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Fungal communities associated with *Eugenia uruguayensis* (Myrtaceae) leaf litter in early stages of decomposition in Uruguay

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Saprobic communities colonizing freshly fallen leaves of *Eugenia uruguayensis* were recovered using incubation of leaf fragments in culture medium and incubation of leaves in moist chambers. Data analysis included species-accumulation curves integrating rarefaction and extrapolation, similarity estimation and diversity assessed with Hill numbers. Our results indicated that both techniques were complementary with regard to species composition, recovering 75 different fungal taxa in total. Communities associated with petioles and blades were significantly different, in contrast to what was previously found for the corresponding endophytic communities from *E. uruguayensis*, suggesting a possible involvement of the abscission process in the community differentiation. One third of the endophytic species were also recovered from freshly fallen leaves, leading us to think they might be completing their life cycle in leaf litter.

Keywords: saprobes, endophytes, petiole, blade, diversity.

Decomposition of plant litter is a key process with regard to nutrient cycling and humus formation in forest soils (Berg & McClaugherty 2008), and is considered to be carried out mainly by saprobic fungi (Kjoller & Struwe 1992, Schneider et al. 2012,Van der Wal et al. 2013). Thus, a full understanding of the diversity and dynamics of fungal communities developing in dead plant material is essential for the identification of factors affecting ecosystem functioning in natural forests. This is particularly relevant in understudied geographic regions (Hawksworth 1991).

Fungal community assembly in plant litter is considered to be mainly driven by the physical and chemical characteristics of the substrate (Polishook et al. 1996). In fact, different litter types support characteristic fungal communities, with plant species identity being a determinant factor (Polishook et al. 1996, Paulus et al. 2006, Hyde et al. 2007). Also, successional changes accompanying the decay process are well documented (e.g. Hudson 1968, Frankland 1992, Voříšková & Baldrian 2013), with distinct pioneer, mature and impoverished fungal communities being recognized (Promputtha et al. 2002, Seephueak et al. 2010, Shanthi & Vittal 2010).

One particular issue that has drawn the researchers' attention in recent years is the role of endophytic fungi in litter decomposition, since it has been hypothesized that they become saprobes after the onset of host tissue senescence (Promputtha et al. 2007, Osono & Hirose 2009, Purahong & Hyde 2011, Yuan & Chen 2014). This would address the dilemma on how these fungi complete their life cycles, and also raises the question of how involved they are in litter mass loss in natural ecosystems (Osono 2006, Hyde & Soytong 2008, Yuan & Chen 2014). While several studies have demonstrated a versatile enzymatic capacity for many endophytic strains, being able to decompose all the major molecular constituents from litter (Koide et al. 2005, Promputtha et al. 2010, Sun et al. 2011, Bettucci & Tiscornia 2013, Yuan & Chen 2014), some other studies have shown that the exclusion of endophytes from litter did not affect the rate of litter mass loss (Koide et al. 2005, Osono 2005). Therefore, it is likely that their involvement in decomposition depends on their persistence in litter through the decay process (Osono 2006).

To our knowledge, there are no studies dealing with litter fungal diversity conducted in Uruguay. Nevertheless, the endophytic communities associated to different tissues of native Myrtaceae have been recently described, particularly for *Eugenia uruguayensis* (Tiscornia et al. 2012, Tiscornia &

Bettucci 2014). This evergreen tree is distributed in tropical and subtropical forests in the south-eastern region of South America (Brazil, Uruguay, Paraguay and Argentinean northeast) (Landrum 1981). It can be found in riparian and ravine zones, growing on various soil types with the exception of saline soils, and being able to withstand droughts and short term floods (Landrum 1981).

The aim of this study was to describe the fungal communities associated with *E. uruguayensis* leaf litter in the early stages of decomposition and to make comparisons with previously described endophytic communities (Tiscornia et al. 2012).

Materials and methods

Study area

The sampling site is located at the park Parador Tajes (34°36′28″ S, 56°28′20″ W) that forms part of the protected wetlands of the Santa Lucía river (Canelones, Uruguay). On the margins of the river a forest can be found with several plant species, including *Eugenia uruguayensis* (for a detailed list see Tiscornia et al. 2012). Although Uruguay belongs to the subtropical region of South America, its climate is considered to be temperate and humid, with a marked seasonality, but without a dry season. The annual average precipitation is 1300 mm and the annual average temperature is nearly 17.5°C (Strahler & Strahler 1992). Precipitations have an irregular distribution along the year in Uruguay.

Collection of leaves and fungal isolation

Freshly fallen leaves of *E. uruguayensis* were collected in April of 2012 from the litter of several trees located throughout the park. The leaves were taken to the laboratory in sealed plastic bags, stored at 5 °C and processed within 24 h.

Two complementary methods were used to reveal the early saprobic community: fragment plating on culture medium (FP) and incubation in moist chambers (MCH). This is specially recommended since each technique imposes a particular filter to fungal diversity (Frankland et al. 1990, Cannon & Sutton 2004, Unterseher & Schnittler 2009).

