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ORIGINAL ARTICLE

Orchid epiphytes do not receive organic substances from living trees through fungi

Alen K. Eskov[1] · Elena Yu. Voronina[2] · Leho Tedersoo[3] · Alexey V. Tiunov[4,5] · Vu Manh[5] · Nikolay G. Prilepsky[2] · Violetta A. Antipina[1] · Tatiana G. Elumeeva[2] · Evgeny V. Abakumov[6] · Vladimir G. Onipchenko[2]

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Abstract

Numerous studies of terrestrial orchids have demonstrated widespread partial mycoheterotrophy, particularly the possibility of obtaining organic matter from surrounding trees through a common fungal network. Fungi are also widespread in epiphytic orchid roots, but there have been no attempts to determine if epiphytes accept organic matter from the living stems of their phorophytes. We hypothesise that such transfer does not exist because epiphytes and phorophytes harbour different fungal communities. To test this hypothesis, we tagged three short *Randia* sp. trees with ^{13}C-enriched CO_2 and examined ^{13}C transfer from the phorophyte into the epiphytic orchids *Grosourdya appendiculata*, *Dendrobium oligophyllum* and *Gastrochilus* sp. in Cat Tien National Park, (South Vietnam, Cat Tien National Park, plot size approx. 1 ha). The coincidence of fungal sequences in the orchid roots and in the branches on which they grew was also examined. We did not detect ^{13}C label moving from phorophytes to epiphytes. Using Illumina sequencing, 162 fungal operational taxonomic units (OTUs) were detected. The fungal communities were significantly different between the roots of epiphytes and branches of phorophytes, although no strict fungal specificity at the species level was found in either epiphytes or phorophytes.

Keywords Orchid epiphytes · Mycorrhizal fungi · Partial mycoheterotrophy · Isotope analysis · Metabarcoding analysis

Introduction

Epiphytes account for nearly 10% of the total biodiversity of vascular plants (Benzing 1990). Orchids dominate the epiphytic group and in the initial stage of their development, they undergo fungal colonisation (Dearnaley 2007). Approximately 235 species of orchids are chlorophyll-free and thus completely dependent on a fungal symbiont for life, i.e. are obligate myco-heterotrophs (MHs) (Merckx et al. 2013). Even most autotrophic terrestrial species of orchids receive C from fungi (Yagame et al. 2012; Merckx 2013; Gebauer and Meyer 2003; Stöckel et al. 2014; Gebauer et al. 2016).

Orchid mycorrhiza and their respective fungal partners can be classified into three categories. The first group includes mycorrhizal orchids with mostly saprotrophic fungi of the polyphyletic rhizoctonia group (Gebauer et al. 2016; Schiebold et al. 2018; Suetsugu et al. 2019). However, recent work has shown that these orchids are not linked via their mycorrhizal fungi to any host trees (May et al. 2020). The second group includes mycorrhizal orchids with fungi simultaneously forming ectomycorrhizas with forest trees. These orchids live as epiparasites in a tripartite network and gain C indirectly from host trees. However, this linkage occurs in the soil through orchid and host tree roots and, thus, is unlikely to be found among epiphytic orchids (Yagame et al. 2012). The final group is composed of mycorrhizal orchids that associate

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✉ Alen K. Eskov
A.K.Eskov@yandex.ru

[1] Tzitzin Main Botanical Garden, Russian Academy of Sciences, 4 Botanicheskaya ul., 117628 Moscow, Russia

[2] Lomonosov Moscow State University, 1-12 Leninskie Gory, 119991 Moscow, Russia

[3] Department of Mycology and Microbiology, University of Tartu, 46 Vanemuise, 51005 Tartu, Estonia

[4] Severtsov Institute of Ecology and Evolution, Russian Academy of Sciences, 33 Leninskij prosp., 119071 Moscow, Russia

[5] The Russian-Vietnamese Tropical Scientific and Technological Centre, Southern Branch, 3 st. 3/2, district 10, Ho Chi Minh 70000, Vietnam

