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Integrating Mycorrhizal Fungi into Orchid Reintroduction: Case Studies, Challenges, and Future Directions

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Abstract: Orchid reintroduction: transplanting propagated orchids into natural or restored habitats is a growing conservation strategy to counter population decline and extinction. However, many efforts have overlooked a critical biological requirement: the reliance of orchids on orchid mycorrhizal fungi (OMF) for seed germination, growth, and survival. Without these fungal partners, restored populations often fail to establish or persist. This review explores how integrating OMF into reintroduction strategies improves outcomes across species, regions, and ecological contexts. Drawing on selected case studies from six biogeographic regions, the study highlights the importance of fungal specificity, source, and ecological matching in shaping reintroduction success. It shows that symbiotic propagation, site selection based on fungal compatibility, and sustained monitoring all contribute to higher survival and reproduction rates. However, major challenges remain, including the technical and financial difficulties of isolating and identifying compatible fungal partners, limited long-term monitoring, and insufficient research on field-based fungal management and inoculation in current efforts. To address these gaps, the review proposes an integrated approach that combines scientific, institutional, and community-based actions. Ensuring broader access to fungal resources, building local capacity, and embedding OMF in conservation planning will be key to making orchid reintroduction more ecologically effective and globally equitable.

Keywords: Orchid mycorrhizal fungi (OMF), symbiotic propagation, conservation, restoration ecology, tropical biodiversity, plant–fungus interactions

1. Introduction

Orchidaceae is the second-largest family of flowering plants, comprising approximately 29,000 described species that occur across a wide range of ecological habitats, from tropical rainforests to temperate and alpine ecosystems (Willis, 2017; Govaerts et al., 2017). Despite their evolutionary success and global distribution, orchids are among the most threatened plant groups. More than half of all wild orchid species face extinction risks, primarily due to habitat loss, climate change, overcollection, and ecological disruption (Fay, 2018; Swarts & Dixon, 2009).

One of the defining characteristics of orchids is their symbiosis with orchid mycorrhizal fungi (OMF). These fungi are essential for seed germination and seedling development, as orchid seeds are minute and lack endosperm requiring colonization by compatible fungi to access nutrients and initiate growth (Rasmussen et al., 2015; Smith & Read, 2008). Upon colonization, the fungi form intracellular pelotons, (coiled hyphal structures) that mediate the exchange of carbon, nitrogen, and other nutrients. Most OMF belong to *Tulasnellaceae*, *Ceratobasidiaceae*, or *Serendipitaceae*, though the degree of specificity varies widely across orchid species and ecosystems (Dearnaley et al., 2012). While some orchids associate with a broad range of fungi, others, particularly terrestrial or mycoheterotrophic species exhibit high specificity, sometimes requiring specific partners at different developmental stages (Liu et al., 2010; Yang et al., 2020).

Despite their critical role, OMF are frequently overlooked in conservation practice. Most reintroduction efforts prioritize habitat restoration, seedling propagation, or pollinator support, often without considering whether compatible fungi are present at the planting site. This omission has led to poor establishment, low survival, and reproductive failure in many reintroduced populations, especially in species with narrow ecological or fungal requirements (Stewart et al., 2003; Phillips et al., 2011).

This integrative approach synthesizes findings from selected case studies across six biogeographic regions to examine how fungal identity, origin, delivery method, and ecological matching influence reintroduction outcomes. It also highlights persistent challenges and offers practical recommendations to guide more effective and ecologically integrated orchid restoration efforts worldwide.

2. Orchid Biology and Symbiotic Requirements

The ecological functioning of orchids is fundamentally shaped by their obligate dependence on mycorrhizal fungi. Unlike most flowering plants, orchids produce minute seeds that lack endosperm and cannot germinate without symbiotic assistance (Fig. 1a). Their survival depends on colonization by a compatible fungal partner, which enables nutrient acquisition during the early stages of development (Smith & Read, 2008; Rasmussen et al., 2015).