A hundred leaves were washed under tap water, then with sterile water and finally air-dried at room temperature (ca. 20 °C). For FP, a 6 mm in diameter blade segment (avoiding the midrib) and a 5 mm petiole segment were cut from every leaf. Segments from each tissue were pooled and surface sterilized by sequential immersion in 80 % ethanol for 1 min, in 4 % NaOCl for 2 min, and finally rinsed in sterile water. Surface sterilization allows the growth of fungi from internal tissues by removing fungal propagules from the leaf surface (Osono & Takeda 1999). A hundred surface-sterilized blade segments and 83 surface-sterilized petiole segments were recovered and plated on maltose extract agar (Malt Extract 20g/L, Agar 20g/L) supplemented with antibiotic (Chloramphenicol, 150 mg/L) (10 segments per Petri dish). Plates were then incubated at 24 °C for 2 weeks and emerging colonies were transferred to fresh medium (MEA). Other media were used when required for later identification, as Potato dextrose agar, PDA (Potato Extract 4g/L, Dextrose 20g/L, agar 15g/L) and Carnation Leaf Agar, CLA (pieces of sterile dry Carnation Leafs, agar 20 g/L). To test the effectiveness of surface sterilization, imprints of all segments were performed on culture medium. For MCH, 38 leaves, including a blade of nearly 4–5 × 2–2.5 cm and a petiole of 5–7 mm, were subjected to the same surface-sterilization protocol and were then incubated in pairs in 19 sterile moist chambers (Keyworth 1951) for a month. Then, the complete adaxial and abaxial surface of leaves was tape-lifted and mounted on slides for direct microscopic observation and identification of fruiting bodies.

Fungal identification

Morphological identification by means of conventional mycological methods was the preferred choice for identifying the isolates obtained from FP, and the only choice for identifying fruiting bodies grown in MCH. Molecular identification was also attempted for non-sporulating isolates from FP and other isolates of inconclusive morphological identification. In those cases, DNA extraction was performed following the protocol described by Lee & Taylor (1990). Amplification of the ITS region, including both internal transcribed spacers and the 5.8 S gene from rDNA, followed the protocol described by Rehner & Uecker (1994) using the fungal specific primers ITS4 and ITS5 (White et al. 1990). Amplicons were then sequenced by MACROGEN (Seoul, South Korea). Finally, taxonomic affiliation of sequences was assessed by conducting a BLAST search against all the reference databases present in MycoBank (http://www.mycobank.org/). In cases in which multiple matches were equally probable, the lowest taxonomic level shared by those matches was recorded. As GenBank remains an incomplete database for fungi, some isolates were named only by the corresponding genus, or were even left as sterile mycelia.

Data analysis

The leaf segments were the sampling units for the culture technique and the pair of leaves for the moist chamber technique. The incidence (presence) of each taxon in each sampling unit was recorded for each category (petiole, blade, leaves). The weight of each taxon in each community was expressed as an incidence frequency (i.e. the sum of incidences per category) in percentage (Chao et al. 2014).

Species accumulation curves integrating rarefaction and extrapolation were carried out using software iNEXT 1.0 (Hsieh et al. 2013) following the recommendations of Colwell et al. (2012). Curves of species richness estimates for a rarefied and extrapolated sample were plotted with respect to sample size on the one hand, and sample coverage on the other. Sample completeness (as measured by sample coverage, from 0 to 1) was also plotted with respect to sample size according with Chao & Jost (2012). For all the curves, 95 % confidence intervals were calculated based on a bootstrap method with 160 replications. For a pair of curves, a non-overlap between intervals was interpreted as a significant difference in the richness of their corresponding communities (Colwell et al. 2012). Comparisons were made in the coverage-based accumulation curve, as recommended by Chao & Jost (2012).

Goodness of fit to the log normal and the log series models were assessed for both distributions using Chi-square with software PAST to know which one provides a better description of the data (Magurran 2004). Non-parametric species richness estimator Chao2 was applied using EstimateS 9.1.0 (Colwell 2013) since it is specially recommended for fungal diversity studies (Unterseher et al. 2008). The estimation is considered informative only when it stabilizes at the reference sample size (Unterseher et al. 2013). Diversity was assessed with PAST using Hill numbers from order one (D_1) and two (D_2), corresponding to the exponential of Shannon entropy and the inverse Simpson concentration, respectively (Hill 1973, Jost 2006). For a friendlier representation of diversity, Hill numbers for each community were integrated in diversity profiles, which not only enable comparisons but also give an insight into the evenness of each community (Leinster & Cobbold 2012). Hill's evenness was also calculated as $E' = D_1/D_2$ (Hill 1973).

Analysis of similarities (ANOSIM) was performed to determine whether *a priori* defined categories (blade and petiole) sustain truly different fungal communities. ANOSIM calculates a *p*-value that is generally accepted as indicating significant differences among groups when below 0.05, and R-value that expresses the degree of dissimilarity (Chapman & Underwood 1999). Similarity percentage analysis (SIMPER) was also performed to know which species contributed more to the differences observed with ANOSIM. Both analyses were conducted with software PAST (Hammer et al. 2001). For the FP technique, sampling units of each category were randomly gathered together in groups of five before performing these analyses, keeping information in terms of presence-absence.

