THE ROLE OF YEASTS IN THE POLLINATION SUCCESS OF A NEOTROPICAL ORCHID

by

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THESIS ABSTRACT

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The Neotropical cloud forest inhabiting orchid *Dracula felix* has long been postulated to be a fungal mimic due to the form of its lower labellum and attraction to it by drosophilid flies that are often found feeding on fungal fruiting bodies in the surrounding area. The low number of co-occurring flowers in the area combined with the high number of fruiting fungi appears to have driven the evolution of the orchid genus *Dracula* to mimic these co-occurring fungi so that pollinators may be recruited. Over several years of working with these orchids we have noticed a particular lapping behavior by the pollinating flies on the labella and sepals of the *Dracula* flowers. In this study we have first surveyed floral yeasts and molds associated with *Dracula* flowers and then investigated the role of these fungi in attracting pollinators and offering a food reward to retain them for pollination purposes. In addition to the floral yeasts, leaf endophytes and root associated fungi were cultured and identified, and their frequencies were determined.

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER I

INTRODUCTION

The *Dracula* genus of orchids has long intrigued botanists with its peculiar shape and odor, which has led to the hypothesis that it is a fungal mimic (Luer 1978, Kaiser 2000). The lower labellum strongly resembles the pileus of a small white gilled mushroom (Luer 1978, Dentinger and Roy 2010), which attracts drosophilid flies that are often found associated with nearby fungi. These flies move pollinia, the pollen packages of orchids, from flower to flower within populations of *Dracula* orchids and sometimes land on real fungal fruitbodies in the process (Endara et al. 2010). This deceptive mating system is successful at attracting flies, sometimes in large numbers to the *Dracula* blooms, though often in the case of some of the more solitarily blooming species the pollinators are few and far between.

While observing the pollinators, we noticed that the flies appeared to be lapping at the surface of the floral parts, as described by Grimaldi (1987) when observing *Zygothrica* aggregating on mushrooms, and that they continued to exhibit this behavior as they moved deeper into the flower where, occasionally, the flower would attach a pollinia packet to their backs. I hypothesized that the flies were attracted to, and consuming yeasts that were growing on the surface of the flowers. Drosophilid flies are well known to be both dispersants and consumers of yeasts (Starmer and Fogleman 1986) and different species have been shown to prefer largely different groupings of yeasts (Heed et al. 1976). The goals of this study were to determine which yeasts were growing on the flowers and in the guts of the flies. Those of us studying this system have often wondered why sometimes the flowers are very attractive and are swarmed with flies, and

other times they seem not to be at all attractive since there are no visitors. Might the flies be sensitive to the presence of particular yeasts? I therefore also gathered some preliminary data on the attraction of the pollinating flies to one of the yeasts.

Most previous floral yeast work has been centered on nectar-inhabiting yeasts (Sandhu and Waraich 1985, Herrera et al. 2008, Belisle et al. 2012, Peay et al. 2012). However, *Dracula*, like many orchids, do not produce nectar (Endara et al. 2010) and thus do not have the fermenting of sugars to attract a certain set of pollinators (Tremblay et al. 2005). The pollinators that they do attract seem to have a more savory palate, being associated with fresh mushrooms in the fungal-rich moist cloud forest environment that harbors this unique system.

CHAPTER II

METHODS

Field Site- Field work was carried out at Los Cedros Biological Reserve (00°18'31.0"N, 78°46'44.6"W), a 6,900 hectare private reserve located in the province of Imbabura, Ecuador that serves as a buffer to the 300,000 hectare Cotacachi-Cayapas Ecological reserve located to the north of Los Cedros. Los Cedros consists mostly of intact primary montane cloud forest that is typical of the southern end of the Chocó phytogeographical zone, an area considered to be one of the most biologically diverse habitats on earth (Myers et al. 2000). Rainfall is heavy and frequent at this elevation (1250-2200m) on the western slope of the Andes, on average 2.9m per year (data collected from 1995-2009 by station manager Jose DeCoux). Work was carried out between January and March of 2012 during the peak of the rainy season, coinciding with the bloom of *Dracula* orchids as well as an abundant fruiting of co-occurring mushrooms.

Flowers- Flowers were harvested and placed in fishing tackle boxes that had been sterilized by wiping with a 5% sodium hypochlorite solution then left to air dry under the flow of a HEPA filter. Flowers were brought back to the field station and under HEPA laminar flow plated on modified YM Acid Agar. This is a solution of 500ml water, 1.5g yeast extract, 1.5g malt extract, 2.5g bacto tryptone, 5g dextrose, 10g agar and pH adjusted to 4 by the addition of 40 drops of juice from lemons at the field station. Contents were autoclaved, plates were poured and cooled under laminar flow from a HEPA-SEP filter model # STD12-12-12-05PEADC50. Sepals and labella were individually removed from each of the harvested flowers with sterilized forceps, pressed

to the surface of the agar media once, and removed. Co-occurring mushrooms and flowers were also harvested and pressed to the surface of the media in the same manner as the *Dracula* flowers. The plates were then labeled and wrapped with plastic wrap to minimize contamination, then checked daily for fungal growth. As yeasts (indistinct edges to colonies, not hyphal) and molds (hyphal) began to grow, they were transferred as somewhat recognizable single isolates onto fresh dishes under the laminar flow hood. These isolated cultures were allowed to grow until they had covered the plate (approximately a month) and sufficient amounts could be harvested for DNA extraction or behavioral experiments.

DNA Extraction- The matured cultures were transferred with a sterile scalpel to Whatman® FTA® Plant Saver cards and smashed into the fiber matrix of the cards with the blunt force of a hammer as in (Dentinger et al. 2010). The cards were dried rapidly by placing them in an airtight box containing silica desiccant. They were then transported to our laboratory in Eugene, OR where a Harris MicroPunch with a 2mm tip was used to remove sections of encrusted fibers. The punch was cleansed between each sample by punching out three disks from sterile filter paper (Dentinger et al. 2010). The sample punches were placed into 96 well plates and 25 μL of extraction buffer from the Sigma Extract-n-Amp Plant PCR kit was added to each well before a 10 minute incubation in a 95 ^{O}C thermocycler. Following incubation 25 μL of Dilution solution from the Sigma kit was added to the samples. From this a 1:29 dilution with sterile DI water was made.

PCR and Sequencing- PCR reactions were carried out in 10μL reactions with 2μL of the 1:29 diluted sample extract added to 8μL of the following mixture: 1μL of buffer, 1μL of

a 25mM $MgCl_2$ solution, 0.2μL of 10mM dNTPs, 0.2 μL Taq (2.5 units/μL), 0.2 μL of each primer (10 μM), and 5.6 μL sterile water. The primer pair ITS 4 and ITS 5 (White et al. 1990) were used to amplify the ITS region of the ribosomal RNA and the primer pair ITS 1(White et al. 1990) and LR3R (Vilgalys 1990) were used to amplify the D1/D2 region of the 26S region of rRNA.

Thermal cycling was completed on an Applied Biosystems Veriti (model 9902) with the following parameters as in (Dentinger et al. 2010): denaturation at 95 ^{o}C for 2 min, five cycles of denaturation 95 ^{o}C for 30 sec, annealing at 60 ^{o}C for 30sec and extension at 72 ^{o}C for 1min; followed by 25 cycles of denaturation at 95 ^{o}C for 30 sec annealing at 55 ^{o}C for 30 sec and extension at 72 ^{o}C for 1 min; a final extension at 72 ^{o}C for 10 min and indefinate refrigeration at 4 ^{o}C. After visualization of positive PCR products on a 1.5% agarose gel, samples were cleaned with 2.8 μL of a master mix that when prepared for a 96 well plate contained 1.3 μL of Exonuclease I, 26 μL shrimp alkaline phosphatase, and 221 μL water. The solutions were mixed and incubated for 15 minutes at 37^{o}C and then for 15 minutes at 80^{o}C in the thermal cycler. Sequencing was carried out at Functional Biosciences on an ABI 3730xl DNA Sequencer with 50cm arrays.

Sequences were viewed, aligned and edited using the Geneious v5.6.4 software package. Nucleotide collection databases at GenBank were queried with the Basic Local Alignment Search Tool (Altschul SF 1990) to look for named species with DNA sequences matching those obtained for our isolates. A matrix of possible matches was created by ranking the top ten hits each for Pairwise ID, Bitscore, and Query Cover, then comparing the most abundant species from each category. The top scoring species that

was most prevalent amongst these three categories was chosen as the sample's identification. See Appendix A.

Behavioral Assay- Over a period of three days in February of 2012 a series of observations were made with cultured yeasts painted on flower models. Surgical silicone models were formed on site using real *D. felix* flowers by an artist (Melinda Barnadas). These replicas were positioned within 5cm of flowering *D. felix* plants and a thin layer of yeast cultured from nearby flowers (ultimately found to be *Debaryomyces hansenii*) was applied to the lower sepal area of the flower models. Observers recorded all approaches and landings on the model as well as a nearby flower for 30-minute sessions several times per day during the morning dry period that correlates with high fly activity. Observers were rotated among the three stations to reduce observer bias. Fly approaches (defined as obvious orientation towards the flower within 10 cm of the flower) and landings were observed. These data were compared with data from a separate experiment in which attraction to the unscented models was compared to real flowers and a material control.

Statistical Analysis-. To determine whether or not the frequencies of surface dwelling fungi were statistically different on labella versus sepals, we used Fisher's exact test with substrate (labellum or sepal) as the explanatory variable and presence/absence as the response variable). To determine whether there were significant differences in the frequency of the different species on *D. felix* labella, sepals and in flies caught from *D. felix*, we performed contingency table analysis on frequencies using the Likelihood Ratio

Chisquare as the test statistic. To determine whether the pollinator flies visited real flowers and yeast painted model flowers at the same rate, I used ANOVA with number of visits per hour (log transformed) as the dependent variable, and treatment (yeast model or true flower) as the explanatory variable. In a separate experiment I examined the differences in visitation rate among real flowers, model flowers without yeast, and model material control, which was a blob of silicone not in flower form. This experiment was also analyzed with ANOVA.

CHAPTER III

RESULTS

Yeasts and molds were cultured out of several different substrates, including flies, flowers and mushrooms. The yeast species found are listed in Table 1 by presence or absence on a particular substrate. The non-yeast species are listed in Table 2 by presence or absence on a particular substrate. We found 22 species from three different fungal classes; twelve Ascomycota, seven Basidiomycota, and three Zygomycota. Most of the yeasts and molds were found on more than one substrate (Tables 1,2).