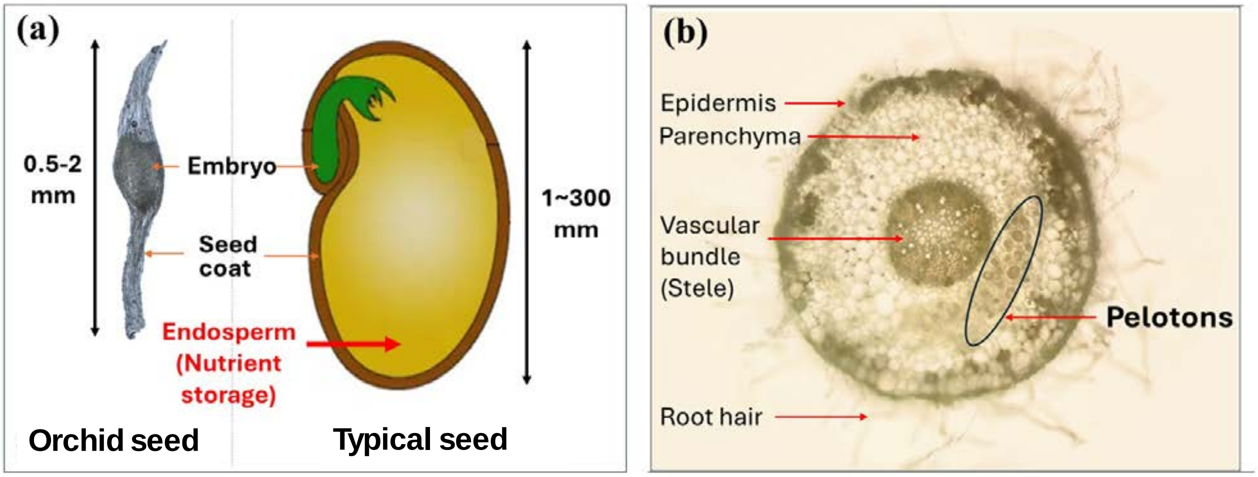

Fig. 1. (a) Comparison of orchid seed (left) and a typical angiosperm seed (right). (b) Cross-sectional microscopic view of an orchid root showing pelotons: coiled hyphal structures formed by orchid mycorrhizal fungi (OMF). Source: Authors.

This symbiotic relationship begins with the formation of pelotons (Fig. 1b) that facilitate the transfer of carbon, nitrogen, and phosphorus to the developing protocorm (Cameron et al., 2006). Importantly, this interaction often continues beyond germination, particularly in nutrient-poor or unstable habitats, where sustained fungal associations enhance orchid survival and ecological performance (Selosse et al., 2022). Terrestrial orchids typically grow in nutrient-poor but more continuously moist soils and rely heavily on a diverse set of OMF (Jacquemyn et al., 2016). In contrast, epiphytic orchids experience intermittent water and nutrient supply in the canopy, use velamen-clad aerial roots to rapidly absorb nutrient-rich rainwater, and often associate with more specialized OMF lineages that colonize only those roots in contact with bark or humus (Suárez et al., 2006).

The main mycorrhizal partners of orchids belong to three Basidiomycota lineages: *Tulasnellaceae*, *Ceratobasidiaceae*, and *Serendipitaceae*, each differing in ecological roles and host ranges (Dearnaley et al., 2012; Bayman et al., 2016). Specificity in these interactions can vary considerably among orchid species and life stages (Ventre Lespiaucq et al., 2021). For instance, *Gastrodia elata*, a mycoheterotrophic terrestrial orchid depends on *Mycena* species for seed germination and *Armillaria* for later vegetative development, demonstrating the dynamic, stage-specific nature of orchid–fungus symbioses (Liu et al., 2010). These intricate partnerships not only define orchid biology but also play a major role in shaping conservation strategies, particularly those involving propagation, habitat restoration, and species reintroduction.

