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## Diversity and substrate partitioning of discomycetes in a cloud forest in Venezuela

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The fungal diversity of discomycetes was surveyed in a cloud forest at El Avila National Park in Venezuela. A systematic collecting scheme based on transects was used for the sampling methodology. Two sites were visited on two occasions during the rainy season (June to October) with a total of 24 samples collected from each site. For each plot, 24 and 27 species were identified respectively. Chao-Sorensen Similarity Coefficient between sites was 43%. Rarefaction curves based on species richness for both sites and the main types of substrates (wood, leaf, bamboo and soil) indicated that there are more species to be found than collected. Similarly, Incidence-based Coverage Estimator of species richness (ICE) used to calculate species richness showed that only 40–55% of the species present were observed during this study. Discomycete species were distributed in the following orders: Ostropales (1.2%), Lecanorales (2.4%), Pezizales (7.3%), Rhytismatales (13.4%), Orbiliales (15.9%) and Helotiales (59.8%) with a total of 42 species. This work adds ten new records of discomycetes to Venezuela and possibly eight new taxa.

**Key words** – biodiversity – ecology – Helotiales – rarefaction curves – South America – species composition

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### Article

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### Introduction

Studies on fungal diversity have increased in recent years due to a growing interest in this area of fungal research. This tendency is necessary since the determination of species diversity has been proposed to be one of the 25 topics to be addressed by science in the present century (Pennisi 2005). Thus far, much of this interest has focused on estimates of the total number of species of fungi in the world (e.g. Hawksworth 2001, Schmit & Mueller 2007). According to Hawksworth's estimate of 1.5 million species of fungi worldwide (1991), at present only 5% of fungal taxa have been described. Since most of the fungal diversity studies have been carried out in temperate regions of the world, fungi in neotropical forests

have been historically under-sampled. This biased approach has been evident in most of the tropical surveys during the last two decades; where the great majority of species represent new records for the regions of study and about 15–30% represent new taxa (May 1996).

Discomycetes are a group of fungi characterized by the presence of ascohymenial apothecia with either inoperculate or operculate unitunicate asci (Kirk et al. 2008). At least five orders can be included in this group; however the phylogenetic relationships among these are still under discussion (see Spatafora et al. 2006). In the traditional sense, the orders of discomycetes are: Helotiales, Orbiliales, Ostropales, Pezizales and Rhytismatales. In these groups, the total number of non-lichenized

discomycetes is approximately 6650 species (Kirk et al. 2008). According to estimations made by Mueller and Schmit (2007), the percentage of known species for Helotiales and Pezizales are 3% and 34%, respectively. From published and unpublished fungal records compiled by Iturriaga and Minter (2006), there are 227 species of discomycetes in Venezuela. The Helotiales are represented by 108 species, followed by the Pezizales with 82 taxa. The Ostropales, Rhytismatales and Orbiliales are represented by 19, 11 and seven species, respectively.

Studies in systematic biodiversity that incorporate standardized methods are important because they allow comparisons between places. Among published systematic studies on tropical microfungi (Bills & Polishook 1994, Lodge & Cantrell 1995) only one, Cantrell (2004), includes the discomycetes. Discomycetes are important decomposers of dead woody and herbaceous debris in tropical forests but their substrate distributional patterns are poorly understood. Some species are specific on a substrate while others may grow on several substrates. Logs and twigs represent major substrates for discomycetes. Leaves are intensively colonized by some particular species. Some species, mainly operculate discomycetes, grow exclusive on soil. Bamboo, if present, also supports species assemblages.

The main objective of the present study was the collection of data on the biodiversity and ecology of discomycetes from a cloud forest in Venezuela. In order to do this, a (i) characterization of the species assemblage, an (ii) estimation of the species diversity and richness and an (iii) analysis of substrates, were also carried out.

## Methods

The present study was carried out during the rainy season of 2007 in Venezuela, northern South America. In all instances, the morphological concept of species was used and nomenclature follows the 10th edition of the Dictionary of the Fungi (Kirk et al. 2008). No samples were isolated in laboratory conditions and voucher specimens have been deposited in the National Herbarium of Venezuela (VEN).