Similarity indices were performed to compare significantly different communities using EstimateS 9.1.0 (Colwell 2013). Chao-Sorensen similarity estimator was used to compare fungal communities associated to petioles and blades. This estimator is recommended for species-rich assemblages and takes into account unseen species, reducing undersampling bias (Chao et al. 2005). The classical Sorensen similarity index was chosen to compare communities obtained here with FP and MCH, because it only takes into account qualitative information.

Results

A total number of 75 different fungal taxa were found to colonize internal tissues of freshly fallen leaves from *Eugenia uruguayensis* with both the FP and the MCH techniques (Tab. 1). Only 13 taxa were recovered with both techniques. In fact, when both inventories were compared using the classical Sorensen similarity index, a value of 0.33 emerged, indicating little overlap between them.

With the FP technique, 203 isolates were obtained from 183 leaf segments and were assigned to 51 taxa. Some taxa were identified or confirmed by means of molecular techniques (Tab. 2). Seven morphotypes were classified as hyaline or dark sterile mycelia. All isolates were found to be asexual morphs from Ascomycota, except for one Oomycete (assigned to Peronosporaceae).

The analysis of similarities (ANOSIM) revealed that petiole and blade associated fungal communities were significantly different but overlapped to some degree ($R = 0.66$; $p \leq 0.01$), something that can also be interpreted from the value of 0.50 given by the Chao-Sorensen similarity estimator. Some descriptive characteristics from each community as the number of isolates, species, singletons, Chao2 estimations of richness and measures of diversity can be found in Tab. 3.

Sampling could not be considered to be complete for the petiole and blade assemblages, as can

Tab. 1. Species list and incidence frequency of fungi associated with freshly fallen leaves of *E. uruguayensis*, obtained using a fragment plating technique (FP) and a moist-chamber technique (MCH).

Taxa*	Incidence frequency (%)		
	FP		MCH
	Petiole	Blade	Leaf
Alternaria alternata (Fr.) Keissl.	6	3	37
Aureobasidium pullulans (de Bary & Löwenthal) G. Arnaud	1		
Bartalinia robillardoides Tassi		1	
Beltrania rhombica Penz.	2		16
Beltraniella portoricensis (F. Stevens) Piroz. & S.D. Patil *	2	2	79
Beltraniella sp. *		8	
Blastomyces dermatitidis Gilchrist & W.R. Stokes			16
Calonectria insularis C.L. Schoch & Crous	4	7	21
Ceratocystis sp. Ellis & Halst.			5
Cercophora samala Udagawa & T. Muroi			11
Chaetomium funicola Cooke			5
Chloridium botryoideum (Corda) S. Hughes			5
Cladoriella eucalypti Crous*	1		
Cladosporium cladosporioides (Fresen.) G.A. de Vries	4	2	11
Cladosporium sp. Link			5
Clonostachys rosea (Link) Schroers, Samuels, Seifert & W. Gams			32
Coleophoma crateriformis (Durieu & Mont.) Höhn.		8	
Coleophoma empetri (Rostr.) Petr.	1	7	26
Colletotrichum dematium (Pers.) Grove		2	21
Colletotrichum gloeosporioides (Penz.) Penz. & Sacc.			16
Coniella castaneicola (Ellis & Everh.) B. Sutton	2	2	5
Coniothyrium sp. Corda	1		5
Corynascus sepedonium (C.W. Emmons) Arx			16
Cytospora chrysosperma (Pers.) Fr.			5
Dactylaria eucalypti Vittal & Dorai			37
Diaporthe foeniculacea Niessl*		9	
Diaporthe infecunda R.R. Gomes, C. Glienke & Crous*	1		
Diaporthe oxe R.R. Gomes, C. Glienke & Crous*	2		
Diaporthe sp. 1 Nitschke*	2		
Diaporthe sp. 2 Nitschke*	1	1	
Diaporthe sp. Nitschke			32
Diaporthe terebinthifolii R.R. Gomes, C. Glienke & Crous*	23	2	
Dictyochaeta assamica (Agnihothr.) Aramb., Cabello & Mengasc.			37
Dinemasporium sp. Lév.			5
Discosia strobilina Lib. ex Sacc.			5
Epicoccum nigrum Link	2	2	
Fusarium dlaminii Marasas, P.E. Nelson & Toussoun	1		
Fusarium incarnatum (Desm.) Sacc.	13		
Fusarium oxysporum Schltdl.	1		
Fusarium sambucinum Fuckel	11		

