Symbiosis
https://doi.org/10.1007/s13199-019-00647-x

SHORT COMMUNICATIONS

Symbiotic fungi undergo a taxonomic and functional bottleneck during orchid seeds germination: a case study on *Dendrobium moniliforme*

Yuan-Yuan Meng[1] · Xu-Li Fan[2] · Lv-Rong Zhou[3] · Shi-Cheng Shao[1] · Qiang Liu[1] · Marc-André Selosse[2,4,5] · Jiang-Yun Gao[2]

Received: 6 July 2019 / Accepted: 10 September 2019

Abstract

In many germinating orchids that rely on fungal partners for their nutrition, fungal diversity decreases during development. We document this pattern in the in situ development of *Dendrobium moniliforme*, where the diversity of cultivable *Tulasnella* species drops from the early germinating stage (6 *Tulasnella* species) to that of emergence of the second leaf (2 species), with species discontinuance and no new species appearing. We investigated the functional aspects of this decay by germinating seeds in vitro with the different strains available and observed, over 60 and 120 days, a perfect match between the stages at which *Tulasnella* spp. occur in situ and the ability to support in vitro development to this stage. The taxonomic bottleneck during germination may result, at least in *D. moniliforme*, from inability of the fungus to support seedling growth beyond a specific stage. Moreover, the isolated *Tulasnella* strains that best supported *D. moniliforme* development did not cluster together phylogenetically. Thus, the interaction between partners, rather than intrinsic fungal traits, may be involved in bottleneck of fungal symbionts during orchid germination.

Keywords Microspermy · Mycoheterotrophy · Partner compatibility · Protocorm · Symbiotic germination · *Tulasnella*

1 Introduction

Orchids rely on fungi not only at the adult stage for mineral nutrition by mycorrhizal interaction, but also for early germination stages (Smith and Read 2008; Rasmussen et al. 2015; Dearnaley et al. 2016). Due to their minute seeds with few nutrient reserves (Arditti and Ghani 2000), orchids depend on fungi mineral and carbon resources to form undifferentiated seedlings called protocorms. Orchids recruit mycorrhizal fungi from the so-called rhizoctonia aggregate, a polyphyletic group of fungi belonging to Tulasnellaceae and Ceratobasidiaceae (in the order Cantharellales), as well as Serendipitaceae (in Sebacinales; Dearnaley et al. 2012; Weiβ et al. 2016). A better understanding of symbiotic germination and seedling establishment is needed for conservation of orchid populations, especially for assisting ex-situ germination of rare or endangered species.

Despite this important link and major research efforts, the dynamics of fungal associations at early germination stages still remains largely unknown. A growing literature shows that fungal association may change during development from

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s13199-019-00647-x) contains supplementary material, which is available to authorized users.

✉ Marc-André Selosse
ma.selosse@wanadoo.fr

✉ Jiang-Yun Gao
jiangyun.gao@ynu.edu.cn

[1] Center for Integrative Conservation, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, Mengla 666303, Yunnan, China

[2] Laboratory of Ecology and Evolutionary Biology, Yunnan University, Kunming 650500, Yunnan, China

[3] Key Laboratory for Plant Diversity and Biogeography of East Asia, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, Yunnan, China

[4] Institut de Systématique, Evolution, Biodiversité (ISYEB), Muséum national d'Histoire naturelle, CNRS, Sorbonne Université, EPHE, CP 39, 57 rue Cuvier, 75005 Paris, France

[5] Faculty of Biology, University of Gdansk, ul. WitaStwosza 59, 80-308 Gdansk, Poland

Published online: 09 November 2019

seedling to adult in some species (Xu and Mu 1990; McCormick et al. 2004; Jacquemyn et al. 2011). Recently, we demonstrated that in the terrestrial orchid *Arundina graminifolia*, one *Tulasnella* species repeatedly isolated from adult mycorrhizas allowed in vitro protocorm formation but not further development, while another *Tulasnella* species isolated from advanced seedlings facilitated development to the advanced seedling stage (Meng et al. 2019). Moreover, fungal association may change even during the successive early developmental stages, i.e. during germination and protocorm development (Bidartondo and Read 2008): fungal diversity was found to be lower in protocorms than in imbibed seeds (seed coat cracked and embryo enlarged) in *Cephalanthera damasonium* and *C. longifolia* (Bidartondo and Read 2008), indicating a bottleneck in fungal symbiont diversity at the protocorm stage. Yet some species do not display such a trend (e.g. in *Epipactis*; Těšitelová et al. 2012), and the species mentioned above are terrestrial, temperate and mixotrophic (Selosse and Roy 2009). The tropical *Cyrtochilum retusum* and *Epidendrum macrum* epiphytes reveal an increase in fungal symbiont diversity between 3 and 12 months after seed sowing in situ (Cevallos et al. 2018). Thus, additional studies are pending to demonstrate, in different phylogenetic and ecological frameworks, mainly tropical, autotrophic and epiphytic orchids that encompass most of the orchid's diversity (Givnish et al. 2015), the existence of a bottleneck in fungal association during orchid germination.

