1 ***Barrenia*, a new genus associated with roots of switchgrass and pine in**
2 **the oligotrophic pine barrens**

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14
15 **ABSTRACT**

16 A new genus *Barrenia* is described based on multi-gene phylogenetic analyses and
17 phenotypic and ecological characters. Isolated from roots of switchgrass and pitch pine in
18 the acidic and oligotrophic New Jersey Pine Barrens in this study, *Barrenia* likely has a
19 wide distribution because its internal transcribed spacer (ITS) sequence has high
20 similarity with a number of GenBank sequences from various ecological studies. The
21 majority of these matching samples were from roots of plants in acidic, nutrient-poor
22 environments, as well as from managed sugarcane plantations. Phylogenetic analyses

based on ITS, LSU and *RPBI* 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. Taxa in PAC also are root endophytes but they have complex phialid arrangements that appear to be lacking in *Barrenia*. Plant-fungal interaction experiments showed that *Barrenia panicia* and *Acidomelania panicicola* significantly promoted root hair growth in switchgrass. Results from this work will facilitate ecological and evolutionary studies on root-associated fungi.

Keywords:

Dark septate endophytes; Grass; Growth promotion; Leotiomycetes; Phylogeny; Taxonomy

1 Introduction

Pine barrens is a drought and fire-prone ecosystem with acidic and oligotrophic soils (Forman 1998; Tedrow 1952). The largest pine barrens in the United States is the 1.4 million acre (57,000 km²) Pine Barrens of New Jersey, which represents one of a series of barrens along the eastern seaboard of the USA and one of a series of similar ecosystems around the world (Forman 1998). Pines and oaks are the most common trees

in the New Jersey Pine Barrens forests, but the disturbed open areas are dominated by grasses (Poaceae), sedges (Cyperaceae), orchids (Orchidaceae), blueberries and other members of the heath family (Ericaceae). The diversity of fungi and their functions in pine barrens ecosystem remain largely unknown (Dighton et al. 2004; Forman 1998).

Dark septate endophytes (DSE) refer to a group of heterogeneous plant root-colonizing ascomycetes that produce melanized, septate hyphae (Jumpponen and Trappe 1998). They have been isolated 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, 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; Sieber and Grünig 2013). 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 due to the use of various experimental designs (Grünig et al. 2008b; Newsham 2011).

In the New Jersey Pine Barrens, we recently uncovered a number of novel fungal lineages from the roots of Poaceae grasses. Two new genera have been described: *Pseudophialophora* in Magnaporthales of Sordariomycetes (Luo et al. 2014b) and

Acidomelania, a novel DSE close to PAC in Leotiomyces (Walsh et al. 2014). In this study we propose another new genus *Barrenia* from apparently healthy switchgrass and pitch pine roots in New Jersey Pine Barrens. *Barrenia* belongs in Helotiales of Leotiomyces and can be distinguished from PAC and other allied fungi based on phylogenetic analyses, as well as ecology and morphology. We also conducted a plant-fungal interaction experiment for *Barrenia* and *Acidomelania* species with switchgrass, in order to understand their roles in the ecosystem.

2 Materials and methods

2.1 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. 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 30 s, 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.

2.2 Morphological study and growth rate

89
90 Fungal isolates were grown on cellophane overlaid 2% MEA (BD Difco, Maryland) and
91 2% water agar (WA). Cultures were incubated at 20 C in the dark with three replicates.
92 Colony diameter was measured after 20 days. The color names of colonies followed
93 Ridgway (1912). The following media also were used to induce sporulation: Cornmeal
94 Agar (CMA, BD Difco, Maryland), Oatmeal Agar (OA, BD Difco, Maryland), Potato
95 Dextrose Agar (PDA, BD Difco, Maryland), Filtered Ground Pine Needle Agar (FGPNA,
96 Luchi et al. 2007), Synthetic Nutrient Agar (SNA, Gams et al. 1998), WA amended with
97 autoclaved switchgrass leaves, WA amended with autoclaved switchgrass roots, WA
98 amended with autoclaved pine needles, and WA amended with autoclaved pine roots.
99 Cultures were incubated at 20 C and 25 C in the dark with three replicates. Colonies were
100 checked weekly for 10 months.

