Endophytic Microorganism From the Endangered Plant Nervilia Fordii (Hance) Schltr. In the Southwest Karst Area of China: Isolation, Genetic Diversity and Potential Functional Discovery

Xiao-Ming Tan
Guangxi University of Chinese Medicine, Guangxi Key Laboratory Cultivation Base of TCM Prevention and Treatment of Obesity
Xin-Feng Yang
Guangxi University of Chinese Medicine, Guangxi Key Laboratory Cultivation Base of TCM Prevention and Treatment of Obesity
Ya-Qin Zhou (zhouyaqin2009@126.com)
Guangxi Botanical Garden of Medicinal Plant
Peng Fu
Guangxi University of Chinese Medicine, Guangxi Key Laboratory Cultivation Base of TCM Prevention and Treatment of Obesity
Shi-Yi Hu
Guangxi University of Chinese Medicine, Guangxi Key Laboratory Cultivation Base of TCM Prevention and Treatment of Obesity
Zhong-Heng Shi
Guangxi University of Chinese Medicine, Guangxi Key Laboratory Cultivation Base of TCM Prevention and Treatment of Obesity

Research Article

Keywords: Nervilia fordii, vulnerable perennial herb, Bletilla striata seeds, various endophytic fungi, orchids

Posted Date: April 27th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-413613/v1

Abstract

The plant *Nervilia fordii* (Hance) Schltr. is known for its antimicrobial and antitumor properties. It is a rare and vulnerable perennial herb of the Orchidaceae family. In this study, 984 isolates were isolated from various tissues of *N. fordii*. and were identified through the sequence analyses of the internal transcribed spacer region of the rRNA gene. Except for 12 unidentified fungi, all others were affiliated to at least 39 genera of 14 orders of Ascomycota (72.66%) and Basidiomycota (19.00%). Antimicrobial activity was determined by using the agar diffusion method. Subsequent assays revealed 20 strains of fungal endophytes exhibited antibacterial activity against at least one pathogenic bacterium or fungus. Moreover, the capability of promoting seed germination was evaluated on the basis of the interaction of *Bletilla striata* seeds with the isolates. Results revealed that the three isolates could promote *B. striata* seed germination. After 21 days, the germination rate under treatment with the best strain was 97.89%, which was higher than that under the control treatment (12.68%). Taken together, the present data suggested that various endophytic fungi of *N. fordii* could be exploited as sources of novel natural antimicrobial agents or used for the artificial breeding of rare orchids.

Introduction

Endophytic fungi have been detected in all plant species investigated thus far[1]. They inhabit living plant tissues for at least parts of their life cycles without causing any apparent disease or injury to their host[2] and are ubiquitous in vascular plant species[3]. All orchids require endophytic fungi. They play a key role in supporting and enhancing plant health and growth[4,5], such as produce different plant hormones to enhancing plant growth[6], protecting the host plant against phytopathogenic microorganisms or pests[7,8] and so on. In addition, they can influence plant ecology; fitness; the evolution of plant community structures; and the diversity of interacting organisms, including nematodes and insects[9,10]. Endophytes represent a relatively underexplored and attractive source of natural products that are suitable as novel sources of bioactive metabolites, including metabolites with anticancer, antimicrobial, antimalarial, and other activities, and have thus elicited considerable attention from many researchers[11,12]. Currently, although many endophytic fungi from various terrestrial plants are gradually being described and explored, they account for less than 10% of the approximately one million known terrestrial endophytes have been investigated[13].

Nervilia fordii (Hance) Schltr. (Fig. 1 a) is a rare and vulnerable perennial herb of the Orchidaceae family; the whole plant (including rhizome, Fig. 1 b) or the aerial part is used as a traditional Chinese medicine called *Qing Tian Kui*[14,15]. This plant species is endemic to the southwest karst area of China (Fig. 1 c) and is mainly distributed at altitudes ranging from 220–1,000 m above sea level in sheltered valley or hillside areas[16,17]. *N. aragoana*, *N. cumberlegii*, *N. fordii*, *N. lanyuensis*, *N. mackinnonii*, *N. plicata* (Andr.) Schltr. *var. plicata*, *N. taiwaniana*, and *N. plicata* (Andr.) Schltr. *var. purpurea* (Hayata) S. S. Ying have been identified as endemic to China in previous studies[18]. *N. fordii* is a traditional Chinese medicine that has long been used in Chinese folk medicine for the treatment of various respiratory diseases, such as bronchitis, stomatitis, acute pneumonia, and acute pharyngitis[19,20]. *N. fordii* has received considerable attention in modern pharmacology research because of its biological behaviors, which include antimicrobial[21], antitumor[22], antiviral[23], and anti-inflammatory[24] behaviors.

Therefore, the present work aimed to investigate the species diversity of the culturable endophytic fungi in *N. fordii* collected from Guangxi Provinces, China, via rDNA internal transcribed spacer (ITS) sequences analysis. The endophytic fungi were screened for antimicrobial activities, and their benefit to seed germination was determined. The results of this report are helpful for exploring the potential sources of novel natural antimicrobial and the actual propagation and conservation of orchids.

Materials And Methods

Ethics Committee approval was obtained from the Institutional Ethics Committee of Guangxi University of Chinese Medicine to the commencement of the study. We confirm that all methods were performed in accordance with the relevant guidelines and regulations.

Collection of plant material. In 2020, he samples of *N. fordii* wild plants were collected from Sanhaung Townlet, Guangxi Province, China (109°66'E; 24°94'N). Healthy *N. fordii* plants were selected. Whole plants were dug out and placed in pots with rhizosphere soil and environmental soil, labeled, and transported to the laboratory within 12 h. The plant specimens were identified by Professor Tan and were preserved in the herbarium of the the Guangxi Botanical Garden of Medicinal Plants (voucher ID: SHNF20200618).

Fungal isolation and cultivation. Endophytic fungi were isolated from the corms, rhizomes, and leaves of the plant specimens. Procedures for the surface sterilization of plant tissues and the isolation and cultivation of fungi have been previously described by Tan *et al*.[3,10]. Briefly, roots, rhizomes, and leaves were separated from the plants; washed thoroughly in running tap water to remove dirt; and surface-sterilized sequentially in 70% ethanol (v/v) for 30 s and sodium hypochlorite solution (2.5%, v/v) for 5 min. All tissues were then rinsed three times with sterile distilled water and were surface-dried with sterile filter paper. Subsequently, 0.5 cm × 0.5 cm pieces were excised by using a sterile blade and placed on PDA containing 50 $\mu g \cdot mL^{-1}$ oxytetracycline and 50 $\mu g \cdot mL^{-1}$ streptomycin. Seven segments were plated per Petri dish (90 mm diameter). The Petri dishes were then wrapped in parafilm and incubated at 25 °C in the dark. They were observed for the growth of fungi from the segments every 2 days for more than 1 week. The colonies were routinely isolated, purified, and maintained in PDA for identification and antimicrobial assays. Pure endophytic fungi were finally photographed and preserved in the Scientific Laboratory Center, Guangxi University of Chinese Medicine.