A total of seventeen yeast and mold species were found on *D. felix* (Tables 3,4). Since I had samples sizes of >15 from *D. felix* flies, sepals and labella, but small samples sizes (5 or fewer) from the rest of the substrates, I performed statistical analyses only on the *D. felix*-associated surface fungi. Six species inhabited both the labellum of *D. felix* flowers as well as the guts of the insects, and two more species were found both in flies and on *D. felix* sepals (Tables 3, 4). Nearly half (8/20) of the species found on *D. felix* flowers were shared with flies (Tables 3, 4). Only one of the yeast species was found more often on one of the substrates; *Bullera ninhbinhesis* was only found on *D. felix* labella, and not on sepals or in flies. There were significantly fewer species cultured from the sepals than the labellum (Fig. 1; Tables 3, 4). Due to the way I sampled, the maximum number of species recovered from any substrate was one. The communities depended on whether labella or sepals were examined; we recovered surface dwelling fungi more often from labella than from sepals (Fig. 1, 2-tailed Fisher's Exact Test, P=0.0008).

I cultured eleven species of surface dwelling fungi from the sixteen flies captured while visiting *D. felix* (Table 2). Of these, eight were also found on *D. felix* flowers (either on labella or sepals). Only two of the eleven fly-gut associated species were not found on *D. felix* flowers (*Ceriporia lacerata* and *Verticillium fungicola*). Only one mold, *P. corylophilium*, was found on all the *D. felix* substrates.

For comparative purposes, I also cultured yeasts and molds from five co-occurring mushrooms, two *Masdevallia* flowers, one Marantaceae (c.f. *Stromanthe*) flower, and a sepal and labellum from one *D. lafleurii* flower. While these sample sizes were small, they were nonetheless informative (Fig. 1). The additional flowers shared yeasts and molds with those already found on flowers or flies. Two of the species found on the mushrooms, however, were only found on mushrooms: *Hanseniaspore uvarum* and *Phanerocaete sordida*.

Finally, to determine whether or not the yeasts were attractive to the pollinator flies, I performed an experiment in which *Debaryomyces hansenii* was cultured from *D. felix* flowers and then painted on to a silicone model. There was no difference in visitation rates to yeasted model flowers and real flowers (Fig. 2.). A separate experiment performed by T. Policha and B. Roy showed that unyeasted model flowers received significantly fewer visits than real flowers did (Fig. 3.)

Table 1. Presence (1) or absence (0) of yeast species (in alphabetical order) from all substrates (flowers, flies and mushrooms) sampled. Sample size for each substrate is the final line of the table. Presence is highlighted.

Phylum	**Class**	**Species**	**Labellum *D. felix***	**Sepal *D. felix***	**Flies from *D. felix***	**Flies from *D. lafleurii***	***D. lafleuri***	**Mushroom**	***Masdevallia***	**Marantaceae**
Basidiomycota	Tremellomycetes	*Bullera ninhbinhensis*	1	0	0	0	0	0	0	0
Ascomycota	Saccaromycetes	*Candida restingae*	1	0	0	0	0	0	0	0
Ascomycota	Saccaromycetes	*Debaryomyces hansenii*	1	0	1	0	0	1	0	0
Ascomycota	Saccaromycetes	*Hanseniaspora uvarum*	0	0	0	0	0	1	0	0
Basidiomycota	Exobasidiomycetes	*Malassezia restricta*	0	1	0	1	0	0	0	0
Basidiomycota	Exobasidiomycetes	*Malassezia uncultured*	0	0	1	0	0	0	0	0
Basidiomycota	Urediniomycetes	*Rhodotorula mucilaginosa*	1	0	0	0	0	1	1	1
		N	**20**	**20**	**16**	**5**	**2**	**5**	**2**	**1**

Table 2. Presence (1) or absence (0) of non-yeast species (in alphabetical order) from all substrates (flowers, flies and mushrooms) sampled. Sample size for each substrate is the final line of the table. Presence is highlighted.

Phylum	**Class**	**Species**	**Labellum *D. felix***	**Sepal *D. felix***	**Flies from *D. felix***	**Flies from *D. lafleurii***	***D. lafleuri***	**Mushroom**	***Masdevallia***	**Marantaceae**
Ascomycota	Sordariomycetes	*Bionectria ochroleuca*	1	0	1	0	0	0	0	0
Basidiomycota	Basidiomycetes	*Ceriporia lacerata*	0	0	1	0	0	0	0	0
Ascomycota	Sordariomycetes	*Cosmospora consors*	0	1	0	0	0	0	0	0
Basidiomycota	Basidiomycetes	*Grammothele sp.*	1	0	0	1	0	0	0	0
Zygomycota	Trichomycetes	*Mucor fragilis*	1	0	0	0	0	0	0	0
Zygomycota	Trichomycetes	*Mucor nederlandicus*	1	0	1	0	0	0	0	0
Zygomycota	Trichomycetes	*Mucor nidicola*	1	1	0	1	0	0	0	0
Ascomycota	Eurotiomycetes	*Penicillium brocae*	0	1	1	0	0	0	0	0
Ascomycota	Eurotiomycetes	*Penicillium corylophilum*	1	1	1	0	1	0	1	0
Ascomycota	Eurotiomycetes	*Penicillium roqueforti*	0	1	1	1	0	0	0	0
Ascomycota	Eurotiomycetes	*Penicillium sumatrense*	1	0	0	0	0	0	0	0
Basidiomycota	Agaricomycetes	*Phanerocaete sordida*	0	0	0	0	0	1	0	0
Ascomycota	Sordariomycetes	*Pochonia bulbillosa*	1	0	1	0	0	0	0	0
Ascomycota	Sordariomycetes	*Verticillium fungicola*	0	0	1	0	1	0	0	0
Ascomycota	Sordariomycetes	*Volutella ciliata*	1	0	1	0	0	0	0	0
		N	**20**	**20**	**16**	**5**	**2**	**5**	**2**	**1**

Table 3. Frequency and sample size (in parentheses) of only those yeasts found on *Dracula felix* labella and sepals, as well as flies visiting *D. felix*. The table is ordered by shared taxa. Cells with yeasts present are highlighted so the pattern of shared species is obvious. Differences in frequencies across substrates were assessed with log-likelihood analysis. Significant P-values are in bold type.

Species	**%*D. felix* Fly (N)**	**%*D. felix* Labellum (N)**	**%*D. felix* Sepal (N)**	**Likelihood ratio**	**P**
Debaryomyces hansenii	12.5 (2)	5 (1)	0	3.40	0.1827
Malassezia uncultured	6.25 (1)	0	0	2.55	0.2792
Rhodotorula mucilaginosa	0	5 (1)	0	2.09	0.3513
Bullera ninhbinhensis	0	20 (4)	0	8.80	**0.0123**
Candida restingae	0	10 (2)	0	4.25	0.1192
Malassezia restricta	0	0	5 (1)	2.09	0.3513
N	**16**	**20**	**20**		

Table 4. Frequency and sample size (in parentheses) of only those non-yeasts found on *Dracula felix* labella and sepals, as well as flies visiting *D. felix*. The table is ordered by shared taxa. Cells with yeasts present are highlighted so the pattern of shared species is obvious. Differences in frequencies across substrates were assessed with log-likelihood analysis. Significant P-values are in bold type.

Species	**%*D. felix* Fly (N)**	**%*D. felix* Labellum (N)**	**%*D. felix* Sepal (N)**	**Likelihood ratio**	**P**
Bionectria ochroleuca	6.25 (1)	5 (1)	0	1.84	0.3996
Mucor nederlandicus	6.25 (1)	5 (1)	0	1.84	0.3996
Pochonia bulbillosa	6.25 (1)	5 (1)	0	1.84	0.3996
Volutella ciliata	6.25 (1)	5 (1)	0	1.84	0.3996
Penicillium corylophilum	25 (4)	10 (2)	15 (3)	3.26	0.1960
Mucor nidicola	0	5 (1)	5 (1)	1.38	0.5280
Penicillium brocae	6.25 (1)	0	5 (1)	1.84	0.3996
Penicillium roqueforti	6.25 (1)	0	5 (1)	1.84	0.3996
Ceriporia lacerata	12.5 (2)	0	0	5.20	0.0743
Malassezia uncultured	6.25 (1)	0	0	2.55	0.2792
Verticillium fungicola	6.25 (1)	0	0	2.55	0.2792
Grammothele sp.	0	10 (2)	0	4.25	0.1192
Mucor fragilis	0	5 (1)	0	2.09	0.3513
Penicillium sumatrense	0	5 (1)	0	2.09	0.3513
Cosmospora consors	0	0	5 (1)	2.09	0.3513
N	**16**	**20**	**20**		

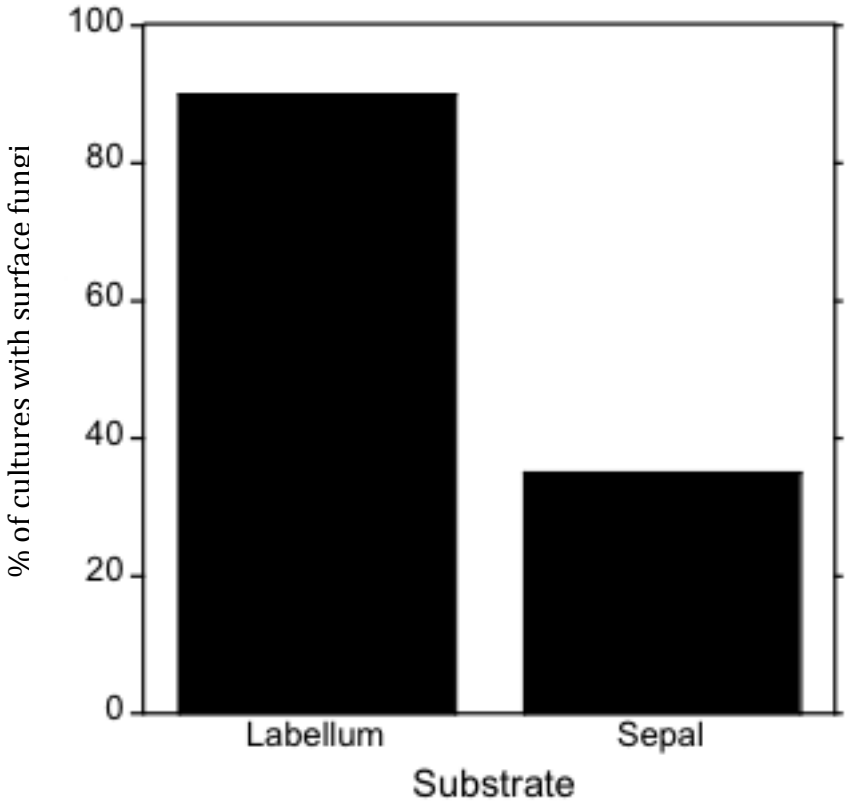
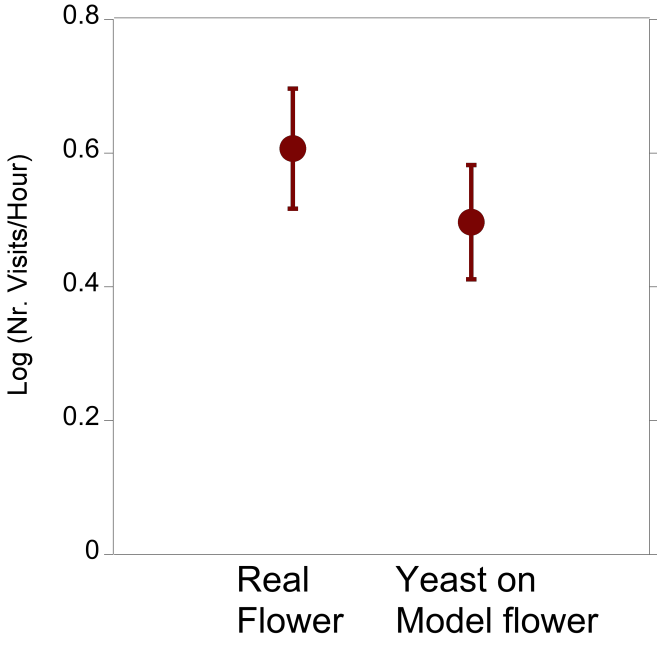
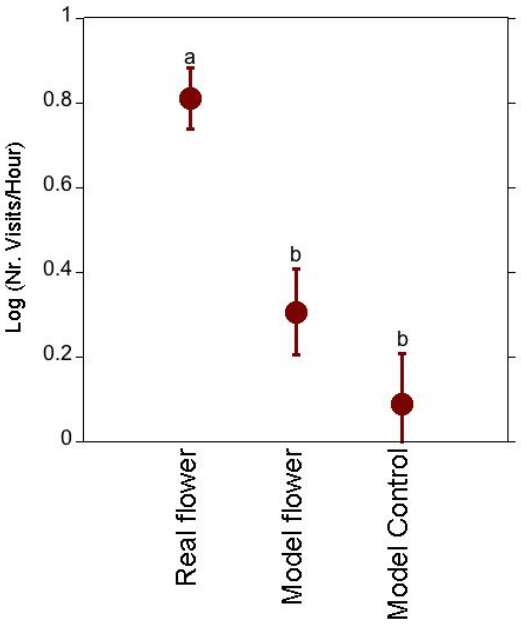


