CZECH MYCOLOGY 73(1): 91–108, MAY 17, 2021 (ONLINE VERSION, ISSN 1805-1421)

Ecological relevance of the endophytic fungal diversity in velamen roots of tropical epiphytic orchids

AZHANTHANILKUNNATHIL SANKUNNY DEEPTHI, JOSEPH GEORGE RAY*

Laboratory of Plant Science and Ecology, School of Biosciences, Mahatma Gandhi University, Kottayam, Kerala, IN-686560, India
*corresponding author: jgray@mgu.ac.in

Deepthi A.S., Ray J.G. (2021): Ecological relevance of the endophytic fungal diversity in velamen roots of tropical epiphytic orchids. – Czech Mycol. 73(1): 91–108.

The velamen roots in epiphytic orchids are rather complex, and their environmental role remains poorly understood. Fungal associates are known in the velamen roots of tropical orchids, but the magnitude of their diversity in particular species remains unknown. The primary aim of this work was to explore endophytic fungal species associated with the velamen roots of three tropical epiphytic orchids.

Velamen roots were collected from 30 plants of *Rhynchostylis retusa* and 25 plants each of *Epidendrum radicans* and *Oncidium sphacelatum*. Endophytic fungi were isolated from 2160 segments. Twenty species of velamen-root-associated fungal endophytes were separated with a culture technique. The isolated strains were grouped into morphotypes, subsequently identified morphologically and by means of ITS DNA barcoding.

Ascomycota were the dominant group with 18 species. *Trichoderma* cf. *asperellum*, *Endomelanconiopsis endophytica*, *Trichoderma* cf. *atroviride* and *Lasiodiplodia theobromae* were the most frequent taxa isolated. A majority of the identified fungi were common to more than one orchid. Colonisation rate, isolation rate, Shannon-Wiener diversity index, species richness and species evenness of the endophytic fungi from different orchids were studied and were tested for significance with the Kruskal-Wallis H test. The colonisation rate and isolation rate of fungal associates in the velamen roots were found to be distinctly the lowest in *Rhynchostylis retusa*.

Key words: epiphytes, *Orchidaceae*, root-associated endophytes, *Trichoderma*, *Endomelanconiopsis*, *Lasiodiplodia*.

Article history: received 5 January 2021, revised 31 March 2021, accepted 16 April 2021, published online 17 May 2021.

DOI: https://doi.org/10.33585/cmy.73107

Deepthi A.S., Ray J.G. (2021): Diverzita endofytických hub ve vzdušných kořenech tropických epifytických orchidejí a její ekologický význam. – Czech Mycol. 73(1): 91–108.

Vzdušné kořeny epifytických orchidejí představují komplexní strukturu, na jejíž úloze v prostředí je stále co zkoumat. Je známo, že vzdušné kořeny tropických orchidejí obsahují též houby, ale rozsah jejich diverzity v konkrétních druzích rostlin zůstává neznámý. Primárním cílem této práce tak bylo zjistit, jaké druhy endofytických hub se vyskytují ve vzdušných kořenech tří tropických druhů epifytických orchidejí.

Vzdušné kořeny byly sebrány z 30 rostlin *Rhynchostylis retusa* a po 25 z *Epidendrum radicans* a *Oncidium sphacelatum*. Endofytické houby byly izolovány z 2160 segmentů. Kultivační technikou bylo vylišeno dvacet druhů endofytických hub, asociovaných se vzdušnými kořeny. Izolované kmeny byly nejprve seskupeny do morfotypů a následně identifikovány morfologicky a sekvenací ITS DNA.

Dominantní skupinou jsou vřeckaté houby (18 druhů), z nichž byly nejčastěji izolovány *Trichoderma* cf. *asperellum*, *Endomelanconiopsis endophytica*, *Trichoderma* cf. *atroviride* a *Lasiodiplodia theobromae*. Většina zjištěných hub se běžně vyskytuje ve více druzích orchidejí. Pro endofyty z různých druhů byla porovnána rychlost kolonizace a izolace, Shannon-Wienerův index diverzity, druhová bohatost a vyrovnanost společenstev; significance byla prověřena Kruskal-Wallisovým H testem. Zřetelně nejnižší rychlost kolonizace a izolace byla zjištěna u hub ze vzdušných kořenů *Rhynchostylis retusa*.

INTRODUCTION

Many plants harbour a large number of hidden microbial communities in their internal tissues, and such microbes not only cause some diseases (Hyde et Soytong 2008) but are often beneficial. Many kinds of endophytic fungal associates have been found in different tissues of diverse taxonomic plant groups (Stone et al. 2000, Rodriguez et Redman 2008). The endophytic fungi present in velamen roots of orchids, either in its outer velamen tissue or in its inner cortical parts, are collectively termed as velamen-root-associated endophytes (Novotná et al. 2018). They include mutualistic mycorrhizae and non-mycorrhizal fungi (Ma et al. 2015). The non-mycorrhizal fungal associates of orchid roots have distinct ecological attributes, acting as saprobes, latent pathogens or symbionts (Ma et al. 2015, Cevallos et al. 2018).

The hanging velamen roots in epiphytic orchids, also found in some terrestrial monocots, are ecologically unique organs in the plant world (Zotz et al. 2017). They are considered suitable ecological niches for many microorganisms (Tsavkelova et al. 2003a, Ram et Shamina 2014, Deepthi et Ray 2018, 2020). The majority of the studies related to endophytic fungi associated with orchid roots deal with the mycorrhizal fungal associates of terrestrial orchid species of tropical and temperate ecosystems (Tao et al. 2008, 2013, Zettler et al. 2013, Ratnaweera et al. 2014, Zhao et al. 2014, Jacquemyn et al. 2015, Těšitelová et al. 2015, Yokoya et al. 2015, Suárez et Kottke 2016). There are only a few investigative reports on the frequency, diversity and ecological roles of velamen-root-associated non-mycorrhizal endophytic fungi, especially of tropical epiphytic orchids (Bayman et Otero 2006, Herrera et al. 2010, Ma et al. 2015, Abraham et Ashakumari 2017, Jin et al. 2017, Cevallos et al. 2018, Deepthi et Ray 2018, Novotná et al. 2018, Shah et al. 2019). Moreover, studies focusing on non-mycorrhizal fungal endophytes in velamen roots of epiphytic orchids of the biodiversity-rich tropical India are relatively rare (Sudheep et Sridhar 2012, Abraham et Ashakumari 2017, Deepthi et Ray 2018).

Muthukumar et Kowsalya (2017) reported the presence of root hairs on velamen roots and Deepthi et Ray (2018) suggested with evidence that the root hairs are produced opportunistically, i.e., when the velamen roots need to be attached to a substratum or to absorb nutrients from the host body or the soil. Because of the unique morphological characteristics of velamen roots, the study of fungal associates is significant in the exploration of fungal biodiversity (Bayman et al. 1997). However, exploring the diversity of fungi in velamen roots is also essential to reveal the unique biological roles of such fungi. It is also necessary to understand the ecological complexities of such roots. Moreover, the study of fungal associates in velamen roots is also valuable because of their importance as potential sources of precious metabolites (Priti et al. 2009, Deepthi et Ray 2019). The nature of velamen roots as a natural habitat for diverse categories of microorganisms, including endophytic fungi (Cevallos et al. 2017, Deepthi et Ray 2018, Novotná et al. 2018) and micro-algae (Tsavkelova et al. 2003b, Deepthi et Ray 2020), offers the possibility of considering velamen roots as an organ of a complex nutritional or other functionally significant 'organ-ecosystem' in epiphytic orchids.

In the above contexts, following a culture technique, the diversity of velamen-root-associated fungi of three common epiphytic orchid species of Kerala State, India, *Rhynchostylis retusa* (L.) Blume, *Epidendrum radicans* Pav. ex Lindl. and *Oncidium sphacelatum* Lindl. have been identified and compared. The primary aims of the present study were (i) to isolate and genetically identify culturable non-mycorrhizal fungi associated with velamen roots of epiphytic orchids; (ii) to analyse and compare the qualitative and quantitative composition of non-mycorrhizal fungi in the velamen roots of epiphytic orchids; (iii) to assess the ecological characteristics of non-mycorrhizal fungal associates of velamen roots of epiphytic orchids. The present report aims to provide sufficient ground for further extensive exploration of endophytic diversity and intensive investigation into the ecological complexities of velamen roots in epiphytic orchids.