DNA extraction, PCR amplification, sequencing, and molecular identification. For the production of fungal mycelia, all strains were grown on PDA plates at 25 °C for 1–2 weeks (the diameter of fungal colony was approximately 4 cm, which could meet the requirement of DNA extraction). Mycelia were scraped by using sterile pipette tips and were then freeze-dried, DNA from endophytic fungi was then extracted using by E.Z.N.A.TM Fungal DNA Mini Kits (Omega Bio-tek, Norcross, USA) were utilized in accordance with the manufacturers' instructions for use as templates in polymerase chain reactions (PCR). The primers ITS1

(5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') were constructed for molecular phylogenetic studies and were used to amplify ribosomal internal transcribed spacers (ITS)[56] The PCR mixture (50 μL) contained 25 μL of 2× SanTaq PCR Mix (Sangon Biotech, Shanghai), 2 μL of each primer (5 μM), and 2–10μL of genomic DNA (20–50 ng·μL^{-1}) and brought to a volume of 50 μL with ddH_2O. PCR was performed on a thermal cycler (BioRAD) as follows: incubation at 94 °C for 3 min; followed by 35 cycles of 94 °C for 30 s, 55 °C for 25 s, 72 °C for 30 s; and then a final extension step at 72 °C for 7 min. Subsequently, PCR products were purified and sequenced at the Shanghai Sangon Biological Engineering Technology & Services Co. Ltd. The sequences were then BLASTed the sequences of known isolates in the NCBI database (http://www.ncbi.nlm.nih.gov)[57]. Only those matching previously published sequences with high similarity were used. All identified isolates were categorized at the genus or family levels in accordance with the ownership criterion as follows: species of the same genera had sequence similarity (SS) ≥ 95% and those of the same families had SS ≤ 95%[58,59].

Antimicrobial activity. Three pathogens, including the fungi *C. tropicalis* and bacteria *E. coli* and *S. aureus*, were used to test the antimicrobial activities of all strains. Inhibitory effects were assayed by using the fungus cake method[60]. Streptomycin (20 μL, 5 mg·mL^{-1}) and tetracycline (20 μL, 5 mg·mL^{-1}) were used as positive antimicrobial controls, and PDA agar plugs were used as the negative control. Antimicrobial activities were determined in accordance with the diameters of ZI. Experiments were repeated three times.

Effects of fungal strains on seed germination. All strains were grown on PDA at 25 °C in the dark. After 10 days, five PDA agar plugs (diameter of 2 mm) with active mycelial growth from the colony margin were inoculated into a Petri dish (diameter 9 mm) containing 15 mL of sterile oatmeal agar (2.5 g·L^{-1} oatmeal, 12 g·L^{-1} agar, and pH of 5.2 prior to autoclaving) with five pieces of nylon cloth (1 × 1 cm), and grown at 25 °C in dark[61,62]. The seeds of the terrestrial orchid plant *B. striata* were selected as the experimental subject for seasonal reasons. The seeds were surface sterilized with sodium hypochlorite solution for 10 min to remove fungal contamination. After 1 week, approximately 150 axenic seeds of *B. striata* (Fig. 3 a) were sown on the surface of each piece of cloth. Each treatment was replicated on five plates. The treatment without fungi was used as the control. All treatments were placed in a tissue culture chamber under 12 h of light exposure at 25 °C for 70 days. A stereomicroscope (LEICA TL3000 Ergo) was used to assess and record seed germination and protocorm development. Stewart *et al.*[63] had divided the seed germination and protocorm development of orchids into six stages (0–5). Three seedling growth stages were added in this study as shown in Table 3 showing as a referencefor assessing the percentage of seed germination and protocorm development of *B. striata*.

Statistical analyses. The IR% of the strains were calculated as follows: IR% = (Ni/Nt) × 100, where Ni represents the number of segments from which the fungal species was isolated, and Nt is the total number of segments incubated[64]. The percentages of seed germination for per stage were calculated by using the following formula[61] :

Percentage of seeds germination

$$= \frac{\text{Number of seeds in per germination stage}}{\text{Total number of viable seeds in the sample}} \times 100.$$

The diversity of fungal species from *N. fordii* was evaluated by using the H' and J with the following formulas:

$H' = -\Sigma(Pi \times \ln Pi) (Pi = ni / N)$

$J = H' / \ln (S)$

where S represents the total species numbers of endophytic fungi in the community, ni represents the numbers of individuals, and N represents the total number of individuals[65,66]. All statistical analyses were performed using by SPSS 19.0 (SPSS Inc., Chicago, IL, USA).

Results

Isolation, sequencing data, and diversity of culturable endophytic fungi. In this study, 984 fungal colonies (isolation rate% [IR] = 78.03 %) were isolated from 1261 tissue segments of *N. fordii* plants. The 984 isolates were assigned to 124 strains in accordance with their culture characteristics on potato dextrose agar (PDA) (Fig. 1 d) and ITS rDNA sequence analyses. They included 51 (41.13%), 48 (38.04%), and 76 (61.29%) strains from rhizome, corm, and leaf tissue segments, respectively (Table 1). Except for 12 unidentified fungi without high similarity in the GenBank database, all 112 isolates were categorized at the species, genus, or family level (Table 1).

At least 39 fungal genera were identified in accordance with the diversity and sequence data of the 112 isolates recovered from *N. fordii* plants. Among the 39 genera, 36 were affiliated with phylum Ascomycota and included 715 isolates (72.66%). Three (19.00%) strains were classified as Basidiomycota, comprising *Epulorhiza*, which is affiliated with Tulasnellaceae; and *Phanerochaete* cf., which is affiliated with Phanerochaetaceae.

Further analyses revealed that most isolates belonged to four classes, including Eurotiomycetes, Dothideomycetes, Sordariomycetes, and Leotiomycetes. Further taxonomic analysis showed that most of the isolates (n = 495) from *N. fordii* belonged to class Sordariomycetes. This class was represented by six orders, namely, Sordariales, Glomerellales, Hypocreales, Xylariales, Diaporthales, and Magnaporthales; and 17 genera: *Amesia*, *Chaetomium*, *Colletotrichum*, *Cylindrocarpon*, *Fusarium*, *Ilyonectria*, *Nectria*, *Volutella*, *Corallomycetella*, *Lecanicillium*, *Arthrinium*, *Obolarina*, *Xylaria*, *Daldinia*, *Diaporthe*, *Phomopsis*, *Leptostroma,* and an unknown Magnaporthaceae genus. 181 isolates were assigned to class Dothideomycetes, comprising the five orders of Pleosporales, Cladosporiales, Muyocopronales, Venturiales and Botryosphaeriales and 15 genera (*Acrocalymma*, *Letendraea*, *Periconia*, *Ascochyta*, *Epicoccum*, *Phoma*, *Alternaria*, *Bipolaris*, *Exserohilum*, *Sclerostagonospora*, *Torula*, *Dictyosporium*, *Cladosporium*, *Mycoleptodiscus* and *Phyllosticta*,). 34 isolates were assigned to

class Eurotiomycetes, and orders Eurotiales and Chaetothyriales, representing the genera *Aspergillus*, *Penicillium*, *Talaromyces*, and *Rhinocladiella*. Finally, five isolates were assigned to class Leotiomycetes and classified as genus *Leptostroma* of the order Rhytismataceae.

The exclusion of 12 unidentified fungi (2.85%) failed to show that the endophytes were widely distributed. The Shannon–Weiner diversity index (*H'*) was estimated on the basis of taxonomic units or morphological characters. ITS sequences showed that the corm segment presented the highest fungal species diversity (2.686), followed by the rhizome (1.923) and leaf (1.976) segments. The corme segment showed a higher Pielou Evenness index (*J*) (0.835) than the rhizome (0.565) and leaf (0.600) segments (Table 1).