Taxa*	Incidence frequency (%)		
	FP		MCH
	Petiole	Blade	Leaf
Fusarium sp. Link			16
Fusarium tricinctum (Corda) Sacc.	1		
Leptostroma sp. Fr.*		1	
Mariannaea elegans (Corda) Samson			5
Microsphaeropsis arundinis (S. Ahmad) B. Sutton*	1		
Mycoleptodiscus sp. Ostaz.			37
Paraconiothyrium fungicola Verkley & Wicklow*	1	1	
Paraconiothyrium sp. Verkley*	7	1	
Periconia byssoides Pers.			5
Peronosporaceae sp. de Bary*		1	
Pestalotiopsis guepinii (Desm.) Steyaert	2	16	53
Phacidium sp. Fr.*		2	
Phaeosphaeria sp. I. Miyake*	1		
Phialemonium sp. W. Gams & McGinnis	1		
Phoma sp. Sacc.	21		
Phyllosticta eucalyptorum Crous, M.J. Wingf., F.A. Ferreira & Alfenas	1		
Pseudoseptoria donacis (Pass.) B. Sutton			11
Ramichloridium australiense Arzanlou & Crous			5
Sarocladium kiliense (Grütz) Summerb.			16
Seimatosporium dilophosporum (Cooke) B. Sutton		2	
Sordaria fimicola (Roberge ex Desm.) Ces. & De Not.			11
Sordariomycetes sp. O.E. Erikss. & Winka*	1		
Sphaeropsis sp. Raf.*	4		
Sporidesmium dioscoreae M.B. Ellis			5
Stemphylium botryosum Sacc.	1		
Sympodiella sp. W.B. Kendr.			5
Trichoderma reesei E.G. Simmons	1		
Wiesneriomyces laurinus (Tassi) P.M. Kirk			32
Xylaria sp. Hill ex Schrank		3	5
Zetiasplozna acaciae Crous*	1		
Hyaline sterile mycelium 1	2		
Hyaline sterile mycelium 2	2	1	
Dark sterile mycelium 1		2	
Dark sterile mycelium 2	1		
Dark sterile mycelium 3		1	
Dark sterile mycelium 4	1		
Dark sterile mycelium 5	2		
Number of sampling units (segments for FP; moist chambers for MCH)	83	100	19

* Species identified or confirmed by molecular methods

Tab. 2. List of fungi identified or confirmed by molecular methods (ITS).

Taxa	Accession number	Nearest match	Database	Similarity (%)	Query cover (%)
Alternaria alternata	KU212348	*Alternaria alternata* MP39	UNITE	100	100
Beltraniella portoricensis	KU212349	*Beltraniella portoricensis* CBS 856.70	FUNCBS	99	100
Beltraniella sp.	KU212350	*Beltraniella portoricensis* CBS 856.70	FUNCBS	97	100
Cladoriella eucalypti	KU212351	*Cladoriella eucalypti* CBS 115890	FUNCBS	100	100
Coniothyrium sp.	KU212360	*Coniothyrium palmicola* CBS 161.37	FUNCBS	97	100
Diaporthe foeniculacea *	KU212352	*Diaporthe neotheicola* ICMP:6987	UNITE	100	100
Diaporthe infecunda *	KU212353	*Phomopsis* sp. CML 1939	UNITE	100	100
Diaporthe oxe *	KU212354	*Diaporthe oxe* CBS 133187	UNITE	100	99
Diaporthe sp. 1 *	KU212355	*Diaporthe terebinthifolii* LGMF907	UNITE	98	99
Diaporthe sp. 2 *	KU212356	*Diaporthe terebinthifolii* CBS 133180	UNITE	99	99
Diaporthe terebinthifolii *	KU212357	*Diaporthe terebinthifolii* LGMF907	UNITE	100	99
Leptostroma sp.	KU212358	*Leptostroma* sp. 20 2*	UNITE	99	100
Microsphaeropsis arundinis	KU212359	*Microsphaeropsis arundinis* CY127	UNITE	100	100
Paraconiothyrium fungicola	KU212362	*Paraconiothyrium fungicola* CBS 113269	FUNCBS	98	100
Paraconiothyrium sp.	KU212361	*Paraconiothyrium sp.* CBS 194.82	FUNCBS	94	99
Peronosporaceae	KU212363	*Phytophthora cryptogea* CBS 130885	FUNCBS	94	100
Phacidium sp.	KU212364	*Phacidium fennicum* CBS 457.83	FUNCBS	99	100
Phaeosphaeria sp.	KU212365	*Phaeosphaeria* sp. CPC 12130	UNITE	97	96
Sordariomycetes sp.	KU212366	Uncultured Sordariomycetes AWB8	UNITE	97	100
Sphaeropsis sp.	KU212367	*Sphaeropsis sapinea* 28 A	UNITE	97	100
Zetiasplozna thuemenii	KU212368	*Zetiasplozna thuemenii* CBS 100230	FUNCBS	99	100

* Species name was assigned according with Gomes et al. (2013).

Tab. 3. Diversity of fungal communities identified from freshly fallen leafs of *E. uruguayensis*.

