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(54) Title: COMPOSITIONS AND METHODS THEREOF INCREASING PLANT GROWTH AND RESISTANCE TO ENVIRONMENTAL STRESS

(57) Abstract: Compositions and methods for enhancing plant growth and resistance to adverse abiotic conditions are disclosed.

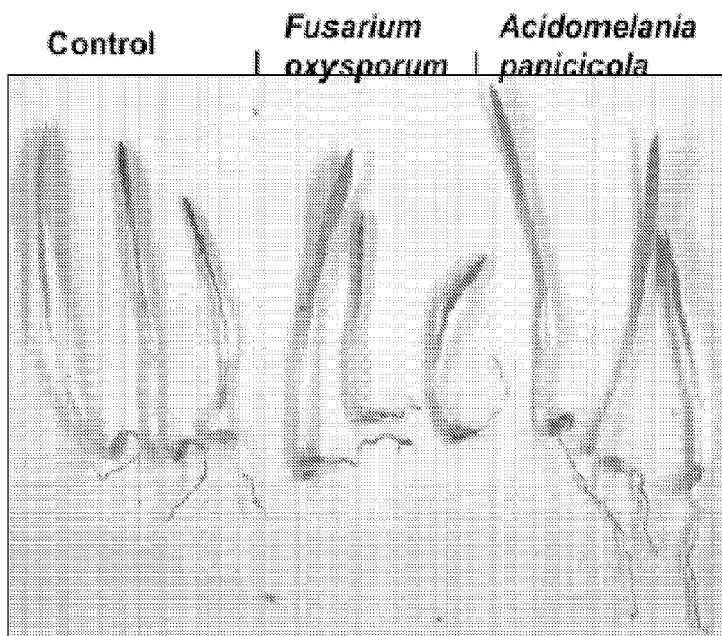


Figure 10



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**COMPOSITIONS AND METHODS THEREOF FOR INCREASING PLANT  
GROWTH AND RESISTANCE TO ENVIRONMENTAL STRESS**

**NING ZHANG**

**CROSS REFERENCE TO RELATED APPLICATIONS**

5           This application claims priority to US Provisional Application No. 62/047,226 filed  
September 8, 2014, the entire contents being incorporated herein by reference.

**FIELD OF THE INVENTION**

          This invention relates to the fields of agriculture and propagation of plants under  
abiotic stress conditions. More specifically, the invention provides methods and microbial  
10   based compositions which facilitate improved plant growth and stress tolerance.

**BACKGROUND OF THE INVENTION**

          Several publications and patent documents are referenced throughout this application  
in order to more fully describe the state of the art to which this invention pertains. The  
disclosure of each of these publications and patent documents is incorporated by reference  
15   herein.

          Pine barrens (pinelands) comprise a unique type of eco-system that is oligotrophic,  
and both drought- and fire-prone. Pine barrens occur throughout northeastern USA from New  
Jersey to Maine (Forman et al. 1998). Pines and oaks are the most common trees in pine  
barrens, while the understory is composed of grasses (Poaceae), sedges (Cyperaceae),  
20   blueberries and other members of heath family (Ericaceae). The largest and most uniform  
area of pine barrens in the United States is the 1.4 million acre pine barrens of New  
Jersey, where the soil is highly acidic, sandy and nutrient poor.

          Dark septate endophytes (DSE) refer to a group of heterogeneous plant root-  
colonizing ascomycetes that produce melanized, septate hyphae. They have been isolated  
25   from over 110 plant families that grow in various environments (Knapp et al. 2012). The best  
studied DSE are the *Phialocephala fortinii*-*Acephala applanata* complex (PAC), a group of  
asexual fungi in Helotiales of Leotiomycetes (Wang et al. 2006). Fungi in PAC are  
characterized by darkly pigmented hyphae, and typically produce branched conidiophores,  
hyaline phialides with collarettes, and intracellular microsclerotia (Grünig et al. 2008a,  
30   2008b; Yu et al. 2001). PAC are the common root associates of many tree species,

specifically conifers in forests of the northern hemisphere (Grünig et al. 2008a, 2008b; Menkis 2004). Despite the global pervasiveness of DSE, their ecological roles, phylogenetic relationships and taxonomy remain poorly understood (Knapp et al. 2012; Mandyam and Jumpponen 2005). DSE fungal-plant interaction studies have yielded variable results, likely  
5 due to the use of differing experimental design strategies (Grünig et al. 2008b).

It is estimated that 30% of the world's total land area consists of acid soils, and 50% of the world's potential arable lands are acidic (Tuininga et al. 2004). In view of these adverse environmental conditions, improved methods to enhance growth of both edible and non-edible plants are needed.

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## SUMMARY OF INVENTION

In accordance with the present invention, a method for enhancing overall plant growth and resistance to adverse abiotic conditions comprising contacting a plant or seed therefrom with a composition comprising a biofertilizer comprising at least one endophytic fungi and optionally bacteria. In one aspect, the fungi is *Acidomelania panicicola* and the  
15 optional bacteria is from the *Burkholderia* genus. The method can include inoculating the seeds with the fungi and, or bacteria in agar or growth medium and placing seeds/agar composition in the soil. In another approach, after mixing the cultures, the seeds and cultures are subjected to drying to form a coating thereon. Vermiculite and rock phosphate may also be included in the composition to enhance plant growth and resistance to abiotic stress. The  
20 method can be applied to both monocots and dicots and can be used on plants which include without limitation, lettuce, corn, rice, soybeans, potatoes, barley, wheat, and carrots. In a particularly preferred embodiment, the plant is a turfgrass plant selected from a Ryegrass, Kentucky Bluegrass, Tall Fescue, Bermuda, St. Augustine or Zoysia plant or any other turfgrass plant.

25

In another aspect of the invention, a biofertilizer composition is provided. An exemplary biofertilizer includes an effective amount of *A. panicicola* and at least one agent or microorganism for promoting plant growth and resistance to abiotic stresses for use in the method described above. In a preferred embodiment, the composition contains *A. panicicola* and at least one *Burkholderia* species in equal concentrations. The composition may also  
30 contain a sun protecting product and a polysaccharide solution. The fungal strains may also be encapsulated in allignate beads.

In yet another aspect of the invention the fungus for use in the method is selected from the *Barrenia* genus. In a preferred embodiment, the fungi is *Barrenia panicia*. This composition may also comprise a bacteria selected from the *Burkholderia* genus.

The biofertilizer composition comprising at least *A. panicicola* and, or *Barrenia* and, optionally, other agents or microbial or fungal species are effective to enhance plant resistance to environmental stresses. Such agents may include gel formulations, agar, vermiculite, sun protectorants, rock phosphate, alginate, which when combined form an efficacious biofertilizer.

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### BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1. Maximum likelihood phylogenetic tree inferred from the large subunit of rRNA gene sequence. Bootstraps higher than 70 have thickened branches.

Figure 2. Maximum likelihood phylogenetic tree inferred from the internal transcribed spacer sequences of rRNA gene. Bootstraps higher than 70 have thickened branches.

15 Figure 3. Maximum likelihood phylogenetic tree inferred from combined ITS, LSU, and RPB1 gene sequence datasets. Bootstraps higher than 70 have thickened branches.

Figure 4. DNA sequence for *A. applanata* AY078151; SEQ ID NO: 1 (Fig. 4A), *A. panicicola* KF874620; SEQ ID NO: 2 (Fig. 4B), *A. panicicola* KF874619; SEQ ID NO: 3 (Fig. 4C), *A. panicicola* AL5m2-2; SEQ ID NO: 4 (Fig. 4D), *A. panicicola* CM11M2; SEQ ID NO: 5 (Fig. 4E), *A. panicicola* WSF1-R37; SEQ ID NO: 6 (Fig. 4F), *A. taeda* CM14-P64; SEQ ID NO: 7 (Fig. 4G), *A. taeda* WSF14-P22; SEQ ID NO: 8 (Fig. 4H), *A. taeda* WSF14-P13; SEQ ID NO: 9 (Fig. 4I), *C. clavus* DQ491502; SEQ ID NO: 10 (Fig. 4J), *D. acerina* AF141164; SEQ ID NO: 11 (Fig. 4K), *H. aureliella* JN943611; SEQ ID NO: 12 (Fig. 4L), *L. virgineum* DQ491485; SEQ ID NO: 13 (Fig. 4M), *L. macrospores* DQ471005; SEQ ID NO: 14 (Fig. 4N), *M. cinerea* DQ491498; SEQ ID NO: 15 (Fig. 4O), *M. laxa* EF153017; SEQ ID NO: 16 (Fig. 4P), *P. fortinii* AB671499; SEQ ID NO: 17 (Fig. 4Q), and *V. truncorum* EU434855; SEQ ID NO: 18 (Fig. 4R).

Figures 5A-5E. Switchgrass seedling two days after inoculation with *Acidomelania panicicola* holotype isolate 61R8 (Fig. 5A); Control (Fig. 5B); three days after inoculation with *Barrenia panicia* holotype isolate WSF1R37 (Fig. 5C); Control (Fig. 5D), *Barrenia taeda* holotype isolate WSF14P22 (Fig. 5E). Bar = 1 mm

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Figures 6A-6C. Representative image of morphological characters of *Barrenia panicia* holotype isolate WSF1R37 (Fig. 6A); Representative image of morphological characters of *Barrenia panicia* holotype isolate WSF1R37 (Fig. 6B); Representative image of morphological characters of *Barrenia panicia* holotype isolate WSF1R37 (Fig. 6C). A-Bar =

5 10  $\mu$ m

Figure 7. Micrographs of control and *Acidomelania panicicola* inoculated switchgrass seedling roots 2 days post inoculation.

Figure 8. Micrographs of control and *Acidomelania panicicola* inoculated rice seedling roots 4 days post inoculation.

10 Figure 9. Micrographs showing control, *Fusarium oxysporum* inoculated and *Acidomelania panicicola* inoculated switchgrass seedling survival percentage.

Figure 10. Micrographs showing control, *Fusarium oxysporum* inoculated and *Acidomelania panicicola* inoculated switchgrass roots.

15 Figure 11. Micrographs showing control and *Acidomelania panicicola* inoculated lettuce roots 4 days post inoculation.

## DETAILED DESCRIPTION OF THE INVENTION

Drought and low nutrient stress typified early terrestrial environments when plant colonization of land occurred and was facilitated by root-symbiotic fungi (Stoyke et al. 1991). Beneficial endophytes encompass bacteria and fungi that have the ability to alleviate  
20 abiotic stresses in combination with plant growth promotion. Endophytes have been reported to enhance early root differentiation, improve drought and salinity tolerance and increased survival rate. These endophytes play critical roles in litter decomposition, nutrient absorption and cycling (Forman et al. 1998; Blackwell et al. 2011).

A group of new fungal species were discovered from switchgrass and other grass  
25 roots in the New Jersey Pine Barrens, which is a dry, highly acidic environment, low in nutrients (P, K, organic matter etc.), with high aluminum toxicity (von Uexkull et al. 1995). Herein we describe two new genera, *Acidomelania* and *Barrenia*, discovered in pine barren switchgrass roots.

*Barrenia* was classified using multi-gene phylogenetic analyses, along with  
30 phenotypic and ecological characteristics. While the new species was isolated from roots of

switchgrass and pitch pine in the acidic and oligotrophic New Jersey Pine Barrens, *Barrenia* likely has a wide distribution as its internal transcribed spacer (ITS) sequence has high similarity with a number of GenBank sequences obtained in various ecological studies. The majority of these similar ITS sequences were obtained from roots in plants growing in acidic, nutrient-poor environments, as well as from managed sugarcane plantations. Phylogenetic analyses of ITS, LSU and RPB1 sequence data strongly support that *Barrenia* is a monophyletic clade in *Helotiales*, distinct from any known taxa. *Barrenia* is phylogenetically close to *Acidomelania*, *Loramyces*, *Mollisia*, and *Phialocephala fortinii* - *Acephala applanata* species complex (PAC), the dark septate endophytes. *Barrenia* can be distinguished from *Loramyces* and *Mollisia* by its association with living plant roots. While taxa in PAC also are root endophytes, they have complex phialid arrangements that appear to be lacking in *Barrenia*.

The present inventors have performed functional studies which demonstrate that application of biofertilizers comprising *Acidomelania panicicola* and *Barrenia panicia* significantly enhanced dense root hair growth in switchgrass. *Acidomelania panicicola* plant-fungal interactions with rice and lettuce seedlings under acidic and poor nutrient conditions also resulted in a significant promotion of root and shoot length.

In one aspect of the invention, a biofertilizer composition is prepared by inoculating seeds with fungi (e.g. *Acidomelania panicicola* or any fungus selected from the genus *Barrenia*) on agar or growth medium and placing seeds and agar in the soil. In another aspect of the invention, a biofertilizer composition is prepared by mixing fungi and bacterial cultures with seeds prior to placing seeds in the soil, the cultures optionally forming a coating around the seeds. In a third aspect of the invention, seeds are mixed with fungi and bacterial cultures and dried. In a fourth aspect of the invention, seeds are grown in fungal inoculated soil formulated with vermiculite and rock phosphate.

### Definitions

An endophyte is an endosymbiont, often a bacterium or fungus, that lives within a plant without causing apparent disease. Endophytes may enhance a plant's growth and improve the plant's ability to tolerate abiotic stresses such as drought or harsh soil conditions. In one embodiment an endophyte useful herein comprises the fungus, *Acidomelania panicicola*. In another embodiment, an endophyte comprises *Barrenia panicia*. Endophytes useful herein include the fungus *Acidomelania panicicola* in combination with certain

bacteria selected from the bacterial species, *Burkholderia*. In yet another approach, the fungi *Acidomelania panicicola* and the fungi *Barrenia panicia* are used in combination to enhance plant growth under abiotic stress conditions.

The term “abiotic” includes non-living chemical and physical parts of the environment that affect ecosystems. An ecosystem's abiotic factors may be classified via "SWATS" (Soil, Water, Air, Temperature, Sunlight).

The term “biofertilizer” comprises at least one substance containing living microorganisms which, when applied to seed, plant surfaces, and/or soil, colonizes the rhizosphere or the interior of the plant and promotes growth by increasing the supply or availability of primary nutrients to the host plant. Biofertilizers can also comprise other agents which enhance the growth of the microorganisms present. Such agents include, without limitation, agar, gel, and minerals.

The term "crop" herein refers to any plant grown to be harvested or used for any economic purpose, including for example human foods, livestock fodder, fuel or pharmaceutical production.

The following materials and methods provided to facilitate the practice of the present invention.

## 20 **Fungal isolation**

Poaceae grass roots were collected from three locations (N 40 12.00, W 74 30.00; N40 04.084, W74 26.696; and N 39 46.136, W 74 40.885) in New Jersey Pine Barrens in 2012 and 2013. Native pitch pine (*Pinus rigida*) roots were collected from two locations (N40 04.084, W74 26.696; and N 39 46.136, W 74 40.885) in New Jersey Pine Barrens in 2014 (Tables 1 and 2). Soil pH of the sampling locations ranged from 4.7 to 5.2. Root samples were rinsed thoroughly to remove soil from the surface, cut into 10-20 mm pieces then surface disinfected with sequential washes of 95% ethanol for 30s, 0.5% NaOCl for 2 min and 70% ethanol for 2 min. After several rinses with sterile water and drying, the root samples were cut into 5 mm pieces and plated on acidified malt extract agar (AMEA, 1.5 ml 85% lactic acid per liter of 2% malt extract agar). Plates were incubated at room temperature with 12 h light and 12 h dark cycles. Fungal cultures were transferred to fresh AMEA and purified by sub-culturing from emergent hyphal tips.