Material and methods

Sample collection. The hanging velamen roots of three epiphytic orchids, *Rhynchostylis retusa*, *Epidendrum radicans* and *Oncidium sphacelatum*, were selected for the present study. *Rhynchostylis retusa*, known as 'foxtail orchid', is a common epiphytic orchid growing in the wild throughout Kerala. *Epidendrum radicans*, commonly known as 'holy cross orchid', and *Oncidium sphacelatum*, generally known as 'dancing lady orchid', are the most common garden species of epiphytic orchids in Kerala.

Orchid velamen root samples were collected in the Pathanamthitta, Kottayam, Idukki, Alappuzha and Kollam districts of Kerala State, India. The sampling locations are situated between 76°15' E to 77°50' E and 8°45' N to 10°15' N. Geographic positions of the study area were recorded, and the data set was imported into geographical information software (ArcGIS10) to display their geographical distribution. Details regarding sampling sites and sampling species are given in Fig. 1.

Sampling was carried out from March 2016 to February 2018. Three roots were collected from 80 plants; 30 from *Rhynchostylis retusa*, 25 each from *Epidendrum radicans* and *Oncidium sphacelatum*. Only medium-sized (20–25 cm long) healthy hanging velamen root samples were collected for fungal isolation. The pieces were placed in polyethylene bags, labelled and transported to the laboratory. All samples were processed within 24 hours of collection.

Isolation of fungal endophytes. Surface sterilisation of the velamen roots and isolation of the endophytic fungi were carried out according to Bayman et al. (1997) and Deepthi et Ray (2018). In brief, the root samples collected from a site were cut into pieces of about 5 cm long and were washed thoroughly with tween 20 (Sigma-Aldrich) followed by running tap water to remove dust and debris and surface-sterilised in a sequence of 70% ethanol for 1 minute, 2.5% $NaClO_2$ for 1 minute and rinsed three times in sterile distilled water. From each piece of root, three 5mm segments were taken using a sterile razor blade. Nine pieces of the roots from each sample were placed on three different media (in triplicates) in 9 separate Petri plates [approximately 15–20 ml of potato dextrose agar medium (PDA) with pH 5.8, malt extract agar medium (MEA) with pH 5.8 and Sabouraud dextrose agar medium (SDA) with pH 5.6] containing 100 $\mu g \cdot ml^{-1}$ streptomycin to prevent bacterial growth. In total, 2160 velamen root segments of the three epiphytic orchids were used to isolate endophytic fungi.

Identification of fungal endophytes. Morphological identification. The fungal isolates were grown on PDA at 28 ± 2 °C in the dark for seven days. The pure cultures were categorised as morphotypes based on their morphological traits such as reverse and obverse colour, texture and shape of the colony. Sporulating cultures were identified using the monographs by Watanabe (2002) and Nagamani et al. (2006).

Molecular identification. DNA isolation and molecular characterisation were carried out according to the procedure by Deepthi et Ray (2018). Primers ITS-1 and ITS-4 (White et al. 1990) were used to amplify the internal transcribed spacer (ITS) sequences. The 'Basic Local Alignment Search Tool' (BLAST) programme in the NCBI GenBank (www.ncbi.nlm.nih.gov) was conducted on all sequences. Compared with reference sequences, the isolates were assigned species level when the database matched for more than 98%. For species identification in

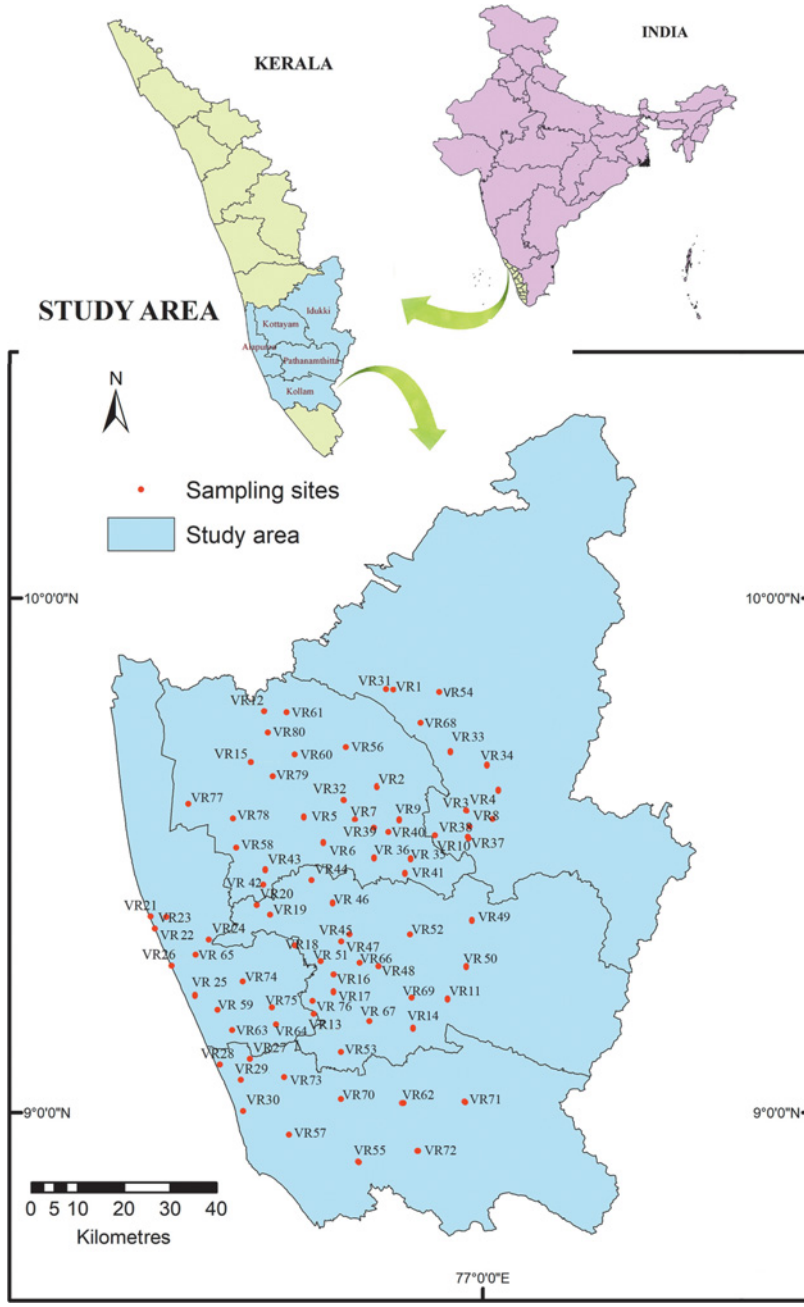

Fig. 1. Study region showing 80 sampling sites in Kerala State, India: VR1–VR30 – sampling sites of *Rhynchostylis retusa*; VR31–VR55 – sampling sites of *Epidendrum radicans*; VR56–VR80 – sampling sites of *Oncidium sphacelatum*.

the genera *Fusarium*, *Trichoderma*, *Aspergillus* and *Colletotrichum* ITS sequencing is not sufficient and multiple gene analysis is needed for exact identification of the species. Therefore, the identified taxa of *Fusarium*, *Trichoderma*, *Aspergillus* and *Colletotrichum* were presented using 'cf.' in their species names.

Data analysis and statistics. The colonisation rate (CR), isolation rate (IR) and relative frequency of isolation (RF%) of fungal associates were calculated following the method by Huang et al. (2008). The colonisation frequency (CF%) was calculated according to Kumaresan et Suryanarayanan (2001). The percentage of dominant fungi (%D) was calculated as the colonisation frequency divided by the total number of endophytes × 100 (Kumaresan et Suryanarayanan 2002). Species diversity indices such as the Shannon-Wiener diversity index (H'), species richness and species evenness were calculated using the PAST software (Hammer et al. 2001). The differences in colonisation rate, isolation rate, Shannon-Wiener diversity index (H'), species richness and species evenness were tested for significance with the Kruskal-Wallis H test using SPSS 20.0 software.

