Diversity and function of fungi associated with the fungivorous millipede, *Brachycybe lecontii*

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Abstract

Fungivorous millipedes (subterclass Colobognatha) likely represent some of the earliest known mycophagous terrestrial arthropods, yet their fungal partners remain elusive. Here we describe relationships between fungi and the fungivorous millipede, *Brachycybe lecontii*. Their fungal community is surprisingly diverse, including 176 genera, 39 orders, four phyla, and several undescribed species. Of particular interest are twelve genera conserved across wood substrates and millipede clades that comprise the core fungal community of *B. lecontii*. Wood decay fungi, long speculated to serve as the primary food source for *Brachycybe* species, were absent from this core assemblage and proved lethal to millipedes in pathogenicity assays while entomopathogenic Hypocreales were more common in the core but had little effect on millipede health. This study represents the first survey of fungal communities associated with any colobognath millipede, and these results offer a glimpse into the complexity of millipede fungal communities.

Keywords

Diplopoda; Colobognatha; *Brachycybe*; millipede; mycophagy; entomopathogenicity; *Apophysomyces*; *Mortierella*; fungal diversity

1. Introduction

The Class Diplopoda, known colloquially as millipedes, represent some of the earliest known terrestrial animals, dating back to the early Devonian period *ca.* 412 million years ago (Wilson & Anderson 2004, Suarez *et al.* 2017). These early representatives were detritivores and likely played a role in early soil formation and the development of terrestrial nutrient cycling (Bonkowski *et al*. 1998, Lawrence & Samways 2003). Detritivorous millipedes continue to play a pivotal role in ecosystem processes, though herbivorous (Marek *et al.* 2012), carnivorous (Srivastava & Srivastava 1967), and fungivorous diets also exist among extant millipedes (Brewer *et al.* 2012, Marek *et al.* 2012).

Most fungivorous millipedes belong to the subterclass Colobognatha, which diverged from detritus-feeding millipedes 200-300 million years ago and possess a primitive trunk-ring architecture composed of a free sternum and/or pleurites (Brewer & Bond 2013, Rodriguez *et al.* 2018). However, these millipedes are characterized by derived rudimentary mouthparts adapted for feeding exclusively on succulent tissues such as fungi (Hopkin & Read 1992, Wilson & Anderson 2004, Wong 2018). Some taxa possess beaks composed of elongate mouthparts encompassing a fused labrum, gnathochilarium, and stylet like mandibles (Read & Enghoff 2018). Members of the Colobognatha are among the most understudied groups in Diplopoda despite their wide geographic distribution and ubiquity in natural history collections (Manton 1961, Hoffman 1980, Read & Enghoff 2009, Shorter *et al.* 2018). The ancient association between millipedes and fungi raises fascinating questions about interactions in early terrestrial ecosystems, and the possible role of fungi in diplopod success.

Most published records of fungal-millipede interactions are cases where millipedes graze on fungi in the environment (Bultman & Mathews 1996, Lilleskov & Bruns 2005) or where parasitic fungi infect millipedes (Kudo *et al.* 2011, Hodge *et al.* 2017). Among the most studied fungal associates of millipedes are specialist ectoparasitic fungi in the Laboulbeniales (Santamaria *et al.* 2014, Enghoff & Santamaria 2015, Reboleira *et al.* 2018) and obligate arthropod gut-associated trichomycetes (Wright 1979). However, in none of these interactions does the millipede strictly depend on fungi for survival as it seemingly does in the fungivorous millipede *B. lecontii* (Diplopoda: Platydesmida: Andrognathidae).

Brachycybe lecontii is most frequently found in multigenerational aggregations in decaying wood with visible fungal growth (Gardner 1975, Shelley *et al.* 2005). The known geographic range of *B. lecontii* extends across 13 U.S. states from eastern Oklahoma to western South Carolina, south to Louisiana, and north to southern West Virginia (Shelley *et al.* 2005, Brewer *et al.* 2012). Within its known range, *B. lecontii* is divided into at least 4 clades that are geographically separated and may represent independent cryptic species (Brewer *et al.* 2012).

Historically, only one study reported *Brachycybe* feeding on an identified fungus, an unknown species of *Peniophora* (Russulales) (Gardner 1975). However, observations of *Brachycybe* species interacting with various fungi (n = 65) from community science websites such as Bugguide.net and iNaturalist.org shows that the fungal communities associated with this genus are more diverse than have been formally described (Supplemental Table 1). Recently, several basidiomycete Polyporales have been confirmed directly from *B. lecontii* and from *B. lecontii*-associated wood (Kasson *et al.* 2016). Given the discovery that Polyporales have helped facilitate the evolution of large, communal colonies with overlapping generations in other arthropods (You *et al*. 2015, Kasson *et al.* 2016, Simmons *et al*. 2016), many interesting questions are raised regarding *Brachycybe* colonies and their association with Polyporales and allied fungi.

In an attempt to uncover which fungi, if any, are consistently associated with *B. lecontii*, this study surveys fungal associates of *B. lecontii* across its known geographic range using culture-based approaches. The use of nuclear ribosomal internal transcribed spacer (ITS) barcoding on collected isolates allowed fine-scale identification and examination of culturable fungal communities. With a primary understanding of these fungal communities, we assessed diversity and applied a network analysis to determine the relationship between genetically and geographically distinct *B. lecontii* populations, their wood substrates, and associated fungal genera.

2. Materials and methods

2.1. Collection sites & field methods

Millipede collection sites were primarily identified through Brewer *et al*. (2012) and Gardner (1975), and additional sites were identified based on the known range of *B. lecontii*. Sampling was targeted to collect millipedes from all four *B. lecontii* clades. Based on previous work by Brewer (2012), individual sites were expected to contain millipedes from a single clade, with no syntopy. In total, 20 sites were sampled, with 18 yielding colonies and solitary individuals, and 2 yielding individuals only. These sites were located in Arkansas, Georgia, Oklahoma, South Carolina, Tennessee, Virginia, and West Virginia (Table 1).

At each site, decaying logs on the forest floor were overturned, examined, and replaced until colonies of *B. lecontii* were located. Colonies are defined as groups of two or more individuals, and were typically found on or near resupinate fungi covering the underside of the logs. When suitable colonies were found, individuals from single colonies were placed together in 25-ml sterile collection vials, often with a piece of the fungus-colonized wood they were on, and stored in a cooler on ice until processing. In addition, cross-sections of logs from which colonies were collected were sampled for wood substrate identification.

2.2. Millipede processing & isolate collection

All millipedes were maintained at 4°C until processing, which typically occurred within 3 d of initial collection. After surface sterilization in 70% ethanol, individuals were sexed, sectioned with a sterilized scalpel to remove tail and gonopod sections (Macias 2017). Tail portions were preserved in 95% ethanol for millipede genotyping using custom markers previously described by Brewer et al. (2012). Gonopods were also preserved from males to permit anatomical study. The remainder of the millipede was macerated in 500 µl of sterile distilled water, and a 50-µl sample was spread on glucose yeast extract agar (GYEA) amended with antibiotics to isolate fungi (Macias 2017). GYEA was made as follows: 1000 ml distilled water, 20 g agar, 2 g yeast extract, and 0.5 g $MgSO_4$ (Fisher Scientific, Pittsburgh, PA, USA), 10 g dextrose (BD and Co., Franklin Lakes, NJ, USA), 1 g KH_2PO_4 (Ward's Science, Rochester, NY, USA), 50 µg thiamine, 10 µg biotin, and 1 mL of microelement solution containing 500 µg Fe^{3+}, 439 µg Mn^{2+}, and 154 µg Zn^{2+}. Antibiotics consisted of: 100 mg tetracycline hydrochloride (Fisher Scientific, Pittsburgh, PA, USA) and 10 mg of streptomycin sulfate (Sigma-Aldrich, St. Louis, MO, USA). Cultures were sealed with parafilm and incubated at ambient conditions until growth was observed. Each colony-forming unit (CFU) was categorized by morphotype, counted, and recorded. One representative of each morphotype from each plate was retained and assigned an isolate number. Culture plates were retained for up to 3 weeks to ensure that slow-growing fungi were counted and sampled. Depending on how rapidly fungi grew in pure culture, isolates were either grown on potato dextrose broth (PDB; BD and Co., Franklin Lakes, NJ, USA) prior to DNA extraction, or mycelium was scraped directly from plates. DNA was extracted from all isolates using a Wizard kit (Promega, Madison, WI, USA). DNA was suspended in 75 ml of Tris-EDTA (TE) buffer preheated to 65°C. For long-term storage, isolates were kept on potato dextrose agar slants (pre-mixed PDA; BD and Co., Franklin Lakes, NJ, USA) at 4°C.

Wood samples were dried at room temperature (~21° C) for several weeks and sanded using an orbital sander equipped with 220-grit paper. Identifications were made by examining wood anatomy in cross section, based on descriptions by Panshin and de Zeeuw (1980).

2.3. Isolate identification

Isolates were identified using the universal fungal barcoding gene, the ribosomal internal transcribed spacer region (ITS), which includes ITS1, 5.8S, and ITS2 (Schoch *et al*. 2012). Primers used in this study were obtained from Integrated DNA Technologies (IDT, Coralville, IA, USA). PCR was conducted using primers ITS4 (5'-TCCTCCGCTTATTGATATGC-3') and ITS5 (5'-GGAAGTAAAAGTCGTAACAAGG-3') (White *et al*. 1990), following the protocol in Macias (2017).

PCR products were visualized via gel electrophoresis on a 1.5% w/v agarose (Amresco, Solon, OH, USA) gel with 0.5% Tris-Borate-EDTA buffer (Amresco, Solon, OH, USA). SYBR Gold (Invitrogen, Grand Island, NY, USA) was used as the nucleic acid stain, and bands were visualized on a UV transilluminator (Syngene, Frederick, MD, USA). PCR products were purified using ExoSAP-IT (Affymetrix, Santa Clara, CA). Products were Sanger sequenced with the same primers used for PCR (Eurofins, Huntsville, AL, USA). Resulting sequences were clipped using the "Clip ends" function in CodonCode Aligner v 5.1.5 and searched in the NCBI GenBank BLASTn database (Altschul *et al.* 1990) and best-match identifications recorded for each isolate.

2.4. Identification of new species

Fungal isolates were considered to represent a putative new species if three or more identical sequences were recovered with identical low percentage (threshold ≤ 95%) BLASTn matches. The large subunit of the ribosomal ITS region (LSU) was also sequenced using primers LR0R (5'-ACCCGCTGAACTTAGC-3') and LR5 (5'-TCCTGAGGGAAACTTCG-3') (Vilgalys and Hester 1990) for each putative new species. PCR conditions were as described in Macias (2017). PCR products were visualized, purified, and sequenced as above.

In the following analyses, the default parameters of each software package were used unless otherwise noted. Two putative new species were confirmed phylogenetically by constructing ITS+LSU concatenated phylogenetic trees for each new species and its known relatives based on a combination of BLAST matches and previously published literature. MEGA7 v. 7.0.16 (Kumar *et al*. 2016) was used to align sequences (CLUSTAL-W, Larkin *et al.* 2007), select a best-fit model for estimating phylogeny, and construct maximum likelihood (ML) and maximum parsimony (MP) trees for each putative new species. For ML and MP analyses, 1000 bootstrap replicates were used. The alignments used default parameters. Initial alignments were trimmed such that all positions with less than 95% site coverage were eliminated. For maximum likelihood analyses, the Tamura 3-parameter substitution model with a gamma distribution (T92+G) was used (Tamura 1992). Both maximum parsimony analyses used the subtree-pruning-regrafting algorithm (Nei and Kumar 2000). Bayesian (BI) trees were constructed using Mr. Bayes v. 3.2.5 (Ronquist *et al.* 2012). Three hot and one cold independent MCMC chains were run simultaneously for 1,000,000 generations, and the first 25% were discarded as a burn-in. The average standard deviation of split frequencies statistic was checked to ensure convergence between chains (Ronquist *et al.* 2012) and was <0.01. Final parameter values and a final consensus tree were generated used the MrBayes "sump" and "sumt" commands respectively. The ML tree was preferred and support for its relationships in the other analyses was determined. Reference sequences used in each phylogenetic analysis are listed in Supplemental Tables 2 and 3, respectively.

2.5. Community and diversity analyses

Community and diversity analyses were used to answer two questions: (1) Are the fungal communities of *B. lecontii* conserved across millipede clade, millipede sex, wood substrate, and/or ecoregion? (2) What genera comprise the core fungal community associated with *B. lecontii*?

The effects of *B. lecontii* clade, sex, wood substrate, and ecoregion on the fungal community composition were analyzed by perMANOVA using the vegan package (Oksanen *et al*. 2018) in R version 3.4.3 (R Core Team 2017). Multilevel pairwise comparisons were performed using the pairwiseAdonis package (Arbizu 2017). Isolates where the fungal order or wood substrate were not identified were removed from the analysis. Additionally, isolates recovered from millipedes that were not part of a colony were removed. Ecoregion 45 was deleted from the analysis because its variance was significantly different from the other ecoregions (checked using function betadisper in vegan). Pairwise comparisons were only made for groups with 20 or more millipedes sampled, and in cases where more than one pairwise comparison was made, Bonferroni-corrected p-values are reported.

In addition, diversity indices were used to provide information about rarity and commonness of genera in the fungal community of *B. lecontii,* by site. Three alpha diversity indices were chosen according to recommendations laid out in Morris *et al.* (2014): number of genera present, Shannon's diversity index, and Shannon's equitability (evenness) index (formulae from Begon *et al.* 1990). The relationship between sample size and the three diversity metrics was examined using Spearman rank correlations.