The fungal diversity bottleneck may reflect low specificity at initiation of germination, with many fungi providing the signals and/or molecules and metabolites required at a very early stage (Vujanovic et al. 2000), while fewer lineages achieve compatibility later, especially at the leafy seedling stages, where higher intimacy and more stringent exchanges are required (Dearnaley et al. 2016). Previous studies have suggested that non-compatible fungi may stimulate germination per se, but not support subsequent seedling development (Bidartondo and Read 2008; Zi et al. 2014); in some cases, germination initiation does not even need the presence of fungi, while their presence is strictly required to establish a protocorm (e.g. in *Microtis media*; Long et al. 2013). The bottleneck in fungal diversity, at both the taxonomical and functional levels, stimulated our interest on its effect on germination, using a fine scale of germination advancement stages to examine fungal diversity during seedling ontogeny. Moreover, in order to understand this fungal bottleneck in a more functional framework, we wanted to test the ability of fungi isolated from different stages to promote germination advancement at different stages.

In the current case study, *Dendrobium moniliforme* (Linnaeus) Swartz, a widely distributed epiphytic orchid, was used to test how the cultivable fungal diversity (i) is affected by the germination stage, and (ii) is related to the ability of fungi to promote germination till this stage and beyond, based on in vitro tests. We restricted our efforts to cultivable fungal diversity because we wanted to test its functional diversity in germination in the second step.

2 Materials and methods

2.1 Study species and sampling site

Dendrobium moniliforme (Linnaeus) Swartz is an epiphytic orchid (tribe Dendrobieae) from the subtropical areas of South-East Asia at elevations from 600 to 3000 m (Chen et al. 2009). Although *D. moniliforme* is one of the *Dendrobium* species used in traditional Chinese medicine ('Shi-Hu' in Chinese) and has already been studied for non-mycorrhizal endophytic fungi (Chen et al. 2011; Shah et al. 2019), its mycorrhizal fungi remain largely unidentified, to the best of our knowledge. *D. moniliforme* grows naturally on shaded tree trunks and flowers between March and May, before fruiting from September to November. Field sampling and experiments were conducted at an evergreen broad-leaved forest at Daxiechang village in Malipo (Yunnan province, China; 23°10′9″N, 104°50′25″E; elevation 1514 m), where medicinal herbs have been introduced in natural conditions, including many *Dendrobium* species.

2.2 Seed collection and in situ fungal baiting

Five fruits of *D. moniliforme* were harvested on September 27th 2015, and seeds were released and stored as in Gao et al. (2014). Seed viability was tested by the TTC method (Vujanovic et al. 2000). Fungi were baited by protocorm formation on supporting trees following our previous methods (Zi et al. 2014): about 350 seeds of *D. moniliforme* were placed in a 4 × 4 cm packet of 45-μm nylon mesh. On June 1st 2016, 40 seed packets were placed at 30 cm around the roots of five adult plants each situated on five different *Platycarya strobilacea* (Juglandaceae) trunks, i.e. 200 seed packets in all. Seed packets were harvested on September 30th 2016 and put on moist sheets of sterilized wet moss for transport to the laboratory.

2.3 Fungi isolation and identification

Seed packets were opened with a scalpel under a dissecting microscope, and seeds were classified into 6 developmental groups derived from Arditti (1967; Table 1; as in Meng et al. 2019), namely: 0, no germination; 1, embryo swells and turns green (an unusual feature observed in lightened protocorm in this species), and testa is propped up (germination); 2, continued embryo enlargement forms a spherule, seed coat is broken (protocorm formation); 3, appearance of protomeristem (protocorm differentiation); 4, advanced seedling with

Table 1 Stage-related features of the 7 isolated fungal OTUs. The black arrow spans the developmental stage(s) (1 to 5) over which each species was isolated (see Table 2). The grey arrow spans the developmental stage(s) (1 to 5) which the species allows to reach, based on our in vitro tests (no germination observed for *Rigidoporus* sp.) over 60 days

