zealand Fungal Herbarium, Auckland, New Zealand; PRM = National Museum Prague of Czech Republic, Prague, Czech Republic; SMH = Sabine M. Huhndorf, Field Museum of Natural History, Chicago, Illinois; UAMH = University of Alberta Microfungus Collection and Herbarium, Toronto, Ontario, Canada; VIC = Universidade Federal de Vicosa, Departamento de Biologia Vegetal, Viçosa, Brazil. Numbers in boldface indicate new sequences from this study.

individual genes and the concatenated three-gene data set were conducted. Genealogical concordance was evaluated with Shimodaira-Hasegawa test (Shimodaira and Hasegawa 1999) and approximately unbiased test (Shimodaira 2002). jModelTest 2.1.10 (Darriba et al. 2012) was used to specify the best-fit nucleotide substitution model under the Bayesian information criterion (BIC). Maximum likelihood (ML) analysis with the selected model was carried out in RAxML 8.1.1 (Stamatakis 2014). Branch support (BS) was assessed with 1000 bootstraps replicates. Bayesian inference (BI) was conducted with the Markov chain Monte Carlo method in MrBayes 3.2.6 (Ronquist et al. 2012) under the selected nucleotide substitution model. Trees were sampled every 1000 generations from 10 000 000 generations. After discarding the first 25% of the trees as the burn-in, the remaining 75% of the trees were used to determine the posterior probability (PP) values. Strong branch support was defined as BS ≥75% from ML and PP ≥0.95 from BI. The sequences obtained in this study and the alignments generated in the phylogenetic analyses were deposited in GenBank (TABLE 1) and TreeBASE (submission number 31122, 31516), respectively. Based on the same data sets used for the phylogeny, sequence similarity analyses were also carried out to evaluate the relationships between orders within Sordariomycetidae. Similarity matrices across 11 orders of Sordariomycetidae were generated by using the average pairwise distance values from all representative taxa of each order in MEGA11 (Tamura et al. 2021).

Plant-fungal interaction experiment.—Three strains of *Pinibarrenia chlamydospora* (PB23.R23, RB23.R23, and RB23.R45) were used in the plant-fungal interaction experiment. *Arabidopsis thaliana* seeds (CS70000; Arabidopsis Biological Resource Center, The Ohio State University, xx, Ohio) were surface-sterilized with 3% NaOCl for 10 min, rinsed 10 times with sterile distilled water, and dried with sterile paper towel. On each MEA plate with 1-week-old fungal culture, 10 surface-sterilized seeds were placed with equal distance on the edge of the fungal colony. Blank MEA plate was used as the negative control. Three plates were used for each treatment, which yielded 30 replicates in total. The seed-fungal cultures were then incubated at 25 C under 12 h light and dark cycle for 14 days.

RESULTS

Phylogenetic analyses.—The 18S and 28S phylogeny (SUPPLEMENTARY FIG. 1) supported that our collections belong to the subclass Sordariomycetidae; thus, a further study focused on Sordariomycetidae was conducted. The genealogical concordance tests indicated that ITS, 28S, and *RPB2* are congruent ($P > 0.05$); thus, they were concatenated. Three single-gene ML phylogenetic trees are provided in SUPPLEMENTARY FIGS. 2–4. The three-gene data set comprised 3350 characters, among which there were 890 nucleotide characters including gaps in the ITS alignment, 1331 in 28S, and 1129 in *RPB2*. SYM+FQ+I was selected as the best nucleotide substitution model for ITS, TN+F+R4 for 28S, and TIM3+F+I+R4 for *RPB2*. The phylogenetic trees were reconstructed using ML and BI methods with partitioned models. Both trees showed similar topology, and only the ML tree is presented in FIG. 1. Our collections grouped with Chaetosphaeriales, Boliniales, Sordariales, Pseudodactylariales, Coniochaetales, Cephalothecales, Phyllachorales, and Meliolales, suggesting that they belong to Sordariomycetidae. The orders Tracyllales and Vermiculariopsiellales also grouped into this clade, which indicated their placement in the subclass. Our collections formed a well-supported clade, which is distinct from other Sordariomycetidae orders.