In the present data, endophytic fungi were highly abundant and diverse in the rhizomes of *N. fordii* and that the most ubiquitous phylum of fungi was Ascomycota, which is reportedly among the most prevalent group of eukaryotes globally[25,26,27]. Eurotiomycetes was the most prevalent class of endophytic fungi, followed by Dothideomycetes. Moreover, 47.87% of endophytic fungi were present in the corm and rhizome of *N. fordii*, and 52.13% of the fungal isolates were found in the leaves of *N. fordii*. The genera of *Epulorhiza*, *Alternaria*, *Phoma*, *Sclerostagonospora*, *Torula*, *Ilyonectria*, *Purpureocillium*, *Amesia*, *Corallomycetella*, and *Daldinia* colonized rhizomes, whereas *Periconia*, *Dictyosporium*, *Volutella*, and *Lecanicillium* were exclusively detected in corms. In addition, *Arthrinium* (26.83%) was the most common fungal genus in *N. fordii* that was abundant in the leaves but was less abundant in corms and rhizomes. The dominant genera of *N. fordii* also included *Colletotrichum* (10.26%, mainly colonizing the leaves) and Tulasnellaceae (16.97%, mainly colonizing the rhizomes).

Antimicrobial activity of culturable endophytic fungi from *N. fordii*. A total of 20 fungal isolates that effectively inhibited pathogen growth were screened out by using the agar diffusion method. The antimicrobial fungi belonged to the genera *Penicillium*, *Aspergillus*, *Epicoccum*, *Alternaria*, *Fusarium*, *Bipolaris*, *Cylindrocarpon*, *Phoma*, *Lecanicillium*, *Amesia*, *Sclerostagonospora*, and *Arthrinium* and included ascomycete and fungal endophyte species (Table 2). Among these fungi, 13 strains (9.33%) showed antibacterial activity against *Escherichia coli*, 14 strains (12.30%) demonstrated activity against *Staphylococcus aureus*, and 2 strains (2.33%) presented activity against *Candida tropicalis*. Notably, *Penicillium* sp. (1151, Fig. 2 a–b) showed the highest activity against all pathogens. The diameter of the inhibition zone (ZI) against *E. coli* was 34.698 mm (Fig. 2 c), which was 1.42 times that against streptomycin and 1.31 times that against tetracycline. The diameter of the ZI against *S.aureus* was 28.478 mm (Fig. 2 d) and was 1.22 and 1.15 times that against the positive control, respectively. However, no activity against *C. tropicalis* was observed. In addition, *Epicoccum* sp. (1243) and *Phoma* sp. (1244, Fig. 1 d) had better antimicrobial effect against *E. coli* (1243 ZI = 18.515 mm; 1244 ZI = 20.690 mm) and *S. aureus* (1243 ZI = 19.540 mm; 1244 ZI = 21.298 mm) than the other strains. Comparison with the positive control group revealed that the antibacterial activities of the strains against the two pathogens exceeded 70%.

Interestingly, the fungi have different antimicrobial activity which isolated from different parts of *N. fordii*. And those that were mainly distributed in the rhizome. However, further studies are required to characterize the dynamic changes in endophytic communities[28] and uncultured fungi[29] and to confirm fungal tissue specificity[30] in *N. fordii*. The results suggested that endophytic fungi from *N. fordii*. are potential sources of natural antimicrobial products.

Seed germination trials. This study aimed to obtain isolates with high seed germination activities from *N. fordii* plants. The seeds of the orchid plant *Bletilla striata* were selected as the experimental subject for seasonal reasons.

The results showed that *Arthrinium* sp. (1130 and 1232) and Tulasnellaceae sp. (1217, Fig. 1 d) had higher seed germination activity than the CK group. After 28 days, tthe germination rate of the seeds treated with the two endophytic Arthrinium species increased by 0.37% compared with that of the CK group. After 3 weeks treatment with the two Arthrinium isolates increased the germination of *B. striata* seeds up to the emergence of first leaf by 27.63% and 2.72%, respectively. *Arthrinium* sp. (1130) promoted seed germination up to the elongation of the secand leaf stage (0.63%) after 4 weeks, and the dominant seed germination stage was the emergence of the second leaf stage (33.54%). The germination rate during this stage increased by 33.14% compared with that under treatment with the CK. After 4 weeks, *Arthrinium* sp. (1232) promoted seed germination up to the emergence of the second leaf stage (0.39%) and stage 4 was the dominant stage (33.54%). The germination rate in the treatment group increased by 18.90% compared with that in the CK group. After 7 weeks, the two strains of *Arthrinium* promoted seed germination up to the appearance of root stage by 73.17% and 21.05%, respectivelybut. Without symbiotic fungi, seed development of *B.striata* was arrested at stage 7 (Fig. 4. a). The above data indicated that the two *Arthrinium* isolates had a certain capability to promote seed germination.

Tulasnellaceae sp. (1217) had the best germination-promoting activity (Fig. 3 a–f). After 3 weeks, the rate of seed germination under treatment with Tulasnellaceae sp. was 97.89%, which was significantly higher than that under treatment with CK (85.21%). After 7 weeks of sowing, roots appeared in 52.41% of *B. striata* seeds, and the uninoculated control showed a germination rate of zero (Fig. 4 b). *B. striata* seedlings in the experimental group had more roots and larger leaves than the seedlings in other groups. The fresh weight and plant height of the *B. striata* seedlings in the experimental group were 1.22 and 3.34 times higher than those of the seedlings in the control group. Most of the seedlings demonstrated root germination. The total germinated root number and root length of the treated seedlings were 13.55 and 6.07 times those of the control, respectively.

Discussion

Endophytes affect the the quality and quantity of the crude drugs through specific fungus–host interactions[31,32]. They are widely considered as valuable natural resources with diverse applications in a variety of areas, such as agriculture and biotechnology[33]. Most endophytic fungi from wild orchids have been studied. However, studies on endophytic fungi from *Nervilia* plants, particularly studies fungi related to biological activity, have rarely been conducted.

In this study, 112 strains of endophytic fungi belonging to tow phyla, five classes, 16 orders, 28 family and 39 genera were obtained from *N. fordii*. Consistent with the findings of Song *et al*.[34], *Arthrinium*, *Colletotrichum*, and Tulasnellaceae were found to be the dominant endophytic fungi of *N. fordii* and accounted for more than 10% of the total endophytes.

The quantities of endophytic fungi varied across different plant tissues from *N. fordii.* The corm of *N. fordii.* harbored the highest number of endophytic fungi. The variation in the quantities of endophytic fungi across different plant tissues might be ascribed to environmental differences between the above- and below-ground parts of plants; for example, the roots are highly susceptible to infestation by soil microorganisms due to their long-term presence and close interaction in the soil[35]. Isolates Ascomycota sp. (1192 and 1299, Fig. 1 d) and Venturiaceae sp. (1300) had darkly pigmented and septate hyphae of thick walls. These strains are often referred to as dark septate fungi (DSE)[36] and are isolated from corms. Mandyam & Jumpponen suggested that DSE–plant symbioses are multifunctional and play unique roles in terrestrial ecosystems[37]. They participate in nutrient acquisition and in the resultant positive host growth responses[38,39]. The isolation of 20 strains exhibiting strong antimicrobial activity indicated that these plants and their endophytes could be potential sources of novel natural antimicrobials. These strains belonged to 12 genera and one to fungal species, thus illustrating the diversity of the distribution of endophytic fungal genera with antibacterial activity. *Phoma*, *Fusarium* and *Penicillium*, which have been isolated from medicinal orchids, exhibit antibacterial activity. Several endophytic fungi with high antioxidant capacities, such as *Alternaria*, *Fusarium*, *Xylaria*, and *Penicillium*, have been isolated from *Dendrobium*[40,41]. Similarly, *Lecanicillium*, *Phoma*, and *Ilyonectria* isolated from *Dysosma versipellis* possess antibacterial activity [3,42]. *Penicillium* sp. (1151) with the best antibacterial activity was screened. The secondary metabolites produced by *Penicillium* sp. have consistently shown good biological activity in antibacterial activities. The well-known antibacterial drugs penicillin and griseofulvin were isolated from *Penicillium chrysogenum*[43] and *Penicillium griseofulvum*[44], which are commonly found in nature. Furthermore, endophytic fungi of *Penicillium* fungal species that have been isolated from some herbal plants, such as *Pogostemon cablin*[45], *Withania somnifera*[46], and *Panax ginseng* Meyer[47] have also been shown to possess a variety of antibacterial activities.

