Analysis of Fungal Community Composition in Root Endosphere and Rhizosphere Soil of Nervilia fordii from Guangxi Karst Region (Postprint)

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Abstract

To investigate the diversity of fungal communities in the roots and rhizosphere soil of *Nervilia fordii* (Orchidaceae), this study utilized Illumina MiSeq high-throughput sequencing technology to analyze the fungal composition in the roots and rhizosphere soil of *N. fordii* from two sampling sites, Daxin (DX) and Longzhou (LZ). The results demonstrated that: (1) Fungal diversity in both roots and rhizosphere soil of *N. fordii* from the two regions was substantial, with rhizosphere soil fungal diversity exceeding that in roots, and fungal diversity in main roots surpassing that in stolons; (2) Sequencing yielded a total of 118,040 effective sequences, comprising 207 operational taxonomic units (OTUs) distributed across 8 phyla, 19 classes, 42 orders, 86 families, and 123 genera; (3) Basidiomycota fungi constituted the dominant fungal group in roots of *N. fordii* from both locations, including Tulasnellaceae, Trimorphomycetaceae, Ceratobasidiaceae, Malasseziaceae, and Marasmiaceae, among which the dominant family and genus were Tulasnellaceae (75%) and *Epulorhiza* (56%), respectively; however, the dominant genus in soil was *Fusarium*. Collectively, these results indicate that fungal communities in *N. fordii* roots differed significantly from the dominant communities in rhizosphere soil, though some shared OTUs were present; furthermore, this study suggests that *Epulorhiza* fungi may exert a crucial influence on seed germination and seedling growth and development of *N. fordii*.

Full Text

Analysis of Fungal Communities in Roots and Rhizosphere Soil of *Nervilia fordii* from Karst Areas of Guangxi

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Abstract

To explore the diversity of fungal communities in the roots and rhizosphere soil of the orchid *Nervilia fordii*, this study employed Illumina MiSeq high-throughput sequencing technology to analyze fungal composition in both root and rhizosphere samples collected from two sites in Daxin (DX) and Longzhou (LZ) counties. The results revealed: (1) Fungal diversity was notably rich in both root and rhizosphere soil samples from both regions, with rhizosphere soil exhibiting higher diversity than roots, and taproots showing greater diversity than stolons. (2) A total of 118,040 valid sequences were obtained, yielding 207 operational taxonomic units (OTUs) across 8 phyla, 19 classes, 42 orders, 86 families, and 123 genera. (3) Basidiomycota fungi represented the dominant group in roots from both locations, including families such as Tulasnellaceae, Trimorphomycetaceae, Ceratobasidiaceae, Malasseziaceae, and Marasmiaceae. The dominant family and genus were Tulasnellaceae (75%) and *Epulorhiza* (56%), respectively. In contrast, *Fusarium* was the dominant genus in rhizosphere soil. These findings demonstrate significant differences in dominant fungal communities between root and rhizosphere compartments, while also revealing shared OTUs. Moreover, the results suggest that *Epulorhiza* fungi may play a crucial role in seed germination and seedling development of *N. fordii*.

Keywords: *Nervilia fordii*, mycorrhizal fungi, high-throughput sequencing, fungal diversity, Funguild

Introduction

Nervilia fordii is a narrowly distributed terrestrial orchid species (family Orchidaceae), also known as "Qingtian Kui," "Dujiao Lian," or "Zhenzhu Ye." It is primarily distributed in Guangxi, Guangdong, and Fujian provinces of China. Studies have shown that its dried leaves or whole plant possess medicinal properties including heat-clearing, lung-moistening, cough-relieving, detoxification, and stasis-dispersing effects, with particularly notable efficacy in treating pediatric cough. The plant is also valued as a high-end culinary ingredient in Hong Kong and Taiwan, attracting considerable attention. However, due to severe habitat destruction from agricultural expansion and low natural seed germination rates, wild *N. fordii* resources are 濒临枯竭 (approaching depletion), severely limiting population expansion and survival.

Research indicates that appropriate mycorrhizal fungi significantly influence orchid seed germination and seedling development. Fungi in orchid rhizosphere soil can colonize root cells through the root system, forming symbiotic relationships with host plants. However, the relationship between rhizosphere soil fungal composition and mycorrhizal fungi within *N. fordii* roots remains unclear. Therefore, investigating the composition and correlation of fungi in *N. fordii* roots and rhizosphere soil is essential for screening and utilizing effective mycorrhizal fungi to restore wild populations and achieve sustainable resource utilization.