3. History and Approaches in Orchid Conservation

Orchid conservation has traditionally focused on species protection through cultivation and habitat preservation, but early methods often failed to consider the biological requirements tied to orchid–fungus symbioses. Ex situ approaches such as seedling propagation in nurseries were frequently disconnected from the ecological conditions needed for successful establishment, especially the

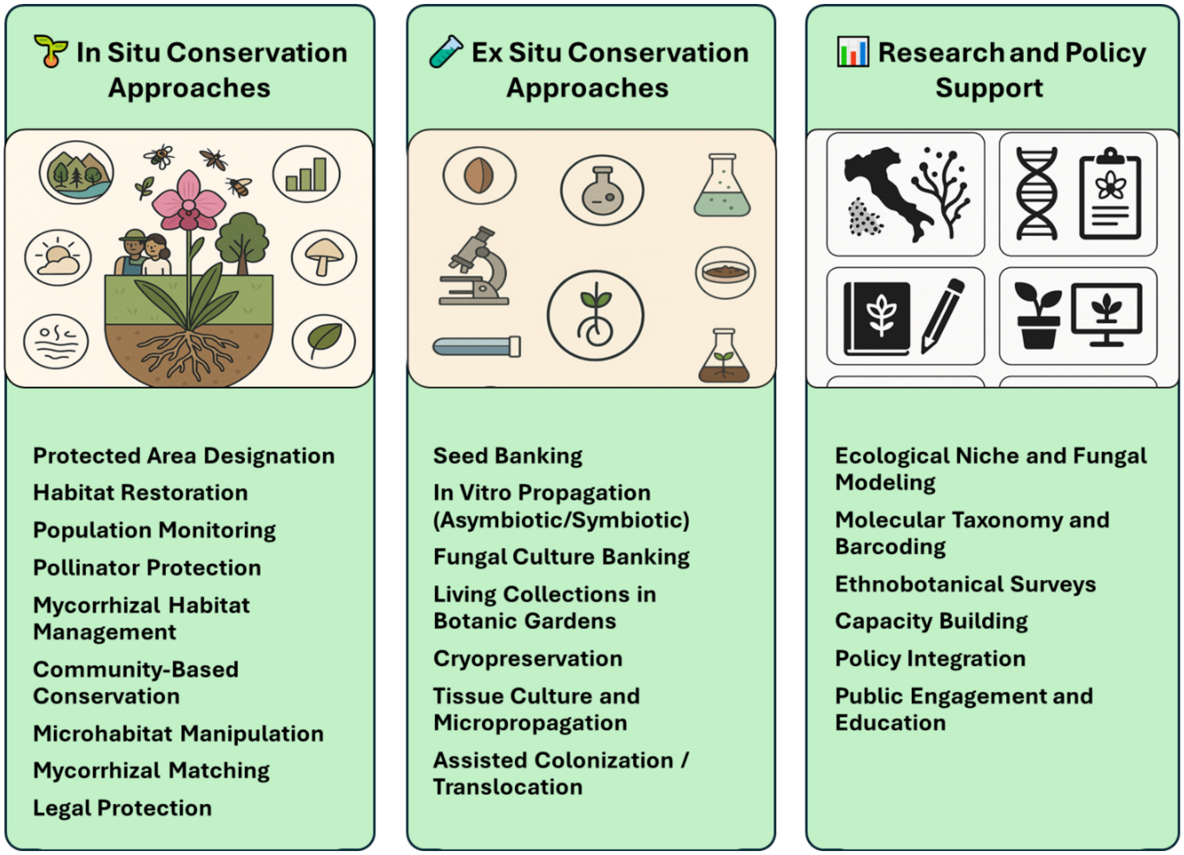

Fig. 2. Overview of major orchid conservation approaches categorized into in situ strategies, ex situ techniques, and research and policy support. Source: Authors.

presence of compatible mycorrhizal fungi (Phillips et al., 2011).