Orchidaceae are typical mycorrhizal plants. Their seeds are tiny, without endosperm and must depend on suitable mycorrhizal fungi to germinate in natural conditions[48]. Symbiotic seed germination has been practically used in orchid recovery projects worldwide and is considered as an effective way for orchid conservation[49,50]. Seed germination trials confirmed the capability of *Arthrinium* sp. (1130 and 1232) and Tulasnellaceae (1217) to enhance the germination of *B. striata*, among which 1217 had the best seed germination-promoting activity. Previous studies have demonstrated that the majority of orchid mycorrhizal symbionts fall under the broad category of *Rhizoctonia*-like fungi, whose members include Tulasnellaceae, and constitute an important class of symbiotic germinating fungi[51,52]. Among the reported orchids, fungi of the Tulasnellaceae can promote seed germination in several species of orchids (approximately 40 species), such as *Dendrobium*, *Epidendrum secundum*, *Chiloglottis*, *Dichromanthus* and so on[53,54,55].

Endophytic fungi were highly abundant in *N. fordii* plants and exhibited a wide range of biological activities. However, their ecological function, relevant metabolic pathways, and secondary metabolites need extensive investigation. These species are potential viable sources for the exploration of novel natural products and the actual propagation of other orchid species.

Declarations

Acknowledgements

This work was supported by the National Natural Science Foundation of China (NSFC, Project No. 31860128), the Guangxi Science Foundation (Project No. 2019GXNSFDA245017) and the Innovation and entrepreneurship training program of Guangxi University of traditional Chinese Medicine (Project No. 202010600176). And we are grateful to Dr. Dawei Zhang (Jiangsu University of Technology) for guiding the revision of this manuscript.

Author Contributions

X.-M. T., P. F. and Y.-Q. Z. conceived and designed the experiments. X.-F. Y., X.-M. T., S.-Y. H. and Z.-H. S. performed the experiments. Y.-Q. Z. and X.-F. Y. analyzed the data. X.-M. T. and X.-F. Y. wrote the manuscript. All authors reviewed the manuscript.

References

1. Hardoim, P. R. *et al.* The Hidden World within Plants: Ecological and Evolutionary Considerations for Defining Functioning of Microbial Endophytes. *Microbiology & Molecular Biology Reviews Mmbr.* **79**, 293–320 https://doi.org/10.1128/mmbr.00050-14 (2015).
2. Wemheuer, F., Wemheuer, B., Daniel, R. & Vidal, S. Deciphering bacterial and fungal endophyte communities in leaves of two maple trees with green islands. *Sci Rep.* **9**, 14183 https://doi.org/10.1038/s41598-019-50540-2 (2019).
3. Tan, X. M. *et al.* Diversity and bioactive potential of culturable fungal endophytes of Dysosma versipellis; a rare medicinal plant endemic to China. *Sci Rep.* **8**, 5929 https://doi.org/10.1038/s41598-018-24313-2 (2018).
4. Hadacek, F. & Kraus, G. F. Plant root carbohydrates affect growth behaviour of endophytic microfungi. *FEMS Microbiol. Ecol.* **41**, 161–170 https://doi.org/10.1016/S0168-6496(02)00292-1 (2002).
5. Sessitsch, C. Plant growth-promoting bacteria in the rhizo- and endosphere of plants: their role, colonization, mechanisms involved and prospects for utilization. *Soil Biology and Biochemistry.* **42**, 669–678 https://doi.org/10.1016/j.soilbio.2009.11.024 (2010).
6. Waqas, M., Khan, A. L., Kamran, M., Hamayun, M. & Lee, I. J. Endophytic fungi produce gibberellins and indoleacetic acid and promotes host-plant growth during stress. *Molecules.* **17**, 10754–10773 (2012).
7. Gunatilaka, A. A. L. Natural products from plant-associated microorganisms: distribution, structural diversity, bioactivity, and implications of their occurrence. *J NAT PROD.* **69**, 509–526 (2006).
8. Estrada, C., Wcislo, W. T. & Bael, S. Symbiotic fungi alter plant chemistry that discourages leaf-cutting ants. *New Phytol.* **198**, 241–251 (2013).
9. Rodriguez, R. J., Jr, J., Arnold, A. E. & Redman, R. S. Fungal endophytes: diversity and functional roles. *New Phytol.* **182**, 314–330 https://doi.org/10.1111/j.1469-8137.2009.02773.x (2009).