101 2.3 DNA extraction, amplification and sequencing

102 Genomic DNA was extracted from fungal mycelium using the UltraClean Soil DNA
103 isolation kit (MoBio, California) following the manufacturer's instructions. PCR was
104 performed with Taq 2X Master Mix (New England BioLabs, Maine), following the
105 manufacturer's instructions. PCR cycling conditions for the internal transcribed spacer
106 (ITS) and the large subunit of ribosomal RNA genes (LSU) consisted of an initial
107 denaturation step at 95 C for 2 min, 35 cycles of 95 C for 45 s, 54 C for 45 s, 72 C for 1.5
108 min, and a final extension at 72 C for 5 min. For the largest subunit of RNA polymerase
109 II (*RPB1*) the cycling conditions included an initial denaturation step at 95 C for 2 min,
110 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).

2.4 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 1) 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 Helotiales. Sequences were aligned with MUSCLE (Edgar 2004). Genealogical concordance was evaluated with the nonparametric Templeton Wilcoxon signed-rank test in PAUP*4.0b10 (Swofford 2002), with 95% bootstrap consensus trees as constraints. No significant conflict was found among the trees based on the individual and combined three-gene datasets. 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-Joining 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. Maximum likelihood analysis using RAxML generated the same tree topologies as those produced by MEGA.

2.5 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 H₂O 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.

3 Results

3.1 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. Despite using a wide variety of media, sporulation has been observed only for isolate WSF14P22.

3.2 Sequence data and phylogeny

There were 1246 characters in the LSU alignment, 573 in ITS, 489 in *RPB1* and 2308 in the three-gene alignment (TreeBASE study number 18173). 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 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 these respective groupings. Based on the molecular phylogenetic analyses, morphological characters and their ecological features, a new genus and two new species are proposed.

3.3 Taxonomy

Barrenia E. Walsh & N. Zhang, **gen. nov.**

MycoBank: MB811715

Etymology: “*Barren*” refers to the pine barrens ecosystem where the fungi were discovered.

Morphological description: Colonies on MEA darkly pigmented, surface fluffy, aerial hyphae thick and light brown. Colonies on WA light brown, sparse aerial hyphae. Sporulation observed in only one species.

Molecular description: *Barrenia* differs from other closely related taxa by the following fixed nucleotides in the largest subunit of RNA polymerase II gene (*RPB1*) from the 3-gene alignment: positions 2055 (T), 2081 (G), 2083 (C), and 2099 (G). (Fig 4)

Type species: *Barrenia taeda*

Habitat: Endophytic in roots of pine.

Known distribution: New Jersey Pine Barrens, United States.

Barrenia panicia E. Walsh & N. Zhang, **sp. nov.** (Fig 5A-C)

MycoBank: MB811716

Etymology: “*panici*” refers to the host.

Morphological description: Colonies on MEA 55 mm diam after 20 d in the dark at 25 C, 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 25 C, Ochraceous Tawny, aerial hyphae sparse, reverse pigmented, Cinnamon Brown.

Warm *Sepia*, paddle-shaped hyphopodium-like structures formed in inoculated switchgrass root tissue.

Molecular description: *Barrenia panicia* differs from *Barrenia taeda* by the following fixed nucleotides of the largest subunit of RNA polymerase II gene (*RPB1*) from the 3-gene alignment: positions 2057 (G), 2069 (C), 2096 (T), 2097 (T), and 2120 (A). (Fig 4)

Holotype: **United States:** *New Jersey:* Wharton State Forest, N 39 45.346, W 074 41.684, 3 m alt., from roots of *Panicum virgatum*, 5 Jun. 2013, *E. Walsh & N. Zhang*, WSF1R37 (RUTPP-WSF1R37).