Stage :	*Rigidoporus* sp.	*Tulasnella* sp. DMG 1	*Tulasnella* sp. DMG 2	*Tulasnella* sp. DMG 3	*Tulasnella* sp. DMG 4	*Tulasnella.* sp. DMG 5	*Tulasnella* sp. DMG 6
1, embryo swells and turns green, testa propped up							
2, embryo enlargement, seed coat broken (protocorm formation)							
3, appearance of protomeristem (protocorm differentiation)							
4, advanced seedling with first leaf							
5, emergence of second leaf and further							

emergence of the first leaf; 5, emergence of the second leaf and further development. For each stage from 1 to 5, 40 germinations were randomly selected for isolation of fungi following the protocol of Zhu et al. (2008). Briefly, samples were surface-sterilized using NaClO (1%) for 5 min, rinsed three times with sterile double-distilled water, and then crumbled into fragments with needles and tweezers. Fragments were immersed in 300 μL of sterile double-distilled water containing 50 μg/mL ampicillin and 50 μg/mL levofloxacin hydrochloride, checked for the presence of pelotons under the dissecting microscope and incubated at 16 °C for ~72 h to allow hyphal growth. Isolates were later purified as in Huang et al. (2018).

All fungal strains were characterized molecularly by internal transcribed spacer (ITS) region sequences of nuclear ribosomal DNA using the ITS1 and ITS4 primers (White et al. 1990) and PCR conditions as in Selosse et al. (2002). Sequences were blasted against the GenBank database (National Center for Biotechnology Information) for identification. In the framework of this study, Operational Taxonomic Units (OTUs) were defined at similarity level of 97% for ITS sequences (as in Linde et al. 2014). Since uncertainties exist in species delineation in Tulasnellaceae, we also repeated the analysis for a 99% similarity threshold. One representative isolate per OTU was randomly selected for the experiments below and to deposit a representative ITS sequence in the GenBank database (MN173009-16).

2.4 Phylogenetic analysis

A phylogenetic relationship of the identified Tulasnellaceae OTU was generated using two different ITS sequences per OTU (using the 99% threshold) since the ITS is known to provide a good phylogenetic signal in Tulasnellaceae (Linde et al. 2014). We added closely related taxa obtained by BLAST searches, and used *Sebacina epigaea* (Sebacinales) AF490397 as an out-group. Sequences were aligned using the MAFFT software (Katoh and Toh 2008), and manually adjusted with the BioEdit software (Hall 1999). Phylogenetic relationships were constructed based on a maximum-likelihood (ML) method implemented in the RAxML version 7.2.6 program (Stamatakis 2006). One thousand bootstrap replicates were performed to obtain the statistical support.

2.5 Test of fungal promotion of seed germination

The effect of each OTU on promotion of seed germination was tested in Petri dishes on each of which ca. 200 sterilized seeds were sown. We used one randomly chosen strain per OTU, using strains isolated at stage 1 (or 2 in the case of *Tulasnella* sp. DMG5). As control, axenic growth was performed for comparison on two media: a nutrient-poor oat agar medium (OMA; 4 g/L ground oat and 8 g/L agar;) and a nutrient-rich Murashige and Skoog medium (MS; Murashige and Skoog 1962). Fungal inoculation was performed on OMA following our previous methods (Zi et al. 2014). Each control or inoculation treatment was replicated in 10 Petri dishes. Cultures were incubated at 25 ± 2 °C with a 12/12-h light/dark cycle. The status of seed germination was assessed 60 days after incubation, according to the criteria of Arditti (1967; see Table 1). We ensured that symbiosis established by looking for the presence of pelotons ten randomly selected germinations at stage >1 per dish. Few Petri dishes survived till 120 days (on average 22%), and we also report below some results, but no statistics could be applied at that time.

2.6 Statistical analysis

Generalized linear models were used to test for the effects of treatment on the percentage of germinations reaching, respectively, stage 1, 2, 3, 4 and 5. To account for multiple comparisons, we used Tukey tests to compare the different treatments. All statistical analyses were performed in R (version 3.3.3), and the alpha-type I error was fixed at 5% (thus, all non-significant differences have $P > 0.05$).

3 Results and discussion

3.1 Fungal diversity during seedling development

Our isolation success averaged 74.5% and continuously increased from 67.5% at stage 1 to 80% at stages 4 and 5 (Table 2). However, although 149 successful isolations were performed, only 7 different OTUs were found (using a threshold of 97% ITS similarity for OTU delineation, Table 2). Each OTU was isolated between 3 and 59 times (Table 2), suggesting some saturation in our sampling. Although isolation success increased, the diversity of OTUs decreased from 6 to a single one during the developmental stages under study (Tables 1 and 2), indicating a bottleneck in fungal diversity during seedling germination and development. This bottleneck resulted from disappearance of species present at earlier stages rather than from a turnover of colonizing species.