Sequence distance comparison.—Sequence distance comparisons were carried out using ITS, 28S, and combined gene data (ITS, 28S, and *RPB2*). The pairwise similarities of ITS sequences between our collections and the 10 orders of Sordariomycetidae (Boliniales, Cephalothecales, Chaetosphaeriales, Coniochaetales, Meliolales, Phyllachorales, Pseudodactylariales, Sordariales, Tracyllales, and Vermiculariopsiellales) ranged from 67.17% to 83.76%, whereas the similarities among these established orders are from 62.01% to 84.06% (SUPPLEMENTARY TABLE 1). The pairwise similarities of 28S sequences between our collections and the 10 orders ranged from 80.01% to 93.92%, whereas the similarities between these orders are from 76.28% to 94.45% (SUPPLEMENTARY TABLE 1). In addition, combined gene data also had similar values between our collections and the 10 orders (75.24%–87.32%) compared with the values among those established orders (72.15%–89.05%).

The ITS sequence similarities among the new collection isolates were 99% to 100%. The within-group phylogenetic relationships of our collections also showed incongruity among different single-gene trees, indicating the occurrence of recombination. Based on the genealogical concordance phylogenetic species recognition (Taylor et al. 2000), and the high ITS sequence similarity, these new collections likely are conspecific. Based on the phylogenetic analyses, sequence divergent

