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# Microfungi from decaying leaves of two rain forest trees in Puerto Rico

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**Fungal species richness and abundance were compared in leaf litter of two tree species, *Guarea guidonia* and *Manilkara bidentata*, in the Luquillo Mountains of Puerto Rico. Four litter samples yielded a total of 3337 isolates, ranging from 591 to 1259 isolates/sample. The number of species/sample ranged from 134 to 228. Many uncommon litter hyphomycetes were recovered as well as coelomycetes, sterile strains, endophytes, and phytopathogens. Species-abundance distributions revealed a typical pattern of a few abundant species and a high proportion of rare species. Similarities in fungal species composition were not correlated with host species or with the site. Replicate samples examined by the moist chamber technique yielded a total of 24 species among the four litter samples. The particle filtration method indicated that leaves of *G. guidonia* were more species-rich, while moist chambers indicated leaves of *M. bidentata* were more species-rich. The moist chamber technique underestimated the number and species of viable fungi.**

**Keywords:** decomposition; microbial diversity; litter preference; spatial heterogeneity; *Guarea guidonia*; *Manilkara bidentata*

## Introduction

This is the third in a series of investigations describing the localized diversity of microfungi associated with decaying leaves of humid neotropical forests [2,3]. We found that mixed litter of primary rain forests in southwestern Costa Rica possesses high levels of species diversity of microfungi and exhibits enormous local variation of fungal species composition. Investigation of decaying leaves from individual plants of *Heliconia mariae* JD Hooker in southeastern Costa Rica demonstrated that levels of fungal diversity in leaves of individuals of one plant species may rival that of mixed forest litter and that local variation in species composition among decaying leaves of a single plant may be as great as variation among small mixed litter samples taken from different areas. Other studies suggested that fungal assemblages on decaying leaves in tropical forests differ considerably between tree species [7,15] and might contribute to the observed high diversity and spatial heterogeneity of decomposer fungi [2,3,15].

This study attempts to dissect the contribution of leaves of individual tree species towards local distribution patterns of the litter mycota by comparing microfungi observed on and isolated from leaves of *Guarea guidonia* (L) Sleumer (Meliaceae) and *Manilkara bidentata* (A DC) Chev (Sapotaceae), growing together in Puerto Rico. The leaves of *G. guidonia* are thin and brittle when dry, while *M. bidentata* leaves are thick and leathery. We asked whether litter of two trees at the same site were colonized by the same taxa, or if microfungal species preferentially colonized one leaf type over the other. Additionally we compared the litter mycota of these decaying leaves using two fundamentally different sampling methods: one, the traditional technique of observing fruiting structures on leaves incubated in moist chambers; and two, particle-filtration, in which tiny washed litter particles are plated on nutrient media and fungi that emerge from these particles are identified based on cultural features. Recently, endophytic fungi associated with *M. bidentata* were investigated [10], but little is known of fungi associated with *G. guidonia* [16]. Thus we present the first in-depth account of the mycota associated with litter of these forest trees.

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## Materials and methods

### *Study area and collection of materials*

Litter samples were collected in a non-seasonal, late secondary subtropical wet forest (392 cm annual rainfall, 350 m elevation) at El Verde in the Luquillo Mountains, Caribbean National Forest, Puerto Rico. The area and its previous mycological studies have been described elsewhere [8]. Collections were made at two sites located 200 m apart where *G. guidonia* and *M. bidentata* grow together. Thus, four samples were analyzed, designated Gg1, Gg2, Mb1 and Mb2. Litter was collected 18 February 1995 at the beginning of the mild dry season.

Leaves representing all stages of decay were selected from the litter layer within a 5-$m^2$ quadrat at each of two sites. Leaves of each species were placed separately in cotton soil-collection bags. Samples were air-dried in bags until transported 2 days later at ambient temperature to Rahway, New Jersey. Upon arrival in Rahway, leaves were refrigerated (4°C) until processed or being placed in moist chambers during the following week of February, 1995.

### *Direct observation of microfungi*

From each bag of decayed leaves, 5 g were removed and wetted with distilled water and placed in moist chambers to observe development of microfungi on leaf surfaces. Moist chambers were plastic boxes (13.2-L volume) with a bottom lining of 2–3 cm of sterile wet vermiculite to retain moisture. Chambers were incubated at room temperature in indirect window light. Leaves were periodically moistened with a mist of distilled water from an atomizer bottle. Leaves were inspected with a dissecting microscope every few days for 8 weeks and fungi present on each sample recorded. Most taxa sporulating on leaves were cultured from conidia in order to determine their cultural morphology. Identifications were made from semi-permanent lactophenol slides.

### *Media*

The media used for isolation and identification were prepared in 1 L of distilled water as follows: malt-cyclosporin medium containing malt extract 10 g, yeast extract 2 g, agar 20 g; cyclosporin A 10 mg was added after the medium cooled [6]; Bandoni's medium containing L-sorbose 4 g, yeast extract 0.5 g, agar 20 g [1]. Chlortetracycline and streptomycin sulfate 50 mg $L^{-1}$ were added as antibacterial agents to both media after they had cooled. MYE is the same as malt-cyclosporin medium but without cyclosporin A or antibacterial agents. Identifications were made from colonies subcultured on oatmeal agar and cornmeal agar with a small piece (1–2 $cm^2$) of autoclaved banana leaf [12,13]. Addition of banana leaves often induced sporulation or affected the form of sporulating structures. *Penicillium* spp were distinguished according to methods described by Pitt [14].