10. Tan, X. M. *et al.* 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**, 140–147 https://doi.org/10.1007/s00284-011-0045-8 (2012).
11. Aly, A. H., De Bbab, A., Kjer, J. & Proksch, P. Fungal endophytes from higher plants: a prolific source of phytochemicals and other bioactive natural products. *Fungal Divers.* **41**, 1–16 https://doi.org/10.1007/s13225-010-0034-4 (2010).
12. Jalgaonwala, R. E., Mohite, B. V. & Mahajan, R. T. A review: Natural products from plant associated endophytic fungi. World J Microbiol Biotechnol. *World J Microbiol Biotechnol.* **1**, 21–32 (2010).
13. Ganley, R. J., Brunsfeld, S. J. & Newcombe, G. A community of unknown, endophytic fungi in western white pine. *PNAS.* **101**, 10107–10112 https://doi.org/10.1073/pnas.0401513101 (2004).
14. Guangdong Food and Drug Administration. *Standard of traditional Chinese medicine in Guangzhou Province* Vol. 1P 122(Guangdong Science and Technology Press, Guangzhou, 2004).
15. Xu, H. H. & Liu, J. M. *'Dictionary of Zhong Cao Yao T u Pu'*P 264(Guangdong Science and Technology Press, Guangzhou, 2007).
16. Delectis florae reipublicae popularis sinicae agendae academiae sinicae edita. Flora Reipublicae Popularis Sinicae (Vol. 18). (Beijing: Science Press, P 21–22, 1999).
17. Liang, Y. S., Gong, L., Huang, Z. H. & Ouyang, P. Y. Analysis of Global Ecological Suitability of Southern Chinese Materia Medica Herba Nervilia fordii. *Subtropical Plant Science.* **46**, 339–342 (2017).
18. Wu, Z. Y., Raven, P. H. & Hong, D. Y. *Flora of China (Vol. 25)* 5760–62(Missouri Botanical Garden Press and Science Press, Beijing, 2009).
19. Mei, Q. X. Advance in the Research of Chemical Constituent Pharmacologic Action and Clinical Application of Herba Nerviliae Plicatae. *Chin Arch Tradit Chin Med.* **26**, 2239–2241 (2008).
20. Huang, M. Q. *et al.* Protection of Total Flavonoid Fraction from Nervilia fordii on Lipopolysaccharide-induced Acute Lung Injury in Rats. *Chinese Herbal Medicines.* **4**, 287–293 https://doi.org/10.3969/j.issn.1674-6348.2012.04.005 (2012).
21. Xiong, M. *et al.* Antibacterial activity of folium Nerviliae in vitro. *Progress in Modern Biomedicine.* **14**, 2753–2756 (2014).
22. Zhen, H. S. *et al.* Study on the anticancer effect in vitro of flavonoids compounds obtained from Nervilia fordii(hance) schltr. *Chinese Journal of Experimental Traditional Medical Formulae.* **14**, 36–38 (2008).
23. Tian, L. W., Pei, Y., Zhang, Y. J., Wang, Y. F. & Yang, C. R. 7-O-methylkaempferol and -quercetin glycosides from the whole plant of Nervilia fordii. *Journal of Natural Products.* **72**, 1057–1060 https://doi.org/10.1021/np800760p (2009).
24. Zhou, G. X., Lu, C. L., Wang, H. S. & Yao, X. S. An acetyl flavonol from Nervilia fordii (Hance) Schltr. *J Asian Nat Prod Res.* **11**, 498–502 https://doi.org/10.1080/10286020902893074 (2009).
25. Sun, Y. *et al.* Endophytic fungi associated with two Sauaeda species growing in alkaline soil in China. *Mycosphere.* **2**, 239–248 (2011).
26. Zare, R. *et al.* Gibellulopsis, a suitable genus for Verticillium nigrescens, and Musicillium, a new genus for V. theobromae. *Nova Hedwigia.* **85** (3–4), 463–489 (2007).
27. Pereiro, M. *et al.* Cutaneous infection caused by Alternaria in patients receiving tacrolimus. *Med. Mycol.* **42** (3), 277–282 (2004).
28. Kõiv, V. *et al.* Microbial population dynamics in response to Pectobacterium atrosepticum infection in potato tubers. *Sci Rep.* **5**, 11606 https://doi.org/10.1038/srep11606 (2015).
29. Tejesvi, M. V. *et al.* Bioactivity and genetic diversity of endophytic fungi in Rhododendron tomentosum Harmaja. *Fungal Divers.* **47**, 97–107 (2011).
30. Singh, D. K. *et al.* Diversity of endophytic mycobiota of tropical tree Tectona grandis Linn.f.: Spatiotemporal and tissue type effects. *Sci Rep.* **7**, 3745 https://doi.org/10.1038/s41598-017-03933-0 (2017).
31. Fagan, F. W. F. Fungal endophytes: common host plant symbionts but uncommon mutualists.*Integrative and Comparative Biology***42**, (2002).
32. Min, J. *et al.* A Friendly Relationship between Endophytic Fungi and Medicinal Plants: A Systematic Review. *FRONT MICROBIOL.* **7**, 1–14 https://doi.org/10.3389/fmicb.2016.00906 (2016).
33. Aly, A. H., Debbab, A. & Proksch, P. Fifty years of drug discovery from fungi. *Fungal Divers.* **50**, https://doi.org/10.1007/s13225-011-0116-y (2011).
34. Song, L. S., Jiang, N., Lan, Z. Z., Guo, X. Y. & Zhang, Z. J. Identification and antibacterial activity of the endophytic fungus of the endangered medicinal plant, Nervilia fordii. *Jiangsu Agricultural Sciences.* **47**, 175–178 https://doi.org/10.15889/j.issn.1002-1302.2019.21.041 (2019).
35. Schulz, B. & Boyle, C. The endophytic continuum. *Mycol. Res.* **109**, 661–686 https://doi.org/10.1017/S095375620500273X (2005).
36. Jumpponen, A. & Trappe, J. M. Dark septate endophytes: a review of facultative biotrophic root-colonizing fungi. *New Phytol.* **140**, 295–310 (1998).
37. Mandyam, K. & Jumpponen, A. Seeking the elusive function of the root-colonising dark septate endophytic fungi. *Stud. Mycol.* **53**, 173–189 (2005).
38. Jumpponen, A. & Trappe, J. M. Dark septate endophytes: a review of facultative biotrophic root-colonizing fungi. *New Phytol.* **140**, 195–310 (1998).
39. Liu, M. J., Zhang, X. T. & Zhao, Z. W. Advances in the research of dark septate endophytes. *Mycosystema.* **028**, 888–894 (2009).
40. Chen, X. M. *et al.* Diversity and Antimicrobial and Plant-Growth-Promoting Activities of Endophytic Fungi in Dendrobium loddigesii Rolfe. *J Plant Growth Regul.* **29**, 328–337 https://doi.org/10.1007/s00344-010-9139-y (2010).
41. Zhang, C. L. & Guo, S. X. Study on Isolation of Endophytic fungi from Five Dendrobium Plants and Its Antimicrobial Activity. *Chinese Pharmaceutical Journal.* **44**, 1540–1543 (2009).
42. Tan, X. M., Yu, L. Y., Zhou, Y. Q. & Isolation Identification and Antimicrobial Activities of Endophytic Fungi of Dysosma versipellis (Hance) M. Cheng. *Chinese Pharmaceutical Journal.* **49**, 363–366 (2014).
43. Makins, J. F., Allsop, A. & Holt, G. Intergeneric cosynthesis of Penicillin by strains of Penicillium chrysogenum, P. chrysogenum/notatum and aspergillus nidulans. J Gen Microbiol 122, 339–343(1981).