Table 1

Species	Mycobank#	Etymology	Morphological description	Type species	Habitat	Known distribution
<b><i>Barrenia</i></b> E. Walsh & N. Zhang, <b>gen. nov.</b>	MB811715	“Barren” refers to the pine barrens ecosystem where the fungi were discovered	Colonies on MEA darkly pigmented, surface fluffy, aerial hyphae thick and light brown. Colonies on WA light brown; sparse aerial hyphae. Sporulation not observed	<i>Barrenia panicia</i>	Endophytic in roots of Poaceae grasses	New Jersey Pine Barrens, United States

Table 2

Species	Mycobank #	Etymology	Morphological Description	Holotype	Other materials examined
<b><i>Barrenia panicia</i></b> E. Walsh & N. Zhang, <b>sp. nov.</b>	MB811716	“panici” refers to the host	Colonies on MEA 55 mm diam after 20 d in the dark at 25C, Cinnamon Brown, surface fluffy, aerial hyphae thick and light brown, reverse pigmented, Warm Sepia. Colonies on WA reaching 51 mm diam after 20 d in the dark at 25C, Ochraceous Tawny, aerial hyphae sparse, reverse pigmented, Cinnamon Brown. Warm Sepia, paddle-shaped hyphopodium-like structures formed in inoculated switchgrass root tissue	<b>United States:</b> <i>New Jersey:</i> Wharton State Forest, N 39 45.346, W 074 41.684, 3 m alt., from roots of <i>Panicum virgatum</i> , 5 Jun. 2013, E. Walsh & N. Zhang, WSF1R37 (RUTPP-WSF1R37)	<b>United States:</b> <i>New Jersey:</i> Assunpink Lake, N 40 12.00, W 74 30.00, 3 m alt., from roots of <i>Digitaria</i> sp., 30 Aug. 2012, E. Walsh & N. Zhang AL5m2; Colliers Mills, N40 04.084 W74 26.696, 5 m alt., from roots of <i>Coix lacryma – jobi</i> , 30 Aug. 2012, E. Walsh & N. Zhang CM11m2
<b><i>Barrenia taeda</i></b> E. Walsh & N. Zhang, <b>sp. nov.</b>	MB811717	“taeda” means pine wood and refers to the host	Colonies on MEA 28 mm diam after 20 d in the dark at 25 C, Cinnamon Brown, surface fluffy, aerial hyphae thick and light brown, reverse pigmented, Mummy Brown. Colonies on WA reaching 31 mm diam after 20 d in the dark at 25C, Buckthorn Brown, sparse	<b>United States:</b> <i>New Jersey:</i> Wharton State Forest N 39 45.346, W 074 41.684, 3m alt., from roots of <i>Pinus rigida</i> , 26 Jun. 2014, E. Walsh & N.	<b>United States:</b> <i>New Jersey:</i> Wharton State Forest, N 39 45.346, W 074 41.684, 3 m alt., from roots of <i>Pinus rigida</i> , 26 Jun. 2014, E. Walsh & N.

			aerial hyphae, reverse pigmented, Buckthorn Brown. Sporulation not observed	Zhang WSF14P22 (RUTPP- WSF14P22)	Zhang WFS14P13; Colliers Mills, N40 04.084 W74 26.696, 5 m alt., from roots of <i>Pinus rigida</i> , 4 Jun. 2014, E. Walsh & N. Zhang CM14P64
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### Morphological study and growth rate

Purified fungal isolates were grown on cellophane overlaid with 2% MEA (BD Difco, Maryland) and 2% water agar (WA). Cultures were incubated at 20°C in the dark with three replicates. Colony diameter was measured after 20 days. The color names of colonies followed Ridgway (1912).

### DNA extraction, amplification and sequencing

Genomic DNA was extracted from fungal mycelium using the UltraClean Soil DNA isolation kit (MoBio, California) following the manufacturer's instructions. PCR was performed with Taq 2X Master Mix (New England BioLabs, Maine), following the manufacturer's instructions. PCR cycling conditions for the internal transcribed spacer (ITS) and the large subunit of ribosomal RNA genes (LSU) consisted of an initial denaturation step at 95°C for 2 min, 35 cycles of 95°C for 45s, 54°C for 45s, 72°C for 1.5 min, and a final extension at 72°C for 5 min. For the largest subunit of RNA polymerase II (RPB1), the cycling conditions included an initial denaturation step at 95°C for 2 min, 35 cycles of 95°C for 60 s, 55°C for 1.5 min, 72°C for 2 min, and a final extension at 72°C for 10 min. Primers used in this study are as follows: ITS1 and ITS4 for the ITS region (White et al. 1990), ITS1 and LR5 for the LSU locus (Rehner and Samuels 1995), and RPB1 Af (Hall and Stiller 1997) and RPB1 CrRev (Matheny et al. 2002) for the RPB1 gene. PCR products were purified with ExoSAP-IT (Affymetrix, California) and sequenced with the PCR primers by Genscript Inc. (Piscataway, New Jersey).

### Sequence alignment and phylogenetic analyses

Six representative isolates of the new taxon (CM11m2, CM14P64, AL5m2, WSF1R37, WSF14P13, and WSF14P22) as well as other reference *Leotiomycetes* species (Table 3) were included in the phylogenetic analyses. The ITS dataset included sequences of

the six new isolates from this study and 15 reference sequences of *Helotiales*. The LSU dataset included the six new sequences and 28 reference sequences of *Helotiales* and *Rhytismatales*. The three-gene (ITS, LSU and RPB1) alignment included the six new sequences and 12 reference sequences of . Sequences were aligned with MUSCLE (Edgar 2004). Maximum likelihood (ML) tree was generated with MEGA 6 (Tamura et al. 2013). Models with the lowest BIC scores (Bayesian Information Criterion) were considered to describe the substitution pattern the best. The best models for LSU, ITS and three-gene datasets were Tamura-3 parameter, Kimura 2-parameter, Kimura 2-parameter, respectively. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood approach, and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites. Bootstrap was computed for 500 replications. All positions containing gaps and missing data were excluded from the analyses.

15

Table 3. Species name, isolate number, host, location and GenBank accession numbers of the fungi used in this study.

Species	Isolate number <sup>a</sup>	Host	Location	ITS	LSU	<i>RPB1</i>
<i>Acephala applanata</i>	CBS109321	<i>Picea abies</i> , root	Büdmerenwald, Switzerland	AY078145	KF951051	AFTOL 3613
<i>Acephala macrosclerotium</i>	CBS123555	<i>Pinus sylvestris</i> , root	Hubertusstock, Germany	HM189719		
<i>Acidomelania panicola</i>	CBS137156	<i>Panicum virgatum</i> , root	New Jersey Pine Barrens, USA	KF874619	KF874622	---
<i>Acidomelania panicola</i>	CM16s1	<i>Schizachyrium scoparium</i> , root	New Jersey Pine Barrens, USA	KF874620	KF874621	---
<i>Barrenia panicia</i>	AL5m2	<i>Digitaria</i> sp., root	New Jersey Pine Barrens,	---	---	---

			USA			
<i>Barrenia panicia</i>	CM11M2	<i>Coix lacryma-jobi</i> , root	New Jersey Pine Barrens, USA	---	---	---
<i>Barrenia panicia</i>	WSF1R37	<i>Panicum virgatum</i> , root	New Jersey Pine Barrens, USA	---	---	---
<i>Barrenia taeda</i>	CM14P64	<i>Pinus rigida</i> , root	New Jersey Pine Barrens, USA	---	---	---
<i>Barrenia taeda</i>	WSF14P13	<i>Pinus rigida</i> , root	New Jersey Pine Barrens, USA	---	---	---
<i>Barrenia taeda</i>	WSF14P22	<i>Pinus rigida</i> , root	New Jersey Pine Barrens, USA	---	---	---
<i>Botryotinia fuckeliana</i>			Oregon, USA		AY544651	
<i>Bulgaria inquinans</i>	CBS118.31		Germany		DQ470960	AFTOL916
<i>Chloroscypha chloromela</i>			Oregon, USA	U92311		
<i>Chlorovibressea</i> sp.			New Zealand		DQ257352	
<i>Collembolispora aristata</i>		foam in stream	Nova Ves, Czech Republic		KC005811	
<i>Cudoniella clavus</i>	AFTOL166	Hemlock cones and small sticks	Benton County, Oregon, USA		DQ470944	DQ471128
<i>Dermea acerina</i>	CBS161.38	<i>Acer rubrum</i>	Bear Island, Ontario, Canada	AF141164	DQ247801	DQ471164

<i>Fabrella tsugae</i>					AF356694	
<i>Hyaloscypha aureliella</i>			Scotland	JN943611	EU940152	JN985241
<i>Hyaloscypha vitreola</i>			Kaarina, Finland	FJ477059	FJ477058	
<i>Lachnum virgineum</i>	AFTOL49	<i>Alnus</i> sp., cones	Oregon, USA	DQ491485	AY544646	DQ842030
<i>Lambertella subsubrenispora</i>	CBS811.85	<i>Aster</i> <i>ageratoides</i> var. <i>ovata</i>	Honshu, Japan		DQ470978	
<i>Leotia lubrica</i>	AFTOL1	<i>Chrysolepis</i> <i>chrysophyla</i>	Oregon, USA		AY544644	
<i>Loramycetes macrosporus</i>	CBS235.53	<i>Equisetum</i> <i>limosum</i>	UK	DQ471005	DQ470957	DQ471149
<i>Microglossum rufum</i>	AFTOL1292		Tennessee, USA		DQ470981	DQ471179
<i>Mollisia cinerea</i>	CBS122029	fallen log	Alsea Falls, Oregon, USA	DQ491498	DQ470942	DQ471122
<i>Mollisia dextrinospora</i>	ICMP18083	<i>Actinidia</i> <i>deliciosa</i> cv. <i>Hayward</i>	New Zealand		HM116757	
<i>Monilinia laxa</i>	CBS122031				AY544670	FJ238425
<i>Neobulgaria lilacina</i>			Finland		EU940141	
<i>Neobulgaria pura</i>	CBS477.97	log with moss	New York, USA		FJ176865	
<i>Neofabrea malicorticis</i>	CBS122030	<i>Malus</i> sp.	Oregon, USA		AY544662	
<i>Phialocephala dimorphospora</i>	CBS300.62	slime in pulp mill		AF486121	AB671465	

<i>Phialocephala fortinii</i>	CBS443.86	<i>Pinus sylvestris</i> , root	Suonenjoki, Finland	AB671499	AB671466	
<i>Phialocephala scopiformis</i>	CBS468.94	<i>Picea abies</i> , bark	Regensburg, Germany	AF486126		
<i>Spathularia velutipes</i>		<i>Tsuga Canadensis</i>	Tennessee, USA		FJ99786	
<i>Varicosporium elodeae</i>			Svalbard, Norway		JN941371	
<i>Vibressea truncorum</i>	CBS258.91	<i>Populus</i> , submerged root	Ontario, Canada	EU434854	FJ176874	FJ238438
<i>Acephala</i> sp. <sup>c</sup>		<i>Cymbidium insigne</i>	China	HQ889709		
<i>Acephala</i> sp. <sup>c</sup>		Sugarcane, root	Brazil	GU973749		
<i>Phialocephala</i> sp. <sup>c</sup>		<i>Rhododendron</i> , root	Smoky Mountain National Park, USA	JQ272328		

<sup>a</sup> AFTOL= Assembling the Fungal Tree of Life project; ATCC = American Type Culture Collection, Manassas, Virginia, USA; CBS = Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; ICMP = International Collection of Micro-organisms from Plants, Lincoln, New Zealand.

<sup>b</sup> Numbers in boldface indicating new sequences from this study.

5 <sup>c</sup> Taxon name was copied from GenBank. Phylogenetic analysis in this study indicated that they belong to *Barrenia*.

### Plant-fungal interaction experiment

Fungal isolates WSF1R37, WSF14P22, and *A. panicicola* isolate 61R8 were used in the seedling inoculation experiment. Switchgrass ('Kanlow') seeds were surface disinfected as follows: 95% ethanol for 30s, 0.5% NaOCl for 1 min, 70% ethanol for 1 min, rinsed with sterile distilled H<sub>2</sub>O and allowed to germinate in the dark at 25° C for 3 days. Agargel (Sigma-Aldrich, USA) plates were made following 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 with visible radicle were then placed on the plugs. Sterile MEA plugs were used as negative control. Cultures were incubated at 25° C under 12 hr light and dark cycle with nine replicates. Root length was measured 7 days after inoculation.

The following examples are provided to illustrate certain embodiments of the invention. They are not intended to limit the invention in any way.

## EXAMPLE I

### ***Barrenia*, a New Genus Associated with Roots of Switchgrass and Pine in the Oligotrophic Pine Barrens, Promotes Root Hair Growth**

#### **A. Culture morphology and growth rate**

Isolate WSF1R37 produced dense Cinnamon Brown mycelium on MEA, and Ochraceous Tawny mycelium on WA. Colony diameter measurements for isolate WSF1R37 after 20 days were 75 mm on average on MEA with standard deviation (SD) of 2.6, and 47 mm on average on WA with SD of 2.6. Isolate WSF14P22 produced dense Cinnamon Brown mycelium on MEA, and Buckthorn Brown mycelium on WA. Colony diameter measurements for isolate WSF14P22 after 20 days were 28 mm on average on MEA with SD of 0.6, and 26 mm on average on WA with SD of 1.0.

#### **B. Sequence data and phylogeny**

There were 173 characters in the LSU alignment, 377 in ITS and 1291 in the three-gene alignment. Maximum likelihood trees based on LSU, ITS and three gene sequences are shown in Figs 1-3. All three phylogenies supported that the new isolates formed a monophyletic clade in *Helotiales* separated from any known taxa. The LSU tree indicated that they were close to *Acidomelania panicicola*, *Loramycetes macrosporus*, *Mollisia cinerea* and PAC. The ITS tree showed that these new isolates were closely related to *A. panicicola*, *M. cinerea*, *Phialocephala scopiformis* and *L. macrosporus*. In the ITS tree, isolates

WSF1R37, AL5m2, and CM11m2 formed a well-supported group, while isolates WSF14P22, WSF14P13, and CM14P64 formed another. The two groups were also recognized and supported by the LSU and RPB1 trees, and variation in the phylogenetic relationships of these isolates only occurred within the groups. DNA sequence information for the different fungal species is displayed in Figs. 4A-4R.

Based on the molecular phylogenetic analyses, morphological characters and their ecological features, a new genus and two new species have been identified. *Barrenia* differs from *Loramyces* by its association with living plant roots while *Loramyces* species are associated with submerged dead plants (Digby and Goos 1987; Ingold and Chapman 1952; Weston 1929). Taxa in the PAC are also root endophytes, but they exhibit complex phialid arrangements that appear to be lacking in *Barrenia*. *Barrenia* also differs from *Mollisia* because of its lack of phialide producing conidia. Moreover, *Barrenia* has 93% or less ITS sequence similarity to the above-mentioned close relatives or any other described species with accessible ITS sequences. The two *Barrenia* species differ from each other on host and growth rate. The pine associated *B. taeda* exhibited slower growth than the grass associated *B. panicia* on both WA and MEA. There is a 96% similarity in ITS sequences between *B. panicia* and *B. taeda*.

### 20 C. Plant-fungal interaction experiment

Switchgrass seedlings inoculated with *A. panicicola* isolate 61R8 and *B. panicia* WSF1R37 produced dense root hairs all the way to the root apical meristem area, while the control seedlings only produced dense root hairs at the region of maturation of the root (Fig 5A and 5C). In addition, the roots inoculated with *A. panicicola* isolate 61R8 and *panicia* WSF1R37 had a serpentine growth pattern, while the control roots were straight. Hyphopodia-like structures were observed on the switchgrass seedling roots inoculated with *B. panicia* WSF1R37 (Fig 6A-6C). Root length for seedlings inoculated with *B. panicia* WSF1R37 after 7 days were 17.4 mm on average with SD of 1.8, not significantly different from the control, which was 19 mm on average with SD of 5.1. Seedlings inoculated with *B. taeda* WSF14P22 showed no difference in root hair production with the control. Root length for *B. taeda* WSF14P22 after 7 days was 8.1 mm on average with SD of 1.6, which was significantly shorter than the control (Fig 5E).