[6] Saint-Petersburg State University, 7/9 Universitetskaya nab., 199034 St. Petersburg, Russia

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with saprotrophic wood- or litter-decomposing fungi. These orchids usually live on dead tree material on forest ground and only seldom interact with living trees; one exception is the fully mycoheterotrophic climbing orchid *Erythrorchis altissima*, which forms stem-borne roots that contact wood-decomposing fungi living in the dead heartwood of host trees (Ogura-Tsujita et al. 2018). Riofrio et al. (2013) found that the epiphytic orchid *Epidendrum rhopalostele* is limited to growth on dead trees and that the main group of root symbionts were wood-decomposing fungi. For example, within the genus *Cymbidium*, for which epiphytic species are also characteristic, myxo- and mycoheterotrophy is shown only for terrestrial species (Motomura et al. 2010). The evolution from autotrophy to complete mycoheterotrophy within the genus goes in the direction from epiphytes to terrestrial plants that tolerate deep shading (Ogura-Tsujita et al. 2012).

Mycorrhizal associations of epiphytic orchids, in general, have remained relatively poorly understood compared with terrestrial species (Lesica and Antibus 1990; Richardson et al. 1993; Liu et al. 2010; Riofrio et al. 2013; Xing et al. 2015; Cevallos et al. 2017; Wang et al. 2017). Research is mainly focusing on the fungal dependency of seed germination and development of protocorm in epiphytic orchids (Alghamdi 2019; Otero et al. 2007; Zettler et al. 2011) and on the context of biological plasticity and adaptation of the species to environmental changes and disturbances (Kartzinel et al. 2013; Otero et al. 2007; Yokoya et al. 2015; Zettler et al. 2013). In particular, the degree of mycorrhizal colonisation of the roots of the epiphytic orchid may depend on the season (Bertolini et al. 2014). In addition, broad specificity associating with diverse mycorrhizal fungi is shown within one species of epiphytic orchid (Kartzinel et al. 2013). Many epiphytic orchids live in the lower canopy layers of the tropical forest, often in deep shade, so theoretically additional C transfer from fungi could be especially profitable for these plants. We are unaware of any definitive biological barriers preventing fungi from colonising epiphytes and phorophytes simultaneously and transferring organic compounds between these symbiotic organisms, but this phenomenon has not been studied in epiphytic orchids. In the fifties of the previous century, J. Ruinen (1953) put forward that a symbiotic consortium of epiphytic orchids and mycorrhizal fungi could be considered as parasitic for the host-tree.

The standard method of demonstrating transfer of organic compounds from fungi to orchids is to compare ^{13}C content with that of authotropic plants. Enrichment of ^{13}C may serve as an indicator of partial mycoheterotrophy (Gebauer and Meyer 2003), but many epiphytic orchids are CAM plants with higher ^{13}C content than common C3 plants as a consequence of this photosynthetic feature. So, in this case, the observation of natural ^{13}C content will not help indicate C transfer, and therefore, the question requires an experimental approach.

We hypothesised that orchid epiphytes, unlike their terrestrial relatives, do not obtain some organic compounds from trees through a fungal partner even when photosynthesis is light-limited (shade environment). To test this hypothesis, we performed a ^{13}C-labelling experiment in a tropical forest of southern Vietnam. We introduced the isotopic label into the phorophyte via ^{13}C-enriched CO_2 to track whether the label enters the epiphytes through possible fungal consortia. Metabarcoding was used to assess the overlap in the fungal communities in the roots of orchids and in the stems (phloem) of the phorophyte branches on which the orchids lived. We proceeded from the fact that if orchids are potentially suspected of having such a connection, then these should be (1) epiphytes growing in deep shadow, (2) having ^{13}C or ^{15}N enrichment.