Figure 1. Percent of cultures from each substrate (labellum or sepal) with surface fungi present.



$F= 0.79_{1,79}$ P=0.3769

Figure 2. Yeasted models attracted flies at the same rate as real *Dracula felix*



$F= 11.60_{4,100}$ P<0.0001

Figure 3. Flies were not attracted to unrewarding model flowers (the same model flowers without yeast painted on them) as they were to real *D. felix*.

CHAPTER IV

DISCUSSION

In the behavioral assay I found that when yeasts were present on a silicone flower model, visitation was indistinguishable from that of true flowers. These results suggest that the flies consume yeasts as they feed on what they perceive to be a mushroom and in the process accidentally perform pollination services for the flowers. These yeasts are not nectar inhabiting since the *Dracula* flowers, as with many orchids, are not nectar producing plants (Endara et al. 2010) instead relying on mimicry for visual attraction of pollinators.

How do the yeasts get to the flowers? The yeasts and molds could be brought to the flowers by the initial visitors when the flowers open, and/or they may be carried to the flowers through air currents. The second possibility is less likely as the air is rather still in the dense cloud forest and the flowers have a downward-facing bell shaped form, making airborne inoculation unlikely. The observation that unopened flowers when opened in the laminar flow of the HEPA filter did not yield any cultivable fungi (albeit a small sample size of 5 flowers) suggests that the fungi are introduced to the flowers after they open. This inoculation can be beneficial for the pollination success of the orchids, but it also runs the risk of being detrimental in that pathogenic fungi, once introduced, may shorten the time that the flower is viable.

An earlier study on a different *Dracula* species suggested that the mimicry could be chemical as well as visual, since fragrance compounds known to occur in mushrooms were detected in the flowers (Kaiser 2006). More recently, T. Policha has been analyzing

the *Dracula* species at my field sites and has consistently found four "mushroom" associated volatile compounds: oct-1-en-3-ol, octan-3-one and octan-3-ol, and oct-1-en-3-one. At least one of the fungi that I isolated, *Penicillium roquefortii*, produces the same "mushroom" volatives (Chalier and Crouzet 1993).What is the contribution of yeasts and molds to the floral odors of *D. felix*? My results suggest that the source of the fungal odor could either be the plant, the yeasts, or that the fragrances produced by the plant are supplemented by the odors produced by the yeasts. Using GCMS, T. Policha (pers. comm.) was able to show that the fragrance of the yeast I used in the behavioral assay (*Debaromyces hanseni*) is different than that of the *D. felix* flowers he tested, but we don't know what the contribution of other fungi was to the floral fragrances he has isolated. However, the fact that the initial fungal fragrance work on these species (Kaiser 2006) was done with greenhouse plants suggests that either the floral yeasts are non-specific, or, more likely that the flower is producing the odors. Nonetheless, when fungi colonize these surfaces, they are likely to add to or change the odor “bouquet” in someway.

Yeast mediated pollination is not a new concept (Sandhu and Waraich 1985), although often overlooked in the framework of more traditional concepts of pollinator recruitment such as nectar rewards and mimicry. As yeasts are often ubiquitous in the environment they may add another layer of reward for the pollinator, or may be a goal unto themselves for the pollinator to consume. Plants have even been shown to mimic the volatiles produced by yeasts to entice fly pollinators to visit the blooms (Goodrich et al. 2006, Stokl et al. 2010). In the situation of the *Dracula* orchids the yeasts may be adding a nutritional reward to the already duped scenario that the Drosophilid flies have found

themselves in. The appearance of yeasts on the already putative fungal mimics add a nutritional reward to the system wherein the flies are able to be retained for a longer period of time while lapping at the yeasts, resulting in a greater chance of pollen transfer. My experiment of adding yeast to a scentless flower model suggested that flies were likely to land and spend time on the model than those flies that were simply given a scentless model. Visual attraction plays a significant role in the recruitment of flies to the flowers (Policha and Roy, Pers. Comm.), but without fragrance (yeast or plant made) they are unlikely to remain long enough to pick up or deposit pollinia. The suggestive evidence that they also eat the yeast is likely to increase the time they spend on flowers.

The wide diversity of yeast and mold species found on the flowers as well as the fly guts speaks to the lack of strong specificity that exists in this relationship. These fungi are commonly isolated from environmental substrates (Morais et al. 1995, Fleet 2001, Hsieh et al. 2010, Glushakova et al. 2011, Chi et al. 2012), including flowers (Lachance et al. 2003, Mushtaq et al. 2007, Herrera et al. 2008, Stokl et al. 2010, Pozo et al. 2011, Belisle et al. 2012, Vadkertiova et al. 2012), and were also isolated from other species of flowers in the study area (Table 1). One explanation for the low diversity and the commonness of the species observed could have been my methods; the sampling technique limited the number isolated to a maximum of one per sample, and culturing and isolating could skew the results towards easily cultivatable species. Culturing was chosen due to financial and logistical constraints and could be improved upon in the future by utilizing tape lifts to parse out separate colonies on the flowers, then picking colonies off of the tape to culture in the lab. This would help elucidate the diversity existing in the floral structure instead of the presence/absence data that was gathered in

this study. This community analysis could also be achieved by incorporating next-generation sequencing techniques, although this was prohibitively expensive at the time of the study. Time spent observing flowers and yeasted models could be expanded as well, as it was relatively small due to an order of operations for other workers utilizing these flowers.

In conclusion, floral surface dwelling fungi may have a role in the pollination efficiency of *Dracula felix* through the attraction and retention of flies, providing a "reward" for visiting these putative deceptive mushroom mimics. The flowers contain a broad array of yeasts and molds that may be introduced to the flowers by the pollinating flies or by airborne means. Given the diversity of fungi I uncovered, it is unlikely that the flies are attracted specifically to one species of surface fungi, but are more likely to be more cosmopolitan in their dining habits.

APPENDIX A

YEAST IDENTITIES BASED ON ITS AND D1/D2 SEQUENCES AND BLAST QUERY RESULTS

Sample Name	ITS 4/5 ID	ITS 4/5 Pairwise ID	D1/D2 Pairwise ID	ITS 4/5 Pairwise ID	Dracula sp.	Substrate
Brl 2.4 fly 1	*Grammothele sp.*	96.3	*Polyporaceae sp.*	99.6	Lefleur	Fly
Brl 2.4 fly 2	*Grammothele sp.*	96.3	*Polyporaceae sp.*	98.5	Lefleur	Fly
BRL 2.4.1	*Verticillium fungicola*	100	*Debaryomyces hansenii*	99.6	Lefleur	Sepal
BRL 2.4.1 lab	*Penicillium corylophilum*	99.8	*Penicillium corylophilum*	100	Lefleur	Labellum
Filo Riv 1	*Hanseniaspora uvarum*	99.1	*Hanseniaspora uvarum*	99.3	Filoboletus	Mushroom
Filo riv 2	*Phanerochaete sordida*	99.5	*Phanerochaete sordida*	99.3	Filoboletus	Mushroom
fpg fly 3	*Uncultured Malassezia*	98.7	*Malassezia restricta*	99	Felix	Fly
FPG L3	*Mucor nidicola*	98.7	*Rhizomucor variabilis*	98.8	Felix	Labellum
FPG L5	*Mucor fragilis*	100	*Mucor fragilis*	98.7	Felix	Labellum
FPG s6	*Cosmospora consors*	99.3	*Cosmospora consors*	100	Felix	Sepal
FPGS5	*Cosmospora consors*	99	*Cosmospora consors*	100	Felix	Sepal
I 10 F fly 1	*Penicillium corylophilum*	100	*Penicillium corylophilum*	100	Felix	Fly
I 10 Flfy2	*Debaryomyces hansenii*	93.9	*Debaryomyces hansenii*	99.8	Felix	Fly
I 10 fly 1	*Penicillium corylophilum*	100	*Penicillium corylophilum*	100	Felix	Fly
I 10 Lab	*Volutella ciliata*	99	*Volutella ciliata*	99	Felix	Labellum

