Acta Botanica Hungarica 58(3–4), pp. 241–256, 2016
DOI: 10.1556/ABot.58.2016.3-4.2

COLOMBIAN VANILLA AND ITS MICROBIOTA. III. DIVERSITY AND STRUCTURE OF THE ENDOPHYTIC COMMUNITY

M. A. Gamboa-Gaitán[1] and J. T. Otero-Ospina[2]

[1]*Laboratorio de Biología Tropical, Departamento de Biología, Universidad Nacional de Colombia, Carrera 30 # 45-03, Bogotá, D. C., Colombia; E-mail: magamboaga@unal.edu.co*
[2]*Grupo de Investigación en Orquídeas, Ecología y Sistemática Vegetal, Universidad Nacional de Colombia, Carrera 32 # 12-00, Palmira, Colombia; E-mail: jtoteroo@unal.edu.co*

(Received 11 April, 2016; Accepted 15 June, 2016)

Endophytic fungal communities are well-recognised entities within plants worldwide. They hold species with potential in medicinal affairs, biological control of pests, industry, and more. Nevertheless, ecological data about structure and dynamics of endophytic communities are scarce. In this study we sampled root, stem and leaf tissues of Colombian vanilla species in order of both, identifying endosymbionts and characterising the community they belong to. An interesting array of endophytic species was found, including taxa that function as pathogens, saprotrophs, and dermatophytes in other plants/scenarios. Ecological parameters show a moderate diversity with a lognormal arrangement of species quite similar to communities of macroorganisms. Many species here reported belong to taxa broadly reported as sources of biologically active compounds, so they are good candidates for bioprospecting research.

Key words: *Colletotrichum*, endophytes, fungal ecology, *Fusarium*, tropical fungi, *Vanilla*, xylariaceous fungi

INTRODUCTION

Endosymbiotic communities of plants gained a lot of attention in recent years due to several factors. First, they are believed to be a very rich source of hidden biodiversity (Arnold *et al.* 2000), and it is expected that biological inventories will increase notably with new microbial species if more plants are studied in this regard. Second, endophytic communities seem to be assembled in the same way communities of macroorganisms are structured, so they are a useful model for ecological community research (and an easier one to work with, Gamboa and Bayman 2001). Third, quite important too, many microbial endophytes are believed to be source of metabolites with potential to be used in medicine, biological control, industry, etc. (Strobel and Long 1998).

A vast majority of plants has not been studied in terms of their endosymbiont communities, and many aspects of the natural history of endophytes are not known. Most studies to date have been performed, as predictable, on angiosperms, and typically studies on dicotyledonous plants are devoted to make the inventory of their endosymbionts and to establish both contribution to biodiversity and potential properties of endophytes (e.g. Arnold *et al.* 2001, Lodge *et al.* 1996, Petrini *et al.* 1982). On the other hand, most studies on monocots have been devoted to the grass-endophyte model in which mutualism has been the recurrent theme (Bacon and de Battista 1991, Clay 1988, Crawford *et al.* 2010, White Jr. 1987).

This paper deals with the endophytic community of vanillas, a group of climbing, tropical monocots, grouped in the genus *Vanilla*. Vanillas are the only edible orchids known to date, and about a hundred species are currently accepted (Soto-Arenas and Cribb 2010). *Vanilla planifolia* is an important aromatic crop that is propagated asexually, so its genetic base is narrow and it is prone to devastating infections by pathogenic microorganisms (Bory *et al.* 2008, Gamboa-Gaitán 2014, Ordóñez *et al.* 2012). Studying naturally-associated endosymbionts of *Vanilla* is important since endophytes have been broadly proposed as potential antagonists of disease-causing microorganisms, so they can play an important role in plant defence and biological control (Herre *et al.* 2007, Mejía *et al.* 2008, Otero *et al.* 2013).

This work was devoted to: 1) study the diversity of endophytic microorganism communities from both wild and cultivated Colombian vanillas, and 2) characterize these communities.