In recent decades, botanic gardens have transformed from passive repositories into active conservation hubs, supporting orchid recovery through seed banking, symbiotic research, and field-based restoration trials (Swarts & Dixon, 2009). Despite this progress, concerns remain regarding artificial selection in controlled propagation environments, which may reduce genetic diversity or lead to maladaptation when plants are reintroduced into the wild (Tremblay et al., 2022). To address these limitations, newer conservation frameworks promote integrated strategies that blend in situ restoration with ex situ tools such as seed banks and cryopreservation (Seaton, 2007; Ortega-Larrocea & Rangel-Villafranco (2007). Brundrett (2020) introduced a practical model that combines population augmentation, habitat enhancement, and co-preservation of mycorrhizal fungi particularly relevant for terrestrial orchids at risk from habitat loss and climate change.

Field studies highlight the importance of ecological matching in reintroduction success. Reiter et al. (2016) found that orchids had higher survival rates when returned to environments that closely mirrored their native ecological conditions, including compatible fungal communities. Delmas et al. (2011) demonstrated how ex situ collections, when guided by taxonomic and historical context, can inform more targeted and effective reintroduction plans. In a broader context, Swarts and Dixon (2009) emphasized that successful orchid recovery requires an understanding of species' full ecological profiles including life history traits, mycorrhizal partnerships, and pollination strategies. This integrated perspective aligns with recent conservation proposals that include orchids in larger-scale habitat and biodiversity recovery frameworks (Mahanayak, 2024).

4. Importance of Mycorrhizal Integration

Increasing evidence demonstrates that orchid reintroductions are significantly more successful when fungal symbionts are incorporated. Beyond initiating seed germination, OMF contribute to seedling establishment, stress tolerance, pathogen resistance, and nutrient acquisition in natural habitats (Rasmussen et al., 2015). Liu et al. (2010) underscored the importance of using ecologically appropriate fungal strains, especially for orchid species with narrow symbiotic preferences.

Conversely, restoration efforts that omit fungal partners often experience low survival rates, delayed development, and reproductive failure, even in seemingly suitable reintroduction sites (Stewart et al., 2003). These limitations have driven a shift toward restoration strategies that actively include mycorrhizal fungal partners. For example, Zhao et al. (2021) proposed sourcing fungi directly from protocorms in wild populations to ensure ecological fidelity. While conceptually appealing, this approach is technically demanding due to the difficulty of locating and isolating protocorms and their associated fungi, and it may also raise conservation concerns if applied at scale, given the potential impact on natural recruitment. Similarly, Reiter et al. (2018) introduced the concept of mycorrhizal matching, advocating for targeted pairing of orchid species with their native fungal partners to improve establishment success.

Methodologies for applying these insights in both laboratory and field contexts have advanced substantially. Brundrett et al. (2001) outlined practical protocols for isolating and culturing orchid fungi, while Batty et al. (2002) provided early empirical evidence linking fungal presence to restoration success. In tropical settings, innovative delivery systems such as alginate-encapsulated fungal inocula have been developed to co-introduce fungi and seeds efficiently (Otero et al., 2013). However, practical implementation has often resulted in low or variable success, suggesting that further optimisation is required before broad application. At the same time, critiques of asymbiotic propagation methods by Jolman et al. (2022) highlight the limitations of fungus-free approaches, particularly in terms of seedling performance and ecological resilience. Such approaches may also have longer-term consequences for restoration, as plants established without fungal partners may fail to develop or transmit genetic or epigenetic traits necessary for effective mycorrhizal interactions across generations.

The value of mycorrhizal integration extends beyond experimental plots and into institutional conservation. Swarts and Dixon (2009) reported that including fungi in propagation protocols within botanic gardens led to increased flowering, seed production, and survival in restored orchid populations. Together, these findings make it clear that orchid conservation efforts are incomplete unless they also support the fungal partners essential for orchid germination, growth, and long-term survival.