Category	No. of samples	No. of occurrences	Q_1	Q_2	S_{obs}	Chao2	D_1	D_2	E'
FP petiole	83	116	20	10	39	56.1	22	13.5	0.61
FP blade	100	87	8	9	25	28.5	16.7	12.1	0.73
MCH	19	125	15	4	37	56.9	26.3	20.3	0.77

FP, fragment plating on culture medium; MCH, moist chambers; Q_1, number of singletons; Q_2, number of doubletons; S_{obs}, observed richness; Chao2, estimated richness; D_1, order 1 diversity (exponential of Shannon entropy); D_2, order 2 diversity (inverse Simpson concentration); E', Hill's evenness.

be seen in the species accumulation curves, which lack an asymptotic behavior at the reference samples sizes (Fig. 1 A). However, completeness levels were quite satisfactory, being 0.91 for the blade community and 0.83 for the petiole one (Fig. 1 B). One of the most important differences between both communities was the greater species richness for the petiole assemblage (Tab. 3). Such difference can be interpreted as significant due to non-overlapping confidence intervals in the coverage-based accumulation curve (Fig. 1 C). Moreover, when the ratio of their Chao2 richness estimation is calculated, it becomes apparent that petioles harbor a community that is twice as rich in species as the blade community ($P_{Chao2}/L_{Chao2} = 2.0$), something that is not evident from the ratio of the observed richness (P_{Sobs}/

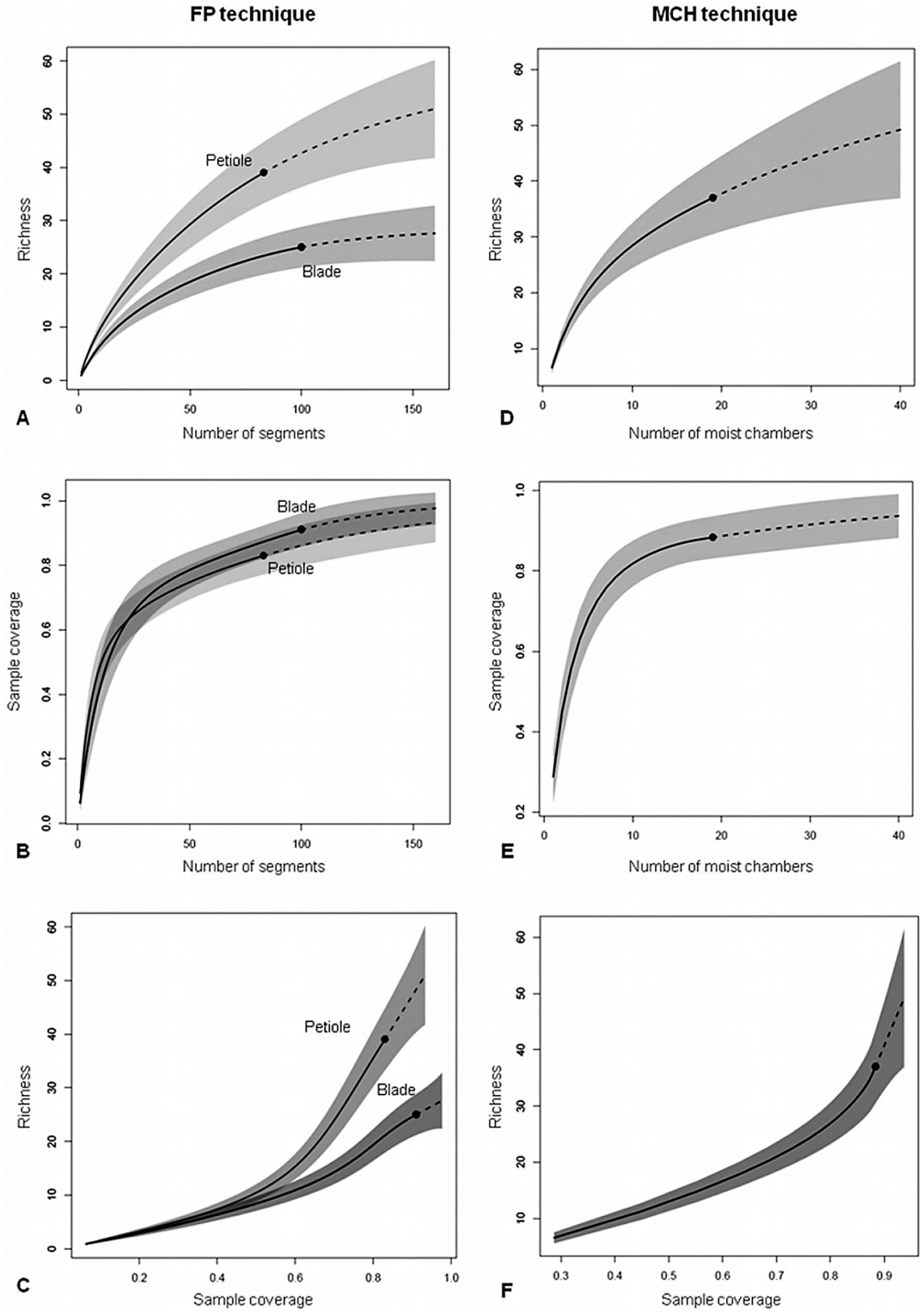

Fig. 1. Species accumulation curves integrating rarefaction and extrapolation for the communities obtained with the fragment plating technique (FP) (petiole and blade communities) and the moist-chamber technique (MCH). **A, D**: sample-based accumulation curves; **B, E**: relationship between sample completeness and sample size; **C, F**: coverage-based accumulation curves. Reference samples are indicated by solid dots, rarefaction by solid lines, extrapolation by dashed lines and 95 % confidence intervals by shaded areas.