Studies have shown that habitats for terrestrial orchid seed germination are more likely to harbor specific symbiotic fungi, whose abundance correlates positively with distance from adult orchid plants. Furthermore, some orchid root-associated fungi are ubiquitous in soil, and such specific fungi may facilitate orchid growth. Fungal communities in orchid roots and rhizosphere soil differ significantly, and these communities are largely independent, with no clear correlation between mycorrhizal fungal types/abundance in roots and those in rhizosphere soil. In addition to basidiomycete mycorrhizal fungi, *Fusarium* species also represent specific dominant fungal groups in both roots and rhizosphere soil of terrestrial orchids.

Based on the current state of orchid mycorrhizal research, this study selected Longzhou and Daxin counties in Guangxi' s karst region as study sites, focusing on wild *N. fordii* populations. Using Illumina MiSeq high-throughput sequencing, we analyzed the composition and differences of root-associated symbiotic fungi and rhizosphere soil fungi across two sample sites to address: (1) the molecular-level floristic composition and dominant endophytic fungal groups in roots and rhizosphere soil of *N. fordii* in karst areas; and (2) whether differences exist in root symbiotic fungal composition between the two sites and potential underlying causes. These results provide important reference value for future systematic studies on mycorrhizal fungal resources of *N. fordii* in Guangxi' s karst region and for applying mycorrhizal technology to achieve seed symbiotic germination and resource conservation of terrestrial medicinal orchids.

Materials and Methods

1.1 Experimental Materials Healthy *N. fordii* plants and soil samples were collected on October 7, 2020, from Longzhou County (106°67 E, 22°52 N; elevation 333 m) and Daxin County (107°05 E, 22°81 N; elevation 311 m) in Chongzuo City, Guangxi Zhuang Autonomous Region. Whole plants were excavated with rhizosphere and environmental soil, placed in ziplock bags, and transported to the laboratory at the Xianhu campus of Guangxi University of Chinese Medicine within 12 hours. Root surfaces were rinsed clean under running water, surface moisture was absorbed with filter paper, and samples were rapidly frozen in liquid nitrogen before storage at -80°C for later use.

Rhizosphere soil was collected from the 0–1 cm layer surrounding *N. fordii* roots

and placed in sterile centrifuge tubes, designated as DXTR (Daxin soil) and LZTR (Longzhou soil). Stolon and taproot portions from Daxin and Longzhou (three replicates each) were placed in sterile centrifuge tubes, designated as DXZG (-1, -2, -3), DXZJ (-1, -2, -3), LZZG (-1, -2, -3), and LZZJ (-1, -2, -3).

1.2 Genomic DNA Extraction and Sequencing Analysis Samples were sent to Guangzhou Genedenovo Biotechnology Co., Ltd. for DNA extraction and ITS rDNA sequencing analysis. Specific primers with barcodes were used to amplify the ITS1 region (primer sequences: ITS1 F KYO2: 5 -TAGAGGAAGTAAAAGTCGTAA-3 ; ITS86R: 5 -TTCAAAGATTCGATGATTCAC-3). Purified PCR products (amplicons) were ligated with sequencing adapters to construct sequencing libraries for Illumina MiSeq high-throughput sequencing and subsequent bioinformatic analysis.

1.3 Data Processing and Analysis Low-quality reads were filtered, paired-end reads were merged into tags, and tags underwent quality filtering to obtain Clean Tags. Usearch software was used for clustering, with chimeric tags detected during clustering removed. Operational taxonomic units (OTUs) were clustered at 97% similarity, and OTU abundance was statistically analyzed based on Effective Tags.

R language vegan package was used for Venn diagram analysis, and Qiime software was employed for alpha diversity indices including Sobs (species number), Shannon index, Simpson index, Chao 1 index, phylogenetic diversity index (pd), and Good' s coverage analysis. Statistical methods were applied for differential feature discovery and significance testing.