44. Dasu, V. & Panda, T. Optimization of microbiological parameters for enhanced griseofulvin production using response surface methodology. *Bioprocess. Eng.* **22**, 45–49 https://doi.org/10.1007/PL00009099 (2000).
45. Wang, Y. *et al.* Study on communities of endophytic fungi from Pogostemon cablin and their antimicrobial activities. *Chinese Pharmaceutical Journal.* **42**, 657–662 (2017).
46. George, T. K., Joy, A., Divya, K. & Jisha, M. S. In vitro and in silico docking studies of antibacterial compounds derived from endophytic penicillium setosum. *Microb. Pathog.* **131**, 87–97 https://doi.org/10.1016/j.micpath.2019.03.033 (2019).
47. Park, Y. H. *et al.* Fungal endophytes inhabiting mountain-cultivated ginseng (Panax ginseng Meyer): Diversity and biocontrol activity against ginseng pathogens. *Sci Rep.* **7**, 16221 https://doi.org/10.1038/s41598-017-16181-z (2017).
48. Chen, Y. H., Xing, X. K. & Guo, S. X. Nutritional relationships between orchids and mycorrhizal fungi: a review. *Mycosystema.* **36**, 807–819 (2017).
49. Batty, A. L., Brundrett, M. C., Dixon, K. W. & Sivasithamparam, K. New methods to improve symbiotic propagation of temperate terrestrial orchid seedlings from axenic culture to soil. *Australian Journal of Botany.* **54**, 367–374 https://doi.org/10.1071/BT04023 (2006).
50. Otero, J. T., Mosquera, A. T. & Flanagan, N. S. Tropical orchid mycorrhizae: potential applications in orchid conservation, commercialization, and beyond. *Lankesteriana.* **13**, 57–63 (2013).
51. Rafter, M. *et al.* Non-specific symbiotic germination of Cynorkis purpurea (Thouars) Kraezl., a habitat-specific terrestrial orchid from the Central Highlands of Madagascar. *Mycorrhiza.* **26**, 541–552 https://doi.org/10.1007/s00572-016-0691-6 (2016).
52. Gao, Y., Guo, S. X. & Xin, X. K. Fungal diversity and mechanisms of symbiotic germination of orchid seeds: a review. *Mycosystema.* **38**, 1808–1825 (2019).
53. Yang, J. W. *et al.* Effects of Tulasnella sp. S7 and its extract on Dendrobium officinale seed germination. *Scientia Sinica (Vitae).* **50**, 559–570 (2020).
54. Xu, L., Tian, J. N., Wang, T. & Li, L. B. Symbiosis Established Between Orchid and Tulasnella spp. *Fungi. Journal of Nuclear Agricultural Sciences.* **31**, 876–883 (2017).
55. Roche, S. A. *et al.* A narrow group of monophyletic Tulasnella (Tulasnellaceae) symbiont lineages are associated with multiple species of Chiloglottis (Orchidaceae): Implications for orchid diversity. *American Journal of Botany.* **97**, 1313–1327 https://doi.org/10.3732/ajb.1000049 (2010).
56. White, T. J. et al. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: PCR protocols: A guide to methods and applications. (Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds)Academic Press, New York.p 315–322, 1990).
57. Altschul, S. F. *et al.* Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic acids research.* **25**, 3389–3402 https://doi.org/10.1093/nar/25.17.3389 (1997).
58. Bosshard, P. P. *et al.* Ribosomal DNA sequencing for identification of aerobic Gram-positive rods in the clinical laboratory (an 18-month evaluation). *Journal of Clinical Microbiology.* **41**, 4134–4140 (2003).
59. Cui, J. L. *et al.* Diversity and antioxidant activity of culturable endophytic fungi from alpine plants of *Rhodiola crenulata*, *R. angusta*, and *R. sachalinensis*. *PLoS ONE* **10**(3), e0118204, https://doi.org/10.1371/journal.pone.0118204 (2015).
60. Ma, Y. M., Tian, C. L. & Zhang, H. C. Isolation and Identification of Endophytic Fungi from Eucommia ulmoides and Screening of Their Antimicrobial Activities. *Lishizhen Medicine and Materia Medica Research.* **22**, 552–554 (2011).
61. Tan, X. M., Wang, C. L., Chen, X. M., Zhou, Y. Q. & Guo, S. X. In vitro seed germination and seedling growth of an endangered epiphytic orchid, dendrobium officinale, endemic to china using mycorrhizal fungi (Tulasnella sp.). *Sci. Hort.* **165**, 62–68 https://doi.org/10.1016/j.scienta.2013.10.031 (2014).
62. Kendon, J. P. *et al.* Recovery of mycorrhizal fungi from wild collected protocorms of Madagascan endemic orchid Aerangis ellisii (B.S. Williams) Schltr. and their use in seed germination in vitro. Mycorrhiza 30, https://doi.org/567-576,10.1007/s00572-020-00971-x(2020).
63. Stewart, S. L., Zettler, L. W., Minso, J. & Brown, P. M. Symbiotic Germination and Reintroduction of Spiranthes brevilabris Lindley, an Endangered Orchid Native to Florida. *Selbyana.* **24** (1), 64–70 https://doi.org/10.2307/41750957 (2003).
64. Maulana, A. F. *et al.* Nitrogen and phosphorus concentrations in growth media affect the relationship between root endophytic fungi and host plant. *Arch Microbiol.* **2**, 1–8 https://doi.org/10.1007/s00203-021-02238-1 (2021).
65. Grafenhan, T. *et al.* An overview of the taxonomy, phylogeny, and typification of nectriaceous fungi in Cosmospora, Acremonium, Fusarium, Stilbella, and Volutella. *Stud. Mycol.* **68**, 79–113 https://doi.org/10.3114/sim.2011.68.04 (2011).
66. Schmeisser, C., Steele, H. & Streit, W. R. Metagenomics, biotechnology with non-culturable microbes. *Applied Microbiology & Biotechnology.* **75**, 955–962 https://doi.org/10.1007/s00253-007-0945-5 (2007).

Tables

Table 1. Fungal endophytes from *N. fordii* and their isolation rate (IR%).

Genus (stated in GenBank)	Phylum; Class; Order Family	Fungal isolate (representative strains number)	Isolate number				IR (%)			
			Leaf	Rhizome	Corm	Total	Leaf	Rhizome	Corm	Tota
Aspergillus	Ascomycota; Eurotiomycetes; Eurotiales; Aspergillaceae	1170, 1171, 1179, 1193, 1237, 1238, 1252,	4	9	8	21	0.78%	2.90%	4.97%	2.13
Unknown	Ascomycota; Eurotiomycetes; Eurotiales; Aspergillaceae	1212	1	0	0	1	0.19%	0	0	0.10
Penicillium	Ascomycota; Eurotiomycetes; Eurotiales; Aspergillaceae	1151, 1263	2	4	0	6	0.39%	1.29%	0	0.61
Talaromyces	Ascomycota; Eurotiomycetes; Eurotiales; Trichocomaceae	1270, 1283	1	0	1	2	0.19%	0	0.62%	0.20
Unknown	Ascomycota; Eurotiomycetes; Eurotiales; Trichocomaceae	1295	0	0	1	1	0	0	0.62%	0.10
Rhinocladiella	Ascomycota; Eurotiomycetes; Chaetothyriales; Herpotrichiellaceae	1185	2	1	0	3	0.39%	0.32%	0	0.30
Acrocalymma	Ascomycota; Dothideomycetes; Pleosporales; Massarineae	1186	1	0	6	7	0.19%	0	3.73%	0.71
Letendraea	Ascomycota; Dothideomycetes; Pleosporales; Massarineae	1239	1	0	0	1	0.19%	0	0	0.10
Periconia	Ascomycota; Dothideomycetes; Pleosporales; Massarineae	1207	0	0	1	1	0	0	0.62%	0.10
Ascochyta	Ascomycota; Dothideomycetes; Pleosporales; Didymellaceae	1213, 1235	3	0	15	18	0.58%	0	9.32%	1.83
Epicoccum	Ascomycota; Dothideomycetes; Pleosporales; Didymellaceae	1154	30	17	1	48	5.85%	5.48%	0.62%	4.88
Phoma	Ascomycota; Dothideomycetes; Pleosporales; Didymellaceae	1244, 1254	0	2	0	2	0	0.65%	0	0.20
Alternaria	Ascomycota; Dothideomycetes; Pleosporales; Pleosporaceae	1211	0	2	0	2	0	0.65%	0	0.20
Bipolaris	Ascomycota; Dothideomycetes; Pleosporales; Pleosporaceae	1198	1	2	0	3	0.19%	0.65%	0	0.30
Exserohilum	Ascomycota; Dothideomycetes; Pleosporales; Pleosporaceae	1205	1	1	0	2	0.19%	0.32%	0	0.20
Sclerostagonospora	Ascomycota; Dothideomycetes; Pleosporales; Pleosporaceae	1303	0	1	0	1	0	0.32%	0	0.10
Torula	Ascomycota; Dothideomycetes; Pleosporales; Torulaceae	1315	0	1	0	1	0	0.32%	0	0.10
Unknown	Ascomycota	1192, 1299, 1310, 1312	0	0	11	11	0	0	6.83%	1.12
Dictyosporium	Ascomycota; Dothideomycetes; Pleosporales; Massarineae	1180	0	0	3	3	0	0	1.86%	0.30
Unknown	Ascomycota; Dothideomycetes; Pleosporales;Sporormiaceae	1272	2	0	0	2	0.39%	0	0	0.20
Unknown	Ascomycota; Dothideomycetes; Pleosporales	1233, 1236	0	0	8	8	0	0	4.97%	0.81
Cladosporium	Ascomycota; Dothideomycetes; Cladosporiales; Cladosporiaceae	1135, 1175, 1277, 1280	8	0	4	12	1.56%	0	2.48%	1.22
Mycoleptodiscus	Ascomycota; Dothideomycetes; Muyocopronales; Muyocopronaceae	1292	1	0	0	1	0.19%	0	0	0.10
Unknown	Ascomycota; Dothideomycetes; Venturiales; Venturiaceae	1300	1	0	0	1	0.19%	0	0	0.10
Unknown	Ascomycota; Dothideomycetes; Magnaporthales	1173	4	0	0	4	0.78%	0	0	0.41
Phyllosticta	Ascomycota; Dothideomycetes; Botryosphaeriales; Phyllostictaceae	1108, 1144, 1159, 1160	52	1	0	53	10.14%	0.32%	0	5.39