### *Sample preparation and particle filtration*

Our strategy for isolation of fungi from filtered litter particles was described previously [2,3]. Decayed leaves were removed from the bags and air-dried for 3–4 h. From each plant, 5 g of leaves, representing all stages of decay, were placed in a sterilized Waring blender and pulverized at high speed for 1 min. The pulverized sample was then washed with a stream of distilled water for 10 min through a 2-mm brass prescreen and through two sterilized polypropylene mesh filters (Spectrum, Houston, TX, 210-$\mu$m and 105-$\mu$m pore size) to remove spores. Plating of this distilled water on MYE with antibacterial antibiotics showed that it was free of fungal propagules. The distilled water was directed from above the pulverized particles, which forced a subset of the particles to be trapped between the 210-$\mu$m and 105-$\mu$m filters. After washing, the 105-$\mu$m filter was removed with forceps and placed into a sterile 50-ml polystyrene centrifuge tube. Fifty milliliters of sterile distilled water were added to the tube which was then vigorously agitated to suspend the particles. The filter was removed from the centrifuge tube with forceps. In an attempt to apply a uniform density of particles from each sample to Petri plates, particles were allowed to settle to the bottom of the conical centrifuge tubes and excess water above the particles was removed. The particles were diluted with a second volume of sterile water to obtain 20 : 1 (v/v) water : particles. Particles were allowed to settle and washed with a 20 : 1 volume of water twice more. Immediately after agitation of the washed particles, 0.1-ml portions of suspended particles were pipetted onto each of ten 100-mm Petri plates of malt-cyclosporin medium and of Bandoni's medium (20 plates total). The particle suspension was spread over the surface of the agar with a flamed bent-glass rod. The plates were incubated at 15°C, 60% relative humidity and 12-h photoperiod under fluorescent lights.

Five replicate 2-ml suspensions from each sample were placed in tared aluminum pans and dried to constant weight to estimate weight of leaf particles applied to plates (Table 1). Starting as soon as 3 days and continuing for up to 30 days, each newly initiated colony was transferred to a MYE slant and incubated at 20°C, 70% relative humidity and 12-h photoperiod. The value of this procedure for colony selection for increasing colonies' densities and species diversity recovered from isolation plates have been explained previously [2,6]. Colonies of some distinctive species were counted directly on plates and were marked from the reverse to avoid double counting.

Once sporulation or sufficient growth occurred to differentiate the isolates, they were sorted into species or morphologically similar groups. Representative slants of a species or isolates from slants where differences in colony morphology were ambiguous were transferred to 60-mm Petri dishes of agar media for identification, at least as far as possible. We grouped isolates into four functional categories: sporulating or nonsporulating species occurring more than once in a sample; coelomycetes that occurred only once in a sample; sterile isolates that occurred only once in a sample: and *Acremonium* spp that occurred only once in a sample (Table 1). The many sterile isolates were problematic because they could not be assigned to any taxonomic category. Sterile isolates were sorted into recognizable taxonomic units or 'morpho-species' based on similarities in colony surface textures, hyphal pigments, exudates, margin shapes, and growth rates when isolates were compared under identical growth conditions. They are referred to as 'sterile species' throughout the text and are assumed to represent different taxonomic species. Because similar strains usually were combined into one 'species', we suggest that if errors in enumeration were made, the actual number of strain types was underestimated. Likewise, we dealt with coelomycetes and the large number of *Acremonium* spp or *Acremonium*-like fungi in a similar fashion. We did not attempt to combine single sterile, *Acremonium*, and coelomycete species between samples because of the uncertainty in their equivalency. Representative cultures and semipermanent lactophenol slides of the more frequent sporulating taxa were deposited at the Center for Forest Mycology Research, Madison, WI, USA (CFMR). However, unidentifiable fungi, eg most coelomycetes, *Acremonium* spp and sterile strains, were retained in the Merck Microbial Resources Culture Collection, Rahway, NJ, USA.

### *Analyses*

Data acquired by particle filtration on fungi from each leaf sample were analyzed separately for species abundances. Comparisons of similarities in fungal assemblages among samples were restricted to species that occurred at least

**Table 1** Number of isolates and species recovered from four samples of decaying leaves of *Guarea guidonia* and *Manilkara bidentata* in the Luquillo Mts, Puerto Rico

| Litter sample | Weight of particles[a] (mg) | Propagules $g^{-1}$ dry leaf litter (×1000) | Total isolates | Sporulating and sterile species[b] | Single sterile species[c] | Single coleomycetes[d] | Single *Acremonium* spp[e] | Total species | $E_{(s200)}$[f] |
|---|---|---|---|---|---|---|---|---|---|
| Gg1 | 4.2 | 141 | 591 | 107 | 23 | 15 | 25 | 170 | 74 |
| Gg2 | 5.4 | 233 | 1259 | 173 | 27 | 22 | 6 | 228 | 76 |
| Mb1 | 4.4 | 165 | 726 | 101 | 16 | 9 | 9 | 134 | 58 |
| Mb2 | 4.4 | 173 | 761 | 118 | 9 | 11 | 12 | 150 | 60 |
| Total | | | 3337 | | | | | | |

[a] Estimated particle weight applied to 20 Petri plates.
[b] Includes all sporulating species and all sterile species that occurred more than once in the sample.
[c] Different sterile individuals of single occurrence.
[d] Different unidentified coelomycetes of single occurrence.
[e] Different unidentified *Acremonium* spp of single occurrence.
[f] $E_{(s200)}$ is the expected number of species in a random sample of 200 isolates taken from the total population of isolates from each sample.

twice. Species abundance ($n$) is expressed as the number of individual isolates of a species. Percent abundance is the number of isolates of a species divided by the total number of isolates recovered from the sample. To compare species richness among samples of unequal size, rarefaction indices were calculated for a random sample of 200 isolates [11]. The expected number of species, $E(s)$, in a random sample of $n$ isolates taken from a total population of $N$ isolates is calculated using the formula:

$$E(s) = \sum_{i=1}^{s} \left\{ 1 - \left[ \binom{N - n_i}{n} \Big/ \binom{N}{n} \right] \right\}$$

where $n_i$ is the number of isolates of the $i$th species. To compare relative abundance of species and to compare abundance of major species occurring in more than one sample, species were sorted in descending order by their abundance and species-abundance distributions were plotted for each sample (Figure 1). To emphasize which fungi were dominant and which were rare species in this study, species are presented in overall descending total abundance in the four samples (Table 2). For the purpose of this discussion, the term 'rare' species signifies that the species was found infrequently among the isolates, rather than that few specimens of the taxon are known to exist. To compare fungal species composition between litter samples from different sites and species, the Jaccard index (JI) of association was calculated pairwise among samples based on presence/absence of a species.

$$JI = a/(a + b + c)$$

where $a$ is the number of species occurring in both samples, $b$ is the number of species unique to the first sample, and $c$ the number unique to the second sample. In addition, the technique of complementarity [5,9], or degree of nonoverlap between species lists, was applied to assess completeness of the sampling between sites (Figure 2a) and leaf types (Figure 2b).