RESULTS

Diversity of velamen-root-associated fungal endophytes

Altogether, 563 fungal cultures were isolated from 2160 velamen root segments of three epiphytic orchid species studied. These were 147 fungal isolates from the velamen root segments of *Rhynchostylis retusa*, 202 fungal isolates from those of *Epidendrum radicans* and 214 fungal isolates from those of *Oncidium sphacelatum*. The 563 fungal cultures obtained were grouped into 38 morphotypes based on their characteristics on PDA. The parameters used for categorising cultures were surface characters, growth pattern and colour of the colony. These morphotypes were further examined morphologically and by means of ITS DNA barcoding, and identified as 20 different taxa (Tab. 1). Two taxa, *Aspergillus* sp. and *Mucor* sp., were identified with morphological methods only. Molecular characterisation of the remaining 36 morphotypes was carried out, grouping them into 18 species. The ITS region of the DNA, amplified using universal primers ITS-1 and ITS-4, provided amplicons of 489–683 base pairs in length. The DNA ITS sequences of the 36 morphotypes divided into 18 species were submitted to GenBank (Tab. 2).

Among the fungal isolates, 94.13% of the members belonged to *Ascomycota* (530 isolates); 15 isolates were *Basidiomycota* and 18 belonged to *Mucoromycota*. In total, 20 fungal species belonging to 13 genera were recovered. The division *Ascomycota* was dominant with 18 species; the *Ascomycota* were represented by seven orders, i.e. *Hypocreales* (36.59%), *Botryosphaeriales* (23.09%),

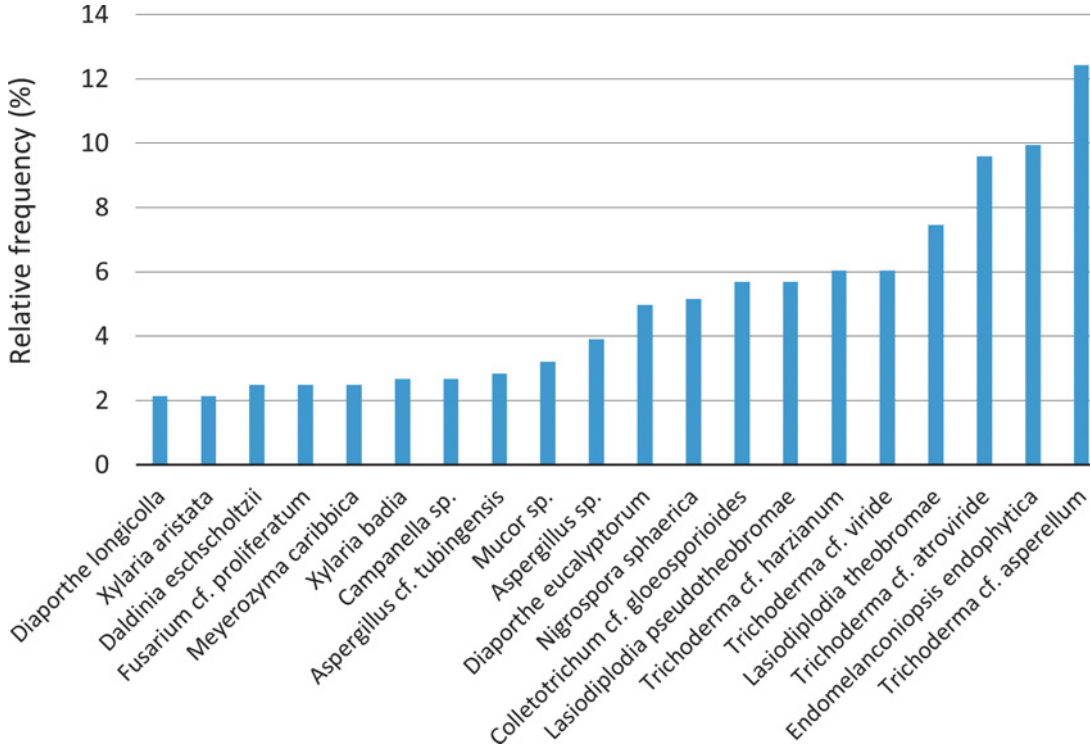

Fig. 2. Overall relative frequency (RF%) of endophytic fungal associates in the velamen roots of epiphytic orchids.

Xylariales (12.43%), *Diaporthales* (7.10%), *Eurotiales* (6.75%), *Glomerellales* (5.68%) and *Saccharomycetales* (2.49%). The divisions *Basidiomycota* and *Mucoromycota* included one species each (Tab. 1).

The most frequent species were *Trichoderma* cf. *asperellum* with 70 isolates with a relative frequency (RF%) of 12.43. Other frequently isolated fungal taxa were *Endomelanconiopsis endophytica* (56 isolates, RF% 9.95), *Trichoderma* cf. *atroviride* (54 isolates, RF% 9.59) and *Lasiodiplodia theobromae* (42 isolates, RF% 7.46). The least frequent taxa were *Diaporthe longicolla* and *Xylaria aristata*, each with an RF% of 2.13 (Fig. 2).

Tab. 1. Colonisation frequency (CF%) and dominance (%D) of fungal associates in the velamen roots of epiphytic orchids.

Fungal associates	**Taxonomic position (division* / order)**	***Epidendrum radicans***		***Oncidium sphacelatum***		***Rhynchostylis retusa***	
		CF%	**%D**	**CF%**	**%D**	**CF%**	**%D**
Aspergillus sp.	A / *Eurotiales*	–	–	4.07	1.90	–	–
Aspergillus cf. *tubingensis* Mosseray	A / *Eurotiales*	–	–	2.96	1.38	–	–
Campanella sp.	B / *Agaricales*	–	–	–	–	2.59	1.76
Colletotrichum cf. *gloeosporioides* (Penz.) Penz. et Sacc.	A / *Glomerellales*	2.22	1.10	2.96	1.38	0.69	0.47
Daldinia eschscholtzii (Ehrenb.) Rehm	A / *Xylariales*	–	–	2.59	1.21	–	–
Diaporthe eucalyptorum Crous et R.G. Shivas	A / *Diaporthales*	3.70	1.83	–	–	1.38	0.94
Diaporthe longicolla (Hobbs) J.M. Santos, Vrandecic et A.J.L. Phillips	A / *Diaporthales*	2.22	1.10	–	–	–	–
Endomelanconiopsis endophytica Rojas et Samuels	A / *Botryosphaeriales*	4.07	2.01	3.51	1.64	2.59	1.76
Fusarium cf. *proliferatum* (Matsush.) Nirenberg ex Gerlach et Nirenberg	A / *Hypocreales*	–	–	–	–	2.41	1.64
Lasiodiplodia theobromae (Pat.) Griffon et Maubl.	A / *Botryosphaeriales*	4.07	2.01	–	–	3.45	2.35
Lasiodiplodia pseudotheobromae A.J.L. Phillips, A. Alves et Crous	A / *Botryosphaeriales*	2.96	1.47	–	–	2.76	1.88
Meyerozyma caribbica (Vaughan-Mart., Kurtzman, S.A. Mey. et E.B. O'Neill) Kurtzman et Suzuki	A / *Saccharomycetales*	–	–	–	–	2.41	1.64
Mucor sp.	M / *Mucorales*	–	–	3.33	1.56	–	–
Nigrospora sphaerica (Sacc.) E.W. Mason	A / *Xylariales*	1.48	0.73	2.96	1.38	0.86	0.59
Trichoderma cf. *asperellum* Samuels, Lieckf. et Nirenberg	A / *Hypocreales*	4.44	2.20	4.81	2.25	3.45	2.35
Trichoderma cf. *atroviride* P. Karst.	A / *Hypocreales*	4.07	2.01	5.93	2.77	–	–
Trichoderma cf. *harzianum* Rifai	A / *Hypocreales*	3.33	1.65	–	–	2.76	1.88
Trichoderma cf. *viride* Pers.	A / *Hypocreales*	2.59	1.28	3.70	1.73	–	–
Xylaria aristata Mont.	A / *Xylariales*	2.22	1.10	–	–	–	–
Xylaria badia Pat.	A / *Xylariales*	–	–	2.77	1.29	–	–