Unknown	Ascomycota; Sordariomycetes; Sordariales	1304	1	0	0	1	0.19%	0	0	0.1(
Amesia	Ascomycota; Sordariomycetes; Sordariales; Chaetomiaceae	1260	0	1	0	1	0	0.32%	0	0.1(
Chaetomium	Ascomycota; Sordariomycetes; Sordariales; Chaetomiaceae	1201, 1209, 1261	0	3	1	4	0	0.97%	0.62%	0.41
Colletotrichum	Ascomycota; Sordariomycetes; Glomerellales; Glomerellaceae	1101, 1107, 1136, 1139, 1147, 1153, 1188, 1203, 1206, 1215, 1242, 1257, 1284	93	4	4	101	18.13%	1.29%	2.48%	10.2
Cylindrocarpon	Ascomycota; Sordariomycetes; Hypocreales; Nectriaceae	1230	0	9	11	20	0	2.90%	6.83%	2.03
Fusarium	Ascomycota; Sordariomycetes; Hypocreales; Nectriaceae	1119, 1133, 1222, 1225, 1286	17	20	21	58	3.31%	6.45%	13.04%	5.89
Ilyonectria	Ascomycota; Sordariomycetes; Hypocreales; Nectriaceae	1265	0	1	0	1	0	0.32%	0	0.1(
Purpureocillium	Ascomycota; Sordariomycetes; Hypocreales;Ophiocordycipitaceae	1314	0	1	0	1	0	0.32%	0	0.1(
Nectria	Ascomycota; Sordariomycetes; Hypocreales; Nectriaceae	1288	1	2	1	4	0.19%	0.65%	0.62%	0.41
Volutella	Ascomycota; Sordariomycetes; Hypocreales; Nectriaceae	1251	0	0	2	2	0	0	1.24%	0.2(
Corallomycetella	Ascomycota; Sordariomycetes; Hypocreales; Nectriaceae	1307	0	1	0	1	0	0.32%	0	0.1(
Lecanicillium	Ascomycota; Sordariomycetes; Hypocreales; Cordycipitaceae	1262	0	0	1	1	0	0	0.62%	0.1(
Arthrinium	Ascomycota; Sordariomycetes; Xylariales; Apiosporaceae	1103, 1105, 1124, 1130, 1163, 1232	202	40	22	264	39.38%	12.90%	13.66%	26.8
Obolarina	Ascomycota; Sordariomycetes; Xylariales; Xylariaceae	1148	11	0	0	11	2.14%	0	0	1.12
Xylaria	Ascomycota; Sordariomycetes; Xylariales; Xylariaceae	1184	1	0	0	1	0.19%	0	0	0.1(
Daldinia	Ascomycota; Sordariomycetes; Xylariales; Hypoxylaceae	1200	0	1	0	1	0	0.32%	0.00%	0.1(
Diaporthe	Ascomycota; Sordariomycetes; Diaporthales; Diaporthae	1204, 1220	0	3	5	8	0	0.97%	3.11%	0.81
Phomopsis	Ascomycota; Sordariomycetes; Diaporthales; Valsaceae	1202	0	3	3	6	0	0.97%	1.86%	0.61
Unknown	Ascomycota; Sordariomycetes; Magnaporthales; Magnaporthaceae	1104	8	1	0	9	1.56%	0.32%	0	0.91
Leptostroma	Ascomycota; Leotiomycetes; Rhytismatales; Rhytismataceae	1167, 1176	5	0	0	5	0.97%	0	0	0.51
Unknown	Basidiomycota; Agaricomycetes;	1129	0	3	15	18	0	0.97%	9.32%	1.83
Epulorhiza	Basidiomycota; Agaricomycetes; Cantharellales; Tulasnellaceae	1158	0	4	0	4	0	1.29%	0	0.41
Unknown	Basidiomycota; Agaricomycetes; Cantharellales; Tulasnellaceae	1217	1	160	2	163	0.19%	51.61%	1.24%	16.5
Phanerochaete cf.	Basidiomycota; Agaricomycetes; Polyporales; Phanerochaetaceae	1141	2	0	0	2	0.39%	0	0	0.2(
Fungal endophyte sp. and fungal sp.		1109, 1120, 1149, 1168, 1172, 1187, 1191, 1228, 1240, 1253, 1255, 1264, 1266, 1278, 1290, 1308, 1305	38	8	8	54	7.41%	2.58%	4.97%	5.49

Unidentified fungi	1106, 1169, 1174, 1183, 1189, 1190, 1195, 1197, 1216, 1229, 1247, 1309	18	4	6	28	3.51%	1.29%	3.73%	2.85
Individual number		513	310	161	984	52.13%	31.50%	16.36%	
Shannon index (*H*)		1.976	1.923	2.686					
Pielou Evenness index (*J*)		0.600	0.565	0.835					

Table 2. Antibacterial activity of 33 endophytic fungi isolated from *N. fordii*. Note："-": no activity.

NO.	Plant part	*Escherichia coli* (inhibitory zone [mm])	*Staphylococcus aureus* (inhibitory zone [mm])	*Candida tropicalis* (inhibitory zone [mm])
1151	Leaf, rhizome	34.698	28.478	-
1154	Leaf, rhizome, corm	22.115	15.702	-
1174	leaf	-	9.657	-
1211	rhizome	-	8.673	-
1221	rhizome	-	9.952	12.107
1224	rhizome	15.675	-	-
1230	rhizome	9.958	-	-
1231	rhizome	12.060	12.520	-
1232	leaf	-	8.740	-
1238	leaf	11.592	-	-
1243	rhizome	18.515	19.540	-
1244	rhizome	20.690	21.298	-
1250	rhizome	9.955	-	-
1252	corm	8.515	-	-
1260	rhizome	-	8.730	-
1262	corm	9.142	9.042	-
1266	leaf, rhizome	-	10.580	-
1273	rhizome, corm	11.075	10.270	-
1303	rhizome	11.078	-	9.838
1306	rhizome, corm	-	9.445	-
5 mg·mL^{-1} 20 μL streptomycin		24.477	23.336	-
5 mg·mL^{-1} 20 μL tetracycline		26.439	24.850	-

Table 3. Seed germination and protocorm development in *N. fordii*[63].