Identification of the fungi by BLAST search revealed one non-rhizoctonia, namely a Meripilaceae belonging to the genus *Rigidoporus* (98% similar in ITS to *R. ginkgonis*, KY131877; Table 1) and occurring only at stage 1. *Rigidoporus* encompasses wood-decaying and parasitic fungi. Since a member is a mycorrhizal associate of the Taiwan mycoheterotrophic orchid *Galeola falconeri* (Lee et al. 2015), we decided to test germinations with this fungus, despite its unexpected ecology and possible contaminant status. The six other OTUs all belonged to the rhizoctonia genus *Tulasnella* (Table 1), commonly associated with many orchids (Dearnaley et al. 2012), especially tropical epiphytic orchids (Martos et al. 2012). These OTUs had diverse positions within the phylogeny of the genus *Tulasnella* (Fig. 1). Use of 99% ITS similarity for OTU delineation did not change the number of OTUs, but divided *Tulasnella* sp. DMG2 into two taxa (*Tulasnella* spp. DMG2.1 and 2.2; see Fig. 1). All OTUs tended to disappear from later developmental stages except *Tulasnella* spp. DMG5 and 6 (Table 1); conversely, all *Tulasnella* OTUs occurred at the earliest developmental stages, except for *Tulasnella* sp. DMG5, which was not isolated from stage 1.

The number of isolated *Tulasnella* spp. was higher than in previous studies of epiphytic *Dendrobium* from this region, e.g. one *Tulasnella* sp. in *Dendrobium aphyllum* (Zi et al. 2014), *D. devonianum* (Huang et al. 2018), and *D. friedericksianum* (Khamchatra et al. 2016). There may be three non-exclusive reasons for this inconsistency. First, our sampling effort was very high (200 attempts; Table 2), likely saturating the *Tulasnella* spp. diversity. Second, previous studies isolated fungi from late germination stages, whereas most of the current diversity occurred at early germination stages (Tables 1 and 2). Third, more than 30 *Dendrobium* species grow at the study site, likely supporting a large abundance of *Tulasnella* spp., which may colonize *D. moniliforme* early germinations non-specifically.

3.2 Promotion of seedling development

At 60 days after incubation, seeds from *Rigidoporus ginkgonis* treatment did not germinate, so this treatment was excluded from the statistical analyses: this fungus fits the view by Cevallos et al. (2018) that many fungi randomly associate during early germination. Moreover, some seeds showed damage (data not shown), suggesting that the presence of this fungus on stage 1 seeds was due to an overlooked parasitic attack. In the past, many endophytic and possible parasites have also been isolated from *D. moniliforme* at the adult stage (Chen et al. 2011; Shah et al. 2019). This was the least often isolated fungus (at stage 1 and in general, Table 2), thus confirming the inability of occasional orchid colonizers to support germination (Meng et al. 2019).

Significant differences among other treatments were recorded for the percentages of germination at stage 1 ($Chi2 = 41.58$, $P < 0.0001$; Fig. S1a), stage 2 ($Chi2 = 38.67$, $P < 0.0001$; Fig. S1b), stage 3 ($Chi2 = 45.00$, $P < 0.0001$; Fig. S1c), stage 4 ($Chi2 = 45.00$, $P < 0.0001$; Fig. S1c) and stage 5 ($Chi2 = 45.00$, $P < 0.0001$; Fig. S1c). Nutrient-rich control treatment (MS) allowed massive germination (92% at stage 1 and above; Fig. S1), leading to further stages, up to stage 5 in rare cases (0.34%; Fig. S1). In nutrient-poor control treatment (OMA),

Table 2 Isolation result from in situ seed baiting experiment (*Tulasnella* OTUs DMG1 to DMG6 were determined at the 97% identity threshold). The value in each cell is the number of successful isolations (out of 40 attempts in each case)

OTU and identity	Stage 1	Stage 2	Stage 3	Stage 4	Stage 5	Total
Tulasnella sp. DMG1	3	6	–	–	–	9
Tulasnella sp. DMG2	9	7	–	–	–	16
Tulasnella sp. DMG3	5	4	–	–	–	9
Tulasnella sp. DMG4	2	3	10	–	–	15
Tulasnella sp. DMG5	–	4	11	19	25	59
Tulasnella sp. DMG6	5	4	9	13	7	38
Rigidoporus sp.	3	–	–	–	–	3
Nb. isolation success	27	28	30	32	32	149
Isolation rate	67.5%	70%	75%	80%	80%	74.5%
Nb. isolated OTUs	6	6	3	2	2	6