## DISCUSSION

Our recent survey on fungi associated with grass roots uncovered a number of novel  
5 DSE in Leotiomyces from the pine barrens ecosystem (Luo et al. 2014a, 2014b; Walsh et  
al. 2014). Leotiomyces are morphologically and ecologically diverse and the phylogenetic  
relationships within this class are not well resolved due to lack of molecular data (Wang et al.  
2006). Based on the multi-locus phylogenetic analyses, the new genus *Barrenia* described  
here belongs to Helotiales, which encompasses plant pathogens, saprobes and endophytes.  
10 The dark, septate hyphal morphology of *Barrenia* spp., their root-colonizing habit and  
phylogenetic closeness to PAC indicate that they likely are also DSE.

The best studied DSE is the PAC, specifically *P. fortinii*. However, the ecological  
functions of PAC and other DSE remain elusive. Host-fungal interaction experiments often  
yielded inconsistent results under various experimental conditions in different laboratories  
15 (Mandyam and Jumpponen 2005). This prompted us to examine the interaction between *B.*  
*panicia*, *B. taeda*, *A. panicicola* and switchgrass, which is the host of *B. panicia* and *A.*  
*panicicola*. Our inoculation results indicated that *A. panicicola* and *B. panicia* remarkably  
promoted the root hair growth in switchgrass. In switchgrass roots, *B. panicia* produced  
hyphopodium-like structures, which may perform penetration and nutrient exchange function  
20 between the fungus and the host plant (Delaux et al. 2013; Walker 1980). *Barrenia taeda*,  
originally isolated from pine roots, had negative effect on root elongation in switchgrass  
seedlings. These results corroborate Mandyam et al. (2010; 2012) that while DSE fungi have  
a broad host range, their effects and characteristics can be considered host specific.

The phylogenetic analysis in this study indicated that *Barrenia* is close to  
25 *Acidomelania*, *Loramyces*, *Mollisia*, and PAC. The phylogenetic proximity of *Mollisia*,  
*Loramyces* and PAC was also supported by Zijlstra et al. (2005) and Wang et al. (2006).  
*Barrenia* can be distinguished from *Loramyces* and *Mollisia* by its association with living  
plant roots. While taxa in PAC also are root endophytes, morphologically they can be  
distinguished from *Barrenia*. In addition, *Barrenia* has 93% or less ITS sequence similarities  
30 to the above-mentioned close relatives or any other described species with accessible ITS  
sequences. The family placement of *Barrenia* is not determined here because the  
Leotiomyces phylogeny is poorly resolved and several families in this class likely are  
polyphyletic (Wang et al. 2006).

The six *Barrenia* isolates from New Jersey Pine Barrens were grouped into two well-supported clades. We delimited the two species based on the genealogical concordance phylogenetic species recognition (Taylor et al. 2000). The BLAST results in GenBank indicated that *Barrenia* might have a wide distribution. Sixteen ITS sequences in GenBank had 97–99% identity with that of *B. panicia* isolate WSF1R37, for example, GU973749 from sugarcane root in Brazil, HQ889709 from *Cymbidium insigne* root in China, and AY599235 from grass root in The Netherlands. Twelve ITS sequences in GenBank had 97–99% identities with that of *B. taeda* isolate WSF14P22, for example, JQ272328 from *Rhododendron* root in USA and KJ817299 from *Vaccinium vitis-idaea* in Inner Mongolia. The host plants of the matched sequences in GenBank are largely Ericaceae, terrestrial orchids, grasses and conifers, usually found in acidic and infertile soils (Keddy 2007). This distribution pattern was also found in *Acidomelania panicicola*, the other root associated fungus frequently isolated from the pine barrens (Walsh et al. 2014).

Additional experiments to uncover fungal-plant interactions included the inoculation of switchgrass seedlings with *A. panicicola* isolate 61R8 and *B. panicia* WSF1R37 produced dense root hairs all the way to the root apical meristem area, while the control seedlings only produced dense root hairs at the region of maturation of the root. In addition, the roots inoculated with *A. panicicola* isolate 61R8 and *B. panicia* WSF1R37 had a serpentine growth pattern, while the control roots were straight. The plant growth promotion effect of *A. panicicola* and *B. panicia* discovered in this study coupled with their distribution pattern indicate that these species may play a role in plant adaptation to acid, low nutrient soils.

In conclusion, we discovered a new genus and two species of root-colonizing fungi associated with plants living in an acidic, nutrient poor environment. The phylogenetic and taxonomic work and the plant-fungal interaction results reported here will aid future ecological and evolutionary studies on root-associated fungi.

## EXAMPLE II

### Endophytic Fungi from Pine Barrens Grasses Promote Plant Growth In Acidic, High Aluminum Toxicity And Low Nutrient Conditions

#### A. Fungal Inoculation of Seeds on Agar

5            In this study, we performed functional studies that demonstrated that *Acidomelania panicocola* inoculation of seeds significantly increased root hair growth in switchgrass, rice and lettuce seedlings compared to the control.

             To assess the effects of *Acidomelania panicicola* inoculation of switchgrass and rice seedlings on root hair abundance, fungus was grown on water agar under room temperature  
10            for 7 days. Seeds were germinated in sterile distilled water in a petri dish under room temperature in the dark for 7 days. Seedlings (roots down) were inserted in the 7 day-old fungal agar culture. Control seedlings were uninoculated but grown under the same conditions. Significant differences in root hair abundance were observed in inoculated seedlings when compared to negative, untreated controls (Figure 7 and 8). These findings  
15            indicate that *Acidomelania panicicola* inoculation enhances root hair abundance.

             To evaluate switchgrass seedling survival percent and root and shoot length, switchgrass seeds were next inoculated with *Acidomelania panicicola* or *Fusarium oxysporum* or uninoculated using the method described above and agar and seedlings were covered with top soil. 6 days post-inoculation, *Acidomelania panicicola* inoculated seedlings  
20            exhibited a significant increase in survival (Figure 9). Root and shoot length of switchgrass seedlings were visualized 8 days after inoculation when enhanced root and shoot length were observed (Figure 10). These results demonstrate that colonization of switchgrass seedlings with *Acidomelania panicicola* enhances switchgrass growth and survival.

             To assess the effects of *Acidomelania panicicola* inoculation on lettuce seed growth,  
25            seeds were inoculated and germinated as described above. Root length was assessed 4 days after inoculation. Increased root growth was observed for the inoculated lettuce seedlings (Figure 11). These findings indicate that *Acidomelania panicicola* can enhance growth of edible plants.

#### 30    B. Bacterial and Fungal Mixing and Inoculation of Seeds

             Fungus (e.g. *Acidomelania panicicola*) is grown on water agar or other growth media under room temperature for 7 days. Bacterium (e.g. *Burkholderia* sp.) is cultured in Luria-

Bertani broth (LB) overnight at 28 °C. Seeds are mixed with the bacterial culture and the fungal cultures (ratio: 500 seeds: 10 mL overnight bacterial culture: 1 Petri dish 7 day old fungal culture) and placed on soil (e.g. Pine Barrens soil or other nutrient-poor soils). Seeds are then covered with top soil and grown under sufficient light.

#### 5 C. Bacterial and Fungal Mixing, Inoculation and Drying of Seeds

Fungus (e.g. *Acidomelania panicicola*) is grown on water agar or other growth media under room temperature for 7 days. Bacteria (e.g. *Burkholderia* sp.) are grown in Luria-Bertani broth (LB) overnight at 28° C. Seeds are mixed with the bacterial culture and the fungal cultures (ratio: 500 seeds: 10 mL overnight bacterial culture: 1 Petri dish 7 day old  
10 fungal culture) and dried. Seeds are placed on soil (e.g. Pine Barrens soil or other nutrient-poor soils). Seeds are then covered with top soil and grown under sufficient light.

#### D. Bacterial and Fungal Mixing, Inoculation of Soil Formulated with Vermiculite and Rock Phosphate

Fungus (e.g. *Acidomelania panicicola*) is grown on water agar or other growth media  
15 under room temperature for 7 days. Bacterium (e.g. *Burkholderia* sp.) are grown in Luria-Bertani broth (LB) overnight at 28°C. Soil formulated with vermiculite and rock phosphate is inoculated with the fungal and bacterial cultures.

### DISCUSSION

Roots were an early development in plant life evolving on land during the Devonian  
20 Period (416 to 360 million years ago; (von Uexkull et al. 1995)). The fossil record and molecular phylogenetic analysis suggest that from the outset, mycorrhizal fungi played a crucial role in facilitating plant invasion of land, which was dry and poor in nutrients at the time of colonization (Gensel et al. 2001). Such drought and low nutrient stress continue to challenge plants living in many extant habitats.

25 We describe herein a novel endophytic fungi, *Acidomelania panicicola*, for use alone, and optionally in the presence bacteria, which enter the root-interior and colonize the tissues of the plant, thereby effectively promoting plant growth and survival. Given that there are limited techniques—both time consuming and cost-intensive—to prevent adverse effects of abiotic stressors on plant growth, the present studies demonstrating that application of a  
30 biofertilizer comprising *Acidomelania* to seeds or seedlings results in increased seedling

survival rate, root hair abundance, and root and shoot length will have great utility for promoting plant growth under adverse environmental conditions.

### EXAMPLE III

#### 5                   **Liquid Formulation of the BioFertilizer for Seed Coating**

This example provides a liquid formulation of biofertilizer, where the formulation consists of two separate solutions that are combined before use as a seed coating.

10                   For the first solution, the fungi are grown in a 1 L flask using an adequate medium and are concentrated by centrifugation in order to separate the solid. This solid is then suspended in a minimum amount of media. A sun protecting product, such as Congo red or green colorant can also be added to the media at 1% (w/v).

15                   According to one preferred embodiment, *A. panificola* only is used for the first solution in similar initial concentrations. In a second embodiment, the first solution contains a fungus from the genus *Barrenia* only (e.g. *B. panicia*). In another embodiment, the first solution is comprised of *A. panificola* and at least one fungus from the genus *Barrenia* (e.g. *B. panicia*). In another embodiment, the first solution contains a mixture of *A. panificola* and at least one bacteria from the genus *Burkholderia*. In another embodiment, a mixture of *B. panicia* and at least one bacteria from the genus *Burkholderia* is contained within the first  
20                   solution.

                    For the second solution, a 1% (w/v) solution of a polysaccharide, such as guar gum, gelan gum, pectin, carboxymetil cellulose, agar-agar, xantan gum (or other food hydrocolloid) is prepared to be used as sticker. The two solutions are then mixed together to treat plant seeds as a coating. The seed should be dried before planting and it is preferable to  
25                   wait at least two hours after application prior to planting.

### EXAMPLE IV

#### **Solid State Formulation of the Biofertilizer of the Invention**

                    This example provides a liquid formulation of a biofertilizer where the fungi and  
30                   optionally bacteria are encapsulated and the fertilizer is in solid form. Alginate beads were prepared as follows:

1 ml of 30% glycerol was added to 1, 1.5 or 2% sodium alginate solution, depending on the alginate properties (M/G ratio) to obtain a final volume of 25 ml. Then, 250 ml of culture (obtained from a culture of *A. panicicola* only, a fungus from the genus *Barrenia* only (e.g. *B. panicia*), or a mixture of *A. panicicola* and *Burkholderia*, or a mixture of *A.*

5 *panicicola* and *Barrenia* (e.g. *B. panicia*) was centrifuged, the cell pellet was washed with saline (0.85% NaCl, w/v) and suspended in 25 ml of alginate mixture and mixed thoroughly. This suspension was added drop wise into a pre-cooled sterile 1.5 or 2% (w/v) aqueous solution of CaCl<sub>2</sub> under mild agitation to obtain the fungal-alginate beads. These beads were allowed to harden for 2-4 h at room temperature. Beads were collected by sieving and were

10 washed several times with sterile water and stored at 4° C. In order to preserve the formulation, the fresh wet beads were frozen at -80 °C prior to lyophilization at -45 ° C. for 15 h. The lyophilized dry beads were stored in sterile glass bottles.

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- 15

While certain of the preferred embodiments of the present invention have been described and specifically exemplified above, it is not intended that the invention be limited to such embodiments. Various modifications may be made thereto without departing from

20 the scope and spirit of the present invention, as set forth in the following claims.

**What is claimed is:**

1. A method for enhancing overall plant growth and resistance to adverse abiotic conditions comprising contacting a plant or seed therefrom with a composition comprising a biofertilizer comprising at least one endophytic fungi and optionally bacteria.
- 5 2. The method of claim 1, wherein the fungi is *Acidomelania panicicola*.
3. The method of claim 2, wherein said composition contains bacteria selected from the *Burkholderia* genus.
4. The method of claim 1, wherein said composition is prepared before use by inoculating said seeds with said endophyte on agar or growth medium and placing seeds and agar in the  
10 soil.
5. The method of claim 1, wherein said composition is prepared before use by mixing said fungi and bacterial cultures with seeds.
6. The method of claim 1, wherein said composition is prepared before use by mixing seeds with said fungi and bacterial cultures and drying said seeds to form a coating thereon.
- 15 7. The method of claim 4-6 wherein said composition comprises vermiculite and rock phosphate.
8. The method of claim 4-6, wherein said biofertilizer is applicable to crop and forest plants.
9. The method of claim 4-6, wherein the crop plants are dicotyledonous plants.
10. The method of claim 4-6, wherein said plant is an edible plant including lettuce, corn,  
20 rice, soybeans, potatoes, barley, wheat, and carrots.
11. The method of claim 4-6, wherein the crop plants are monocotyledonous plants.
12. The method of claim 4-6 wherein said plant is a turfgrass plant selected from a Ryegrass, Kentucky Bluegrass, Tall Fescue, Bermuda, St. Augustine or Zoysia plant or any other turfgrass plant.
- 25 13. A biofertilizer composition, comprising an effective amount of *A. panicicola* and at least one agent or microorganism for promoting plant growth and resistance to abiotic stresses for use in the method of claim 1.

14. The biofertilizer composition of claim 13, wherein the composition contains *A. panicicola* and at least one *Burkholderia* species in equal concentrations.
15. The biofertilizer composition of claim 14, comprising a culture suspension comprising the  
5 fungal strain and optionally a bacterial strain, a sun protecting product and a polysaccharide solution.
16. The biofertilizer composition of claim 15, wherein the polysaccharide is selected from the  
10 group consisting of guar gum, gelan gum, pectin, carboxymetil cellulose, agar, and xantan gum and contains 1% w/v of polysaccharide.
17. The biofertilizer composition of claim 13, wherein the composition is for seed coating.
18. The biofertilizer composition of claim 13, wherein the composition improves seed  
15 germination.
19. The biofertilizer composition of claim 13, wherein the fungal strains are encapsulated in allignate beads.
20. The biofertilizer composition of claim 13, wherein the biofertilizer is applicable to edible  
20 and non edible plants.
21. The method of claim 1, wherein said composition contains fungi selected from the *Barrenia* genus.
22. The method of claim 21, wherein the fungi is *Barrenia panicia*.
23. The method of claim 21, wherein said composition contains bacteria selected from the  
25 *Burkholderia* genus.
24. The method of claim 21, wherein said composition is prepared before use by inoculating said seeds with said endophyte on agar or growth medium and placing seeds and agar in the soil.
25. The method of claim 1, wherein said composition is prepared before use by mixing said  
30 fungi and bacterial cultures with seeds.