* A – *Ascomycota*, B – *Basidiomycota*, M – *Mucoromycota*

Tab. 2. Molecular identification of isolated morphotypes to species, their closest match in the NCBI database with their accession number, similarity (%ID) and query coverage (%QC).
Abbreviations: RRVR – velamen-root-associated fungi isolated from *Rhynchostylis retusa*, ERVR – velamen-root-associated fungi isolated from *Epidendrum radicans*, ONVR – velamen-root-associated fungi isolated from *Oncidium sphacelatum*. ▶

Morpho-type acronym	Num-ber of iso-lates	Species identification	GenBank accession number	Number of base pairs sub-mitted	Reference accession number	ID/QC (%)	Reference
RRVR 01	4	*Lasiodiplodia theobromae*	MF100156.1	591	KX464366.1	100/100	Yang et al. (2017)
RRVR 02	3	*Lasiodiplodia theobromae*	MH259842.1	489	LN907507.1	99/99	Valenzuela-Lopez et al. (2017)
RRVR 03	6	*Lasiodiplodia theobromae*	MH260583.1	489	LN907507.1	99/99	Valenzuela-Lopez et al. (2017)
RRVR 04	15	*Endomelanconiopsis endophytica*	MH260585.1	605	EU683630.1	99/100	Rojas et al. (2008)
RRVR 05	9	*Lasiodiplodia pseudotheobromae*	MH259847.1	494	MF410073.1	99/100	Li et al. (2018)
RRVR 06	15	*Campanella* sp.	MH846120.1	597	AF261340.1	99/100	Moncalvo et al. (2002)
RRVR 07	20	*Trichoderma* cf. *asperellum*	MH260587.1	611	KC800921.1	100/100	Singh et al. (2014)
RRVR 08	16	*Trichoderma* cf. *harzianum*	MH260588.1	591	KY643781.1	100/100	Deepthi et Ray (2018)
RRVR 09	14	*Meyerozyma caribbica*	MH259848.1	560	KF268354.1	98/100	Romi et al. (2014)
RRVR 10	7	*Lasiodiplodia pseudotheobromae*	MH259862.1	563	MF410073.1	99/100	Li et al. (2018)
RRVR 11	7	*Lasiodiplodia theobromae*	MH259863.1	565	KX464366.1	99/100	Yang et al. (2017)
RRVR 12	14	*Fusarium* cf. *proliferatum*	MH260586.1	554	FN868470.1	99/100	Sankar et Babu (2012)
RRVR 13	5	*Nigrospora sphaerica*	MH259869.1	591	KM921666.1	99/99	Wang et al. (2017)
RRVR 14	8	*Diaporthe eucalyptorum*	MH260584.1	565	KY643784	100/100	Deepthi et Ray (2018)
RRVR 15	4	*Colletotrichum* cf. *gloeosporioides*	MF100149.1	591	KT282939.1	98/100	Unpublished
ERVR 01	12	*Xylaria aristata*	MK142801.1	576	AB376716.1	99/93	Okane et al. (2008)
ERVR 02	12	*Colletotrichum* cf. *gloeosporioides*	MK182361.1	581	KT282939.1	99/100	Unpublished
ERVR 03	24	*Trichoderma* cf. *asperellum*	MK182360.1	567	KY643785.1	99/100	Deepthi et Ray (2018)
ERVR 04	14	*Trichoderma* cf. *viride*	MK182364.1	561	AJ230682.1	100/99	Lieckfeldt et al. (1999)
ERVR 05	8	*Nigrospora sphaerica*	MK182363.1	585	KM921666.1	98/99	Wang et al. (2017)
ERVR 06	22	*Lasiodiplodia theobromae*	MK182366.1	593	KX464366.1	99/100	Yang et al. (2017)
ERVR 07	18	*Trichoderma* cf. *harzianum*	MK182367.1	591	JN939832.1	100/100	Schoch et al. (2012)
ERVR 08	12	*Diaporthe longicolla*	MK182365.1	565	JF704166.1	100/99	Unpublished
ERVR 09	22	*Endomelanconiopsis endophytica*	MK182373.1	605	EU683630.1	100/99	Rojas et al. (2008)
ERVR 10	20	*Diaporthe eucalyptorum*	MK182372.1	571	NG042675.1	100/100	Crous et al. (2012)
ERVR 11	22	*Trichoderma* cf. *atroviride*	MK182382.1	554	KT 323351.1	100/100	Unpublished
ERVR 12	16	*Lasiodiplodia pseudotheobromae*	MK182381.1	593	MF410073.1	99/99	Li et al. (2018)
ONVR 01	14	*Daldinia eschscholtzii*	MK142808.1	555	KP012954.1	100/99	Unpublished
ONVR 02	22	*Aspergillus* sp.	Morphology	–	–	–	–
ONVR 03	18	*Mucor* sp.	Morphology	–	–	–	–
ONVR 04	26	*Trichoderma* cf. *asperellum*	MK142811.1	581	KC800921.1	99/100	Singh et al. (2014)
ONVR 05	20	*Trichoderma* cf. *viride*	MK142815.1	567	AJ230682.1	100/99	Lieckfeldt et al. (1999)
ONVR 06	32	*Trichoderma* cf. *atroviride*	MK142817.1	567	KT323351.1	100/100	Unpublished
ONVR 07	16	*Colletotrichum* cf. *gloeosporioides*	MK142814.1	561	JQ754058.1	99/100	Unpublished
ONVR 08	15	*Xylaria badia*	MK142821.1	683	JQ862643.1	98/84	Chen et al. (2013)
ONVR 09	16	*Nigrospora sphaerica*	MK142820.1	591	KM921666.1	99/99	Wang et al. (2017)
ONVR 10	19	*Endomelanconiopsis endophytica*	MK142816.1	605	EU683630.1	100/99	Rojas et al. (2008)
ONVR 12	16	*Aspergillus* cf. *tubingensis*	MK142819.1	560	KX958024.1	100/100	Liu et al. (2017)

Comparison of velamen-root-associated fungal endophytes between the orchid species

Altogether 12 species of endophytic fungi were found in the velamen roots of *Epidendrum radicans*, whereas 11 species each in the velamen roots of both *Rhynchostylis retusa* and *Oncidium sphacelatum*. Among the total fungal isolates from the three orchids observed, four fungal species, *Trichoderma* cf. *asperellum*, *Colletotrichum* cf. *gloeosporioides*, *Endomelanconiopsis endophytica* and *Nigrospora sphaerica*, were found to be common to all the three epiphytic orchid species studied. This means that one third of the endophytic fungal associates in the velamen roots of the three orchids have no host specificity at all. However, some others were common to only two orchids, and a few were found host-specific to one orchid. Five fungal isolates, *Aspergillus* sp., *A.* cf. *tubingensis*, *Mucor* sp., *Daldinia eschscholtzii* and *Xylaria badia*, were host-specific to velamen roots of the orchid *Oncidium sphacelatum*, whereas two fungal isolates, *Diaporthe longicolla* and *Xylaria aristata*, were host-specific to velamen roots of *Epidendrum radicans*, and three fungal species such as *Campanella* sp., *Fusarium* cf. *proliferatum* and *Meyerozyma caribbica* were found to be host-specific to the velamen root of *Rhynchostylis retusa*.

The Kruskal-Wallis H test showed a statistically significant low colonisation rate (CR; H = 10.370, P = 0.006) with a mean rank CR score of 13.6 for *Epidendrum radicans*, 5.5 for *Rhynchostylis retusa* and 18.2 for *Oncidium sphacelatum*. The isolation rate (IR; H = 7.995, P = 0.016) with a mean rank IR score for *Epidendrum radicans* was 14.4, for *Rhynchostylis retusa* 6.08, and for *Oncidium sphacelatum* 17.05 (Tab. 3).