MATERIALS AND METHODS

Vanillas used in this study were *Vanilla planifolia*, *V. calyculata*, *V. odorata*, and four unidentified species from the Colombian Chocó region (Figs 1–2). Vouchers consist of photographs since specimens found were either too small or weak, and a greater sample would mean killing the individual (vanillas are very scarce, they have low densities and are an endangered group). Furthermore, wild individuals were all of them sterile. Isolation of endophytes was performed as published in Gamboa and Bayman (2001), and Gamboa *et al.* (2002). In brief, fresh, healthy plant tissues from roots, stems, and leaves were taken to the lab and washed externally with detergent and tap water. Although samples from diseased leaves and a fruit were surveyed too, they were not included in ecological analysis, and are only shown for registering its presence in vanillas. Superficial sterilisation was performed submerging tissues in ethanol 70% (1 min), commercial hypochlorite 50% (3 min), and ethanol 70% (30 s). They were then washed in distilled water and cut into 4 mm × 4 mm squares that were plated in Petri dishes containing potato-based growth medium (PDA: 20 g of potato dextrose agar Scharlau® in 1 L of water, autoclaved 15 min at 125 °C and 1.25 kg/cm^2). Cultures were kept at room temperature and observed daily. Growing mycelia were transferred to Petri dishes containing PDA as they emerged, and observed for further identification. Strains were transferred to malt extract agar (MEA) to induce sporulation or facilitate identification, as needed (MEA: 20 g of malt agar Scharlau® in 1 L of water, autoclaved 15 min at 125 °C and 1.25 kg/cm^2). Purified strains were later transferred to potato dextrose broth (PDA without agar) and kept two weeks at room temperature and slow agitation. Mycelia were lyophilised at the Plant Physiology Laboratory at Universidad Nacional de Colombia-Bogotá, and sent to the Molecular Biology Laboratory at Pontificia

Fig. 1. Macroscopical view of some vanillas used in this study. A and B = vanillas from Bahía Málaga, courtesy of Néstor D. Jiménez, Universidad Nacional de Colombia-Bogotá; C and D = vanillas from Colombian Pacific coast courtesy of Nicola Flanagan, Pontificia Universidad Javeriana-Cali

Universidad Javeriana-Cali for DNA extraction. Sequencing of the ITS region was performed at Macrogen (Korea). Microscopical observation, keys, fungal experts' advice, and ITS sequences were used for species identification. The term morphospecies is here used to refer to any isolated strain, even if its scientific name is not known.

Endophytic fungal communities were characterised using diversity indices of Shannon–Wiener (H'), and evenness (J') H'(Krebs 1989, Zar 1999):

$$H' = -\sum_{i=1}^{k} p_i \log p_i \qquad H'_{\max} = \log k \qquad J' = \frac{H'}{H'_{\max}}$$

where p_i is proportion of each morphospecies (number of individuals of that taxon over total number of isolated individuals), and k is number of categories, in this case, number of morphospecies. For estimating further community parameters, i.e. sampling effort and distribution patterns, species accumulation curves (curve showing species in a decreasing arrangement), and Preston octave's graph were made.