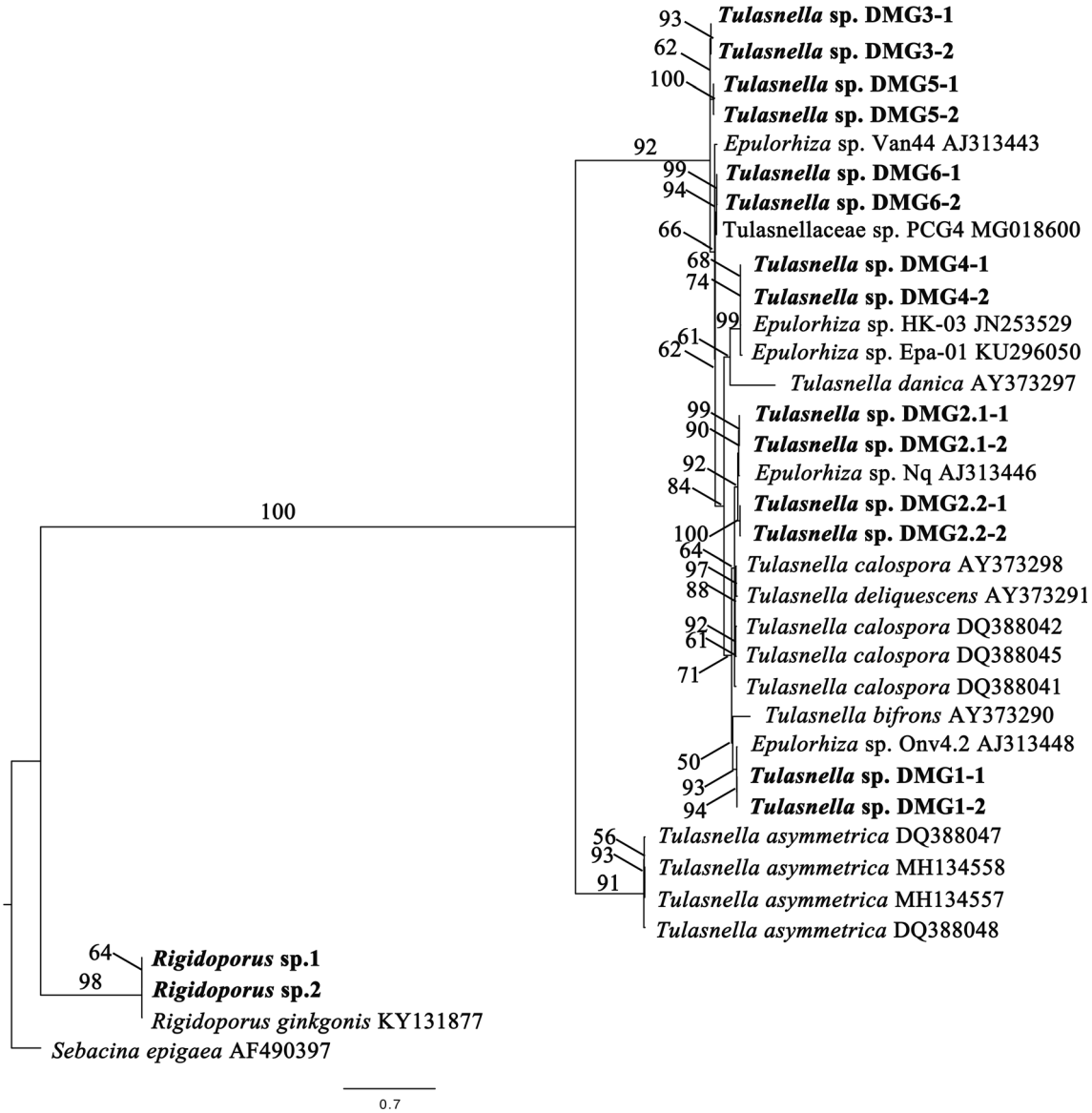

Fig. 1 Phylogenetic positions of the fungal OTUs recovered from *Dendrobium moniliforme* seedlings, with *Sebacina epigaea* (Sebacinales) AF490397 as an out-group. Maximum-likelihood tree constructed using ITS sequences; numbers above branches represent bootstrap probabilities (whenever ≥50%) out of 1000 replicates

only a small proportion (3.63%) of seeds germinated at stage 1 and fewer (0.05%) developed above this stage (Fig. S1). *Tulasnella* sp. DMG2.1 and 2.2 did not show significantly different ability to promote seed germination (Fig. S1), so that considering a 97% or a 99% threshold for OTU delineation did not modify the conclusions of our functional approach.