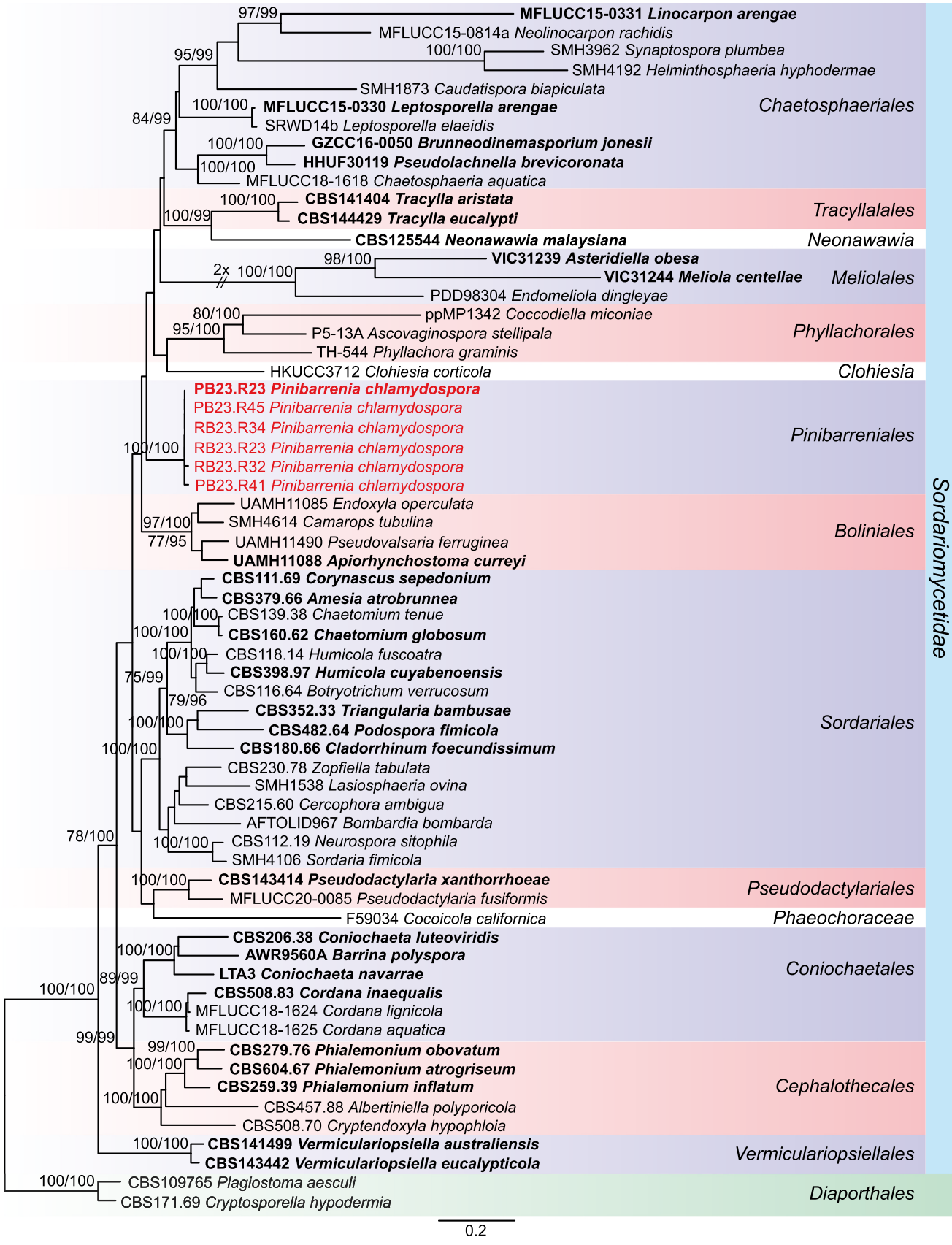

Figure 1. The maximum likelihood tree inferred from the combined ITS, 28S, and *RPB2* sequence data sets. Strong branch support is defined and noted when ML bootstrap proportion (MLBP) is ≥75%, and BI posterior probability (BIPP) ≥0.95. New species proposed in this study are in red. Ex-type strains are in bold.

comparison, and morphological and ecological characteristics, a new species, a new genus, a new family, and a new order were proposed.

Inoculation experiment.—The fungus had a noticeable inhibiting effect on the seedling's growth (FIG. 2). Compared with the control, *Arabidopsis thaliana* seeds inoculated with PB23.R23, RB23.R23, and RB23.R45 exhibited significantly lower germination rates (FIG. 3).

TAXONOMY

Pinibarreniales J. Luo & N. Zhang, ord. nov.
MycoBank MB852094

Morphological description: Asexual state hyphomycetous. Hyphae branched, septate. Conidiophores micronematous, simple, unbranched to branched, septate. Conidiogenous cells phialidic. Conidia ellipsoidal, aseptate, straight to curved. Chlamydospores terminal or intercalary, usually in long chains or clusters, ovoid to subglobose. Sexual state unknown.

Pinibarreniaceae J. Luo & N. Zhang, fam. nov.
MycoBank MB852095

Morphological description: Asexual state hyphomycetous. Hyphae branched, septate, smooth. Conidiophores micronematous, simple, erect, unbranched to branched, septate. Conidiogenous cells erect, terminal, phialidic, cylindrical to ampulliform. Conidia ellipsoidal, aseptate, straight to curved. Chlamydospores terminal or intercalary, usually in long chains or clusters, ovoid to subglobose, smooth, thick-walled. Sexual state unknown.

Pinibarrenia J. Luo & N. Zhang, gen. nov.
MycoBank MB852096

Morphological description: Asexual state hyphomycetous. Hyphae branched, septate, hyaline to light brown, smooth. Conidiophores micronematous, simple, solitary, erect, unbranched to branched, cylindrical to subcylindrical, septate, hyaline. Conidiogenous cells erect, terminal, phialidic, cylindrical to ampulliform, without a conspicuous collarette. Conidia ellipsoidal, aseptate, straight to curved, hyaline, smooth. Chlamydospores terminal or intercalary, usually in long chains or clusters, ovoid to subglobose, hyaline to light brown, smooth, thick-walled. Sexual state unknown.

Type species: Pinibarrenia chlamydospora J. Luo & N. Zhang, sp. nov.

Etymology: The generic name refers to the pine barrens ecosystem where the fungus was uncovered.

Pinibarrenia chlamydospora J. Luo & N. Zhang, sp. nov. FIG. 2
MycoBank MB852097

Typification: USA. NEW JERSEY: New Jersey Pine Plains (39.70570, −74.37407, altitude of 40 m), from roots of *Vaccinium corymbosum*, 30 Jan 2023, *J. Luo* (holotype RUTPP-PB23.R23). Ex-type culture PB23.R23 = NRRL 64832.

Etymology: The specific epithet refers to chlamydospores of the fungus.