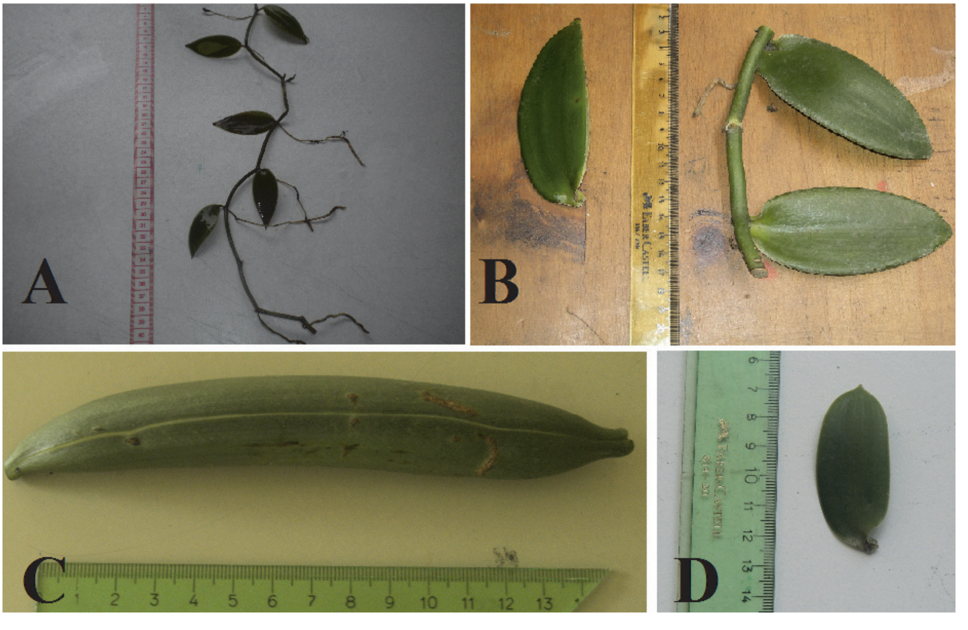

Fig. 2. Macroscopical view of some vanillas used in this study. A = vanilla from Colombian Pacific coast courtesy of Nicola Flanagan, Pontificia Universidad Javeriana-Cali; B = cultivated vanilla (*V. planifolia*); C = wild vanilla (*V. calyculata*), from lowlands in Departamento del Valle, courtesy of Guillermo Reina; D = vanilla from private garden courtesy of professor Huertas, Universidad Nacional de Colombia-Palmira

RESULTS

General aspects

In this study 1055 plant fragments that totalised 4,220 mm^2 were sampled, and 56 morphospecies of endosymbiont microorganisms were found in tissues of seven species of Colombian vanillas (Table 1). Most morphospecies were fungi (54 out of 56) and included 13 mycelia sterilia. Additionally, one actinomycete and one Gram-negative bacterium were found. A total of 525 isolates yielded a community density of 1.3 species per cm^2, while individual endophytic density was 0.124 microorganisms per mm^2. The most frequent microorganism found was the Gram-negative bacterium, but neither this nor the actinomycetes were included in community characterisation analysis, since the objective of this report is to characterise the fungal endophytic community in vanillas.

All organs sampled, i.e. roots, stems, leaves and fruits, yielded endophytic microorganisms. Just two foliar epiphytes were identified: *Biscogniauxia atropunctata* (Xylariales) and *Cosmospora* sp. (Hypocreales), although they appeared as endophytes, too. Fungal taxa reported here are new records for endosymbiont mycoflora of vanillas, with the exception of *Fusarium oxysporum* and *Colletotrichum gloeosporoides*, which have already been reported as inhabitants of *Vanilla* (Gamboa-Gaitán 2013, Ordóñez-Castillo 2011, Ordóñez *et al.* 2012, Talubnak and Soytong 2010). Along with *Alternaria alternata*, *Bionectria* sp., and *Colletotrichum boninense*, those taxa are well recognised as plant pathogens, but all of them were recovered from healthy tissues here. From both healthy and diseased leaves *Fusarium solani* was isolated along with other yet unidentified species of *Fusarium* (Table 2).

Community analysis

Diversity, as measured by the Shannon–Wiener index was $H' = 1.577701$, maximal diversity was $H'_{max} = 1.732393$ and evenness was $J' = 0.91$. Cumulative curves of species abundance showed a pattern typical of lognormal distribution (Fig. 3), with few abundant species and many species with few individuals. Frequencies of observed number of species can be grouped in classes or octaves according to Preston (1948, 1962), and this study shows a tendency to a bimodal pattern of distribution (Fig. 4) of the endophytic fungal community. The modal octave is well included within distribution (2–4 octaves, Fig. 4).

Fungal endophytes in Colombian vanillas

Fifty-four taxa of endophytic fungi were recovered in this study. Sterile morphospecies are treated as either mycelia sterilia if no reproductive structure and no somatic trait is notable for referring to them, or they are described by some phenotypic character like colour or texture (Tables 1 and 2). Most strains are stored at the Laboratorio de Biología Tropical ceparium. Although all morphospecies were grown in liquid medium and then lyophilised for DNA extraction and ITS sequence amplification, not all of them were successfully identified this way. Keys and fungal expert advice helped to assign names to species here presented.