Table 1. Summary of orchid species reintroduced with mycorrhizal fungi across different regions.

Country / Region	Orchid species	Conservation status	Fungal partner	Method / setting / duration	Key outcomes	Reference
China	*Gastrodia elata*	Not reported	*Mycena* sp.	In vitro symbiotic seed germination; 1 year	77.3% germination by month 3	Kim et al. (2006)
China	*Dendrobium devonianum* *	Not reported	*Tulasnella* sp.	In situ symbiotic seed germination; 3 months	Significant recruitment; 1.4% survival	Shao et al. (2017)
China	*Paphiopedilum spicerianum*	Critically endangered	*Tulasnella* sp.	Ex situ seed baiting for fungal isolation; in vitro seed germination; assisted colonization using seed–fungus propagules; 4 months	35% seedling survival; successful assisted colonization	Yang et al. (2020)
Singapore	Multiple species	Not reported	Not specified (natural colonization)	Ex situ asymbiotic propagation; 13 years	80% survival; 100% flowering of surviving plants	Yam & Arditti (2011)
India	*Dactylorhiza hatagirea*	Endangered	*Ceratobasidium* sp.	In vitro symbiotic seed germination; 3 months	Rapid germination; well-developed seedlings (100%)	Aggarwal & Zettler (2010)
Australia	*Diuris fragrantissima*	Endangered	*Tulasnella* sp.	In vitro symbiotic seed germination; ex situ seedling raising; in situ transplant; 1.5 years	Fungal introduction improved reintroduction reliability	Smith et al. (2010)
Australia	*Caladenia colorata*	Endangered	*Serendipita* sp.	In vitro symbiotic propagation; in situ transplant; 6 years	77% survival; 84% population growth	Reiter & Menz (2022)
Australia	*Thelymitra epipactoides*	Endangered	*Tulasnella* sp.	In vitro symbiotic seed germination; greenhouse co-planting; 1 year	Recruitment influenced by site-specific fungi and temperature	Reiter et al. (2018)
USA	*Spiranthes brevilabris*	Endangered	*Epulorhiza* sp.	In vitro symbiotic seed germination; ex vitro	50% germination; 100% survival; 9.9% flowering	Stewart et al. (2003)

				seedling raising; in situ transplant; ~2 years		
USA	*Epidendrum nocturnum* *	Endangered	*Epulorhiza repens*	In vitro symbiotic seed germination; ex vitro seedling raising; in situ transplant; 1.5 years	90% survival; successful field establishment	Zettler et al. (2007)
USA	*Dendrophylax lindenii* *	Endangered	*Ceratobasidium* sp.	In vitro symbiotic seed germination and seedling development; 2 years	84% germination; seedlings viable for 2 years with occasional flowering	Hoang et al. (2017)
USA	*Platanthera holochila*	Endangered	*Epulorhiza* sp.	In vitro symbiotic seed germination; ex situ asymbiotic propagation; 3 years	Symbiotic approach ineffective; asymbiotic reintroduction successful	Zettler et al. (2011)
UK	*Dactylorhiza incarnata* subsp. *ochroleuca*	Critically endangered	*Tulasnella* sp.	In vitro symbiotic seed germination; ex vitro seedling raising; in situ transplant; 1.5 years	100% survival pre-introduction; 42% post-introduction	Sarasan et al. (2021)
Brazil	*Schomburgkia crispa* *	Not reported	Not specified (natural colonization)	In vitro asymbiotic seed germination; in situ transplant; 2.3 years	73.3% survival	Soares et al. (2020)
Mexico	*Bletia urbana*	Endangered	*Epulorhiza* sp.	In vitro symbiotic seed germination; in situ transplant; in situ seed baiting; 6 years	90% germination; 50% survival with flowering	Rangel-Villafranco & Ortega-Larrocea (2007)
Mexico	*Guarianthe skinneri* *	Threatened	*Nigrospora* sp., *Coprinellus* sp., *Fusarium* sp.	In vitro symbiotic seed germination; ex vitro seedling raising; in situ transplant; 1 year	3.3% survival after 1 year	Emeterio-Lara & Damon (2024)
Madagascar	*Aerangis ellisii* *	Endangered	*Ceratobasidium* sp.	In vitro symbiotic seed germination; 6 months	Improved germination and seedling growth	Kendon et al. (2020)