## Study sites

Two sites located in a premontane cloud forest on the south side of the El Avila National Park were selected. The first site is located near Humboldt Peak (10°32'45"N, 66°52'23"W, 2000 m asl – above sea level) and will be referred to in the present study as Boqueron. The second site is located approximately 700 m east of the first site (10°32'42"N, 66°52'01"W, 2250 m asl) and will be referred to as Lagunazo.

The structure of the forest in both sites is characterized by more than three non-discrete layers of vegetation. The most abundant canopy trees include the dominant *Clusia multiflora*, *Podocarpus pittieri* and *P. oleifolius*. Other common species in the canopy are *Chimarris microcarpa*, *Cordia alliodora*, *Dictyocaryum fuscum*, *Ecclinusa abbreviata*, *Euterpe precatoria*, *Guapira olfersiana*, *Inga corucans*, *I. marginata*, *Licania cruegeiana*, *Ectandra kunthiana*, *Platymiscium polystachyum* and *Protium araguense*. Three of the most common species in the intermediate-sized layer are the palms *Catoblastus praemorsus*, *Ceroxylon klopstockia* and *Geonoma pinnatifrons*. Tree ferns of the genus *Cyathea* are dominant in the understory. Some open areas or gaps are dominated by *Arthrostylidium venezuelae*, a species of bamboo.

## Sampling method

The sampling methodology included the establishment of two study areas per study site. On each of these areas, two transects were used to sample the fungi following the method described by Cantrell (2004). Each transect consisted of a 60 m long imaginary line with a series of twelve 1×1 m plots located at 5 m intervals. These plots were randomly positioned on either side of the transect sides.

With this methodology, a total of 24 plots from each study site were established and fruiting bodies corresponding to discomycetes were collected twice during each collecting period on all plots. Specimens of discomycetes were collected on three different types of dead plant material and on the ground (abbreviated as S for soil). For the former, wood (W), ground litter (L) and bamboo (B) were examined. If possible, all samples were collected on the same day and examined using a compound

light microscope following the protocol described by Iturriaga and Korf (1990).

### Data Analysis

A number of general analyses were carried out with the obtained dataset. To characterize the assemblages, the number of species per genus (S/G) was calculated from both collecting sites and from the total data set, as recommended by Stephenson et al. (1993). A low value for S/G implies a higher diversity than a high value. Similarly, an abundance-based categorization of observations was constructed following the method described by Stephenson et al. (1993) for the complete dataset. Four categories were established and species abundances were assigned to one of these by means of their relative abundance. In this way, those species regarded as “rare” were represented by <2.0% of the total number of collections, “occasional” between >2.0% and <3.8% of the total, “common” between >3.8% and <5.8% and “abundant” species were represented by >5.8% of the total. Similarly, the incidence (presence or absence) of the species was used as a measurement of abundance in each plot as recommended by Schmit et al. (1999). The Shannon-Wiener index (SI) was obtained for both study sites as well as for the entire assemblage of discomycetes recorded in this study.

In addition to the latter, a comparison of species richness values across study sites and among the main types of substrates, was carried out by constructing rarefaction-based species accumulation curves along with their 95% confidence interval curves using EstimateS, version 8.2.0 (Colwell 2006). These curves were based on the values obtained for the Chao 2 estimator, as recommended by Unterseher et al. (2008). The same program was used for the Incidence-based Coverage Estimator of species richness (ICE) for both sites and all types of substrates, to estimate the sampling efficiency as recommended by Chao et al. (2000). The similarity between both sites was determined using the Chao-Sorensen Similarity Coefficient (Chao et al. 2005).

### Results

A total of 76 specimens representing 42 species of discomycetes were recorded being

*Chlorencoelia torta*, *Hyalorbilia inflatula*, *Orbilia delicatula* and several species of the genus *Lachnum* the most abundant taxa. Out of the total number of species, 24 were recorded from Boqueron and 27 from Lagunazo. The S/G values calculated for each site and for the total dataset ranged from 1.85 to 2 (see Table 1). The SI value calculated for the total assemblage of species was 3.57 (see Table 1). A list of discomycete species and their abundance category are given in Table 2.