Xylariaceous fungi were represented by *Biscogniauxia atropunctata*, *Pestalotiopsis theae* and *Xylaria* sp. These taxa were isolated from healthy stem and leaf tissues. Other conspicuous endosymbionts were dark septate endophytes (DSE) from roots, traditionally referred to as members of the *Phialocephala* spe-

Table 1
Endophytic microorganisms found in Colombian vanillas and their frequency. Some identifications are dubious (*). Proportion was calculated for fungi only

Isolate	Frequency	Proportion (p_i)
Actinomycetes	3	–
Alternaria sp.	12	0.033
Yellow	8	0.022
Yellow metallic	2	0.005
Arthrographis sp.	11	0.031
Aspergillus niger	5	0.014
Bacillus (Gram–)	169	–
Bionectria sp.	10	0.028
Biscogniauxia atropunctata	8	0.022
White 1	4	0.011
White 2	18	0.050
White 3	2	0.005
White wetty (“*Colletotrichum*”)*	12	0.033
Brown	1	0.002
Colletotrichum sp1	7	0.019
Colletotrichum sp2	5	0.014
Colletotrichum boninense	9	0.025
Colletotrichum gloeosporoides	10	0.028
Cosmospora sp.	5	0.014
Diaporthe eucalyptorum	5	0.014
Fusarium oxysporum	12	0.033
Fusarium solani	26	0.073

Table 1 (continued)

Isolate	Frequency	Proportion (p_i)
Grey greenish	1	0.002
Cream-coloured	5	0.014
Hypocrea lixii	3	0.008
Hypocrea virens	5	0.014
Lasiodiplodia sp.	4	0.011
Lasiodiplodia venezuelensis	3	0.008
Mycelia sterilia	3	0.008
Mycelia sterilia 2	4	0.011
Mycelia sterilia 3	3	0.008
Mycelia sterilia 4	4	0.011
Mycelia sterilia 5	3	0.008
Mycelia sterilia 6	4	0.011
Mycelia sterilia 7	3	0.008
Mycelia sterilia 8	4	0.011
Mycelia sterilia 9	3	0.008
Mycelia sterilia 10	4	0.011
Mycelia sterilia 11	3	0.008
Mycelia sterilia 12	3	0.008
Mycelia sterilia 13	3	0.008
Kinky	1	0.002
Kinky 2	1	0.002
Neofusicoccum sp.	10	0.028
Penicillium sp.	16	0.045
Pestalotia sp.	11	0.031
Pestalotiopsis theae	12	0.033
Phialocephala sp. (EOS)*	15	0.042
Phomopsis sp.	9	0.025
Rhizoctonia sp.	1	0.002
Reddish (“*Fusarium*”)*	2	0.005
Trichoderma sp.	13	0.036
Trichoderma harzianum	11	0.031
Volutella sp.	3	0.008
Xylaria sp.	5	0.014
Total	525 (fungi 353)	

cies complex. Two more species complexes already mentioned are *Fusarium* and *Colletotrichum*. Two forma specialis of *F. oxysporum* were identified: loti and melonis. Additionally, *F. solani* and a strain from a sick leaf, probably *F. oxysporum* f. sp. *vanillae* (Table 2), were isolated, too. On the other hand, *Colletotrichum* was present in both healthy and sick tissues of leaves and stems: *C.*

Table 2
Endophytes in Colombian vanillas showing organs in which they were found and possible role according to literature