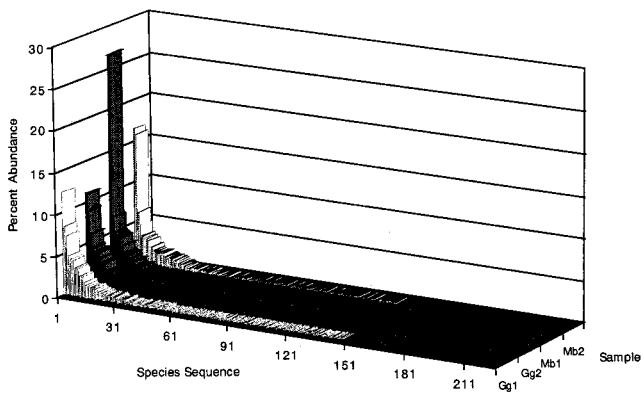


**Figure 1** Species-abundance distributions of microfungi isolated from decaying leaves of *Guarea guidonia* and *Manilkara bidentata* in the Luquillo Mts, Puerto Rico. Species in each sample ordered from most abundant at left to least abundant at right as a percentage of the total isolates of each sample. See methods for sample locations.

## Results

### *Particle filtration*

A total of 3337 isolates were recovered from the four litter samples, ranging from 591 to 1259 isolates/sample (mean = 834) (Tables 1, 2). The number of species/sample ranged from 134 to 228. The list of species from each litter sample and their abundance are given in Table 2. The numbers of isolates and species fell within an order of magnitude of each other, indicating that we extracted comparable numbers of fungal propagules from each sample. Sample Gg2 yielded more than twice as many isolates (1259) as Gg1 (591) possibly because of its higher propagule density and because more particles were applied to the isolation plates (Table 1). Samples of *M. bidentata* litter yielded similar numbers of isolates (Table 1). Rarefaction indices based on the number of species expected in a random subsample of 200 isolates from each sample indicated that samples were relatively uniform in species richness (Table 1).

The species-abundance distributions for each sample (Figure 1) show that only a few species were abundant (those accounting for more than 5% of the total isolates)

**Table 2** Abundance of fungi isolated from four samples of decaying leaves of *Guarea guidonia* and *Manilkara bidentata* in the Luquillo Mts, Puerto Rico. Species ordered by decreasing number of total isolates recovered

| Taxon | Samples | | | | Total isolates |
|---|---|---|---|---|---|
| | Gg1 | Gg2 | Mb1 | Mb2 | |
| *Pestalotiopsis maculans* (Corda) Nag Raj | 42 | 50 | 193 | 125 | 410 |
| *Volutella minima* Höhnel | 73 | 113 | 1 | 26 | 213 |
| *Idriella lunata* PE Nelson & K Wilheim | 9 | 138 | 9 | 12 | 168 |
| *Pestalotiopsis theae* (Saw) Stey | | 6 | 26 | 120 | 152 |
| Sterile fungus A | 49 | 51 | 5 | 12 | 117 |
| *Sclerophoma* sp | 8 | 19 | 34 | 48 | 109 |
| *Microsphaeropsis* sp A | 25 | 43 | 13 | 22 | 103 |
| *Colletotrichum acutatum* JH Simmonds | | 76 | | 2 | 78 |
| *Colletotrichum gloeosporioides* (Penz) Penz & Sacc in Penz | 7 | 22 | 33 | | 62 |
| *Phoma* sp B | | 57 | | | 57 |
| Unidentified hyphomycete F | 1 | | 18 | 36 | 55 |
| *Microsphaeropsis* sp B | 2 | 50 | | | 52 |
| Sterile fungus K | | | 50 | | 50 |
| *Phomopsis* sp | 19 | 13 | 16 | 1 | 49 |
| *Scolecobasidium constrictum* Abbott | 14 | 7 | 13 | 13 | 47 |
| *Trichoderma hamatum* (Bonord) Bainier | 20 | 16 | | 9 | 45 |
| *Verticillium* sect *albo-erecta* | | 37 | | 4 | 41 |
| *Cladosporium cladosporioides* (Fresen) De Vries | 8 | 15 | 9 | 5 | 37 |
| Sterile fungus L | | | 36 | | 36 |
| Unidentified coelomycete C | | 35 | | | 35 |
| *Beltraniella portoricensis* (F Stevens) Pirozynski & SD Patil | | | 32 | 2 | 34 |
| *Acremonium terricola* ser A | | 11 | 20 | | 31 |
| *Clonostachys* sp | 26 | | 1 | 1 | 28 |
| Unidentified coelomycete K | 28 | | | | 28 |
| *Chaetopsina* cf *fulva* Rambelli | | | 7 | 17 | 24 |
| *Chaetosticta* sp B | | | | 24 | 24 |
| Unidentified coelomycete D | | 24 | | | 24 |
| *Penicillium olsonii* Bain & Sartori | | | | 24 | 24 |
| Sterile fungus D | 7 | | 9 | 8 | 24 |
| *Volutella* sp | | 6 | | 17 | 23 |
| *Dendrodochium* sp A | | 21 | | 1 | 22 |
| *Penicillium soppii* Zaleski | | 10 | 2 | 9 | 21 |
| *Paecilomyces* sp A | 20 | | | | 20 |
| *Beltrania rhombica* O Penzig | | | 18 | 1 | 19 |
| *Dactylaria* sp C | 5 | 14 | | | 19 |
| Xylariales Group J | | 5 | 2 | 12 | 19 |
| *Penicillium multicolor* Grigorieva-Manoilova & Poradielova | 1 | 14 | | 3 | 18 |
| *Sarcopodium coffeanum* Nag Raj & George | 15 | 1 | 2 | | 18 |
| *Chaetosticta* sp A | 8 | 2 | | 7 | 17 |
| *Ramichloridium clavulisporum* Matsushima | 1 | 4 | | 10 | 15 |
| *Trichoderma harzianum* Rifai | 2 | 3 | 2 | 8 | 15 |
| *Acremonium* sp A | 6 | 6 | | 2 | 14 |
| *Acremonium* sp B | 5 | | | 9 | 14 |
| *Fusarium* sp A | | | | 14 | 14 |
| *Penicillium steckii* Zaleski | 4 | 8 | 2 | | 14 |
| Unidentified coelomycete A | | 4 | | 10 | 14 |
| *Aspergillus flavus* Link:Fr | 1 | | | 12 | 13 |
| *Mucor hiemalis* Wehmer | 5 | 5 | | 3 | 13 |
| *Mycoleptodiscus* sp A | | 12 | | | 12 |
| *Rosellinia* sp (anamorph) | 2 | 10 | | | 12 |
| *Xylaria* cf *multiplex* (Kunze) Fr sensu Dennis | 1 | 8 | 3 | | 12 |
| *Acremonium* sp C | 4 | | 7 | | 11 |
| *Leptographium* sp | 4 | 2 | 2 | 3 | 11 |
| *Paecilomyces* sp B | | 10 | 1 | | 11 |
| *Septomyrothecium uniseptata* Matsushima | 8 | 2 | 1 | | 11 |
| *Sesquicillium microsporum* (Jaap) Veenbas-Rijks & W Gams | 11 | | | | 11 |
| *Acremonium terricola* ser B | | 10 | | | 10 |
| *Aposphaeria* sp | | | 10 | | 10 |
| Xylariales Group H | 1 | 6 | 2 | 1 | 10 |
| *Acremonium* sp D | | | 4 | 5 | 9 |
| *Cyphellophora* cf *taiwanensis* Matsushima | | 8 | 1 | | 9 |
| *Gonytrichum chamydosporium* Barron & Bhatt | 1 | 8 | | | 9 |
| *Sesquicillium* sp A | 1 | | 7 | 1 | 9 |
| Unidentified hyphomycete E | | | 3 | 6 | 9 |

