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Root associated *Fusarium solani* Species Complex (FSSC) in epiphytic and terrestrial orchids

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Abstract. *Sukarno N, Mursidawati S, Listiyowati S, Nugraha NH, Fadillah WN, Waite M. 2023. Root associated* Fusarium solani *Species Complex (FSSC) in epiphytic and terrestrial orchids. Biodiversitas 24: 2577-2586.* Members of the *Fusarium solani* Species Complex (FSSC) are ecologically diverse, covering human and plant pathogens, saprobes, and endophytic fungi of economically important plants. The role of FSSC on tropical orchids, however, has received little attention. This research aimed to isolate and identify the FSSC found as endophytes in roots of the epiphytic orchids *Cymbidium finlaysonianum* Lindl. and *Vanda tricolor* Lindl., and the terrestrial orchids *Calanthe triplicata* (Willemet) Ames and *Phaius tankervilleae* (Banks) Blume. Fungal isolation was obtained from peloton structures within the root. Fungal identification was done using combined morphological and molecular characteristics using ITS rDNA sequences. Four isolates of *Fusarium* were identified based on morphological characteristics. The BLAST analysis showed that all the isolates were in the *Fusarium solani* Species Complex (FSSC). Further phylogenetic relationship analysis indicated that all the FSSC isolates belonged to FSSC5 lineage or *Fusarium solani* sensu stricto, which is nested in FSSC clade 3 as a subgroup. The fungi *F. solani* sensu stricto V2 and CF34 were isolated from the epiphytic orchids, and *F. solani* sensu stricto C5 and P44 were isolated from the terrestrial orchids. It is interesting that the FSSC5 isolated from the epiphytic orchids form different groups than those of the terrestrial orchids. This is the first report showing the tropical orchids are the host of the FSSC5 as endophytes and this broadens the known host range and ecological behavior of the FSSC5.

Keywords: Endophytic fungi, *Fusarium solani* sensu stricto, ITS rDNA, morphology, phylogenetic analysis

INTRODUCTION

Fusarium is a large genus of fungi in the Ascomycota (Nectriaceae, Hypocreales). Based on molecular phylogenetic relationships, the genus *Fusarium* consists of 20 species complexes, one of which is the *Fusarium solani* Species Complex (FSSC) (O'Donnell et al. 2015; Al-Hatmi et al. 2018). The FSSC contains more than 60 phylogenetic species (O'Donnell et al. 2015; Coleman 2016; Schroers et al. 2016; Crous et al. 2021). Molecular phylogenetic relationships analysis shows that the FSSC consists of three distinct subgroups: clades 1, 2, and 3 (Al-Hatmi et al. 2018). The largest clade of FSSC is clade 3. Most FSSC living in soils, in humans as pathogens, and in plants as endophytes and pathogens are members of FSSC clade 3 (Al-Hatmi et al. 2018). This clade consists of the most common and diverse groups, containing all seven mating populations defined by Matuo and Snyder (1973) and all human pathogen phylogenetic lineages (Chehri 2014; Chitrampalam and Nelson 2016). The FSSC has recently been segregated into a separate genus *Neocosmospora* (Lombard et al. 2015), with newer work further detailing the phylogenetic and morphological characters distinguishing it from the genus *Fusarium* (Sandoval-Denis et al. 2019; Crous et al. 2021). However, O'Donnell et al. (2020), and Geiser et al. (2013, 2021) argue for maintaining the FSSC within the genus *Fusarium* to maintain continuity with existing literature in a wide variety of fields, such as plant pathology and human medicine. In this study, the name *F. solani* species complex is used but acknowledge that the case for recognizing it as the separate genus *Neocosmospora* is strong.

Indonesia is one of the world's largest archipelagos and home to more than 5000 species, or one-third of all known orchids in the world. Many orchids are economically, medicinally, and ethnobotanically important in Indonesia, such as the epiphytic orchids *Vanda tricolor* Lindl. and *Cymbidium finlaysonianum* Lindl., and the terrestrial orchids *Phaius tankervilleae* (Banks) Blume and *Calanthe triplicata* (Willemet) Ames. *V. tricolor* is widely spread in Southeast Asia, including in Java and Bali (Handoyo 2012). This orchid has ornamental value by having white petals with brown, red, or purplish-red spots and a red, purple, or purplish-red labellum. Some species of *Vanda* have medicinal uses. The root and leaves of *V. parviflora* are used to cure nerve disorders, rheumatic conditions, and as an antidote for scorpion stings. The flower of *V. spathulata* is used for asthma (Hossain 2011). *C. finlaysonianum* ranges throughout Southeast Asia, including Indonesia. This orchid has long inflorescences from a pseudobulb with yellow or brown petals. *C. finlaysonianum* has been reported to be used for curing ear infections (Teoh 2019). *P. tankervilleae* is a terrestrial orchid that has ornamental

and medicinal uses. This orchid has purplish-brown petals with a pink labellum (Devadas et al. 2019). The pseudobulb, roots and leaves are used as a natural dye, while the tubers are used for curing arm and leg swellings (Kanwal 2014; Buragohain et al. 2016). *Calanthe triplicata* is a terrestrial orchid with relatively slow growth. The orchid has white flowers with the base of the labellum yellow or purple (Kurzweil 2010) and is used as an ornamental plant. All parts of this orchid have medicinal value. The roots are used for curing arm swelling and diarrhea, and the flowers for reducing headaches and stomachaches (De et al. 2015).

As a consequence of their seeds in very small size, orchid seeds lack sufficient nutrition to support the growth and development of the embryo during germination (Jiang et al. 2019; Lawrie et al. 2021). Therefore, the seeds depend on mycorrhizal or endophytic fungi for germination (Vujanovic et al. 2000; Smith and Read 2008; Pant et al. 2017; Duffy et al. 2019). The fungi provide mineral nutrition and plant growth regulators such as auxin, cytokinin, and gibberellin. There is much less known about the orchid seed and fungi relationship amongst tropical species than amongst temperate species (Rasmussen et al. 2015; McCormick et al. 2018).

The fungal partners commonly reported with orchid roots are *Rhizoctonia*-like fungi (Ding et al. 2014; Meng et al. 2019). However, non-*Rhizoctonia*-like fungi such as *Fusarium oxysporum* have also been reported in orchid roots as mycorrhizal fungi (Jiang et al. 2019) while *Fusarium sp.* reported as endophytic fungi (Vujanovic et al. 2000). However, research on fungi belonging to FSSC as fungal partners is still rare. Despite its richness in orchid diversity, only a few attempts have been made to isolate endophytic and mycorrhizal fungi from terrestrial native Indonesian orchids (Suryantini et al. 2015; Agustini et al. 2016; Sufaati et al. 2016; Mahfut 2021). There are a few reports of *Fusarium* as endophytes in orchids from China, Madagascar, and Southern Ecuador (Tan et al. 2012; Zettler et al. 2017; Salazar et al. 2020; Sarsaiya et al. 2020), but none of these reported finding the FSSC. The main objective of this research was to isolate and analyze the FSSC associates in the roots of *V. tricolor*, *C. finlaysonianum*, *P. tankervilleae*, and *C. triplicata*. The information gained from this study will contribute to understanding FSSC and the sustainable cultivation and conservation of these orchids.