Microorganism	Species	Tissue	Role suggested
Alternaria alternata	*V. planifolia*	leaf, root	pathogen
Arthrographis sp.	*Vanilla* sp.	root	human pathogen
Aspergillus niger	*Vanilla* sp.	stem	endophyte, saprotroph
Bacterium (bacilo Gram–)	*V. planifolia*	leaf	?
Bionectria sp.	*V. odorata*	leaf	pathogen
Biscogniauxia atropunctata	*Vanilla* sp.	leaf epiphyte	saprotroph, epiphyte, pathogen
Colletotrichum boninense	*V. planifolia*	stem	pathogen
Colletotrichum gloeosporioides	*V. planifolia*	leaf	endophyte, pathogen
Colletotrichum sp.	*V. planifolia*, *Vanilla* sp.	sick leaf, healthy leaf, stem	endophyte
Cosmospora sp.	*Vanilla* sp.	leaf	epiphyte, polyphyletic, *volutella*?
Diaporthe eucalyptorum	*Vanilla* sp.	stem	pathogen
Fusarium oxysporum f. sp. *loti*	*V. odorata*	leaf	pathogen
Fusarium oxysporum f. sp. *melonis*	*V. planifolia*	leaf	pathogen
Fusarium solani	*V. planifolia*	sick leaf, healthy leaf	pathogen
Fusarium solani	*V. calyculata*	root fruit	pathogen
Fusarium sp. (probably *F. oxysporum* f. sp. *vanillae*)	*V. planifolia*	sick leaf	
Pathogen endophyte			
Fusarium sp.	*V. odorata*	leaf	endophyte
Fusarium sp.	*Vanilla* spp.	stem, root	endophyte
Hypocrea virens	*Vanilla* sp.	stem	saprotroph
Lasiodiplodia venezuelensis	*Vanilla* sp.	stem	pathogen, endophyte
Mycelia sterilia	*Vanilla* spp.	leaf, stem, root	endophyte
Neofusicoccum sp.	*V. calyculata*	fruit, leaf	pathogen

Table 2 (continued)

Microorganism	Species	Tissue	Role suggested
Penicillium spp.	*V. odorata*	leaf	endophyte
Penicillium spp.	*Vanilla* spp.	stem, root	endophyte
Pestalotia sp.	*V. planifolia*	sick leaf	endophyte, pathogen
Pestalotia sp.	*V. calyculata*	healthy leaf	endophyte, pathogen
Pestalotia sp.	*Vanilla* sp.	fruit	endophyte, pathogen
Pestalotiopsis theae	*Vanilla* sp.	leaf	
Phialocephala sp.	*V. odorata* *Vanilla* sp.	root	endophyte
Phomopsis sp.	*V. planifolia*, *Vanilla* sp.	root	pathogen, endophyte
Rhizoctonia sp.	*Vanilla* sp.	root	pathogen, endophyte, mycorrhiza
Trichoderma harzianum	*V. planifolia*	root	saprotroph
Trichoderma sp.	*V. odorata*	leaf	endophyte
Trichoderma sp.	*Vanilla* spp.	stem, root	endophyte
Volutella sp.	*Vanilla* sp.	leaf	pathogen
Xylaria spp.	*V. planifolia*	sick leaf	saprotroph, endophyte
Xylaria spp.	*Vanilla* spp.	root	saprotroph, endophyte

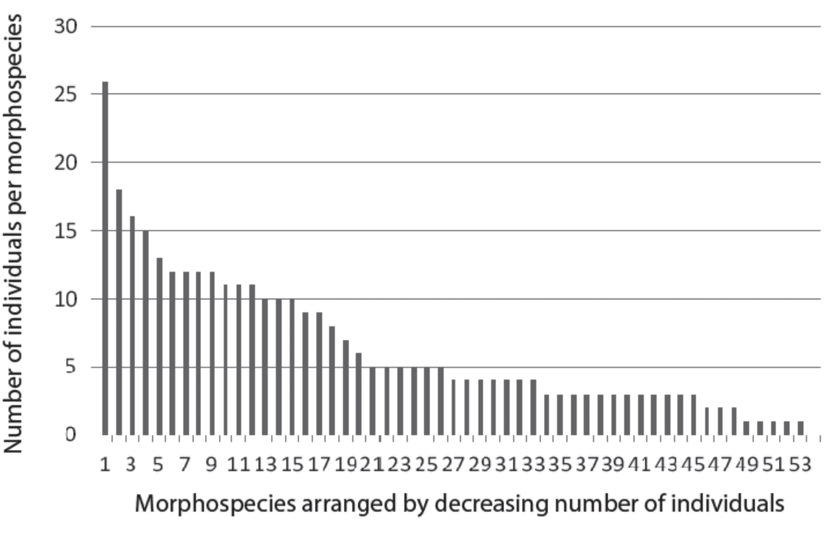

Fig. 3. Community structure of fungal endophytic morphospecies from vanillas. Few species are abundant while most taxa are represented by few individuals