Other materials examined: **United States:** *New Jersey:* Assunpink Lake, N 40 12.00, W 74 30.00, 3 m alt., from roots of *Digitaria* sp., 30 Aug. 2012, *E. Walsh & N. Zhang* AL5m2; Colliers Mills, N40 04.084 W74 26.696, 5 m alt., from roots of *Coix lacryma - jobi*, 30 Aug. 2012, *E. Walsh & N. Zhang* CM11m2.

Barrenia taeda *E. Walsh & N. Zhang*, **sp. nov.** (Fig 6A-D)

MycoBank: MB811717

Etymology: “*taeda*” means pine wood and refers to the host.

Morphological description: 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 25 C, Buckthorn Brown, sparse aerial hyphae, reverse pigmented, Buckthorn Brown. Conidia formed on WA with autoclaved switchgrass leaves after 10 months at 20–25 C in dark or light. Conidiophores simple, hyaline, and straight. Conidiogenous cells

cylindrical, 27.25– 43.68 x 0.89– 1.94 μm (n = 20), tapered at the top 0.53– 1.16 μm , and wider at the base, 1.47– 2.46 μm (n = 20). Conidia aggregated in slimy heads, fusiform, aseptate, hyaline, smooth, 1.92– 4.04 x 0.7– 1.67 μm (n = 50).

Molecular description: *Barrenia taeda* differs from *Barrenia panicia* by the following fixed nucleotides in the largest subunit of RNA polymerase II gene (*RPB1*) from the 3-gene alignment: positions 2057 (A), 2069 (A), 2096 (C), 2097 (C), and 2120 (T). (Fig 4)

Holotype: **United States:** *New Jersey:* Wharton State Forest N 39 45.346, W 074 41.684, 3 m alt., from roots of *Pinus rigida*, 26 Jun. 2014, *E. Walsh & N. Zhang* WSF14P22 (RUTPP-WSF14P22).

Other materials examined: **United States:** *New Jersey:* Wharton State Forest, N 39 45.346, W 074 41.684, 3 m alt., from roots of *Pinus rigida*, 26 Jun. 2014, *E. Walsh & N. Zhang* WSF14P13; Colliers Mills, N40 04.084 W74 26.696, 5 m alt., from roots of *Pinus rigida*, 4 Jun. 2014, *E. Walsh & N. Zhang* CM14P64.

Notes:

Barrenia differs from *Loramycetes* and *Mollisia* by its association with living plant roots because *Loramycetes* and *Mollisia* species are associated with dead or decaying above-ground plant parts (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*. Moreover, *Barrenia* has 93% or less ITS sequence similarities 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*.

3.4 Plant-fungal interaction experiment

Switchgrass seedlings inoculated with *A. panivicola* isolate 61R8 and *B. panicia* WSF1R37 produced dense root hairs all the way to the root apical meristem area, whilst the control seedlings only produced dense root hairs at the region of maturation of the root (Fig 7). In addition, the roots inoculated with *A. panivicola* isolate 61R8 and *B. panicia* WSF1R37 had a serpentine growth pattern, whilst the control roots were straight. Hyphopodia-like structures were observed on the switchgrass seedling roots inoculated with *B. panicia* WSF1R37 (Fig 5). 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 those inoculated with *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 7).

4 Discussion

Our recent survey on fungi associated with grass roots uncovered a number of novel 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* proposed here belongs to Helotiales, which encompasses plant pathogens, saprobes and endophytes. 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 (Mandyam and Jumpponen 2005). This prompted us to examine the interaction between *B. panicia*, *B. taeda*, *A. pannicola* and switchgrass, which is the host of *B. panicia* and *A. pannicola*. Our inoculation results indicated that *A. pannicola* 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 between the fungus and the host plant (Delaux et al. 2013; Walker 1980). *Barrenia taeda*, originally isolated from pine roots, had a negative effect on root elongation in switchgrass seedlings. These results corroborate Mandyam et al. (2010; 2012) that although DSE fungi have a broad host range, their effects and characteristics can be considered host specific. Further studies are needed to understand the molecular mechanisms of the observed different interactions between these DSE and plants.