Results

2.1 Sequencing Results Analysis High-throughput sequencing of the fungal rDNA ITS1 region was performed on 14 samples including taproots, stolons, and rhizosphere soil from wild *N. fordii* in Daxin and Longzhou counties. Results showed that post-quality control, each sample yielded over 110,000 tags (Table 1). Rarefaction curves constructed from sequencing depth versus OTU number approached saturation (Figure 1), indicating that sequencing depth adequately covered the majority of fungal species in the samples. Good' s coverage indices for all samples reached 0.999 (Table 1), demonstrating extremely low probability of undetected sequences and qualified sequencing depth.

Chao 1 and Ace indices evaluate community richness, while Shannon index reflects community diversity, influenced by both species richness and evenness. For Daxin taproot (DXZG), Chao 1, Ace, and Shannon indices were 302.67 $\pm$ 7.23, 316.04 $\pm$ 3.41, and 1.10 $\pm$ 0.23, respectively (Figure 2A). For Longzhou taproot (LZZG), these indices were 212.72 $\pm$ 143.99, 215.14 $\pm$ 145.47, and 2.31 $\pm$ 0.15, respectively. The Shannon index, reflecting community diversity, was significantly higher in Longzhou taproot ($P = 0.0035$), while Chao 1 and

Ace indices, representing species richness, showed no significant differences ($P > 0.05$). For Daxin stolon (DXZJ), Chao 1, Ace, and Shannon indices were 205.41 ± 113.57, 210.70 ± 118.47, and 0.72 ± 0.25, respectively (Figure 2B), while Longzhou stolon (LZZJ) showed values of 209.37 ± 47.62, 212.68 ± 73.34, and 0.69 ± 0.11, respectively. Thus, fungal species richness and diversity in Longzhou stolon were lower than in Daxin stolon, but differences were not significant ($P > 0.05$).

2.2 Root-Associated Fungal Diversity and Correlation Analysis This study detected numerous OTUs from two rhizosphere soil samples (DXTR, LZTR), six stolon samples (DXZJ, LZZJ), and six taproot samples (DXZG, LZZG). Rhizosphere soil analysis revealed 411 OTUs in DXTR, slightly more than the 406 in LZTR. Shannon, Simpson, Chao 1, and pd indices were all slightly higher in Daxin rhizosphere soil (Table 1), indicating greater richness and diversity compared to Longzhou. OTU-Venn diagrams visually reflect shared and unique OTUs between samples. The Venn diagram for DXTR and LZTR (Figure 3) showed 705 total OTUs, with 112 shared, 299 unique to DXTR, and 294 unique to LZTR. These results demonstrate that rhizosphere fungal communities from both sites were compositionally rich but exhibited differences in composition, structure, and relative abundance.

Analysis of different plant parts showed Daxin taproot (DXZG) contained 236 OTUs and stolon (DXZJ) contained 151 OTUs, while Longzhou taproot (LZZG) had 158 OTUs and stolon (LZZJ) had 151 OTUs. Taproots consistently showed higher OTU numbers and alpha diversity indices than stolons (Table 1), indicating greater symbiotic fungal richness and diversity in taproots. Venn diagrams for DXZJ vs. DXZG and LZZJ vs. LZZG (Figure 4) revealed 221 total OTUs in Longzhou root samples, with 126 shared between stolon and taproot, 59 unique to taproot, and 36 unique to stolon. Daxin root samples contained 289 total OTUs, with 113 shared, 109 unique to taproot, and 67 unique to stolon. Combined with alpha diversity indices (Table 1), these findings indicate rich symbiotic fungal composition in both Daxin and Longzhou *N. fordii* roots, with taproots showing higher fungal composition, structure, and relative abundance than stolons.

2.3 Fungal Community Structure in Roots and Rhizosphere Soil From *N. fordii* roots and rhizosphere soil, we obtained 118,040 valid sequences representing 207 OTUs across 8 phyla, 19 classes, 42 orders, 86 families, and 123 genera. The top 10 most abundant species were analyzed, with other known species grouped as "others" and unknown sequences marked as "unclassified." Community structure variations were analyzed at phylum, class, order, family, genus, and species levels (Figure 5).

