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Diversity of Mycorrhizal Fungi Associated with Tropical Epiphytic Orchids in Ecuador

Juan Pablo Suárez[1]*, Stefania Cevallos[1], Paulo Herrera[1]

[1]Ecología y Evolución de Sistemas Microbianos, Departamento de Ciencias Biológicas y Agropecuarias, Universidad Técnica Particular de Loja, Loja, Ecuador

*Autor para Correspondencia / Corresponding Author, e-mail: jpsuarez@utpl.edu.ec

Diversidad de hongos micorrízicos asociados con orquídeas epífitas tropicales en Ecuador

Abstract

This review summarizes the studies carried out by the authors, aiming to understand the diversity of mycorrhizal fungi associated with tropical epiphytic orchids, particularly in Ecuador, and how these vital partnerships contribute to the ecology of orchids. It is based on studies conducted across several sites in Ecuador, including the Reserva Biológica San Francisco, Podocarpus National Park, Cajas National Park, and other locations, and compares methodologies for characterizing root-associated fungal communities while synthesizing current knowledge on the ecological roles of orchid mycorrhizal fungi.

Samples were collected from numerous orchid species, including *Stelis* spp., *Pleurothallis* spp., *Teagueia* spp., *Cyrtochilum* spp., *Epidendrum* spp., and others. Additionally, a seedling-trap experiment was conducted using seedlings obtained from *in-vitro* culture. The most significant findings revealed that all sampled epiphytic orchids consistently formed mycorrhizal associations, underscoring the fungi's critical role in their survival. Through various analytical methods, including light and electron microscopy, fungal isolation, and advanced DNA sequencing techniques, the reviewed studies identified key fungal groups like Serendipitaceae, Tulasnellaceae, Ceratobasidiaceae, and Atractiellales as major orchid mycobionts.

The comparison showed that methodological approaches, particularly the choice of DNA primers for PCR amplification, significantly influenced the observed fungal diversity, with different primers favoring the detection of distinct fungal groups. These findings are crucial for conservation, as identifying appropriate fungal partners is essential for programs aimed at enhancing seed germination and propagating endangered Ecuadorian orchid species, thereby contributing to the persistence of the extraordinary orchid diversity currently threatened by deforestation and climate change.

Keywords: Symbiosis, saprophytic, DNA sequencing, ecology, tropical montane rainforest.

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Resumen

Esta revisión resume los estudios realizados por los autores con el objetivo de comprender la diversidad de los hongos micorrícicos asociados con las orquídeas epífitas tropicales, particularmente en Ecuador, y cómo estas asociaciones fundamentales contribuyen a la ecología de las orquídeas. Se basa en estudios realizados en varios sitios de Ecuador,

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entre ellos la Reserva Biológica San Francisco, el Parque Nacional Podocarpus, el Parque Nacional Cajas y otras localidades, y compara metodologías para la caracterización de las comunidades fúngicas asociadas a las raíces, al tiempo que sintetiza el conocimiento actual sobre los roles ecológicos de los hongos micorrícicos de las orquídeas.

Se recolectaron muestras de numerosas especies de orquídeas, incluyendo *Stelis* spp., *Pleurothallis* spp., *Teagueia* spp., *Cyrtochilum* spp., *Epidendrum* spp., entre otras. Además, se llevó a cabo un experimento de plántulas trampa, utilizando plántulas obtenidas mediante cultivo *in vitro*. Los principales hallazgos mostraron que todas las orquídeas epífitas muestreadas establecieron consistentemente asociaciones micorrícicas, lo que pone de manifiesto el papel fundamental de estos hongos en su supervivencia. Mediante diversos enfoques analíticos, que incluyeron microscopía óptica y electrónica, aislamiento de hongos y técnicas avanzadas de secuenciación de ADN, los estudios revisados identificaron grupos fúngicos clave, como Serendipitaceae, Tulasnellaceae, Ceratobasidiaceae y Atractiellales, como los principales micobiontes de las orquídeas.

La comparación mostró que los enfoques metodológicos, en particular la elección de los cebadores (primers) de ADN para la PCR, influyeron significativamente en la diversidad fúngica observada, ya que diferentes cebadores favorecieron la detección de distintos grupos de hongos. Estos hallazgos son fundamentales para la conservación, ya que la identificación de socios fúngicos adecuados es esencial para los programas destinados a mejorar la germinación de semillas y la propagación de las especies de orquídeas ecuatorianas amenazadas, contribuyendo así a la persistencia de la extraordinaria diversidad de orquídeas, actualmente amenazada por la deforestación y el cambio climático.