MATERIALS AND METHODS

Fungal isolation

Fungal isolation was conducted on the roots of *V. tricolor*, *C. finlaysonianum*, *P. tankervilleae*, and *C. triplicata* (Figure 1). The orchids from the collection of the Bogor Botanical Garden, Indonesia were used in this study. The orchid had been grown in the garden for seven years since their collection from the wild. Fungal isolation followed the method reported by Fujimori et al. (2019) with modification. The roots of each species of orchid were carefully cleaned of soil and organic debris. The roots then washed with sterile distilled water and blotted on sterile filter paper. The roots were cut into five pieces of 1 cm lengths, soaked in 1.5% sodium hypochlorite for 10 min, rinsed with sterile distilled water three times, and blotted on sterile filter paper for about 1 h to dry the sample. The roots were sliced longitudinally and observed for pelotons. The inner parts of the roots containing pelotons were collected and spread in three spots on a Petri dish containing a Fungal Isolation Medium (FIM) for 15 replicates (Alghamdi 2019). The plates were incubated at 25°C for seven days. Each emerging colony was purified by transferring the hyphal tip onto Potato Dextrose Agar (PDA) plates to obtain pure cultures.

Fungal identification

Morphological identification

Morphological identification was done by following Burgess et al. (1994) and Short et al. (2013). Colony morphology and microscopic characteristics of the isolates were observed on culture grown on PDA at 25°C for 10-14 days. The colony characteristics such as growth rate, colour of the surface, underside, and pigmentation of the colony were observed. The colour was determined using the Methuen handbook of colour (Kornerup and Wanscher 1987). Colony diameter of the fungal growth was measured on the culture grown on PDA at 25°C daily for seven days. Microscopic features such as hyphal characteristics, shape, and size of macroconidia, presence or absence of microconidia, shape, and size of microconidia, conidiogenous cell bearing microconidia, and size and characteristics of chlamydospores were also measured.

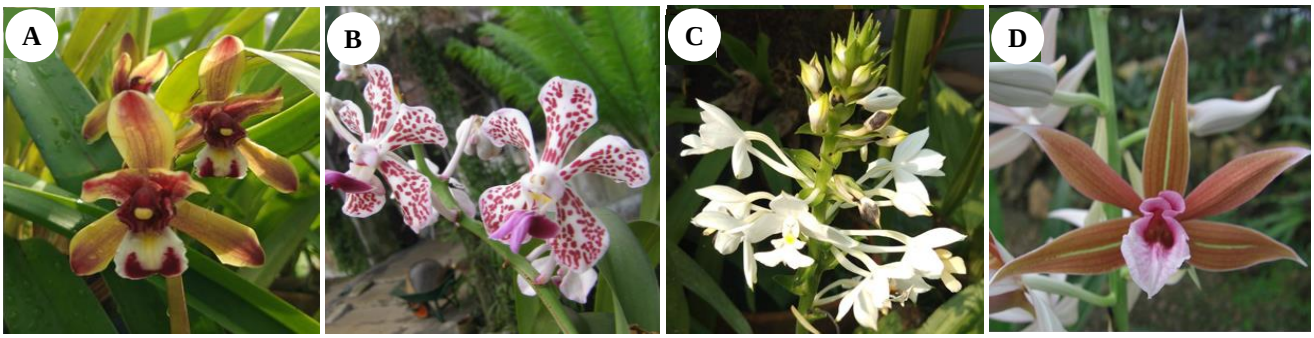

Figure 1. Habitus of the orchid species used for fungal isolation. A. *Cymbidium finlaysonianum*, B. *Vanda tricolor*, C. *Calanthe triplicata*, D. *Phaius tankervilleae*. These orchids are in the collection of the Bogor Botanical Garden, Indonesia

Molecular identification

Molecular identification was carried out using fungal Internal Transcribed Spacer ribosomal DNA (ITS rDNA) sequences. The fungi were grown on cellophane membrane on PDA medium for 7-14 days of incubation. The fungal DNA genome was obtained by using DNA extraction method with Cetyltrimethylammonium Bromide (CTAB) buffer lysis solution and ethanol precipitation described by Fadillah et al. (2021). The sequences were obtained by PCR amplification of fungal DNA genome using ITS1F (forward) and ITS4 (reverse) primers (White et al. 1990) with PCR conditions as follows: pre-denaturation at 95°C for 10 min, 35 cycles of denaturation at 95°C, annealing at 55°C, and elongation at 72°C for 1 min each, followed by post elongation at 72°C for 10 min. Successfully amplified PCR products were sent to 1st BASE Laboratories (Seri Kembangan, Malaysia) for sequencing.

Chromatograms of the generated DNA sequences were checked for their quality by analyzing the sharpness of the peaks using SeqTrace 0.9.0 to assure sequence quality (Stucky 2012). The consensus sequences were then submitted to Basic Local Alignment Search Tool nucleotides (BLASTn) on the National Center for Biotechnology Information (NCBI) website for homology analysis (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The homologous sequences obtained from BLAST were then aligned and analyzed in MEGA 6 (Tamura et al. 2013).

Phylogenetics analysis

Fungal datasets of ITS rDNA sequences were used for the phylogenetic tree analysis in MEGA 6 (Tamura et al. 2013) and inferred using the Neighbour-Joining method. The datasets used to ascertain the isolates obtained belong to which group among FSSC clade 3. The datasets are obtained from NCBI as also being used by Migheli et al. (2010), Chehri (2014), Chehri et al. (2015) and Al-Hatmi et al. (2018). The optimal tree was drawn with the percentage of the bootstrap test of 1000 replicates shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the p-distance method. *Fusarium oxysporum* as a member of *Fusarium oxysporum* Species Complex (FOSC) used as an outgroup (Geiser et al. 2021).

RESULTS AND DISCUSSION

Fungal isolation and morphological identification

A total of four different isolates of *Fusarium* were obtained from the roots of the four species of orchid, *C. finlaysonianum*, *V. tricolor*, *C. triplicata*, and *P. tankervilleae*. The colony and micromorphological characteristics of all four isolates obtained in this study were observed (Figure 2; Figure 3; Figure 4; Figure 5; Table 1). All four isolates produced septate hyphae and aerial mycelium, with whitish cream to light purplish pigmentation on PDA. Only one of the four isolates, *Fusarium* sp. CF34, produced dark pigmentation on the underside of the colony. The *Fusarium* sp. CF34 isolated from *C. finlaysonianum* formed dark brown pigmentation in the center of the colony underside. All four isolates formed fusoid macroconidia with the dorsal (upper) side more curve than the ventral side, as is typical of the genus *Fusarium*. The septa number of the macroconidia varied between 2 to 5. The size of the macroconidia also varied. The *Fusarium* sp. C5 had a shorter range of macroconidia lengths compared to the other three *Fusarium* isolates. In addition, all isolated fungi also formed abundant microconidia arising from long phialide-like structures. The microconidia were oval to cylindrical, hyaline, with and without one septum, and formed one to two cells. The *Fusarium* sp. V2 had a shorter range of microconidia lengths compared to the other three *Fusarium* isolates. All four isolates produced aerial mycelium and chlamydospores. The chlamydospores were hyaline to slightly grey (Table 1). Sexual structures were not observed in this study.