Comparing the results of this experiment with the stages at which *Tulasnella* spp. were isolated (Table 1) revealed a striking correlation between isolation stages and fungal effect on seed germination. Except for *Tulasnella* sp. DMG5 at stage 1 and *Tulasnella* sp. DMG6 at stage 5, all OTUs allowed development at all stages from which they were isolated within 60 days, but never above the latest stage of isolation (Table 1). After 120 days, on the few Petri dishes that survived, some protocorms developed from stage 3 into seedlings (stage 4 or stage 5; data not shown), but only in MS treatments or with *Tulasnella* spp. DMG5 and DMG6.

Tulasnella spp. DMG5 and 6 could both be isolated from seedling stages (stages 4 and 5). Yet, their effect on germination differed in vitro beyond early germination, for which they were similarly very efficient. *Tulasnella* sp. DMG5 produced a small proportion of protocorms at stages 2 and 3 (resp. 3.3 and 8.3%) and a larger proportion of leafy seedlings at stages 4 and 5 (20.9 and 65.9%) at 60 days; conversely *Tulasnella* sp. DMG6 promoted a large proportion of protocorms at stages 2 and 3 (21.1% and 65.21%), but a smaller proportion of leafy seedlings at stage 4 (6.5%; Fig. S1). At 120 days, *Tulasnella* sp. DMG6 allowed a small portion of seedlings at stage 5,

which was 9.8 times lower than that with *Tulasnella* sp. DMG5 (20 versus 196 Petri dishes; data not shown). Thus, *Tulasnella* sp. DMG5 more effectively promoted seedling development to later stages.

Despite these differences, *Tulasnella* spp. DMG5 and 6 shared an ability to support late stages of development, despite different phylogenetic placements (Fig. 1). We compared in more detail the phylogenetic distance to the effect on seed germination of *Tulasnella* spp., by a Mantel test comparing this distance (inferred from phylogeny; Fig. 1) to consistency of the latest stage reached (counted as a Boolean value of 1 when it was the same versus 0 when it differed). This test (Fig. S2) confirmed that phylogenetic placement did not relate to germination function of fungi. For example, the closest related fungi of *Tulasnella* sp. DMG3 and 5 differed in promoting seed germination: the latter was the most effective fungus in promoting seed germination to seedlings, while the former only supported stage 2 (Figs.1 and S1). Thus, there was no phylogenetic signal in the ability of *Tulasnella* spp. to germinate *D. moniliforme* and the interaction between partners, rather than any intrinsic fungal traits, may be involved.

3.3 Bottleneck in *Tulasnella* diversity during seedling development

We observed a perfect match between the stages at which *Tulasnella* spp. occur in situ and the ability to support in vitro development to this stage (Table 1). We never isolated one strain from a stage it was not able to support in vitro at 60 or even 120 days. This is evidence that the taxonomic bottleneck observed in nature largely results from the inability of some species to assist seedling development beyond a certain point, and that only some strains allow full development. Here, *Tulasnella* sp. DMG5 and 6 were the only ones covering all the developmental stages under study, while DMG6 may be of limited efficiency in promoting further stages. Unfortunately, no data is available from adult roots of *D. moniliforme* to assess whether *Tulasnella* sp. DMG5 is able to assist the whole lifespan of this orchid.

This bottleneck corroborates data obtained for terrestrial *Cephalathera* species (Bidartondo and Read 2008). At first glance, it may be seen as contradictory with the report of Cevallos et al. (2018) that the tropical epiphytic *Cyrtochilum retusum* and *Epidendrum macrum* undergo increase in fungal symbiont diversity between 3 and 12 months after seed sowing in situ, but these author may simply report the recruitment of the initial diversity, before any bottleneck occurs. Yet, no evidence for bottleneck in fungal symbiont diversity was found in terrestrial *Epipactis* species (Bidartondo and Read 2008; Těšitelová et al. 2012). Thus, this process may not be universal and its determinism and conditions of occurrence are unknown. Previous studies of *Microtis media* showed that the presence of suitable fungi was necessary to reach stage 3 (Long et al. 2013), which fits well with our results indicating a functional checkpoint at this stage (protocorm differentiation). A transition to specific, different transfers to the protocorm, possibly more demanding in quantity or quality of fungal resources, may occur around this time. We speculate that, after stage 3, better functional matching with the fungus is required to allow the enhanced carbon flow required for development of bigger, more demanding seedlings. Thus, the shift in fungal diversity might reflect that germination is initiated with relatively unspecific symbiont and generalist mechanisms (also present in non-mycorrhizal fungi such as *Fusarium*; Vujanovic et al. 2000), followed by subsequent protocorm development only in the presence of more specific fungi.