A co-occurrence network was constructed using Gephi (Bastian *et al.* 2009) for fungal isolates obtained from *B. lecontii* at the genus level. Betweenness-centrality was used to measure relative contribution of each node (single fungal genus) to connectivity across the whole network. High betweenness-centrality values are typically associated with nodes located in the core of the network (Greenblum *et al.* 2011), which in this system are defined as fungal genera with multiple edges connecting multiple *B. lecontii* clades and multiple wood substrates. Low betweenness-centrality values indicate fungal genera with a more peripheral location in the network, with fewer edges connecting clades and wood substrates (Greenblum *et al.* 2011).

2.6. Pathogenicity testing

Twenty-one isolates (Table 2) representing the diversity of all collected isolates were initially chosen for live plating pathogenicity assay (hereafter referred to as "full-diversity assay") with *Brachycybe lecontii.* A second assay (hereafter referred to as "Polyporales assay") using nineteen isolates in the Polyporales (Table 3) were tested in a separate experiment. Three isolates from the full-diversity assay were re-tested in the second assay, for a total of 37 isolates used between both assays.

Isolates were grown on GYEA and scraped to generate inoculum suspensions in sterile water. An ~500 µl aliquot of suspension was spread onto fresh GYEA plates at incubated at room temperature (21° C). After all plates were covered by fungal growth (~3 weeks), the millipedes were introduced for 7-d pathogenicity trials. Five individuals were placed on each plate. In the full-diversity assay, 15 millipedes were used for each treatment, while 10 were used in the Polyporales assay. For a negative control, millipedes were placed on sterile GYEA plates that were changed each time contaminating fungal growth was observed. These plates required replacement due to inadvertent inoculation by the millipede's phoretic contaminants and gut microbes. Observations were made every 12 h for the first 36 h and then every 4 h for an additional 108 h until 7 d were complete. Mortality was assessed by failure of millipedes to move in response to external stimuli (Panaccione & Arnold 2017). At the end of the assay, samples of deceased individuals were preserved for chemical analyses. Surviving millipedes were returned to laboratory colonies for future studies.

Statistical analysis of survivorship was performed using the “Survival / Reliability” function in JMP 13.1.0 (SAS Institute Inc., Cary, NC). Post-hoc pairwise comparisons to the control treatment were performed for treatments that reached at least 25% mortality by the end of the trials (five treatments in the full-diversity assay, and seven in the Polyporales assay). Both log-rank and Wilcoxon tests were used. Log-rank tests score mortality at all time points evenly, while Wilcoxon tests score early mortality more heavily. For pairwise comparisons, Bonferroni corrections were applied such that the P-value reported by the analysis was multiplied by the number of comparisons made in each experiment.

3. Results and discussion

3.1. Diversity and community structure

A total of 301 millipedes were collected from 3 of 4 known *B. lecontii* clades (Brewer *et al.* 2012) and from 20 sites across 7 states (Table 1). Our study recovered 102 males and 146 mature females. Most millipedes were engaged in feeding behavior, with their heads buried in fungus growing on the log (Figure 1).

Brachycybe lecontii was found to associate with a large and diverse community of fungi, including at least 176 genera in 39 fungal orders from four phyla (Supplemental Table 4). A majority of these fungi (59%) were members of Ascomycota. Of all the genera of fungi found in this study, 40% were represented by a single isolate, and only 13% had 10 or more isolates. The most common order was the Hypocreales, containing 26% of all isolates resolved to at least order. The five next most common orders were the Polyporales (9% of all isolates), Chaetothyriales (8%), Xylariales (6%), Capnodiales (5%), and Eurotiales (5%). All other orders contained fewer than 50 isolates (<5%).

Alpha diversity was assessed at the genus level by millipede clade, wood substrate, and site. Clade 1 included 31 fungal genera, clade 3 included 156, and clade 4 included 69. The number of fungal genera obtained from each wood substrate were as follows: *Liriodendron* (114 genera), *Quercus* (74), *Betula* (54), *Carya* (45), *Fagus* (33), *Ulmus* (19), *Acer* (18), *Pinus* (11), *Carpinus* (10), and *Fraxinus* (10). However, the number of millipedes sampled for Clade 1, and all wood substrates except *Quercus* and *Liriodendron*, are likely not sufficient to capture the full diversity of the communities with culture-based methods (n<20).

At each site, fungal alpha diversity varied from 3 genera at SC2 to 63 genera at WV1 with a mean of 23 per site (Table 4). Shannon's diversity index and Shannon's equitability were also calculated for each site. Shannon's diversity index ranged from 0.95 in SC2 to 3.79 in WV2. Shannon's equitability ranged from 0.977 in AR1 and VA2 to 0.848 in TN1 (Table 4). Sites with the five highest Shannon's diversity index values did not overlap with sites with the five highest site equitability values (Table 4). Three diversity metrics were found to be correlated with sample size (alpha diversity r = 0.70, Shannon's diversity index r = 0.58, Shannon's equitability index r = -0.28), indicating that many more millipedes would be needed to capture the full fungal diversity using culture-based methods. The wide ranges of the three diversity metrics across sites raises questions about functional redundancies in the fungal communities.

To statistically explore relationships between fungal community composition and millipede sex, millipede clade, wood substrate, and ecoregion, perMANOVAs and pairwise multilevel comparisons were used. No relationship was found between millipede sex and fungal community composition ($p = 0.353$, $R^2 = 0.005$), but relationships were found for millipede clade ($p = 0.045$, $R^2 = 0.006$), wood substrate ($p = 0.002$, $R^2 = 0.049$), and ecoregion ($p = 0.001$, $R^2 = 0.012$). However, effect size is very small for each of these factors, indicating that while there are significant relationships between these factors and the fungal community, the strength of those relationships is weak. Pairwise multilevel corrections indicate that there are significant differences between the fungal communities of Clade 1 and 3 ($p = 0.009$, $R^2 = 0.015$) and 4 and 3 ($p = 0.003$, $R^2 = 0.015$), but not 1 and 4 ($p = 0.096$, $R^2 = 0.014$). For wood substrate, only the fungal communities of *Liriodendron* and *Quercus* were compared (see Methods), and they were significantly different ($p = 0.045$, $R^2 = 0.015$). For ecoregion, there are significant differences between the fungal communities of ecoregions 36 and 39 ($p = 0.018$, $R^2 = 0.014$) and 66 and 69 ($p = 0.006$, $R^2 = 0.018$), but not 36 and 37 ($p = 1.000$, $R^2 = 0.007$), 36 and 66 ($p = 0.084$, $R^2 = 0.025$), 37 and 66 ($p = 0.462$, $R^2 = 0.029$), or 37 and 69 ($p = 0.300$, $R^2 = 0.010$). For all of these pairwise comparisons, the effect size was small. Increased sampling depth should help clarify whether any of these factors truly impact the fungal community composition.

A network analysis and betweenness-centrality scores were used to examine how the structure of the fungal community is affected by different millipede clades and wood substrates (Figure 2). As a whole, community structure was heterogeneous across millipede clades and wood substrates. However, some genera of fungi were consistently associated with most clades and wood substrates, as indicated by their betweenness-centrality scores. Twelve fungal genera showed high connectivity across the whole network (betweenness-centrality values > 0.5) (Figure 2). These included 1) *Phialophora* (1.55), 2) *Ramichloridium* (1.44), 3) *Mortierella* (1.28), 4) *Trichoderma* (1.03), 5) *Mucor* (1.02), 6) *Verticillium* (0.90), 7) *Phanerochaete* (0.89), 8) *Fonsecaea* (0.84), 9) *Penicillium* (0.75), 10) *Umbelopsis* (0.73), 11) *Cosmospora* (0.68), and 12) *Xylaria* (0.63). All other fungal genera fell below a 0.5 threshold, including 144 genera with betweenness-centrality values of 0.0, indicating low presence across millipede communities.

The betweenness-centrality scores from the network revealed that the core of the *Brachycybe*-associated fungal community is comprised of a small group of fungal genera that is fairly representative of the diversity in the broader millipede-associated fungal community. The structure of the network indicates that these core fungi are consumed by many individuals from different lineages of *B. lecontii* across its reported range and across many wood substrates. As such, these fungi may be the preferred fungal food source for *B. lecontii*. Alternatively, these fungi may readily survive gut passage, which would result in them being over-represented after culturing.

Only a single member of the order Polyporales, *Phanerochaete*, falls in the core of the fungal network, a highly unexpected result given that the majority of community science records of *Brachycybe* interacting with fungi appear to show the millipedes associating with Polyporales and closely allied decay fungi (Supplemental Table 1). It is possible that these fungi may serve a vital role, despite their near-absence from the core network. One such role may be to precondition substrates for arthropod colonization. For example, vascular wilt fungi predispose trees to attack by wood-boring ambrosia beetles (Hulcr & Stelinski 2017). The Verticillium wilt pathogen, *V. nonalfalfae*, predisposes tree-of-heaven to mass colonization by the ambrosia beetle

Euwallacea validus. However, the fungal community recovered from surface-disinfested beetles does not include *V. nonalfalfae* (Kasson *et al.* 2013). As such, *V. nonalfalfae* might be overlooked in its role to precondition substrates for arthropod colonization. A second possibility is that millipedes do actively utilize wood decay fungi but do not exhibit strict fidelity with single species or rely disproportionately on individual fungal community members (Kasson *et al.* 2013, Jusino *et al.* 2015, Jusino *et al.* 2016). Nevertheless, these results, much like studies examining fungal communities in red-cockaded woodpecker excavations, may indicate millipedes are either: (1) selecting degraded logs with a pre-established preferred fungal community, or (2) selecting fresh logs without any evidence of decay, then subsequently facilitating colonization by specific fungi (Jusino *et al.* 2015, Jusino *et al.* 2016).

3.2. Pathogenicity testing

To determine how members of the Polyporales and other fungi interact with *B. lecontii*, millipedes were challenged with pure cultures of a representative set of fungal isolates for 7 d. Only four of 21 isolates caused significant mortality after 7 d (Table 2): *Metarhizium flavoviride* (Hypocreales; Log-rank $p < 0.0005$), *Bjerkandera adusta* (Polyporales; Log-rank $p < 0.0005$), *Irpex lacteus* (Polyporales; Log-rank $p = 0.014$), and *Trametopsis cervina* (Polyporales; Log-rank $p < 0.0005$). Interestingly, the known virulent entomopathogens *Lecanicillium attenuatum*, *Pochonia bulbillosa*, and *Verticillium insectorum* caused little to no mortality to *Brachycybe* after 7 d of continuous exposure (Table 2).

Since three of the four pathogenic fungi were in the Polyporales, a follow-up experiment was performed using 18 isolates from the Polyporales, and one isolate of *Peniophora* (Russulales), the only fungus reported in the literature to be in association with *Brachycybe*

(Gardner 1975). Only three of these isolates were significantly more pathogenic than the sterile agar control (Table 3): *Gloeoporus pannocinctus* (Polyporales; Log-rank $p < 0.0007$), and two isolates of *Trametopsis cervina* (Polyporales; Log-rank p for both <0.0007). The *Bjerkandera* isolate and the *Irpex* isolate that were significantly pathogenic in the first assay were not significantly pathogenic in the second. However, the number of individuals used in the second assay was 10 per treatment, as compared to 15 per treatment in the first assay, and two individuals in the control treatment in the second assay were dead by the end of the assay. Together, these factors suggest that the results of the second assay are less reliable.

The results of the two pathogenicity assays indicate that several members of the Polyporales, including *Bjerkandera, Irpex, Trametopsis,* and *Gloeoporus*, may be pathogenic to *Brachycybe* millipedes (Figure 3), while three of the four entomopathogenic Hypocreales were not pathogenic. Additionally, seven other fungal orders caused little to no mortality (Table 2).

It is unclear how *B. lecontii* resists the well-documented entomopathogenic effects of many Hypocreales with the exception of *Metarhizium* (Hajek & St. Leger 1994). Parallel studies by Macias (2017) demonstrated that the Hypocrealean isolates used in the *Brachycybe* pathogenicity assays were pathogenic to insects. In contrast, the high incidence of pathogenicity among *Brachycybe*-associated Polyporales was unexpected. In a separate study, insects challenged with these same Polyporales were completely unaffected (Macias 2017).

One hypothesis is that Polyporales, depending on whether they are in a growth phase or a fruiting phase (Calvo *et al.* 2002, Lu *et al.* 2014), may produce chemicals that inadvertently harm millipedes. Since fruiting bodies were never observed in culture, it is likely that the fungi used in the pathogenicity assays were in a growth phase, which proved detrimental to *B. lecontii*. A second hypothesis that may explain pathogenicity among the Polyporales is that experiments relying on pure cultures of a single fungus do not account for fungus-fungus interactions or interactions between fungi and other organisms (Li & Zhang 2014, Macias 2017).

3.3. New species

At least seven putative new species were identified, but only two were investigated in this study. The five not examined are "aff. *Coniochaeta"* (Coniochaetales), "aff. *Leptodontidium*" (Helotiales), "*Pseudonectria* aff. *buxi*" (Hypocreales), "aff. *Fonsecaea* sp." (Chaetothyriales) and "aff. *Oidiodendron*" (Onygenales). The two examined species were from the phylum Mucoromycota (Spatafora *et al*. 2017), in the orders Mortierellales and Mucorales.