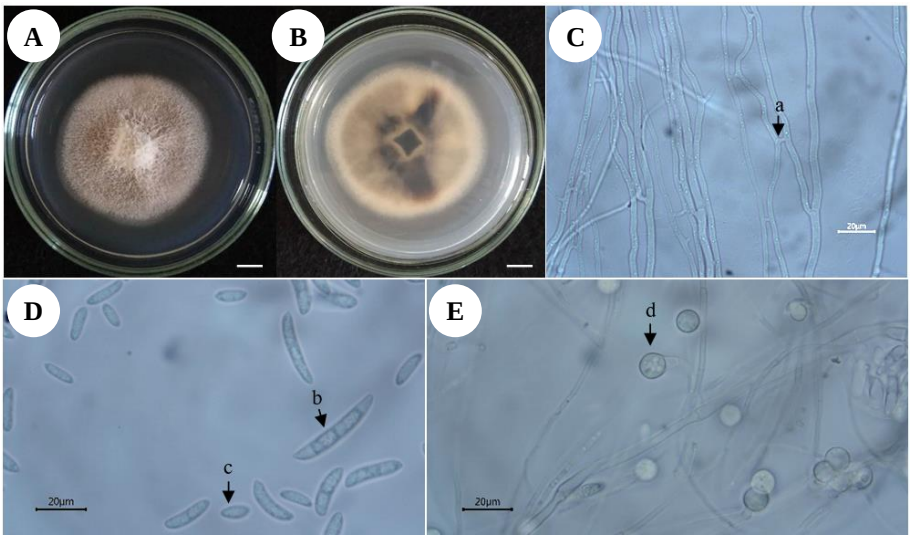

Figure 2. Colony and microscopic structures of *Fusarium solani* CF34 isolated from *Cymbidium finlaysonianum* grown on PDA, the microscopic structures observed at 10 days after inoculation. (A-B) Top and underside view of *Fusarium solani* CF34 colonies, a. Hyphal anastomosis, b. Macroconidium, c. Microconidium, d. Chlamydospore. Scale bars are 1 cm (A-B) and 20 µm (C-E)

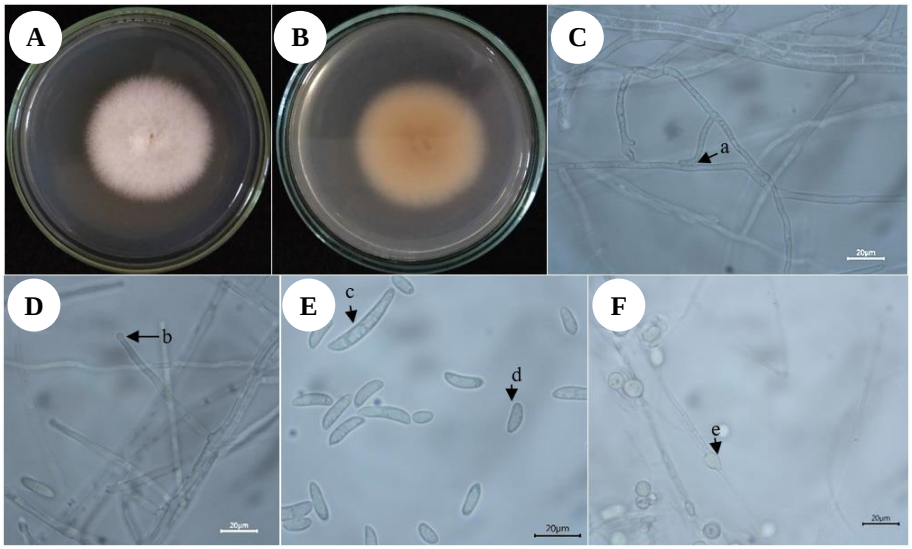

Figure 3. Colony and microscopic structures of *Fusarium solani* V2 isolated from *Vanda tricolor* grown on PDA, the microscopic structures observed at 10 days after inoculation. (A-B) Top and underside view of *Fusarium solani* V2 colonies, a. Hyphal anastomosis, b. Phialide, c. Macroconidium, d. Microconidium, e. Chlamydospore. Scale bars are 1 cm (A-B) and 20 µm (C-F)

Table 1. Colony and microscopic characteristics of FSSC strains isolated from roots of *Cymbidium finlaysonianum*, *Vanda tricolor*, *Calanthe triplicata*, and *Phaius tankervilleae*

Characters		***Fusarium* sp. CF34**	***Fusarium* sp. V2**	***Fusarium* sp. C5**	***Fusarium* sp. P44**
Pigmentation on PDA		Purplish pink	Whitish cream	Purplish cream	Light pink
Colony	Top view	Colony light purplish cream with white colour in the center of colony, cottony	Colony whitish cream with white colour in the center, lightly cottony and distinct radial hyphal growth	Colony white purplish cream, thick velvety	Colony light cream, velvety
	Underside	Purplish light brown with dark brown in the center of colony	Whitish cream	Cream	Light pinkish brown
Mycelium		Septate, hyaline, smooth with diameter 2.57-5.8 µm	Septate, hyaline, smooth with diameter 2.31-5.55 µm.	Septate, hyaline, smooth with diameter 1.7-4.16 µm	Septate, hyaline, smooth with diameter 1.42-4.15 µm
Aerial mycelium		Present	Present	Present	Present
Macroconidia	Shape	Fusoid with dorsal (upper) side more curved than ventral, basal cell barely notched, blunt apical cell, hyaline, septate, 4-5 cells	Fusoid with dorsal (upper) side more curved than ventral, basal cell barely notched, blunt apical cell, hyaline, septate, 3-6 cells	Fusoid with dorsal (upper) side more curved than ventral, basal cell barely notched, blunt apical cell, hyaline, septate, 3-6 cells	Fusoid with dorsal (upper) side more curved than ventral, basal cell barely notched, blunt apical cell, hyaline, septate, 3-6 cells
	Septa	3-4	2-5	2-5	2-4
	Length (µm)	22.69-46.29	24.76-46.22	29.08-45.71	26.42-44.85
	Width (µm)	Basal 2.17-3.78; middle 4.36-6.41; apical 2.25-3.81	Basal 2.29-4.23; middle 5.12-7.59; apical 2.29-5.65	Basal 2.49-4.50; middle 4.83-6.90; apical 2.84-5.30	Basal 2.06-4.06; middle 4.41-6.91; apical 2.31-4.27
Microconidia	Shape	Oval to cylindrical, hyaline	Oval, hyaline	Oval to cylindrical, hyaline	Oval to cylindrical, hyaline
	Septa	0-1	0-1	0-1	0-1
	Length (µm)	11.58-18.38	15.66-23.98	13.34-26.29	10.88-22.04
	Width (µm)	3.25-6.18	4.24-8.03	4.42-8.47	3.47-6.32
Chlamydospore	Shape and colour	Globose, intercalary and terminal, hyaline to slightly grey	Globose to oval, intercalary and terminal, hyaline to slightly grey	Globose to oval, intercalary and terminal, hyaline	Globose, intercalary and terminal, slightly brownish grey
	Size (µm)	5.26-9.99 x 5.79-9.99	6.28-12.07 x 7.25-13.62	6.70-9.95 x 6.99-9.99	6.17-9.90 x 6.36-12.14
Host		*Cymbidium finlaysonianum*	*Vanda tricolor*	*Calanthe triplicata*	*Phaius tankervilleae*

All four *Fusarium* isolates were categorized as fast-growing fungi with the *Fusarium* sp. V2 grew slower compared to the other three isolates. The three isolates reached 9 cm growth diameter in 5 days after inoculation, except the *Fusarium* sp. V2 (Table 2). Based on morphological features described by Burgess et al. (1994) and Short et al. (2013), all four isolates obtained in this study were identified as *Fusarium* based on the observed morphological characteristics.