I 26 Lab	*Penicillium sumatrense*	100	*Penicillium chrysogenum*	100	Felix	Labellum
I 34.1 fly 1	*Ceriporia lacerata*	98.9	*Penicilium atramentosum*	99.8	Felix	Fly
I 34.1 lab	*Bullera ninhbinhensis*	92.6	*Bullera ninhbinhensis*	92.2	Felix	Labellum
I 7 lab	*Candida restingae*	96.4	*Candida restingae*	92.6	Felix	Labellum
I10 S	*Penicillium brocae*	100	*Penicillium brocae*	96.4	Felix	Sepal
I34.1Lab	*Rhodotorula mucilaginosa*	99.8	*Rhodotorula mucilaginosa*	100	Felix	Labellum
Marant Fl1	*Rhodotorula mucilaginosa*	99.8	*Rhodotorula mucilaginosa*	96.3	Marantaceae	Sepal
Mas RG 1.4	*Rhodotorula mucilaginosa*	99.8	*Rhodotorula mucilaginosa*	96.3	Masdevallia	Sepal
Mas RG 1.4	*Penicillium corylophilum*	100	*Penicilium toxicarium*	100	Masdevallia	Sepal
Oso 12.1 lab 2	*Bionectria ochroleuca*	99.8	*Candida sp.*	100	Felix	Labellum
Oso 12.1 lab 3	*Mucor nederlandicus*	96.8	*Debaryomyces hansenii*	99.8	Felix	Labellum
Oso 12.1L	*Debaryomyces hansenii*	100	*Debaryomyces hansenii*	99.8	Felix	Labellum
Oso 18.12.1 lab	*Candida restingae*	97.4	*Candida sp.*	96.8	Felix	Labellum
Oso 18.12.2 lab	*Bullera ninhbinhensis*	92.7	*Bullera ninhbinhensis*	98.9	Felix	Labellum
Oso 18.12.3 lab	*Bullera ninhbinhensis*	92.8	*Bullera ninhbinhensis*	100	Felix	Labellum
Oso 18.14 lab	*Bullera ninhbinhensis*	92.2	*Bullera ninhbinhensis*	98.7	Felix	Labellum
Oso 48.1 lab	*Pochonia bulbillosa*	99.8	*Pochonia bulbillosa*	100	Felix	Labellum
Oso 51.1 F Fly 2	*Penicillium corylophilum*	100	*Penicillium corylophilum*	98.9	Felix	Fly
oso 51.1 F Fly 3	*Bionectria ochroleuca*	99.8	*Bionectria ochroleuca*	96.9	Felix	Fly

Oso 51.1 Ffly 1	*Penicillium corylophilum*	100	*Penicillium corylophilum*	100	Felix	Fly
Oso 51.1 Fly 4	*Penicillium roquefortii*	99.9	*Penicillium roquefortii*	99.8	Felix	Fly
Oso 51.1 lab 1	*Grammothele sp.*	96.3	*Grammothele sp.*	99.9	Felix	Labellum
Oso 51.1 lab 2	*Grammothele sp.*	96.3	*Geotrichum carabidarum*	100	Felix	Labellum
Oso 51.1 lab 3	*Penicillium corylophilum*	100	*Candida intermedia*	96.3	Felix	Labellum
Oso 51.1 S1	*Penicillium corylophilum*	100	*Penicillium corylophilum*	96.3	Felix	Sepal
Oso 51.1 S2	*Penicillium roqueforti*	100	*Debaryomyces hansenii*	100	Felix	Sepal
Oso F com gar	*Penicillium corylophilum*	100	*Penicillium corylophilum*	93.9	Felix	Sepal
PG FF 10	*Verticillium fungicola*	100	*Lecanicillium fusisporum*	97.4	Felix	Fly
pg FF 9	*Volutella ciliata*	99	*Dactylaria longispora*	92.7	Felix	Fly
PG FF1	*Debaryomyces hansenii*	100	*Debaryomyces hansenii*	92.8	Felix	Fly
PG FF8	*Penicillium brocae*	99.6	*Penicillium brocae*	99.8	Felix	Fly
PG LF 2	*Penicillium roquefortii*	99.6	*Penicillium roquefortii*	99.6	Lefleur	Fly
PG LF 4	*Mucor nidicola*	98.5	*Mucor nidicola*	99	Lefleur	Fly
Pg LF 7	*Malassezia restricta*	99.6	*Malassezia restricta*	100	Lefleur	Fly
pgf fly 5	*Ceriporia lacerata*	98.9	*Ceriporia lacerata*	99.6	Felix	Fly
PGF fly 6	*Mucor nederlandicus*	96.9	*Mucor ellipsoideus*	99.8	Felix	Fly
PGF S3	*Malassezia restricta*	99.3	*Malassezia restricta*	99.8	Felix	Sepal
Plut Riv CG 4	*Rhodotorula mucilaginosa*	99.8	*Rhodotorula mucilaginosa*	99.8	Pluteus	Mushroom

Plut RV CG 3	*Debaryomyces hansenii*	99.8	*Debaryomyces hansenii*	100	Pluteus	Mushroom
Pluteus River CG 1	*Rhodotorula mucilaginosa*	99.6	*Rhodotorula mucilaginosa*	99.8	Pluteus	Mushroom
Poo gar F Fly 7	*Pochonia bulbillosa*	99.8	*Pochonia bulbilosa*	99.1	Felix	Fly
Poo gar sepal F1	*Mucor nidicola*	99.3	*Rhizomucor variabilis*	99.5	Felix	Sepal

APPENDIX B

FUNGI FROM LEAVES AND ROOTS OF *DRACULA* ORCHIDS

Introduction

All orchids are mycoheterophillic organisms and are completely dependent on resident fungal hyphae to obtain the nutrients needed for germination on their substrate. Some maintain a connection to these fungi as they mature into photosynthetic plants and these become either mycorrhizal partners with the plant, endophytic inhabitants of their tissue, or pathogenic saprobes. I set out to determine what fungi might be associating with the *Dracula* orchids both in their roots and in their leaves and how that may shift amongst species of these orchids that are reasonably restricted to certain microhabitats. Roots and leaves from three separate species, *D. lefleur, D. felix, D. pubescens* representing low, mid, and high elevation respectively were cultured under sterile conditions to isolate fungi that were inhabiting the plant's tissues.

Root methods- Two roots of each orchid were cross-sectioned to make a disk of approximately 0.5mm in thickness. Several disks were immersed in analine blue for 1 minute, then taken out of the dye, excess dye was absorbed with Kimwipes and the root disks were immersed in DI water to wash off any excess dye. Slides were prepared with this material and viewed under a 60X light microscope for fungal pelotons. After recognizing the root surface patterns that often correlated with peloton presence three 2cm sections of roots that appeared to harbor infection were sliced, velamen removed and surface sterilized by 1 minute immersion in 2% sodium hypochlorite followed by 1

minute immersion in 95% ethanol followed by two consecutive baths in autoclaved water. These roots were allowed to dry on autoclaved filter paper under the clean flow of air from a HEPA filter and then plated on 2% malt agar. Plates were labeled and wrapped with plastic wrap to minimize contamination, then checked daily for fungal growth. As fungi began to emerge, they were transferred onto fresh dishes under the laminar flow hood for isolated growth. These isolated cultures were allowed to grow for several weeks to a month until they had covered the plate and sufficient amounts could be harvested for DNA extraction.

Leaf methods- Three 1cm diameter punches of *Dracula* leaves were taken and surface sterilized by 1 minute immersion in 2% sodium hypochlorite followed by 1 minute immersion in 95% ethanol followed by two consecutive baths in autoclaved water. These leaf punches were allowed to dry on autoclaved filter paper under the clean flow of air from a HEPA filter and then plated on 2% malt agar. Plates were labeled and wrapped with plastic wrap to minimize contamination, then checked daily for fungal growth. As fungi began to emerge, they were transferred onto fresh dishes under the laminar flow hood for isolated growth. These isolated cultures were allowed to grow for several weeks to a month until they had covered the plate and sufficient amounts could be harvested for DNA extraction.

DNA extraction- The matured cultures were unwrapped and the mycelium scraped with a sterile scalpel, then transferred to Whatman FTA Plant Saver cards and smashed into the fiber matrix of the cards with the blunt force of a hammer as in Dentinger (2009). These cards were immediately dried by placing them in an airtight box containing silica desiccant for several days. They were then transported to our laboratory in Eugene, OR

where a Harris MicroPunch with a 2mm tip was used to remove sections of encrusted fibers. The punch was cleansed between each sample by punching out three disks from sterile filter paper. The sample punches were placed into 96 well plates and 25 μL of extraction buffer from the Sigma Extract-n-Amp Plant PCR kit was added to each well before a 10 minute incubation in a 95 ^{O}C thermocycler. Following incubation 25 μL of Dilution solution from the Sigma kit was added to the samples. From this a 1:29 dilution with sterile DI water was made.

PCR and sequencing- PCR reactions were carried out in 10μL reactions with 2μL of the 1:29 diluted sample extract added to 8μL of the following mixture: 1μL of buffer, 1μL of a 25mM $MgCl_2$ solution, 0.2μL of 10mM dNTPs, 0.2 μL Taq (2.5 units/μL), 0.2 μL of each primer (10 μM), and 5.6 μL water. Primers pairs used for these reactions were the ITS 1-F and ITS 4.

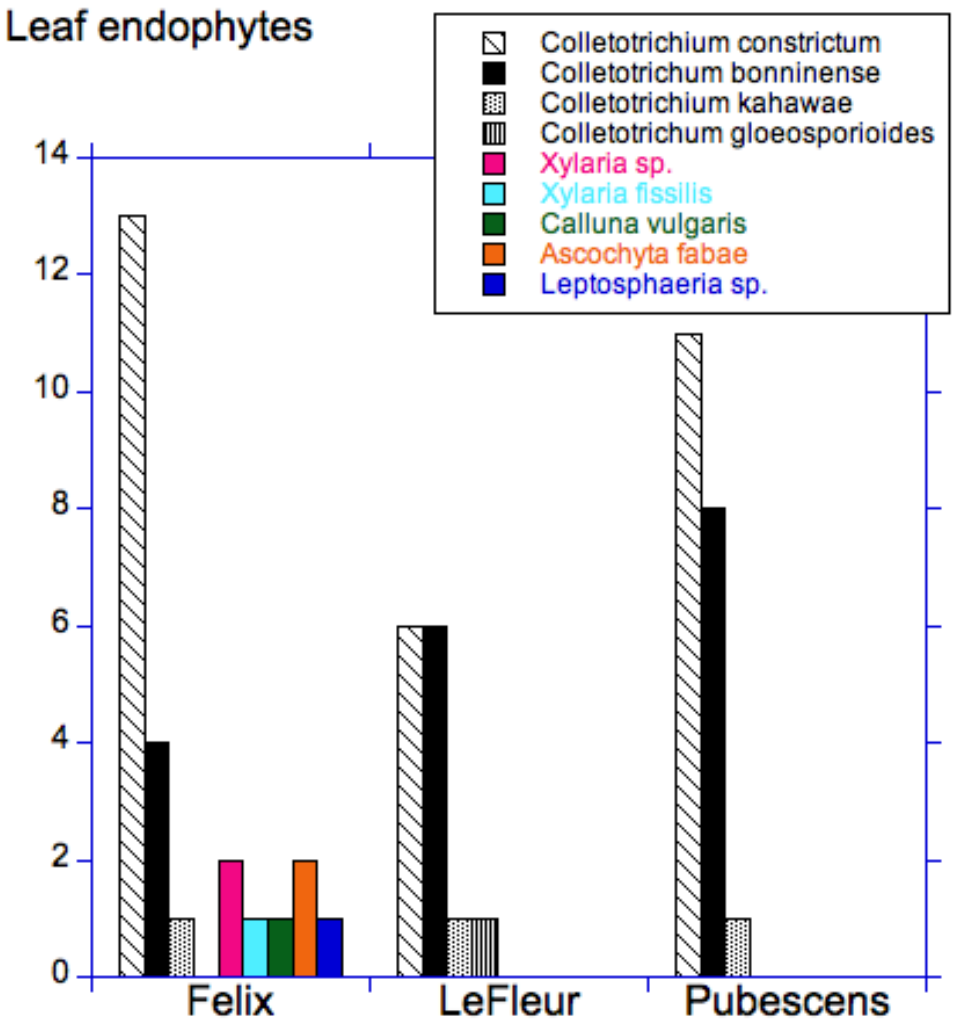
Thermal cycling was completed on an Applied Biosystems Veriti (model 9902) with the following parameters as in Dentinger *et al.* (2009): denaturation at 95 ^{o}C for 2 min, five cycles of denaturation 95 ^{o}C for 30 sec, annealing at 60 ^{o}C for 30sec and extension at 72 ^{o}C for 1min; followed by 25 cycles of denaturation at 95 ^{o}C for 30 sec annealing at 55 ^{o}C for 30 sec and extension at 72 ^{o}C for 1 min; a final extension at 72 ^{o}C for 10 min and indefinate refrigeration at 4 ^{o}C. After visualization of positive PCR products on a 1.5% agarose gel, samples were cleaned with 2.8 μL of a master mix that when prepared for a 96 well plate contained 1.3 μL of Exonuclease I, 26 μL shrimp alkaline phosphatase, and 221 μL water. The solutions were mixed and incubated for 15 minutes at 37^{o}C and then

for 15 minutes at 80^{o}C in the thermal cycler. Sequencing was carried out at Functional Biosciences on an ABI 3730xl DNA Sequencer with 50cm arrays.