Mortierella aff. *ambigua* is represented by 27 clonal isolates from seven widespread collection sites (AR1, AR3, AR4, VA3, WV2, WV4, and WV5), five wood substrates (*Acer*, *Fagus*, *Fraxinus*, *Liriodendron*, and *Quercus*), and two millipede clades (Clade 3 and 4). These isolates are 92% identical to strain "*Mortierella ambigua* CBS 450.88" and were deposited as GenBank accessions MH971275 and MK045304 (Supplemental Table 2 & 4). All isolates of "*Mortierella* aff. *ambigua*" produced large gemmae (Embree 1963, Aki *et al.* 2001) as the cultures aged past 7 d. These structures grew to at most half a centimeter across and were present on the surface and embedded in the media (Figure 4). Sporangia were not observed in any of the *Mortierella* aff. *ambigua* isolates so comparisons with known *Mortierella* sporangial morphology could not be made. More in-depth morphological studies are needed before a formal description can be made.

The initial alignment included 1149 characters and the final dataset was reduced to 834 characters, and the maximum parsimony analysis yielded 7 most-parsimonious trees with a length of 732. Phylogenetic analysis of a concatenated ITS+LSU 17-isolate dataset including 5

Mortierella aff. *ambigua* confirmed placement of this novel species sister to *M. ambigua* sensu stricto (Figure 4) and inside the previously described Clade 5 of *Mortierella* (Wagner *et al.* 2013). Clade 5 *Mortierella* species are common from soil but have also been associated with amphipods and invasive mycoses in humans. More distantly related species such as *Mortierella beljakovae* and *Mortierella formicicola* have known associations with ants but the nature of this relationship remains unclear (Wagner *et al.* 2013).

The second putative new species, aff. *Apophysomyces* sp., is represented by five isolates from one site (OK1), one wood substrate (*Quercus*), and one millipede clade (Clade 4). These isolates are 84% identical to strain "*Apophysomyces ossiformis* strain UTHSC 04-838" and were deposited as GenBank accessions MH971276 and MK045305 (Supplemental Table 3 & 4). Sporangial morphology of these isolates aligns with described features for this genus (Alvarez *et al*. 2010, Bonifaz *et al.* 2014), but more in-depth morphological studies are needed.

The initial alignment included 1258 characters and the final dataset was reduced to 935 characters, and the maximum parsimony analysis yielded 3 most-parsimonious trees with a length of 1101. Phylogenetic analysis of a concatenated ITS+LSU 16 isolate dataset including three aff. *Apophysomyces* sp. isolates confirmed placement of this novel species sister to the clade containing known species of *Apophysomyces* sp. (Figure 5). The combined branch length among all known species of *Apophysomyces* (0.0589 substitutions/site) is less than the branch length separating our putative new species and these known species (0.0903 substitutions/site), providing evidence that our novel species is in fact, a novel genus. The genus *Apophysomyces* has been isolated from soil but is also known to cause severe mycoses in immunocompetent humans (Alvarez *et al*. 2010, Etienne *et al.* 2012, Bonifaz *et al.* 2014).

Despite the use of more classical culture-based approaches, the recovery of seven putative new species highlights the vast amount of undescribed fungal biodiversity associated with millipedes. Culture-independent approaches will undoubtedly uncover many additional new species, possibly including some from unculturable lineages of fungi.

3.4. Summary

Brachycybe lecontii associates with a large and diverse community of fungi, including at least 176 genera in 39 fungal orders from four phyla. Significant differences in the fungal community among wood substrates, millipede clades, and ecoregions indicate that these factors influence the composition of millipede-associated fungal communities, while millipede sex does not. Follow-up studies will help determine if these factors remain important determinants in fungal community composition. One putative new species and one putative new genus of fungi were found and examined in this study, and there is evidence for several additional new species that remain to be assessed phylogenetically. Additional loci and morphological studies are needed to assess the phylogenetic placement of these fungal taxa.

The core fungal community of *B. lecontii* consists of fungi from at least nine orders, primarily members of phylum Ascomycota. While community science records of *Brachycybe* show the millipedes interacting with almost entirely Basidiomycota, especially Polyporales, only one genus from that order occurs in the core of the network. The disagreement between the bulk of community science records and the core network identified in this study can be explained by the fact that observers often overlook the dozens to hundreds of microfungi that co-occur on and in the large Polyporales fruiting bodies.

Four genera in the Polyporales were found to be pathogenic to *Brachycybe* in live-plating assays, while three genera of notorious entomopathogens from the Hypocreales did not cause significant mortality in millipedes. Only a single fungus outside the Polyporales caused significant mortality. However, the results of simple culture-based experiments may not be accurate representations of what happens when these organisms interact in nature.

In less than a decade, the research on arthropod-fungus interactions has accelerated and led to the discoveries of several new associations (Voglmayr *et al.* 2011, Menezes *et al.* 2015**,** You *et al*. 2015). This study demonstrates that the complexity of millipede-fungus interactions has been underestimated and these interactions involve many undescribed species. This paper represents the first comprehensive survey of fungal communities associated with any member of the millipede subterclass Colobognatha. We anticipate that future studies of millipede-fungus interactions will yield countless new fungi and clarify the ecology of these interactions.

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Author contributions

A.M.M., P.E.M., E.M.M., M.S.B., D.G.P., R.V.M.R., and M.T.K. conceived of the study. A.M.M., D.P.G.S., C.M.S., K.L.W., M.C.B., A. M. M., V.W., T.H.J., and M.T.K. performed laboratory work with the help/advice of P.E.M., M.C.B., D.G.P., J.E.S., G.R.B. A.M.M., P.E.M., E.M.M., and M.T.K. analyzed data. A.M.M., P.E.M., E.M.M., M.S.B., J.E.S., G.R.B, R.V.M.R., and M.T.K. wrote the manuscript with input from all coauthors.

Supplementary data

Supplementary data related to this article include Tables S1-S4.

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Figure Legends

Figure 1. *Brachycybe lecontii* colony feeding on the white-rot fungus *Irpex lacteus* (Basidiomycota: Polyporales). Mature adults (larger individuals in the photo) range from 1.5 cm

– 2 cm in length.

Figure 2. Fungal community network across *B. lecontii* clades and wood substrates. Small unlabeled nodes represent fungal genera, color-coded by taxonomic order. Orders with fewer than 10 isolates are lumped into "Other". Genus nodes with betweenness-centrality scores >0.5 are labeled with the rank of their betweenness-centrality score (1-12). White and black nodes represent clades and wood substrates, respectively. For these, the size of the node represents the relative sample size. Edges represent interactions between a fungal genus and a wood substrate/clade. Edge boldness indicates the strength of the interaction.

Figure 3. Representative outcomes of live-plating assay with Polyporales. A-C show *B. lecontii* with no outward disease symptoms after 7 d of exposure to the indicated fungus, and D-F show millipedes that were killed by the indicated fungus. In all three examples of mortality, fungal hyphae are growing over the millipede. A: *Phlebia livida* (BC0629), B: *Ceriporia lacerata* (BC1158), C: *Scopuloides rimosa* (BC1046), D: *Trametopsis cervina* (BC0494), E: *Bjerkandera adusta* (BC0310), F: *Irpex lacteus* (BC0523).

Figure 4. Concatenated ITS+LSU phylogenetic tree of *Mortierella* aff. *ambigua* and close relatives. Bootstrap support and posterior probabilities are indicated near each node (ML/MP/BI), and nodes with >50% support are labeled. Dashes indicate that a particular node did not appear in the indicated analysis. The grey box indicates the isolates belonging to *Mortierella* aff. *ambigua*. A representative culture of the fungus with gemmae is shown in the upper left.

Figure 5. Concatenated ITS+LSU phylogenetic tree of aff. *Apophysomyces* sp. and close relatives. Bootstrap support and posterior probabilities are indicated near each node (ML/MP/BI), and nodes with >50% support are labeled. Dashes indicate that a particular node did not appear in the indicated analysis. The grey box indicates the isolates belonging to aff. *Apophysomyces* sp. A representative culture of the fungus is shown in the upper left.

Figure 1

Figure 2

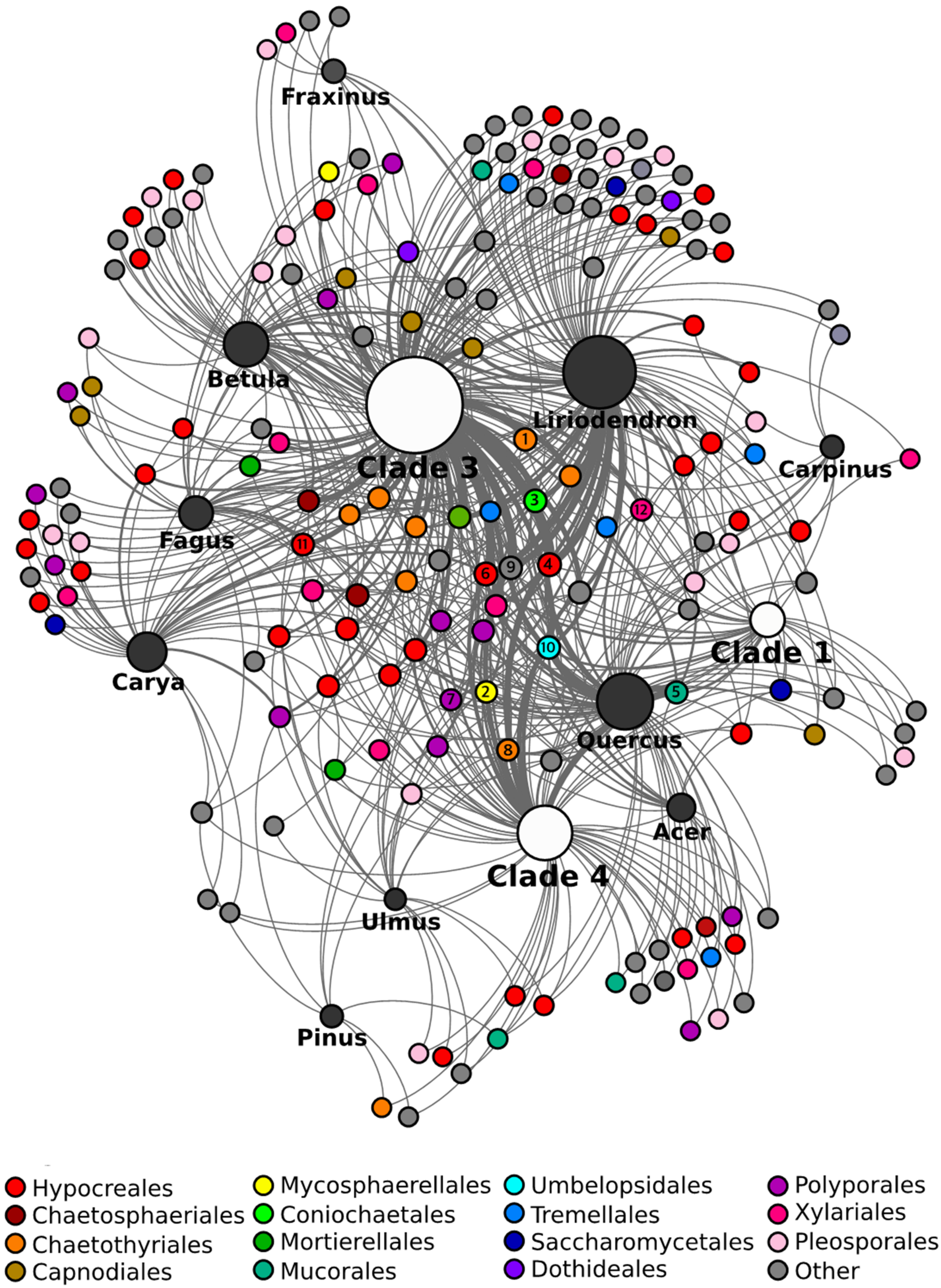

Fraxinus
Betula
Clade 3
Liriodendron
Carpinus
Fagus
Carya
Clade 1
Quercus
Acer
Clade 4
Ulmus
Pinus
Hypocreales
Chaetosphaeriales
Chaetothyriales
Capnodiales
Mycosphaerellales
Coniochaetales
Mortierellales
Mucorales
Umbelopsidales
Tremellales
Saccharomycetales
Dothideales
Polyporales
Xylariales
Pleosporales
Other