Molecular analysis of *Fusarium* isolates

A single band of DNA fragments of approximately 650 bp was successfully amplified for the ITS1-5.8S-ITS2 region of rDNA for all four *Fusarium* isolates obtained in this study. All four isolate sequences were analyzed for their homology determination using BLASTn (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The results of BLAST analysis showed that all four isolates have close similarity with *F. solani* with query coverage of 99 to 100%, 0.0 E-value, and 99 to 100% identity (Table 3). Since *F. solani* was a species complex, therefore further analysis was conducted by constructing a phylogenetic tree using sequences of FSSC strains available in GenBank.

The molecular phylogenetic relationship among the strains indicated that all four *F. solani* obtained in this study belong to the FSSC group (Figure 6). The FSSC group consists of 3 clades: clade 1, clade 2, and clade 3. The *F. solani* obtained in this research belong to FSSC clade 3 and clustered further into FSSC5, which is known as *F. solani* sensu stricto. The four isolates formed 3 different groups within FSSC5. The *F. solani* C5 isolated from *C. triplicata* is in the same group with *F. solani* P44 isolated from *P. tankervilleae*, both hosts are terrestrial orchids. The *F. solani* C5 differed by only several base pair from *F. solani* P44, therefore they considered to form a single group (Figure 6). In contrast, the *F. solani* V2 and *F. solani* CF34 that were isolated from the epiphytic orchids *V. tricolor* and *C. finlaysonianum* were in a separate group from the isolates derived from terrestrial orchids (Figure 6). Furthermore, the *F. solani* V2 and the *F. solani* CF34 were in separate subgroups.

Table 3. Results of BLAST analysis of *Fusarium* spp. isolated from roots of *Cymbidium finlaysonianum, Vanda tricolor, Calanthe triplicata*, and *Phaius tankervilleae*

Isolate	IPB Culture Collection Code	Assigned accession number	BLAST result	Query (%)	Similarity index (%)	BLAST result accession number
Fusarium CF34	IPBCC 21.1527	OP204856	*Fusarium solani* CBS 101018	99	98.23	NR 154227.1
Fusarium V2	IPBCC 21.1529	OP204902	*Fusarium solani* CBS 140079	100	99.89	NR 163531.1
Fusarium C5	IPBCC 21.1530	OP204992	*Fusarium solani* CBS 140079	100	98.82	NR 163531.1
Fusarium P44	IPBCC 21.1538	OP216264	*Fusarium solani* CBS 140079	100	99.89	NR 163531.1

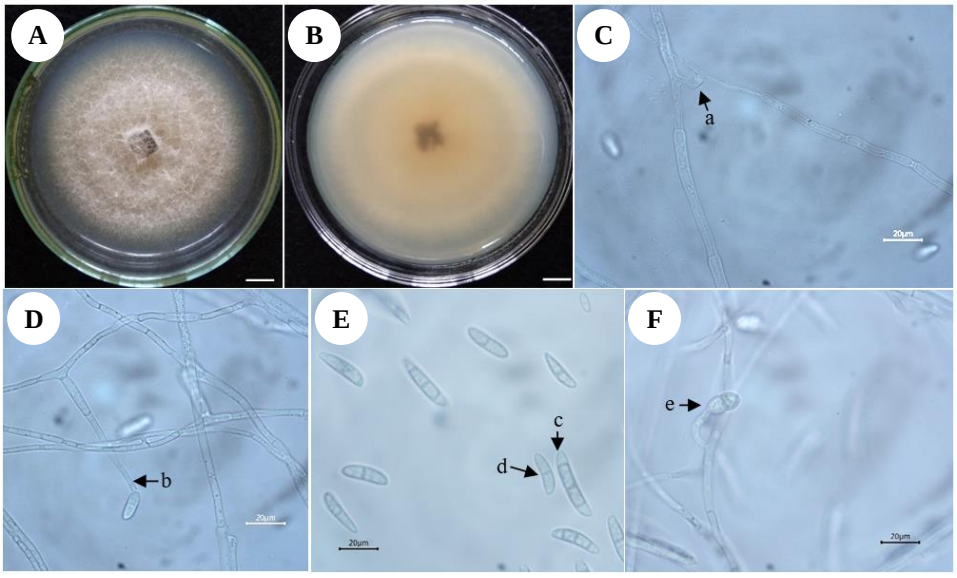

Figure 4. Colony and microscopic structures of *Fusarium solani* C5 isolated from *Calanthe triplicata* grown on PDA, the microscopic structures observed at 10 days after inoculation. (A-B) Top and underside view of *Fusarium solani* C5 colonies, a. Hyphal anastomosis, b. Phialide bearing microconidium, c. Macroconidium, d. Microconidium, e. Chlamydospore. Scale bars are 1 cm (A-B) and 20 µm (C-F)

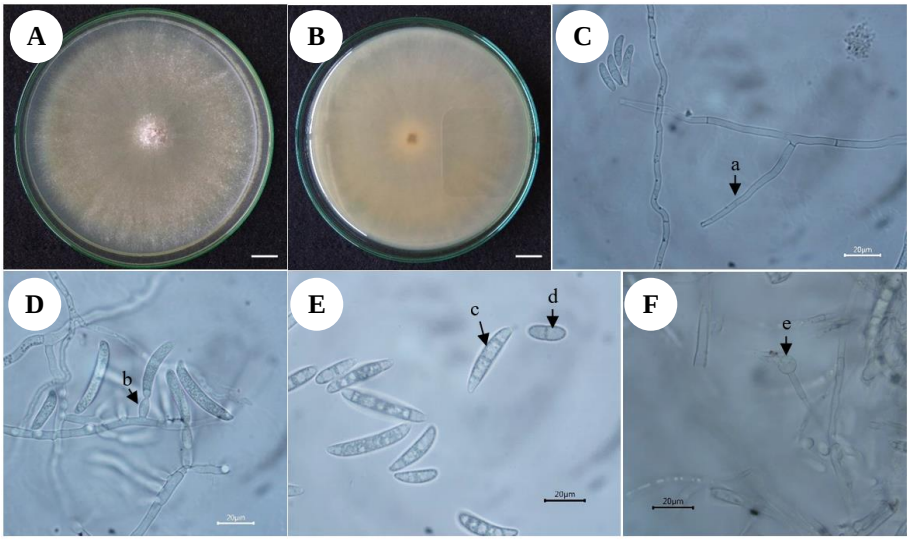

Figure 5. Colony and microscopic structures of *Fusarium solani* P44 isolated from *Phaius tankervilleae* grown on PDA, the microscopic structures observed at 10 days after inoculation. (A-B) Top and underside view of *Fusarium solani* P44 colonies, a. Phialide, b. Phialide bearing macroconidium, c. Macroconidium, d. Microconidium, e. Chlamydospore. Scale bars are 1 cm (A-B) and 20 µm (C-F)

Table 2. Colony growth diameter of four *Fusarium* isolates derived from four orchid species grown on PDA for 5 days