Palabras clave: Simbiosis, saprofítico, secuenciación de DNA, ecología, bosque montano tropical.

INTRODUCTION

Orchids rely on mycorrhizal fungi, especially during the critical stages of seed germination and early development when they lack photosynthetic capabilities [1]. This dependence often continues throughout their lives, as these fungi inhabit the root cortical cells of both green (photosynthetic) and non-photosynthetic adult orchids. Within these cells, the fungi form coiled hyphal structures known as pelotons, the defining feature of orchid mycorrhizae [2]. The pelotons are thought to be the site of nutrient transfer between symbionts [1]. Beyond carbon, these fungi also facilitate the transfer of essential nutrients like nitrogen and phosphorus to orchids [3, 4, 5, 6]. The mutualistic nature of this relationship is further supported by demonstrated bidirectional carbon transfer between orchids and their fungal partners [5].

Functionally, orchid mycorrhizal fungi (OMF) are broadly categorized into saprophytic mycobionts and ectomycorrhizal mycobionts based on their carbon acquisition strategies. Saprophytic fungi, which show enzyme activities capable of degrading complex organic compounds like cellulose, are thought to obtain carbon from humus [7, 8]. Conversely,

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ectomycorrhizal fungi, supply carbon obtained from the roots of woody plants to achlorophyllous, myco-heterotrophic, or green, mixotrophic orchids [9, 10, 11].

Historically, the identification of OMF relied on fungal isolation from orchid roots or protocorms, followed by morphological descriptions of their anamorphic states [12] and occasionally inducing their teleomorphs [13, 14]. Re-infection experiments have been crucial for distinguishing true mycobionts from other endophytes [15]. Transmission electron microscopy (TEM) of both, isolates and *in situ* hyphae, revealed diagnostic ultrastructural features, including dolipores and parenthesomes, that enabled the distinction of major orchid mycobiont groups such as Sebacinales and Tulasnellales [16, 17, 18].

More recently, DNA sequencing of mycobionts directly from roots, combined with molecular phylogeny, has significantly advanced our understanding of OMF diversity. This approach has revealed an unexpected diversity of fungal genotypes, including groups previously unrecognized [18]. Direct sequencing is critical because many symbiotic fungi are difficult to culture, and traditional isolation methods can be biased by the presence of numerous non-symbiotic fungi in the velamen or epidermal cells [16, 19].

Plant community assembly, including the highly diverse orchids found across tropical environments, is driven by species dispersal, environmental filtering, and essentially, mutualistic interactions. The diversity of OMF has a strong impact on the niche and life cycle of their orchid hosts [20]. A distinctive feature of tropical orchid ecology is that many OMF are also saprotrophic [21], meaning OMF community assembly is not solely determined by orchid distribution. Moreover, the functional roles of these plant-associated fungi can dynamically shift, even from endophytic associations to mutualism [22]. Despite these established ecological principles, we still have limited understanding of the factors shaping the community structure of epiphytic tropical orchids and how various OMF groups overlap within different orchid species and sites in megadiverse ecosystems like Ecuador.

Ecuador is recognized as one of the world's major centers of orchid diversity, most of which is represented by epiphytic species inhabiting tropical montane forests [23]. Early investigations of the mycorrhizal status of tropical epiphytic orchids yielded conflicting results [24]; however, subsequent studies consistently demonstrated mycorrhizal colonization in roots attached to the substrate [16, 17], establishing the fundamental role of these symbioses in epiphytic orchid ecology. As one of the most biodiverse regions for epiphytic orchids, Ecuador provides an exceptional system for investigating orchid–fungus interactions [23, 25]. This knowledge is particularly important given the increasing threats posed by deforestation and climate change, as identifying the fungal partners associated with epiphytic orchids is essential for understanding their ecology and for developing effective conservation and propagation strategies.

MATERIALS AND METHODS

Sites and sampled species

Our investigations focused on root-associated fungi (RAF), that refer to any fungal species that are found colonizing or inhabiting the roots of orchids, including OMF. Our

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primary focus was the mycorrhizal partners of epiphytic orchid species geographically concentrated in key biodiversity hotspots in the south of Ecuador. The initial studies examined a few common orchid species from the genera *Stelis* and *Pleurothallis* in the tropical montane rainforest of the Reserva Biológica San Francisco (RBSF), Zamora-Chinchipe, Ecuador [16, 17, 19].