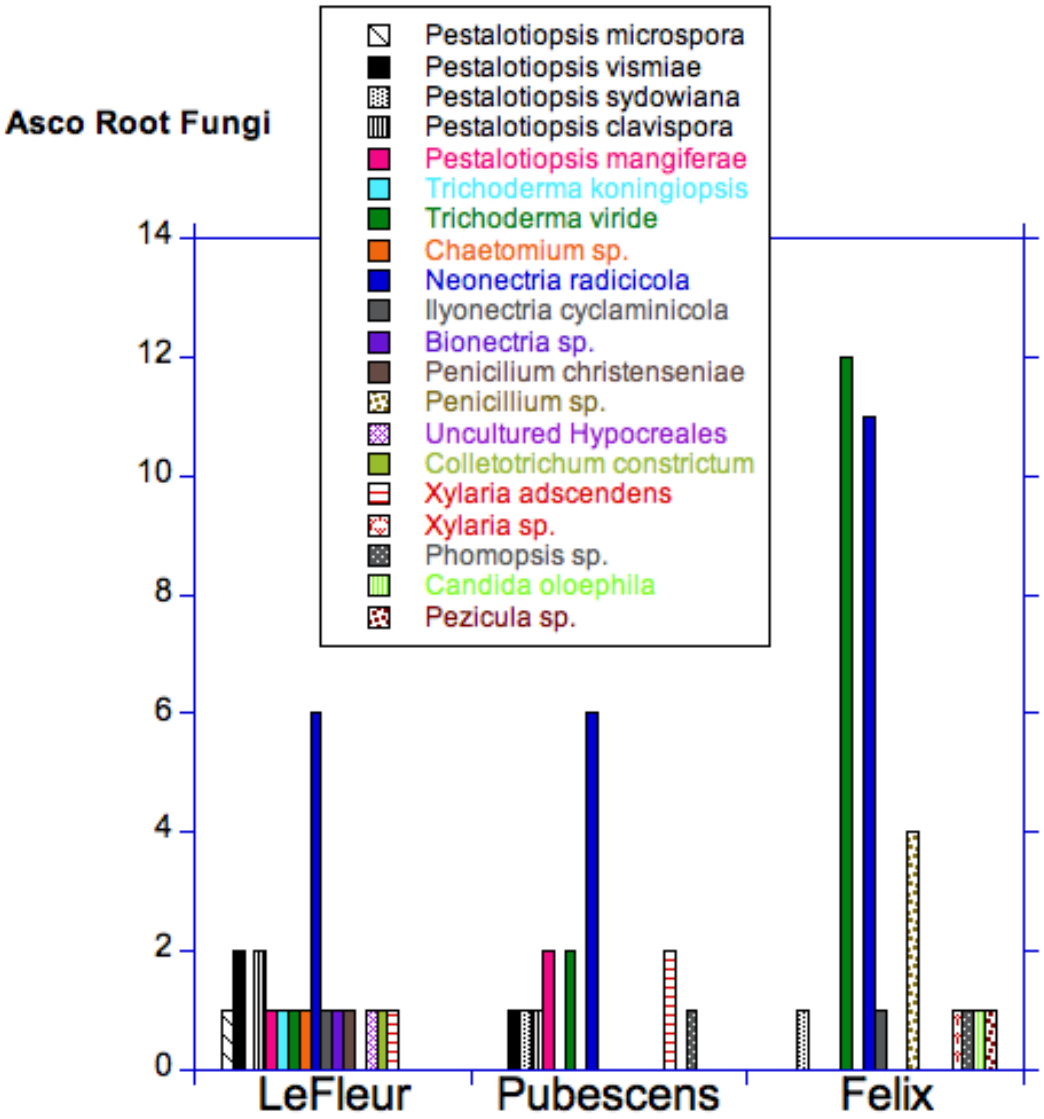
Sequences were viewed, edited and searched against the BLAST database using the Geneious v5.6.4 software package. A matrix of possible matches was created by ranking the top ten hits each for Pairwise ID, Bitscore, and Query Cover, then comparing the most abundant species from each category. The top scoring species that was most prevalent amongst these three categories was chosen as the sample's identification.

Results

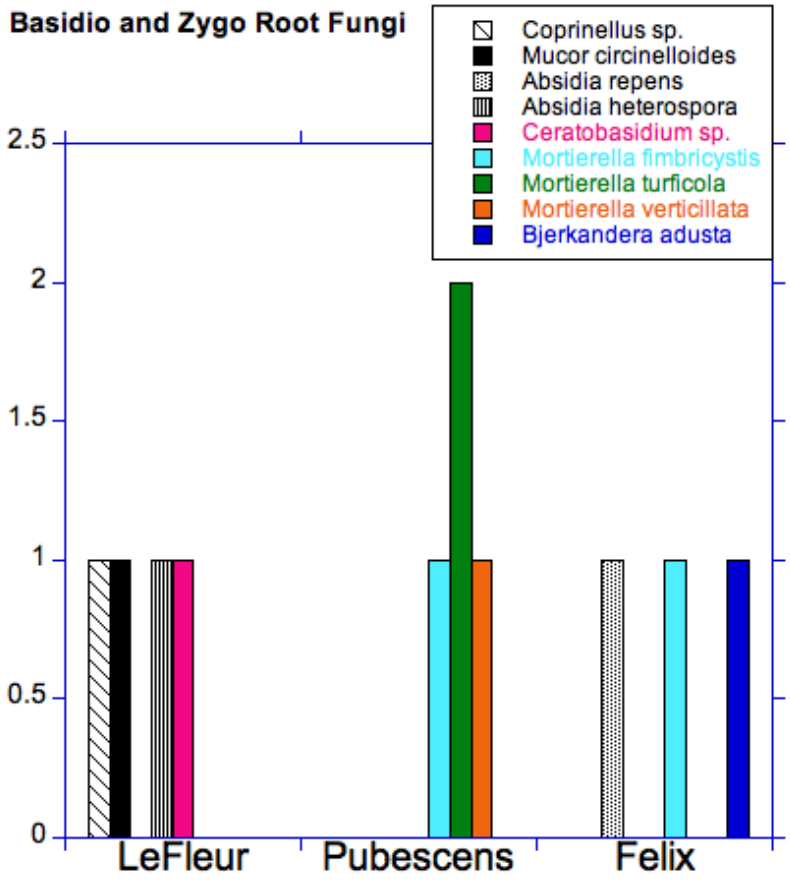
Figure B1 shows the endophytic fungi. Within leaves the endophytic fungi in the genus *Colletotrichium* is ubiquitously distributed throughout the landscape and was the dominant group of fungi found. The roots, however , contain a wider range of inhabitants, most of which are pathogenic and decomposer fungi. Figure B2 illustrates the frequency of various ascomycetes in the roots and Figure B3 shows the basiomycetes and zyogomycetes. Few true mycorrrhizal associates were found.



Appendix B Figure B1. Frequency of leaf endophytes amongst the three species of *Dracula* orchids



Appendix B Figure B2. Frequency of Ascomycetous root associating fungi amongst the three species of *Dracula.*



Appendix B Figure B3. Frequency of Basidiomycetous and Zygomycetous root associating fungi in the three *Dracula* species

Appendix B Table B1. Endophytic fungi isolated from the leaves.

Sample #	Organism / Pairwise ID	Hits-ID	Pairwise ID	Accession # ID	Organism / Bit score	Hits-bit	Bit score	Accession # Bit	Organism / Query cover	Hits-query	Query Cover	Accession #Query	Dracula sp.
B 1.3 .1	*Colletotrichium kahawae*	4	99.8	JX01 0229	*Colletotrichum gloeosporioides*	2	980.5 16	AJ30 1907	*Colletotrichum gloeosporioides*	3	100	JX25 8693	Lefl eur
B 1.3 .3	*Colletotrichum boninense*	5	98.1	JX01 0292	*Fungal endophyte*	3	1058. 06	HM5 3704 4	*Colletotrichum gloeosporioides*	1	99.84	AJ30 1974	Lefl eur
B 16. 37. 1	*Colletotrichium constrictum*	1	99	JQ00 5238	*Colletotrichum sp.*	1	940.8 41	AJ30 1939	*Fungal endophyte*	3	99.65	IM537 33	Lefl eur
B 16. 37. 2	*Colletotrichium constrictum*	1	98.5	JQ00 5238	*Colletotrichum boninense*	4	944.4 48	JX01 0292	*Colletotrichum boninense*	8	100	JQ93 6175	Lefl eur
B 2.2 .1	*Colletotrichum boninense*	5	100	JX25 8799	*Fungal endophyte*	3	1113. 96	HM5 3704 4	*Colletotrichum boninense*	6	99.84	EU8 2280 2	Lefl eur
B 2.2 .2	*Colletotrichium constrictum*	1	98.7	JQ00 5238	*Fungal endophyte*	3	1041. 83	HM5 3704 4	*Colletotrichum gloeosporioides*	1	99.84	AJ30 1974	Lefl eur
B 2.2 .3	*Colletotrichum boninense*	4	100	JX25 8799	*Fungal endophyte*	1	1090. 52	HM5 3704 4	*Fungal endophyte*	2	99.67	HM5 3704 4	Lefl eur
B 2.4 .1	*Penicilium christenseniae*	1	100	JN61 7674	*Penicilliu m manginii*	1	762.3 08	JN90 3566	*Penicilliu m manginii*	1	100	JN90 3566	Lefl eur
B 2.4 .2	*Colletotrichum boninense*	10	100	FN56 6869	*Fungal endophyte*	3	1108. 55	HM5 3704 4	*Colletotrichum boninense*	5	99.52	EU8 2280 3	Lefl eur
B 2.6 .2	*Colletotrichium constrictum*	1	98.7	JQ00 5238	*Fungal endophyte*	2	955.2 68	HM5 3704 4	*Colletotrichum boninense*	5	99.83	EU8 2280 3	Lefl eur