Materials and methods

Field studies

Field studies were performed in a lowland monsoon tropical forest in Cat Tien National Park (Vietnam) in 2015 and 2016 (Fig. 1). The subequatorial monsoon climate of the National Park is characterised by two seasons: wet/rainy (May–October) and dry (November–April). The annual precipitation is approximately 2500 mm, and the average annual temperature is 26.2 °C (Deshcherevskaya et al. 2013). Cat Tien forest grows on rich soils of volcanic origin, defined as Vertisol Folic and Vertisol Hyperskeletic (Khokhlova et al. 2017). The sample plot occupied approximately 1 ha (11° 26′ 14″ N and 107° 25′ 26″ E, ca. 120 m a.s.l.). Canopy trees are 30–35 m in height.

We determined the isotopic carbon signature of all types of available epiphytic orchids. We identified three species of small orchid epiphytes, which had a deviating carbon signature (resembling CAM plants) but also grew in shade: *Grosourdya appendiculata* (Blume) Rchb.f., *Dendrobium oligophyllum* Gagnep. and *Gastrochilus* sp. The number of CAM epiphytes with increased ^{13}C content tends to increase with the height of the host tree (Silvera and Lasso 2016). The presence of plants enriched by ^{13}C in shadow is considered as abnormal and this phenomenon could suggest partial mycoheterotrophy of those plants. Therefore, we used this group of epiphytes. The study was conducted at the beginning of the dry season, when it is not raining daily, but dominant trees have not yet shed their leaves. The microclimate at height of epiphytic growth (about at the height of human lenght) shows minimal fluctuations in relative humidity (≥ 90%) throughout the year (Deshcherevskaya et al. 2013). Thus, the fungal community of epiphytic orchids in the shaded understory should suffer little from periodic drying during the year maintaining functional mycorrhizal networks if present.

Fig. 1 Addition of ^{13}C-CO_2 to the Phorophyte-Epiphyte system, demonstrating a branch of the lower-story phorophyte *Randia* sp. insulated with a bag into which labelled CO_2 was introduced (**a**); experimental species included *Dendrobium oligophyllum* Gagnep. (**b**), *Gastrochilus* sp. (**c**) and *Grosourdya appendiculata* (Blume) Rchb.f (**d**); confocal microscopy image of fungi colonisation of a flattened living aerial root of *Gastrochilus* sp. adjoining the bark of the branches (**e**); in the upper right corner at larger magnification is the cell with fungal hyphae (green-blue)

Isotope enrichment

We selected three small phorophytes belonging to *Randia* sp. that grow in shaded habitats in the lowest canopy layer (2–5 m in height) (Fig. 1a). These phorophytes were selected because the abundance of the orchid epiphytes *D. oligophyllum* (Fig. 1b), *Gastrochilus* sp. (Fig. 1c) and *G. appendiculata* (Fig. 1d). Before labelling, leaf specimens were collected from all three species of epiphytes and the leaves of phorophytes. Subsequently, one of the large branches of each of the trees was dressed in a dense transparent 150-L plastic bag (Fig. 1a). The bag was equipped with a plastic capsule containing 500 mg of 99% ^{13}C-$CaCO_3$. A sulphuric acid solution was injected through the wall of the capsule, which was added until the carbon dioxide evolution ceased. The labelling lasted from 10 a.m. to 5 p.m., after which the bags were removed. The experiment with Isotope enrichment was carried out on a day without rain. Samples of green leaves from phorophytes and epiphytes were collected after 2, 4, 6, 9 and 12 days from untreated tree branches.

Stable isotope analysis Dried samples were pulverised in the Retsch MM 200 mill (Retsch GmbH, Germany) and wrapped in tin foil. The sample weight was approximately 1.5 mg. The isotopic composition of C was measured using a Thermo Flash 1112 elemental analyser and Thermo Delta V Plus isotopic mass-spectrometer (Thermo, USA). The isotopic composition of C was expressed in δ-notation relative to the international standard (VPDB) using the equation:

$$\delta^{13}C(‰) = \left[\left(R_{\text{sample}}/R_{\text{standard}}\right)-1\right] \times 1000$$

in which R is the ratio of ^{13}C to ^{12}C. Samples were analysed with reference gas calibrated against the IAEA reference

materials USGS 40 and USGS 41 (glutamic acid). Drift was corrected using the internal laboratory standards (casein, alfalfa). The standard deviations of the $\delta^{13}C$ values of reference materials ($n = 8$) were less than 0.2‰.