Morphological description: On CMA, hyphae branched, septate, hyaline to light brown, smooth, 2–4 µm (mean = 3 µm, n = 30) diam. Conidiophores simple, solitary, erect, straight or curved, unbranched or sparsely branched, 1–5-septate (mean = 3-septate, n = 50), hyaline, smooth, 5.5–21.5 × 1.5–3.5 µm (mean = 15 × 2.5 µm, n = 50). Conidiogenous cells phialidic, cylindrical to ampulliform, erect, terminal, straight, hyaline, smooth, 5.5–13.5 × 1.5–3 µm (mean = 10 × 2 µm, n = 50). Conidia aggregated in slimy heads, ellipsoidal, straight or slightly curved, aseptate, hyaline, smooth, 2–4.5 × 1–3 µm (mean = 3.5 × 2.5 µm, n = 50), 1–3.5 µm (mean = 2.5 µm, n = 50) wide at the base, 0.5–1.5 µm (mean = 1 µm, n = 50) wide near the apex. Chlamydospores terminal or intercalary, usually in long chains or clusters, ovoid to subglobose, hyaline to light brown, smooth, thick-walled, 4.5–10 × 3.5–9 µm (mean = 8.5 × 6.5 µm, n = 50). Sexual state unknown.

Cultural characteristics: Colonies on CMA reaching 2.5 cm after 7 days in dark at 25 C; surface flat, marguerite yellow; aerial mycelium sparse; reverse ivory yellow. Colonies on MEA 2.6 cm diam after 7 days at 25 C in dark; surface felty to floccose, cream buff; aerial mycelium yellowish; reverse chamois. Colonies on PDA 2.8 cm diam after 7 days at 25 C in dark; surface felty to floccose, colonial buff; aerial mycelium yellowish; reverse honey yellow.

Additional materials examined: USA. NEW JERSEY: New Jersey Pine Plains (39.70570, −74.37407, altitude of 40 m), from roots of *Vaccinium corymbosum*, 30 Jan 2023, *J. Luo, RUTPP-PB23.R32, RUTPP-PB23.R41*; New Jersey Pine Barrens (39.69727, −74.36865, altitude of 28 m), from roots of *Vaccinium corymbosum*, 30 Jan 2023, *J. Luo, RUTPP-RB23.R23, RUTPP-RB23.R34, RUTPP-RB23.R45*.

Hosts/substrates: From roots of *Vaccinium corymbosum*.

Distribution: USA (New Jersey).

Notes: This taxon is characterized in the subclass Sordariomycetidae by having abundant, ovoid to subglobose, and light-colored chlamydospores, phialophora-like conidia, simple and hyaline conidiophores, phialidic conidiogenous cells without a collarette, and on Ericaceae