Fungal isolate	Host	Colony diameter (cm)
Fusarium CF34	*Cymbidium finlaysonianum*	9.0 ± 0.0[a]
Fusarium V2	*Vanda tricolor*	8.5 ± 0.09[b]
Fusarium C5	*Calanthe triplicate*	9.0 ± 0.0[a]
Fusarium P44	*Phaius tankervilleae*	9.0 ± 0.0[a]

Note: Values of colony diameter are means of five replications and standard errors. Values followed by the same letter are not significantly different in DMRT. The growth of most colonies had reached the diameter of the plate (9 cm) used in day 5

Discussion

The growth of orchids is dependent on endophytic or mycorrhizal fungi. The level of orchid dependency on the fungal partner is varies among species (Suetsugu and Matsubayashi 2021). The dependency of achlorophyllous orchids on the fungi is absolute because they lack photosynthetic tissue (Suetsugu et al. 2022). While chlorophyllous orchids are not fully reliant on fungal partners for carbon and other nutrients after their leaves and roots developed (Smith and Read 2008; Jiang et al. 2019). Once the symbiotic connection is established, the fungus will widen its role in the orchid's further growth and development, particularly in providing resistance to biotic and abiotic stress from the environment (Otero et al. 2013). Orchid species also vary in how much they depend on one or a few specific fungal partners or if they can more flexibly associate with many different fungal species (Suarez and Kottke 2016; Xing et al. 2019; Lespiaucq et al. 2021). Thus, the orchids are able to colonize a wider spectrum of habitats.

FSSC is an extremely diverse assemblage of fungi with respect to hosts, substrate, pathogenicity, geographic distribution, morphological characteristics such as presence or absence of microconidia, macroconidia characteristics, ascospore size as well as homo-and heterothallic sexual stages (Schroers et al. 2016). So far, the sexual cycle of the FSSC is only known for about one-third of the total taxa. As endophytes, members of the FSSC have been isolated from various plants in Indonesia, including from orchids. The fungus has been isolated from rhizomes of turmeric (*Curcuma longa* Linn.) (Septiana et al. 2017), roots of ginger (*Zingiber officinale* Roscoe) (Ginting et al. 2013), leaves of Asiatic pennywort (*Centella asiatica* L.) (Sukarno et al. 2021) and roots of the orchid *P. tankervilleae* (Sufaati et al. 2016). However, the phylogenetic entities of these Indonesian FSSC isolates, particularly those isolated from tropical orchids, have yet to be described.

Four FSSC isolates were obtained in this research from two epiphytic orchids, *C. finlaysonianum* and *V. tricolor*, and two terrestrial orchids, *C. triplicata* and *P. tankervilleae*. The morphological characteristics of all four isolates matched *F. solani* (Zakaria et al. 2009; Chehri et al. 2015). They had microconidia arising from long phialide-like structures and had fusoid macroconidia with the dorsal (upper) side more curved than the ventral, basal cells barely notched and blunt apical cells, and produced chlamydospores. The macroconidia shape's characteristics are in agreement with that of the FSSC5 strain reported from Malaysia, which was isolated from *Echinochloa colonum* (Chehri et al. 2015). Interestingly, the macroconidia characteristics obtained in this study are similar to those reported from temperate regions rather than to the macroconidia from tropical strains. The macroconidia of tropical strains commonly tend to be narrower and longer, with a more distinct basal cell than those of temperate isolates (Burgess et al. 1994). The long phialides observed in this study and the formation of chlamydospores are typical characteristics of *F. solani*.

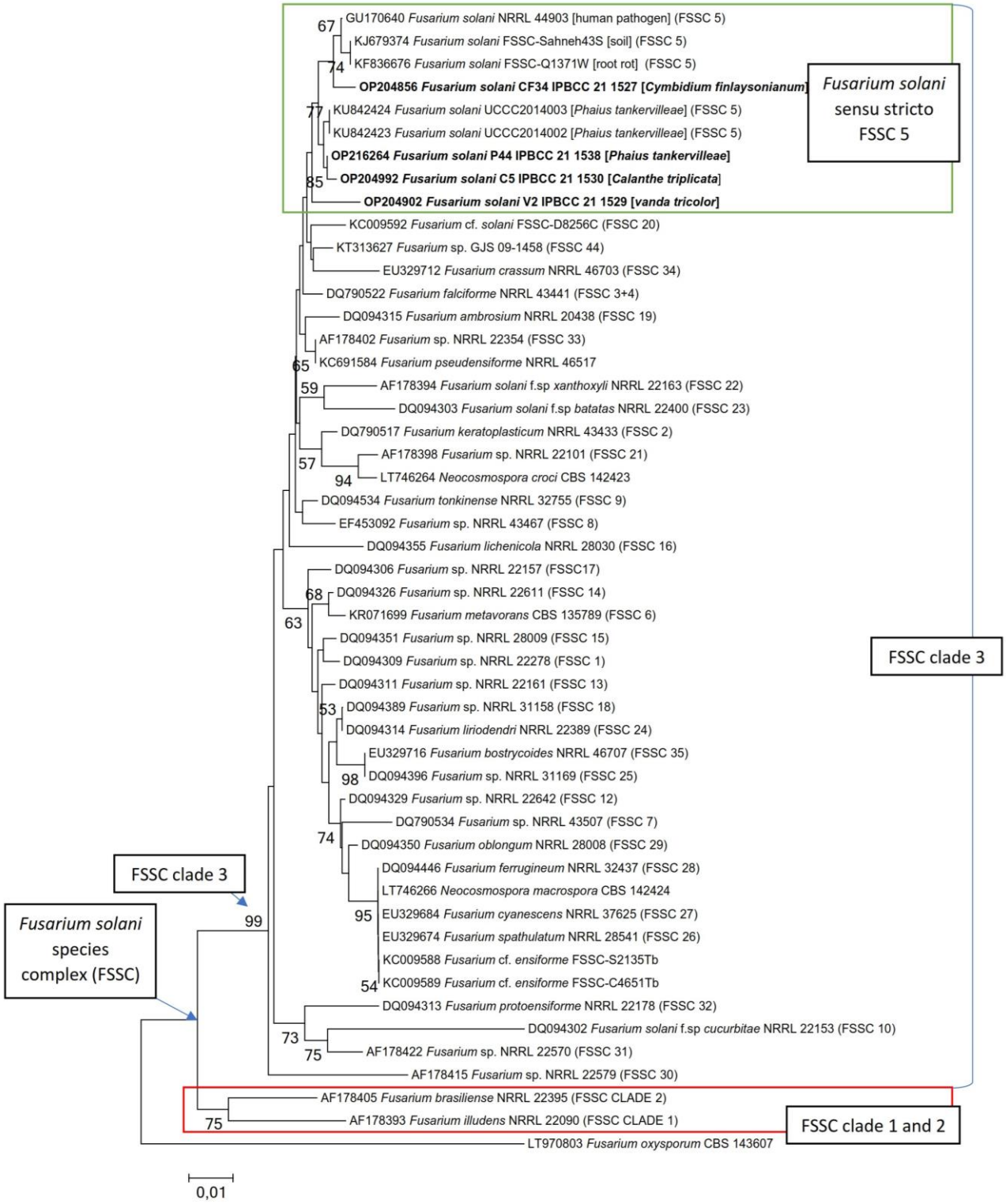

Figure 6. Phylogenetic analysis of *Fusarium solani* Species Complex (FSSC) based on ITS rDNA sequences. Statistical support values (≥50%) are shown at nodes and presented as bootstrap values performed with 1000 replication. *Fusarium oxysporum* CBS 143607 used as an outgroup

The results of BLASTn analysis using DNA sequences from ITS rDNA also indicates that all isolates are *F. solani* (Table 3). Results of the phylogenetic analysis showed that the four endophytic *F. solani* obtained from the orchids in this research are in FSSC5 (*F. solani* sensu stricto). The phylogenetic tree shows that two isolates, *F. solani* C5 and P44 (Figure 6), which were isolated from terrestrial orchids from Central Java, Indonesia, are closely related to endophytic *Fusarium* sp. strain UCCC2014002 and UCCC2014003 isolated from terrestrial *P. tankervilleae*

orchids growing in Papua, Indonesia (Sufaati et al. 2016). This suggests that the relationship between these FSSC5 strains and terrestrial orchid is important over a broad geographic range.