Of all the species recorded, ten represent new records for Venezuela, five are new records for the Neotropics and eight may represent undescribed species, which for practical purposes were assigned to the genera *Dascycephella* (1 sp), *Dimorphotricha* (1 sp), *Chlorencoelia* (1 sp), *Lachnum* (3 spp) and *Strossmayeria* (2 spp, see Table 2). Overall, the relative abundance of collections in each order was: Ostropales (1%), Lecanorales (2.4%), Pezizales (7.3%), Rhytismatales (13.4%), Orbiliales (15.9%) and Helotiales (59.8%). The species-abundance distribution (Table 2) shows that there were few abundant species (16%) and a high proportion of species with intermediate abundance or rare (60%).

The ascomata of most wood-inhabiting species were collected on logs, twigs and leaf litter; and correspond to 71% of the total collections. Similarly, species growing on leaf litter represented 17% and the rest of the species were almost equally divided between bamboo-inhabiting species (7%) and soil-inhabiting species (5%).

Species accumulation curves for both sites are shown in Fig 1, with effort measured as the number of individuals collected on both study sites. Although species richness estimates for Lagunazo are higher than for Boqueron, differences are clearly not significant because the confidence intervals overlap.

The species accumulation curves for the most common substrates (wood and leaf litter), appear to indicate that wood-inhabiting species are more diverse than leaf litter species. This difference is not significant on the basis of the confidence intervals (Fig 2), even when the number of individuals is higher on wood than on litter.

None of the species accumulation curves (for both sites and substrates) appear to be

**Table 1** Summary of discomycete species diversity recorded in the present study.

Parameter	Study sites		Total
	Boqueron	Lagunazo	
Total number of collection	43	38	81
Number of genera	13	14	21
Number of species	24	27	42
S/G value	1.85	1.93	2.00
SI value	2.93	3.22	3.57

leveling off (Figs 1 and 2). These results are comparable with the ICE values for the maximum number of expected species in both sites and in different substrates (Fig 3). According to the latter estimations the studied plots contained about 40% of the species present in Boqueron, 55% in Lagunazo, 40% of wood-inhabiting species, 75% of species on leaf litter, 75% of species on bamboo and 50% of species growing on soil.

The Chao-Sorensen similarity coefficient between sites was 0.43. Six species were found in both areas: *Chlorociboria aeruginosa*, *Chlorencoelia torta*, *Hyalorbilia inflatula*, *Lachnum brasiliensis*, *Lachnum pseudoesclerotii* and *Lachnum* sp.1. The low number of common species found on both sites seems to indicate that species composition in each site is moderately different.

## Discussion

The study sites surveyed herein yielded a total of 24 and 27 species of Discomycetes. In a previous study carried out in the tropical forests of Puerto Rico and Dominican Republic (Cantrell 2004) using the same sampling method, the number of species in two sites per country were 31 and 30 (Puerto Rico) and 25 and 26 (Dominican Republic). The number of total records was higher for Puerto Rico (180 and 127 for each site). Differences in the number and frequency of sampling periods may account for some of the discrepancies in the number of records. While only two visits were made to each collecting site in the present study, Cantrell (2004) made twelve visits to each site. Although there are differences in the number of records, the number of species is similar. The mean number of species per genus for Boqueron and Lagunazo was 1.85 and 1.93, respectively. The values obtained for the data set from each site in Puerto Rico and Dominican Republic was 2.2, 2.8, 2.1 and 1.86,

respectively. It seems clear that the values obtained in the present study are lower than those obtained in the study by Cantrell (2004).