Assessment of the scale of mycorrhizal colonisation

We collected living root systems of adult plants with a total weight of 3–5 g per sample and five replications for each species. The roots were fixed with a mixture of 96% ethanol:formalin:glacial acetic acid (18:1:1). The fixed roots were washed for 30 min in running water and macerated in a 15% solution of KOH in a thermostat at 85 °C for 3 h. For staining, 0.1 g of aniline blue was dissolved in 100 mL of 33% lactic acid; the preparations were kept in the dye solution for about 1 h, differentiated in a 33% lactic acid solution for 30 min and washed for another 30 min with running water. Then, samples were placed in a 25% glycerol solution for contrast. Squeezed preparations were studied by light microscopy. The incidence rate of mycorrhizal colonisation was evaluated as a percentage of the number of fields of view with mycorrhizal colonisation to the total number of fields of view (Akhmetzhanova et al. 2012; Selivanov 1981).

Collecting samples for identification of fungi

To identify mycorrhizal and endophytic fungi, all three orchid species (*G. appendiculata*, *D. oligophyllum* and *Gastrochilus* sp.) were investigated. Roots of orchids were collected, together with the branches on which they grew, in five replications. A layer of the most superficial fouling was carefully removed from the phorophyte branches without damage to the phloem. Next, the roots of epiphytes and their corresponding branches were processed in concentrated hydrogen peroxide for 10 min to degrade surface biota. Samples were dried at room temperature over silica gel.

Metabarcoding analyses

Before DNA extraction, ~ 0.5 g of a dried sample was powdered using two 3-mm steel beads in the Mixer Mill MM400 (Retsch GmbH, Haan, Germany) at 30 Hz for 10 min. Altogether, 0.2 g of root and branch tissue powder was subjected to DNA extraction using the PowerSoil DNA Isolation Kit (MoBio, Carlsbad, CA, USA) following the manufacturer's instructions. For amplification, we used a mixture of forward primers (ITS5mix1–5) in equimolar concentration (Tedersoo et al. 2014) in combination with the indexed reverse primer ITS4ngsUni (Tedersoo and Lindahl 2016). Positive and negative controls were included for PCR and sequencing. The amplicons were pooled and subjected to ligation of sequencing adaptors using the TruSeq DNA PCR-free HT Sample Prep kit (Illumina Inc., San Diego, CA, USA). The single library was sequenced on an Illumina MiSeq platform using 2 × 300 paired-end chemistry. The resulting single-end reads were merged, demultiplexed, checked for chimaeras and trimmed to include only ITS2 sequences, following Tedersoo et al. (2015). Operational taxonomic units (OTUs) were separated based on 97% sequence similarity using CD-HIT v4.6.1 (Fu et al. 2012). Fungal taxa were identified using blastN searches against UNITE v7.0beta reference data set (Nilsson et al. 2019).

Statistical analyses

The detrended correspondence analysis (DCA) was run to visualise spectra of fungal taxa between orchid species and bark of phorophytes. The analysis was run in the *vegan* package in the R statistical environment (Oksanen et al. 2016).

Results

Stable isotope patterns

All three species of epiphytic orchids *G. appendiculata*, *D. oligophyllum* and *Gastrochilus* sp. had a high content of ^{13}C ($\delta^{13}C \approx -15$‰), suggesting CAM metabolism. The leaves of the phorophytes *Randia* sp. had $\delta^{13}C$ values of approximately −31‰, which is typical of understory plants. Two days after labelling, the $\delta^{13}C$ values in the phorophyte leaves (taken outside the branches on which labelling was performed) had increased by more than an order of magnitude. The $\delta^{13}C$ values decreased significantly within the subsequent 10 days but remained ca. three times higher than the background level. In contrast, the $\delta^{13}C$ values of epiphytes did not change after labelling (Table 1).