**Table 2** Continued

| Taxon | Samples | | | | Total isolates |
|---|---|---|---|---|---|
| | Gg1 | Gg2 | Mb1 | Mb2 | |
| *Exophiala* sp A | 2 | 5 | 1 | | 8 |
| *Microsphaeropsis* sp C | | 8 | | | 8 |
| *Septofusidium* sp | | 2 | 6 | | 8 |
| Xylariales Group B | 2 | 6 | | | 8 |
| *Acremonium* sp E | 3 | 4 | | | 7 |
| *Graphium penicilliodes* Corda | 2 | 4 | | 1 | 7 |
| *Linodochium* sp | | | 6 | 1 | 7 |
| Sterile fungus J | | 7 | | | 7 |
| Unidentified coelomycete B | | 7 | | | 7 |
| *Xylaria mellissii* (Berk) Dennis | 1 | 4 | 1 | 1 | 7 |
| *Nodulisporium* sp A | 3 | 1 | 1 | 1 | 6 |
| *Penicillium* sp nov A (JP3427) | 4 | 1 | 1 | | 6 |
| *Phoma* sp C | 2 | | 4 | | 6 |
| *Sphaeropsis* sp | 2 | 4 | | | 6 |
| Xylariales Group F | | 2 | 1 | 3 | 6 |
| *Ceuthospora* sp B | | 5 | | | 5 |
| *Dendrodochium* sp B | 4 | 1 | | | 5 |
| *Mortieriella ramanniana* (A Moller) Linnemann var *angulispora* (Naumov) Linnemann | | 5 | | | 5 |
| *Penicillium citrinum* Thom | | 3 | | 2 | 5 |
| *Rhinocladiella atrovirens* Nannf | | 5 | | | 5 |
| *Sporothrix* sp | 1 | 1 | 2 | 1 | 5 |
| Sterile fungus G | | 5 | | | 5 |
| Sterile fungus L | | | 5 | | 5 |
| *Triposporium elegans* Corda | | | 3 | 2 | 5 |
| Unidentified ascomycete A | | | 5 | | 5 |
| Unidentified coelomycete G | | 5 | | | 5 |
| *Verticillium* sect *prostrata* | | 1 | | 4 | 5 |
| Xylariales Group A | 3 | 1 | 1 | | 5 |
| Xylariales Group 1 | 2 | 3 | | | 5 |
| *Penicillium* sp nov B (JP3430) | 2 | 1 | | 1 | 4 |
| *Acrodontium crateriforme* (van Beyma) De Hoog | | | 2 | 2 | 4 |
| *Ceuthospora* sp A | 2 | 1 | 1 | | 4 |
| *Fusarium* cf *lateritium* Nees:Fr | 1 | 1 | | 2 | 4 |
| *Gliocladiopsis tenuis* (Bugnicourt) Crous & Wingfield | 1 | | 2 | 1 | 4 |
| *Gliocladium roseum* Bainier | 3 | | 1 | | 4 |
| *Mortieriella* sp | | | 4 | | 4 |
| *Myrothecium leucotrichum* (Peck) Tulloch | 1 | 3 | | | 4 |
| *Ophiostoma* sp | | 1 | 1 | 2 | 4 |
| *Penicillium radulatum* G Smith | | 4 | | | 4 |
| *Sesquicillium candelabrum* (Bonord) W Gams | 4 | | | | 4 |
| Sterile fungus N | 4 | | | | 4 |
| *Thozetella* sp | 2 | 1 | 1 | | 4 |
| *Tolypocladium* cf *inflatum* W Gams | 2 | 2 | | | 4 |
| Unidentified coelomycete H | | 4 | | | 4 |
| Unidentified hyphomycete A | | 4 | | | 4 |
| Unidentified hyphomycete H | | 1 | 3 | | 4 |
| *Verticillium* sp A | | | 2 | 2 | 4 |
| *Acremonium* cf *falciforme* (Carrión) W Gams | | 3 | | | 3 |
| *Acremonium* sp F | | 3 | | | 3 |
| *Cylindrosympodiella* sp | | 3 | | | 3 |
| *Dactylaria* sp A | 1 | 1 | 1 | | 3 |
| *Dactylaria* sp B | | | 3 | | 3 |
| *Fusarium solani* (Mart) Sacc | 2 | | | 1 | 3 |
| *Gliocladium viride* Matr | 3 | | | | 3 |
| *Glomerella* sp A | | | 3 | | 3 |
| *Hypoxylon* sp | | | 2 | 1 | 3 |
| *Menisporiopsis theobromae* Hughes | | 3 | | | 3 |
| *Microsphaeropsis* sp D | | 3 | | | 3 |
| *Mirandina* sp A | | 3 | | | 3 |
| *Mirandina* sp B | | 3 | | | 3 |
| *Mycoleptodiscus terrestris* (Gerd) Ostazeski | 1 | 2 | | | 3 |
| *Paecilomyces marquandii* (Massee) Hughes | 2 | 1 | | | 3 |
| *Penicillium kazachstanicum* Novobranova | | 2 | 1 | | 3 |
| *Phoma* sp A | | 3 | | | 3 |
| *Scolecobasidium* sp A | | | 3 | | 3 |