B 2.7 .1	*Colletotrichium constrictum*	1	98.7	JQ005238	*Colletotrichum boninense*	4	1023.8	JX010292	*Colletotrichum boninense*	5	99.67	JX258700	Lefleur
B 5.1 .1	*Colletotrichium constrictum*	1	99	JQ005238	*Colletotrichum boninense*	3	931.825	JX010292	*Colletotrichum boninense*	6	99.82	DQ286170	Lefleur
B 5.1 .2	*Colletotrichum bonninense*	3	100	JX624301	*Colletotrichum boninense*	4	1005.76	JX010292	*Colletotrichum boninense*	5	100	EU822802	Lefleur
B 5.1 .3	*Colletotrichum bonninense*	7	100	JX624301	*Fungal endophyte*	1	1002.16	HM537044	*Colletotrichum boninense*	7	99.82	JX258675	Lefleur
B 5.1 .3	*Colletotrichum gloeosporioides*	4	100	GU066671	*Colletotrichum gloeosporioides*	4	1000.35	JN715837	*Colletotrichum gloeosporioides*	2	99.8	GU066671	Lefleur
C 4.1 3.1	*Colletotrichum bonninense*	6	97.6	JX010292	*Fungal endophyte*	3	1052.65	HM537044	*Fungal endophyte*	3	99.84	HM537033	Pubescens
C 4.1 3.2	*Colletotrichum bonninense*	7	99	JX624301	*Colletotrichum boninense*	2	1013.34	AJ301939	*Colletotrichum boninense*	7	100	AJ301939	Pubescens
C 4.1 3.3	*Colletotrichium constrictum*	6	98.6	JQ005238	*Colletotrichium constrictum*	3	936.185	JQ005238	*Colletotrichium constrictum*	3	99.84	JQ005238	Pubescens
C 4.2 0.1	*Colletotrichum bonninense*	9	99.8	EU482210	*Colletotrichum sp.*	4	985.926	AJ301939	*Colletotrichum boninense*	8	100	JX258768	Pubescens
C 4.2 0.2	*Colletotrichium constrictum*	1	98.7	JQ005238	*Colletotrichum boninense*	4	1032.81	JX010292	*Colletotrichum boninense*	3	100	EU822801	Pubescens
C 4.2 1.1	*Colletotrichum bonninense*	4	97.6	JX010292	*Fungal endophyte*	2	1040.03	HM537044	*Colletotrichum boninense*	6	100	JX258729	Pubescens
C 4.2 1.2	*Colletotrichium constrictum*	1	98.7	JQ005238	*Colletotrichum boninense*	6	1027.4	JX010292	*Colletotrichum gloeosporioides*	1	99.67	AJ301974	Pubescens
C 4.2	*Colletotrichium*	2	98.7	JQ00	*Colletotrichium*	3	1045.	JQ00	*Colletotrichium*	4	99.84	JQ00	Pubesce

2.1	*constrictum*			5238	*constrictum*		458	5238	*constrictum*			5238	ns
C 4.2 2.2	*Colletotrichium constrictum*	3	98.7	JQ005238	*Colletotrichium constrictum*	4	936.293	JQ005238	*Colletotrichium constrictum*	5	100	JQ005238	Pubescens
C 4.2 2.3	*Colletotrichium constrictum*	1	98.7	JQ005238	*Colletotrichium constrictum*	2	934.223	JQ005238	*Colletotrichium constrictum*	3	100	JQ005238	Pubescens
C 4.3 .1	*Colletotrichium constrictum*	7	98.4	JQ005238	*Colletotrichium constrictum*	4	945.856	JQ005238	*Colletotrichium constrictum*	4	99.52	JQ005238	Pubescens
C 4.4 .1	*Colletotrichum bonninense*	3	100	JX624301	*Colletotrichum boninense*	1	993.139	AJ301939	*Colletotrichum boninense*	3	99.6	AJ301939	Pubescens
C 4.4 .2	*Colletotrichum bonninense*	4	99.8	JX624301	*Colletotrichum boninense*	5	980.516	AJ301939	*Colletotrichum boninense*	2	99.8	AJ301939	Pubescens
C 4.5 .1	*Colletotrichium kahawae*	4	99.9	JX010229	*Colletotrichum gloeosporioides*	2	993.139	AJ301907	*Colletotrichum gloeosporioides*	2	99.8	JX258693	Pubescens
C 4.5 .2	*Colletotrichum bonninense*	1	99.6	JX258799	*Colletotrichum sp.*	6	996.746	AJ301939	*Colletotrichum boninense*	7	100	JQ936175	Pubescens
C 4.6 .1	*Colletotrichium constrictum*	1	98.7	JQ005238	*Colletotrichum gloeosporioides*	2	1023.8	JX258743	*Colletotrichum boninense*	6	99.67	JX258700	Pubescens
C 4.6 .2	*Colletotrichum bonninense*	4	99.5	JX010292	*Colletotrichum boninense*	7	1034.62	EU822802	*Colletotrichum gloeosporioides*	2	100	AJ301974	Pubescens
C 5.1 .1	*Colletotrichium constrictum*	1	98.6	JQ005238	*Colletotrichum boninense*	5	1021.99	JX010292	*Colletotrichum sp.*	1	99.67	FJ466723	Pubescens
C 5.1 .2	*Colletotrichium constrictum*	1	98.6	JQ005238	*Fungal endophyte*	2	1031.01	HM537044	*Fungal endophyte*	2	99.52	HM537031	Pubescens
C 5.1 .3	*Colletotrichium constric*	1	98.6	JQ005238	*Fungal endophyte*	2	1031.01	HM537044	*Colletotrichum gloeospori*	1	99.52	AJ301974	Pubescens

	tum								*oides*				
I 13.1	*Leptosphaeria sp.*	2	100	EN38476 2	*Leptosphaeria sp.*	4	1008.23	EN38476 2	*Leptosphaeria sp.*	3	98.1	EN38476 2	Felix
I 21.1	*Ascochyta fabae*	1	99.1	PB004378	*Ascochyta fabae*	4	1031.78	PB004378	*Ascochyta fabae*	1	98.6	PB004378	Felix
I 24.1	*Ascochyta fabae*	2	99.5	PB004378	*Ascochyta fabae*	3	944.38	PB004380	*Ascochyta fabae*	1	99.3	PB004378	Felix
I 26.1	*Colletotrichium constrictum*	6	100	JQ005238	*Colletotrichium constrictum*	5	923.345	JQ005238	*Colletotrichium constrictum*	4	98.6	JQ005238	Felix
I 26.2	*Colletotrichium constrictum*	6	98.6	JQ005238	*Colletotrichium constrictum*	2	1039.5	JQ005238	*Colletotrichium constrictum*	6	99.6	JQ005238	Felix
I 31.1	*Colletotrichium constrictum*	1	98.7	JQ005238	*Colletotrichium constrictum*	6	1035.45	JQ005238	*Colletotrichium constrictum*	4	99.82	JQ005238	Felix
I 31.1.1	*Colletotrichium constrictum*	1	98.7	JQ005238	*Colletotrichum sp.*	1	955.268	AJ301939	*Colletotrichum sp.*	1	99.83	AJ301939	Felix
I 31.1.2	*Colletotrichium constrictum*	1	98.7	JQ005238	*Colletotrichum boninense*	6	1027.4	JX010292	*Colletotrichum boninense*	6	99.67	JX010292	Felix
I 31.2	*Colletotrichium constrictum*	3	98.7	JQ005238	*Colletotrichium constrictum*	6	986.496	JQ005238	*Colletotrichium constrictum*	2	100	JQ005238	Felix
I 34.1	*Colletotrichium constrictum*	2	98.5	JQ005238	*Colletotrichium constrictum*	5	978.345	JQ005238	*Colletotrichium constrictum*	1	99.67	JQ005238	Felix
I 34.2	*Xylaria sp.*	5	90	TF398642	*Xylaria sp.*	1	931.825	TF398642	*Xylaria sp.*	6	99.8	TF398642	Felix
I 40.1	*Colletotrichium constrictum*	8	99.7	JQ005238	*Colletotrichium constrictum*	4	980.567	JQ005238	*Colletotrichium constrictum*	2	100	JQ005238	Felix
I 9.2	*Ascochyta fabae*	2	99.5	PB004378	*Ascochyta fabae*	5	1028.85	PB004379	*Ascochyta fabae*	3	99.6	PB004378	Felix

O 1.1	*Xylaria fissilis*	3	98	TF39 7612	*Xylaria sp.*	3	940.8 41	TF39 7612	*Xylaria fissilis*	3	99.6	TF39 7612	Felix
O 18. 10. 1	*Colletot richium constric tum*	7	98.6	JQ00 5238	*Colletotri chium constrictu m*	1	993.6 52	JQ00 5238	*Colletotri chium constrictu m*	1	100	JQ00 5238	Felix
O 18. 10. 2	*Colletot richum bonnine nse*	2	98.1	JX62 4301	*Colletotri chum boninense*	3	1058. 06	AJ30 1941	*Colletotri chum boninense*	4	98.6	AJ30 1941	Felix
O 18. 12. 2	*Colletot richum bonnine nse*	1	98.6	JX62 4301	*Colletotri chum boninense*	3	1108. 55	AJ30 1941	*Colletotri chum boninense*	1	99	AJ30 1941	Felix
O 18. 12. 3	*Xylaria sp.*	3	98.7	DS27 6591	*Xylaria sp.*	2	945.8 56	DS27 6591	*Xylaria sp.*	4	100	DS27 6591	Felix
O 18. 13	*Colletot richium constric tum*	7	98.7	JQ00 5238	*Colletotri chium constrictu m*	1	1039. 35	JQ00 5238	*Colletotri chium constrictu m*	4	100	JQ00 5238	Felix
O 18. 14. 1	*Colletot richium constric tum*	5	98.6	JQ00 5238	*Colletotri chium constrictu m*	2	1036. 352	JQ00 5238	*Colletotri chium constrictu m*	3	100	JQ00 5238	Felix
O 18. 2.1	*Colletot richum bonnine nse*	1	100	JX62 4301	*Colletotri chum boninense*	2	1113. 96	AJ30 1941	*Colletotri chum boninense*	4	99.8	AJ30 1941	Felix
O 18. 2.2	*Colletot richium kahawa e*	5	100	JX01 0229	*Fungal endophyte*	3	1013. 34	JX01 0229	*Colletotri chium kahawae*	2	100	JX01 0229	Felix
O 18. 9.1	*Colletot richum bonnine nse*	4	99.8	JX62 4301	*Colletotri chum boninense*	3	1090. 52	AJ30 1941	*Colletotri chum boninense*	6	100	AJ30 1941	Felix
O 21. 1.1	*Colletot richium constric tum*	3	98.4	JQ00 5238	*Colletotri chium constrictu m*	5	983.3 56	JQ00 5238	*Colletotri chium constrictu m*	6	99.8	JQ00 5238	Felix
O 3.1	*Colletot richium constric tum*	6	98.7	JQ00 5238	*Colletotri chium constrictu m*	2	1034. 67	JQ00 5238	*Colletotri chium constrictu m*	3	99.8	JQ00 5238	Felix

Appendix B Table B2. Fungi isolated from *Dracula* orchid roots.