26. The method of claim 1, wherein said composition is prepared before use by mixing seeds with said fungi and bacterial cultures and drying said seeds to form a coating thereon.
27. The method of claims 24-66 wherein said composition comprises vermiculite and rock phosphate.
- 5 28. The biofertilizer composition, according to claim 13, wherein said at least one microorganism is of the genus *Barrenia*.
29. The biofertilizer of claim 28, wherein the fungi is *Barrenia panicia*.
30. The biofertilizer composition of claim 13, wherein the composition contains *A. panicipicola* and at least one *Barrenia* species in equal concentrations.
- 10 31. The biofertilizer composition of claim 28, further comprising a sun protecting product and a polysaccharide solution.
32. The biofertilizer composition of claim 26, wherein the polysaccharide is selected from the group consisting of guar gum, gelan gum, pectin, carboxymetil cellulose, agar, and xantan  
15 gum and contains 1% w/v of a polysaccharide.
33. The biofertilizer composition of claim 32 applied to seeds as a coating
34. The biofertilizer composition of claim 28, wherein the fungal strains are encapsulated in  
20 allignate beads.

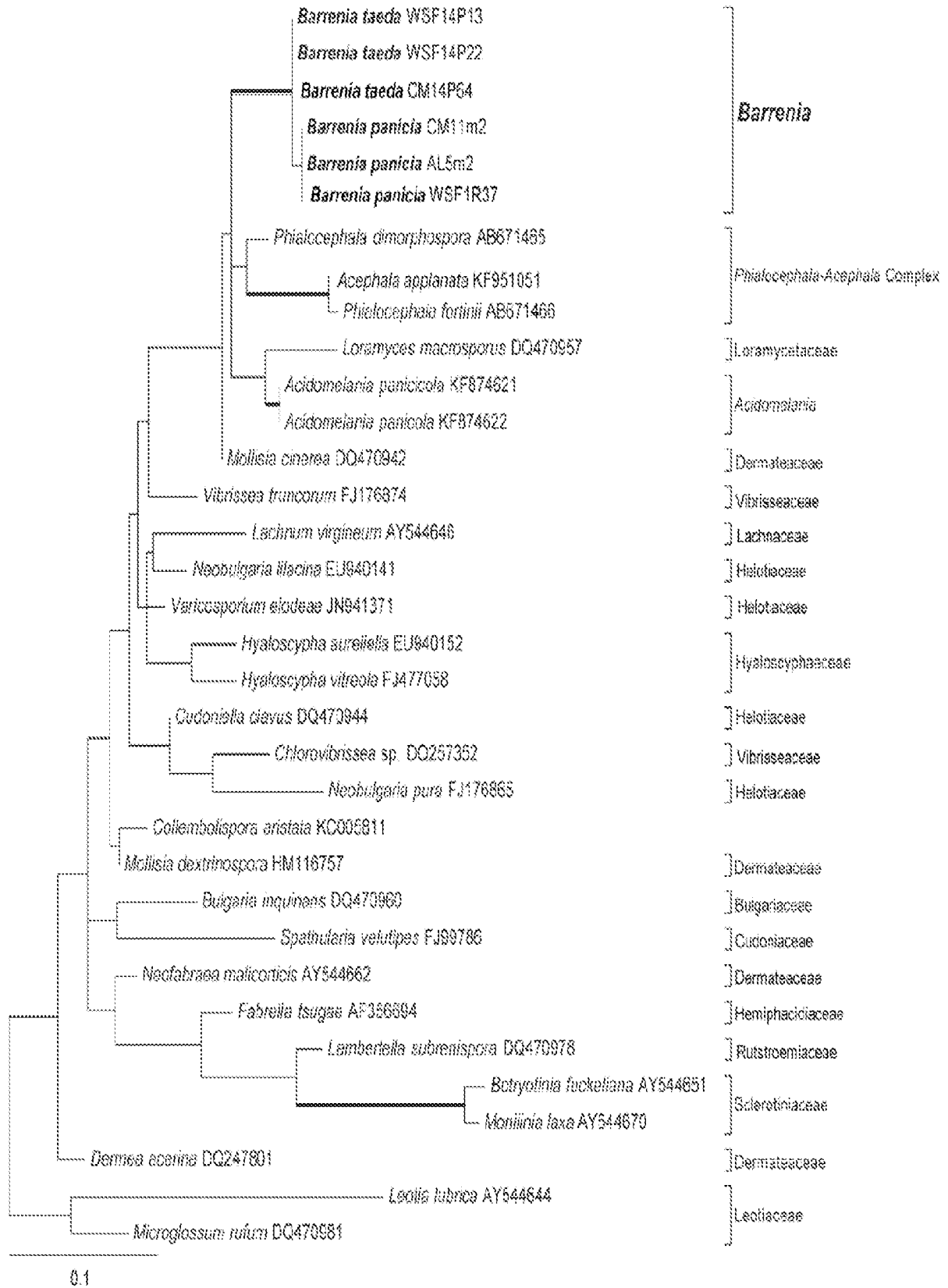


Figure 1



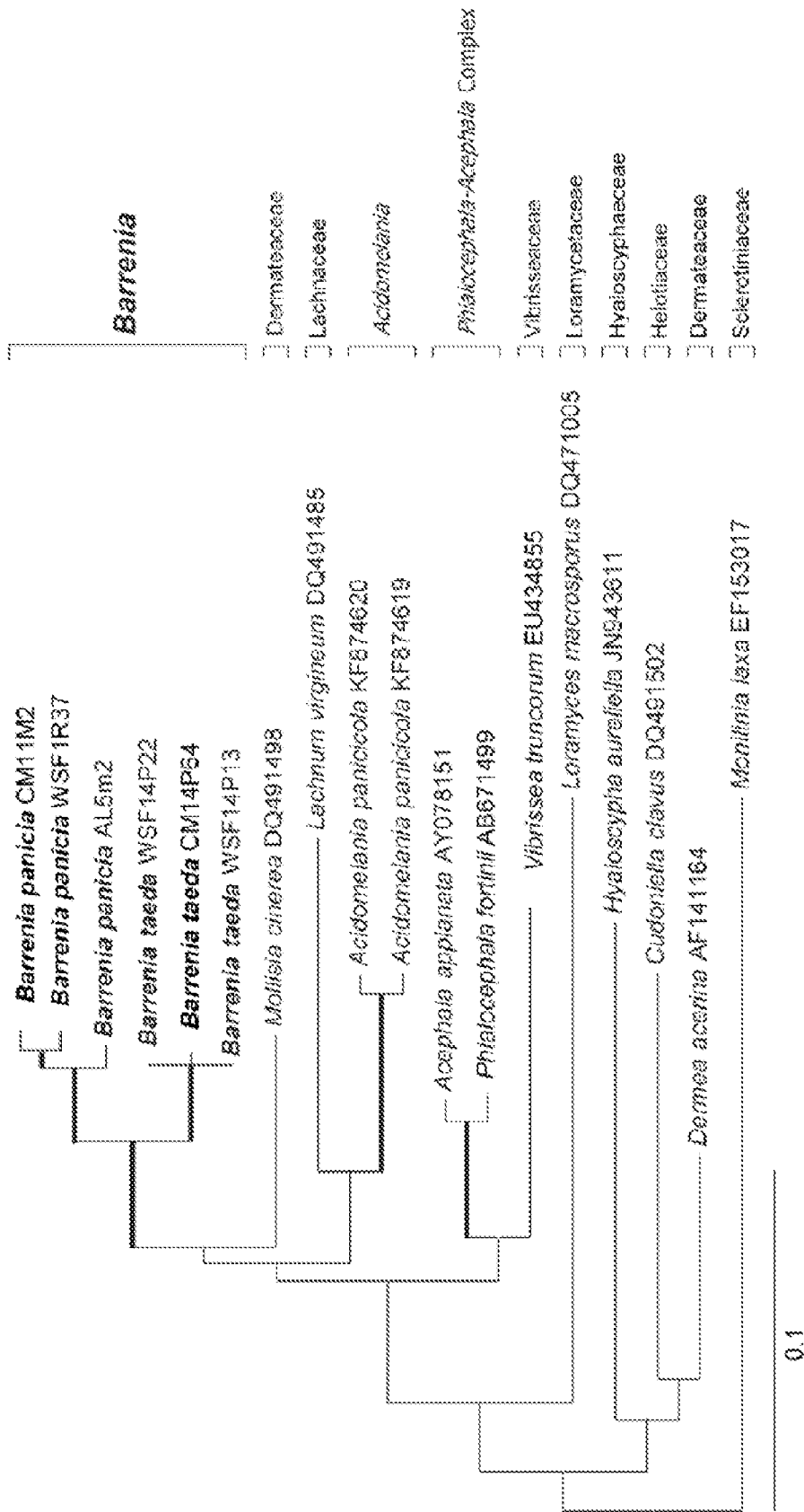


Figure 3

Acephala\_applanata\_AY078151

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GTGTTTA-----
-----
-----CATACTATTGT-TGCTTTGGCGGGCCGTGA-CCT-CCAC--TGC----GGGCTC
TGCTCGT-----GTGTGCCCGCCAGAGGACC---AAACTCTGAATGTTAGTGATGTCTGA
GTACTATCTAATAGTTAAAACCTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGA
ACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTG
AACGCACATTGCGCCCTGTGGTATTCCGCAGGGCATGCCTGTTGAGCGTCATT-TAACC
ACTCACGCCTGGCGTGGTATTGGGGT-ACGCGGT--CTCGCGGCCCTCAAATCAGTGGC
GGTGCCGGTG-GGCTCTAAGCGTAGTAC-ATACTCCCGCTATAGAGTTC-----
-----CC-----CCGGTGGCTCGC--
-----ACCCGCTGAACTTAAGCATATCAATAAGCGGAGGAA
AAGAAACCAACAGGGATTACCTCAGTAACGGCGAGTGAAGCGGTAACAGCTCAAATTTGA
AAGCTGCC-----AACAGGCCGCGTTGTAATTTGTAGARGCTGCTTTGGGTGTGGCCCG
GTCTAAGTTCCTTGGAACAGGACGTCATAGAGGGTGAGAATCCCGTATGTGATCGG-TGC
CGTTGCCCGTGTAAGCGCTTTCGACGAGTCGAGTTGTTGGGAATGCAGCTCAAATGG
GTGGTAAATTTTCATCTAAAGCTAAATATTGGCCAGAGACCGATAGCGCACAAGTAGAGTG
ATCGAAAGATGAAAAGCACTTTGGAAAGAGAGTTAAACAGTACGTGAAATGTTGAAAGG
GAAGCGCTTGCAACCAGACTTGCGGGCGGTTCGATCATCCGAGGTTT-TCCCCGGTGC ACT
CGATCGTTC-TCAGGCCAGCATCGGTTTCCGGGGTGGGATAAAGGCGGTGGGAATGTGGC
TC--TTC-----GGAGTGTTATAGCCACCGTGCAATGCCGCCACCGGGGACCGAGGAC
CGCGCTTC-GGCTAGGATGCTGGCGTAATGGTTGTAAGCGACCCGTCTTGAAACACGGAC
CAAGGAGTCTAACATCTATGCGAGTGTGTTGGGTGTCAAACCCATACGCGTAATGAAAGTG
AACGGAGGTAAGAGCCCTTTAGGGTGCATTATCGACCGATCCTGATGTCTTCGGATGGAT
TTGAGTAAGAGCATAGCTGTTGGGACCCGAAAGATGGTGAACATGCGTGAATAGGGTGA
AGCCAGAGGAAACTCTGGTGGAGGCTCGCAGCGGTTCTGACGTGCAAATCGATCGTC-AA
ATTTGCGTATAGGGC-GAAAGACTAATCGGCTAGACAGAGTCAGTTCG---CCCGATGA
GGGTGGCAGATCTACT--TGTTT--TYGTGCTGACATGAGT-ATCTCAGAGTAATCCGGC
CTTCAAAGCAGCTGTTTCCATTCGAGACCCGAAAGCGTAGGTTTCGATACGATTTGGCGACT
TTGCAAGCCCAAGATGATCTGCGATAGCGACGTTTCTGCGGACGATCAAGAATTCGGTGG
CGATCCAAAGGAAGCCGTGAAG---CGCTCTCATGGAGGCTGTGGAAATACTCAGCCCGA
GGTGCGCCAGCAGGCTCTGCAGCTTTGGGGTACATGGAAGATGCCTAAGGACGAGGAGAA
CGAGGG-----AAGCCAATCCGAGAAGAGACAAATCACTCCAGAGATGGCTCTGAACGT
CTTCCGAAGCATGTCTACTGCTGAGATTCGCGACCTTGGGTTGAGCAACGATTATGCCCG
ACCCGACTGGCTGATCATCACAGTCTTCCAGTTCCTCCTCCGCCGGTTCGACCAAGTAT
CTCAATGGATGGCACAAGCACAGGCATGCGTGGAGARGATGATTTGACGTACAAGCTCGG
TGATATCATCCGTGCGAACGGCAATGTCAAGC-AGGCACAACAGGAAGG

```

Figure 4A

Acidomelania\_panicicola\_KF874620

GTGTCTA-----  
-----  
-----CATACTCTTGT-TGCTTTGGCAGGCCGTGG-CCTCCCAC--TGT----GGGCTC  
AGCCTGC-----ATGTGCCTGCCAGAGGACC---AAACTCTGAATGTTACTGATGTCTGA  
GTACTATATAATAGTTAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGA  
ACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTG  
AACGCACATTGCACCCGGTGGTATTCCGCCGGGTATGCCTGTTGAGCGTCATTACAACC  
ACTCAAGCCTGTCTTGGTGTGGGGA-TTGCGAAT-CTCGCAGCCCTAGAGTCCAGTAGC  
GTCACCTTTA-GGTCCTAAGCGTAGTAATTTCTCCTCGCTACAGAACCT-----  
-----GCCGGTGGATAGTATAAATCCAGTTAAGTCTGGTATCCCGC-G  
GTTGACCTCGGATCAAGTAGGGATACCCGCTGAACTTAAGCATATCAATAAGCGGAGGAA  
AAGAAACCAACAGGGATTACTTTAGTAACGGCGAGTGAAGCGGTAAGTCTCAAATTTGA  
AAGCTGCC-----AACAGGCCGCGTTGTAATTTGTAGAAGATGCTTTGGGTGTGGCCCA  
GTCTAAGTTCCTTGGAACAGGACGTCATAGAGGGTGAGAATCCCGTATGTGATTGG-TGC  
CGTCCCCCGTGTAAAGCTCTTTCGACGAGTCGAGTTGTTTGGGAATGCAGCTCAAATGG  
GTGGTAAATTTTCATCTAAAGCTAAATATTGGCCAGAGACCGATAGCGCACAAGTAGAGTG  
ATCGAAAGATGAAAAGCACTTTGGAAAGAGAGTTAAACAGTACGTGAAATTTGTTGAAAGG  
GAAGCGCTTGCAATCAGACTTGCAGGCGGTTGATCATCCGAGGTTT-TCCCCGGTGCCT  
CGATCGTCT-TCAGGCCAGCATCGGTTTCAGTGGTGGGATAAAGGCTGTGAGAACGTGGC  
TC--TTC-----GGAGTGTATAGCTCACGGTGAATGCCGCTACTGGGACCGAGGAC  
CGCGCTTC-GGCTAGGATGCTGGCGTAATGGTTGTAAGCGACCCGTCTTGAAACACGGAC  
CAAGGAGTCTAACATCTATGCGAGTGTGGGTGTCAAACCCATACGCGTAATGAAAGTG  
AACGGAGGTGAGACCCATTAGGGCGCATCATCGACCGATCCTGATGTCTTCGGATGGAT  
TTGAGTAAGAGCATAGCTGTTGGGACCCGAAAGATGGTGAAGTATGCGTGAATAGGGTGA  
AGCCAGAGGAAACTCTGGTGGAGGCTCGCAGCGGTTCTGACGTGCAAATCGATCGTC-AA  
ATTTGCGTATAGGGC-GAAAGACTAATCGGTTAGACAGGGTCAGTTCA---CCCCTATA  
GGGTGGTGGCATCTCT--TGCAT-CTTGTGCTGACATGAAT-ATCTCAGAGTAACCCGCA  
ATACAAGGCAGCTGTTTCTATTCGGGACCCAAAGCGTAGATTTCGACACCATTTGGCGACT  
TTGCAAGCCCAAGATGATCTGCGACAGTGTTCCTAATGACGAC---GAATTCGGAGG  
TGATCCAAAGGAGGCTGTGAAG---CGTTCGCATGGAGGATGTGGAAATACGCAACCTGA  
GGTGCGCCAGCAAGCTTTGCAGCTTTGGGGAACATGGAAGATGCCAAAAGATGAAGAGAA  
TGAGGGTGG--CAACACT----GAGAAGCGACAAATTACTCCAGAGATGGCTCTCAATGT  
CTTCCGGTCCATGTCTTCCGATGAGATTCGCGATCTCGGTTTGAGCAACGACTATGCGCG  
TCCTGACTGGTTGATCATCACTGTTCTTCCAGTTCACCTCCTCCCGTTCGCCCCAGTAT  
TTCTATGGATGGTACAAGCACAGGAATGCGCGGAGAGGATGATTTGACCTACAAGCTAGG  
TGATATCATTCGTGCCAACGGCAATGTCAAGC-AGGCACAGCAAGAAGG

Figure 4B

Acidomelania\_panicicola\_KF874619  
 GTGTCTA-----  
 -----  
 -----CATACTCTTGT-TGCTTTGGCAGGCCGTGG-CCTCCCAC--TGT----GGGCTC  
 AGCCTGC-----ATGTGCCTGCCAGAGGACC---AAACTCTGAATGTTAGTGATGTCTGA  
 GTACTATATAATAGTTAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGA  
 ACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTG  