**Table 2** Continued

| Taxon | Samples | | | | Total isolates |
|---|---|---|---|---|---|
| | Gg1 | Gg2 | Mb1 | Mb2 | |
| *Stachybotryna* sp | 1 | 1 | | 1 | 3 |
| Sterile fungus K | | 3 | | | 3 |
| Sterile fungus B | | | | 3 | 3 |
| *Tilletiopsis* sp | | | | 3 | 3 |
| Unidentified coelomycete F | | 3 | | | 3 |
| *Verticillium psalliotae* Treschow | 1 | 2 | | | 3 |
| Xylariales Group C | 2 | | | 1 | 3 |
| Xylariales Group D | | 3 | | | 3 |
| Xylariales Group E | 1 | 1 | | 1 | 3 |
| *Acremonium* sp G | | 2 | | | 2 |
| *Acremonium* sp H | | 2 | | | 2 |
| *Botryosphaeria* sp A | | 2 | | | 2 |
| *Botryosphaeria* sp B | | 2 | | | 2 |
| *Chloridium lignicola* (Mangenot) W Gams & Hol-Jech | 2 | | | | 2 |
| *Chloridium phaeosporum* var *cubense* Hol-Jech | | 1 | 1 | | 2 |
| *Cladobotryum mycophilum* (Oudem) W Gams et Hoozem | 2 | | | | 2 |
| *Colletotrichum* sp | | 2 | | | 2 |
| *Fusarium decemcellularae* C Brick | | 1 | 1 | | 2 |
| *Heteroconium* sp | | | 2 | | 2 |
| *Paecilomyces carneus* (Duchen & Heim) AHS Brown & G Sm | | 1 | 1 | | 2 |
| *Paecilomyces* sp C | 1 | 1 | | | 2 |
| *Penicillium daleae* Zaleski | 1 | 1 | | | 2 |
| *Pseudobotrytis terrestris* (Timonin) Subram | | 2 | | | 2 |
| *Pseudorobillardia sojae* Uecker & Kulik | | 2 | | | 2 |
| *Ramichloridium* sp | | | 2 | | 2 |
| *Sagenomella verticilliata* Gams & Söderstrom | | 1 | | 1 | 2 |
| *Sesquicillium* sp B | | 2 | | | 2 |
| Sterile fungus C | | | | 2 | 2 |
| Sterile fungus E | | | | 2 | 2 |
| Sterile fungus F | | 2 | | | 2 |
| Sterile fungus H | | 2 | | | 2 |
| Sterile fungus I | | 2 | | | 2 |
| Sterile fungus M | | | 2 | | 2 |
| *Stibella* sp | 1 | | | 1 | 2 |
| *Thozetella havenensis* Casteñada-Ruiz | | | | 2 | 2 |
| *Torulomyces lagena* Delitsch | | 2 | | | 2 |
| *Tritriachrium* sp | 1 | | | 1 | 2 |
| Unidentified ascomycete B | 2 | | | | 2 |
| Unidentified coelomycete E | | 2 | | | 2 |
| Unidentified coelomycete I | | 2 | | | 2 |
| Unidentified coelomycete J | | | 2 | | 2 |
| Unidentified hyphomycete G | | 2 | | | 2 |
| *Verticimonosporium ellipticum* Matsushima | 2 | | | | 2 |
| *Actinostilbe* state of *Nectria flavolanata* Berkley & Broome | | 1 | | | 1 |
| *Alternaria* sp | | | 1 | | 1 |
| *Angulisopora* sp | | | | 1 | 1 |
| *Aspergillus janus* Raper & Thom | | 1 | | | 1 |
| *Aureobasidium* sp | | 1 | | | 1 |
| *Botryosporium longibrachiatum* (Oudem) Maire | | | 1 | | 1 |
| *Botryotrichum* sp | | 1 | | | 1 |
| *Chaetophoma* sp | | 1 | | | 1 |
| *Chaetopsina* sp A | | 1 | | | 1 |
| *Chaetopsina* sp B | | 1 | | | 1 |
| *Chaetopsina* sp C | | | 1 | | 1 |
| *Chalara cylindrosperma* (Cda) Hughes | | | 1 | | 1 |
| *Chloridium* sp | | | | 1 | 1 |
| *Circinotrichum maculiforme* CG Nees ex Persoon | | 1 | | | 1 |
| *Cladobotryum* sp | | | | 1 | 1 |
| *Clonostachys* sp | | | 1 | | 1 |
| *Coccomyces* sp | | | 1 | | 1 |
| *Coniothyrium* sp | | 1 | | | 1 |
| *Cryptophiale* sp | | | | 1 | 1 |
| *Cylindrocarpon ianothele* Wollenweber | 1 | | | | 1 |
| *Cylindrocarpon* sp | | | | 1 | 1 |
| *Cytospora* sp | | | | 1 | 1 |