At the phylum level (Figure 5A), eight fungal phyla were detected across 14 root and rhizosphere samples: Basidiomycota, Ascomycota, Glomeromycota, Mortierellomycota, Kickxellomycota, Mucoromycota, Chytridiomycota, and En-

tomophthoromycota, with unclassified sequences accounting for 18.24%. Root endophytic fungi were predominantly Basidiomycota (76%), comprising 66.89%, 78.68%, 97.03%, and 96.39% in DXZG, LZZG, DXZJ, and LZZJ groups, respectively. Ascomycota was the second most abundant phylum (10%), with significant differences in mean abundance between DXZJ and DXZG ($P = 0.0179$) and between DXZJ and LZZJ ($P = 0.02$). Mortierellomycota showed significant differences between LZZG and DXZG ($P = 0.01$), while Basidiomycota differed significantly between LZZJ and LZZG ($P = 0.02$). Rhizosphere soil from the two locations showed distinct dominant phyla: Ascomycota (59.34%) in Daxin and Basidiomycota (36.63%) in Longzhou.

At the class level (Figure 5B), dominant groups included Agaricomycetes (58% total), Sordariomycetes (5%), Tremellomycetes (3%), Eurotiomycetes (1%), Archaeorhizomycetes, Malasseziomycetes, Dothideomycetes, Glomeromycetes, and Saccharomycetes, with unclassified sequences at 34.90% and others at 0.35%. Significant differences ($P < 0.05$) were observed in fungal class proportions between rhizosphere soils from different locations and between root samples, particularly for Agaricomycetes ($P = 0.03$ between LZZJ and LZZG) and Archaeorhizomycetes and Mortierellomycetes (both $P = 0.01$ between DXZG and LZZG).

At the order level (Figure 5C), dominant taxa included Cantharellales (57%), Tremellales (3%), Hypocreales (2%), Eurotiales (1%), Archaeorhizomycetales, Malasseziales, Glomerellales, Sordariales, Agaricales, and Capnodiales. At the family level (Figure 5D), Tulasnellaceae dominated Basidiomycota (75%), with minor contributions from Trimorphomycetaceae, Ceratobasidiaceae, Malasseziaceae, and Marasmiaceae. Ascomycota families included Nectriaceae, Archaeorhizomycetaceae, Aspergillaceae, Chaetomiaceae, and Glomerellaceae.

At the genus level (Figure 5E), dominant taxa included *Epulorhiza*, *Saitozyma*, *Fusarium*, *Archaeorhizomyces*, *Malassezia*, *Aspergillus*, *Tetrapyrgos*, *Humicola*, *Colletotrichum*, and *Penicillium*. *Fusarium* was the significantly dominant genus in rhizosphere soil, while *Epulorhiza* dominated *N. fordii* roots (56% of isolated fungi) and was also abundant in rhizosphere soil (4% in Daxin, 14% in Longzhou). At the species level (Figure 5F), dominant species included *Panus conchatus*, *Nigrospora oryzae*, *Meyerozyma guilliermondii*, *Staphylotrichum coccosporum*, *Septoria cretae*, *Sodiomyces magadii*, *Humicola olivacea*, *Tetrapyrgos subcinerea*, *Malassezia restricta*, and *Saitozyma podzolica*. Overall, fungal communities at different taxonomic levels showed compositional, structural, and abundance differences between rhizosphere soils from the two locations and between different root parts, with taproots exhibiting higher fungal diversity than stolons.

Discussion and Conclusion

This study employed high-throughput sequencing to compare and analyze fungal community structures in roots and rhizosphere soil of the terrestrial orchid

N. fordii from Guangxi' s karst region. The results identified *Epulorhiza* (sexual stage: *Tulasnella*) as the absolutely dominant fungal genus in roots from both Daxin and Longzhou sites. Research suggests that terrestrial orchid seeds undergo prolonged development in soil after dispersal, during which they rely on symbiotic fungi for stable, persistent carbon sources. Consequently, terrestrial orchids often form highly specific mycorrhizal relationships, with most associating with *Tulasnella* group fungi, as observed in *Arundina* and *Cypripedium* species. Moreover, *Tulasnella* represents not only the dominant group in orchid mycorrhizal fungal communities but also constitutes important symbiotic germination fungi. Therefore, *Epulorhiza* likely plays a crucial role in seed germination and corm development of *N. fordii*, though further evidence from symbiotic germination experiments is needed. Additionally, higher *Epulorhiza* abundance in taproots than stolons may relate to longer taproot growth periods versus shorter stolon growth cycles focused on reproductive propagation.