 AACGCACATTGCACCCGGTGGCATTCCGCCGGGTATGCCTGTTTCGAGCGTCATTATAACC  
 ACTCAAGCCTGTCTTGGTGTGGGGA-TTGCGAAT-CTCGCAGCCCTAGAGTCCAGTAGC  
 GTCACCTGTG-GGTCCTAAGCGTAGTAATTTCTCCTCGCTACAGAGCCT-----  
 -----GCTCGTGGATAGTGTAAATCCAGTTCGGTCTGGTATCCCGC-G  
 GTTGACCTCGGATCAAGTAGGGATACCCGCTGAACTTAAGCATATCAATAAGCGGAGGAA  
 AAGAAACCAACAGGGATTAC--TAGTAACGGCGAGTGAAGCGGTAAGTCTCAAATTTGA  
 AAGCTGCC-----AACAGGCCGCGTTGTAATTTGTAGAAGATGCTTTGGGTGTGGCCCA  
 GTCTAAGTTCCTTGGAACAGGACGTCATAGAGGGTGAGAATCCCGTATGTGATTGG-TGC  
 CGTCCCCCGTGTAAAGCTCTTTCGACGAGTCGAGTTGTTTGGGAATGCAGCTCAAATGG  
 GTGGTAAATTTTCATCTAAAGCTAAATATTGGCCAGAGACCGATAGCGCACAAGTAGAGTG  
 ATCGAAAGATGAAAAGCACTTTGGAAAGAGAGTTAAACAGTACGTGAAATTTGTTGAAAGG  
 GAAGCGCTTGCAATCAGACTTGCAGGCGGTTGATCATCCGAGGTTT-TCCCCGGTGCCT  
 CGATCGTCT-TCAGGCCAGCATCGGTTTCAGTGGTGGGATAAAGGCTGTGAGAACGTGGC  
 TC--TTC-----GGAGTGTATAGCTCACGGTGAATGCCGCCTACTGGGACCGAGGAC  
 CGCGCTTC-GGCTAGGATGCTGGCGTAATGGTTGTAAGCGACCCGTCTTGAAACACGGAC  
 CAAGGAGTCTAACATCTATGCGAGTGTGGGTGTCAAACCCATACGCGTAATGAAAGTG  
 AACGGAGGTGAGACCCATTAGGGCGCATCATCGACCGATCCTGATGTCTTCGGATGGAT  
 TTGAGTAAGAGCATAGCTGTTGGGACCCGAAAGATGGTGAAGTATGCGTGAATAGGGTGA  
 AGCCAGAGGAAACTCTGGTGGAGGCTCGCAGCGGTTCTGACGTGCAAATCGATCGTC-AA  
 ATTTGCGTATAGGGGC-GAAAGACTAATCGGTTAGACAGGGTCAGTTCA---CCCCTATA  
 GGGTGGTGGCATCTCT--TGCAT-CTTGTGCTGACATGAGT-ATCTCAGAGTAACCCGCA  
 ATACAAGGCAGCTGTTTCTATTCGGGATCCAAAGCGTAGATTTCGACACCATTTGGCGACT  
 TTGCAAGCCCAAGATGATCTGCGACAGTGTTCCTAATGACGAC---GAATTCGGAGG  
 TGATCCAAAGGAGGCTGTGAAG---CGTTCCCATGGAGGATGTGGAAATACGCAACCTGA  
 GGTGCGCCAGCAAGCCTTGCAGCTTTGGGGAACATGGAAAATGCCAAAGGATGAAGAGAA  
 TGAGAGTGG--CAACACT----GAGAAGCGACAAATTACTCCAGAGATGGCTCTCAATGT  
 CTTCCGGTCCATGTCTTCCGATGAGATTCGCGATCTCGGTTTGAGCAACGACTATGCGCG  
 TCCTGACTGGTTGATCATCACTGTTCTTCCAGTTCACCTCCTCCTGTTTCGCCCCAGTAT  
 TTCTATGGATGGTACAAGCACAGGAATGCGCGGAGAGGATGATTTGACCTACAAGCTGGG  
 TGATATCATTCGCGCCAACGGCAATGTCAAGC-AGGCACAGCAAGAAGG

Figure 4C

Acidoradicia\_panicicola\_AL5m2-2

GTGTCTA-----  
-----  
-----TCTACTCTTGT-TGCTTTGGCAGGCCGTGG-CCT-CCAC--CGC----GGGCTC  
TGCCCTGC-----GTGTGCCTGCCAGAGGACC---AAACTCTGAATTTTAGTGATGTCTGA  
GTACTATATAATAGTTAAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGA  
ACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTG  
AACGCACATTGCGCCCGGTGGTATTCCGCCGGGCATGCCTGTTTCGAGCGTCATTATAACC  
ACTCAAGCCTAGCTTGGTATTGGGGT-TCGCGGT--CCCGCGGCCCTAAAATCAGTGGC  
GGTGCCGGTG-GGCTCTAAGCGTAGTAA-ATCTCCTCGCTATAGGGTCC-----  
-----CC-----CCGGTTGCCCGC-G  
GTTGACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAGCATATCAATAAGCGGAGGAA  
AAGAAACCAACAGGGATTAC-TCAGTAACGGCGAGTGAAGCGGTAACAGCTCAAATT-GA  
AAGCTGCC-----AACAGGCCGCGTTGTAATTTGTAGAAGATGCTTTGGGGGTAGGCCA  
GTCTAAGTTCCTTGGAACAGGACGTCATAGAGGGTGAGAATCCCGTATGTGATTAG-TGC  
CTGCTCCCGTGTAAGCTCTTTCGACGAGTCGAGTTGTTTGGGAATGCAGCTCAAAATGG  
GTGGTATATTTTCATCTAAAGCTAAATATTGGCCAGAGACCGATAGCGCACAAGTAGAGTG  
ATCGAAAGATGAAAAGCACTTTGGAAAGAGAGTTAAACAGTACGTGAAATTGTTGAAAGG  
GAAGCGCTTGCAACCAGACTTGCAGGGCGGTTCGATCATCCGAGGTTTCCCGGTGCACT  
CGATCGTCT-TCAGGCCAGCATCGGTTTTGGTGGCGGGATAAAGGCTCTAGGAATGTGGC  
TC--TTC-----GGAGTGTATAGCCTAGGGTGCAATGCCGCCTACCGGGACCGAGGAC  
CGCGCTTC-GGCTAGGATGCTGGCGTAATGGTTGTAAGCGGCCCGTCTTGAAACACGGAC  
CAAGGAGTCTAACATCTATGCGAGTGTGGGTGTCAAACCCATACGCGTAATGAAAGTG  
AACGGAGGTGAGAGCCCTTTAGGGCGCATCATCGACCGATCCTGATGTCTTCGGATGGAT  
TTGAGTAAGAGCATAGCTGTTGGGACCCGAAAGATGGTGAACATGCGTGAATAGGGTGA  
AGCCAGAGGAAACTCTGGTGGAGGCTCGCAGCGGTTCTGACGTGCAAATCGATCGTC-AA  
ATTTGCGTATAGGGC-GAAAGACTAATCGGCTAGACAGGGTCAGTTTCG---CCCGAAA  
GGGTGGCGGATCTACT--TGTTT-TTTGTGCTGACATGAGT-TTCTCAGAGTAATCCGGC  
ATACAAGGCAGCCGTTTCGATTCGAGACCCGAAGCGTAAGTTTCGATAACCATATGGCGACT  
TTGCAAGCCCAAGATGATCTGCGACAGCGATGTCCCTAACGACGAT---GAATTTGGTGG  
TGATCCAAAGGAAGCTGTTAAA--CGTTCTCATGGAGGTTGTGGCAATACTCAACCCGA  
GGTTCGCCAACAAGCTTTACAGCTCTGGGGAACATGGAAGATGCCCAAGGATGAAGAAA  
CGAGGGTG---CGACTCAA---GAAAAGAGACAGATTACTCAAAGATGGCTCTGAATGT  
CTTCCGCAGCATGTCCCTCGGCTGAGATTTCGCGATTTGGGCTTGAGCAATGACTATGCACG  
CCCTGACTGGCTTATCATTACTGTCTTCCTGTTCCCTCCCCGCCTGTTTCGACCGAGTAT  
CTCCATGGATGGTACAAGCACAGGAATGCGCGGAGAGGATGATTTGACATAACAAGCTTGG  
TGATATTATTCGTGCAAACGGAAACGTTAAGC-AAGCCCAACAAGAGGG

Figure 4D

Acidoradicia\_panicicola\_CM11M2

GTGTCTA-----  
-----  
-----TCTACTCTTGT-TGCTTTGGCAGGCCGTGG-CCT-CCAC--CGC----GGGCTC  
TGCCCTGC-----GTGTGCCTGCCAGAGGACC---AAACTCTGAATTTTGTAGTGTCTGA  
GTACTATATAATAGTTAAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGA  
ACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTG  
AACGCACATTGCGCCCCGGTGGTATTCCGCCGGGCATGCCTGTTTCGAGCGTCATTATAACC  
ACTCAAGCCTAGCTTGGTATTGGGGT-TCGCGGT--CCCGCGGCCCTAAAATCAGTGGC  
GGTGCCGGTG-GGCTCTAAGCGTAGTAA-ATCTCCTCGCTATAGGGTCC-----  
-----CC-----CCGTTGCCCGC-G  
GTTGACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAGCATATCAATAAGCGGAGGAA  
AAGAAACCAACAGGGATTACCTCAGTAACGGCGAGTGAAGCGGTAACAGCTCAAATTTGA  
AAGCTGCC-----AACAGGCCGCGTTGTAATTTGTAGAAGATGCTTTGGGGGTTCGGCCTA  
GTCTAAGTTCCTTGGAACAGGACGTCATAGAGGGTGAGAATCCCGTATGTGATTAG-TGC  
CGGCTCCCGTGTAAGCTCTTTCGACGAGTCGAGTTGTTTGGGAATGCAGCTCAAATGG  
GTGGTATATTTTCATCTAAAGCTAAATATTGGCCAGAGACCGATAGCGCACAAGTAGAGTG  
ATCGAAAGATGAAAAGCACTTTGGAAAGAGAGTTAAACAGTACGTGAAATTTGTTGAAAGG  
GAAGCGCTTGCAACCAGACTTGCAGGCGGTTCGATCATCCGAGGTTTCCCGGTGCACT  
CGATCGTCT-TCAGGCCAGCATCGGTTTTGGTGGCGGGATAAAGGCTCTAGGAATGTGGC  
TC--TTC-----GGAGTGTTATAGCCTAGGGTGCAATGCCGCTACCGGGACCGAGGAC  
CGCGCTTC-GGCTAGGATGCTGGCGTAATGGTTGTAAGCGGCCCGTCTTGAAACACGGAC  
CAAGGAGTCTAACATCTATGCGAGTGTGGGTGTCAAACCCATACGCGTAATGAAAGTG  
AACGGAGGTGAGAGCCCTTTAGGGCGCATCATCGACCGATCCTGATGTCTTCGGATGGAT  
TTGAGTAAGAGCATAGCTGTTGGGACCCGAAAGATGGTGAACATATGCGTGAATAGGGTGA  
AGCCAGAGGAACTCTGGTGGAGGCTCGCAGCGGTTCTGACGTGCAAATCGATCGTC-AA  
ATTTGCGTATAGGGGC-GAAAGACTAATCG-----  
-----GACATGAGT-TTCTCAGAGCAATCCGGC  
ATACAAGGCAGCCGTTTCAATTCGAGACCCGAAGCGTAGGTTTCGATACGATATGGCGACT  
TTGCAAGCCCAAGATGATCTGCGACAGCGATGTCCCTAATGATGAT---GAATTTGGTGG  
TGATCCAAAAGAAGCTGTTAAA--CGTTCTCATGGAGGTTGTGGCAATACTCAACCCGA  
GGTTCGCCAGCAAGCTTTACAGCTCTGGGGAACATGGAAGATGCCCAAGGATGAAGAAAA  