Mycorrhizal associations

We found a small degree of mycorrhizal colonisation of the epiphyte roots (Table 1). The occurrence of mycorrhiza did not exceed 10%. Mycorrhizal fungi were located mainly in the proximal part of the roots, adjoining the bark of the branches (Fig. 1e).

Metabarcoding

We detected 162 fungal operational taxonomic units (OTUs) in the studied orchid species and corresponding phorophyte branches. The fungal OTUs belonged to 19 orders, 26 families and 34 genera from Ascomycota (Dothideomycetes, Eurotiomycetes, Lecanoromycetes, Pezizomycetes, Saccharomycetes, Sordariomycetes) and Basidiomycota (Agaricomycetes, Microbotryomycetes, Tremellomycetes). Only OTUs assigned to the rank of order or lower ($n = 98$)

Table 1 The occurrence of mycorrhiza (F) in roots of the studied epiphytes and the isotopic composition of carbon ($\delta^{13}C$ values) in the leaves of epiphytes and phorophytes before labelling and 2 and 12 days after labelling

	Species	Mycorrhiza in the roots		^{13}C labelling			
		F ± SD%	*n*	$\delta^{13}C$ ± SD‰ before labelling	$\delta^{13}C$ ± SD‰ after 2 days	$\delta^{13}C$ ± SD‰ after 12 days	*n*
Epiphytes	*Grosourdya appendiculata*	8.4 ± 3.6	5	− 15.6 ± 0.3	− 16.5 ± 1.0	− 16.1 ± 0.4	3
	Dendrobium oligophyllum	9.0 ± 2.5	5	− 15.1 ± 0.5	− 14.9 ± 0.4	− 15.1 ± 0.5	6
	Gastrochilus sp.	7.8 ± 2.4	5	− 15.9	− 16.2	− 15.7	1
Phorophytes	*Randia* sp.	—	—	− 31.6 ± 0.7	475 ± 279	68 ± 30	3

are discussed further in the text. There were 65 OTUs assigned to family, 57 identified to genus and 29 to the species level. Most of these ($n = 75$) were detected both in orchid roots and their respective phorophyte branches, 11 were restricted to orchids and 12 were found in phorophytes only. The composition of the fungal communities was significantly different between the roots of epiphytes and branches of phorophytes (Fig. 1 Suppl.). No fungal taxa were unique to a particular orchid species (see Supplementary Table S1). Xylariales and Capnodiales appeared to be the most diverse orders comprising 29 and 26 OTUs, respectively. Species of *Annulohypoxylon*, *Astrocystis*, *Daldinia*, *Hypoxylon*, *Neopestalotiopsis*, *Nodulisporium*, *Pseudopestalotiopsis*, *Whalleya* and *Xylaria* and some unidentified Xylariales were shown as common orchid root endophytes (see Supplementary Table S2). Most of the taxa were detected in phorophyte branches as well, but the number of Xylariales OTUs was higher in orchids (Fig. 2 Suppl.). Other orders were represented by five or less different OTUs. The most frequent fungal orders (with maximal OTU counts detected) from all hosts were Ostropales ($n = 12{,}458$ sequences), Pyrenulales ($n = 11{,}314$) and Cantharellales ($n = 9782$). Phorophyte branches were predominantly colonised by Ostropales ($n = 12{,}404$), Pyrenulales ($n = 11{,}312$) and Capnodiales ($n = 2526$). The main orchid root endophytes were Cantharellales ($n = 9736$), Xylariales ($n = 6504$), Pezizales ($n = 4977$) and Sebacinales ($n = 4000$) (Supplementary Table S1).