Subsequent investigations progressively expanded the geographic coverage and taxonomic scope to include additional orchid species from southern Ecuador, encompassing RBSF, Podocarpus National Park (PNP), El Cajas National Park and the Upper Pastaza watershed [18, 26, 27, 28, 29, 30, 31, 32]. Collectively, these studies included more than 500 orchid individuals representing over 50 orchid species. Additionally, an *in situ* seedling-trap experiment was conducted using 400 *in vitro*-produced seedlings to avoid pre-existing fungal colonization [33]. A comparative summary of the study sites, orchid species, sampling effort, methodologies, and key findings is provided in Table 1.

Light and transmission electron microscopy (TEM)

A combination of light and transmission electron microscopy (TEM) has been fundamental for confirming mycorrhizal colonization. Light microscopy was used to select material with fungal coils. For that, transverse sections were cut from the middle part of each root sample by hand using a razor blade. Sections were stained by Methyl blue 0.05 % solution [16]. TEM was indispensable to observe the anatomy of mycorrhizae and ultrastructure of hyphae. Ultrastructural details were needed to demonstrate the presence of an active symbiosis [16, 17, 18].

Fungal isolation

Fungal isolation was performed by extracting pelotons from surface sterilized roots [16, 17, 30]. Pelotons were grown in Petri plates containing Fungal Isolation Medium [30]. All fungal isolates obtained have been deposited in the fungal culture collection of UTPL.

Molecular identification of fungi

Molecular approaches evolved substantially over the course of these studies, from Sanger sequencing of the ITS and 28S ribosomal DNA regions to Illumina metabarcoding of the ITS2 region. Early investigations combined Sanger sequencing with phylogenetic analyses, with some studies incorporating molecular cloning to resolve multiple fungal symbionts within individual roots [16, 17, 18, 26, 27, 31]. Later studies adopted Illumina MiSeq sequencing and OTU-based analyses, greatly expanding the detection of root-associated fungal diversity and enabling comparisons of fungal communities across orchid species, habitats, and developmental stages [28, 29, 32, 33].

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Table 1. Comparative summary of studies on orchid mycorrhizal fungi associated with tropical epiphytic orchids in Ecuador.

Reference	Study site(s)	Orchid species	Sample size (N)	Methodology	Main fungal groups detected	Key finding
Suárez et al. [16]	RBSF	*Stelis spp., Pleurothallis lilijae.*	77	LM, TEM, fungal isolation, ITS, 28S, Sanger, phylogeny.	Tulasnellaceae	First characterization of *Tulasnella* associated with Ecuadorian epiphytic orchids.
Suárez et al. [17]	RBSF	*Stelis spp., Pleurothallis lilijae.*	83	LM, TEM, ITS, 28S, Sanger, phylogeny	Sebacinales	First evidence of Serendipitaceae (formerly Sebacinales subgroup B) as mycorrhizal partners of Neotropical epiphytic orchids.
Kottke et al. [18]	Southern Ecuador	Multiple orchid genera	117	TEM, ITS, 28S, molecular cloning, Sanger, phylogeny	Atractiellales	First report of Atractiellales as orchid mycorrhizal fungi.
Herrera et al. [19]	RBSF	*Stelis spp., Pleurothallis lilijae.*	83	LM, fungal isolation, ITS, 28S, Sanger, phylogeny	Ascomycota (root endophytes)	Distinguished root endophytes from orchid mycorrhizal fungi.
Riofrío et al. [26]	Southern Ecuador	*Epidendrum rhopalostele*	25	LM, ITS, molecular cloning, Sanger, phylogeny	Tulasnellaceae	Linked mycorrhizal fungi to orchid population ecology.
Suárez et al. [27]	Upper Pastaza watershed	*Teagueia* spp.	11	LM, ITS, 28S, molecular cloning, Sanger, phylogeny	Tulasnellaceae, Atractiellales	Mycorrhizal specialization does not explain *Teagueia* radiation.
Cevallos et al. [28]	Podocarpus National Park region	*Cyrtochilum spp., Maxillaria calantha*	68	Illumina MiSeq (ITS2), OTUs	Serendipitaceae, Tulasnellaceae and others	Detected 55 OMF OTUs. Introduced Illumina metabarcoding for Ecuadorian epiphytic orchid OMF.
Novotná et al. [30]	RBSF; Reserva Arcoiris	*Cyrtochilum myanthum, Scaphyglottis punctulata and Stelis superbiens*	110	fungal isolation, ITS, 28S, molecular cloning, Sanger, OTUs	Ascomycota, Tulasnellaceae, Ceratobasidiaceae	Revealed limitations of culture-dependent approaches for OMF detection.
Cevallos et al. [29]	Southern Ecuador	Multiple epiphytic orchid species	130	Illumina MiSeq (ITS2), OTUs	Root-associated fungal community	Detected 3,492 root-endophyte OTUs. Host species and environment shaped root-associated fungal communities.
Cevallos et al. [33]	RBSF	*Cyrtochilum retusum, Epidendrum macrum*	400	Seedling-trap experiment, Illumina MiSeq (ITS2), OTUs	Early colonizing OMF	First *in situ* seedling-trap study of tropical epiphytic orchids.
Herrera et al. [31]	RBSF	52 orchid species	114	ITS, molecular cloning, Sanger, OTUs, network analysis	Tulasnellaceae	Detected 29 Tulasnellaceae OTUs. Generalist Tulasnellaceae dominated orchid–fungus interaction networks.
Herrera et al. [32]	El Cajas National Park	*Epidendrum marsupiale, Cyrtochilum pardinum*	83	Illumina MiSeq (ITS2), OTUs	Serendipitaceae, Ceratobasidiaceae, Atractiellales and Tulasnellaceae	Detected 108 OMF OTUs. Revealed broad fungal sharing and low mycorrhizal specialization.