CGAGGGTG---CGACTCAA---GAAAAGAGACAGATTACTCCAGAGATGGCTCTGAACGT  
CTTCCGCAGCATGTCTCGGCTGAGATTCGCGATTTGGGCTTGAGCAATGACTATGCACG  
CCCTGACTGGCTTATCATTACTGTCTTCCCGTTCCTCCCCACCTGTTTCGACCAAGTAT  
TTCCATGGATGGTACAAGCACAGGAATGCGCGGAGAGGATGATTTGACATACAAGCTTGG  
TGATATTATCCGTGCAAATGGTTTCATTAAGC-AAGCCCAACAAGAGGG

Figure 4E



Acidoradicia\_taeda\_CM14-P64

GTGTCTA-----  
-----  
-----TTTACTCTTGT-TGCTTTGGCAGGCCGTGG-CCT-CCAC--CGT----GGGCTC  
TGCTCTAC-----GCGTGTCTGCCAGAGGACC---AAACTCTGAATTTTAGTGATGTCTGA  
GTACTATAACAATAGTTAAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGA  
ACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTG  
AACGCACATTGCGCCCCGGTGGTATTCCGCCGGGCATGCCTGTTTCGAGCGTCATTATAACC  
ACTCAAGCCTGGCTTGGTATTGGGGT-ACGCGGC--TTCGCGGCTCCTAAAATCAGTGGC  
GGTGCCGGTG-GGCTCTAAGCGTAGTAA-ATCTCCTCGCTATAGGGTTC-----  
-----CT-----CTGGTTGCTTGC--  
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-----GGTGC  
ACT  
CGATCGTCT-TCAGGCCAGCATCGGTTTCGGTGGCGGGATAAAGGCTCTAGGAATGTGGC  
TC--TTC-----GGAGTGTATAGCCTAGGGTGCAATGCCGCTACCGGGACCGAGGAC  
CGCGCTTC-GGCTAGGATGCTGGCGTAATGGTTGTAAGCGGCCCGTCTTGAAACACGGAC  
CAAGGAGTCTAACATCTATGCGAGTGTGGGGTGTCAAACCCATACGCGTAATGAAAGTG  
AACGGAGGTGAGAGCCCTTTAGGGCGCATCATCGACCGATCCTGATGTCTTCGGATGGAT  
TTGAGTAAGAGCATAGCTGTTGGGACCCGAAAGATGGTGAACATGCGTGAATAGGGTGA  
AGCCAGAGGAAACTCTGGTGGAGGCTCGCAGCGTTCGACGTGCAAATCGATCGTC-AA  
ATTTGCGTATAGGGGC-GAAAGACTAATCGGCTAGACAGGGTCAGTTCG---CCCGAATA  
GGGTGGCAGATCTACT--TGTTCT-TTTGTGCTGACATGAGT-ATCTCAGAGTAATCCGGC  
GTACAAGGCAGCTGTTTCGATTCGGGACCCGAAGCGTAGGTTTCGATACGATATGGCGACT  
TTGCAAGCCCAAGATGATCTGCGACAGCGATGTCCCTAACGACGAT---GAATTTGGTGG  
TGATCCAAAGGAAGCTGTCAAG---CGTTCTCATGGAGGTTGTGGTAATACTCAGCCCGA  
GGTTCGTCAGCAGGCTCTACAGCTCTGGGGTACATGGAAGATGCCAAAGGATGAAGAAA  
TGAGGGGT---CAAGTCAA---GAAAAGAGACAAATCACTCCAGAGATGGCTTTAAATGT  
CTTCCGAAGCATGTCTCGGCTGAGATTCGCGACCTGGGCCTGAGCAACGACTACGCTCG  
TCCCGACTGGCTCATCATTACAGTCCTTCCTGTTCCCTCCTCCGCCCGTTCCGCCCTAGTAT  
TTCTATGGATGGCACAAGCACGGGAATGCGTGGAGAAGATGATTTGACCTACAAGCTTGG  
TGATATAATTCGTGCCTACGGCAACGTTATGCAAAGCACAACAAGAATG

Figure 4G





Cudoniella\_clavus\_DQ491502

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-----AAAAGTCGTAACAAGGTTTCCGTAG
GTGAACCTGCGGAAGGATCATTACAGTGTTCCCTGCCCTCACGGGTAGAAACGCCACCCT
TGTATATATTATCTTGT-TGCTTTGGCGGGCCGCCT-TTAGGCAC----T----GGCTTC
GGCTGGC-----TCGCGCCCGCCAGAGAACCCC-AAACTCTAAATGTTAGTGTCTGTCTGA
GTACTATCTAATAGTTAAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGA
ACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTG
AACGCACATTGCGCCCCTTGGTATTCCGGGGGGGCATGCCTGTTTCGAGCGTCATTTAAACC
AATCCAGCAT-GCTGGGTCTTGGGCCCTTCGCCTC--TGGGCGGGCCTCAA AATTAGTGGC
GGTGCCACCT-GGCTCTACGCGTAGTAA-TTCTTCTCGCGATGGAGTCCCAGGTGGAAGC
TTGCCAACAAACCCCAAATTCTTTTAAAGGTTGACCTCGGATCAGGTAGGGATAACCCGC--
-----CTAAGCATATCAATAAGCGGAGGAA
AAGAA?CCAACAGGGATTACCTCAGTAACGGCGAGTGAAGCGGTAACAGCTCAAATTTGA
AATCTGGCTCTTTCAGGGTCCGAGTTGTAATTTGTAGAAGATGCTTCGGGTGTGGCTCCG
GTCTAAGTTCCTTGGAACAGGACGTCATAGAGGGTGAGAATCCCGTATGTGACTGGTTGC
CTTCGCCCATGTGAAGCTCTTTCGACGAGTCGAGTTGTTTGGGAATGCAGCTCTAAATGG
GTGGTAAATTTTCATCTAAAGCTAAATATTGGCCAGAGACCGATAGCGCACAAAGTAGAGTG
ATCGAAAGATGAAAAGCACTTTGGAAAGAGAGTTAAACAGTACGTGAAATTTGTTGAAAGG
GAAGCGCTTGCAACCAGACTTGCACGTCGTCGATCATCCTCAGTTC-TCTGGGGTGC ACT
CGGCGGTGT-TCAGGCCAGCATCGGTTTTCGGTGGTGGGATAAAGGCCTTGGGAATGTGGC
TCCTCTC----GGGGAGTGTATAGCCCTCGGTGCAATGCCGCCTACTGGGACCGAGGAC
CGCGCTTC-GGCTAGGATGCTGGCGTAATGGTTGTAAGCGACCCGTCTTGAAACACGGAC
CAAGGAGTCTAACATCTATGCGAGTGTGTTGGGTGTTAAACCCATACGCGTAATGAAAGTG
AACGGAGGTGAGAACCCTTTAGGGTGCATCATCGACCGATCCTGATGTCTTCGGATGGAT
TTGAGTAAGAGCATAGCTGTTGGGACCCGAAAGATGGTGA ACTATGCCTAAATAGGGTGA
AGCCAGAGGAAACTCTGGTGGAGGCTCGCAGCGTTCTGACGTGCAAATCGATCGTC-AA
ATTTGGGTATAGGGGC-GAAAGACTAATCGACTAGACAGGGTCAGTTGG--CCC GCATA
GCGTGGCATGTCT-TT--TGTGCCTTTGTGCTAACA-GAATGATTTTCAGAGCAATCCAGC
GTACAAGGCAGCTGTTTCTATCCGAGATCCAAAGCGTAGATTTGATACAATATGGCGACT
GTGCAAGCCGAAGATGATTTGCGAGGGTGATGTGCAGGCGAATGAGGAAGAATTTGATCC
CAACCAAAAAGAACC---GAAG---CCGTCGCACGGAGGGTGTGGTAATTCTCAGCCTGA
AGTGCGTCAGACTGCTTTGCAACTATGGGGAACATGGAAAGTGCCTAAGGACGAAGATAA
CGAGAGTCAGTCGCCG-----GAAAAGAGGCAGATTACTCCCGAAATGGCTCTGGCTGT
CTTCCGAAGCATTTCACGGAAGAAATCTTC?ACCTTGGCCTGAGTAATGATTATGCGCG
TCCCGAATGGATGATCATAACGTTTCTCCAGTTCCTCCACCACCTGTTTCGACCCAGTAT
TTCAATGGATGGCACTGGTCAGGGCATGCGCGGAGAGGACGATTTGACATATAAGTTGGG
AGATATCATCCGGGCAAACGGCAATGTGCGGC-AAGCTCAGCAGGAAGG

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Figure 4J

Dermea\_acerina\_AF141164

GTGTCGT-----  
-----  
-----TATACCTTCGT-TGCTTTGGTGGGCCGCTGGGCTTCGGCCTGGCTCCTGGCTCC  
GGCTAGG-----GAGTGCCCGCCAGAGGACC-TTAAACCTGAA-GTTAGTGTCTGCTCTGA  
GTACTATACAATAGTTAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGA  
ACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTG  
AACGCACATTGCGCCCCTTGGTATTCCGGGGGGCATGCCTGTTTCGAGCGTCATTACAACC  
-CTCAAGCTCTGCTTGGTATTGGGCG-TCACCGGGTTCGGTGTGCCTTAAAATCAGTGGC  
GGCGCCGTCT-GGCTCTAAGCGTAGTAC-ATACTCTCGCTATGGACGCC-----  
-----TG-----GCGGATGCTTGC--  
-----  
-----GA  
AATCTGGGTCTTTTAGGCTCCGAGTTGTAAT?TGTAGAAGATGCTTCGGGTGCGGCTCCG  
GTCTAAGTTCCTTGGAACAGGACGTCATAGAGGGTGAGAATCCCGTATGTGATCGGGGGC  
TTGCGCCCATGTGAAGCTCTTTCGACGAGTCGAGTTGTTTGGGAATGCAGCTCAAAATGG  
GTGGTATATTTTCATCTAAAGCTAAATATTGGCCAGAGACCGATAGCGCACAAGTAGAGTG  
ATCGAAAGATGAAAAGCACTTTGGAAAGAGAGTTAAACAGTACGTGAAATTTGTTGAAAGG  
GAAGCGCTTGCAACCAGACTTGGGCGGGGTTGATCATCTAGGGTTC-TCCCTAGTGCCT  
CGACCTCGC-ACAGGCCAGCATCGGTTCCGGTGGTTGGATAAAGGCCTTGGGAATGTAGC  
TTCTTTC---GGGGAGTGTATAGCCCTCGGTGCAATGCAGCCTACTGGGACCGAGGAC  
CGCGCTTC-GGCTAGGATGCTGGCGTAATGGTTGTAAGCGACCCGTCTTGAAACACGGAC  
CAAGGAGTCTAACATCTATGCGAGTGTGGGTGTCAAACCCATACGCGTAATGAAAGTG  
AACGGAGGTGAGAACCCTTAAGGGTGCATCATCGACCGATCCTGATGTCTTCGGATGGAT  
TTGAGTAAGAGCATAGCTGTTGGGACCCGAAAGATGGTGAACCTATGCCTGAATAGGGTGA  
AGCCAGAGGAAACTCTGGTGGAGGCTCGCAGCGGTTCTGACGTGCAAATCGATCGTC-AA  
ATTTGGGTATAGGGC-GAAAGACTAATCGGCTAGACAGGGTCAGTTCT---CCCACGTA  
GCGTGGGGAGCATGAT--TGTTTCTGTGTGCTAACGTCAAT-ATCTCAGAGTAATCCAGC  
GTACAAAGCAGCCGTTTCGATTCGAGACCCGAAAGCGTAGGTTTCGATACGATCTGGCGACT  