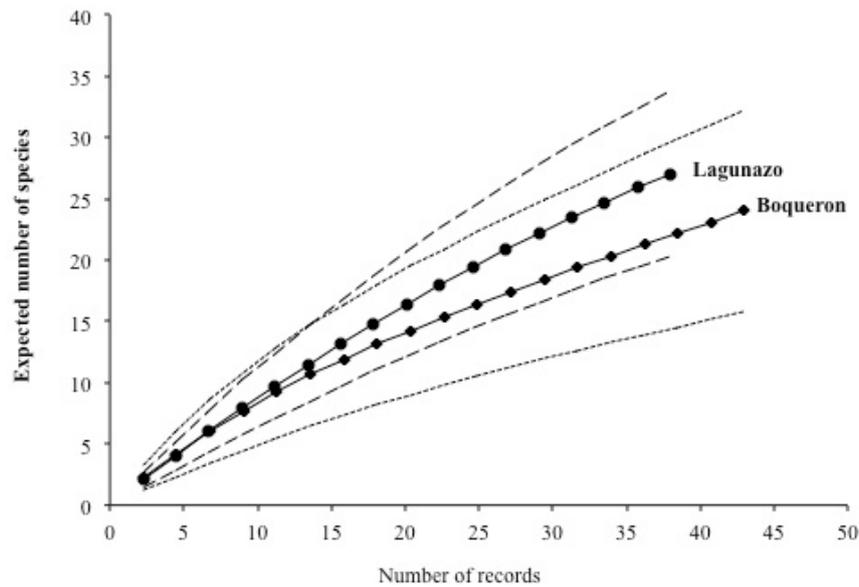
Our results show that the four main types of substrates support assemblages of discomycetes. The most productive substrate, both in terms of records and species present, was decaying wood. Leaf litter occupied the second place, followed by bamboo and soil. In general, members of the Orbiliales and Helotiales tend to be commonly associated with woody substrates, while members of the Rhytismatales were almost always collected from leaf litter, specifically on leaves of *Clusia multiflora*. Several authors have recognized specificity of some species of Rhytismatales with this host in the tropics (Sherwood 1980, Johnston 1989a, b). Bamboo-inhabiting discomycetes form a distinct assemblage of a few but highly specialized species. Some of the taxa that have been previously reported growing only on bamboo include species such as *Lachnum pseudoesclerotii* and *Strossmayeria jamaicensis*. Interestingly, all the species present on soil (Fig. 3), belong to the order Pezizales. This is not surprising, considering that this group seems to be more abundant in temperate forests (Lodge et al. 1995). Furthermore, members of this order often fruit in habitats with high pH and low content of organic matter (Hansen & Pfister 2006). According to Ataroff (2001) soils in El Avila National Park are characterized by having low pH and high content of organic matter.

In both of the species accumulation curves the asymptote is far from being reached. This implies that there are more species to be found. According to the ICE values, the number of expected species for Boqueron and Lagunazo were 62 and 49 respectively. These values indicate that between 40–55 % of the expected total number of species are yet to be found. Despite the similar number of species

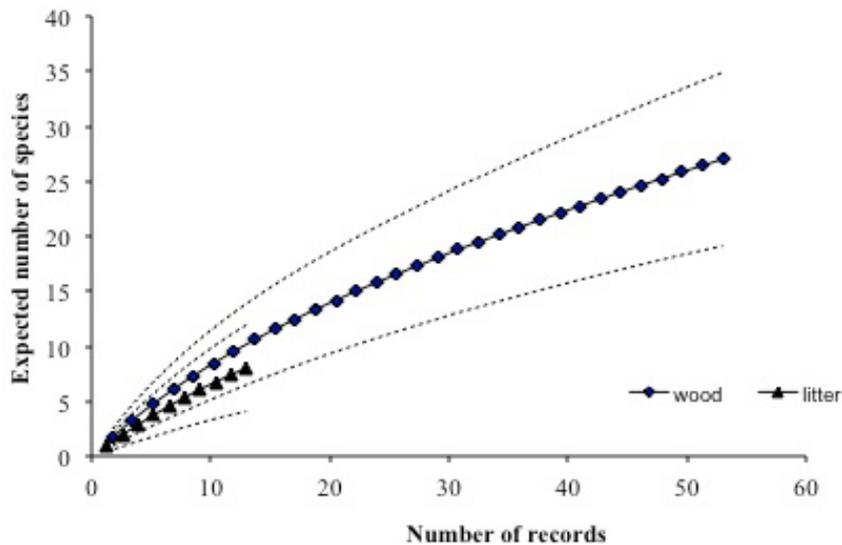
**Table 2** Species of discomycetes recorded in the present study and their occurrence on the studied substrates.