Figure 3

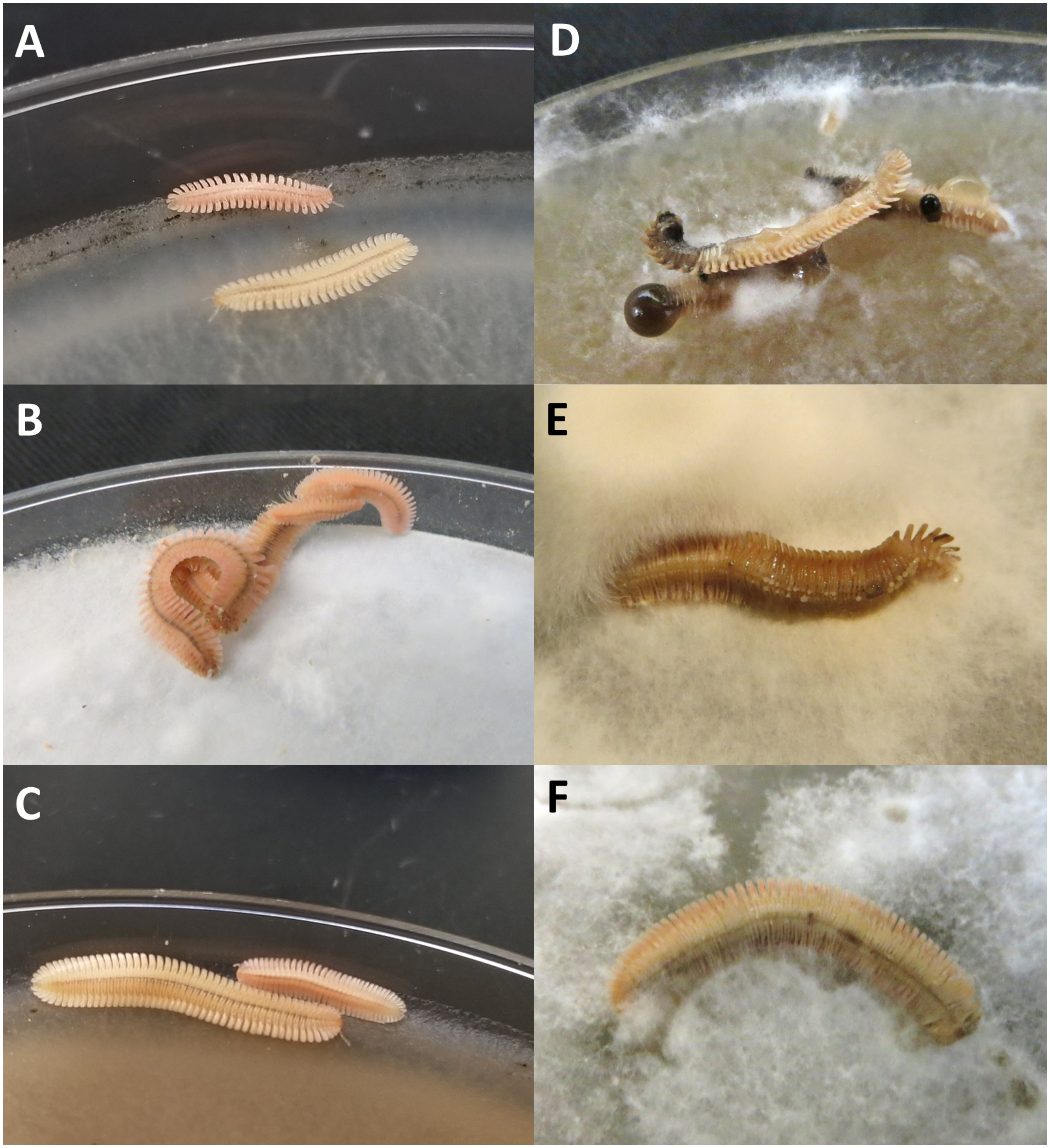
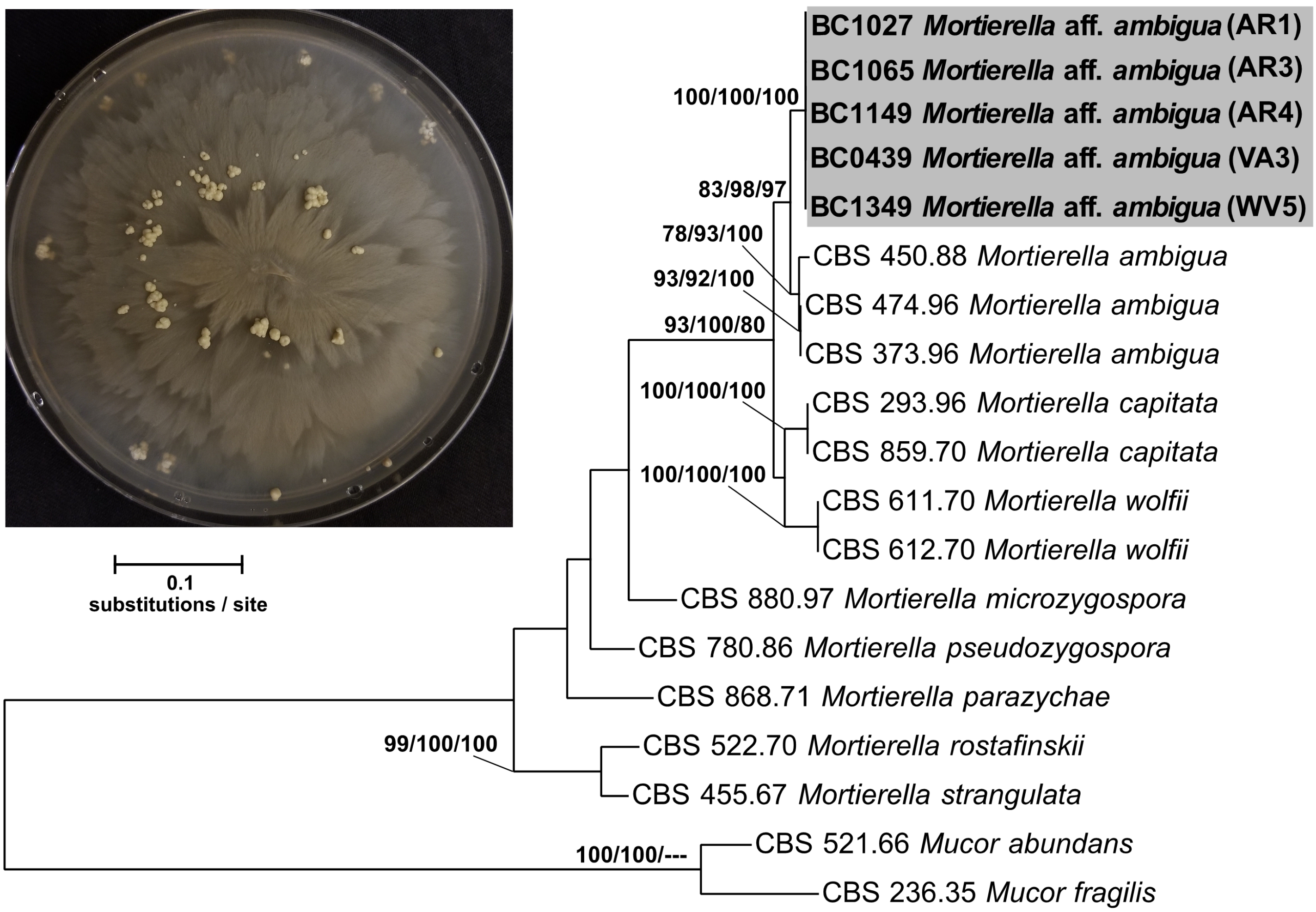


Figure 4



0.1
substitutions / site
100/100/100
83/98/97
78/93/100
93/92/100
93/100/80
100/100/100
100/100/100
99/100/100
100/100/---
BC1027 Mortierella aff. ambigua (AR1)
BC1065 Mortierella aff. ambigua (AR3)
BC1149 Mortierella aff. ambigua (AR4)
BC0439 Mortierella aff. ambigua (VA3)
BC1349 Mortierella aff. ambigua (WV5)
CBS 450.88 Mortierella ambigua
CBS 474.96 Mortierella ambigua
CBS 373.96 Mortierella ambigua
CBS 293.96 Mortierella capitata
CBS 859.70 Mortierella capitata
CBS 611.70 Mortierella wolfii
CBS 612.70 Mortierella wolfii
CBS 880.97 Mortierella microzygospora
CBS 780.86 Mortierella pseudozygospora
CBS 868.71 Mortierella parazychae
CBS 522.70 Mortierella rostafinskii
CBS 455.67 Mortierella strangulata
CBS 521.66 Mucor abundans
CBS 236.35 Mucor fragilis

Figure 5

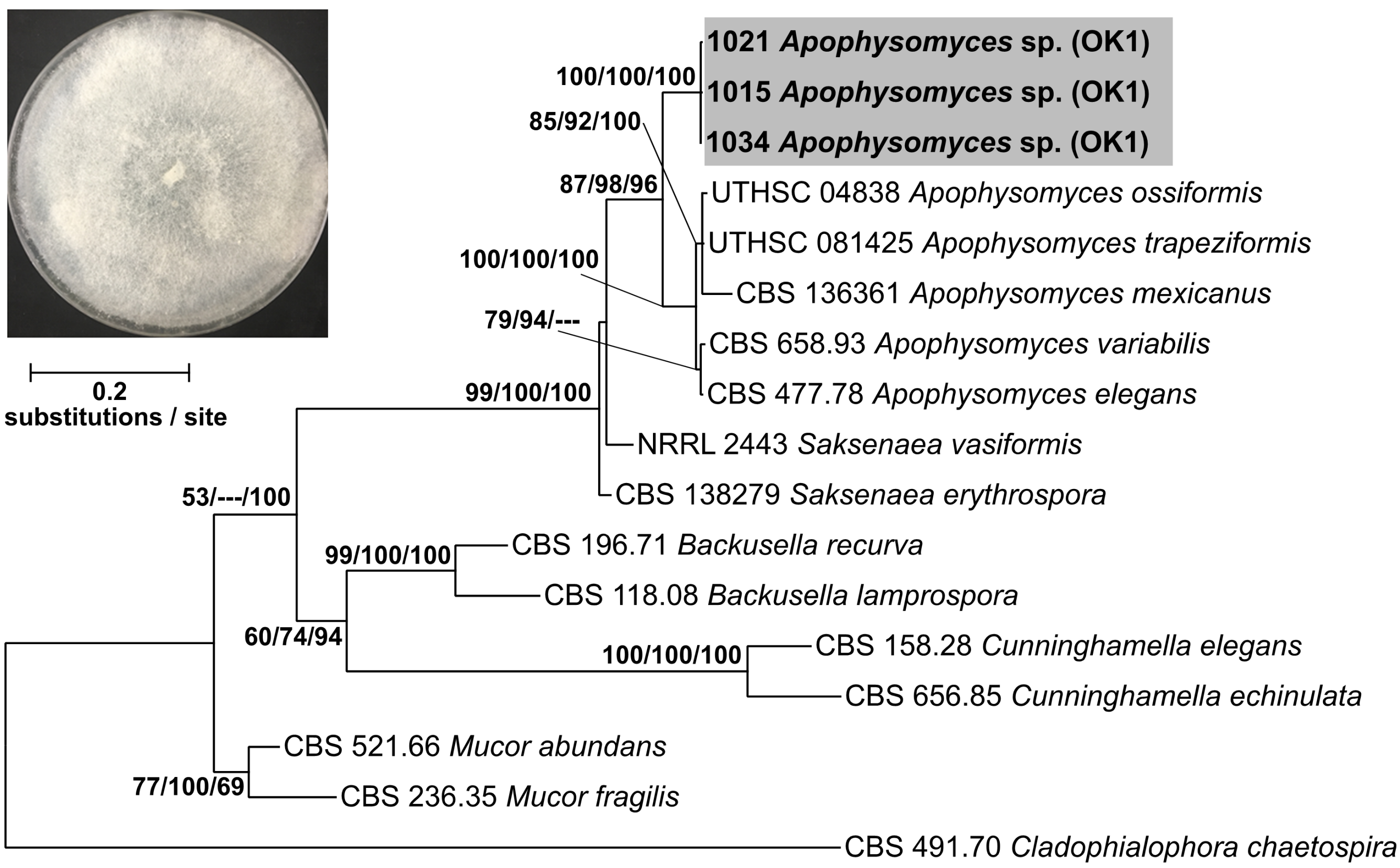

0.2
substitutions / site
1021 Apophysomyces sp. (OK1)
1015 Apophysomyces sp. (OK1)
1034 Apophysomyces sp. (OK1)
UTHSC 04838 Apophysomyces ossiformis
UTHSC 081425 Apophysomyces trapeziformis
CBS 136361 Apophysomyces mexicanus
CBS 658.93 Apophysomyces variabilis
CBS 477.78 Apophysomyces elegans
NRRL 2443 Saksenaea vasiformis
CBS 138279 Saksenaea erythrospora
CBS 196.71 Backusella recurva
CBS 118.08 Backusella lamprospora
CBS 158.28 Cunninghamella elegans
CBS 656.85 Cunninghamella echinulata
CBS 521.66 Mucor abundans
CBS 236.35 Mucor fragilis
CBS 491.70 Cladophialophora chaetospira
100/100/100
85/92/100
87/98/96
100/100/100
79/94/---
99/100/100
53/---/100
99/100/100
60/74/94
100/100/100
77/100/69

Table 1. *Brachycybe lecontii* collection sites.

Site	Collection reference	Millipede clade	Level 4 ecoregion	# millipedes sampled
AR1	Gardner 1975	4	37A	6
AR2	--------------	4	36B	6
AR3	--------------	4	36C	13
AR4	Brewer *et al.* 2012	4	37A	22
AR5	--------------	4	36B	14
AR6	--------------	4	36B	12
GA1	Gardner 1975	1	66D	9
OK1	Brewer *et al.* 2012	4	36D	14
SC1	Brewer *et al.* 2012, Gardner 1975	1	66D	22
SC2	Gardner 1975	1	45E	4
TN1	Gardner 1975	3	69D	42
VA1	Brewer *et al.* 2012, Gardner 1975	3	69D	4
VA2	--------------	3	67I	8
VA3	Gardner 1975	3	69D	15
VA4	--------------	1	67H	5
WV1	Brewer *et al.* 2012, Gardner 1975	3	69D	29
WV2	--------------	3	69D	24
WV3	--------------	3	69D	4
WV4	--------------	3	69D	12
WV5	--------------	3	69D	36

Table 2. Results of pathogenicity assays using a representative set of fungal community members isolated from *B. lecontii.* HPP = Hours post-plating. Asterisks denote significantly faster and greater mortality in a treatment, compared to the negative control: * = p<0.05, ** = p<0.01, *** = p<0.001. Both Log-rank P and Wilcoxon P are Bonferroni-corrected. † Denotes isolates tested again in the Polyporales pathogenicity assay.