gloeosporoides and *C. boninense*, plus an unidentified strain. Two anamorphic fungi *Aspergillus niger* and a triverticilated *Penicillium* were found as endophytes, too.

Hypocreales fungi were represented by *Bionectria* sp., *Cosmospora* sp., *Hypocrea virens, H. lixii* and its anamorph *Trichoderma harzianum*. *Volutella* sp., anamorphic state of *Pseudonectria*, was found, too. Among Botryosphaeriales we found *Lasiodiplodia* and *Neofusicoccum*. Other groups of vanilla endophytes here reported are *Arthrographis* (Eurotiales), *Diaporthe eucalyptorum* (Diaporthales) and *Alternaria alternata* (Pleosporales). All of them were isolated from healthy tissues along with the most common endosymbiont here isolated, a Gram-negative bacillus.

DISCUSSION

This study was performed on vanillas collected from southwest Colombia, and both wild and cultivated vanillas were sampled. *Vanilla planifolia*, *V. calyculata*, *V. odorata* and four unidentified species were employed. This report is novel in two aspects: it represents the first inventory of endosymbiont microorganisms of vanillas and it is the first attempt to characterise the endophytic fungal community in genus *Vanilla*. Given that vanilla density in the wild is so low, it was possible to obtain samples of more than one individual only from cultivated vanillas. This is why we present the analysis of the fungal community of vanillas as a unit.

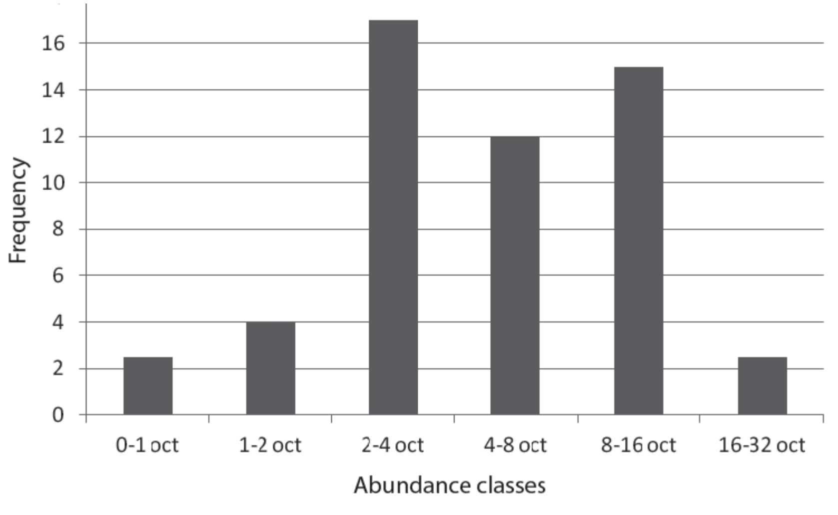

Fig. 4. Frequency of observed number of species grouped in abundance classes (Preston octaves). There is a tendency to a bimodal pattern of distribution and the modal octave (2–4 octaves) is well included in distribution

General aspects

The number of morphospecies found (54 fungi, one actinomycetes and one bacillus, Table 1) is similar to other records in tropical and subtropical plants. For example cultivated vanillas (55 morphospecies, Ordóñez-Castillo 2011), *Guarea guidonia* (38 morphospecies, Gamboa and Bayman 2001), *Opuntia humifusa* (17 morphospecies, Silva-Hughes *et al.* 2015) and adds evidence to the hypothesis that all plant species harbour endosymbionts in healthy tissues. Presence of many unidentified species, including 13 mycelia sterilia, also adds support to the hypothesis that tropical plants can be considered hot spots of biodiversity due to their cryptic endosymbiont microbiota (Arnold *et al.* 2000). Although PDA was the only growth medium used for endophyte isolation, an important amount of endosymbionts was recovered and this supports the idea that a diversification of both sampling methods and growing techniques are needed for better sampling and understanding endophytic communities (Gamboa-Gaitán 2006).