Sample Name	**Dracula sp.**	**Organism-Pairwise**	**Hits**	**Pairwise ID**	***Organism-Bitscore***	**Hits**	**Bit score**	***Organism-Query cover***	**Hits**	**Query Cover**	**Primer**	**Primer**
B 1.3.1	Lefleur	Hypocrea rufa	7	100.00%	*Hypocrea rufa*	4	936.185	*Hypocrea rufa*	6	98.10 %	ITS1	ITS4
B 1.3.2	Lefleur	Pestalotiopsis microspora	6	99.80%	*Pestalotiopsis microspora*	2	1021.99	*Pestalotiopsis microspora*	2	99.20 %	ITS1	ITS4
B 1.3.3	Lefleur	Pestalotiopsis vismiae	4	99.70%	*Pestalotiopsis vismiae*	4	1008.23	*Pestalotiopsis vismiae*	1	98.00 %	ITS1	ITS4
B 1.3.4	Lefleur	Pestalotiopsis vismiae	1	100.00%	*Pestalotiopsis vismiae*	1	1031.78	*Pestalotiopsis vismiae*	6	98.60 %	ITS1	ITS4
B 1.3.5	Lefleur	Pestalotiopsis clavispora	1	99.80%	*Pestalotiopsis clavispora*	3	993.139	*Pestalotiopsis clavispora*	3	100.00 %	ITS1	ITS4
B 1.3.6	Lefleur	Trichoderma koningiopsis	6	99.10%	*Trichoderma koningiopsis*	6	978.345	*Trichoderma koningiopsis*	5	99.30 %	ITS1	ITS4
B 2.2.2	Lefleur	Chaetomium sp.	5	98.00%	*Chaetomium sp.*	1	1108.55	*Chaetomium sp.*	5	98.90 %	ITS1F	ITS4
B 2.6.2	Lefleur	Neonectria radicicola	3	98.60%	*Neonectria radicicola*	5	940.841	*Neonectria radicicola*	3	99.70 %	ITS1	ITS4
B 2.6.3	Lefleur	Uncultured Ceratobasidiaceae	1	93.50%	*Uncultured Ceratobasidiaceae*	5	1013.34	*Uncultured Ceratobasidiaceae*	5	100.00 %	ITS1	ITS4
B 2.6.4	Lefleur	Ilyonectria cyclaminicola	7	99.80%	*Ilyonectria cyclaminicola*	7	1027.4	*Ilyonectria cyclaminicola*	2	100.00 %	ITS1	ITS4
B 2.6.5	Lefleur	Neonectria	5	100.00%	*Neonectria radicicola*	5	1036.352	*Neonectria*	1	100.00 %	ITS	ITS

		radicicola						*radicicola*			1	4
B 2.7.1	Lefleur	Bionectria sp.	6	97.10%	*Bionectria sp.*	4	944.448	*Bionectria sp.*	4	99.80 %	ITS1	ITS4
B 2.7.3	Lefleur	Penicilium christenseniae	1	100.00%	*Penicilium christenseniae*	3	1047.23	*Penicilium christenseniae*	3	98.70 %	ITS1F	ITS4
B 3.1.4	Lefleur	Neonectria radicicola	2	98.80%	*Neonectria radicicola*	2	1058.06	*Neonectria radicicola*	2	98.60 %	ITS1	ITS4
B 3.1.5	Lefleur	Neonectria radicicola	3	99.40%	*Neonectria radicicola*	6	945.856	*Neonectria radicicola*	5	100.00 %	ITS1	ITS4
B 3.1.6	Lefleur	Neonectria radicicola	2	99.40%	*Neonectria radicicola*	4	1028.85	*Neonectria radicicola*	5	99.80 %	ITS1	ITS4
B1.2	Lefleur	Unculture d Hypocreales	5	99.60%	*Uncultured Hypocreales*	6	1090.52	*Unculture d Hypocreales*	3	100.00 %	ITS1	ITS4
B2.2.1	Lefleur	Colletotrichum constrictum	6	98.70%	*Colletotrichum constrictum*	6	1023.8	*Colletotrichum constrictum*	5	97.10 %	ITS1	ITS4
B2.2.3	Lefleur	Xylaria adscendens	5	100.00%	*Xylaria adscendens*	3	983.356	*Xylaria adscendens*	5	100.00 %	ITS1	ITS4
B2.21.1	Lefleur	Colletotrichum constrictum	1	98.60%	*Colletotrichum constrictum*	2	931.825	*Colletotrichum constrictum*	7	99.40 %	ITS1	ITS4
B2.4.1	Lefleur	Pestalotiopsis mangiferae	5	100.00%	*Pestalotiopsis mangiferae*	5	996.746	*Pestalotiopsis mangiferae*	5	100.00 %	ITS1	ITS4
B2.4.2	Lefleur	Pestalotiopsis clavispora	2	100.00%	*Pestalotiopsis clavispora*	2	993.139	*Pestalotiopsis clavispora*	2	99.00 %	ITS1	ITS4
B2.4.3	Lefleur	Coprinellus sp.	1	100.00%	*Coprinellus sp.*	5	1002.16	*Coprinellus sp.*	4	93.20 %	ITS	ITS

											ITS1	ITS4
B2.6.1	Lefleur	Unculture d Ceratobasidiaceae	1	95.00%	*Uncultured Ceratobasidiaceae*	3	1113.96	*Unculture d Ceratobasidiaceae*	4	99.80 %	ITS1	ITS4
B2.7.2	Lefleur	Coprinellus sp.	2	99.80%	*Coprinellus sp.*	5	1005.76	*Coprinellus sp.*	3	100.00 %	ITS1	ITS4
B3.1.1	Lefleur	Mucor circinelloides	1	99.80%	*Mucor circinelloides*	2	996.746	*Mucor circinelloides*	2	99.40 %	ITS1	ITS4
B3.1.3	Lefleur	Neonectria radicicola	4	99.60%	*Neonectria radicicola*	6	978.345	*Neonectria radicicola*	3	99.60 %	ITS1	ITS4
B5.1.1	Lefleur	Absidia heterospora	3	94.00%	*Absidia heterospora*	4	1058.06	*Absidia heterospora*	4	99.10 %	ITS1	ITS4
C 4.22.1	Pubescens	Trichoderma viride	4	99.90%	*Trichoderma viride*	2	1058.06	*Trichoderma viride*	3	99.70 %	ITS1	ITS4
C 4.22.3	Pubescens	Ceratobasidium sp.	4	98.10%	*Ceratobasidium sp.*	5	762.308	*Ceratobasidium sp.*	1	100.00 %	ITS1	ITS4
C 4.24.1	Pubescens	Pestalotiopsis mangiferae	2	100.00%	*Pestalotiopsis mangiferae*	5	1034.62	*Pestalotiopsis mangiferae*	1	100.00 %	ITS1	ITS4
C 4.24.2	Pubescens	Pestalotiopsis vismiae	4	100.00%	*Pestalotiopsis vismiae*	3	944.38	*Pestalotiopsis vismiae*	2	99.40 %	ITS1	ITS4
C 4.25.1	Pubescens	Neonectria radicicola	5	99.80%	*Neonectria radicicola*	4	1039.35	*Neonectria radicicola*	1	98.90 %	ITS1	ITS4
C 4.25.2	Pubescens	Neonectria radicicola	6	100.00%	*Neonectria radicicola*	5	1113.96	*Neonectria radicicola*	6	100.00 %	ITS1	ITS4
C 4.25.3	Pubescens	Neonectria radicicola	6	99.80%	*Neonectria radicicola*	5	944.38	*Neonectria radicicola*	4	100.00 %	ITS1	ITS4

C 4.25.5	Pubescens	Trichoderma viride	3	99.30%	*Trichoderma viride*	3	931.825	*Trichoderma viride*	6	100.00%	ITS1F	ITS4
C 4.5.2	Pubescens	Neonectria radicicola	4	99.80%	*Neonectria radicicola*	1	1090.52	*Neonectria radicicola*	6	100.00%	ITS1	ITS4
C 4.8.2	Pubescens	Xylaria adscendens	1	99.30%	*Xylaria adscendens*	3	1034.67	*Xylaria adscendens*	2	98.50%	ITS1	ITS4
C 4.8.2 PT 2	Pubescens	Xylaria adscendens	3	100.00%	*Xylaria adscendens*	1	1047.23	*Xylaria adscendens*	4	99.70%	ITS1	ITS4
C4.13.1	Pubescens	Phomopsis sp.	1	99.00%	*Phomopsis sp.*	2	1035.45	*Phomopsis sp.*	5	100.00%	ITS1	ITS4
C4.13.2	Pubescens	Trichoderma viride	1	99.40%	*Trichoderma viride*	2	1028.85	*Trichoderma viride*	3	99.80%	ITS1	ITS4
C4.20.1	Pubescens	Pestalotiopsis mangiferae	3	100.00%	*Pestalotiopsis mangiferae*	3	1023.8	*Pestalotiopsis mangiferae*	2	100.00%	ITS1	ITS4
C4.20.2	Pubescens	Mortierella fimbricystis	5	99.70%	*Mortierella fimbricystis*	6	934.223	*Mortierella fimbricystis*	3	98.60%	ITS1	ITS4
C4.20.3	Pubescens	Pestalotiopsis clavispora	2	100.00%	*Pestalotiopsis clavispora*	3	980.516	*Pestalotiopsis clavispora*	1	100.00%	ITS1	ITS4
C4.20.4	Pubescens	Mortierella turficola	2	99.70%	*Mortierella turficola*	5	993.139	*Mortierella turficola*	6	99.80%	ITS1	ITS4
C4.20.5	Pubescens	Mortierella turficola	5	99.70%	*Mortierella turficola*	1	980.516	*Mortierella turficola*	2	100.00%	ITS1	ITS4
C4.22.2	Pubescens	Pestalotiopsis sydowiana	3	100.00%	*Pestalotiopsis sydowiana*	5	1031.01	*Pestalotiopsis sydowiana*	6	98.20%	ITS1	ITS4