Name	Order	Isolate	Time to 50% mortality	% dead at 7 days HPP	Log-rank P	Wilcoxon P
Negative control	----------------------	----------	---------------	0%	-------------	-------------
Pestalotiopsis microspora	Amphisphaeriales	BC0630	---------------	20%	-------------	-------------
Chaetosphaeria myriocarpa	Chaetosphaeriales	BC0320	---------------	0%	-------------	-------------
Capronia dactylotricha	Chaetothyriales	BC1244	---------------	0%	-------------	-------------
Fonsecaea sp.	Chaetothyriales	BC1147	---------------	0%	-------------	-------------
Phialophora americana	Chaetothyriales	BC1193	---------------	7%	-------------	-------------
Lecanicillium attenuatum	Hypocreales	BC0678	---------------	0%	-------------	-------------
Metarhizium flavoviride	Hypocreales	BC1163	136 HPP	87%	<.0005***	<.0005***
Pochonia bulbillosa	Hypocreales	BC0029	---------------	0%	-------------	-------------
Trichoderma viride	Hypocreales	BC1216	---------------	7%	-------------	-------------
Verticillium insectorum	Hypocreales	BC0482	---------------	0%	-------------	-------------
Mortierella aff. *ambigua*	Mortierellales	BC1150	---------------	0%	-------------	-------------
Mortierella sp.	Mortierellales	BC0530	---------------	27%	0.173	0.175
aff. *Apophysomyces* sp.	Mucorales	BC1015	---------------	0%	-------------	-------------
Mucor abundans	Mucorales	BC1010	---------------	0%	-------------	-------------
Ramichloridium anceps	Mycosphaerellales	BC0329	---------------	0%	-------------	-------------
Bjerkandera adusta †	Polyporales	BC0310	36 HPP	80%	<.0005***	<.0005***
Irpex lacteus †	Polyporales	BC0523	72 HPP	47%	0.014*	0.0155*
Trametopsis cervina †	Polyporales	BC1143	36 HPP	100%	<.0005***	<.0005***
Umbelopsis angularis	Umbelopsidales	BC0529	---------------	13%	-------------	-------------
Umbelopsis isabellina	Umbelopsidales	BC1290	---------------	0%	-------------	-------------
Umbelopsis ramanniana	Umbelopsidales	BC1028	---------------	0%	-------------	-------------
Overall	----------------------	----------	---------------	-------	<.0001***	<.0001***

Table 3. Results of pathogenicity assays using a representative set of Polyporales isolated from *B. lecontii* . HPP = Hours post-plating. Asterisks denote significantly faster and greater mortality in a treatment, compared to the negative control: * = p<0.05, ** = p<0.01, *** = p<0.001. Both Log-rank P and Wilcoxon P are Bonferroni-corrected. † Denotes isolates used in initial pathogenicity assay.

Name	Order	Isolate	Time to 50% mortality	% dead at 7 days HPP	Log-rank P	Wilcoxon P
Negative control	---------------	----------	---------	20%	---------	---------
Bjerkandera adusta †	Polyporales	BC0310	36 HPP	50%	0.8484	0.4907
Ceriporia lacerata	Polyporales	BC1158	---------	10%	---------	---------
Ceriporiopsis gilvescens	Polyporales	BC0174	---------	0%	---------	---------
Gloeoporus pannocinctus	Polyporales	BC0042	12 HPP	100%	<.0007***	<.0007***
Irpex lacteus	Polyporales	BC1038	---------	30%	1.0000	1.0000
Irpex lacteus †	Polyporales	BC0523	72 HPP	50%	0.7119	0.5033
Junghuhnia nitida	Polyporales	BC0528	---------	10%	---------	---------
Phanerochaete cumulodentata	Polyporales	BC0709	---------	0%	---------	---------
Phanerochaete sordida	Polyporales	BC0691	84 HPP	60%	0.2352	0.1603
Phlebia acerina	Polyporales	BC1331	---------	0%	---------	---------
Phlebia fuscoatra	Polyporales	BC1014	---------	0%	---------	---------
Phlebia livida	Polyporales	BC0629	---------	0%	---------	---------
Phlebia subserialis	Polyporales	BC0054	---------	0%	---------	---------
Phlebiopsis flavidoalba	Polyporales	BC1426	---------	10%	---------	---------
Phlebiopsis gigantea	Polyporales	BC1049	---------	0%	---------	---------
Scopuloides rimosa	Polyporales	BC1046	---------	0%	---------	---------
Trametopsis cervina †	Polyporales	BC1143	36 HPP	100%	<.0007***	<.0007***
Trametopsis cervina	Polyporales	BC0494	36 HPP	100%	<.0007***	<.0007***
Peniophora pithya	Russulales	BC1467	---------	0%	---------	---------
Overall	---------------	----------	---------	-------	<.0001***	<.0001***

Table 4. Collection information and genus-level diversity indices for each site.

Site	# millipedes sampled	Alpha diversity	Shannon's diversity index	Shannon's equitability
AR1	6	11	2.342	0.977
AR2	6	14	2.497	0.946
AR3	13	17	2.590	0.914
AR4	22	26	3.064	0.941
AR5	14	19	2.801	0.951
AR6	12	18	2.583	0.894
GA1	9	13	2.378	0.927
OK1	14	18	2.737	0.947
SC1	22	14	2.262	0.857
SC2	4	3	0.950	0.865
TN1	42	42	3.170	0.848
VA1	4	34	3.301	0.936
VA2	8	11	2.342	0.977
VA3	15	24	3.043	0.957
VA4	5	7	1.787	0.918
WV1	29	63	3.784	0.913
WV2	24	57	3.790	0.938
WV3	4	16	2.599	0.937
WV4	12	24	3.033	0.954
WV5	36	34	3.045	0.864

Supplemental Table 1. Community science records of *Brachycybe* from BugGuide and iNaturalist websites. Habit and fungus columns were based on subjective

Species	Database	URL	Year	Contributor	Habit	Fungus?
B. lecontii	BugGuide	https://bugguide.net/node/view/45/bgpage	2002	Troy Bartlett	Colony on bark	Not apparent
B. lecontii	BugGuide	https://bugguide.net/node/view/49/bgpage	2000	Troy Bartlett	Colony on bark	Not apparent
B. lecontii	BugGuide	https://bugguide.net/node/view/57554/bgpage	1969	Steven Barney	Individual on dead leaf	Not apparent
B. lecontii	BugGuide	https://bugguide.net/node/view/213351/bgpage	2008	Tim Nichols	Colony on bark (?)	Not apparent
B. lecontii	BugGuide	https://bugguide.net/node/view/595215/bgpage	2011	David J. Thomas	Individual on rock	Not apparent
B. lecontii	BugGuide	https://bugguide.net/node/view/1178937/bgpage	2016	Jonathan Carpenter	Individual on dead wood	Not apparent
B. lecontii	BugGuide	https://bugguide.net/node/view/1517909/bgpage	2018	John Lampkin	Individual on rock	Not apparent
B. lecontii	iNaturalist	https://www.inaturalist.org/observations/17512358	2018	"wjneely"	(Individual in collection)	Not apparent
B. lecontii	iNaturalist	https://www.inaturalist.org/observations/17283380	2018	"rhinoclemmys"	Individual on dead wood	Not apparent
B. lecontii	iNaturalist	https://www.inaturalist.org/observations/13439380	2018	"kevinfitzpatrick"	(Individual in collection)	Not apparent
B. lecontii	iNaturalist	https://www.inaturalist.org/observations/12280088	2018	"cavemander17 "	Individual on rock	Not apparent
B. lecontii	iNaturalist	https://www.inaturalist.org/observations/10718361	2018	"paulmarek"	Individual on dead wood	Not apparent
B. lecontii	iNaturalist	https://www.inaturalist.org/observations/8057119	2017	"hazelsnail"	(Individual in collection)	Not apparent
B. lecontii	iNaturalist	https://www.inaturalist.org/observations/6737379	2017	"beschwar"	Colony on rotten wood	Not apparent
B. lecontii	iNaturalist	https://www.inaturalist.org/observations/6604286	2017	"mark_swanson"	Individual on dead wood	Not apparent
B. lecontii	iNaturalist	https://www.inaturalist.org/observations/6537354	2017	"libbing_life"	Individual on dead wood	Not apparent
B. lecontii	iNaturalist	https://www.inaturalist.org/observations/6451167	2017	"reallifeecology"	Individual on dead wood	Not apparent
B. lecontii	iNaturalist	https://www.inaturalist.org/observations/6451129	2017	"muir"	Individual on dead wood	Not apparent
B. lecontii	iNaturalist	https://www.inaturalist.org/observations/5524007	2017	"chinquapin"	Individual on wood	Not apparent
B. lecontii	iNaturalist	https://www.inaturalist.org/observations/5012087	2017	"reallifeecology"	Individual on dead wood	Not apparent
B. lecontii	iNaturalist	https://www.inaturalist.org/observations/4534657	2016	"reallifeecology"	Individual on rock	Not apparent
B. lecontii	iNaturalist	https://www.inaturalist.org/observations/3402635	2016	"mhedin"	Colony on rotten wood	Not apparent
B. lecontii	iNaturalist	https://www.inaturalist.org/observations/3007821	2016	"reallifeecology"	(Individual in collection)	Not apparent
B. lecontii	iNaturalist	https://www.inaturalist.org/observations/2877858	2016	"damontighe"	Large colony on dead wood	Not apparent
B. lecontii	iNaturalist	https://www.inaturalist.org/observations/453193	2013	"eric_hunt"	Individual on dead wood	Not apparent
B. lecontii	iNaturalist	https://www.inaturalist.org/observations/134815	2009	"rcurtis"	(Individual in collection)	Not apparent
B. lecontii	iNaturalist	https://www.inaturalist.org/observations/4308248	2014	"michaelskvarla"	Colony on dead wood	Yes; grey phlebioid crust
B. lecontii	iNaturalist	https://www.inaturalist.org/observations/11292069	2018	"teriyaki12"	Colony on rotten wood	Yes; tan poroid crust
B. lecontii	iNaturalist	https://www.inaturalist.org/observations/2360344	2015	"eric_hunt"	Colony on dead wood	Yes; *Terana caerulea*
B. lecontii	iNaturalist	https://www.inaturalist.org/observations/13127260	2018	"rdandekar"	Large colony on dead wood	Yes; *Trichoderma*-infected white phlebioid crust

B. lecontii	iNaturalist	https://www.inaturalist.org/observations/11265140	2018	"goody"	Large colony on dead wood	Yes; well-rotted black fungus
B. lecontii	iNaturalist	https://www.inaturalist.org/observations/12606811	2018	"jeremysouthers1"	Individual on pine bark	Yes; white crust
B. lecontii	iNaturalist	https://www.inaturalist.org/observations/9988932	2017	"rdandekar"	Large colony on dead wood	Yes; white crust
B. lecontii	iNaturalist	https://www.inaturalist.org/observations/5322157	2005	"larry14"	Colony on dead wood	Yes; white crust
B. lecontii	iNaturalist	https://www.inaturalist.org/observations/5212886	2017	"muddynaturalist"	Colony on dead wood	Yes; white hyphal cords
B. lecontii	iNaturalist	https://www.inaturalist.org/observations/3915382	2016	"chris184"	Large colony on dead wood	Yes; white hyphal mat and cup fungus
B. lecontii	iNaturalist	https://www.inaturalist.org/observations/13292468	2018	"daniel_folds "	Large colony on snag	Yes; white poroid bracket
B. lecontii	BugGuide	https://bugguide.net/node/view/53529/bgimage	2006	Jonathan Burishkin	Large colony on dead wood	Yes; white-grey crust
B. petasata	BugGuide	https://bugguide.net/node/view/270869/bgimage	2009	Rob Craig	Colony under rotten log	Yes; phlebioid white crust
B. producta	BugGuide	https://bugguide.net/node/view/1027823/bgimage	2014	Sam McNally	Individual on dead wood	Not apparent
B. producta	iNaturalist	https://www.inaturalist.org/observations/10618906	2018	"damarisb"	Individual on wood	Not apparent
B. producta	iNaturalist	https://www.inaturalist.org/observations/9480009	2018	"kueda"	Individual on dead wood	Not apparent
B. producta	iNaturalist	https://www.inaturalist.org/observations/9465211	2018	"tiwane"	Individual on dead wood	Not apparent
B. producta	iNaturalist	https://www.inaturalist.org/observations/8771365	2017	"mazer"	Colony on rotten wood	Not apparent
B. producta	iNaturalist	https://www.inaturalist.org/observations/5540144	2017	"richardwasson"	(Individual in collection)	Not apparent
B. producta	iNaturalist	https://www.inaturalist.org/observations/5536177	2017	"dominic"	Colony on rotten wood	Not apparent
B. producta	iNaturalist	https://www.inaturalist.org/observations/4502473	2016	"icosahedron"	Colony on dead wood	Not apparent
B. producta	iNaturalist	https://www.inaturalist.org/observations/1404122	2015	"loarie"	Individual on dead wood	Not apparent
B. producta	iNaturalist	https://www.inaturalist.org/observations/1117299	2014	"biosam"	Individual on dead wood	Not apparent
B. producta	iNaturalist	https://www.inaturalist.org/observations/1108594	2011	"temminicki"	Individual on dead wood	Not apparent
B. producta	BugGuide	https://bugguide.net/node/view/514775/bgimage	2011	Timothy Boomer	Colony on rotten wood	Yes; brown crust
B. producta	iNaturalist	https://www.inaturalist.org/observations/4551536	2016	"m_patton"	Colony on dead wood	Yes; brown crust
B. producta	iNaturalist	https://www.inaturalist.org/observations/5406781	2017	"catchang"	Colony on rotten wood	Yes; grey hyphae
B. producta	iNaturalist	https://www.inaturalist.org/observations/5471277	2017	"loarie"	Colony on rotten wood	Yes; tan phlebioid crust
B. producta	iNaturalist	https://www.inaturalist.org/observations/10189738	2018	"katewread"	Colony on rotten wood	Yes; white crust
B. producta	iNaturalist	https://www.inaturalist.org/observations/9556670	2018	"lisahug"	Individual on dead wood	Yes; white crust
B. producta	iNaturalist	https://www.inaturalist.org/observations/2701345	2016	"loarie"	Individual on dead wood	Yes; white hyphal mat
B. producta	iNaturalist	https://www.inaturalist.org/observations/6908225	2017	"biosam"	Individual on rotten wood	Yes; white-green hyphal mat
B. producta	iNaturalist	https://www.inaturalist.org/observations/4784269	2016	"icosahedron"	Colony on dead wood	Yes; white-tan crust
B. rosea	BugGuide	https://bugguide.net/node/view/169590/bgpage	2007	Jim McClarin	Rotting oak wood	Not apparent
B. rosea	BugGuide	https://bugguide.net/node/view/418663/bgpage	2010	Debbi Brusco	Individual on dead wood	Not apparent