Species	Abundance	Substrates				Species	Abundance	Substrates			
		Wood	Litter	Bamboo	Soil			Wood	Litter	Bamboo	Soil
<i>Arachnopeziza colachna</i> <sup>b</sup>	Rare	X				<i>Lachnum</i> undescribed 2	Rare	X			
<i>Bisporella discedens</i>	Rare	X				<i>Lachnum</i> undescribed 3	Occasional			X	
c.f. <i>arachnopeziza</i>	Rare	X				<i>Lophodermium platyplacum</i>	Rare		X		
<i>Cerion leucophaeum</i>	Rare	X				<i>Lophodermium</i> sp.1	Rare		X		
<i>Chlorencoelia</i> undescribed	Rare	X				<i>Mollisia</i> sp.1	Rare	X			
<i>Chlorencoelia torta</i>	Common	X				<i>Mollisia</i> sp.2	Rare	X			
<i>Chlorociboria aeruginosa</i>	Common	X				<i>Niptera</i> c.f. <i>stictoidea</i>	Rare	X			
<i>Coccomyces limitatus</i>	Occasional		X			<i>Niptera</i> sp.1	Rare		X		
<i>Coccomyces radiatus</i>	Rare		X			<i>Niptera</i> sp.2	Rare		X		
<i>Dactylospora stygia</i> var. <i>striata</i> <sup>a</sup>	Rare	X				<i>Niptera trichophoricola</i> <sup>b</sup>	Common	X		X	
<i>Dactylospora stygia</i> var. <i>tenuispora</i>	Rare	X				<i>Orbilbia delicatula</i>	Abundant	X			
<i>Dascycephella</i> undescribed <sup>b</sup>	Rare	X				<i>Orbilbia gaillardi</i>	Occasional	X			
<i>Dimorphotricha</i> undescribed <sup>b</sup>	Rare	X				<i>Plectania melastoma</i> <sup>a</sup>	Occasional	X			
<i>Hyalorbilia inflatula</i>	Abundant					<i>Plectania rhytidia</i>	Rare				X
<i>Lachnum abnorme</i>	Rare	X				<i>Poculum</i> c.f. <i>bambusae</i>	Rare		X		
<i>Lachnum brasiliensis</i>	Abundant	X				<i>Pseudoplectania nigrella</i> <sup>a</sup>	Occasional				X
<i>Lachnum calosporum</i>	Rare	X				<i>Pulvinula orichalcea</i>	Rare				X
<i>Lachnum correae</i> <sup>b</sup>	Common		X			<i>Stictis radiata</i>	Rare	X			
<i>Lachnum pseudoesclerotii</i> <sup>b</sup>	Abundant	X		X		<i>Strossmayeria jamaicensis</i> <sup>a</sup>	Rare			X	
<i>Lachnum sclerotii</i>	Occasional	X				<i>Strossmayeria</i> undescribed 1	Rare			X	
<i>Lachnum</i> undescribed 1	Rare	X				<i>Strossmayeria</i> undescribed 2	Rare	X			

<sup>a</sup>New record for Venezuela<sup>b</sup>New record for the Neotropics



**Fig. 1** – Species accumulation curves for discomycetes from the study sites in the present study. Dashed lines indicate 95% confidence intervals.