Abbreviations: RBSF, Reserva Biológica San Francisco; LM, light microscopy; TEM, transmission electron microscopy; ITS, Internal transcribed spacer region of nuclear ribosomal DNA; 28S, Nuclear large subunit ribosomal DNA (LSU) region; Sanger, Sanger sequencing; OMF, orchid mycorrhizal fungi; OTUs, Operational taxonomic units.

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RESULTS AND DISCUSSION

Mycorrhizal status

All sampled epiphytic orchids were consistently mycorrhizal, highlighting the crucial role of mycobionts in their survival [16, 17]. Anatomy of mycorrhizae and ultrastructure of hyphae allowed unambiguous distinctions among Sebacinales, Tulasnellales and Ceratobasidiales due dolipore (septa) features [34] and the observation of simple septal pores and symplechosomes in the hyphal coils indicated members of Atractiellomycetes [18]. The use of TEM allowed the observation of pelotons in living cortical cells, strongly indicating that the fungal group studied is forming mycorrhizae.

Dominant OMF lineages

rDNA ITS sequencing and phylogenetic analysis identified Serendipitaceae (Sebacinales), Tulasnellaceae, Ceratobasidiaceae (Cantharellales), and Atractiellomycetes (Atractiellales) [35] as the main groups of fungi that form mycorrhizae in tropical epiphytic orchids in southern Ecuador.

Serendipitaceae (Sebacinales): Formerly Sebacinales clade B, primarily act as mycobionts for green orchids, including epiphytes [35] and as endophytes in various other plant species [36]. While the closely related Sebacinaceae often form ectomycorrhizae in tripartite associations with achlorophyllous and mixotrophic orchids [37]. Research has consistently revealed the significant diversity of Serendipitaceae associated with orchids. Suárez et al. [17] identified eight operational taxonomic units (OTUs) in four epiphytic orchid species in southern Ecuador. Further studies by Martos et al. [38] detected 23 OTUs across 77 orchid species on Reunion Island, and Kartzinel et al. [39] found five OTUs with *Epidendrum firmum* in Costa Rica. More recent Next-generation sequencing approaches have uncovered even greater richness, with 32 OTUs detected by Cevallos et al. [28] and 66 OTUs by Herrera et al. [32] in Ecuadorian orchids. Despite their widespread prevalence, members of Serendipitaceae are notoriously difficult to culture; to date, only a single isolate from *Stanhopea connata* is known [40].

Tulasnellaceae (Cantharellales): These fungi are generally saprotrophs or mutualistic biotrophs [41] and are considered frequent and widespread mycobionts of autotrophic orchids [35]. Numerous Tulasnellaceae members have been detected in tropical orchids through culture-independent methods. Suárez et al. [16] found 13 OTUs and Herrera et al. [32] found 29 OTUs in epiphytic orchids in southern Ecuador. Other studies report two OTUs associated with *Epidendrum rhopalostele* [26], four OTUs with *Teagueia* spp. [27], and six *Tulasnella* strains with *E. firmum* [39]. Large-scale studies, like Martos et al. [38] on Reunion Island, detected 58 OTUs. Next-generation sequencing by Cevallos et al. [28] and Herrera et al. [32] each detected nine Tulasnellaceae OTUs in different Ecuadorian locations.