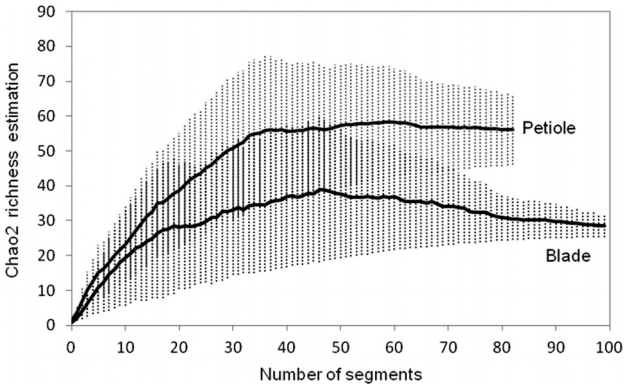

Fig. 2. Estimated Chao2 species richness and the standard deviation for the petiole and blade communities obtained with the fragment plating technique.

L_{Sobs} = 1.6). Chao2 estimates for both communities leveled-off at the reference sample sizes, and thus were considered informative (Fig. 2). Another important difference comes from the percentage of segments colonized by at least one fungus for each category: for 95 % of petiole segments and 69 % of blade segments.

On the other hand, both petiole and blade communities share a similar distribution of frequencies with a few frequent species and a long tail of rare ones (Fig. 3). Also, both fitted the log series model (Petiole: p = 0.66; Blade: p = 0.99) but not the log normal model ($p \leq 0.01$ for both communities). However, they do not share exactly the same proportion of rare species: when different order diver-