Among the fungal isolates of the velamen roots of the three orchids, *Trichoderma* cf. *asperellum* was the dominant one in the velamen roots of *Epidendrum radicans* (%D 2.20), *Trichoderma* cf. *atroviride* (%D 2.77) in *Oncidium sphacelatum*, and *Lasiodiplodia theobromae* and *Trichoderma* cf. *asperellum* (%D 2.35) in *Rhynchostylis retusa* (Tab. 1).

A statistically significant low Shannon-Wiener diversity index was observed for the velamen-root-associated endophytic fungi in *Rhynchostylis retusa* (H = 9.660, P = 0.002 between *Rhynchostylis retusa* and *Epidendrum radicans*; H = 7.467, P = 0.006 between *Rhynchostylis retusa* and *Oncidium sphacelatum*). Simultaneously, no statistically significant variation in the species evenness and the species richness of the velamen-root-associated endophytic fungi was observed between the velamen roots of the epiphytic orchid species (Tab. 4).

Tab. 3. Colonisation rate (CR) and isolation rate (IR) of endophytic fungal associates in the velamen roots of epiphytic orchids.
CR and IR are given as mean ± SD. Values with a different letter in the same row are significantly different at $P < 0.05$ after the Kruskal-Wallis H test.

	Epidendrum radicans	*Oncidium sphacelatum*	*Rhynchostylis retusa*
Colonisation rate (%)	25.41 ± 9.59^{a}	28.73 ± 5.46^{a}	15.09 ± 6.24^{b}
Isolation rate (%)	0.21 ± 0.11^{a}	0.28 ± 0.17^{a}	0.10 ± 0.03^{b}
Total number of isolates	202	214	147

Tab. 4. Diversity parameters of fungal associates in the velamen roots of epiphytic orchids.
Data are given as mean ± SD. Values with a different letter in the same row are significantly different at $P < 0.05$ after the Kruskal-Wallis H test; ns = statistically non-significant differences.

	Epidendrum radicans	*Oncidium sphacelatum*	*Rhynchostylis retusa*
Shannon-Wiener diversity index	1.47 ± 0.71^{b}	1.49 ± 0.41^{b}	0.47 ± 0.12^{a}
Species evenness	0.94 ± 0.04^{ns}	0.91 ± 0.04^{ns}	0.97 ± 0.02^{ns}
Species richness	1.90 ± 1.08^{ns}	1.79 ± 0.59^{ns}	1.53 ± 0.12^{ns}

DISCUSSION

Diversity of fungal endophytes in orchids

A total of 20 fungal species isolated from the velamen roots of the three epiphytic orchids in the present investigation endorses the fact that endophytic fungal diversity in orchids is generally relatively high (Chen et al. 2010). The present finding also implies that velamen roots provide a better niche for colonising microorganisms (Tsavkelova et al. 2001) when compared to leaves and stems of orchids.

The 20 species of fungi isolated from the three orchids belonged to 13 different genera. The fungal endophytes are probably part of a mutually beneficial association, but the specific benefits of the plant are not yet fully understood (Kia et al. 2017). Even though the ecological characteristics of these fungal communities are not yet clear, a polyphyletic group of fungi has been reported from many ecological groups of orchids, including epiphytes (Cevallos et al. 2018, Novotná et al. 2018, Deepthi et Ray 2018). However, the velamen roots are structures hanging in the air and grow continuously with the option of getting attached to the ground or a host surface. The fungal associates in such roots may remain part of its being prepared to act as an organ of nutrient absorption. Consequently, they may affect plant ecology (Bever et al. 2012), resistance to pathogen control

(Chen et al. 2013), nutritional requirements, and biochemical components essential in the physiology of environmental resistance, indispensable for survival and ecological competence of the species.

All 20 species of the fungi identified in the present investigation belonged to *Ascomycota*, *Basidiomycota*, and *Mucoromycota*. *Ascomycota* is already a well-known dominant group of fungal endophytes in many groups of plants, including orchids (Yuan et al. 2009, Sudheep et Sridhar 2012, Ma et al. 2015, Novotná et al. 2018). In contrast to observations in the present study, fungi belonging to the order *Agaricales* of the division *Basidiomycota* remain the dominant fungal partners in the roots of certain epiphytic orchids (Cevallos et al. 2018). The inability of basidiomycetes to develop in cultures (Singh et al. 2017) might be one of the reasons for the observation of fewer *Basidiomycota* as orchid-root associates in the present investigation as a culture-dependent fungal isolation study. Moreover, Cevallos et al. (2018) are of the opinion that a large number of fungi belonging to this division can be detected by molecular characterisation of the endophytic mycelia in velamen roots. However, just as in the present study, *Mucoromycota* have occasionally been reported as orchid-root associates in previous studies (Zhao et al. 2014, Novotná et al. 2018).

The fungal associates of the velamen roots of the three orchids identified in the present study belong to seven orders of *Ascomycota*. Among them, members of the *Botryosphaeriales* and *Hypocreales* included nearly 60% of the total isolates. Herrera et al. (2010) reported the presence of *Hypocreales* in the roots of neotropical orchids, and Martos et al. (2012) reported their presence in epiphytic orchids. We also found fungal isolates from the order *Xylariales* in the present investigation. It is well-known that xylariaceous fungi are the dominant endophytic fungal taxa in the epiphytic orchid *Dendrobium* spp. (Chen et al. 2013, Whalley et al. 2015). Sawmya et al. (2013) reported frequent occurrence of xylariaceous fungi in Indian epiphytic orchids. However, in the present study these members were found to be a rare category.

In terms of genera, many of the isolated species observed in the current investigation agree with those reported in other studies of fungal endophytes in orchids and other plants (Gazis et Chaverri 2010, Schoch et al. 2012, Tan et al. 2012, Chen et al. 2013). In general, fungi of the genera *Fusarium*, *Colletotrichum*, *Nigrospora* and *Xylaria* have already been identified as root colonisers in orchids (Chen et al. 2013). As observed in the present study, the fungi *Colletotrichum* cf. *gloeosporioides*, well known as a plant pathogen (Rojas et al. 2010, Munir et al. 2016), and *Diaporthe* spp. have already been identified as endophytes in the roots of the epiphytic orchid *Holcoglossum* sp. (Tan et al. 2012). The same fungi have been detected as a dominant endophytic fungal partners in *Madhuca indica* (Verma et al. 2014). However, *Campanella* sp. observed in the present study has not yet been reported as an endophyte from any other plants.

Biological role of the explored endophytes

A majority of the isolated species have well-defined biological functions in plants, including orchids, but have mostly been demonstrated in in-vitro experimental studies. *Diaporth*e sp. and *Nigrospora* sp. are known to help in the biotransformation of vanillin in *Vanilla planifolia* (Khoyratty et al. 2015). The fungal species *Aspergillus* cf. *tubingensis* and *Xylaria* sp. have been observed to be producers of antibacterial and antifungal compounds (Oliveira et al. 2011, Ratnaweera et al. 2014, Verma et al. 2014, Rajulu et al. 2016). Two *Trichoderma* species are already identified as plant growth promoters (Altomare et al. 1999, Yedidia et al. 2001, Hermosa et al. 2012). However, Deepthi et Ray (2019) have demonstrated that fungus species such as *Trichoderma* cf. *asperellum*, *Trichoderma* cf. h*arzianum*, *Endomelanconiopsis endophytica*, *Diaporthe eucalyptorum* and *Trichoderma* cf. *atroviride* are plant growth promoters in the epiphytic orchid *Dendrobium* sp. These authors also have observed the species mentioned above as potential in-vitro producers of the plant growth hormone indole acetic acid (IAA). Maor et al. (2004) reported the fungus species *Colletotrichum gloeosporioides* to be a producer of IAA.

Fungal colonisation of different orchids

The colonisation rate and isolation rate of fungal associates in velamen roots were found to be the lowest in the velamen roots of *Rhynchostylis retusa*. These rates reflect the degree of fungal colonisation in roots (Chen et al. 2010). Therefore, from the above observations it has become clear that among the three species of orchids studied, the degree of infection and colonisation of the fungal associates in the velamen roots was the lowest in *Rhynchostylis retusa*. An analysis of the species evenness index of the three orchid species showed values close to the maximum value of 1, which indicates that the endophytic fungal communities in the velamen roots of all three orchid species examined in the present study have an even distribution.