Ceratobasidiaceae (Cantharellales): This family includes genera like *Ceratobasidium* and *Thanatephorus*, known both as crop pathogens and mycorrhizal partners of orchids and trees [42]. Delimitation at the species level through ITS sequencing is often unclear. Many members of Ceratobasidiaceae have been successfully isolated from tropical orchids, four OTUs from nine orchid species in Puerto Rico [43], and diverse members from *Vanilla*

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spp. [44]. In Colombia, 12 strains of Ceratobasidiaceae were isolated from several orchids [45]. Culture-independent methods have also detected their high diversity. Martos et al. [38] detected 14 OTUs, and Cevallos et al. [28] and Herrera et al. [32] each detected 14 and 22 OTUs, respectively.

Atractiellales (Pucciniomycotina): While Pucciniomycotina primarily comprised parasites, members of Atractiellales are now confirmed to form mycorrhizae with terrestrial and epiphytic tropical orchids [18]. Riofrío et al. [26] and Suárez et al. [27] detected one OTU, while Herrera et al. [32] found 11 OTUs using Next-generation sequencing.

Ecological patterns and methodological considerations

Our studies in southern Ecuador have shown complex patterns in the orchid mycorrhizal interaction. Despite the wide geographical distribution of some OMF-OTUs, such as Tulasnellaceae [35, 41], their presence or abundance was not always correlated with the most frequent OMF-OTUs in our data sets. This is likely because OMF distribution is not solely determined by orchid distribution [41], even though orchids rely on specific OMF for seed germination and establishment. This highlights a complex interplay where orchids might utilize distinct strategies to associate with OMF, even when coexisting in the same environment. A key finding is the spectrum of specificity; some orchid species appear to be highly specialized, while others are generalists [31, 32]. For example, in the PNP, *Epidendrum rhopalostele* associated with only two Tulasnellaceae OTUs and one Atractiellales OTU [26], whereas *Epidendrum marsupiale* was associated with 65 OMF-OTUs across multiple fungal groups [32]. Our results suggested that populations of studied epiphytic orchids have site-adjusted mycorrhizal communities structured around keystone fungal species [28].

Methodological biases can significantly impact our understanding of OMF diversity. Studies using Sanger sequencing with nested-PCR [16, 26, 27] often found Tulasnellaceae to be the richest group, likely due to the use of specific tulasnelloid primers [46]. Conversely, recent Next-generation sequencing studies using broader fungal-specific primers (e.g., ITS86F and ITS4) [28, 32] tend to identify Serendipitaceae as the richest OMF group. So, our understanding of OMF communities is method-dependent, not only by primer choice, but also by PCR strategies, sequencing methods, bioinformatic analyses, etc., which underscores the need for actualized strategies in future studies.

CONCLUSIONS

The identification of mycobionts is vital for conservation efforts, especially for endemic and endangered epiphytic orchid species in Ecuadorian tropical forests. Using appropriate mycobionts to enhance seed germination and facilitate *in vitro* inoculation of orchid plantlets is a critical step for future rehabilitation programs. Further research with advanced sequencing technologies and rigorous testing of new primer combinations is necessary to comprehensively capture the diversity of fungal groups like Tulasnellaceae in tropical orchids.

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AUTHORS' CONTRIBUTIONS

Juan Pablo Suárez, Stefania Cevallos, and Paulo Herrera designed and conceived the manuscript. Juan Pablo Suárez wrote the manuscript, and Juan Pablo Suárez, Stefania Cevallos, and Paulo Herrera edited, validated, and finalized the document.

DECLARATION OF GENERATIVE AND AI-ASSISTED TECHNOLOGIES IN THE WRITING PROCESS

For this work, the authors used ChatGPT (OpenAI) for language editing and to improve the clarity and readability of the manuscript, as English is not the authors' first language. Afterwards, the authors reviewed and edited the content as deemed necessary and take full responsibility for the final version and the published content.

DATA AVAILABILITY STATEMENT

Data are available upon reasonable request to the corresponding author (in exceptional cases).

CONFLICT OF INTEREST

The authors have no conflicts of interest regarding this research.

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REFERENCES

[1] Smith, S. E., & Read, D. J. (2008). Mycorrhizal Symbiosis. 3rd ed. Academic Press, London. https://doi.org/10.1016/B978-0-12-370526-6.X5001-6

[2] Dearnaley, J., Perotto, S., & Selosse, M.-A. (2016). *Structure and development of orchid mycorrhizas, in Molecular Mycorrhizal Symbiosis*. ed. F. Martin (Hoboken, NJ: John Wiley and Sons), 63–86. https://doi.org/10.1002/9781118951446.ch5

[3] Smith, S. E. (1967). Carbohydrate translocation into orchid mycorrhizas. *New Phytologist,* 66, 371-378. https://doi.org/10.1111/j.1469-8137.1967.tb06016.x