Stage	Description
0	No germination, viable embryo
1	Enlarged embryo, production of rhizoid(s) (=germination)
2	Continued embryo enlargement, testa rupture, further rhizoid production
3	Appearance of the protomeristem
4	Emergence of first leaf
5	Elongation of first leaf
6	Emergence of second leaf
7	Elongation of second leaf
8	Appearance of root

Figures

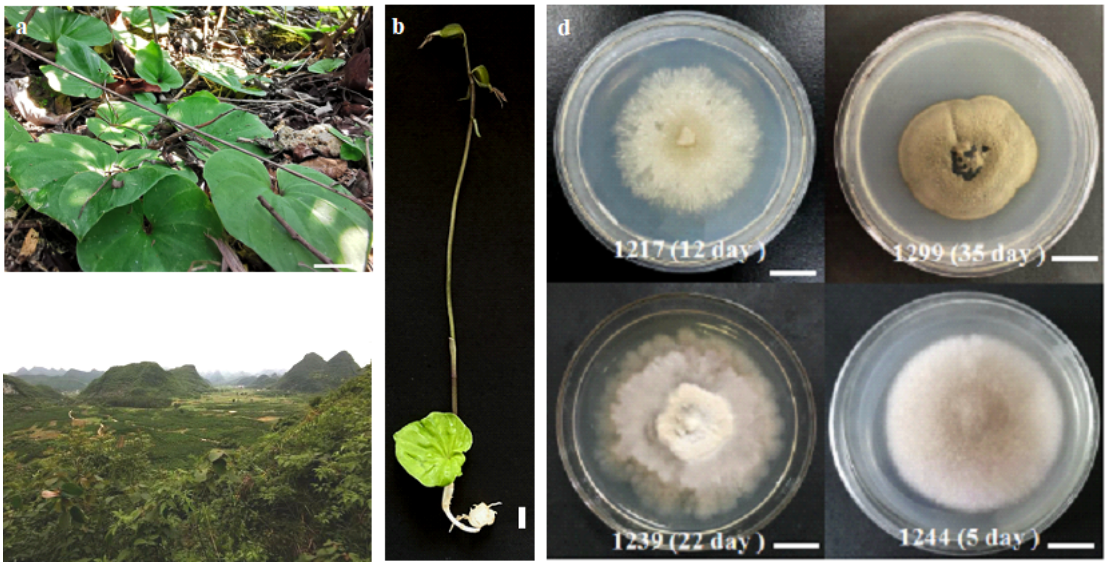

Figure 1

Wild N. fordii plant samples and their endophytic fungi. (a) Plants of N. fordii growing among on a hillside, bar =1cm; (b) the whole plant (including rhizome), bar =1cm; (c) habitat; (d) representative fungal morphotypes isolated from N. fordii grown on potato dextrose agar at 26 °C, bar =0.5cm.

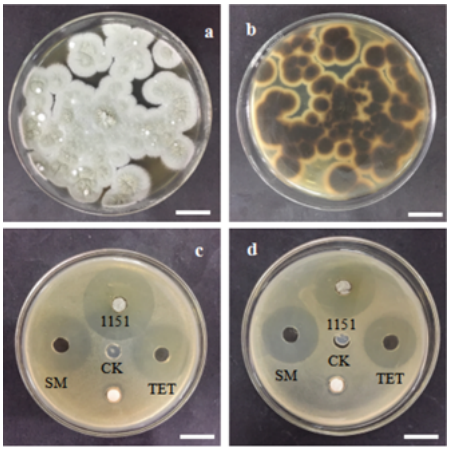

Figure 2

Antimicrobial activity of some strains. (a–b) morphological characteristics of strain 1151; (c) Antibacterial activity of 1151 against E. coli; (d) antibacterial activity of 1151 against S. aureus. Bar: a–d=1cm.

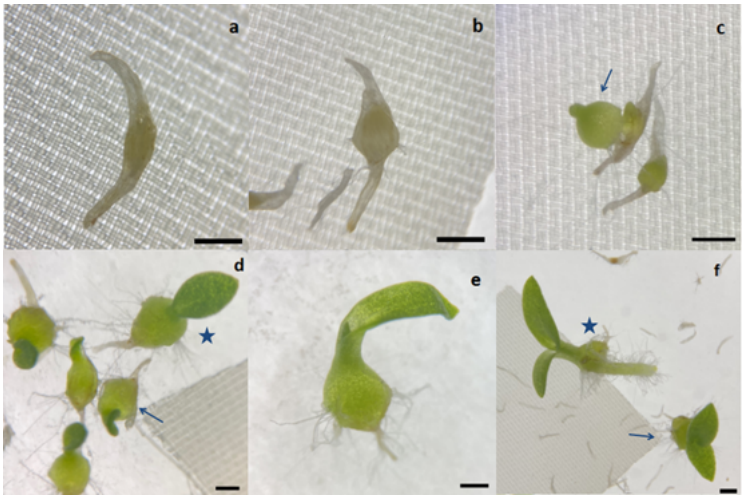

Figure 3

Symbiotic seed germination and protocorm developmental stages of B.striata cultured with the mycorrhizal fungal strain 1217 (Tulasnellaceae sp.) cultured on oatmeal agar at 10 weeks after sowing; (a) stage 0; (b) stage 1 (shown by arrow); stage 2 (shown by star); (c) stage 3 (shown by arrow); (d) stage 4 (shown by arrow); stage 5 (shown by star) of protocorm development (note the elongation of the first leaf); (e) stage 6; (f) tage 7 (shown by arrow); and stage 8 (shown by star). Bar: a–f = 500 μm.

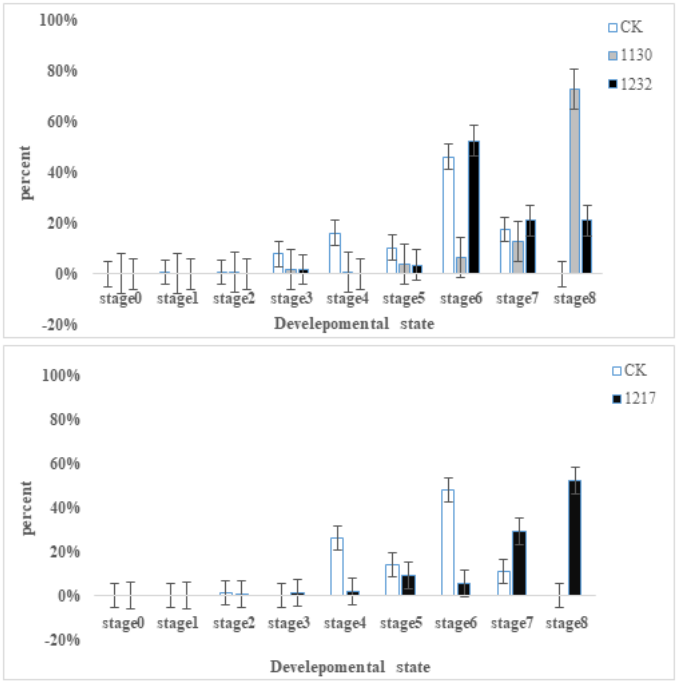

Figure 4

Effects of mycorrhizal fungi on the seed germination of B.striata 7 weeks after sowing. (a) Arthrinium sp. (1130 and 1232); (b) Tulasnellaceae sp. (1217). Note: the results show the mean of three replicates with bars indicating the standard error.