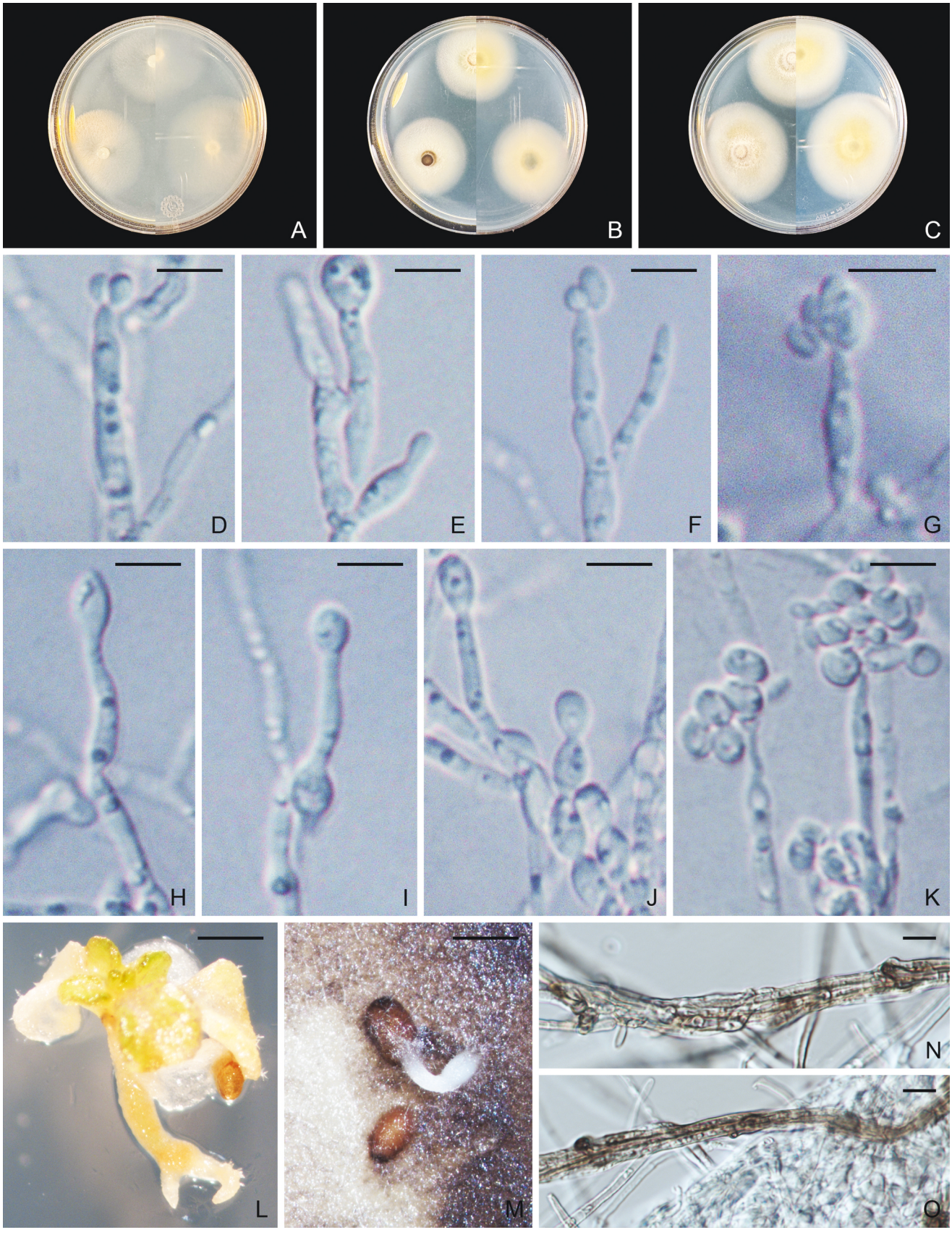

Figure 2. *Pinibarrenia chlamydospora* (PB23.R23). A–C. Colonies (front and reverse views) on CMA, MEA, and PDA after 2 weeks. D–G. Conidiophores and conidia. E, H–K. Chlamydospores. L–M. An *Arabidopsis thaliana* (CS70000) seed (the control) and the seeds treated with the fungus (PB23.R23) after 2 weeks. N–O. Fungal hyphae on inoculated *Arabidopsis thaliana*. Bars: D–G = 5 µm; H–K, N–O = 10 µm; L–M = 1000 µm.

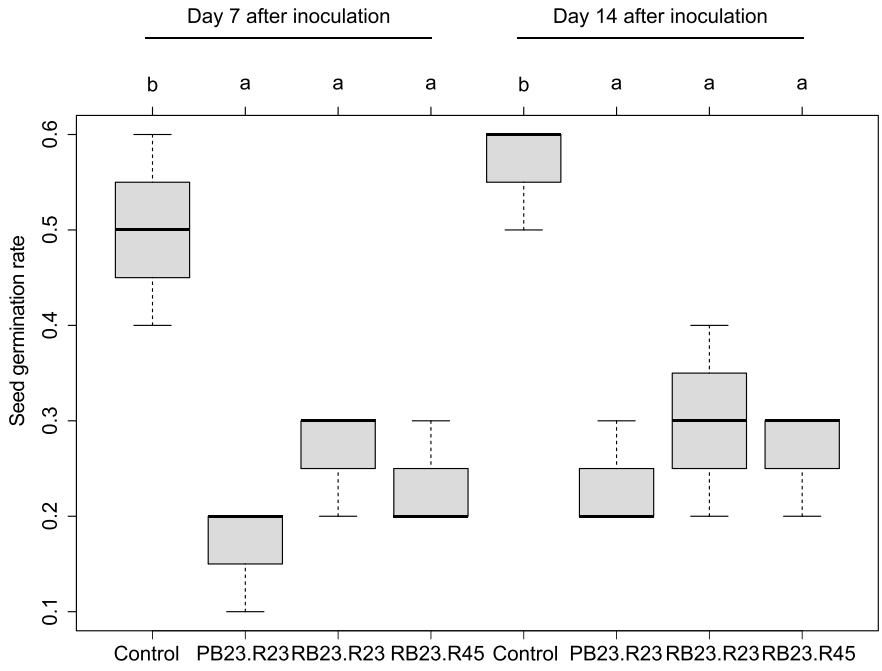

Figure 3. Seed germination rates for control and *Pinibarrenia chlamydospora* (isolates PB23.R23, RB23.R23, and RB23.R45) treated *Arabidopsis thaliana* (CS70000) seeds at day 7 and day 14 after inoculation (n = 30).