Fungal community composition and abundance significantly regulate plant ecosystem functions, with core fungal groups and keystone species being hot topics in fungal ecology. Based on FunGuild database functional prediction, Symbiotroph was the primary nutritional type in both taproots and stolons, with relative abundances of 63% and 92% (Longzhou) and 36% and 60% (Daxin). Other nutritional types (Pathotroph-Saprotroph-Symbiotroph, Pathotroph, and Pathotroph-Symbiotroph) were less abundant (<2%). Rhizosphere soil fungal communities showed similar nutritional profiles, with Pathotroph-Saprotroph-Symbiotroph at 17%, Saprotroph and Symbiotroph at ~4% in Daxin, and Symbiotroph and Pathotroph-Saprotroph-Symbiotroph at 14.43% and 13.31% in Longzhou. Functionally, both locations were dominated by ectomycorrhizal functional groups centered on *Epulorhiza*, with minor contributions from undefined saprotrophs, animal pathogens, arbuscular mycorrhizal fungi, and endomycorrhizal-plant pathogen-undefined saprotroph groups, reflecting fungal diversity in roots and rhizosphere.

Furthermore, total OTU numbers were higher in rhizosphere soil than in roots from both locations, with shared OTUs present between rhizosphere soil and roots, indicating more complex fungal assemblages in rhizosphere soil and overlapping communities between compartments. Similar results were reported by Jiang et al. (2019) in studies of nine orchid species. Dominant phyla in rhizosphere soil differed significantly between locations: Ascomycota in Daxin and Basidiomycota in Longzhou. Significant differences in Ascomycota abundance between root samples and in Mortierellomycota abundance between DXZG and LZZG groups suggest that both root and rhizosphere fungal communities are rich but exhibit location- and compartment-specific differences in composition, structure, and abundance. These variations likely relate to environmental factors including altitude, soil moisture, pH, nutrient conditions, monsoon climate, and vegetation type.

In summary, modern molecular methods like high-throughput sequencing provide effective tools for comprehensively analyzing fungal communities associated

with *N. fordii* roots, offering scientific reference for targeted isolation of culturable mycorrhizal fungi to study orchid seed symbiotic germination and mycorrhizal mechanisms. This has important practical significance for conserving endangered *N. fordii* resources through mycorrhizal technology. However, despite obtaining numerous sequences, incomplete NCBI database coverage prevented full bioinformatic interpretation, representing a limitation requiring future improvement.

References

BUNCH WD, COWDEN CC, WURZBURGER N, et al., 2013. Geography and soil chemistry drive the distribution of fungal associations in lady' s slipper orchid, *Cypripedium acaule*[J]. *Botany*, 91(12): 850-856.

CHEN J, MENG ZX, XING YM, et al., 2017. Isolation and identification of endophytic fungi from five medicinal plants species of Orchidaceae[J]. *Chin Pharm J*, 52(4): 267-271.

CHEN YH, XING XK, GUO SX, 2017. Nutritional relationships between orchids and mycorrhizal fungi: a review[J]. *Mycosystema*, 36(7): 807-819.

CHEN YH, XING XK, GUO SX, 2018. The endophytic fungal community composition of *Gymnadenia conopsea* in Beijing[J]. *Mycosystema*, 37(1):35-42.

FAY MF, 2016. Orchid conservation: further links[J]. *Annals of Botany*, 118(1): 89-91.

FU YJ, ZHANG JL, HOU XQ, 2019. Comparative analysis of fungi diversity in rizospheric and non-rhizospheric soil from *Cypripedium macranthum* estimated via high-throughput sequencing[J]. *Acta Agric Boreal-Occident Sin*, 28(02): 253-259.

HUANG MQ, XIE YL, LAI XP, et al., 2012. Protection of total flavonoid fraction from *Nervilia fordii* on lipopolysaccharide-induced acute lung injury in rats[J]. *Chin Med Mat*, 4(4): 337-340.

JACQUEMYN H, BRYS R, WAUD M, et al., 2015. Mycorrhizal networks and coexistence in species-rich orchid communities[J]. *New Phytolt*, 206(3): 1127-1134.

Jacquemyn H, Merckx V, Brys, et al., 2011. Analysis of network architecture reveals phylogenetic constraints on mycorrhizal specificity in the genus *Orchis* (Orchidaceae)[J]. *New phytologist*, 192(2):518-528.

JIANG YL, CHEN XH, MIAO Q, et al., 2019. Difference in fungal communities between in roots and in root-associated soil of nine orchids in Liaoning, China[J]. *Chin J Plant Ecol*, 43(12):1079-1090.