The possibility of such a transition calls for further research and, in this respect, proteome (López-Chávez et al. 2016) or transcriptome (Perotto et al. 2014; Chen et al. 2017) methods offer promising perspectives to test for it, if used in fine-scale developmental series of symbiotic germination with early- or late-stage fungi. If our interpretation is correct, orchid rarity is not a result of early development stages, but at least from a transition to stage 3 and beyond. Indeed, other transitions may occur later, especially at the onset of photosynthesis after the first leaves develop, which also requires investigation. The transition to autotrophic growth, often assumed to be a demographic bottleneck and a shock similar to that of metamorphosis in animals (Těšitelová et al. 2012), may entail overlooked changes in optimal symbionts, explaining why fungi isolated from adults are not always optimal partners for germination (e.g. Meng et al. 2019). Finally, our results were obtained in vitro, and in situ investigations, on *D. moniliforme* and other species, are needed.

4 Conclusions

By comparing the fungal assemblages at different stages of germination and testing their effects on the germination sequence in *D. moniliforme*, we confirmed that symbiotic fungi undergo a taxonomic and functional bottleneck during germination in this species. This adds support to the growing literature suggesting that in some orchid species, at least, fungal diversity decreases from early germination stages to leafy seedling stages. Our results also suggest that this relates to the ability of the fungus to promote seedling growth to different stages. They improve our understanding of fungi-orchid symbiosis in early life stages of the plant and should be valuable for conservation and management efforts targeting *D. moniliforme* and other rare orchids. We call for more investigations of germination stages on a fine temporal scale and for the use of fungi isolated from protocorms to enhance in vitro germination in orchids.

Acknowledgments This work was supported by the National Natural Science Foundation of China (U1702235) and the Ministry and Province Joint Construction Project of Yunnan University (C176280109).

References

Arditti J (1967) Factors affecting the germination of orchid seeds. Bot Rev 33:1–97

Arditti J, Ghani AKA (2000) Numerical and physical properties of orchid seeds and their biological implications. New Phytol 145:367–421

Bidartondo MI, Read DJ (2008) Fungal specificity bottlenecks during orchid germination and development. Mol Ecol 17:3707–3716

Cevallos S, Declerck S, Suárez JP (2018) *In situ* orchid seedling-trap experiment shows few keystone and many randomly-associated mycorrhizal fungal species during early plant colonization. Front Pl Sci 9:1664

Chen SC, Liu ZJ, Zhu GH et al (2009) Orchidaceae. In: Wu ZY, Raven PH, Hong DY (eds) Flora of China, vol 25. Science Press, Beijing, pp 381–382

Chen J, Hu KX, Hou XQ, Guo SX (2011) Endophytic fungi assemblages from 10 *Dendrobium* medicinal plants (Orchidaceae). World J Microbiol Biotechnol 27:1009–1016

Chen J, Liu SS, Kohler A, Yan B, Luo HM, Chen XM, Guo SX (2017) iTRAQ and RNA-Seq analyses provide new insights into regulation mechanism of symbiotic germination of *Dendrobium officinale* seeds (Orchidaceae). J Proteome Res 16:2174–2187

Dearnaley JDW, Martos F, Selosse MA (2012) Orchid mycorrhizas: molecular ecology, physiology, evolution and conservation aspects. In: Hock B (ed) The Mycota, vol 9. Springer, Berlin, pp 207–230

Dearnaley JDW, Perotto S, Selosse MA (2016) Structure and development of orchid mycorrhizas. In: Martin F (ed) Molecular mycorrhizal symbiosis. Wiley-Blackwell, Hoboken, pp 63–86

Gao JY, Liu Q, Yu DL (2014) Orchids in Xishuangbanna: diversity and conservation. China Forestry Publishing House, Beijing (in Chinese)

Givnish TJ, Spalnik D, Ames M, Lyon SP, Hunter SJ, Zuluaga A, Iles WJD, Clements MA, Arroyo MTK, Leebens-Mack J, Endara L, Kriebel R, Neubig KM, Whitten WM, Williams NH, Cameron KM (2015) Orchid phylogenomics and multiple drivers of their extraordinary diversification. Proc R Soc B. https://doi.org/10.1098/rspb.2015.1553

Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucl Acid S 41:95–98

Huang H, Zi XM, Lin H, Gao JY (2018) Host-specificity of symbiotic mycorrhizal fungi for enhancing seed germination, protocorm formation and seedling development of over-collected medicinal orchid, *Dendrobium devonianum*. J Microbiol 56:42–48

Jacquemyn H, Brys R, Cammue BPA, Honnay O, Lievens B (2011) Mycorrhizal associations and reproductive isolation in three closely related *orchis* species. Ann Bot 107:347–356