**Table 2** Continued

| Taxon | Samples | | | | Total isolates |
|---|---|---|---|---|---|
| | Gg1 | Gg2 | Mb1 | Mb2 | |
| *Dactylella* sp | | | 1 | | 1 |
| *Dendrodochium* sp C | | | | 1 | 1 |
| *Dendrodochium* sp D | | 1 | | | 1 |
| *Dendrodochium* sp E | | 1 | | | 1 |
| *Dendrodochium* sp F | | | 1 | | 1 |
| *Dicrandion fragile* Harkn | | | | 1 | 1 |
| *Eriocercophora balladynae* (Hansf) Deighton | | | | 1 | 1 |
| *Exophiala jeanselmii* (Langer) McGinnis & Padhye | | 1 | | | 1 |
| *Exophiala* sp B | | 1 | | | 1 |
| *Exophiala* sp C | | | | 1 | 1 |
| *Fusarium* sp B | | | | 1 | 1 |
| *Fusarium* sp C | | | 1 | | 1 |
| *Fusarium* sp D | | 1 | | | 1 |
| *Fusarium* sp E | | 1 | | | 1 |
| *Gliocladium* sp | | | | 1 | 1 |
| *Haplographium* sp | 1 | | | | 1 |
| *Hyalodendron* sp | | | 1 | | 1 |
| *Idriella* cf *ramosa* Matsushima | | | 1 | | 1 |
| *Idriella* sp A | | | | 1 | 1 |
| *Idriella* sp B | | | 1 | | 1 |
| *Isthmolongispora minima* Matsushima | | | | 1 | 1 |
| *Kazulia vagans* (Speg) Nag Raj | | | | 1 | 1 |
| *Leptodiscella* sp | | 1 | | | 1 |
| *Leptosphaeria* sp | 1 | | | | 1 |
| *Libertella* sp | | 1 | | | 1 |
| *Microdochium* sp | | | | 1 | 1 |
| *Monieliella* sp | | 1 | | | 1 |
| *Mortieriella isabellina* Oudem | | 1 | | | 1 |
| *Mycena* sp | 1 | | | | 1 |
| *Mycoenterlobium platysporum* Goos | | 1 | | | 1 |
| *Mycoleptodiscus* sp B | | 1 | | | 1 |
| *Naemania* or *Hypoxylon* sp | | 1 | | | 1 |
| *Nectria rugulosa* Patouillard & Gaillard | | | 1 | | 1 |
| *Sesquicillium* state of *Nectria* sp | | 1 | | | 1 |
| *Nodulisporium* sp B | | | | 1 | 1 |
| *Nodulisporium* sp C | | 1 | | | 1 |
| *Nodulisporium* sp D | | 1 | | | 1 |
| *Nodulisporium* sp E | | | 1 | | 1 |
| *Nodulisporium* sp F | | | | 1 | 1 |
| *Papularia* sp | | | | 1 | 1 |
| *Penicillium janczewski* Zaleski | | 1 | | | 1 |
| *Penicillium* sp C | | | | 1 | 1 |
| *Phaeoisaria clematidis* (Fuckel) Hughes | | | | 1 | 1 |
| *Phialamonium* sp A | | 1 | | | 1 |
| *Phialamonium* sp B | | | | 1 | 1 |
| *Phialophora clavispora* Gams | | 1 | | | 1 |
| *Phialophora* sp A | | 1 | | | 1 |
| *Phialophora* sp B | | 1 | | | 1 |
| *Phialophora* sp C | | 1 | | | 1 |
| *Phialophora* sp D | | | 1 | | 1 |
| *Phialophora* sp E | | 1 | | | 1 |
| *Phialophora* sp F | | | 1 | | 1 |
| *Phialophora* sp G | | | | 1 | 1 |
| *Polycephalomyces* sp | | 1 | | | 1 |
| *Ramichloridium* sp | | | 1 | | 1 |
| *Ramichoridium* cf *schulzeri* (Sacc) de Hoog | | | 1 | | 1 |
| *Ramularia* sp | | 1 | | | 1 |
| *Rhinocladiella* sp | | | 1 | | 1 |
| *Scolecobasidium* sp | | 1 | | | 1 |
| *Trinacrium gracile* Matsushima | | | 1 | | 1 |
| Unidentified ascomycete C | | | | 1 | 1 |
| Unidentified ascomycete D | | 1 | | | 1 |
| Unidentified filamentous yeast | | | | 1 | 1 |
| Unidentified hyphomycete C | | 1 | | | 1 |
| Unidentified hyphomycete D | | 1 | | | 1 |
| Unidentified hyphomycete B | | 1 | | | 1 |

**Table 2** Continued

| Taxon | Samples | | | | Total isolates |
|---|---|---|---|---|---|
| | Gg1 | Gg2 | Mb1 | Mb2 | |
| Unidentified hyphomycete G | | 1 | | | 1 |
| *Verticillium catenulatum* (Kamyschko ex Barron & Onions) W Gams | | 1 | | | 1 |
| *Verticillium* cf *aranearum* (Petch) W Gams | | 1 | | | 1 |
| *Verticillium* sp B | | 1 | | | 1 |
| *Verticillium* sp C | | 1 | | | 1 |
| *Verticillium* sp D | | 1 | | | 1 |
| *Verticillium* sp E | | 1 | | | 1 |
| *Volucrispora aurantiaca* Haskins | | | | 1 | 1 |
| *Xenobotrytis* sp | | | | 1 | 1 |
| *Xylaria allantoidea* (Berk) Fr | | 1 | | | 1 |
| *Xylaria coccophora* Mont | | 1 | | | 1 |
| Xylariales Group G | | 1 | | | 1 |
| *Zalerion* sp | | | | 1 | 1 |
| Individual sterile isolates | 23 | 27 | 16 | 16 | 82 |
| Individual coelomycetes | 15 | 22 | 9 | 11 | 57 |
| Individual *Acremonium* spp | 26 | 7 | 9 | 15 | 52 |
| Totals | 591 | 1259 | 726 | 761 | 3337 |

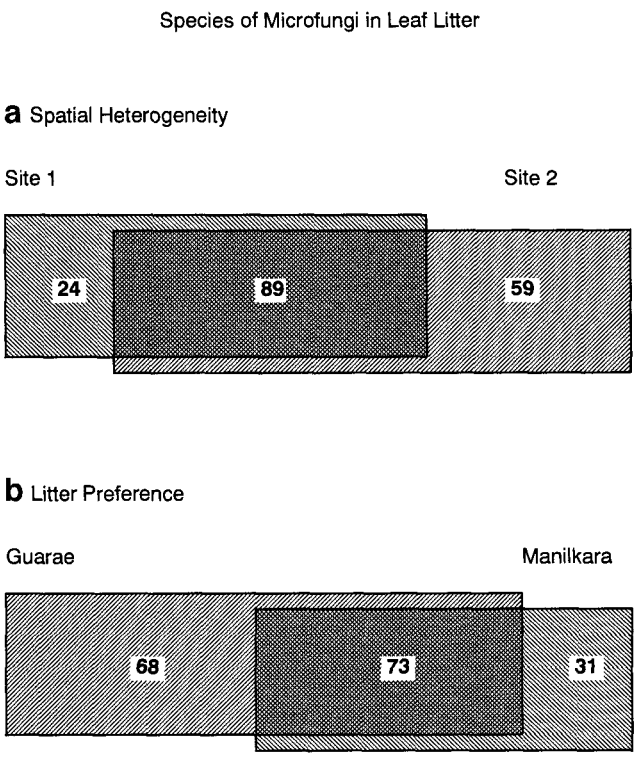


**Figure 2** Complementarity of species composition of microfungi obtained by particle filtration occurring at least twice in samples of leaf litter. (a) Spatial heterogeneity is indicated by species of fungi occurring in only one of the two sample sites, located 200 m apart (number of species unique to each site and common to both sites are shown in boxes). (b) Substrate preference is indicated by fungi occurring in only one of the two leaf litter species (number of fungal species unique to each litter type, or common to both leaf species are shown in boxes).

and a high proportion of species were encountered rarely. Among the four litter samples, the single most abundant species accounted for only 26% (Mb1) of the total isolates from the sample (Figure 1, Table 2). All samples yielded many species of intermediate abundance (4%–0.5% of the total isolates) as well as many rare isolates that occurred only once.