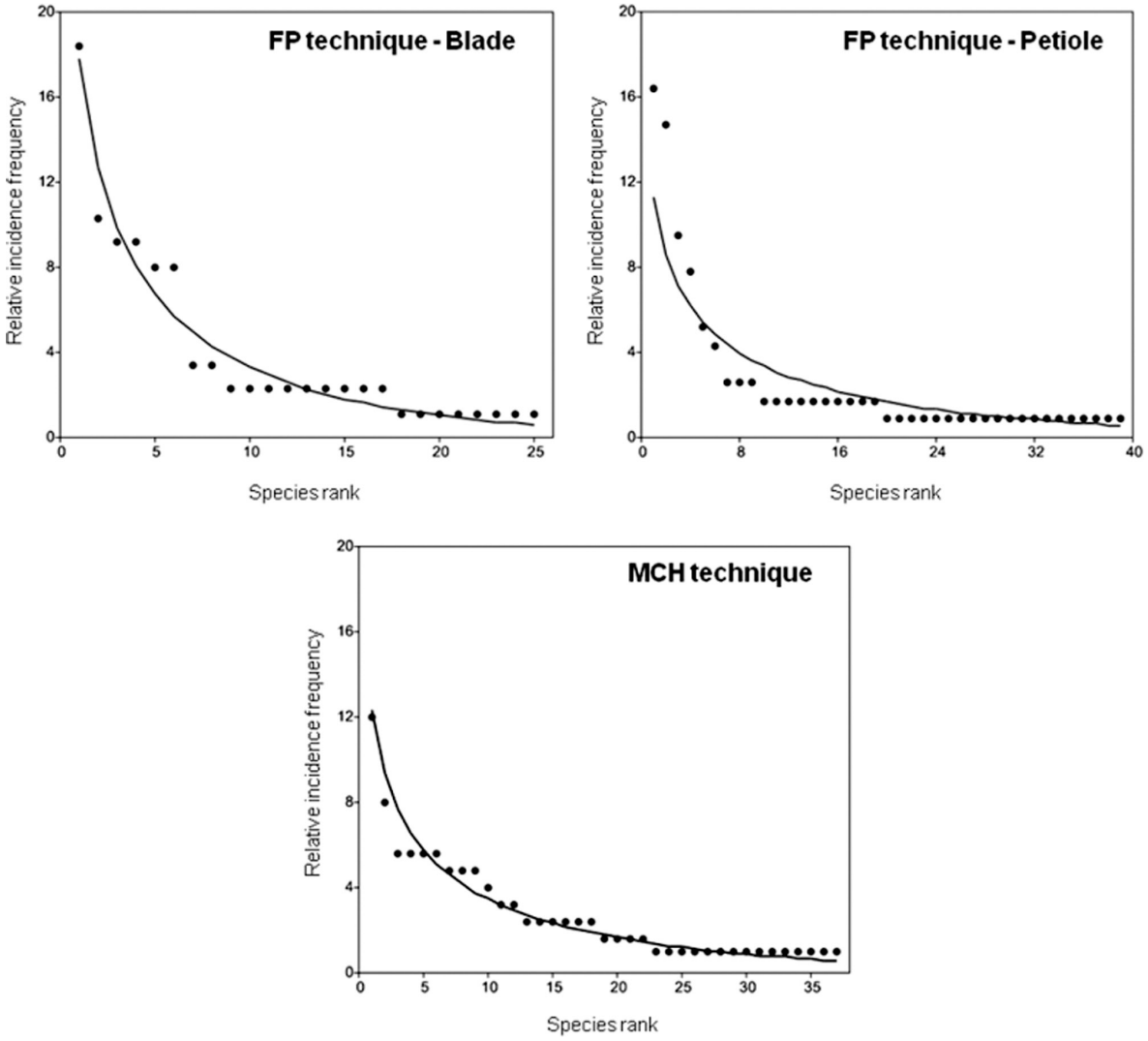

Fig. 3. Distribution of relative incidence frequencies in descending rank order for the communities obtained with the moist-chamber technique (MCH) and the fragment plating technique (FP). Goodness of fit to the log-series model (MCH technique: p = 1; FP technique-Petiole: p = 0.66; FP technique – Blade: p = 0.99).

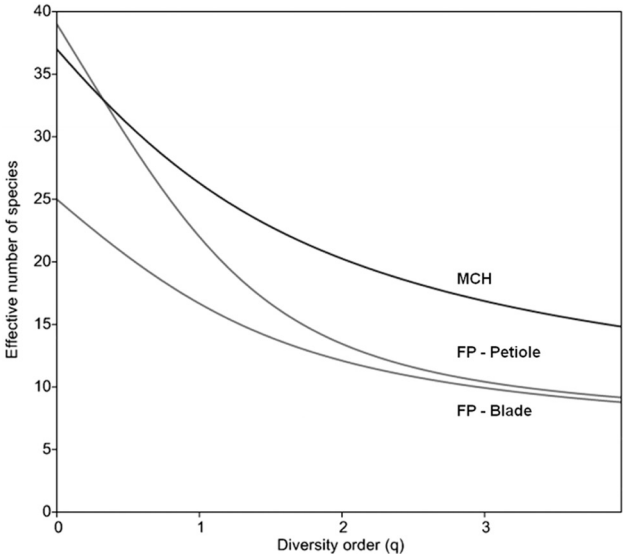

Fig. 4. Diversity profiles for the communities obtained with the fragment plating technique (FP) and the moist-chamber technique (MCH).

sities are regarded as a whole at the diversity profiles for petiole and blade assemblages (Fig. 4), it can be seen that both curves start running separately and then gather together with increasing diversity order (q). This indicates that both communities differ in the amount of rare species but share virtually the same effective number of dominant ones, and also that the blade community is more even than the petiole one. However, from a qualitative point of view, those frequent species are completely different. While the six more frequent species in the petiole community were *Diaporthe terebinthifolii*, *Phoma* sp., *Fusarium incarnatum*, *F. sambucinum*, *Paraconiothyrium* sp. and *Alternaria alternata*; the blade community was characterized by another set of six more frequent species: *Pestalotiopsis guepinii*, *Diaporthe foeniculacea*, *Beltraniella* sp., *Coleophoma crateriformis*, *C. empetri* and *Calonectria insularis*. In fact, the similarity percentage analysis (SIMPER) found that *Phoma* sp., *P. guepinii*, *D. terebinthifolii* and *F. incarnatum* were the species that contributed more to the differences observed between both assemblages (Tab. 4). Nevertheless, none of these frequent species was truly dominant in neither of both assemblages, blade and petiole, being that none exceeded an incidence frequency of 23 %, corresponding to *Diaporthe terebinthifolii* (Tab. 1).

From the MCH technique, 37 taxa were found to be colonizing internal tissues of 38 freshly fallen leaves (Tab. 1). All of the fruiting bodies directly observed belonged to Ascomycota, most of them corresponding to asexual morphs. Sampling could not be considered complete for the MCH technique, as can be seen by the lack of an asymptote in the sample-based species accumulation curve from Fig. 1 D. Nevertheless, a completeness level of 0.88 was achieved (Fig. 1 E). In agreement with the results obtained with the FP technique, the distribution of frequencies fitted well the log series model ($p = 1$) but not the log normal model ($p \leq 0.05$) (Fig. 3). The species that clearly dominated the MCH community were *Beltraniella portoricensis* and *Pestalotiopsis guepinii*. Other frequent species were *Mycoleptodiscus* sp., *Alternaria alternata*, *Dactylaria eucalypti* and *Dictyochaeta assamica*.

Richness estimation using Chao2 did not reach an asymptote for the MCH reference sample and thus could not be considered informative. Different order diversities (Tab. 3) and its correspondent diversity profile (Fig. 4) show that the saprobic community obtained with MCH was more diverse than the FP communities, at least for diversity orders greater than one. Hill's evenness for MCH resembled more that of the blade community than that of the petiole one (Tab. 3).

Tab. 4. Species contribution to the dissimilarity between communities associated with blades and petioles of *E. uruguayensis* early leaf litter as assessed by the Similarity Percentage Analysis (SIMPER). Only species with the highest contribution to the average dissimilarity are listed.