host. Moreover, it has ≤83.76% sequence similarity in ITS or ≤93.92% sequence similarity in 28S to any described Sordariomycetidae orders. Phylogenetically, it showed some affinities to Phyllachorales and Boliniales. However, Phyllachorales differ by their dark black stromata, heavily melanized perithecia, deliquescent paraphyses, pycnidial asexual states, such as *Linochora*, angiosperm leaf or stem habitats, and 27.5% sequence dissimilarity in ITS and 12.01% sequence dissimilarity in 28S (Mardones et al. 2017; this study); Boliniales differs by its stromatic and perithecial ascomata, carbonaceous ascomatal wall, small asci, ellipsoidal and flattened ascospores with apical germ pores absence of asexual state, bark and wood habitats, and 20.01% sequence dissimilarity in ITS and 6.08% sequence dissimilarity in 28S (Untereiner et al. 2013; this study).

DISCUSSION

Delimitation of Sordariomycetidae has not been stable. Based on 18S, 28S, *TEF1*, and *RPB2*, Maharachchikumbura et al. (2015) found that Boliniales, Cephalothecales, Chaetosphaeriales, Phyllachorales, Sordariales, and Meliolales grouped together but the support was not strong. Their following study revealed that the same six orders as well as Coniochaetales grouped with low support (Maharachchikumbura et al. 2016). Based on the same genes, Luo et al. (2019) and Hyde et al. (2020) found similar results that Boliniales, Cephalothecales, Chaetosphaeriales, Coniochaetales, Phyllachorales, Sordariales, and Meliolales as well as Pseudodactylariales, Tracyllales, and Vermiculariopsiellales were closely related but the statistical support still was low. Based on 18S, 28S, *RPB1* (the largest subunit of RNA polymerase II), and *RPB2*, Wijayawardene et al. (2022) reconstructed a well-supported monophyletic group comprising these 10 orders. Our findings further supported this group representing Sordariomycetidae, and we proposed the inclusion of Pinibarreniales, Tracyllales, and Vermiculariopsiellales in Sordariomycetidae. Morphological and ecological features of Pinibarreniales also support that they belong to Sordariomycetidae, e.g., hyphomycetous asexual state, simple and hyaline conidiophores, endophytic lifestyle, and terrestrial habitat. Unlike other Sordariomycetidae having hyphomyceteous asexual state, Tracyllales have coelomycetous asexual state (Crous et al. 2018). However, their phialidic conidiogenesis and endophytic, saprobic, and pathogenic lifestyles on leaves of grasses and trees indicated the placement in Sordariomycetidae. Vermiculariopsiellales is an asexual order represented by phialidic dematiaceous hyphomycetes (Hernández-Restrepo et al. 2022; Réblová and Nekvindová 2023). Their saprobic lifestyle on leaf and twig litters and chloridium-like asexual state fit the concept of Sordariomycetidae.

Another challenge for the classification of Sordariomycetidae is that the relationships among the orders in the subclass remain obscure. Our results suggested that Cephalothecales, Tracyllales, and Coniochaetales formed a monophyletic clade.