Discussion

In this study, we applied two methodological approaches traditionally used to detect mycoheterotrophy in orchids: assessment of carbon flow from trees or fungi to orchids (McKendrick et al. 2000; Gebauer and Meyer 2003; Trudell et al. 2003) and the detection of identical fungal ITS sequences in the roots of orchids and donor trees (Selosse et al. 2004; Bidartondo et al. 2004; Girlanda et al. 2006; Abadie et al. 2006). Mycoheterotrophy was previously shown for orchid climbers (Ogura-Tsujita et al. 2018) and epiphytes (Riofrio et al. 2013) growing on dead wood. But is it possible in adult epiphytic orchids growing on living trees? One can imagine that adult orchids growing on thin branches with very thin cork (phellema) in the shade form mycorrhizal networks that penetrate into the phloem of branches and intercept some of the organic substances from the tree. The search for a hypothetical mycoheterotrophy (except for the self-evident connection with saprotrophic fungi on dead wood) is justified under the field conditions that we investigated. Saprotrophic mycoheterotrophy cannot be considered typical of epiphytes, because the growth on dead wood is more likely an exception for epiphytes. Dead branches quickly break off and falling epiphytes die on the ground under conditions of darkening and hyperhumidification. Thus, growing on dead wood is not a typical lifelong strategy for most epiphytes.

No movement of the ^{13}C label from phorophyte to epiphyte was detected, whereas ITS sequences in the roots and branches were significantly different (Supplementary Fig. S1). So, our hypothesis was confirmed, and we can conclude that epiparasitic mycoheterotrophy did not occur, even in deep shade, in the specific epiphytic orchids studied. The minimal annual fluctuations in the relative humidity in the lowest layer of the forest, that we studied, should be favourable for the year-round functioning of mycorrhizal networks. Nevertheless, it was previously noted that the presence of functional mycorrhiza in roots of epiphytic orchids may depend on seasonality (Bertolini et al. 2014).

Our data confirm the scantiness of the mycorrhizal colonisation in the roots of epiphytic orchids and how it can be limited to particular root sections (Lesica and Antibus 1990; Richardson et al. 1993). Recent studies indicate that the composition of the mycorrhizal fungal community in a same species may differ among epiphytic and lithophytic lifestyles (Xing et al. 2015). Furthermore, the orchid root fungal composition maybe strongly affected by the host tree species (Wang et al. 2017).

Metabarcoding of the mycobiota of epiphytes and phorophytes demonstrated its complex structure. Although no fungal taxon was unique to any plant species, there was a tendency for particular fungi to be more common either in phorophytes or in epiphytic orchids (Supplementary Fig. S2). The 'phorophyte-preferring' fungal taxa were

Pyrenulales, Ostropales, Pleosporales, Russulales, Hypocreales, Saccharomycetales and Eurotiales. Pyrenulales (both OTUs placed within Pyrenulaceae) are known as saprobic or lichenised bark-dwelling fungi not involved in mycorrhizal interactions (Cannon and Kirk 2007). The most common, *Pyrenula quassiicola*, is a lichenised pantropical species that grows on bark (Aptroot 2012). All Ostropales OTUs were identified at the rank of order, and xylotrophic mode of life is typical for that order (Kirk et al. 2008). Pleosporales comprise epiphytes, endophytes and plant pathogens. A *Paraconiothyrium* sp. was detected in phorophyte branches only, but there is evidence that the genus associates with epiphytic orchids such as *Stanhopea tigrina* (Salazar-Cerezo et al. 2018) and *Holcoglossum* spp. (Tan et al. 2012). *Megacapitula villosa* was discovered both in phorophyte and orchid (*G. appendiculata*) tissues, but according to published data, it inhabits mainly fallen decaying petioles (Prabhugaonkar and Bhat 2011). Russulales were represented by a single species, *Heterobasidion annosum*, a well-acknowledged tree pathogen (Núñez and Ryvarden 2001). Hypocreales OTUs included representatives of *Aschersonia*, *Nectria* and *Trichoderma*. Although the two former genera are more frequent in phorophyte branches, *Trichoderma* sp. appeared to have an inclination to *G. appendiculata* roots. This genus is known to comprise ubiquitous soil, and plant remnant dwellers, saprobes and mycopathogens, but some *Trichoderma* species have been previously reported as endophytes from both tropical epiphytic and boreal terrestrial orchids (Supplementary Table S2).