Community analysis

Diversity index (H′ = 1.57) was low for fungal endophytes of vanilla as compared to other tropical plant in which the same index was used for studying endophytic fungi: H′ = 3.18 (Gamboa and Bayman 2001). Nevertheless, maximal diversity (H'_{max} = 1.73) is close to H′ and can be interpreted as undersampling was low. This is also supported by Preston octaves graph (Fig. 4), which shows modal octave well placed within distribution. Given that evenness was high (J′ = 0.91, maximal value is 1.0), it is obvious to ask why diversity appears so low in this community. A possible answer is that the most common endophyte, a Gram-negative bacillus, was not included in community analysis. In fact, this bacterium was present in one third of vanilla fragments that were plated and it showed the ability of restricting fungal growth (other than *Fusarium*). Possible fungistatic effects of this bacterium are not known, and using other artificial growing media that exclude it could lead to a higher number of fungal isolates. Comparing endophytic fungal communities of vanillas from this study with those of other plants, such as *Guarea guidonia*, requires caution. Vanillas are tropical climbing monocot herbs, while *G. guidonia* is a dicot tree that can reach 30 m tall. Although diversity index was much higher in *G. guidonia*, evenness is higher in vanilla and this is difficult to interpret. A tree 30 m tall spans a more variable range of environments than climbing vanillas that are restricted to the understory. Vertical stratification of trees in terms of their endosymbiont community has already been demonstrated (Gamboa and Bayman 2001, Lodge *et al.* 1996).

Finding few abundant species of endophytic fungi and more species with few individuals, indicates a lognormal distribution of species, typical of macroorganism communities that was already found for endophytes in a tropical timber tree (*Guarea guidonia*, Gamboa and Bayman 2001). This is the first report of a fungal endosymbiont community structure for a tropical monocot.

Fungal endophytes in Colombian vanillas

The fungal endophytic community found here shows a very interesting composition and most species are new records of vanilla endophytes (Tables 1 and 2). A study with cultivated vanillas (Ordóñez-Castillo 2011, Ordóñez *et al.* 2012), showed a few genera in common but there is no species overlap. The inclusion of wild vanillas made it possible to get data from preserved habitats that are richer in biodiversity than commercial plantations. This implies that the inoculum source is more diverse and this is essential for obtaining greater fungal diversity in terms of both richness and variety (Rodrigues 1994, Gamboa and Bayman 2001).

Xylariaceous fungi are common endophytic inhabitants of plants (Petrini *et al.* 1995) and they were represented in this study by *Biscogniauxia atropunctata*, *Pestalotiopsis theae* and *Xylaria* sp. These taxa were isolated from healthy stem and leaf tissues although the first two taxa are mostly reported as plant pathogens. In fact, *B. atropunctata* is a pathogen in oaks (Williamson 2010) and *P. theae* is the causal agent of tea gray blight (Yang and Zhang 2012). *Xylaria* spp. are mostly reported as endophytes or saprotrophs (Gamboa and Bayman 2001, Lodge *et al.* 1996, Taylor *et al.* 1999). One of the most intriguing aspects of fungal endophytes is the fact that they can be completely asymptomatic in a plant species and pathogenic to other plants or even to a species of another kingdom (*Coccidioides posadasii*, *Sporothrix schenckii*, Arnold 2007). We found *Arthrographis*, a genus in which notorious dermatophyte species have been reported (Sugiura and Hironaga 2010).