[4] Alexander, C., & Hadley, G. (1985). Carbon movement between host and mycorrhizal endophyte during the development of the orchid *Goodyera repens* Br. *New phytologist*, 101(4), 657–665. https://doi.org/10.1111/j.1469-8137.1985.tb02871.x

[5] Cameron, D. D, Leake, J. R., & Read, D. J. (2006). Mutualistic mycorrhiza in orchids: evidence from plant-fungus carbon and nitrogen transfer in the green-leaved terrestrial orchid *Goodyera repens. New Phytologist,* 171, 405-416. https://doi.org/10.1111/j.1469-8137.2006.01767.x

[6] Cameron, D. D., Johnson, I., Leake, J. R., & Read, D. J. (2007). Mycorrhizal acquisition of inorganic phosphorus by the green-leaved terrestrial orchid *Goodyera repens. Annals of Botany,* 99, 831-834. https://doi.org/10.1093/aob/mcm018

[7] Hadley, G. (1969). Cellulose as a carbon source for orchid mycorrhiza. *New Phytologist,* 68, 933-939. https://doi.org/10.1111/j.1469-8137.1969.tb06492.x

[8] Midgley, D. J., Jordan, L. A., Saleeba, J. A., & McGee, P. A. (2006). Utilisation of carbon substrates by orchid and ericoid mycorrhizal fungi from Australian dry sclerophyll forests. *Mycorrhiza,* 16, 175-182. https://doi.org/10.1007/s00572-005-0029-2

[9] McKendrick, S. L., Leake, J. R., & Read, D. J. (2000). Symbiotic germination and development of myco-heterotrophic plants in nature: transfer of carbon from ectomycorrhizal *Salix repens and Betula pendula to the orchid Corallorhiza trifida* through shared hyphal connections. *New Phytologist,* 145, 539-548. https://doi.org/10.1046/j.1469-8137.2000.00592.x

[10] Gebauer, G., & Meyer, M. (2003). 15N and 13C natural abundance studies of autotrophic and myco-heterotrophic orchids provides insight into nitrogen and carbon gain from fungal associations. *New Phytologist,* 160, 209-223. https://doi.org/10.1046/j.1469-8137.2003.00872.x

[11] Trudell, S. A., Rygiewicz, P. T., & Edmonds, R. I. (2003). Nitrogen and carbon stable isotope abundance support the myco-heterotrophic nature and host-specificity of certain achlorophyllous plants. *New Phytologist,* 160, 391-401. https://doi.org/10.1046/j.1469-8137.2003.00876.x

[12] Roberts P. (1999). Rhizoctonia-forming Fungi: a taxonomic guide. Royal Botanic Gardens, Kew

[13] Warcup, J. H., & Talbot, P. H. B. (1967). Perfect states of Rhizoctonias associated with orchids. *New Phytologist,* 66(4), 631-641. https://doi.org/10.1111/j.1469-8137.1967.tb05434.x

[14] Cruz, D., Suárez, J. P., & Piepenbring, M. (2016). Morphological revision of Tulasnellaceae, with two new species of *Tulasnella* and new records of *Tulasnella* spp. for Ecuador. *Nova Hedwigia*, 102(3-4), 279-338. https://doi.org/10.1127/nova_hedwigia/2015/0304

[15] Bonnardeaux, Y., Brundrett, M., Batty, A., Dixon, K., Koch, J., & Sivasithamparam, K. (2007). Diversity of mycorrhizal fungi of terrestrial orchids: compatibility webs, brief encounters, lasting relationships and alien invasions. *Mycological research*, 111(1), 51-61. https://doi.org/10.1016/j.mycres.2006.11.006

[16] Suárez, J. P., Weiss, M., Abele, A., Garnica, S., Oberwinkler, F., & Kottke, I. (2006). Diverse tulasnelloid fungi form mycorrhizas with epiphytic orchids in an Andean cloud forest. *Mycological Research,* 110, 1257–1270. https://doi.org/10.1016/j.mycres.2006.08.004

[17] Suárez, J. P., Weiss, M., Abele, A., Oberwinkler, F., & Kottke, I. (2008). Members of Sebacinales subgroup B form mycorrhizae with epiphytic orchids in a neotropical mountain rain forest. *Mycological Progress*, *7*(2), 75–85. https://doi.org/10.1007/s11557-008-0554-4

[18] Kottke, I., Suárez, J. P., Herrera, P., Cruz, D., Bauer, R., Haug, I., & Garnica, S. (2010). Atractiellomycetes belonging to the 'rust'lineage (Pucciniomycotina) form mycorrhizae with terrestrial and epiphytic neotropical orchids. *Proceedings of the Royal Society B*: Biological Sciences, 277(1685), 1289-1298. https://doi.org/10.1098/rspb.2009.1884