B. rosea	BugGuide	https://bugguide.net/node/view/1508463/bgimage	2018	"Madratter"	Individual on dead leaf	Not apparent
B. rosea	BugGuide	https://bugguide.net/node/view/904123/bgimage	2014	Sharon Hadley	Individual on dead wood	Not apparent
B. rosea	iNaturalist	https://www.inaturalist.org/observations/6543298	2017	"biosam"	(Colony in collection)	Not apparent
B. rosea	iNaturalist	https://www.inaturalist.org/observations/5914163	2017	"leptonia"	Individual on dead wood	Not apparent
B. rosea	iNaturalist	https://www.inaturalist.org/observations/3067933	2016	"rebeccafay"	Colony on rotten wood	Not apparent
B. rosea	iNaturalist	https://www.inaturalist.org/observations/2896872	2016	"mhedin"	Individual on wood	Not apparent
B. rosea	iNaturalist	https://www.inaturalist.org/observations/1310859	2015	"kueda"	Colony on dead wood	Not apparent
B. rosea	iNaturalist	https://www.inaturalist.org/observations/1205992	2015	"kueda"	Individual on dead wood	Not apparent
B. rosea	iNaturalist	https://www.inaturalist.org/observations/23559	2011	"biosam"	Colony on dead wood	Not apparent
B. rosea	iNaturalist	https://www.inaturalist.org/observations/4039035	2016	"damontighe"	Individual on dead wood	Yes; white hyphal mat
B. sp.	BugGuide	https://bugguide.net/node/view/1459279/bgimage	2017	Bob Kipfer	Individual on dead wood	Not apparent
B. sp.	BugGuide	https://bugguide.net/node/view/1421004/bgimage	2017	"Hobo Joe A.K.A. Insect Lover"	Individual on dead wood	Not apparent
B. sp.	BugGuide	https://bugguide.net/node/view/1104425/bgimage	2015	Jonathan Carpenter	(Individual in collection)	Not apparent
B. sp.	BugGuide	https://bugguide.net/node/view/865459/bgimage	2013	Mark H Brown	Individual on rock	Not apparent
B. sp.	BugGuide	https://bugguide.net/node/view/843152/bgimage	2013	William Hull	Individual on dead wood	Not apparent
B. sp.	BugGuide	https://bugguide.net/node/view/784265/bgimage	2013	"BugCatcher10"	(Individual in collection)	Not apparent
B. sp.	BugGuide	https://bugguide.net/node/view/620056/bgimage	2012	Marvin Smith	Colony on soil	Not apparent
B. sp.	BugGuide	https://bugguide.net/node/view/413806/bgimage	2010	Scott Cox	Individual on wood	Not apparent
B. sp.	BugGuide	https://bugguide.net/node/view/176887/bgimage	2008	Natalie McNear	Individual on dead wood	Not apparent
B. sp.	BugGuide	https://bugguide.net/node/view/172005/bgimage	2008	Natalie McNear	Colony on rotten wood	Not apparent
B. sp.	iNaturalist	https://www.inaturalist.org/observations/17666548	2018	"sageharmon"	(Individual in collection)	Not apparent
B. sp.	iNaturalist	https://www.inaturalist.org/observations/17422879	2018	"ecology2"	(Individual in collection)	Not apparent
B. sp.	iNaturalist	https://www.inaturalist.org/observations/17418303	2018	"jainitastic"	(Individual in collection)	Not apparent
B. sp.	iNaturalist	https://www.inaturalist.org/observations/12322132	2018	"apteryxrowi"	(Individual in collection)	Not apparent
B. sp.	iNaturalist	https://www.inaturalist.org/observations/12152016	2018	"lorri-gong"	Individual on dead wood	Not apparent
B. sp.	iNaturalist	https://www.inaturalist.org/observations/11718802	2018	"magicicada"	Individual on dead wood	Not apparent
B. sp.	iNaturalist	https://www.inaturalist.org/observations/11510349	2018	"kestrel"	Individual on dead wood	Not apparent
B. sp.	iNaturalist	https://www.inaturalist.org/observations/11095607	2018	"garmonb0zia"	Individual on dead wood	Not apparent
B. sp.	iNaturalist	https://www.inaturalist.org/observations/10940260	2018	"christopher13"	Colony on rotten wood	Not apparent
B. sp.	iNaturalist	https://www.inaturalist.org/observations/10178482	2018	"christopher13"	Individual on wood	Not apparent
B. sp.	iNaturalist	https://www.inaturalist.org/observations/9963155	2018	"l0000_hz_legend"	Colony on rotten wood	Not apparent
B. sp.	iNaturalist	https://www.inaturalist.org/observations/9307728	2017	"irislane"	Individual on dead wood	Not apparent

B. sp.	iNaturalist	https://www.inaturalist.org/observations/9307713	2017	"irislane"	Individual on dead wood	Not apparent
B. sp.	iNaturalist	https://www.inaturalist.org/observations/9281269	2017	"temminicki"	Individual on dead wood	Not apparent
B. sp.	iNaturalist	https://www.inaturalist.org/observations/8993568	2017	"easmeds"	Colony on bark	Not apparent
B. sp.	iNaturalist	https://www.inaturalist.org/observations/7609652	2017	"tonytravlos"	Colony on rotten wood	Not apparent
B. sp.	iNaturalist	https://www.inaturalist.org/observations/6937088	2017	"biosam"	Colony on rotten wood	Not apparent
B. sp.	iNaturalist	https://www.inaturalist.org/observations/6253831	2017	"chinquapin"	Colony on soil	Not apparent
B. sp.	iNaturalist	https://www.inaturalist.org/observations/6207443	2017	"friel"	Individual on rotten wood	Not apparent
B. sp.	iNaturalist	https://www.inaturalist.org/observations/5722971	2017	"geodani"	(Individual in collection)	Not apparent
B. sp.	iNaturalist	https://www.inaturalist.org/observations/5714347	2017	"lorri-gong"	Individual on dead wood	Not apparent
B. sp.	iNaturalist	https://www.inaturalist.org/observations/5534690	2017	"christopher13"	Colony on rotten wood	Not apparent
B. sp.	iNaturalist	https://www.inaturalist.org/observations/5282983	2016	"sclerobunus"	Individual on dead wood	Not apparent
B. sp.	iNaturalist	https://www.inaturalist.org/observations/5228142	2017	"vermfly"	Individual on dead wood	Not apparent
B. sp.	iNaturalist	https://www.inaturalist.org/observations/5189159	2017	"christopher13"	Individual on dead wood	Not apparent
B. sp.	iNaturalist	https://www.inaturalist.org/observations/2936552	2016	"allisonpeters"	Individual on rotten wood	Not apparent
B. sp.	iNaturalist	https://www.inaturalist.org/observations/2730609	2016	"marionanoiram"	Individual on dead wood	Not apparent
B. sp.	iNaturalist	https://www.inaturalist.org/observations/1522694	2015	"beeboy"	Colony on dead wood	Not apparent
B. sp.	iNaturalist	https://www.inaturalist.org/observations/1333933	2015	"damontighe"	Individual on dead leaf	Not apparent
B. sp.	iNaturalist	https://www.inaturalist.org/observations/158981	2007	"kucycads"	Individual on dead wood	Not apparent
B. sp.	iNaturalist	https://www.inaturalist.org/observations/18981	2010	"tapbirds"	Individual on dead wood	Not apparent
B. sp.	iNaturalist	https://www.inaturalist.org/observations/10674619	2006	"henkwallays2"	Individual on wood	Yes; black-orange crust
B. sp.	iNaturalist	https://www.inaturalist.org/observations/1275824	2015	"robberfly"	Colony on dead wood	Yes; cup fungus
B. sp.	iNaturalist	https://www.inaturalist.org/observations/9013075	2017	"easmeds"	Individual on dead wood	Yes; gelatinous grey crust
B. sp.	iNaturalist	https://www.inaturalist.org/observations/14057280	2018	"clinchriverdreams"	Individual on dead wood	Yes; grey crust
B. sp.	iNaturalist	https://www.inaturalist.org/observations/363144	2013	"tonyg"	Colony on dead wood	Yes; grey crust
B. sp.	iNaturalist	https://www.inaturalist.org/observations/9722536	2018	"kookamongus"	Individual on dead wood	Yes; grey phlebioid crust
B. sp.	iNaturalist	https://www.inaturalist.org/observations/5823516	2017	"leenash"	Individual on dead wood	Yes; large brown crust
B. sp.	iNaturalist	https://www.inaturalist.org/observations/12265670	2018	"athensalive"	Colony on rotten wood	Yes; *Phlebia* sp.
B. sp.	iNaturalist	https://www.inaturalist.org/observations/595969	2014	"moonlittrails"	Colony on dead wood	Yes; phlebioid crust and cup fungus
B. sp.	iNaturalist	https://www.inaturalist.org/observations/5408733	2017	"damontighe"	Individual on dead wood	Yes; phlebioid grey crust and white hyphal mat
B. sp.	iNaturalist	https://www.inaturalist.org/observations/11719631	2018	"magicicada"	Individual on dead wood	Yes; pink-white hyphal mat

B. sp.	iNaturalist	https://www.inaturalist.org/observations/8870068	2017	"debk"	Individual on rotten wood	Yes; tan bracket fungus and white poroid crust
B. sp.	iNaturalist	https://www.inaturalist.org/observations/12367836	2018	"dgreenberger"	Colony on rotten wood	Yes; tan crust
B. sp.	iNaturalist	https://www.inaturalist.org/observations/5257850	2016	"eccentric_entomophile"	Individual on dead wood	Yes; tan crust
B. sp.	iNaturalist	https://www.inaturalist.org/observations/10698727	2006	"henkwallays2"	Large colony on dead wood	Yes; tan poroid crust
B. sp.	iNaturalist	https://www.inaturalist.org/observations/5452255	2017	"damontighe"	Individual on dead wood	Yes; very rotten yellow crust
B. sp.	BugGuide	https://bugguide.net/node/view/851644/bgimage	2011	Sam McNally	Large colony on dead wood	Yes; white crust
B. sp.	iNaturalist	https://www.inaturalist.org/observations/9736972	2018	"twillrichardson"	Colony on pine log	Yes; white crust
B. sp.	iNaturalist	https://www.inaturalist.org/observations/9281266	2017	"temminicki"	Individual on dead wood	Yes; white crust
B. sp.	iNaturalist	https://www.inaturalist.org/observations/5920644	2017	"leslie_flint"	Colony under rotten log	Yes; white crust
B. sp.	iNaturalist	https://www.inaturalist.org/observations/5229632	2017	"tomv"	Colony on dead wood	Yes; white crust
B. sp.	iNaturalist	https://www.inaturalist.org/observations/2801341	2016	"robberfly"	Colony on rotten wood	Yes; white crust
B. sp.	iNaturalist	https://www.inaturalist.org/observations/5095109	2016	"mazer"	Colony on dead wood	Yes; white crust and phlebioid grey crust
B. sp.	iNaturalist	https://www.inaturalist.org/observations/9964190	2018	"10000_hz_legend"	Large colony on dead wood	Yes; white hyphae and orange crust
B. sp.	iNaturalist	https://www.inaturalist.org/observations/5408600	2017	"robberfly"	Colony on rotten wood	Yes; white hyphal cords
B. sp.	iNaturalist	https://www.inaturalist.org/observations/3520995	2016	"biosam"	Colony on rotten wood	Yes; white hyphal cords
B. sp.	iNaturalist	https://www.inaturalist.org/observations/3198564	2014	"eccentric_entomophile"	Colony on rotten wood	Yes; white hyphal cords
B. sp.	iNaturalist	https://www.inaturalist.org/observations/1110059	2014	"lindynik"	Colony on dead wood	Yes; white hyphal cords
B. sp.	iNaturalist	https://www.inaturalist.org/observations/1066156	2014	"bapeck8"	Individual on dead wood	Yes; white hyphal cords
B. sp.	iNaturalist	https://www.inaturalist.org/observations/9812105	2018	"amacedo"	Colony on rotten wood	Yes; white hyphal mat
B. sp.	iNaturalist	https://www.inaturalist.org/observations/8858584	2008	"robirwin"	Individual on dead wood	Yes; white hyphal mat
B. sp.	iNaturalist	https://www.inaturalist.org/observations/7489413	2008	"robirwin"	Individual on dead wood	Yes; white hyphal mat
B. sp.	iNaturalist	https://www.inaturalist.org/observations/6682063	2017	"biosam"	Colony on rotten wood	Yes; white hyphal mat
B. sp.	iNaturalist	https://www.inaturalist.org/observations/5278798	2017	"jessefurrow"	Individual on dead wood	Yes; white hyphal mat
B. sp.	iNaturalist	https://www.inaturalist.org/observations/5225939	2017	"lenaz"	Individual on dead wood	Yes; white hyphal mat
B. sp.	iNaturalist	https://www.inaturalist.org/observations/4780656	2016	"lorri-gong"	Colony on dead wood	Yes; white hyphal mat
B. sp.	iNaturalist	https://www.inaturalist.org/observations/1275884	2015	"metsa"	Colony on dead wood	Yes; white hyphal mat
B. sp.	iNaturalist	https://www.inaturalist.org/observations/4505555	2016	"dutchflatterry"	Large colony on dead wood	Yes; white hyphal mat, tan bracket, slime mold

B. sp.	BugGuide	https://bugguide.net/node/view/945769/bgimage	2014	Jonathan Carpenter	Colony on rotten wood	Yes; white poroid crust
B. sp.	BugGuide	https://bugguide.net/node/view/633418/bgimage	2012	Alan Rockefeller	Colony on rotten wood	Yes; *Xylodon* sp.
B. sp.	iNaturalist	https://www.inaturalist.org/observations/5786153	2017	"daniam"	Individual on dead wood	Yes; yellow slime mold and white hyphal cords
B. sp.	iNaturalist	https://www.inaturalist.org/observations/4902929	2017	"grayson"	Colony on dead pine wood	Yes; yellow slime mold or cup fungus

Supplemental Table 2. Strains and their associated NCBI Genbank reference numbers for isolates of *Mortierella* aff. *ambigua* and close relatives used in phylogenetic analysis.