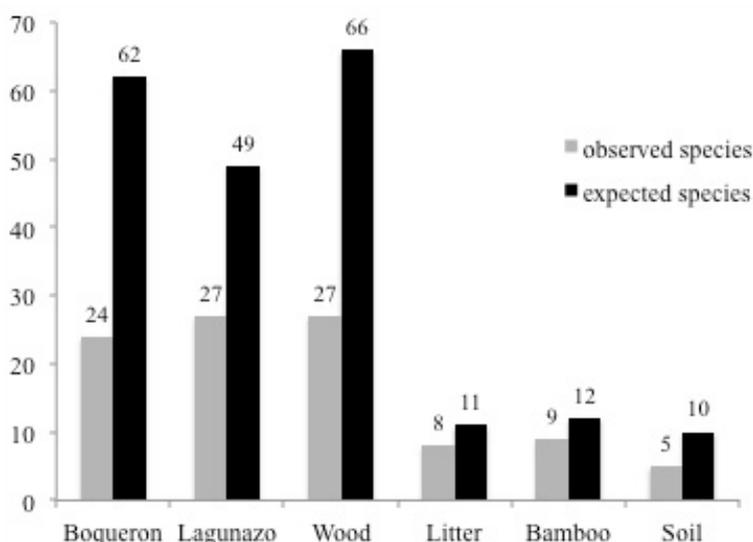
**Fig. 2** – Species accumulation curves for discomycetes associated with wood and leaf litter in the present study. Species richness values (solid lines) were calculated using the Chao 2 estimator with 95% confidence intervals (dash lines).

found on both sites, the structure of the data suggests that the Boqueron site has higher species richness. The ICE values for the maximum number of species indicate that the survey was more complete for woody substrates than for leaf litter, bamboo and soil. These estimates however, may represent underestimations simply because all of the different substrates were not sampled with equal intensity.

The ICE values for expected species conform with the results obtained in the curve analyses. Both results suggest that a greater sampling effort is required to find the remain-

ing species. In both cases, the nonparametric estimators magnified the differences in species richness.

The Chao-Sorensen Similarity Coefficient between sites was 0.43. This value suggests low similarity between the assemblages of discomycetes at both study sites, which may indicate that both sites were established at appropriate distances as to represent independent samples of the same community (Lodge & Cantrell 1995). This value may also be an indicative of the high diversity of species in El Avila National Park, since only six species of



**Fig. 3** – Observed versus expected species richness values for both sites and all type of substrates, using the Incidence-based Coverage Estimator (ICE). Numbers over columns indicate the actual value.

the total of 45, were shared between sites. Similar results were obtained by Cantrell (2004) for Puerto Rico and Dominican Republic (0.5 and 0.40 respectively).

Records of new taxa – The information on the diversity of discomycetes in tropical ecosystems is scarce. The present study represents a useful set of data to further understand the diversity and biogeography of discomycetes in tropical areas. Our study has increased the number of species known from Venezuela to 244. Two genera are recorded for the first time for the Neotropics: *Dascycyphella* sp. and *Dimorphotricha* sp, as well as three species: *Arachnopeziza colachna*, *Lachnum pseudoescerotii* and *Lachnum correae*. *Dascycyphella* occurs predominantly in temperate areas, and none of its 22 described species have been reported for tropical areas (Raitviir 2002). *Dimorphotricha* is a monotypic genus reported only for Australia (Spooner 1987) and this is the second report in the world for this genus. Similarly *Lachnum correae* has also been reported for Australia (Spooner 1987). Twenty-eight percent of the total number of species collected represent new reports for Venezuela. This is an interesting fact since the study of Discomycetes in this country is relatively ahead of the situation in other countries of the region.

In summary, the data obtained in the present study indicate that the assemblages of discomycetes present in the study sites conform

to similar patterns reported for other areas in the Neotropics. Although more research is needed to determine a good estimate of the true number of discomycetes species, we conclude that this study adds to the small amount of data previously known of discomycetes diversity in the Neotropics. However, the lack of fungal studies with the group and the disparities in methodology difficult comparisons among studies and large-scale patterns of tropical fungal diversity remain difficult to make reliable conclusions. We envision that more research, based on consistent methods, would help determine the degree of gamma diversity present in tropical areas. At the same time, studies assessing substrates preference help elucidate ecological patterns of these fungi. At present, we continue collecting data in the study sites described in the present study with the goal of obtaining more accurate information to estimate the number of species present in these sites over a longer period of time.

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