The phylogenetic analysis in this study indicated that *Barrenia* is close to *Acidomelania*, *Loramyces*, *Mollisia*, and PAC. The phylogenetic proximity of *Mollisia*, *Loramyces* and

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

302 The six *Barrenia* isolates from New Jersey Pine Barrens were grouped into two well-
 303 supported clades. We delimited the two species based on the ITS, LSU and 3-gene trees.
 304 The BLAST results in GenBank indicated that *Barrenia* might have a wide distribution.
 305 Sixteen ITS sequences in GenBank had 97–99% identities with that of *B. panicia* isolate
 306 WSF1R37, for example, GU973749 from sugarcane root in Brazil, HQ889709 from
 307 *Cymbidium insigne* root in China, and AY599235 from grass root in The Netherlands.
 308 Twelve ITS sequences in GenBank had 97–99% identities with that of *B. taeda* isolate
 309 WSF14P22, for example, JQ272328 from *Rhododendron* root in USA and KJ817299
 310 from *Vaccinium vitis-idaea* in Inner Mongolia. The host plants of the matched sequences
 311 in GenBank are largely Ericaceae, terrestrial orchids, grasses and conifers, usually found
 312 in acidic and infertile soils (Keddy 2007). This distribution pattern was also found in
 313 *Acidomelania panicicola*, the other root associated fungus frequently isolated from the
 314 pine barrens (Walsh et al. 2014). The plant root hair growth promotion effect of *A.*
 315 *panicicola* and *B. panicia* discovered in this study coupled with their distribution pattern
 316 suggest that these species may play a role in plant adaptation to acid, low nutrient soils.

In this study we named a new genus and two species of root-colonizing fungi associated with plants living in 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.

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Figure Legends:

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

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

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

Fig 4 – Partial *RPB1* gene alignment of *Barrenia panicia* (WSF1R37), *B. taeda* (WSF14P22), and closely related members of Leotiomyces: *Acidomelania panicicola*, *Mollisia cinerea* and *Lachnum virgineum*. Black highlights indicate differences between

the genus *Barrenia* and related Leotiomyces, while gray highlights indicate differences between *B. panicia* and *B. taeda*.

Fig 5 – Morphological characters of *Barrenia panicia* holotype isolate WSF1R37. A-C.

Pseudohyphopodia on inoculated switchgrass seedling roots. Bar = 10 µm

Fig 6 – Morphological characters of *Barrenia taeda* holotype isolate WSF14P22. A-D

Conidia and conidiogenous cells. Bar = 10 µm

Fig 7 – Switchgrass seedling two days after inoculation with A. *Acidomelania panicicola*

holotype isolate 61R8 B. Control; three days after inoculation with C. *Barrenia panicia*

holotype isolate WSF1R37, D. Control, E. *Barrenia taeda* holotype isolate WSF14P22.

Bar = 1 mm

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453

454

Figure 1

Figure 2

Figure 3

Figure 4

<i>Barrenia panicia</i>	TCCAGAGATGGCTTTGAATGTCTTCCGCAGCATGTCCTCGGCTGAGATTCGCGATTTGGGCTTGAGCAATGACTATGCA
<i>Barrenia taeda</i>	TCCAGAGATGGCTTTAAATGTCTTCCGAAGCATGTCCTCGGCTGAGATTCGCGACCTGGGCCTGAGCAACGACTACGCT
<i>Acidomelania panicicola</i>	TCCAGAGATGGCTTCAATGTCTTCCGGTCCATGTCTTCGATGAGATTCGCGATCTCGGTTTGAGCAACGACTATGCG
<i>Mollisia cinerea</i>	TCCAGAGATGGCTTCAATGTCTTCAAAGCATGTCCACCAAGGAGATCTACGATCTTGGCTTGAACAACGACTACGCT
<i>Lachnum virgineum</i>	CCCAGAGGCAGCTGTGCACATCTTCCG AAGCATTTCTCTCGACGAGATTCGCGACTTAGGCCTGAGCAATGATTATGCG

Figure 5

Figure 6

Figure 7