CONCLUSION

Altogether, 20 different fungal endophytes were isolated with a culture technique from the velamen roots of three epiphytic tropical orchids. A majority of the isolated fungi were not associated explicitly with a single orchid species. It indicated that certain orchids have preferences for widely distributed fungal groups as their velamen root associates.

In general, the present study has high relevance in the ecological understanding of velamen roots and sustainable, eco-friendly cultivation of ornamental or-

chids. The study also emphasises the importance of root-associated endophytic fungal communities having potential biological roles in the aerial velamen roots of epiphytic tropical orchids. Overall, the present findings have revealed the need of further in-depth investigations into the ecological complexities of velamen roots of orchids, especially into the specific roles of individual non-mycorrhizal endophytic fungi.

ACKNOWLEDGEMENTS

The first author wishes to acknowledge the financial support provided to her by the University Grants Commission (UGC), Government of India, under its FDP programme (Order No: FIP/12th plan/KLMG016 TF 02, dated 6th January 2015) and the leave granted to her by the Management of the Catholicate College, Pathanamthitta, Kerala, for conducting the research work.

REFERENCES

ABRAHAM C.M., ASHAKUMARI K. (2017): Isolation and identification of fungal endophytes from velamen roots of epiphytic orchids. – Paripex – Indian Journal of Research 6: 184–185.

ALTOMARE C., NORVELL W.A., BJÖRKMAN T., HARMAN G.E. (1999): Solubilisation of phosphates and micronutrients by the plant-growth-promoting and biocontrol fungus *Trichoderma harzianum* Rifai. – Applied and Environmental Microbiology 65: 2926–2933. DOI: https://doi.org/10.1128/AEM.65.7.2926-2933.1999

BAYMAN P., LEBRÓN L.L., TREMBLAY R.L., LODGE D.J. (1997): Variation in endophytic fungi from roots and leaves of *Lepanthes* (*Orchidaceae*). – New Phytologist 135: 143–149. DOI: https://doi.org/10.1046/j.1469-8137.1997.00618.x

BAYMAN P., OTERO J.T. (2006): Microbial endophytes of orchid roots. – In: Schulz B., Boyle C., Sieber T., eds., Microbial root endophytes, pp. 153–181. Springer Verlag, Berlin, Heidelberg. DOI: https://doi.org/10.1007/3-540-33526-9_9

BEVER J.D., PLATT T.G., MORTON E.R. (2012): Microbial population and community dynamics on plant roots and their feedbacks on plant communities. – Annual Review of Microbiology 66: 265–283. DOI: https://doi.org/10.1146/annurev-micro-092611-150107

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. DOI: https://doi.org/10.1016/j.funeco.2018.05.002

CEVALLOS S., SÁNCHEZ-RODRÍGUEZ A., DECOCK C., DECLERCK S., SUÁREZ J.P. (2017): Are there keystone mycorrhizal fungi associated with tropical epiphytic orchids? – Mycorrhiza 27: 225–232. DOI: https://doi.org/10.1007/s00572-016-0746-8

CHEN J., ZHANG L.-C., XING Y.-M., WANG Y.-Q., XING X.-K., ZHANG D.-W., LIANG H.-Q., GUO S.-X. (2013): Diversity and taxonomy of endophytic xylariaceous fungi from medicinal plants of *Dendrobium* (*Orchidaceae*). – PLoS One 8: e58268. DOI: https://doi.org/10.1371/journal.pone.0058268

CHEN X.M., DONG H.L., HU K.X., SUN Z.R., CHEN J., GUO S.X. (2010): Diversity and antimicrobial and plant-growth-promoting activities of endophytic fungi in *Dendrobium loddigesii* Rolfe. – Journal of Plant Growth Regulation 29: 328–337. DOI: https://doi.org/10.1007/s00344-010-9139-y

CROUS P.W. et al. (2012): Fungal Planet description sheets: 107–127. – Persoonia – Molecular Phylogeny and Evolution of Fungi 28: 138–182. DOI: https://doi.org/10.3767/003158512X652633

DEEPTHI A.S., RAY J.G. (2018): Endophytic diversity of hanging velamen roots in the epiphytic orchid *Acampe praemorsa*. – Plant Ecology and Diversity 11: 649–661. DOI: https://doi.org/10.1080/17550874.2019.1610911

DEEPTHI A.S., RAY J.G. (2019): Applications of endophytic-fungal-isolates from velamen root of wild orchids in floriculture. – Brazilian Journal of Biological Sciences 6: 577–589. DOI: https://doi.org/10.21472/bjbs.061409

DEEPTHI A.S., RAY J.G. (2020): Algal associates and the evidence of cyanobacterial nitrogen fixation in the velamen roots of epiphytic orchids. – Global Ecology and Conservation 22: p.e00946. DOI: https://doi.org/10.1016/j.gecco.2020.e00946

GAZIS R., CHAVERRI P. (2010): Diversity of fungal endophytes in leaves and stems of wild rubber trees (*Hevea brasiliensis*) in Peru. – Fungal Ecology 3: 240–254. DOI: https://doi.org/10.1016/j.funeco.2009.12.001

HAMMER Ø., HARPER D.A.T., RYAN P.D. (2001): PAST: Paleontological statistics software package for education and data analysis. – Palaeontologia Electronica 4: 1–9

HERMOSA R., VITERBO A., CHET I., MONTE E. (2012): Plant-beneficial effects of *Trichoderma* and its genes. – Microbiology 158: 17–25. DOI: https://doi.org/10.1099/mic.0.052274-0

HERRERA P., SUÁREZ J.P., KOTTKE I. (2010): Orchids keep the ascomycetes outside: a highly diverse group of ascomycetes colonising the velamen of epiphytic orchids from a tropical mountain rainforest in Southern Ecuador. – Mycology 1: 262–268. DOI: https://doi.org/10.1080/21501203.2010.526645

HUANG W.Y., CAI Y.Z., HYDE K.D., CORKE H., SUN M. (2008): Biodiversity of endophytic fungi associated with 29 traditional Chinese medicinal plants. – Fungal Diversity 33: 61–75.

HYDE K.D., SOYTONG K. (2008): The fungal endophyte dilemma. – Fungal Diversity 33: 163–173.

JACQUEMYN H., BRYS R., WAUD M., BUSSCHAERT P., LIEVENS B. (2015): Mycorrhizal networks and coexistence in species-rich orchid communities. – New Phytologist 5: 75–77. DOI: https://doi.org/10.1111/nph.13281

JIN Z., LI D., LIU T., YU F., ZHANG Z., SU C., WANG Y., GUO Q., LIU Z. (2017): Cultural endophytic fungi associated with *Dendrobium officinale*: identification, diversity estimation and their antimicrobial potential. – Current Science 112: 1690–1697.

KHOYRATTY S., DUPONT J., LACOSTE S., PALAMA T.L., CHOI Y.H., KIM H.K., PAYET B., GRISONI M., FOUILLAUD M., VERPOORTE R., KODJA H. (2015): Fungal endophytes of *Vanilla planifolia* across Réunion Island: isolation, distribution and biotransformation. – BMC Plant Biology 15: 1–19. DOI: https://doi.org/10.1186/s12870-015-0522-5

KIA S.H., GLYNOU K., NAU T., THINES M., PIEPENBRING M., MACIÁ-VICENTE J.G. (2017): Influence of phylogenetic conservatism and trait convergence on the interactions between fungal root endophytes and plants. – ISME Journal 11: 777–790. DOI: https://doi.org/10.1038/ismej.2016.140

KUMARESAN V., SURYANARAYANAN T.S. (2001): Occurrence and distribution of endophytic fungi in a mangrove community. – Mycological Research 105: 1388–1391. DOI: https://doi.org/10.1017/S0953756201004841

KUMARESAN V., SURYANARAYANAN T.S. (2002): Endophyte assemblages in young, mature and senescent leaves of *Rhizophora apiculata*: evidence for the role of endophytes in mangrove litter degradation. – Fungal Diversity 6: 81–91. DOI: https://doi.org/10.1017/S0953756201004841

LI G.Q., LIU F.F., LI J.Q., LIU Q.L., CHEN S.F. (2018): *Botryosphaeriaceae* from *Eucalyptus* plantations and adjacent plants in China. – Persoonia – Molecular Phylogeny and Evolution of Fungi 40: 63–95. DOI: https://doi.org/10.3767/persoonia.2018.40.03

LIECKFELDT E., SAMUELS G.J., NIRENBERG H.I., PETRINI O. (1999): A morphological and molecular perspective of *Trichoderma viride*: is it one or two species? – Applied and Environmental Microbiology 65: 2418–2428.