Name	Strain	ITS	LSU
Mortierella ambigua	CBS 450.88	JX976067	KC018411
Mortierella ambigua	CBS 474.96	JX976056	KC018416
Mortierella ambigua	CBS 373.96	JX976062	JX976147
Mortierella capitata	CBS 293.96	JX976123	KC018334
Mortierella capitata	CBS 859.70	JX976008	KC018395
Mortierella microzygospora	CBS 880.97	NR_111569	HQ667394
Mortierella parazychae	CBS 868.71	HQ630283	HQ667362
Mortierella pseudozygospora	CBS 780.86	JX975880	JX976143
Mortierella wolfii	CBS 611.70	JN943806	JN940863
Mortierella wolfii	CBS 612.70	MH859876	HQ667381
Mortierella rostafinskii	CBS 522.70	NR_111586	NG_042570
Mortierella strangulata	CBS 455.67	HQ630359	HQ667437
Mucor abundans	CBS 521.66	JN206110	JN206457
Mucor fragilis	CBS 236.35	JN205979	FN650671

Supplemental Table 3. Strains and their associated NCBI Genbank reference numbers for isolates of aff. *Apophysomyces* sp. and close relatives used in phylogenetic analysis.

Name	Strain number	ITS	LSU
Apophysomyces variabilis	CBS 658.93	NR_130683	HM849695
Apophysomyces elegans	CBS 477.78	JN206280	JN206536
Apophysomyces ossiformis	UTHSC 04-838	NR_137035	FN554252
Apophysomyces trapeziformis	UTHSC 08-1425	NR_137034	FN554261
Apophysomyces mexicanus	CBS 136361	HG974255	HG974256
Saksenaea vasiformis	NRRL 2443	FR687327	HM776679
Saksenaea erythrospora	CBS 138279	KM102733	KM102734
Mucor abundans	CBS 521.66	JN206110	JN206457
Mucor fragilis	CBS 236.35	JN205979	FN650671
Backusella circina	CBS 128.70	NR_103649	JN206529
Backusella recurva	CBS 196.71	JN206265	JN206523
Backusella lamprospora	CBS 118.08	NR_145291	JN206531
Cunninghamella echinulata	CBS 656.85	JN205896	JN206598
Cunninghamella elegans	CBS 158.28	JN205888	JN206602
Cunninghamella bertholletiae	CBS 190.84	JN205878	HM849701
Cladophialophora chaetospira	CBS 491.70	EU035405	EU035405

Supplemental Table 4. Fungi recovered from *B. lecontii* based on ITS barcoding and arranged taxonomically. †LSU sequences are deposited for *Mortierella* aff. *ambigua* (NCBI Genbank accession MK045304) and aff. *Apophysomyces* sp. (NCBI Genbank accession MK045305).

Name		Example isolate	Best match	Query coverage (%)	Identity (%)	Deposited ITS sequence #
Ascomycota						
Amphisphaeriales						
Discosia	sp.	----------	---------------	----	----	--------------
Neopestalotiopsis	sp.	----------	---------------	----	----	--------------
Pestalotiopsis	*crassiuscula*	BC1288	AY687868.1	100	99	--------------
	jesteri	BC1501	KT000165.1	95	99	--------------
	knightiae	BC0084	KM199311.1	99	99	--------------
	mangiferae	BC1427	KP074973.1	100	100	--------------
	microspora	BC1439	MH707065.1	100	100	--------------
	sp.	----------	---------------	----	----	--------------
Annulatascales						
Conlarium	*duplumascospora*	BC1222	JN936997.1	98	85	--------------
Rhodoveronaea	*varioseptata*	BC0645	KF823603.1	99	82	--------------
Capnodiales						
Acrocalymma	*aquatica*	BC0359	JX276951.1	83	83	--------------
Cladosporium	*cladosporioides*	BC1475	MH714552.1	100	100	--------------
	sp.	----------	---------------	----	----	--------------
Passalora	*brachycarpa*	BC1621	GU214664.1	100	98	--------------
Ramularia	*coryli*	BC1480	KX287391.1	100	98	--------------
	endophylla	BC1635	KP894243.1	98	98	--------------
	interstitiales	BC1540	KX287458.1	98	98	--------------
	rumicicola	BC1601	KX287503.1	100	96	--------------
	sp.	----------	---------------	----	----	--------------
Septoria	*hyperici*	BC1571	NR 147271.	99	97	--------------
Sphaerulina	*berberidis*	BC1578	LC206672.1	100	99	--------------
Trichomerium	*foliicola*	BC0315	NR 144963.	100	95	--------------
Chaetosphaeriales						

	Chaetosphaeria	*chloroconia*	BC1533	AF178542.1	98	99 --------------
		myriocarpa	BC0320	MH107883.1	98	99 --------------
	Chloridium	*virescens*	BC1507	EF029220.1	99	100 --------------
		sp.	----------	---------------	----	---- --------------
	Codinaea	*acaciae*	BC1318	KY965397.1	100	96 --------------
Chaetothyriales						
	Capronia	*dactylotricha*	BC1244	NR 137136.	91	86 --------------
		leucadendri	BC0548	NR 156212.	98	98 --------------
		pilosella	BC1634	DQ826737.1	98	96 --------------
		sp.	----------	---------------	----	---- --------------
	Cladophialophora	*chaetospira*	BC1539	KF359558.1	100	92 --------------
		potulentorum	BC1241	EU035410.1	99	90 --------------
		sp.	----------	---------------	----	---- --------------
	Cyphellophora	*gamsii*	BC1562	NR 156306.	100	95 --------------
		olivacea	BC1633	KX302010.1	95	98 --------------
		oxyspora	BC0325	MF196874.1	99	99 --------------
		sp.	----------	---------------	----	---- --------------
	Exobasidium	*otanianum*	BC1069	AB180343.1	96	96 --------------
	Exophiala	*moniliae*	BC1410	HE605213.1	100	97 --------------
		xenobiotica	BC1574	KY434151.1	97	92 --------------
		sp.	----------	---------------	----	---- --------------
	Fonsecaea	*pedrosoi*	BC1176	AB114131.1	98	98 MH971241
		sp. (new)	BC1045	JN999999.1	93	94 MH971242
		sp.	----------	---------------	----	---- --------------
	Phialophora	*americana*	BC1388	U31840.1	99	99 MH971243
		sessilis	BC1631	GU981736.1	100	99 MH971244
		sp.	----------	---------------	----	---- --------------
	Rhinocladiella	*anceps*	BC0327	AF050284.1	100	99 MH971245
		atrovirens	BC0550	AB091215.1	98	98 MH971246
		quercus	BC1534	NR 155728.	97	99 MH971247
Coniochaetales						
	Coniochaeta	*cephalothecoides*	BC0639	KY064029.1	97	99 --------------
		sp.	----------	---------------	----	---- --------------

Cordanales						
Cordana	*pauciseptata*	BC0927	HE672147.1	93	99	--------------
Diaporthales						
Cryptodiaporthe	*hystrix*	BC0433	KX776446.1	100	98	--------------
Diaporthe	*phaseolorum*	BC0083	FJ441609.1	99	98	--------------
Gnomonopsis	sp.	----------	---------------	----	----	--------------
Phaeoacremonium	*iranianum*	BC1423	KF764529.1	100	98	--------------
	mortoniae	BC1555	EU427312.1	82	99	--------------
Togninia	*minima*	BC0539	KP083231.1	100	100	--------------
Dothideales						
Aureobasidium	*pullulans*	BC1444	MF497401.1	100	100	--------------
	sp.	----------	---------------	----	----	--------------
Dothiora	*sorbi*	BC0422	KY929146.1	100	98	--------------
	pyrenophora	BC1629	KU728514.1	97	98	--------------
Eurotiales						
Aspergillus	*versicolor*	BC1564	KU318416.1	98	99	--------------
Paecilomyces	*carneus*	BC1457	HQ660442.1	100	99	--------------
	inflatus	BC0451	KU702692.1	99	99	--------------
	javanicus	BC1300	AB099944.1	100	100	--------------
Penicillium	*carneum*	BC1278	NR 111551.	100	100	MH971248
	chrysogenum	BC1254	MH778149.1	99	100	MH971249
	daleae	BC1205	MH854984.1	100	100	MH971250
	fellutanum	BC1326	HM469425.1	100	100	MH971251
	glabrum	BC1095	MF803957.1	100	100	MH971252
	herquei	BC1214	MF663569.1	100	100	MH971253
	nodositatum	BC1031	NR 103703.	100	100	MH971254
	oxalicum	BC1327	MG733762.1	100	100	MH971255
	pancosmium	BC1499	MF803943.1	100	100	MH971256
	pinophilum	BC0544	EF488397.1	100	99	MH971257
	steckii	BC0778	MG554368.1	100	100	MH971258
	sumatraense	BC1530	JX140874.1	100	100	MH971259
	sp.	----------	---------------	----	----	--------------
Thysanophora	*penicilloides*	BC1421	JQ272462.1	99	100	--------------

Glomerellales						
Glomerella	*acutata*	BC1479	JN697577.1	100	100	--------------
Helotiales						
Cadophora	*malorum*	BC0073	DQ404350.1	100	98	--------------
Catenulifera	*brachyconia*	BC1570	AB190384.1	100	97	--------------
Hyalodendriella	sp.	----------	---------------	----	----	--------------
Hymenoscyphus	*dehlii*	BC0543	LC206621.1	94	94	--------------
Hyphodiscus	*hymeniophilus*	BC0581	DQ227258.1	99	97	--------------
Idriella	*rara*	BC1306	KC775737.1	91	98	--------------
Leptodontidium	*elatius*	BC1257	AM981224.1	100	95	--------------
	sp.	----------	---------------	----	----	--------------
Leptodontium	sp.	----------	---------------	----	----	--------------
Pezicula	*cinnamomea*	BC1553	KR859145.1	98	83	--------------
Pilidium	*concavum*	BC1478	MF776047.1	100	100	--------------
Rhexocercosporidium	sp.	----------	---------------	----	----	--------------
Scytalidium	*lignicola*	BC1391	GQ272634.1	98	99	--------------
	sp.	----------	---------------	----	----	--------------
Synchaetomella	*acerina*	BC1299	NR 111811.	99	93	--------------
Xenopolyscytalum	sp.	----------	---------------	----	----	--------------
Hypocreales						
Acremonium	*variecolor*	BC0031	HE608648.1	99	99	--------------
	sp.	----------	---------------	----	----	--------------
Atractium	*stilbaster*	BC1404	KM231792.1	100	87	--------------
Beauveria	*brongniartii*	BC0062	JX110373.1	99	99	--------------
	caledonica	BC1250	DQ350137.1	100	99	--------------
Calcarisporium	*arbuscula*	BC1182	LC145810.1	100	99	--------------
Clonostachys	*rosea*	BC1297	KX421414.1	99	99	--------------
	sp.	----------	---------------	----	----	--------------
Cordyceps	*militaris*	BC1419	KY407763.1	100	99	--------------
	sp.	----------	---------------	----	----	--------------
Cosmospora	*butyri*	BC1580	JQ070093.1	99	100	MH971260
	flavoviridis	BC0394	HQ897791.1	100	99	MH971261
	berkeleyana	BC0428	MH859583.1	99	96	MH971262

	sp.	----------	---------------	----	----	--------------
Cylindrium	*elongatum*	BC1446	KM231852.1	100	99	--------------
Dialonectria	sp.	----------	---------------	----	----	--------------
Fusarium	*equiseti*	BC1447	GQ365157.1	100	100	--------------
	tricinctum	BC1510	JX045791.1	99	100	--------------
	sp.	----------	---------------	----	----	--------------
Fusicolla	*melogramma*	BC0649	NR 155096.	99	99	--------------
Geosmithia	sp.	----------	---------------	----	----	--------------
Gibberella	sp.	----------	---------------	----	----	--------------
Gliomastix	*murorum*	BC0156	AB540558.1	100	98	--------------
Hirsutella	sp.	----------	---------------	----	----	--------------
Hypocrea	*lutea*	BC1265	AF359264.1	99	99	--------------
	pachybasioides	BC0321	AY240843.1	100	98	--------------
Hypomyces	*aurantius*	BC1020	AB591044.1	100	98	--------------
Isaria	*farinosa*	BC0416	MH191137.1	100	99	--------------
	fumosorosea	BC0014	FJ177460.1	100	99	--------------
Lasionectria	sp.	----------	---------------	----	----	--------------
Lecanicillium	*araneogenum*	BC0675	KX845704.1	99	98	--------------
	attenuatum	BC1503	MH231313.1	100	100	--------------
	fungicola	BC1022	KX379184.1	99	100	--------------
	fusisporum	BC1518	AB378517.1	99	100	--------------
	psallotiae	BC0015	AB360367.1	99	99	--------------
	saksenae	BC1455	KY320616.1	100	97	--------------
Mariannaea	*elegans*	BC1489	KX028787.1	99	99	--------------
	samuelsii	BC1156	KM231757.1	99	100	--------------
Metacordyceps	*chlamydosporia*	BC0618	AB214654.1	100	99	--------------
Metarhizium	*anisopliae*	BC1203	MG786739.1	100	100	--------------
	flavoviride	BC1163	KX380789.1	100	99	--------------
Monocillium	sp.	----------	---------------	----	----	--------------
Nectria	sp.	----------	---------------	----	----	--------------
Niesslia	*pulchriseta*	BC0033	MG827040.1	96	99	--------------
Pochonia	*bulbillosa*	BC1436	AB709835.1	100	99	--------------
	chlamydosporia	BC1165	AB713184.1	99	100	--------------