Species isolated from all litter samples were *Cladosporium cladosporioides*, *Idriella lunata*, *Leptographium* sp, *Microsphaeropsis* sp, *Nodulisporium* sp, *Pestalotiopsis maculans*, *Phomopsis* sp, an unidentified sterile fungus, *Sclerophoma* sp, *Scolecobasidium constrictum*, *Trichoderma harzianum*, *Sporothrix* sp, *Volutella minima*, *Xylaria mellisii*, and an unidentified xylariaceous anamorph (Table 2).

Abundant species found in two or more of the samples and with an abundance of at least 1% of the total isolates include *Beltraniella portoricensis*, *Colletotrichum acutatum*, *C. gloeosporioides*, an unidentified sporodochial hyphomycete, a second *Microsphaeropsis* sp, *Pestalotiopsis theae*, *Trichoderma hamatum*, and *Verticillium* sp sect *albo-erecta*. Although the four litter samples shared many species, each was distinct with respect to the species composition and to the relative abundance among species (Figure 1, Table 2).

Pairwise comparisons of similarities in species composition of the 172 fungal species that were isolated at least twice by particle filtration were calculated for both leaf species at both sites. Although no clear-cut pattern of association emerged, samples of the same litter species in different areas tended to have higher similarities (JI = 0.38 and 0.34) than samples of different leaf species from the same area (JI = 0.32 and 0.26). In descending order of similarity were samples of: *G. guidonia* collected at different sites (JI = 0.38); *M. bidentata*/site 2 – *G. guidonia*/site 1

(JI = 0.37); *M. bidentata* collected at different sites (JI = 0.34); *M. bidentata* and *G. guidonia* at site 1 (JI = 0.32); *M. bidentata*/site 1 – *G. guidonia*/site 2 (JI = 0.27); *M. bidentata* and *G. guidonia* at site 2 (JI = 0.26). Pairwise complementarity between fungal species isolated at least twice in this study ranged from 68–74% (26–32% overlap) among samples of different leaf species collected at the same site. Complementarity shows that less overlap in fungal species composition occurred between leaf species (Figure 2b) than the two collection sites (Figure 2a).

### *Direct observation in moist chambers*

Replicate samples of litter used for particle filtration, incubated in moist chambers for 8 weeks, yielded 24 species of fungi, mostly hyphomycetes (Table 3). In contrast to results with particle filtration, more species sporulated on litter of *M. bidentata* from both sites than on litter of *G. guidonia*. *Idriella lunata*, *Menisporiopsis theobromae*, *Verticillium* spp, and *V. minima* fruited in all moist chambers on both species. *Beltrania rhombica*, *Beltraniella portoricensis*, *Chaetopsina* cf *fulva*, and *Linodochium* sp were abundant on leaves of *M. bidentata* at both sites. Nearly all species could be identified to at least genus, because unlike artificially cultivated isolates obtained by particle filtration, we observed normal sporulating structures as described in identification guides. We suspect that many of the unidentifiable species in Table 3 are undescribed. Of the 24 species, 19 (79%) were isolated at least once by particle filtration. Highest similarities as measured by Jaccard indices were between samples of the same species from different sites (*M. bidentata*, JI = 0.44; and *G. guidonia*, JI = 0.50), while indices of similarity between different species within each site were lower (JI = 0.28 and 0.25 at sites 1 and 2, respectively).

## Discussion

Data from isolations which involved the moist chamber suggest a significant degree of substrate preference among microfungal decomposers on leaf litter, supporting what microfungal taxonomists intuitively have long known [7,13]. Thus, collectors looking for diversity of fungal decomposers should examine as many different leaf litter species as possible. Based on our limited number of samples, fungal preferences for particular leaf species probably contributed significantly to the high diversity of saprobic fungi that was previously found in individual 0.2-$m^2$ samples of mixed litter of a wet tropical forest in Costa Rica [2]. Fungal substrate preferences may have also contributed to the very low overlap in species composition among samples of the same plant species taken from different sites in the same study [3] (15–28% species overlap; [9]). For fungal species isolated at least twice in this study, we found 26–32% overlap (ie 68–74% complementarity) in species composition among samples of different leaf

**Table 3** Fungi observed fruiting on leaves of *Manilkara bidentata* (Mb) and *Guarea guidonia* (Gg) in humid chambers[a]. Leaves are from two sites in the Luquillo Mts, Puerto Rico

| Taxon | Sample | | | | Isolated from particles[b] |
|---|---|---|---|---|---|
| | Mb1 | Gg1 | Mb2 | Gg2 | |
| *Beltrania rhombica* | 1 | | 1 | | yes |
| *Beltraniella portoricensis* | 1 | | 1 | | yes |
| *Chaetopsina* cf *fulva* | 1 | | 1 | | yes |
| *Coccomyces* sp | | | 1 | | yes |
| *Cryptophiale* sp | 1 | 1 | | | yes |
| *Cryptophiale udagawae*[c] Pirozynski & Ichinoe | 1 | | | | unsure |
| *Gliocladiopsis tenuis* | 1 | | | | yes |
| *Idriella lunata* | 1 | 1 | 1 | 1 | yes |
| *Lauriomyces pulchra* Castañeda-Ruiz & Kendrick | | | 1 | | no |
| *Linodochium* sp | 1 | | 1 | | yes |
| *Menisporiopsis theobromae* | 1 | 1 | 1 | 1 | yes |
| *Myrothecium leucotrichum* | | | | 1 | yes |
| *Pestalotiopsis maculans* | 1 | | | | yes |
| *Polyscylatum* sp | 1 | | | | no |
| *Polyscytalina* sp | | | 1 | | no |
| *Septomyrothecium uniseptata* | | | | 1 | yes |
| *Sesquicillium* sp (citrine) | | 1 | | | yes |
| *Stachybotryna* sp | | 1 | | 1 | yes |
| *Thozetella* sp | 1 | | | | yes |
| *Trichoderma hamatum* | | 1 | | 1 | yes |
| Unidentified cupulate hyphomycete | 1 | | | | yes |
| *Verticillium* spp | 1 | 1 | 1 | 1 | yes |
| *Virgatospora echinofibrosa* Finley | | | | 1 | no |
| *Volutella minima* | 1 | 1 | 1 | 1 | yes |
| Total taxa observed | 15 | 8 | 11 | 9 | |