LIU C.-H., HUANG X., XIE T.-N., DUAN N., XUE Y.-R., ZHAO T.-X., LEVER M.A., HINRICHS K.-U., INAGAKI F. (2017): Exploration of cultivable fungal communities in deep coal-bearing sediments from ~1.3

to 2.5 km below the ocean floor. – Environmental Microbiology 19: 803–818. DOI: https://doi.org/10.1111/1462-2920.13653

MA X., KANG J., NONTACHAIYAPOOM S., WEN T., HYDE K.D. (2015): Non-mycorrhizal endophytic fungi from orchids. – Current Science 1098: 1–16.

MAOR R., HASKIN S., LEVI-KEDMI H., SHARON A. (2004): In planta production of indole-3-acetic acid by *Colletotrichum gloeosporioides* f. sp. *aeschynomene*. – Applied and Environmental Microbiology 70: 1852–1854. DOI: https://doi.org/10.1128/AEM.70.3.1852-1854.2004

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: 5098–5109. DOI: https://doi.org/10.1111/j.1365-294X.2012.05692.x

MONCALVO J.M., VILGALYS R., REDHEAD S.A., JOHNSON J.E., JAMES T.Y., AIME M.C., HOFSTETTER V., VERDUIN S.J., LARSSON E., BARONI T.J., THORN R.G., JACOBSSON S., CLÉMENÇON H., MILLER O.K. JR. (2002): One hundred and seventeen clades of euagarics. – Molecular Phylogenetics and Evolution 23: 357–400. DOI: https://doi.org/10.1016/S1055-7903(02)00027-1

MUNIR M., AMSDEN B., DIXON E., VAILLANCOURT L., WARD GAUTHIER N.A. (2016): Characterization of *Colletotrichum* species causing bitter rot of apple in Kentucky orchards. – Plant Disease 100: 2194–2203. DOI: https://doi.org/10.1094/PDIS-10-15-1144-RE

MUTHUKUMAR T., KOWSALYA A. (2017): Comparative anatomy of aerial and substrate roots of *Acampe praemorsa* (Rox.) Blatt. & Mc Cann. – Flora 226: 17–28. DOI: https://doi.org/10.1016/j.flora.2016.11.001

NAGAMANI A., KUNWAR I.K., MANOHARACHARY C. (2006): Handbook of soil Fungi. – I.K. International, New Delhi.

NOVOTNÁ A., BENÍTEZ Á., HERRERA P., CRUZ D., FILIPCZYKOVÁ E., SUÁREZ J.P. (2018): High diversity of root-associated fungi isolated from three epiphytic orchids in southern Ecuador. – Mycoscience 59: 24–32. DOI: https://doi.org/10.1016/j.myc.2017.07.007

OKANE I., SRIKITIKULCHAI P., TOYAMA K., LÆSSOE T., SIVICHAI S., HYWEL-JONES N., NAKARGIRI A., POTACHAROEN W., SUZUKI K. (2008): Study of endophytic *Xylariaceae* in Thailand: diversity and taxonomy inferred from rDNA sequence analyses with saprobes forming fruit bodies in the field. – Mycoscience 49: 359–372. DOI: https://doi.org/10.1007/S10267-008-0440-6

OLIVEIRA C.M., REGASINI L.O., SILVA G.H., PFENNING L.H., YOUNG M.C.M., BERLINCK R.G.S., BOLZANI V.S., ARAUJO A.R. (2011): Dihydroisocoumarins produced by *Xylaria* sp. and *Penicillium* sp., endophytic fungi associated with *Piper aduncum* and *Alibertia macrophylla*. – Phytochemical Letters 4: 93–96. DOI: https://doi.org/10.1016/j.phytol.2010.11.003

PRITI V., RAMESHA B.T., SINGH S., RAVIKANTH G., GANESHAIAH K.N., SURYANARAYANAN T.S., SHAANKER R.U. (2009): How promising are endophytic fungi as an alternative source of plant secondary metabolites? – Current Science 97: 477–478.

RAJULU G., SURYANARAYANAN T., TANGJANG S. (2016): Endophytic fungi of orchids of Arunachal Pradesh, North-Eastern India. – Current Research in Environmental & Applied Mycology 6: 293–299. DOI: https://doi.org/10.5943/cream/6/4/7

RAM A., SHAMINA M. (2014): Cyanobacterial association on the roots of epiphytic orchids. – International Journal of Recent Scientific Research 5: 2014–2016.

RATNAWEERA P.B., WILLIAMS D.E., DILIP DE SILVA E., WIJESUNDERA R.L.C., DALISAY D.S., ANDERSEN R.J. (2014): Helvolic acid, an antibacterial nortriterpenoid from a fungal endophyte, *Xylaria* sp. of orchid *Anoectochilus setaceus* endemic to Sri Lanka. – Mycology 5: 23–28. DOI: https://doi.org/10.1080/21501203.2014.892905

RODRIGUEZ R., REDMAN R. (2008): More than 400 million years of evolution and some plants still can't make it on their own: plant stress tolerance via fungal symbiosis. – Journal of Experimental Botany 59: 1109–1114. DOI: https://doi.org/10.1093/jxb/erm342

ROJAS E.I., HERRE E.A., MEJIA L.C., ARNOLD A.E., CHAVERRI P., SAMUELS G.J. (2008): *Endomelanconiopsis*, a new anamorph genus in the *Botryosphaeriaceae*. – Mycologia 100: 760–775. DOI: https://doi.org/10.3852/07-207

ROJAS E.I., REHNER S.A., SAMUELS G.J., VAN BAEL S.A., HERRE E.A., CANNON P., CHEN R., PANG J., WANG R., ZHANG Y., PENG Y.Q., SHA T. (2010): *Colletotrichum gloeosporioides* s.l. associated with *Theobroma cacao* and other plants in Panama: multilocus phylogenies distinguish host-associated pathogens from asymptomatic endophytes. – Mycologia 102: 1318-1338. DOI: https://doi.org/10.3852/09-244

ROMI W., KEISAM S., AHMED G., JEYARAM K. (2014): Reliable differentiation of *Meyerozyma guilliermondii* from *Meyerozyma caribbica* by internal transcribed spacer restriction fingerprinting. – BMC Microbiology 14: 1–11. DOI: https://doi.org/10.1186/1471-2180-14-52

SANKAR N.R., BABU G.P. (2012): First report of *Fusarium proliferatum* causing rot of garlic bulbs (*Allium sativum*) in India. – Plant Disease 96: 290–290. DOI: https://doi.org/10.1094/PDIS-08-11-0649

SAWMYA K., VASUDEVAN T.G., MURALI T.S. (2013): Fungal endophytes from two orchid species – pointer towards organ specificity. – Czech Mycology 65: 89–101. DOI: https://doi.org/10.33585/cmy.65107

SCHOCH C.L., SEIFERT K.A., HUHNDORF S., ROBERT V., SPOUGE J.L., LEVESQUE C.A., CHEN W., and FUNGAL BARCODING CONSORTIUM (2012): Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. – Proceedings of the National Academy of Sciences 109: 6241–6246. DOI: https://doi.org/10.1073/pnas.1117018109

SHAH S., SHRESTHA R., MAHARJAN S., SELOSSE M., PANT B. (2019): Isolation and characterisation of plant growth-promoting endophytic fungi from the roots of *Dendrobium moniliforme*. – Plants 8: 5–15. DOI: https://doi.org/10.3390/plants8010005

SINGH A., SHAHID M., SRIVASTAVA M. (2014): Phylogenetic relationship of *Trichoderma asperellum* Tasp/8940 using internal transcribed spacer (ITS) sequences. – International Journal of Advanced Research 2: 979–986.