C4.25.4	Pubescens	Neonectria radicicola	6	100.00%	*Neonectria radicicola*	1	1035.45	*Neonectria radicicola*	4	100.00%	ITS1	ITS4
C4.4.1	Pubescens	Neonectria radicicola	2	100.00%	*Neonectria radicicola*	2	1027.4	*Neonectria radicicola*	3	99.80%	ITS1	ITS4
C4.5.1	Pubescens	Mortierella verticillata	3	100.00%	*Mortierella verticillata*	4	993.139	*Mortierella verticillata*	4	100.00%	ITS1	ITS4
D. and 2.1	Andina	Neonectria radicicola	3	100.00%	*Neonectria radicicola*	1	1039.5	*Neonectria radicicola*	7	99.70%	ITS1F	ITS4
D. dod 1.1.1	Dodsonii	Nigrospora oryzae	5	100.00%	*Nigrospora oryzae*	1	983.356	*Nigrospora oryzae*	3	100.00%	ITS1F	ITS4
D. dod 3.1	Dodsonii	Nigrospora sp.	6	100.00%	*Nigrospora sp.*	7	1034.67	*Nigrospora sp.*	2	99.80%	ITS1F	ITS4
D. dod 4.1	Dodsonii	Rhexocercosporididum sp.	3	98.90%	*Rhexocercosporidium sp.*	2	1027.4	*Rhexocercosporidium sp.*	5	93.50%	ITS1F	ITS4
D. dod 6.1	Dodsonii	Neonectria radicicola	1	100.00%	*Neonectria radicicola*	6	1008.23	*Neonectria radicicola*	2	100.00%	ITS1F	ITS4
D. dod 7.1	Dodsonii	Xylaria sp.	5	99.40%	*Xylaria sp.*	6	955.268	*Xylaria sp.*	1	99.80%	ITS1F	ITS4
D. dod 7.1.2	Dodsonii	Xylaria sp.	2	99.40%	*Xylaria sp.*	6	1023.8	*Xylaria sp.*	6	98.70%	ITS1F	ITS4
D. dod 8.1.1	Dodsonii	Penicilium corylophilium	3	100.00%	*Penicilium corylophilium*	3	1027.4	*Penicilium corylophilium*	7	100.00%	ITS1	ITS4

											F	
D. sod 1.1	Sodoroi	Trichoderma koningiopsis	1	98.50%	*Trichoderma koningiopsis*	2	986.496	*Trichoderma koningiopsis*	3	100.00 %	ITS1F	ITS4
D. sod 1.2.1	Sodoroi	Neonectria radicicola	2	100.00%	*Neonectria radicicola*	3	1031.78	*Neonectria radicicola*	1	99.40 %	ITS1F	ITS4
D. ves 1.1.1	Vespertillo	Cylindrobasidium sp.	5	98.20%	*Cylindrobasidium sp.*	7	1000.35	*Cylindrobasidium sp.*	2	99.80 %	ITS1F	ITS4
D. ves 1.2	Vespertillo	Leptosphaerulina chartarum	1	100.00%	*Leptosphaerulina chartarum*	2	1045.458	*Leptosphaerulina chartarum*	5	100.00 %	ITS1F	ITS4
D. ves 1.2.1	Vespertillo	Leptosphaerulina chartarum	5	100.00%	*Leptosphaerulina chartarum*	3	936.293	*Leptosphaerulina chartarum*	2	100.00 %	ITS1F	ITS4
D. ves 2.5 1.1	Vespertillo	Pleurotus sp.	3	100.00%	*Pleurotus sp.*	3	955.268	*Pleurotus sp.*	1	98.90 %	ITS1F	ITS4
D. ves 2.5 1.1.1	Vespertillo	Neonectria radicicola	2	99.20%	*Neonectria radicicola*	2	923.345	*Neonectria radicicola*	5	99.40 %	ITS1F	ITS4
I 13.1	Felix	Trichoderma viride	1	98.60%	*Trichoderma viride*	1	1036.352	*Trichoderma viride*	6	99.40 %	ITS1	ITS4
I 24.1	Felix	Pezicula sp.	4	98.00%	*Pezicula sp.*	3	923.345	*Pezicula sp.*	1	100.00 %	ITS1F	ITS4
I 24.1.1	Felix	Pestalotiopsis sydowiana	2	100.00%	*Pestalotiopsis sydowiana*	2	1031.01	*Pestalotiopsis sydowiana*	2	99.30 %	ITS1	ITS4
I 24.1.	Felix	Mortierell	2	100.00%	*Mortierella*	5	945.8	*Mortierell*	5	100.00	IT	IT

2		a sp.			*sp.*		56	*a sp.*		%	S1	S4
I 31.1.1	Felix	Hypocrea rufa	6	99.80%	*Hypocrea rufa*	2	1032.81	*Hypocrea rufa*	6	100.00%	ITS1	ITS4
I 31.1.2	Felix	Hypocrea rufa	6	100.00%	*Hypocrea rufa*	1	1052.65	*Hypocrea rufa*	3	100.00%	ITS1	ITS4
I 33.2	Felix	Trichoderma viride	5	99.00%	*Trichoderma viride*	2	945.856	*Trichoderma viride*	5	100.00%	ITS1	ITS4
I 40.1	Felix	Trichoderma viride	2	100.00%	*Trichoderma viride*	6	1108.55	*Trichoderma viride*	4	99.80%	ITS1	ITS4
I 40.1.1	Felix	Trichoderma viride	5	100.00%	*Trichoderma viride*	2	980.516	*Trichoderma viride*	1	100.00%	ITS1F	ITS4
I 7.1	Felix	Absidia repens	2	98.70%	*Absidia repens*	2	940.841	*Absidia repens*	2	100.00%	ITS1F	ITS4
I 9.1	Felix	Hypocrea rufa	3	100.00%	*Hypocrea rufa*	1	985.926	*Hypocrea rufa*	4	100.00%	ITS1	ITS4
I 9.2	Felix	Candida oloephila	3	99.50%	*Candida oloephila*	6	1090.52	*Candida oloephila*	6	99.30%	ITS1	ITS4
I21.1	Felix	Neonectria radicicola	6	100.00%	*Neonectria radicicola*	1	931.825	*Neonectria radicicola*	5	100.00%	ITS1	ITS4
I26.2	Felix	Ilyonectria cyclaminicola	2	100.00%	*Ilyonectria cyclaminicola*	6	1040.03	*Ilyonectria cyclaminicola*	5	99.00%	ITS1	ITS4
I34.3	Felix	Penicilliu m paxilli	2	100.00%	*Penicillium paxilli*	5	945.856	*Penicilliu m paxilli*	2	100.00%	ITS1	ITS4
I7.2	Felix	Neonectria	5	99.80%	*Neonectria*	1	955.2	*Neonectria*	5	98.60	IT	IT

		radicicola			*radicicola*		68	*radicicola*		%	S1 S4
I9.1	Felix	Neonectria radicicola	3	100.00%	*Neonectria radicicola*	5	986.496	*Neonectria radicicola*	3	99.80 %	ITS1 ITS4
Mas @ I2	Masdevallia	Colletotrichum bonninense	2	93.20%	*Colletotrichum bonninense*	5	955.268	*Colletotrichum bonninense*	5	100.00 %	ITS1F ITS4
O 1.2	Felix	Penicilium godlewskii	6	99.00%	*Penicilium godlewskii*	3	1045.458	*Penicilium godlewskii*	2	100.00 %	ITS1F ITS4
O 1.3	Felix	Colletotrichum constrictum	5	100.00%	*Colletotrichum constrictum*	6	1041.83	*Colletotrichum constrictum*	3	100.00 %	ITS1F ITS4
O 12.2	Felix	Neonectria radicicola	3	100.00%	*Neonectria radicicola*	2	1023.8	*Neonectria radicicola*	6	100.00 %	ITS1F ITS4
O 18.10.3	Felix	Phomopsis columnaris	6	100.00%	*Phomopsis columnaris*	5	1039.5	*Phomopsis columnaris*	6	100.00 %	ITS1F ITS4
O 18.12.1	Felix	Trichoderma viride	5	100.00%	*Trichoderma viride*	1	1039.35	*Trichoderma viride*	2	100.00 %	ITS1 ITS4
O 18.14.2	Felix	Neonectria radicicola	2	100.00%	*Neonectria radicicola*	6	1034.62	*Neonectria radicicola*	2	99.00 %	ITS1F ITS4
O 18.2.1	Felix	Neonectria radicicola	7	99.80%	*Neonectria radicicola*	1	1013.34	*Neonectria radicicola*	2	100.00 %	ITS1 ITS4
O 18.2.2	Felix	Xylariales sp.	1	98.90%	*Xylariales sp.*	3	931.825	*Xylariales sp.*	1	100.00 %	ITS1 ITS4
O 21.1.	Felix	Neonectria	6	98.60%	*Neonectria radicicola*	6	993.652	*Neonectria*	1	100.00 %	ITS ITS

1		radicicola						*radicicola*			1	4
O 21.1.2	Felix	Neonectria radicicola	4	99.20%	*Neonectria radicicola*	2	1108.55	*Neonectria radicicola*	6	94.00 %	ITS1	ITS4
O 21.1.4	Felix	Neonectria radicicola	6	100.00%	*Neonectria radicicola*	3	1021.99	*Neonectria radicicola*	3	98.80 %	ITS1F	ITS4
O 21.1.4	Felix	Neonectria radicicola	5	100.00%	*Neonectria radicicola*	4	1031.01	*Neonectria radicicola*	1	99.90 %	ITS1F	ITS4
O 21.4.1	Felix	Penicilium nothofagi	5	100.00%	*Penicilium nothofagi*	5	934.223	*Penicilium nothofagi*	3	99.50 %	ITS1F	ITS4
O 3.1	Felix	Penicilium lividum	4	99.80%	*Penicilium lividum*	4	936.293	*Penicilium lividum*	3	99.80 %	ITS1F	ITS4
O 42.1.1	Felix	Neonectria radicicola	2	99.30%	*Neonectria radicicola*	3	1031.01	*Neonectria radicicola*	5	99.60 %	ITS1F	ITS4
O 42.1.2	Felix	Trichoderma viride	3	98.90%	*Trichoderma viride*	5	980.567	*Trichoderma viride*	6	98.00 %	ITS1F	ITS4
O12.3	Felix	Bjerkandera adusta	1	98.70%	*Bjerkandera adusta*	2	1113.96	*Bjerkandera adusta*	1	99.20 %	ITS1	ITS4
O18.10.2	Felix	Trichoderma viride	6	100.00%	*Trichoderma viride*	4	940.841	*Trichoderma viride*	2	100.00 %	ITS1	ITS4
O18.14.1	Felix	Trichoderma viride	3	100.00%	*Trichoderma viride*	1	993.652	*Trichoderma viride*	1	98.70 %	ITS1	ITS4
O18.14.3	Felix	Neonectria radicicola	4	99.40%	*Neonectria radicicola*	6	980.567	*Neonectria radicicola*	1	100.00 %	ITS	ITS

											1	4
O56.2.1	Felix	Hypocrea rufa	2	100.00%	*Hypocrea rufa*	1	1013.34	*Hypocrea rufa*	6	95.00%	ITS1	ITS4

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