Taxa	Contribution %	Cumulative contribution %
Phoma sp.	7.81	7.81
Pestalotiopsis guepinii	7.76	15.57
Diaporthe terebinthifolii	7.39	22.96
Fusarium incarnatum	5.15	28.11
Fusarium sambucinum	4.69	32.81
Coleophoma crateriformis	4.53	37.33
Diaporthe foeniculacea	4.37	41.71

Discussion

A community of 75 different fungal taxa was recovered with the FP and the MCH techniques, most of them being asexual morphs of Ascomycetes. Although sampling completeness was quite satisfactory for both methods, the inventories obtained were rather different. This was an expected result considering that each technique imposes a different filter to fungal diversity (Paulus et al. 2006). For instance, the FP inventory has a bias in favor of the

fast-growing fungi, while the MCH inventory neglects sterile fungi (Cannon & Sutton 2004).

Characteristics that are considered typical of pioneer fungal communities were observed for the FP assemblages, such as supporting a large number of different taxa with no obvious dominant species (Seephueak et al. 2010), or being composed mostly of phyllosphere fungi and primary saprobes as *Pestalotiopsis guepinii*, *Phoma* sp., *Alternaria alternata* and several *Diaporthe* species (Osono 2006). In contrast, the MCH assemblage had a close resemblance to what is considered a mature community (Promputtha et al. 2002, Seephueak et al. 2010), having two clearly dominant species in nearly all samples (*Beltraniella portoricensis* and *P. guepinii*). In addition, MCH community had a strong presence of well recognized secondary saprobes as *B. portoricensis*, *Beltrania rhombica*, *Dictyochaeta assamica* and *Wiesneriomyces laurinus* (Shanthi & Vittal 2010). These results suggest that the inocula of secondary saprobes was already present in the internal tissues of *E. uruguayensis* freshly fallen leaves. Under these conditions, we can assume that the FP technique would provide a better representation of the early saprobic community.

Tiscornia et al. (2012) described the endophytic fungal communities associated with living tissues of *E. uruguayensis*, by sampling in the same study site and using the same FP technique as this work. When comparing those pre-abscission communities with the post-abscission ones found here, several differences became apparent. First of all, richness was substantially greater for saprobic communities, notably for the petiole associated community, being that only 15 species were found in the endophytic communities. In fact, the saprobic community from petioles was six fold more diverse than the endophytic community from the same tissue when order one diversities (D_1) are compared, while the blade saprobic community was only twofold more diverse than its endophytic counterpart.

The 33 % of all endophytes persisted in the freshly fallen leaves. *Beltrania rhombica*, *Cladosporium cladosporioides*, *Colletotrichum gloeosporioides*, *Diaporthe oxe* and *Diaporthe infecunda* were the species found in both assemblages. Species of *Diaporthe* were particularly frequent in both communities and relationship between strains were confirmed by homology of their ITS regions. The persistence of endophytic fungi observed here in fallen leaves goes in line with the idea that foliar endophytes complete their entire life cycles on leaves, considering that reproduction by means of spores in leaf litter would allow reinfection of living leaves (Osorio & Stephan 1991, Osono 2006, Promputtha et al. 2007). However, this third of endophytic species persisting in leaf litter accounts for half of what is expected by Osono (2006) after summarizing results from several studies. One factor that could be underestimating the endophytic persistence observed is the possibility that there was some degree of turnover in endophytic communities over the two-year time lag between the two sampling events. Nevertheless, the composition of these communities has been proposed to be quite stable (Guo et al. 2008). In addition, persistence of endophytes in litter could increase notoriously after floods in riparian forests, as the one studied here, with the endophytic communities possibly being a reservoir of litter degrading fungi.

The present study assessed whether portions of leaves (blade and petiole) influenced the fungal community assembly. Indeed, we found that distinct communities were associated with blades and petioles from *E. uruguayensis* freshly fallen leaves, and that the fungal community from petioles was twofold more diverse than that from blades. This was also observed by Pinruan et al. (2007) for decaying leaves of *Licuala longicalycata*, who suggested that more complex fungal communities develop in petioles because of their higher nutrient value and greatest moisture retention. Several authors have also observed the existence of tissue preferences among saprobic fungi associated to leaf litter (Hyde & Alias 2000, Yanna et al. 2001, Hyde et al. 2007).

In contrast to what was observed for saprobic communities obtained here, the endophytic communities associated with blades and petioles (Tiscornia et al. 2012) were rather similar to each other, with the blade community showing a greater fungal diversity. This led us to hypothesize that the abscission process could also be involved in the differentiation of blade and petiole fungal communities. Abscission takes place in the so called abscission layer at the base of petioles, with the concomitant development of a protective layer at the stem side, but not at the petiole side (Addicott 1982). Hence, the petiole would be exposed to fungal colonization by airborne spores during the time the leaf remains attached to the stem only by the vascular elements (Pandey & Chadha 1996). This would explain the almost complete colonization of petioles segments, and the great proportion of rare species found in fallen petioles. More importantly, it would explain the differential increase in fungal diversity observed when comparing pre- and post-abscission communities from both anatomical portions (the six fold increase for petioles and the twofold in-

crease for blades). Therefore, we propose that petioles could be a gateway to access leaf litter, at least for those pioneer fungal species that would have difficulties breaking through the thick cuticles typical of *E. uruguayensis* leaves.

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