TTGCAAGCCCAAGATGATCTGTGATAGCGATTTGACTGCCGCCGATGATGATTTCAATGC  
AGACCCGAAGGAAGCCGAAAG---CGCTCGCACGGTGGATGTGGAAATACTCAGCCTGA  
GGTGCGCCAGTCGGCCTTGCAGCTGTGGGGTACATGGAAGGTTCCAAAGGATGAAGACAA  
TGATGGTGTACTGCC-----GAAAAGAAGCAGATCACTGCAGAGATGGCCCTGAATGT  
CTTCCGAAGCATTTCCTTCTGAGATCCAAGACCTTGGCTTGAGTACTGACTATGCGCG  
ACCTGAATGGATGATCATTACGTTCTTCCAGTTCCTCCTCCACCTGTTTCGACCGAGTAT  
TTCGATGGACGGCAAGGCATGCGAGGAGAGGATGATTTGACATACAAGCTTGG  
CGATATCATTCGTGCGAATGGCAACGTTTCGAC-AGGCCAGCAAGAAGG

Figure 4K

Hyaloscypha\_aureliella\_JN943611  
 GTGCCTGGTCTAAGATATAGTCCGGTCCCGGCCCGAAAGGGCCGGGGAACAGCGTCCGTAG  
 GTGAACCTGCGGAAGGATCATTACAGAGTTCATGCCCTCACGGGTAGATCTCCACCCTT  
 GAATACCTTACCTTTGTCTGCTTTGGCGGGCCACGT-----CCGCG-TGC----CGGCTC  
 CGGCTGG-----TTGCGCCCGCCAGAGGACC--CAAACCTTTTTGTTTAGTGATGTCTGA  
 GTACTATATAATAGTTAAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGA  
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 ACTCAAGCCTGGCTTGGTGTGGGGT-CCGCGGT--CCGCGGCCCTTAAAATCAGTGGC  
 GCGCCATCT-GGCTCTCAGCGTAGTAA-TACTCCTCGCTACAGGGTCC-----  
 -----C--  
 -----  
 -----ACGGCGAGTGAAGCGGCAACAGCTCAAATTTGA  
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 CTCCGTTTCGTGTAAGCTCTTTCGACGAGTTCGAGTTGTTTGGGAATGCAGCTCAAATGG  
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 CGGCAGCGA-TCAGGCCAGCATCGGTTCTGGTGGCGGATAAAGGCCTTGGGAATGTAGC  
 TTCTTC----GGGGAGTGTATAGCCCTCGGTGCAATGCGCCCTACCGGGACCGAGGAC  
 CGCGCTTC-GGCTAGGATGCTGGCGTAATGGTTGTAAGCGACCCGTCTTGAAACACGGAC  
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 AGCCAGAGGAAACTCTGGTGGAGGCTCGCAGCGGTTCTGACGTGCAAATCGATCGTC-AA  
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 AGTGCGCCAGAGCGCCCTTCAGCTCACCGGCACTTTTAAGCCTTCGAAGGAAGAACTCAG  
 CGAGGGC-----ATGCAGCCAGAAAAGAAGTTAATCACCCAGAGGCAGCTCTGCACAT  
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 CCCGGAATGGATGATCATCACAGTCCTTCCCGTGCCTCCTCCTCCTGTTTCGGCCCAGTAT  
 TTCTATGGATGGCACTGGTCAAGGTATGCGAGGAGAGGATGATTTGACATACAACTTGG  
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Figure 4L

Lachnum\_virgineum\_DQ491485

GTATCAT-----  
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GATTCGCGTCGAGCGCGCCCGCCAGAGGACCCCTAAACTSTGAATGTTAGTGTCTGTCTGA  
GTACTATTAATAGTTAAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGA  
ACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTG  
AACGCACATTGCGCCCCTTGGTATTCCGGGGGGCATGCCTGTTTCGAGCGTCATTTATAACC  
AATCTARCTGGCTAGGTGTTGGGCC-TCGCCAG--TTGGCGGGCCTTAAAAC TAGTGGC  
GGTGCTCTTC-AGCTCTACGCGTAGTAA--TTTTCTCGCTATAGGGTCT-----  
-----GGGGAGATGCTTGC--  
-----  
-----CCACAGGGATTACCTCAGTAACGGCGAGTGAAGCGGTAACAGCTCAAATTTGA  
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GTGGTAAATTTTCATCTAAAGCTAAATATTGGCCAGAGACCGATAGCGCACAAGTAGAGTG  
ATCGAAAGATGAAAAGCACTTTGGAAAGAGAGTTAAACAGTACGTGAAATTTGTTGAAAGG  
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CGGTAGTAT-CTAGGCCAGCATCGGTTTGGGTGGTGGGATAAAGGCCTTGGGAATGTAGC  
TTCTTTC---GGGGAGTGTATAGCCCTCGGTGCAATGCCGCCTACCCGGACCGAGGAC  
CGCGCTTC-GGCTAGGATGCTGGCGTAATGGTTGTAAGCCACCCGTCTTGAAACACAGAC  
CAAGGAGTCTAACATCTATGCGAGTATTTGGGTGTTAAACCCATATGCGTAATGAAAGTG  
AACGGAGGTGAGAACCCTTAAGGGTGCATCATCGACCGATCCTGATGTCTTCGGATGGAT  
TTGAGTAAGAGCATAGCTGTTGGGACCCGAAAGATGGTGAACCTATGCCTAAATAGGGTGA  
AGCCAGAGGAAACTCTGGTGGAGGCTCGCAGCGTTCTGACGTGCAAATCGATCGTC-AA  
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GTACAAGGCAGCTGTTTCTATTCGTGATCCAAAGCGTAGATTTCGATACAATCTGGCGACT  
TTGCAAGCCCAAGATGATCTGCGATAGCGATGTCCCTAATGAAGAT---GAATTCGGTGG  
TGATCCAAAGGAAGCTGTGAAG---CGTTCGCATGGAGGATGTGGAAATACACAACCTGA  
GGTCCGCCAACAGGCATTGCAGCTCTGGGGAACATGGAAGATGCCAAAGGATGAGGAGAA  
CGAGGGTGG--CAACTCG---GAGAAGAGACAAATTACACCAGAGATGGCTCTCAATGT  
CTTCAGATCCATGTCTTCTGAAGAAATTCGCGACCTCGGTCTCAGCAACGATTATGCACG  
TCCTGACTGGTTGATTATTACAGTTCTTCCAGTTCGGCCTCCTCCTGTTTCGACCCAGTAT  
TTCCGTGGACGGCACGAGCACAGGTATGCGCGGAGAGGATGATTTGACATACAAGCTTGG  
TGATATCATTCGTGCCAACGGCAATGTGAAGC-AGGCTCAACAAGAAGG

Figure 4M



Mollisia\_cinerea\_DQ491498

GTGTCTA-----  
-----  
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TGCCCTAC-----ATGTGCCTGCCAGAGGACC---AAAATCTGAATTTTAGTGATGTCTGA  
GTACTATATAATAGTTAAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGA  
ACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTG  
AACGCACATTGCGCCCTGTGGTATTCCGCAGGGCATGCCTGTTTCGAGCGTCATTATAACC  
ACTCAAGCCTGGCTTGGTATTGGAGT-TTGCGGT--TCCGCAGCTCCTAAAATCAGTGGC  
GGTGCCGGTGTGGCTCTACGCGTAGTAA-TTCTTCTCGCGATGGAGTTC-----  
-----CC-----CTGGTTGCTTGC--  
-----  
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CAGCCCCCGTGTAAGCTCTTTCGACGAGTTCGAGTTGTTTGGGAATGCAGCTCAAATGG  
GTGGTAAATTTTCATCTAAAGCTAAATATTGGCCAGAGACCGATAGCGCACAAGTAGAGTG  
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CGATTGTCT-TCAGGCCAGCATCGGTTTCGGTGGTGGGATAAAGGCTGTGGGAATGTGGC  
TC--TTC-----GGAGTGTATAGCCCACGGTGCAATGCCGCCCTACCGGGACCGAGGAC  
CGCGCTTC-GGCTAGGATGCTGGCGTAATGGTTGTAAGCGACCCGTCTTGAAACACGGAC  
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GGGTGGCAGATCTACT--TGTTC-TTTGTGCTGACATGAGT-ATCTCAGAGTAATCCGGC  
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TGATCCTAAGGAAGCTGTCAAG---CGCTCACATGGAGGTTGTGGAAACACTCAGCCCCGA  
AGTGCGTCAACAATCTCTCCAACCTTTGGGGTACATGGAAGATGCCAAAGGATGAGGAGAA  
CGAGGGCGGTGCAACGCAG---GAGAAGAAACAAATTACTCCAGAGATGGCTCTCAATGT  
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TCCTGACTGGTTGATTATCACTGTTCTTCCAGTTCACCTCCCCAGTCCGACCAAGTAT  
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TGATATCATTCGTGCAAATGGAAACGTTAAGC-AGGCACAACAGGAAGG

Figure 40

Monilinia\_laxa\_EF153017

GTGGAAGT-----AAAAGTCGTAACAAGGTTTCCGTAG  
 GTGAACCTGCGGAAGGATCATTACAGAGTTCATGCCCGAAAGGGTAGACCTCCACCCTT  
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 TG-----TATGCTCGCCAGAGAATAATCAAACCTTTTTTATTAATGTCGTCTGA  
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 ACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTG  
 AACGCACATTGCGCCCCTTGGTATTCCGGGGGGCATGCCTGTTTCGAGCGTCATTTCAACC  
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 GGCGCCGCTG-GGTCCTGAA-----  
 -----  