Katoh K, Toh H (2008) Recent developments in the MAFFT multiple sequence alignment program. Brief Bioinform. https://doi.org/10.1093/bib/bbn013

Khamchatra NM, Dixon K, Chayamarit K, Apisitwanich S, Tantiwiwata S (2016) Using *in situ* seed baiting technique to isolate and identify endophytic and mycorrhizal fungi from seeds of a threatened epiphytic orchid, *Dendrobium friedericksianum* Rchb.f. (Orchidaceae). Agric Nat Resour 50:8–13

Lee YI, Yang CK, Gebauer G (2015) The importance of associations with saprotrophic non-Rhizoctonia fungi among fully mycoheterotrophic orchids is currently under-estimated: novel evidence from subtropical Asia. Ann Bot 116:423–435

Linde CC, Phillips RD, Crisp MD, Peakall R (2014) Congruent species delineation of Tulasnella using multiple loci and methods. New Phytol 201:6–12

Long JRD, Swarts ND, Dixon KW, Egertonwarburton LM (2013) Mycorrhizal preference promotes habitat invasion by a native Australian orchid: *Microtis media*. Ann Bot 111:409–418

López-Chávez MY, Guillén-Navarro K, Bertolini V et al (2016) Proteomic and morphometric study of the in vitro interaction between *Oncidium sphacelatum* Lindl. (Orchidaceae) and *Thanatephorus* sp. RG26 (Ceratobasidiaceae). Mycorrhiza 26:353–365

Martos F, Munoz F, Pailler T, Kottke I, Gonneau C, Selosse MA (2012) The role of epiphytism in architecture and evolutionary constraint within mycorrhizal networks of tropical orchids. Mol Ecol 21:5098–5109

Mccormick MK, Whigham DF, O'Neill J (2004) Mycorrhizal diversity in photosynthetic terrestrial orchids. New Phytol 163:425–438

Meng YY, Zhang WL, Selosse MA, Gao JY (2019) Are fungi from adult orchids roots the best symbionts at germination? A case study, Mycorrhiza (in press)

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant 15:473–497

Perotto S, Rodda M, Benetti A, Sillo F, Ercole E, Rodda M, Girlanda M, Balestrini R (2014) Gene expression in mycorrhizal orchid protocorms suggests a friendly plant–fungus relationship. Planta 239:1337–1349

Rasmussen HN, Dixon KW, Jersákova J, Těšitelová T (2015) Germination and seedling establishment in orchids: a complex of requirements. Ann Bot 116:391–402

Selosse MA, Roy M (2009) Green plants eating fungi, facts and questions about mixotrophy. Trends Plant Sci 14:64–70

Selosse MA, Weiβ M, Jany JL, Tillier A (2002) Communities and populations of sebacinoid basidiomycetes associated with the achlorophyllous orchid *Neottia nidus-avis* (L.) L.C.M. Rich. and neighbouring tree ectomycorrhizae. Mol Ecol 11:1831–1844

Shah S, Shrestha R, Maharjan S, Selosse MA, Pant B (2019) Isolation and characterization of plant growth-promoting endophytic fungi from the roots of *Dendrobium moniliforme*. Plants. https://doi.org/10.3390/plants8010005

Smith SE, Read DJ (2008) Mycorrhizal symbiosis, 3rd edn. Academic Press, San Diego

Stamatakis A (2006) RAxML-VI-HPC: maximum-likelihood- based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 22:2688–2690

Těšitelová T, Těšitel J, Jersáková J, Říhová G, Selosse MA (2012) Symbiotic germination capability of four *Epipactis* species (Orchidaceae) is broader than expected from adult ecology. Am J Bot 99:1020–1032

Vujanovic V, St-Arnaud M, Barabé D, Thibeault G (2000) Viability testing of orchid seed and the promotion of colouration and germination. Ann Bot 86:79–86

Weiβ M, Waller F, Zuccaro A, Selosse MA (2016) Sebacinales - one thousand and one interactions with land plants. New Phytol 211:20–40

White TJ, Bruns T, Lee S, Taylor JW (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 methods and applications. Academic, NewYork, pp 315–322

Xu JT, Mu C (1990) The relation between growth of *Gastrodia elata* protocorms and fungi. Acta Bot Sin 32:26–31

Zhu GS, Yu ZN, Gui Y, Liu ZY (2008) A novel technique for isolating orchid mycorrhizal fungi. Fungal Divers 33:123–137

Zi XM, Sheng CL, Goodale UM, Shao SC, Gao JY (2014) In situ seed baiting to isolate germination-enhancing fungi for an epiphytic orchid, *Dendrobium aphyllum* (Orchidaceae). Mycorrhiza 24:487–499