	suchlasporia	BC0017	AB214658.1	99	99	--------------
Protocrea	sp.	----------	---------------	----	----	--------------
Pseudonectria	sp.	----------	---------------	----	----	--------------
Purpureocillium	*lilacinum*	BC1070	FJ765024.1	100	98	--------------
Sepedonium	*chalcipori*	BC1058	KT946847.1	100	90	--------------
Simplicillium	*lamellicola*	BC1496	KT004573.1	100	97	--------------
	lanosoniveum	BC0027	AB758126.1	99	99	--------------
	sp.	----------	---------------	----	----	--------------
Stilbella	sp.	----------	---------------	----	----	--------------
Tolypocladium	*album*	BC1113	MH137667.1	100	98	--------------
	inflatum	BC0499	KT693271.1	100	99	--------------
Trichoderma	*atroviride*	BC1215	MF871528.1	100	100	MH971263
	harzianum	BC1204	MG132085.1	100	100	MH971264
	lixii	BC1322	MF782824.1	100	100	MH971265
	melanomagnum	BC1271	KU738454.1	100	100	MH971266
	pleuroticola	BC1277	MF687194.1	100	100	MH971267
	viride	BC1433	AJ230676.1	99	100	MH971268
	sp.	----------	---------------	----	----	--------------
Verticillium	*fungicola*	BC0488	FJ810136.1	100	99	MH971269
	insectorum	BC1005	AB214655.1	100	99	MH971270
	leptobactrum	BC1341	EF641871.1	99	99	MH971271
	sp.	----------	---------------	----	----	--------------
Xenoacremonium	*falcatus*	BC1488	MH062972.1	98	100	--------------
Mycosphaerellales						
Mycosphaerella	sp.	----------	---------------	----	----	--------------
Ramichloridium	sp.	----------	---------------	----	----	--------------
Onygenales						
Oidiodendron	sp.	----------	---------------	----	----	--------------
Ophiostomatales						
Ophiostoma	*dentifundum*	BC1140	AY495435.1	97	93	--------------
	grandicarpum	BC1638	NR 147600.	99	90	--------------
Sporothrix	*inflata*	BC0434	AY495432.1	100	99	--------------
	sp.	----------	---------------	----	----	--------------

Pleosporales						
Coniothyrium	*fuckelii*	BC0181	AB665314.1	100	100	--------------
Curvularia	*geniculata*	BC1177	MH517581.1	100	100	--------------
	senegalensis	BC1151	MH517581.1	100	100	--------------
Dictyosporium	*australiense*	BC0100	DQ018092.1	97	98	--------------
Helminthosporium	*asterinum*	BC1248	AF073917.1	99	99	--------------
	velutinum	BC1385	AB551948.1	98	99	--------------
Leptosphaerulina	*chartarum*	BC0086	GU566269.1	100	99	--------------
Lophiostoma	sp.	----------	---------------	----	----	--------------
Neocucurbitaria	*vachellia*	BC1535	NR 156363.	100	95	--------------
	acerina	BC1494	MF795767.1	99	98	--------------
Paraconiothyrium	*brasiliense*	BC1485	JF502455.1	99	99	--------------
	hawaiiense	BC1456	HM751092.1	100	99	--------------
	sp.	----------	---------------	----	----	--------------
Paraphaeosphaeria	*neglecta*	BC1617	JX496038.1	99	99	--------------
Periconia	*pseudobyssoides*	BC0452	KC954161.1	100	99	--------------
	sp.	----------	---------------	----	----	--------------
Peyronellaea	*glomerata*	BC0592	MG832565.1	99	99	--------------
Phaeosphaeria	sp.	----------	---------------	----	----	--------------
Phoma	*bellidis*	BC1498	JF502444.1	100	100	--------------
	sp.	----------	---------------	----	----	--------------
Preussia	sp.	----------	---------------	----	----	--------------
	sp.	----------	---------------	----	----	--------------
Stagonosporopsis	*cucurbitacearum*	BC1500	LC168795.1	100	99	--------------
Teichospora	*quercus*	BC1412	MH107920.1	98	96	--------------
Saccharomycetales						
Candida	*aglyptina*	BC1600	FJ196775.1	76	98	--------------
	boleticola	BC1585	KY102001.1	100	99	--------------
	sp.	----------	---------------	----	----	--------------
Pichia	*jadinii*	BC0555	FJ865435.1	100	95	--------------
	sp.	----------	---------------	----	----	--------------
Yamadazyma	*mexicana*	BC0590	KY105944.1	100	99	--------------
Sordariales						

Apodus	*deciduus*	BC1170	NR 145141.	100	96	--------------
Cercophora	*sulphurella*	BC1619	AY587913.1	99	96	--------------
Chaetomium	sp.	----------	---------------	----	----	--------------
Fimetariella	*rabenhorstii*	BC1129	KX869958.1	98	98	--------------
	sp.	----------	---------------	----	----	--------------
Phialemonium	*dimorphosporum*	BC0511	FJ441614.1	100	98	--------------
Spadicoides	*bina*	BC0107	JF340260.1	97	99	--------------
Trichosphaetiales						
Nigrospora	*oryzae*	BC1017	HQ608152.1	100	100	--------------
Tubeufiales						
Thaxteriellopsis	*lignicola*	BC1594	JN865207.1	99	78	--------------
Venturiales						
Venturia	*viennotii*	BC0500	KF793787.1	97	96	--------------
Xylariales						
Ascovirgaria	*occulta*	BC0589	AB740957.1	100	97	--------------
Biscogniauxia	*atropunctata*	BC0106	JX507799.1	97	99	--------------
	sp.	----------	---------------	----	----	--------------
Diatrype	*disciformis*	BC1565	KR092795.1	99	99	--------------
Eutypella	*vitis*	BC1453	KU320620.1	99	99	--------------
Hansfordia	*nebularis*	BC1173	KF893290.1	99	100	--------------
Hypoxylon	*crocopeplum*	BC0178	JN673047.1	99	99	--------------
	fendleri	BC1400	JN979417.1	100	92	--------------
	fuscum	BC1159	JN979424.1	100	96	--------------
	lenormandii	BC1352	KM610287.1	99	96	--------------
	perforatum	BC1305	JQ009308.1	100	99	--------------
	rubiginosum	BC1438	MH178694.1	91	99	--------------
	truncatum	BC1079	AF201716.2	98	99	--------------
	sp.	----------	---------------	----	----	--------------
Nemania	*diffusa*	BC1521	MH633932.1	99	91	--------------
	serpens	BC1093	KU683860.1	99	99	--------------
	sp.	----------	---------------	----	----	--------------
Spegazzinia	sp.	----------	---------------	----	----	--------------
Virgaria	*nigra*	BC1406	AB670716.1	100	98	--------------

Xylaria	*heliscus*	BC1355	JQ761642.1	96	95	MH971272
	sp.	----------	---------------	----	----	--------------
incertae sedis						
Acrodontium	*crateriforme*	BC1302	KX287270.1	99	97	--------------
Barbatosphaeria	*dryina*	BC1060	KM492892.	92	96	--------------
	varioseptata	BC1188	NR 132089.	92	97	--------------
Geomyces	sp.	----------	---------------	----	----	--------------
Leohumicola	sp.	----------	---------------	----	----	--------------
Pseudogymnoascus	sp.	----------	---------------	----	----	--------------
Ramimonilia	*apicalis*	BC1546	NR 144959.	89	93	--------------
Veronaea	*japonica*	BC1623	NR 111277.	98	97	--------------
Xylomelasma	sp.	----------	---------------	----	----	--------------
Basidiomycota						
Agaricales						
Schizophyllum	*commune*	BC1449	MF554593.1	100	99	--------------
Cantharellales						
Sistotrema	*brinkmanii*	BC1078	KX527870.1	99	99	--------------
	oblongisporum	BC1508	KP814309.1	99	99	--------------
Tulasnella	sp.	----------	---------------	----	----	--------------
Dacrymycetales						
Calocera	sp.	----------	---------------	----	----	--------------
Dacrymyces	*australis*	BC1195	MH858261.1	96	84	--------------
Exobasidiales						
Meira	sp.	----------	---------------	----	----	--------------
Hymenochaetales						
Hydnochaete	*olivacea*	BC1560	KJ140712.1	100	82	--------------
Peniophorella	*pubera*	BC1138	KP715565.1	97	84	--------------
Malasseziales						
Malassezia	sp.	----------	---------------	----	----	--------------
Microbotryales						
Curvibasidium	*cygneicollum*	BC1606	KY102973.1	98	98	--------------
	pallidicorallinum	BC0542	JX188149.1	100	99	--------------
Microstromatales						

Microstroma	*juglandis*	BC1607	EU069498.1	95	90	--------------
Polyporales						
Bjerkandera	*adusta*	BC1316	MF161298.1	100	99	--------------
Ceriporia	*lacerata*	BC1158	KP135024.1	99	100	--------------
	sp.	----------	---------------	----	----	--------------
Ceriporiopsis	*gilvescens*	BC0174	KY948761.1	100	100	--------------
Gloeoporus	*pannocinctus*	BC0042	JQ673102.1	100	100	--------------
Irpex	*lacteus*	BC1038	JX290579.1	100	99	--------------
	sp.	----------	---------------	----	----	--------------
Junghuhnia	*nitida*	BC0528	KP135323.1	97	99	--------------
Phanerochaete	*cumulodentata*	BC0709	KP994373.1	100	100	MH971273
	sordida	BC0691	KP135074.1	100	100	MH971274
	sp.	----------	---------------	----	----	--------------
Phlebia	*acerina*	BC1331	KP135373.1	100	99	--------------
	fuscoatra	BC1014	KY948754.1	99	97	--------------
	lividina	BC0629	KY948756.1	100	99	--------------
	subserialis	BC0054	HQ607954.1	100	99	--------------
	tremellosa	BC0184	GU062266.1	99	99	--------------
Phlebiopsis	*flavidoalba*	BC1426	KP135401.1	100	100	--------------
	gigantea	BC1049	KX028786.1	100	100	--------------
Scopuloides	*rimosa*	BC1046	KP135348.1	99	100	--------------
Trametopsis	*cervina*	BC1143	JX463662.1	99	99	--------------
Russulales						
Peniophora	*pithya*	BC1467	MH857635.1	100	95	--------------
	sp.	----------	---------------	----	----	--------------
Stereum	*complicatum*	BC1428	MF161283.1	98	100	--------------
	sp.	----------	---------------	----	----	--------------
Sporidiobolales						
Rhodotorula	*eucalyptica*	BC1579	EU075186.1	99	87	--------------
	sp.	----------	---------------	----	----	--------------
Tremellales						
Cryptococcus	*dimennae*	BC0392	KM246197.1	98	98	--------------
	sp.	----------	---------------	----	----	--------------

Kockovaella	*fuzhouensis*	BC1536	KY103850.1	97	93	--------------
	sichuanensis	BC1542	KY103859.1	100	99	--------------
Tremella	*subalpina*	BC1424	NR 155908.	91	85	--------------
incertae sedis						
Acaromyces	*ingoldii*	BC1417	HM595575.1	100	100	--------------
Hamamotoa	*singularis*	BC1050	AF444600.1	100	87	--------------
Mucoromycota						
Mortierellales						
Mortierella	aff. *ambigua* †	BC1065	JX976067.1	100	92	MH971275
	sp.	----------	---------------	----	----	--------------
Mucorales						
aff. *Apophysomyces*	sp.†	BC1021	NR 137035.	91	78	MH971276
Backusella	*circina*	BC1287	JQ979443.1	100	94	--------------
	recurva	BC1267	JN206265.1	99	98	--------------
Cunninghamella	*elegans*	BC1044	MH857146.1	99	99	--------------
Mucor	*abundans*	BC1003	MH855716.1	99	99	MH971277
	circinelloides	BC1285	MH856522.1	99	100	MH971278
	fragilis	BC1294	KY047150.1	100	99	MH971279
	genevensis	BC1002	JN206046.1	97	98	MH971280
	luteus	BC1107	MH859851.1	98	98	MH971281
	mucedo	BC1056	JQ319046.1	100	98	MH971282
	racemosus	BC1295	MF356581.1	100	99	MH971283
	sp.	----------	---------------	----	----	--------------
Umbelopsidales						
Umbelopsis	*angularis*	BC0529	MH859191.1	99	99	MH971284
	isabellina	BC1080	KM044070.1	100	99	MH971285
	ramanniana	BC1097	MH864647.1	100	100	MH971286
	sp.	----------	---------------	----	----	--------------
Zoopagomycota						
Entomophthorales						
Basidiobolus	*ranarum*	BC1232	EF392530.1	100	99	--------------