Wijayawardene et al. (2022) also found that Cephalothecales and Coniochaetales were close but distant from Tracyllales. Cephalothecales and Coniochaetales share saprobic and pathogenic lifestyles on plants and humans, mostly with hairy ascomata, and phialophora-like asexual state (Damm et al. 2010; Hyde et al. 2020). However, the phylogeny generated from this study, like those from other multigene studies (Hongsanan et al. 2017; Hyde et al. 2020; Wijayawardene et al. 2022), cannot resolve the

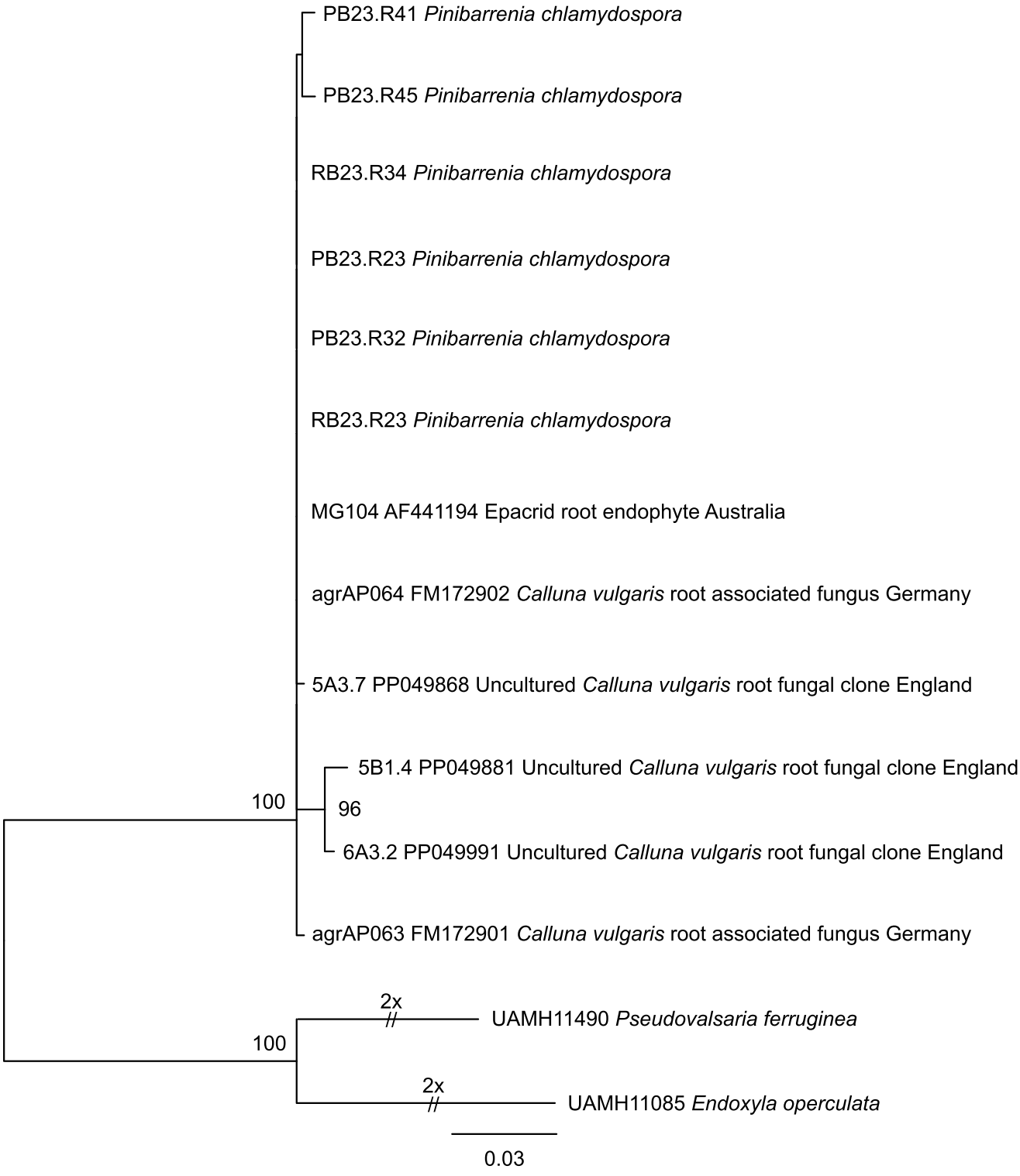

Figure 4. The maximum likelihood tree inferred from the ITS sequences of *Pinibarrenia chlamydospora* and closely related environmental sequences from GenBank. MLBPs ≥75% are shown at branches.

relationships among other orders due the lack of strong branch supports. It was estimated that at least 20 unlinked genes or 8000 randomly selected orthologous nucleotides are required to reconstruct a robust systematic framework (Rokas et al. 2003). Recent phylogenomic study at the genome scale has greatly expanded our knowledge of Sordariomycetes (Hensen et al. 2023). Chen et al. (2023) conducted a phylogenomic investigation of three Sordariomycetidae orders, identifying that Coniochaetales was the basal lineage while Phyllachorales and Sordariales were later-diverging lineages. More taxon sampling in phylogenomic analysis is needed to infer a more robust classification of Sordariomycetes.