Dark septate endophytes (DSE) are conspicuous endosymbionts commonly found as endophytes, especially in roots, and they have traditionally been assigned to the *Phialocephala* species complex (Sieber and Grünig 2006). This is particularly true for temperate species; almost nothing is known about tropical DSE. Two more important species complex were found as vanilla endophytes: *Fusarium* and *Colletotrichum*. *Fusarium* was present as *F. oxysporum* with two forma specialis: loti and melonis. Additionally, *F. solani* and a strain from a diseased leaf, probably *F. oxysporum* f. sp. *vanillae*, were isolated, too. The presence of *Fusarium* as both endophyte and pathogen has been well documented (Bacon and Yates 2006), and we report here and elsewhere (Gamboa-Gaitán 2013) both roles for *Fusarium* species in vanillas. Another important species complex is *Colletotrichum*, here represented by *C. gloeosporoides* and *C. boninense*, plus an unidentified strain. Again, taxa were recovered from both healthy and sick tissues of leaves and stems. Both complexes *C. gloeosporoides* and C. *boninense* are cited as endophytes and anthracnose-causing agents (Damm *et al.* 2012), but they were not recorded as disease causing agents in vanilla here.

Two anamorphic fungi well recognised as cosmopolitan species were isolated as vanilla endophytes: *Aspergillus niger* and a triverticillate *Penicillium*. Hypocreales fungi were represented by *Bionectria* sp., *Cosmospora* sp., *Hypocrea virens*, *H. lixii* and its anamorph *Trichoderma harzianum*. This taxon was reported as antagonistic to *Fusarium oxysporum* (Naik *et al*. 2010), but such a property was not recorded here. Similarly, Danielsen and Jensen (1999), did not find an inhibitory action of *Trichoderma* against *F. verticillioides*, so it appears that the role of this taxon in biological control awaits further confirmation. *Volutella* sp. is an anamorphic state of *Pseudonectria* that is commonly reported as a plant pathogen (Douglas 2008), and it was found asymptomatic here, too. Other plant pathogenic fungi that appeared asymptomatic here are two genera of Botryosphaeriales: *Lasiodiplodia* and *Neofusicoccum*. Other vanilla endophytes here reported are *Arthrographis* (Eurotiales), *Diaporthe eucalyptorum* (Diaporthales) and *Alternaria alternata* (Pleosporales). All of them were isolated from healthy tissues; vanillas appear to harbour them without any disease symptoms.

The most common endosymbiont of vanillas here isolated was a Gram-negative bacillus (Table 1). Bacteria are well-recognised endosymbiont microorganisms of plants (Chanway 1996), including vanillas (White Jr. *et al.* 2014), and the one found here was isolated from all organs sampled: roots, stems, and leaves. Some filamentous fungi growing in the vicinity of this bacterium were inhibited when they entered in contact with it, and this could be a consequence of compounds produced by this bacillus. It is clear that this bacterium deserves further studies. Endophytic microbes are well recognised as a potential source of bioactive compounds (Strobel 1996, Strobel and Long 1998), and many taxa here reported have also been recognised in this regard: *Hypocrea* (*Trichoderma*), *Cosmospora*, *Bionectria*, *Fusarium* spp., *Aspergillus niger*, *Trichoderma* spp., xylariaceous fungi, *Colletotrichum* spp., etc. (Tables 1 and 2). Thus, next obvious step in this research is bioprospecting this notable set of endosymbionts. They could also be the source of some metabolites found in vanillas that contribute to the flavour and aroma.

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Acknowledgements – Many people helped us with logistics, to all of them many thanks: professor Huertas, Héctor K. Chávez, Miguel Tascón, Guillermo Reina and Fernando Ramos (Universidad Nacional de Colombia, Palmira campus); Nicola Flanagan (Pontificia Universidad Javeriana, Cali campus); Philip Silverstone-Sopkin (CUVC herbarium, Universidad del Valle); Luz Marina Melgarejo, Carolina Becerra, Julio Betancur y Néstor D. Jiménez (Universidad Nacional de Colombia, Bogotá campus); Paul Bayman (University of Puerto Rico). Funding was provided by Grupo de Investigación en Orquídeas y Ecología Vegetal, and División de Investigaciones de la Universidad Nacional de Colombia, Palmira campus (DIPAL) and Bogotá campus (DIB). Comments from anonymous reviewers were very useful, too.

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