The FSSC5 have been reported as general saprobes and plant pathogens of diverse hosts, but surprisingly they have also been found in orchids as endophytic fungi. Thus, an important result of this research is to increase the number of known orchid species serving as hosts of FSSC5 and this, in turn, suggests a broader role of FSSC5 in orchid ecology.

There is a report of orchid mycorrhiza formed by *F. oxysporum*, but none from FSSC5 so far. The four orchids used in this study are not rare species. Therefore, the fungal partners of these orchids are likely common fungi. Rare orchids usually form symbiotic relationships with rare fungi (Batty et al. 2002). Both chlorophyllous and achlorophyllous orchids are highly dependent on mutualistic symbiosis with fungi to form Orchid Mycorrhiza (OM), at least during their early development. However, study on orchid-fungal relationship, either endophyte or mycorrhiza, is scarce in comparison with the study on orchid diversity (Zettler et al. 2017). The study of OM has so far concentrated on *Rhizoctonia*-like fungi (Jaquemyn et al. 2017). Non-*Rhizoctonia* fungi have also been found as true mycobionts in chlorophyllous orchids such as *Cymbidium* spp. (Li et al. 2016) and *Arundina graminifolia* (Meng et al. 2019), *Vanda cristata* (Chand et al. 2020), and *Cremastra variabilis* (Yagame et al. 2021). Therefore, research on non-*Rhizoctonia* orchid-associated fungi such as FSSC needs to be encouraged. It is likely that the diversity and role of non-*Rhizoctonia* fungi are much underestimated because of taxonomic and geographical bias in orchid mycobiont research so far (Jaquemyn et al. 2017). This research suggests that tropical orchids have a wider array of mycobionts in addition to *Rhizoctonia*-like fungi.

Application of sensitive and accurate molecular techniques was needed to identify the FSSC5 strains as the fungal endophytes of the orchids in this study because many important fungal taxa in the species complex share morphological characteristics. Accuracy is important in understanding fungal-orchid interaction in natural habitats. The orchid's dependency on suitable mycobionts for growth and development means that the conservation of the orchids also depends on the conservation of the fungi in appropriate habitats. Mapping of Indonesia's native orchids and their mycobionts is needed for the conservation, cultivation, and utilization of both organisms, the mycobionts, and orchids. Indonesia's great diversity of orchids deserves further exploration over the coming years. In this research, the FSSC in the roots of two tropical epiphytic orchids, *C. finlaysonianum* and *V. tricolor*, and two terrestrial orchids, *C. triplicata* and *P. tankervilleae* were analyzed. As has been demonstrated in this paper that the orchids studied are the hosts of FSSC5, identified as *F. solani* sensu stricto. This is the first report showing the tropical orchids are the host of the FSSC5 as endophytes and this broadens the known host range and ecological behavior of the FSSC5. *Fusarium* deserve further investigation since some member of the genus were recorded to have potential physiological and ecological advantage as endophytic fungi in orchids.

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REFERENCES

Agustini V, Sufaati S, Suharno, Suwannasai N. 2016. Short Communication: Rhizoctonia-like fungi isolated from roots of *Dendrobium lancifolium* var. *papuanum* and *Calanthe triplicata* in Papua, Indonesia. Biodiversitas 17 (1): 377-383. DOI: 10.13057/biodiv/d170151.

Alghamdi SA. 2019. Influence of mycorrhizal fungi on seed germination and growth in terrestrial and epiphytic orchids. Saudi J Biol Sci 26 (3): 495-502. DOI: 10.1016/j.sjbs.2017.10.021.

Al-Hatmi AMS, Ahmed SA, van Diepeningen AD, Drogari-Apiranthitou M, Verweij PE, Meis JF, de Hoog GS. 2018. *Fusarium metavorans* sp. nov.: The frequent opportunist 'FSSC6'. Med Mycol 56: S144-S152. DOI: 10.1093/mmy/myx107.

Batty AL, Dixon KW, Brundrett MC, Sivasithamparam K. 2002. Orchid conservation and mycorrhizal association. In: Sivasithamparam K, Dixon KW, Barret RL (eds.). Microorganism in Plant Conservation and Biodiversity. Springer, Dordrecht. DOI: 10.1007/0-306-48099-97.

Buragohain B, Chaturvedi SK, Puro N. 2016. Pollination biology of *Phaius tankervilleae* (Banks ex L' Herit) Bl. (Orchidaceae). Intl J Plant Reprod Biol 8 (1): 75-81. DOI: 10.14787/ijprb.2016 8.1.75-81.

Burgess LW, Summerell BA, Bullock S, Gott KP, Backhouse D. 1994. Laboratory Manual for *Fusarium* Research 3rd Edition. University of Sydney and Royal Botanic Gardens, Sydney (AU).

Chand K, Shah S, Sharma J, Paudel MR, Pant B. 2020. Isolation, characterization, and plant growth-promoting activities of endophytic fungi from a wild orchid *Vanda cristata*. Plant Signal Behav 15 (5): 1744294. DOI: 10.1080/15592324.2020.1744294.

Chehri K, Salleh B, Zakaria L. 2015. Morphological and phylogenetic analysis of *Fusarium solani* species complex in Malaysia. Microb Ecol 69 (3): 457-471. DOI: 10.1007/s00248-014-0494-2.

Chehri K. 2014. Molecular phylogeny of the *Fusarium solani* species complex (FSSC) isolated from soils in Iran. Botany 92 (11): 815-820. DOI: 10.1139/cjb-2014-0096.

Chitrampalam P, Nelson B Jr. 2016. Multilocus phylogeny reveals an association of agriculturally important *Fusarium solani* species complex (FSSC) 11, and clinically important FSSC 5 and FSSC 3 + 4 with soybean roots in the north-central United States. Antonie Van Leeuwenhoek 109 (2): 335-47. DOI: 10.1007/s10482-015-0636-7.

Coleman JJ. 2016. The *Fusarium solani* species complex: Ubiquitous pathogens of agricultural importance. Mol Plant Pathol 17 (2): 146-158. DOI: 10.1111/mpp.12289.

Crous PW, Lombard L, Sandoval-Denis M, et al. 2021. *Fusarium*: More than a node or a foot-shaped basal cell. Stud Mycol 98: 100116. DOI: 10.1016/j.simyco.2021.100116.