SINGH D.K., SHARMA V.K., KUMAR J., MISHRA A., VERMA S.K., SIEBER T.N., KHARWAR R.N. (2017): Diversity of endophytic mycobiota of tropical tree *Tectona grandis* Linn. f.: Spatiotemporal and tissue type effects. – Scientific Reports 7: 1–14. DOI: https://doi.org/10.1038/s41598-017-03933-0

STONE J.K., BACON C.W., WHITE J.F. JR. (2000): An overview of endophytic microbes: endophytism defined. – In: Bacon C.W., White J.F. Jr., eds., Microbial endophytes, pp. 6–12. Marcel Dekker, New York.

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: 299–305. DOI: https://doi.org/10.15517/lank.v16i2.26014

SUDHEEP N.M., SRIDHAR K.R. (2012): Non-mycorrhizal fungal endophytes in two orchids of Kaiga forest (Western Ghats), India. – Journal of Forestry Research 23: 453–460. DOI: https://doi.org/10.1007/s11676-012-0284-y

TAN X.-M., CHEN X.-M., WANG C.-L., JIN X.-H., CUI J.-L., CHEN J., GUO S.-X., ZHAO L.-F. (2012): Isolation and identification of endophytic fungi in roots of nine *Holcoglossum* plants (*Orchidaceae*) collected from Yunnan, Guangxi, and Hainan provinces of China. – Current Microbiology 64: 140–147. DOI: https://doi.org/10.1007/s00284-011-0045-8

TAO G., LIU Z., HYDE K., LUI X., YU Z. (2008): Whole rDNA analysis reveals novel and endophytic fungi in *Bletilla ochracea* (*Orchidaceae*). – Fungal Diversity 33: 101–122.

TAO G., LIU Z.Y., LIU F., GAO Y.H., CAI L. (2013): Endophytic *Colletotrichum* species from *Bletilla ochracea* (*Orchidaceae*), with descriptions of seven new species. – Fungal Diversity 61: 139–164. DOI: https://doi.org/10.1007/s13225-013-0254-5

TĚŠITELOVÁ T., KOTILÍNEK M., JERSÁKOVÁ J., JOLY F.-X., KOŠNAR J., TATARENKO I., SELOSSE M.-A. (2015): Two widespread green *Neottia* species (*Orchidaceae*) show a mycorrhizal preference for *Sebacinales* in various habitats and ontogenetic stages. – Molecular Ecology 24: 1122–1134. DOI: https://doi.org/10.1111/mec.13088

TSAVKELOVA E.A., CHERDYNTSEVA T.A., LOBAKOVA E.S., KOLOMEITSEVA G.L., NETRUSOV A.I. (2001): Microbiota of the orchid rhizoplane. – Microbiology 70: 492–497. DOI: https://doi.org/10.1023/A:1010402715376

TSAVKELOVA E.A., LOBAKOVA E.S., KOLOMEITSEVA G.L., CHERDYNTSEVA T.A., NETRUSOV A.I. (2003a): Localisation of associative cyanobacteria on the roots of epiphytic orchids. – Microbiology 72: 86–91. DOI: https://doi.org/10.1023/A:1022286225013

TSAVKELOVA E.A., LOBAKOVA E.S., KOLOMEITSEVA G.L., CHERDYNTSEVA T.A., NETRUSOV A.I. (2003b): Associative cyanobacteria isolated from the roots of epiphytic orchids. – Microbiology 72: 92–97. DOI: https://doi.org/10.1023/A:1022238309083

VALENZUELA-LOPEZ N., SUTTON D.A., CANO-LIRA J.F., PAREDES K., WIEDERHOLD N., GUARRO J., STCHIGEL A.M. (2017): Coelomycetous fungi in the clinical setting: morphological convergence and cryptic diversity. – Journal of clinical microbiology 55: 552–567. DOI: https://doi.org/10.1128/JCM.02221-16

VERMA S.K., GOND S.K., MISHRA A., SHARMA V.K., KUMAR J., SINGH D.K., KUMAR A., GOUTAM J., KHARWAR R.N. (2014): Impact of environmental variables on the isolation, diversity and antibacterial activity of endophytic fungal communities from *Madhuca indica* Gmel. at different locations in India. – Annals of Microbiology 64: 721–734. DOI: https://doi.org/10.1007/s13213-013-0707-9

WANG M., LIU F., CROUS P.W., CAI L. (2017): Phylogenetic reassessment of *Nigrospora*: ubiquitous endophytes, plant and human pathogens. – Persoonia – Molecular Phylogeny and Evolution of Fungi 39: 118–142. DOI: https://doi.org/10.3767/persoonia.2017.39.06

WATANABE T. (2002): Pictorial atlas of soil and seed fungi: Morphologies of cultured fungi and a key to species, 2nd ed. – CRC Press, Boca Raton, London, New York, Washington. DOI: https://doi.org/10.1201/9781420040821

WHALLEY A.J., SUWANNASAI N., RUCHIKACHORN N., SANGVICHIEN E., SIHANONTH P. (2015): Endophytic *Xylariaceae* from Thai plants: a research review. – Suan Sunandha Science and Technology Journal 2: 11–17.

WHITE T.J., BRUNS T.D., LEE S.B., TAYLOR J.W. (1990): Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. – In: Innis M.A., Gelfand D.H., Sninsky J.J., White T.J., eds., PCR protocols: a guide to methods and applications, pp. 315–322. Academic Press, San Diego.

YANG T., GROENEWALD J.Z., CHEEWANGKOON R., JAMI F., ABDOLLAHZADEH J., LOMBARD L., CROUS P.W. (2017): Families, genera, and species of *Botryosphaeriales*. – Fungal Biology 121: 322–346. DOI: https://doi.org/10.1016/j.funbio.2016.11.001

YEDIDIA I., SRIVASTAVA A.K., KAPULNIK Y., CHET I. (2001): Effect of *Trichoderma harzianum* on microelement concentrations and increased growth of cucumber plants. – Plant and Soil 235: 235–242. DOI: https://doi.org/10.1023/A:1011990013955

YOKOYA K., ZETTLER L.W., KENDON J.P., BIDARTONDO M.I., STICE A.L., SKARHA S., COREY L.L., KNIGHT A.C., SARASAN V. (2015): Preliminary findings on the identification of mycorrhizal fungi from diverse orchids in the Central Highlands of Madagascar. – Mycorrhiza 25: 611–625. DOI: 10.1007/s00572-015-0635-6

YUAN Z.L., CHEN Y.C., YANG Y. (2009): Diverse non-mycorrhizal fungal endophytes inhabiting an epiphytic, medicinal orchid (*Dendrobium nobile*): estimation and characterisation. – World Journal of Microbiology and Biotechnology 25: 295–303. DOI: https://doi.org/10.1007/s11274-008-9893-1

ZETTLER L.W., COREY L.L., JACKS A.L., GRUENDER L.T., LOPEZ A.M. (2013): *Tulasnella irregularis* (*Basidiomycota*: *Tulasnellaceae*) from roots of *Encyclia tampensis* in south Florida, and confirmation of its mycorrhizal significance through symbiotic seed germination. – Lankesteriana 13: 119–128. DOI: https://doi.org/10.15517/lank.v0i0.11552

ZHAO X.-L., YANG J.-Z., LIU S., CHEN C.-L., ZHU H.-Y., CAO J.-X. (2014): The colonisation patterns of different fungi on roots of *Cymbidium hybridum* plantlets and their rebankective inoculation effects on growth and nutrient uptake of orchid plantlets. – World Journal of Microbiology and Biotechnology 30: 1993–2003. DOI: 10.1007/s11274-014-1623-2

ZOTZ G., SCHICKENBERG N., ALBACH D. (2017): The velamen radicum is common among terrestrial monocotyledons. – Annals of Botany 120: 625–632. DOI: https://www.doi.org/10.1093/aob/mcx097