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 CCTCTC----GGGGGTGTTATAGCTCTAGGTGCAATGTAGCCTACCTGG??TGAGG?C  
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 CAAGGAGTGTACCTAATATGCGAGTGTGGGTGTT-AACCCATACGCGTAATGAAAGTG  
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 CTGCAAGTCCAAGATGATTTGTGATAGTGACGTCCAGGCGAACGAGGAGGAATTC AATGG  
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 CCCAGAATGGATGATTATTACCGTTCTACCAGTACCACCGCCTCCCGTTTCGACCAAGTAT  
 TTCCATGGATGGTACTGGTCAGGGTATGCGAGGAGAGGATGATTTGACATACAAGTTGGG  
 TGATATCATTCGTGCCAATGGTAACGTTTCGTC-AAGCACAACAAGAAGG

Figure 4P

Phialocephala\_fortinii\_AB671499

GTGTTTA-----  
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TGCTCGT-----GTGTGCCCGCCAGAGAACC---AAACTCTGAATGTTAGTGATGTCTGA  
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-----GTAACGGCGAGTGAAGCGGTAACAGCTCAAATTTGA  
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Figure 4Q

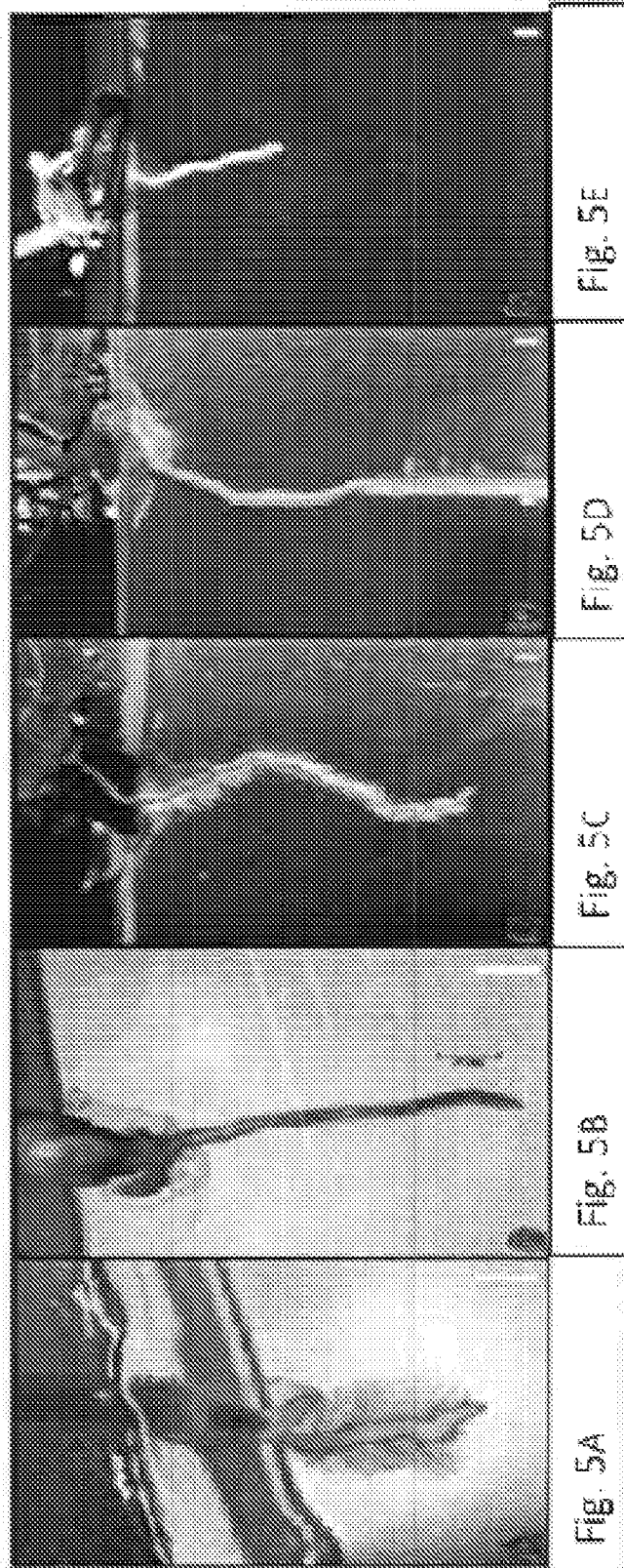
Vibrissea\_truncorum\_EU434855

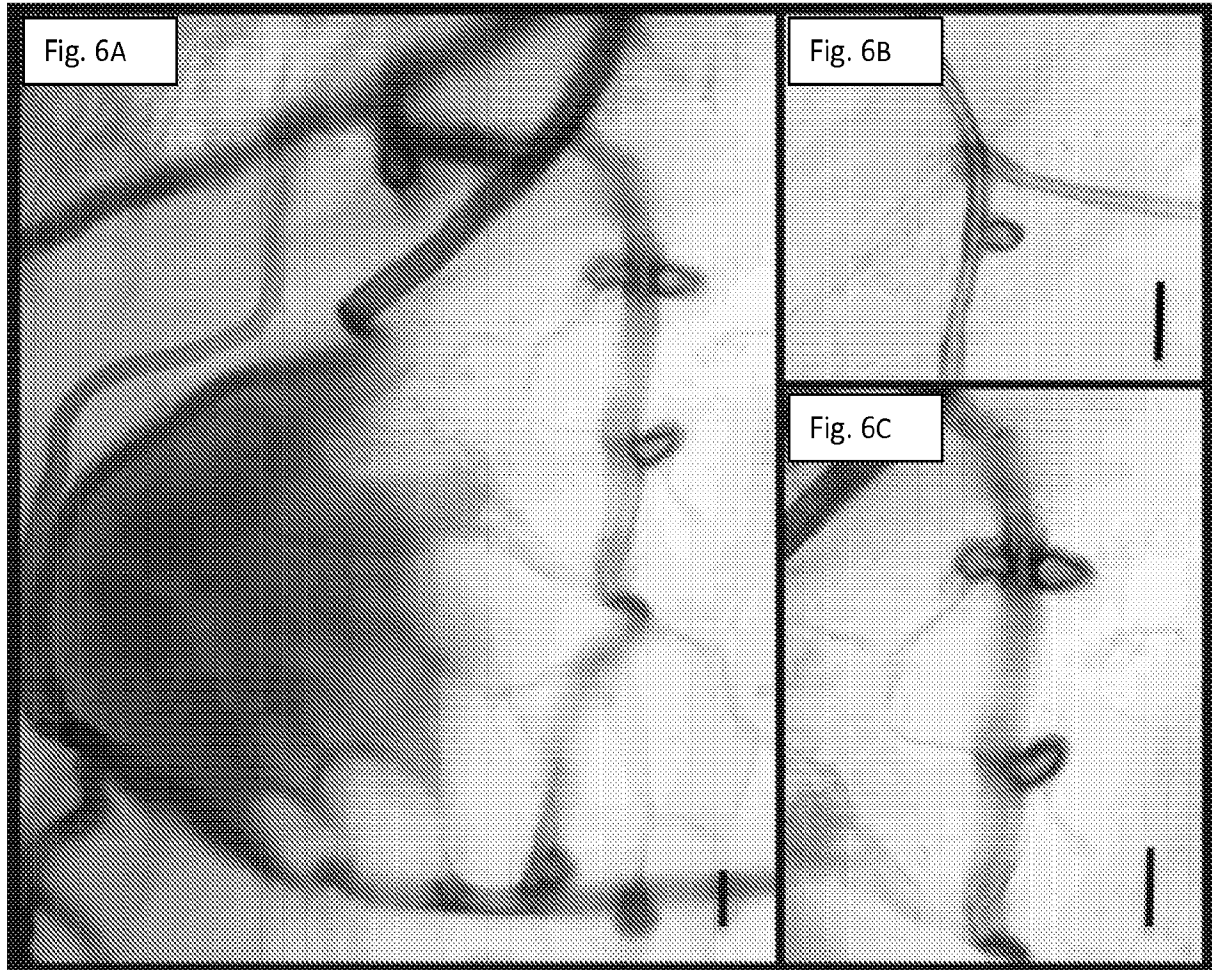
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GATGCCGGTT-GGCTCTAAGCGTAGTAA-CTTCTCTCGCTATAGATGTC-----
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-----
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AAGCTGCC-----AACAGGCCGCATTGTAATTTGTAGAGGATGCTTTGGGGGTTGGCCCG
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Figure 4R





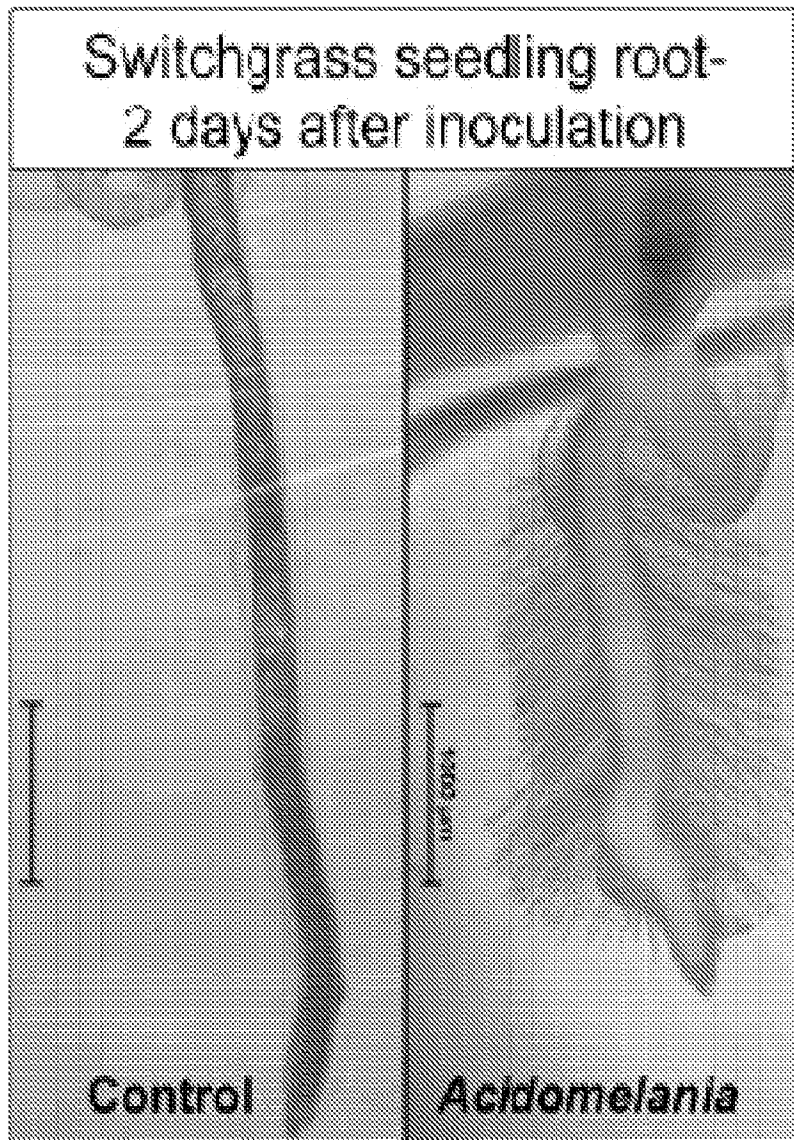


Figure 7

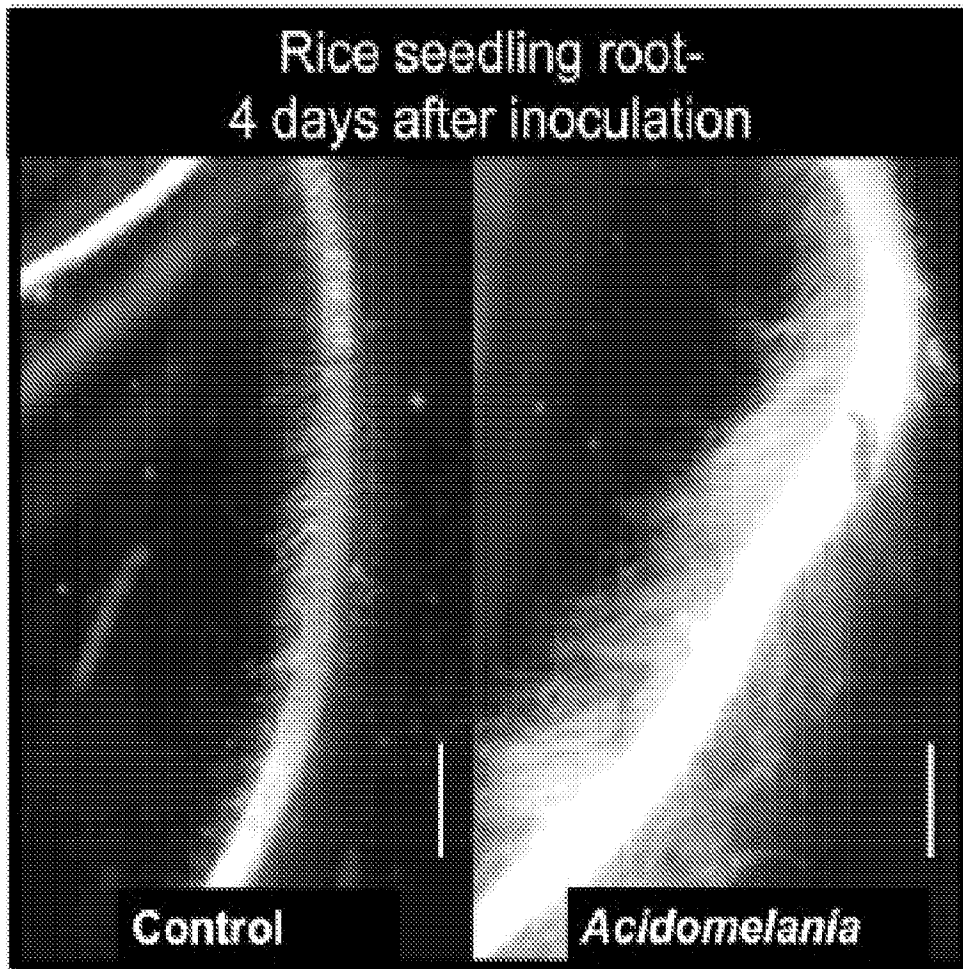


Figure 8

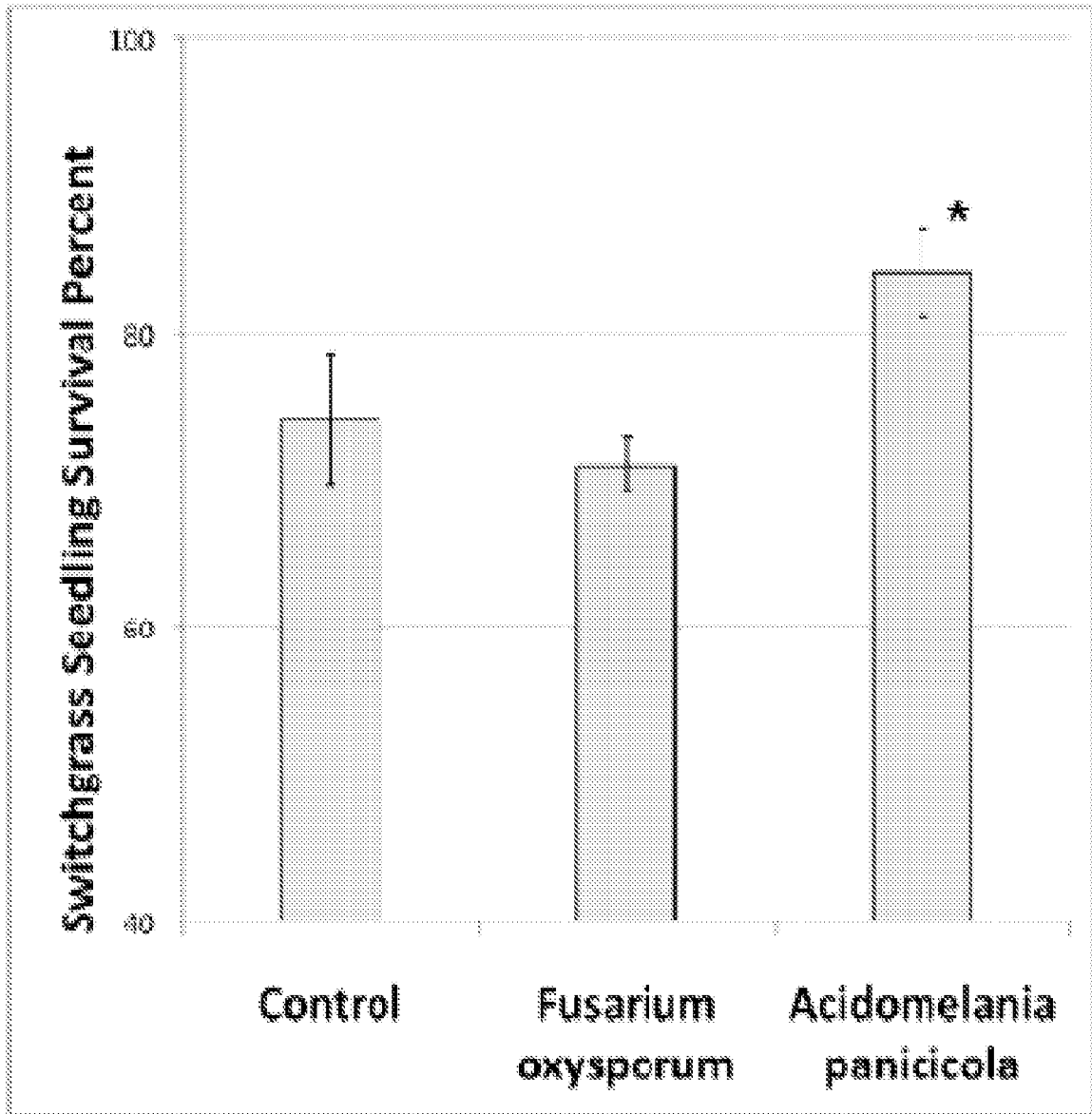


Figure 9

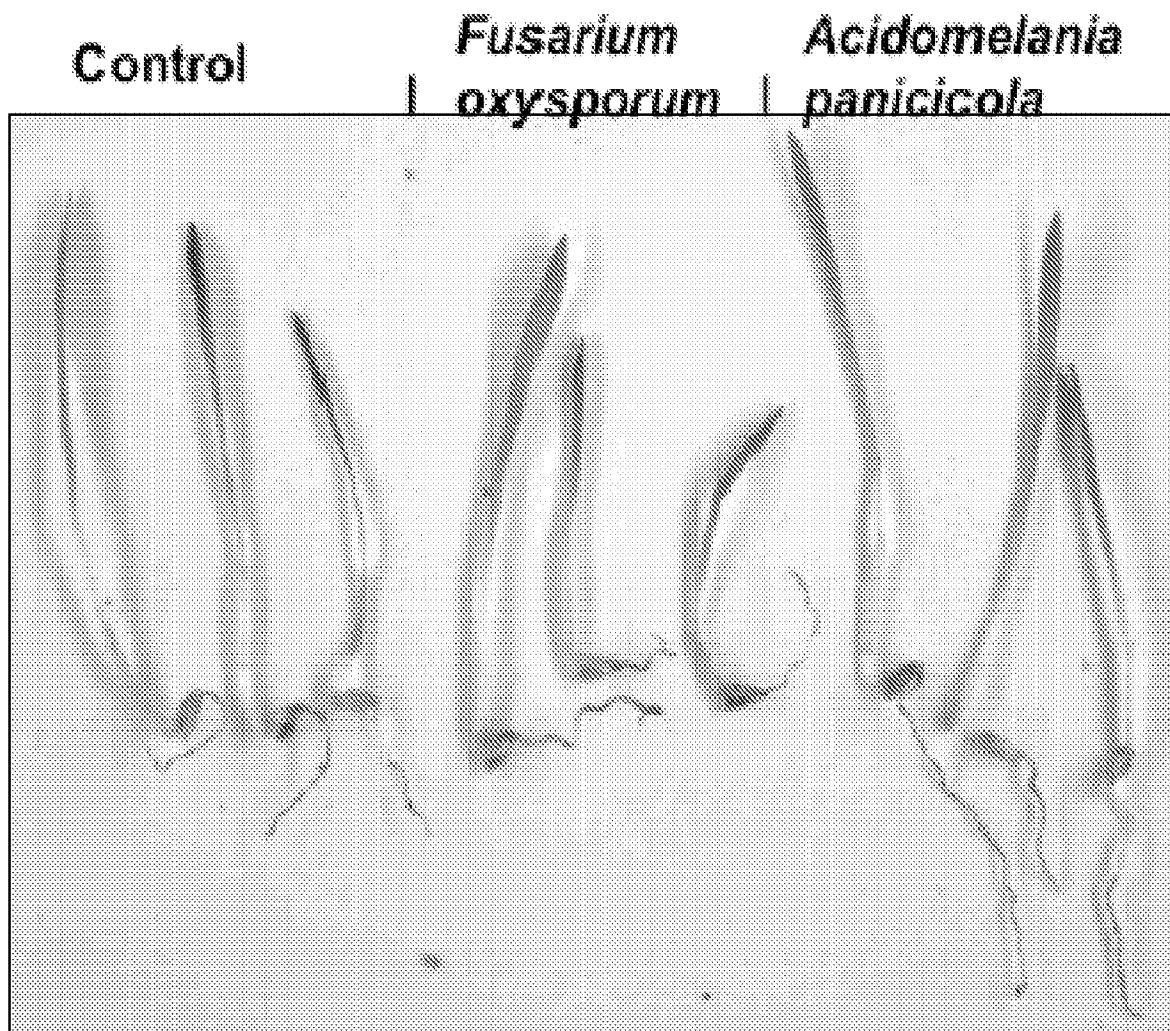
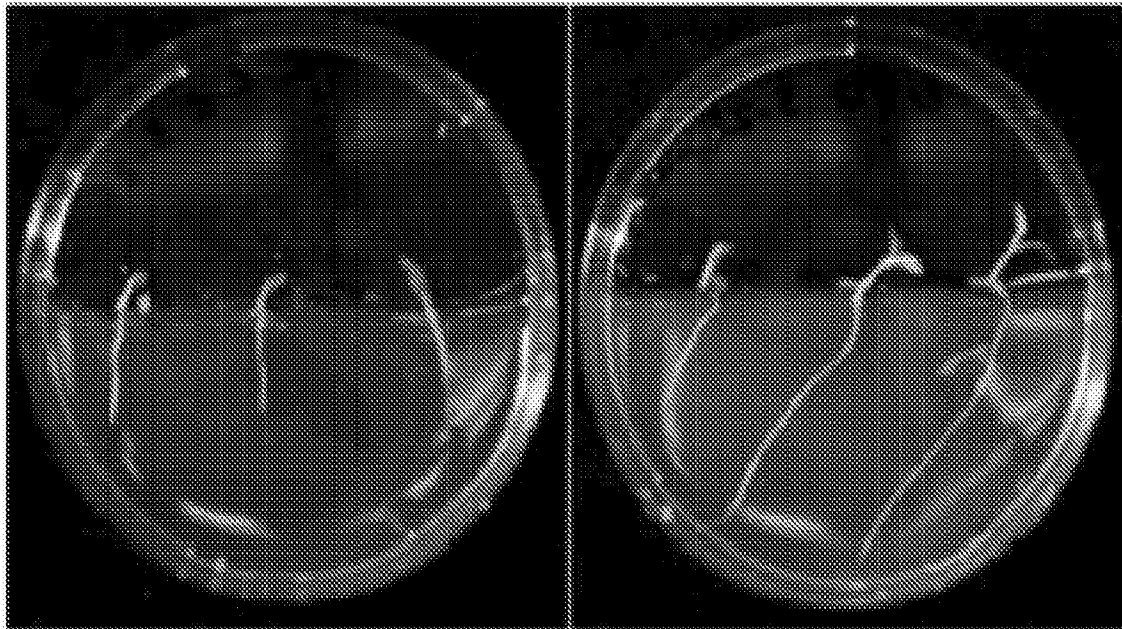


Figure 10

# Lettuce



**Control**

***Acidomelania*, 4 days  
after inoculation**

Figure 11

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US15/48889

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - C05F 11/08; C12N 1/14; C08L 5/06 (2015.01)

CPC - C05F 11/08; C12N 1/14; C08L 5/06

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC(8): C05F 11/08; A01C 21/00; C12N 1/14, 1/20; A01H 17/00; C08L 5/04, 5/06, 5/12; A01N 63/04 (2015.01)

CPC: C05F 11/08; C12N 1/14, 1/20; A01G 1/048; C08L 5/04, 5/06, 5/12; A01C 21/00; A01N 63/04

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Patseer (US, EP, WO, JP, DE, GB, CN, FR, KR, ES, AU, IN, CA, INPADOC, RU, AT, CH, TH, BR, PH); PubMed; EBSCO; Google; Google Scholar; Google Patents: biofertilizer, fertilizer, bacteria, fungi, endophytic, 'Acidomelania panicicola', 'Acidomelania,' 'Burkholderia,' 'Barrenia panicia,' 'Barrenia,' 'dark septate,' symbiotic, mycorrhizae

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- A	WO 2013/090628 A1 (SYNTHETIC GENOMICS, INC.) June 20, 2013; paragraphs [0010]-[0012], [0059], [0060], [0070]-[0077], [00164]-[00166]	1, 4-6, 25, 26 ----- 2, 3, 13-24, 28-34
A	✓ WALSH, E et al. <i>Acidomelania panicicola</i> gen. et sp. nov. from Switchgrass Roots In Acidic New Jersey Pine Barrens. <i>Mycologia</i> . 02 June 2014, Vol. 106, No. 4; pages 856-864; abstract; DOI: 10.3852/13-377.	2, 3, 13-24, 28-34
A	✓ ZIJLSTRA, JD et al. Diversity Of Symbiotic Root Endophytes Of The Helotiales In Ericaceous Plants And The Grass, <i>Deschampsia flexuosa</i> . <i>Studies in Mycology</i> . 2005, Vol. 53; pages 147-162; abstract; page 158, first column, third paragraph; DOI: 10.3114/sim.53.1.147.	2, 3, 13-24, 28-34
A	✓ MATHEW, A et al. The Evidence Of Mycorrhizal Fungi And Dark Septate Endophytes In Roots Of <i>Chlorophytum borivillanum</i> . <i>Acta Botanica Croatica</i> . 2008 [retrieved on 12 November 2015], Vol. 67, No. 1; pages 91-96; Retrieved from the Internet: <URL: <a href="http://hrcak.srce.hr/file/35449">http://hrcak.srce.hr/file/35449</a> >.	1-6, 13-26, 28-34
A	WO 2013/098829 A1 (THE ENERGY AND RESOURCES INSTITUTE (TERI)) July 04, 2013; abstract	1-6, 13-26, 28-34

 Further documents are listed in the continuation of Box C. See patent family annex.

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&amp;" document member of the same patent family

Date of the actual completion of the international search

13 November 2015 (13.11.2015)

Date of mailing of the international search report

14 DEC 2015

Name and mailing address of the ISA/

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents  
P.O. Box 1450, Alexandria, Virginia 22313-1450

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PCT Helpdesk: 571-272-4300  
PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US15/48889

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
- a.  forming part of the international application as filed:
    - in the form of an Annex C/ST.25 text file.
    - on paper or in the form of an image file.
  - b.  furnished together with the international application under PCT Rule 13ter.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.
  - c.  furnished subsequent to the international filing date for the purposes of international search only:
    - in the form of an Annex C/ST.25 text file (Rule 13ter.1(a)).
    - on paper or in the form of an image file (Rule 13ter.1(b) and Administrative Instructions, Section 713).
2.  In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

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**Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

- 1.  Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
  
- 2.  Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
  
- 3.  Claims Nos.: 7-12, 27  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

- 1.  As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
- 2.  As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
- 3.  As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
  
- 4.  No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

- Remark on Protest**
- The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
  - The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
  - No protest accompanied the payment of additional search fees.