The tendency to colonise epiphytic orchid roots rather than the phorophyte tissues was noted for Tremellales (detected in orchids only), Sordariales, Sebacinales, Pezizales, Polyporales, Xylariales, Cantharellales, Chaetothyriales, Microascales and Botryosphaeriales (Fig. 2 Suppl). *Kockovaella prillingeri*, a single species of Tremellales revealed in *G. appendiculata* and *Gastrochilus* sp., was not previously found in association with orchids (Supplementary Table S2). *Gelasinospora calospora* from Sordariales was also not reported from orchids, being a common soil saprobe, but in our case, the species was found as endophyte in all three orchid species studied. Sebacinales OTUs were identified only at order level. The order exhibits a wide range of mutualistic interactions with plants, including orchid and ectomycorrhizas (Smith and Read 2008). Pezizales are ecologically heterogeneous, with some ecto- and orchid mycorrhizal species but with saprobes prevailing (Kirk et al. 2008). *Phillipsia carnicolor* was detected as endophyte in all three orchid species, and this genus was also not reported as orchid-associated. Polyporales were presented by *Flavodon flavus* and *Ganoderma* sp. Both species are wood-decaying but were more frequently detected in orchids rather than in its phorophytes, and mentioned as non-mycorrhizal endophytes in orchids (Supplementary Table S2). *Ganoderma* species were reported as endophytes for achlorophyllous climber orchid *Erythrorchis altissima* (Bayman and Otero 2006), but were treated (e.g. Rasmussen 2002) as physiological (rather than ecological) symbionts able to influence seed germination and colonise seedlings.

Cantharellales including the Ceratobasidiaceae family are known to be mycorrhizal with Orchidaceae and some other plants, but other species are plant-pathogens or saprobes (Cannon and Kirk 2007). In our case, they were common endophytes in *G. appendiculata* but were absent or rare in the other two orchid species. Chaetothyriales were presented by a single species, *Cyphellophora guyanensis*, which occurred in both orchids and its phorophytes. This species was previously described in orchids; it was described as a leaf litter saprobe (Decock et al. 2003). There are some endophyte species in this and related genera (Réblová et al. 2013). Microscales OTUs were detected in all orchid species studied, but the order comprises primarily saprobic fungi in soil, rotting vegetation, dung and marine environments (Zhang and Wang 2015). *Microascus murinus* (found in *D. oligophyllum* and *G. appendiculata*) was not reported from orchids, but congeneric species were mentioned as orchid endophytes (Supplementary Table S2). Botryosphaeriales were represented by *Lasiodiplodia theobromae* detected in *Gastrochilus* sp. Being known as a non-specific plant pathogen, it was often reported from non-affected roots of a wide range of orchids. According to a vast review of many experimental studies by Bayman and Otero (2006), *L. theobromae* belongs to the most frequent endophytes in tropical epiphytic orchids.

To date, no adult epiphytic orchid species have been discovered to engage in myco-heterotrophy, and our study suggests that this type of nutrition is unlikely for epiphytes. However, it seems that periodic drying of roots and growth substrates (even under deep shadow) makes it impossible to develop fungal networks that are functionally similar to those in the soil. However, searches in other groups of epiphytes may yield different results. In particular, humus and nesting epiphytes, which form suspended soils, may give a greater chance of detecting epiparasitic mycoheterotrophy with respect to the host tree. A promising direction is the study of different indicators (for example, signatures H, N, O, see Gebauer et al. 2016). In this sense, our work is the first in the search for partial mycoheterotrophy in orchid epiphytes on living hosts (and not on dead wood) and does not pretend to a final solution to the problem.

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