 DOI: https://doi.org/10.18272/aci.3990

[19] Herrera, P., Suárez, J. P., & Kottke, I. (2010). Orchids keep the ascomycetes outside: a highly diverse group of ascomycetes colonizing the velamen of epiphytic orchids from a tropical mountain rainforest in southern Ecuador. *Mycology*, 1(4), 262-268. https://doi.org/10.1080/21501203.2010.526645

[20] Li, T., Wu, S., Yang, W., Selosse, M. A., & Gao, J. (2021). How mycorrhizal associations influence orchid distribution and population dynamics. *Frontiers in Plant Science, 12*, 647114. https://doi.org/10.3389/fpls.2021.647114

[21] Kohler, A., Kuo, A., Nagy, L. G., Morin, E., Barry, K. W., Buscot, F., et al. (2015). Convergent losses of decay mechanisms and rapid turnover of symbiosis genes in mycorrhizal mutualists. *Nature Genetics,* 47(4), 410-415. https://doi.org/10.1038/ng.3223

[22] Selosse, M. A., Petrolli, R., Mujica, M. I., Laurent, L., Perez-Lamarque, B., et al. (2022). The waiting room hypothesis revisited by orchids: were orchid mycorrhizal fungi recruited among root endophytes? *Annals of Botany*, 129(3), 259-270. https://doi.org/10.1093/aob/mcab134

[23] Vitt, P., Taylor, A., Rakosy, D., Kreft, H., Meyer, A., Weigelt, P., & Knight, T.M. (2023) Global conservation prioritization for the Orchidaceae. *Scientific Reports,* 13, 6718. https://doi.org/10.1038/s41598-023-33823-7

[24] Bermudes, D., & Benzing, D.H. (1989). Fungi in neotropical epiphyte roots. *BioSystems,* 23, 65–73. https://doi.org/10.1016/0303-2647(89)90009-9

[25] Zotz, G., Weigelt, P., Kessler, M., Kreft, H., & Taylor, A. (2021). EpiList 1.0: a global checklist of vascular epiphytes. *Ecology* ,102, e03326. https://doi.org/10.1002/ecy.3326

[26] Riofrío, M. L., Cruz, D., Torres, E., De la Cruz, M., Iriondo, J. M., & Suárez, J. P. (2013). Mycorrhizal preferences and fine spatial structure of the epiphytic orchid *Epidendrum rhopalostele. American Journal of Botany*, *100*(12), 2339–2348. https://doi.org/10.3732/ajb.1300069

[27] Suárez, J. P., Eguiguren, J. S., Herrera, P., & Jost, L. (2016). Do mycorrhizal fungi drive speciation in *Teagueia* (Orchidaceae) in the upper Pastaza watershed of Ecuador? *Symbiosis*, *69*(3), 161–168. https://doi.org/10.1007/s13199-016-0399-6

[28] Cevallos, S., Sánchez-Rodríguez, A., Decock, C., Declerck, S., & Suárez, J. P. (2017). Are there keystone mycorrhizal fungi associated to tropical epiphytic orchids? *Mycorrhiza, 27*, 225-232. https://doi.org/10.1007/s00572-016-0746-8

[29] Cevallos, S., Herrera, P., Sánchez-Rodríguez, A., Declerck, S., & Suárez, J. P. (2018). Untangling factors that drive community composition of root associated fungal endophytes of Neotropical epiphytic orchids. *Fungal Ecology*, *34*, 67-75. https://doi.org/10.1016/j.funeco.2018.05.002

[30] Novotná, A., Benitez, A., Herrera, P., Cruz, D., Filipczykova, E., & Suárez, J. P. (2018). High diversity of root-associated fungi isolated from three epiphytic orchids in southern Ecuador. *Mycoscience*, 59(1), 24-32. https://doi.org/10.1016/j.myc.2017.07.007

[31] Herrera, P., Kottke, I., Molina, M. C., Mendez, M., & Suárez, J. P. (2018). Generalism in the interaction of Tulasnellaceae mycobionts with orchids characterizes a biodiversity hotspot in the tropical Andes of southern Ecuador. *Mycoscience*, *59*(1), 38-48. https://doi.org/10.1016/j.myc.2017.08.003