[a] Leaves were incubated 8 weeks at ambient temperature and light in plastic boxes. See methods.
[b] Isolated at least once by particle filtration. Compare species list with Table 2.
[c] Does not sporulate or form distinctive colonies when cultured from conidia. Therefore we were uncertain if it was isolated from particles.

species collected at the same site. Such low levels of overlap among samples indicates that many fungal species were missed, thereby underestimating the total diversity of microfungi at the site by an unknown amount [5,9]. However, the high overlap in species composition we found between samples of the same leaf species at different sites (34–38% overlap, 62–66% complementarity) suggest one or two additional samples of the same leaf species would have produced a fairly complete inventory for that tree species. Furthermore, by limiting the number of leaf species to two, and using closely matched replicate sites, we found a 52% overlap in total species complementation between sites — or, close to the ideal sampling intensity. Thus, a complete inventory of microfungi in decomposing leaves might be approached by sampling leaf species individually, while recognizing that there is a high degree of overlap among common saprobic microfungi.

Few techniques work well for determining species richness in highly diverse groups of organisms [5,9]. Complementarity analyses [9] might be more useful than species discovery curves in designing sampling regimes for determining the diversity of saprobic microfungi in tropical forests if each leaf type is inventoried separately. We suspect that some fungi that appear to be host-specific are not, but could be found on leaves of other species with similar structure, texture, or chemistry (ie functional groups). Extensive sampling of leaf species would be needed to test this hypothesis.

In addition to leaf species-specificity, we found a larger-scale spatial component of heterogeneity that contributed to differences in microfungal composition of samples collected 200 m apart (Figure 2a). Therefore, collectors of these fungi in tropical forests can increase the total number of species in their samples by collecting litter from widely separated sites. We have observed similar variation in fungi associated with litter of the same plant species collected 50 m apart [3], and among mixed litter samples collected 1 km apart in Costa Rica [2]. Similarly, in dry to mesic conifer-hardwood forests in Wisconsin the principal microfungi of soil were highly restricted in occurrence among stands, but there was little spatial heterogeneity of microfungi in prairie soils [4].

The levels of species richness in this study were comparable to those recovered from mixed primary forest leaf litter or from decayed leaves of *H. mariae*, a monocot, in Costa Rica where $E_{s(200)}$ of litter samples ranged between 40–84 species (compare with Table 1). The distribution of abundance among species recovered from decayed *G. guidonia* and *M. bidentata* leaves yielded the same patterns as in previous studies in humid tropical forest litter [2,3] and are consistent with our experiences isolating microfungi by particle filtration from humid tropical forests worldwide. Likewise, the overall floristic composition was similar to that obtained from leaf litter in Costa Rica [2,3]. Hyphomycetes and coelomycetes were the most abundant types of fungi isolated as well as observed directly on leaves. The mycota of tropical leaf litter appears to be highly varied and extensive, although some consistent features are evident. Certain cosmopolitan and pantropical species eg *Beltrania rhombica*, *B. portoricensis*, *C. cladosporioides*, *Colletotrichum gloeosporioides*, *Fusarium solani*, *Gliocladium roseum*, *Idriella lunata*, *Paecilomyces marquandii*, *Lasiodiplodia theobromae*, *Scolecobasidium constrictum*, *M. hiemalis*, *Pestalotiopsis maculans*, *Phomopsis* spp, *Trichoderma* spp *V. minima*, and *Weisneromyces laurinus* are inevitably isolated, even during cursory examination, from nearly any litter collection from humid tropical forests. The genera *Chaetopsina*, *Chloridium*, *Clonostachys*, *Cryptophiale*, *Dactylaria*, *Dictyochaeta*, *Myrothecium*, *Scolecobasidium*, *Stachybotrys*, and many anamorphs of Hypocreales and Xylariales are richly represented in the mycota of tropical litter, and their isolation is favored by the particle filtration method. Superimposed on these ubiquitous elements, a high degree of regional or local endemism probably exists, as evidenced by high numbers of species described from limited collections or specific for hosts of restricted geography. A group of species is frequently isolated in Puerto Rico, Costa Rica, and southern Mexico (Bills and Polishook, unpublished data) or have been reported from Cuba, including *Chloridium phaeosporum* var *cubense*, *Cylindrosympodiella* sp, *Cyphellophora* cf *taiwanensis*, *Thozetella havenensis*, *Lauriomyces pulchra*, and *Solheimia costaspora*; these fungi may be characteristic of a Caribbean litter mycota. Except for some of the xylariaceous and *Phomopsis* species, the endophytic fungi found in healthy leaves of *M. bidentata* [10] were not generally recovered from the decomposing leaves of that species, and vice versa.

Reliance on isolation methods for evaluating fungal diversity has some serious limitations in that many fungi will not sporulate once established in culture, hence use of identification methods based on sporulating structures may be impossible. Additionally, the simultaneous isolation of hundreds of species of fungi can present formidable difficulties for identification. In this study, we found it difficult to characterize all the species. The problem was exacerbated because basic taxonomic survey data are lacking for saprobic microfungi of Central America; thus to carry out the identifications we were forced to rely on regional floristic works from Asia, Africa, Cuba, on general identification manuals, and on specialized monographs.

## Conclusions

Based on our results, sampling design to capture the greatest diversity of microfungi for the collection of litter decomposers in humid tropical forests should include litter of many different tree species or leaf types, as well as samples collected at widely spaced sites. For a single time-point sample, many more species of microfungi were recovered from leaf litter using particle filtration rather than moist chambers. The use of complementarity analysis can overcome some of the difficulties in the design of sampling regimes for determining the high biodiversity of microfungi of tropical leaf litter.

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