Epiphytic species are indicated by an asterisk (*). Conservation status follows IUCN or national listings where reported. Information on whether species were native or exotic to the study area was inconsistently reported across studies and therefore could not be standardized for inclusion in the table.

5. Comparative Analysis and Thematic Insights

Drawing on selected case studies from six regions (Table 1), this section synthesizes key patterns in orchid reintroduction efforts that integrate mycorrhizal fungi. The following subsections focus on fungal partner selection, implementation strategies, and broader ecological and logistical factors influencing restoration success.

5.1 Which Fungal Partners Work Best and Why

Across case studies, successful reintroductions were most often associated with fungal partners that were either generalist and broadly compatible or specialist but ecologically adapted to the target orchid and reintroduction site. Generalist fungi, such as *Epulorhiza* and *Ceratobasidium* spp., were frequently used in both epiphytic orchids like *Epidendrum nocturnum* and *Dendrophylax lindenii* and terrestrial orchids like *Spiranthes brevilabris* and *Dactylorhiza hatagirea*. These fungi supported high survival rates in greenhouse and semi-natural conditions due to their ease of isolation and adaptability across orchid species (Aggarwal & Zettler, 2010; Hoang et al., 2017; Stewart et al., 2003; Zettler et al., 2007). In contrast, specialist fungi, particularly OTU-specific strains of *Tulasnella* and *Serendipita*, were more effective for terrestrial orchids with narrow habitat preferences. For example, *Caladenia colorata* responded exclusively to local *Serendipita* OTUs, and *Dactylorhiza incarnata* subsp. *ochroleuca* showed improved seedling performance when inoculated with *Tulasnella* isolates collected from its native habitat (Reiter & Menz, 2022; Sarasan et al., 2021).

The origin of the fungal isolation played a decisive role. Fungi collected from protocorms, seedling roots, or closely related species in the same habitat typically resulted in better compatibility and colonization. For instance, *Dactylorhiza incarnata* subsp. *ochroleuca* achieved long-term survival and flowering when inoculated with *Tulasnella* from a fen-adapted congener (*D. praetermissa*) (Sarasan et al., 2021). These findings highlight that even within compatible mycorrhizal families, ecological adaptation and local co-evolution are key determinants of successful symbiosis.

5.2 Reintroduction Methods Involving Fungi

Orchid reintroduction projects have employed a range of fungal integration strategies shaped by species biology, resource availability, and field context (Table 2). While differing in complexity,

Table 2. Overview of key methods for integrating mycorrhizal fungi into orchid reintroduction.