[32] Herrera, P., Suárez, J. P., Sánchez-Rodríguez, A., Molina, M. C., Prieto, M., & Méndez, M. (2019). Many broadly-shared mycobionts characterize mycorrhizal interactions of two coexisting epiphytic orchids in a high elevation tropical forest. *Fungal Ecology*, 39, 26-36. https://doi.org/10.1016/j.funeco.2018.11.003

[33] Cevallos, S., Declerck, S., & Suárez, J. P. (2018). In situ orchid seedling-trap experiment shows few keystone and many randomly associated mycorrhizal fungal species during early plant colonization. *Frontiers in plant science*, *9*, 1664. https://doi.org/10.3389/fpls.2018.01664

[34] Kottke, I., & Suárez, J. P. (2009). Mutualistic, root-inhabiting fungi of orchids identification and functional types. In Proceedings of the Second Scientific Conference on Andean Orchids (pp. 84-99). Loja, Ecuador: Universidad Técnica Particular de Loja.

[35] Suárez, J. P., & Kottke, I. (2016). Main fungal partners and different levels of specificity of orchid mycorrhizae in the tropical mountain forests of Ecuador. *Lankesteriana, 16*(2), 299–305. https://doi.org/10.15517/lank.v16i2.26014

[36] Weiß, M., Waller, F., Zuccaro, A., & Selosse, M. A. (2016). Sebacinales–one thousand and one interactions with land plants. *New Phytologist*, 211(1), 20-40. https://doi.org/10.1111/nph.13977

[37] Julou, T., Burghardt, B., Gebauer, G., Berveiller, D., Damesin, C., & Selosse, M. A. (2005). Mixotrophy in orchids: insights from a comparative study of green individuals and nonphotosynthetic individuals of *Cephalanthera damasonium*. *New Phytologist*, 166(2), 639-653. https://doi.org/10.1111/j.1469-8137.2005.01364.x

[38] Martos, F., Munoz, F., Pailler, T., Kottke, I., Gonneau, C., & Selosse, M. A. (2012). The role of epiphytism in architecture and evolutionary constraint within mycorrhizal networks of tropical orchids. *Molecular Ecology*, 21(20), 5098-5109. https://doi.org/10.1111/j.1365-294X.2012.05692.x

[39] Kartzinel, T. R., Trapnell, D. W., & Shefferson, R. P. (2013). Highly diverse and spatially heterogeneous mycorrhizal symbiosis in a rare epiphyte is unrelated to broad biogeographic or environmental features. *Molecular Ecology*, 22(23), 5949-5961. https://doi.org/10.1111/mec.12536

[40] Novotna, A., & Suárez, J. P. (2018). Molecular detection of bacteria associated with *Serendipita* sp., a mycorrhizal fungus from the orchid *Stanhopea connata* Klotzsch in southern Ecuador. *Botany Letters*, 165(2), 307-313. https://doi.org/10.1080/23818107.2018.1436087

[41] Oberwinkler, F., Cruz, D., & Suárez, J. P. (2017). Biogeography and ecology of Tulasnellaceae, in *Biogeography of Mycorrhizal Symbiosis: Ecological Studies*. ed. L. Tedersoo (Switzerland, Cham: Springer), 237–271. https://doi.org/10.1007/978-3-319-56363-3_12

[42] Tedersoo, L., May, T. W., & Smith, M. E. (2010). Ectomycorrhizal lifestyle in fungi: global diversity, distribution, and evolution of phylogenetic lineages. *Mycorrhiza*, 20(4), 217–263. https://doi.org/10.1007/978-3-319-56363-3_12

[43] Otero, J. T., Ackerman, J. D., & Bayman, P. (2002). Diversity and host specificity of endophytic *Rhizoctonia*-like fungi from tropical orchids. *American Journal of Botany*, 89(11), 1852–1858. https://doi.org/10.3732/ajb.89.11.1852

[44] Porras-Alfaro, A., & Bayman, P. (2007). Mycorrhizal fungi of *Vanilla*: diversity, specificity and effects on seed germination and plant growth. *Mycologia*, 99(4), 510–525. https://doi.org/10.1080/15572536.2007.11832545

[45] Mosquera-Espinosa, A. T., Bayman, P., & Otero, J. T. (2010). *Ceratobasidium* como hongo micorrízico de orquídeas en Colombia. *Acta Agronómica*, 59(3), 316–326.

[46] Rammitsu, K., Kajita, T., Imai, R., & Ogura-Tsujita, Y. (2021). Strong primer bias for Tulasnellaceae fungi in metabarcoding: Specific primers improve the characterization of the mycorrhizal communities of epiphytic orchids. *Mycoscience*, 62(6), 356-363. https://doi.org/10.47371/mycosci.2021.06.005