LIANG YS, GONG L, HUANG ZH, et al., 2017. Analysis of global ecological suitability of southern chinese materia medica herba *Nervilia fordii*[J]. *Subtrop Plant Sci*, 46(4): 339-342.

LIN M, XIONG H, XIANG X, et al., 2020. The effect of plant geographical location and developmental stage on root-associated microbiomes of *Gymnadenia conopsea*[J]. *Front Microbiol*, 11(1257): 1-17.

MA XT, XING XK, GUO SX, 2016. Correlation between distribution of *Dendrobium chrysotoxum* and mycorrhizal fungal community composition[J]. *Mycosystema*, 35(7): 814-821.

MCCORMICK MK, JACQUEMYN H, 2014. What constrains the distribution of orchid populations?[J]. *New Phytol*, 202(2): 392-400.

MCCORMICK MK, TAYLOR DL, WHIGHAM DF, et al., 2016. Germination patterns in three terrestrial orchids relate to abundance of mycorrhizal fungi[J]. *J Ecol*, 104(3): 744-754.

MEI QX, 2008. Advance in the research of chemical constituent pharmacologic action and clinical application of herba *Nerviliae Plicatae*[J]. *Chin Arch of Trad Chin Med*, (10): 2239-2241.

MENG YY, ZHANG WL, SELOSSE MA, et al., 2019. Are fungi from adult orchid roots the best symbionts at germination? A case study[J]. *Mycorrhiza*, 29(5): 541-547.

OGURA-TSUJITA Y, YUKAWAT, 2008. High mycorrhizal specificity in a widespread mycoheterotrophic plant, *Eulophia zollingeri* (Orchidaceae)[J]. *Amer J Bot*, 95(1): 93-97.

OKTALIRA FT, WHITEHEAD MR, LINDE CC, 2019. Mycorrhizal specificity in widespread and narrow-range distributed *Caladenia* orchid species[J]. *Fungal Ecol*, 42(C): e100869.

PANG MX, JIN G, HUANG ZL, et al., 2021. Diversity of fungi associated with *Caulerpa lentillifera* in the South China Sea[J]. *Mycosystema*, 40(8): 2171-2180.

QIU L, XU LY, MIU JH, et al., 2011. Study advances on chemical constituents from *Nervilia fordii* (Hance) Schltr. and its bioactivities[J]. *Lishizhen Med Mater Med Res*, 22(9): 2258-2260.

RAFTER M, YOKOYA K, SCHOFIELD EJ, et al., 2016. Non-specific symbiotic germination of *Cynorkis purpurea* (Thouars) Kraezl., a habitat-specific terrestrial orchid from the Central Highlands of Madagascar[J]. *Mycorrhiza*, 26(6): 541-52.

RASMUSSEN HN, RASMUSSEN FN, 2009. Orchid mycorrhiza: implications of a mycophagous life style[J]. *Oikos*, 118(3): 334-345.

SCHLOSS PD, WESTCOTT SL, RYABIN T, et al., 2009. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities[J]. *Appl Environ Microbiol*, 5(23): 7537-7541.

WANG XG, LI XL, DONG Y, et al., 2021. Difference in fungal communities between in roots and in rhizosperic soil of *Paphiopedilum hirsutissimum*[J]. *SW Chin J Agric*, 34(4):762-769.

WAUD M, WIEGAND T, BRYS R, et al., 2016. Nonrandom seedling establishment corresponds with distance-dependent decline in mycorrhizal abundance in two terrestrial orchids[J]. *New Phytol*, 211(1): 255-264.

XIONG D, OU J, LI LP, et al., 2020. Community composition and ecological function analysis of endophytic fungi in the roots of *Rhododendron simsii* in *Pinus massoniana* forest in central Guizho[J]. *Acta Ecol Sin*, 40(4):1228-1239.

XU LL, ZHAO MY, LI J, et al., 2019. Correlation between vertical distribution of three *Cypripedium* species and composition of orchid mycorrhizal fungal community[J]. *Mycosystema*, 38(6): 811-821.

YUAN L, YANG ZL, LI SY, et al., 2010. Mycorrhizal specificity, preference, and plasticity of six slipper orchids from south western China[J]. *Mycorrhiza*, 20(8): 559-568.

Note: Figure translations are in progress. See original paper for figures.