De LC, Rao AN, Rajeevan PK, Pathak P, Singh DR. 2015. Medicinal and aromatic orchids-an overview. Intl J Curr Res 7 (9): 19931-19935.

Devadas R, Pamarthi RK, Meitei AL, Pattanayak SL, Sherpa R. 2019. Morphological description of novel *Phaius* primary hybrid (Orchidaceae). J Exp Biol Agric Sci 7 (2): 138-147. DOI: 10.18006/2019.7(2).138.147.

Ding R, Chen XH, Zhang LJ, Yu XD, Qu B, Duan R, Xu YF. 2014. Identity and specificity of *Rhizoctonia*-like fungi from different

populations of *Liparis japonica* (Orchidaceae) in Northeast China. PloS One 9 (8): e105573. DOI: 10.1371/journal.pone.0105573.
Duffy KJ, Waud M, Schatz B, Petanidou T, Jacquemyn H. 2019. Latitudinal variation in mycorrhizal diversity associated with a European orchid. J Biogeogr 46: 968-980. DOI: 10.1111/jbi.13548.
Fadillah WN, Sukarno N, Iswantini D, Rahminiwati M, Hanif N, Waite M. 2021. In vitro pancreatic lipase inhibition by marine fungi *Purpureocillium lilacinum* associated with *Stylissa* sp. sponge as anti-obesity agent. Hayati J Biosci 29 (1):76-86. DOI: 10.4308/hjb.29.1.76-86.
Fujimori S, Abe JP, Okane I, Yamaoka Y. 2019. Three new species in the genus *Tulasnella* isolated from orchid mycorrhiza of *Spiranthes sinensis* var. *amoena* (Orchidaceae). Mycoscience 60 (1): 71-81. DOI: 10.1016/j.myc.2018.09.003.
Geiser DM, Al-Hatmi AMS, Aoki T, et al. 2021. Phylogenomic analysis of a 55.1-kb 19-gene dataset resolves a monophyletic *Fusarium* that includes the *Fusarium solani* species complex. Phytopathology 111 (7): 1064-1079. DOI: 10.1094/PHYTO-08-20-0330-LE.
Geiser DM, Aoki T, Bacon CW, et al. 2013. One fungus, one name: defining the genus *Fusarium* in a scientifically robust way that preserves longstanding use. Phytopathology 103 (5): 400-408. DOI: 10.1094/PHYTO-07-12-0150-LE.
Ginting RCB, Sukarno N, Widyastuti U, Darusman LK, Kanaya S. 2013. Diversity of endophytic fungi from red ginger (*Zingiber officinale* rosc.) plant and their inhibitory effect to *fusarium oxysporum* plant pathogenic fungi. Hayati J Biosci 20 (3): 127-137. DOI: 10.4308/hjb.20.3.127.
Handoyo F. 2012. Orchid of Indonesia, Vol.1. Perhimpunan Anggrek Indonesia, Jakarta. [Indonesian]
Hossain MM. 2011. Therapeutic orchids: Traditional uses and recent advances-an overview. Fitoterapia 82 (2): 102-140. DOI 10.1016/j.fitote.2010.09.007.
Jacquemyn H, Duffy KJ, Selosse MA. 2017. Biogeography of orchid mycorrhizas. In: Tedersoo L (eds.). Biogeography of Mycorrhizal Symbiosis. Ecological Studies, vol 230. Springer, Cham. DOI: 10.1007/978-3-319-56363-3_8.
Jiang J, Zhang K, Cheng S, Nie Q, Zhou SX, Chen Q, Zhou J, Zhen X, Li XT, Zhen TW, Xu M, Hsiang T, Sun Z, Zhou Y. 2019. *Fusarium oxysporum* KB-3 from *Bletilla striata*: an orchid mycorrhizal fungus. Mycorrhiza 29 (5): 531-540. DOI: 10.1007/s00572-019-00904-3.
Kanwal KS. 2014. Conservation of *Phaius tankervilleae* a valuable orchid of Arunachal Pradesh, India. Indian For 140 (12): 1263-1264.
Kornerup A, Wanscher JH. 1978. Methuen handbook of colour. 3rd edition. Eyre Methuen, London (UK).
Kurzweil H. 2010. A precursory study of the *Calanthe* group (Orchidaceae) in Thailand. Adansonia 32 (1): 57-107. DOI: 10.5252/a2010n1a4.
Lawrie MD, Layina Z, Ningtias DR, Alifianto FN, Indrianto A, Purwantoro A, Semiarti E. 2021. In vitro germination and flowering of *Dendrobium capra* J.J. Smith, an endemic orchid of Java. Hayati J Biosci 28 (2): 172-180. DOI: 10.4308/hjb.28.2.172.
Lespiaucq AV, Jacquemyn H, Rasmussen HN, Méndez M. 2021. Temporal turnover in mycorrhizal interactions: A proof of concept with orchids. New Phytol 230 (5): 1690-1699. DOI: 10.1111/nph.17291.
Li J, Wang R, Wang Z, Kuang P. 2016. The phylogenetic relationship and non-specific symbiotic habit of mycorrhiza fungi from a terrestrial orchid (*Cymbidium*). Nord J Bot 34 (3): 343-348. DOI: 10.1111/njb.00935.
Lombard L, van der Merwe NA, Groenewald JZ, Crous PW. 2015. Generic concepts in *Nectriaceae*. Stud Mycol 80: 189-245. DOI: 10.1016/j.simyco.2014.12.002.
Mahfut. 2021. Morphological identification of mycorrhizal fungi isolated from native orchids of Indonesia. Jordan J Biol Sci 14 (5): 1031-1034. DOI: 10.54319/jjbs/140523.
Matuo T, Snyder WC. 1973. Use of morphology and mating populations in the identification of formae speciales in *Fusarium solani*. Phytopathol 63: 562-565. DOI: 10.1094/Phyto-63-562.
McCormick MK, Whigham DF, Canchani-Viruet A. 2018. Mycorrhizal fungi affect orchid distribution and population dynamics. New Phytol 219 (4): 1207-1215. DOI: 10.1111/nph.15223.
Meng YY, Zhang WL, Selosse MA, Gao JY. 2019. Are fungi from adult orchid roots the best symbionts at germination? A case study. Mycorrhiza 29 (5): 541-547. DOI: 10.1007/s00572-019-00907-0.
Migheli Q, Balmas V, Harak H, Sanna S, Scherm B, Aoki T, O'Donnell K. 2010. Molecular phylogenetic diversity of dermatologic and other human pathogenic fusarial isolates from hospitals in northern and central Italy. J Clin Microbiol 48 (4): 1076-1084. DOI: 10.1128/JCM.01765-09.
O'Donnell K, Ward TJ, Robert VARG, Crous PW, Geiser DM, Kang S. 2015. DNA sequence-based identification of *Fusarium*: Current status and future directions. Phytoparasitica 43: 583-595. DOI: 10.1007/s12600-015-0484-z.
O'Donnell K, Al-Hatmi AMS, Aoki T, et al. 2020. No to *Neocosmospora*: Phylogenomic and practical reasons for continued inclusion of the *Fusarium solani* species complex in the genus *Fusarium*. mSphere 5 (5): e00810-20. DOI: 10.1128/mSphere.00810-20.
Otero JT, Mosquera AT, Flanagan NS. 2013. Tropical orchid mycorrhizae: Potential applications in orchid conservation, commercialization, and beyond. Lankesteriana 13 (1-2): 57-63. DOI: 10.15517/lank.v0i0.11537.
Pant B, Shah S, Shrestha R, Pandey S, Joshi PR. 2017. An overview on Orchid endophytes. In: Varma A, Prasad R, Tuteja N (eds.). Mycorrhiza-Nutrient uptake, biocontrol, ecorestoration: Fourth edition. Springer International Publishing, New York. DOI: 10.1007/978-3-319-68867-1_26.
Rasmussen HN, Dixon KW, Jersáková J, Těšitelová T. 2015. Germination and seedling establishment in orchids: A complex of requirements. Ann Bot 116 (3): 391-402. DOI: 10.1093/aob/mcv087.
Salazar JM, Pomavilla M, Pollard AT, Chica EJ, Peña DF. 2020. Endophytic fungi associated with roots of epiphytic orchids in two Andean forests in southern Ecuador and their role in germination. Lankesteriana 20 (1): 37-47. DOI: 10.15517/lank.v20i1.41157.
Sandoval-Denis M, Lombard L, Crous PW. 2019. Back to the roots: A reappraisal of *Neocosmospora*. Persoonia 43 (1): 90-185. DOI: 10.3767/persoonia.2019.43.04.
Sarsaiya S, Jain A, Jia Q, Fan X, Shu F, Chen Z, Zhou Q, Shi J, Chen J. 2020. Molecular identification of endophytic fungi and their pathogenicity evaluation against *Dendrobium nobile* and *Dendrobium officinale*. Intl J Mol Sci 21 (1): 316. DOI: 10.3390/ijms21010316.
Schroers HJ, Samuels GJ, Zhang N, Short DPG, Juba J, Geiser DM. 2016. Epitypification of *Fusisporium* (*Fusarium*) s*olani* and its assignment to a common phylogenetic species in the *Fusarium solani* species complex. Mycologia 108 (4): 806-819. DOI: 10.3852/15-255.
Septiana E, Sukarno N, Sukarno, Simanjuntak P. 2017. Endophytic fungi associated with turmeric (*Curcuma longa* L.) can inhibit histamine-forming bacteria in fish. Hayati J Biosci 24 (1): 46-52. DOI: 10.4308/j.hjb.2017.05.004.
Short DPG, O'Donnell K, Thrane U, Nielsen KF, Zhang N, Juba JH, Geiser DM. 2013. Phylogenetic relationships among members of the *Fusarium solani* species complex in human infections and the descriptions of *F. keratoplasticum* sp. nov and *F. petroliphilum* stat. nov. Fungal Genet Biol 53: 59-70. DOI: 10.1016/j.fgb.2013.01.004.
Smith SE, Read DJ. 2008. Mycorrhizal Symbiosis 3rd Edition. Academic Press, London (UK).
Stucky BJ. 2012. SeqTrace: A graphical tool for rapidly processing DNA sequencing chromatograms. J Biomol Tech 23 (3): 90-93. DOI: 10.7171/jbt.12-2303-004.
Suárez JP, Kottke I. 2016. Main fungal partners and different levels of specificity of orchid mycorrhizae in the tropical mountain forests of Ecuador. Lankesteriana 16 (2): 26014. DOI: 10.15517/lank.v16i2.26014.
Suetsugu K, Haraguchi TF, Tayasu I. 2022. Novel mycorrhizal cheating in a green orchid: *Cremastra appendiculata* depends on carbon from deadwood through fungal associations. New Phytol 235 (1): 333-343. DOI: 10.1111/nph.17313.
Suetsugu K, Matsubayashi J. 2021. Subterranean morphology modulates the degree of mycoheterotrophy in a green orchid *Calypso bulbosa* exploiting wood-decaying fungi. Funct Ecol 35 (10): 2305-2315. DOI: 10.1111/1365-2435.13864.
Sufaati S, Agustini V, Suharno S. 2016. *Fusarium* as endophyte of some terrestrial orchid from Papua, Indonesia. Biodiversitas 17 (1): 366-371. DOI: 10.13057/biodiv/d170149.
Sukarno N, Ginting RCB, Widyastuti U, Darusman LK, Kanaya S, Batubara I, Aryantha INP, Waite M. 2021. Endophytic fungi from four Indonesian medicinal plants and their inhibitory effect on plant pathogenic *Fusarium oxysporum*. Hayati J Biosci 28 (2): 152-171. DOI: 10.4308/hjb.28.2.152.
Suryantini R, Wulandari RS, Kasiamdari RS. 2015. Orchid mycorrhizal fungi: Identification of *Rhizoctonia* from Kalimantan. Microbiol Indones 9 (4): 157-162. DOI: 10.5454/mi9.4.3.
Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. 2013. MEGA 6: Molecular Evolutionary Genetics Analysis version 6.0. Mol Biol Evol 30 (12): 2725-2729. DOI: 10.1093/molbev/mst197.