The new order status of Pinibarreniales was based on the multigene phylogeny and the ITS and 28S sequence distance/similarity comparisons with other taxa in Sordariomycetidae. In fungal systematics, pairwise sequence similarity is a useful tool for ranking taxa (Schoch et al. 2012). Tedersoo et al. (2014, 2017) suggested 80% ITS similarity as delimitation threshold at the order level for soil fungi. Vu et al. (2019) proposed 81.2% ITS sequence similarity and 94.7% 28S sequence similarity as delimitation threshold at the order level for filamentous fungi. The pairwise similarities of ITS sequences among the 10 established orders in Sordariomycetidae range from 62.01% to 84.06%, whereas the similarities between our collections and the 10 orders are from 67.17% to 83.76%, which fall into the range of order-level distance/similarity. The 28S sequence similarities between our collections and the 10 Sordariomycetidae orders (76.28%–94.45%) are also comparable to the order-level distinction. Because fungi are highly diverse, we do not expect that all fungal lineages will fit a universal threshold. Therefore, our taxonomic treatment here is based on a variety of evidence, including morphology, ecology, molecular phylogeny, as well as sequence similarity.

The GenBank BLAST results and phylogenetic analyses (FIG. 4) indicated that Pinibarreniales fungi likely have a wide distribution. Seventy-three environmental ITS sequences in GenBank had 97% to 99% identities to that of *Pinibarrenia*, for example, AF441194 from *Woollsia pungens* root growing in sandy soil in Australia; FM172901 and FM172902 from *Calluna vulgaris* root in a raise bog in Germany; PP049868, PP049881, and PP04999 from *Calluna vulgaris* root in a heathland in southern England. Interestingly, the host plants of these samples are all in Ericaceae, and their habitats are similar to the edaphic conditions of the New Jersey Pine Barrens where *Pinibarrenia* were isolated. These observations indicate that *Pinibarrenia* may represent a lineage of fungi that have formed association with Ericaceae plants and adapted to acidic and oligotrophic environments.

In addition to *Pinibarrenia*, we have uncovered a number of other root-associated fungi from the pine barrens ecosystem that favor acidic and oligotrophic conditions. Plant-fungal interaction experiments indicated that they may have different effects on plants. For example, *Acidomelania panicicola* and *Barrenia panicia* (*Leotiomycetes*) from the wild switchgrass root promoted switchgrass root hair growth under low-nutrient conditions (Walsh et al. 2015, 2014); *Pseudophialophora angusta, P. eragrostis, P. magnispora, P. schzachyrii, P. tarda*, and *P. whartonensis* (Sordariomycetes) had negative effects on the growth of switchgrass seedlings (Luo et al. 2015); *Pygmaeomyces thomasii* and *P. pinuum* (*Mucoromycetes*) had no significant effect on the growth of switchgrass but the inoculated *Kalmia* seedlings had root nodules and darker green–colored leaves than the control plants (Walsh et al. 2021). The *Pinibarrenia* cultures were isolated from apparently healthy blueberry plants, but the *Arabidopsis* interaction experiment indicated that it is a potential plant pathogen. A pathogenic lifestyle was also seen in certain fungi that were isolated as endophytes from symptomless roots in other studies (Collinge et al. 2022; Manzotti et al. 2020). This type of fungi may evolve to accommodate both lifestyles. They may have a stage in their life cycle when they live mutualistically in the host as endophytes, but under particular situations, depending on the plant and its biotic habitats, they can convert to pathogenicity for their own benefits. Further plant-fungal interaction experiments are needed to have a better understanding of their functions in nature.

DISCLOSURE STATEMENT

No potential conflict of interest was reported by the author(s).

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ORCID

Ning Zhang http://orcid.org/0000-0002-2680-1354

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