Tan XM, Chen XM, Wang CL, Jin XH, Cui JL, Chen J, Guo SX, Zhao LF. 2012. Isolation and identification of endophytic fungi in roots of nine *Holcoglossum* plants (Orchidaceae) collected from Yunnan, Guangxi, and Hainan Provinces of China. Curr Microbiol 64 (2): 140-147. DOI: 10.1007/s00284-011-0045-8.

Teoh ES. 2019. Orchids as Aphrodisiac, Medicine or Food. Springer Nature Switzerland AG, Gewerbestrasse (CH). DOI: 10.1007/978-3-030-18255-7.

Vujanovic V, St-Armaud M, Barabe D, Thibeault G. 2000. Viability testing of orchid seed and the promotion of colouration and germination. Ann Bot 86 (1): 79-86. DOI: 10.1006/anbo.2000.1162.

White TJ, Bruns T, Lee S, Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds). PCR Protocols: a guide to method and applications. Academic Press, San Diego (US). DOI: 10.1016/B978-0-12-372180-8.50042-1.

Xing X, Jacquemyn H, Gai X, Gao Y, Liu Q, Zhao Z, Guo S. 2019. The impact of life form on the architecture of orchid mycorrhizal networks in tropical forest. Oikos 128 (9): 1254-1264. DOI: 10.1111/oik.06363.

Yagame T, Lallemand F, Selosse MA, Funabiki E, Yukawa T. 2021. Mycobiont diversity and first evidence of mixotrophy associated with *Psathyrellaceae* fungi in the chlorophyllous orchid *Cremastra variabilis*. J Plant Res 134 (6): 1213-1224. DOI: 10.1007/s10265-021-01337-w.

Zakaria L, Zain NHMD, Salleh B, Zakaria M. 2009. Morphological and RAPD analysis of *Fusarium* species associated with root and stem rot of *Dendrobium* orchid in Northern Peninsula Malaysia. Hayati J Biosci 16 (2): 64-68. DOI: 10.4308/hjb.16.2.64.

Zettler LW, Rajaovelona L, Yokoya K, Kendon JP, Stice AL, Wood AE, Sarasan V. 2017. Techniques for the collection, transportation, and isolation of orchid endophytes from afar: A case study from Madagascar. Bot Stud 58 (1): 54. DOI: 10.1186/s40529-017-0209-3.