Method	Description	Advantages	Drawbacks	Examples
1. In Vitro Symbiotic Propagation	Seeds are germinated with fungi under sterile lab conditions, then seedlings are acclimatized.	-Controlled environment -High-quality seedlings -Ensures early colonization	-Labor and resource-intensive -Requires skilled personnel and lab facilities	Epiphytic: *Aerangis ellisii* (Kendon et al., 2020)
2. Seed–Fungus Packets	Seeds mixed with fungi and packaged in biodegradable material; placed in natural habitat.	-Cost-effective -Field-based germination -Can be deployed at scale	-Sensitive to microsite conditions -Unpredictable germination rates in harsh environments	Terrestrial: *Paphiopedilum spicerianum* (Yang et al., 2020)
3. Soil Inoculation	Fungal inoculum is applied to soil at reintroduction sites before or during transplant.	-Strengthens soil fungal communities -May aid natural colonization	-Risk of fungal dilution or mortality -Difficult controlling fungal spread or dominance	Terrestrial: *Anacamptis pyramidalis* (Tesitelova et al., 2022)
4. Tuber / Root Inoculation	Fungi applied directly to tubers or roots before planting in the field.	-Targeted and efficient for mycoheterotrophic species -Scalable for some medicinal orchids	-Less suitable for very young seedlings -May not ensure full root colonization	Terrestrial (mycoheterotrophic): *Gastrodia elata* (Liu et al., 2010)
5. Microhabitat Manipulation	Abiotic conditions are optimized to support fungal presence (e.g., shading, moisture, litter).	-Improves long-term site suitability -Benefits both orchid and fungus ecological needs	-Requires detailed habitat knowledge -Not feasible for heavily altered or urban sites	Terrestrial: *Caladenia colorata* (Reiter & Menz, 2022)

these methods all aim to facilitate or maintain mycorrhizal relationships crucial for seed germination, seedling development, and long-term survival.

Among the reviewed studies, controlled methods such as in vitro symbiotic propagation remain the most widely used, particularly for epiphytic, terrestrial or mycoheterotrophic orchids with high fungal specificity. By introducing compatible fungi during early seed germination, this technique improves colonization success and seedling vigor prior to acclimatization and planting (Liu et al., 2010; Kendon et al., 2020). For field-based applications, seed–fungus packets offer a practical and scalable solution. These biodegradable packets, combining orchid seeds with fungal inoculum, have yielded positive outcomes under natural conditions. Their use in *Paphiopedilum spicerianum* enabled symbiotic germination directly in situ without the need for greenhouse infrastructure (Yang et al., 2020). Less frequently reported but ecologically promising is soil inoculation, where fungal cultures are applied directly to reintroduction sites (Tesitelova et al., 2022).

In *Schomburgkia crispa*, survival success in the Brazilian Cerrado was attributed, at least in part, to potential recolonization by native fungi, although direct inoculation was not confirmed (Soares et al., 2020). Lastly, several studies emphasize the role of microhabitat manipulation in supporting fungal persistence. Modifying substrate conditions, litter depth, shading, or host tree presence can improve fungal viability and symbiosis. For example, *Caladenia colorata* demonstrated improved recruitment in sites with favorable microsite characteristics that aligned with the ecological needs of both orchid and fungus (Reiter & Menz, 2022). While each method offers distinct advantages and limitations, their effectiveness depends on careful alignment with the species' lifeform, mycorrhizal specificity, and logistical feasibility. As illustrated in Table 2, method selection should be approached not as a fixed protocol but as a flexible design decision shaped by ecological and practical realities.

5.3 Factors Influencing Success of OMF influenced Orchid Reintroduction

The success of orchid reintroduction programs involving mycorrhizal fungi depends on the interplay of biological, ecological, methodological, and logistical factors (Fig. 3). Each of these dimensions shapes conservation outcomes and must be integrated thoughtfully into species-specific strategies.

Biologically, orchid lifeform, fungal specificity, and mycorrhizal dependency significantly influence reintroduction potential. Terrestrial orchids like *Diuris fragrantissima* and *Dactylorhiza incarnata* often require narrowly defined fungal partners and habitats (Smith et al., 2010; Sarasan et al., 2021), while epiphytes such as *Epidendrum nocturnum* and *Guarianthe skinneri* are more flexible, commonly associating with generalist fungi (Emeterio-Lara & Damon, 2024; Zettler et al., 2007). Mycoheterotrophic orchids, notably *Gastrodia elata*, demonstrate complete fungal dependency and demand sustained symbiotic support (Liu et al., 2010). Success also correlates with fungal origin—strains sourced from the same species or local habitat outperform unrelated or commercial isolates (Yang et al., 2020; Reiter & Menz, 2022).

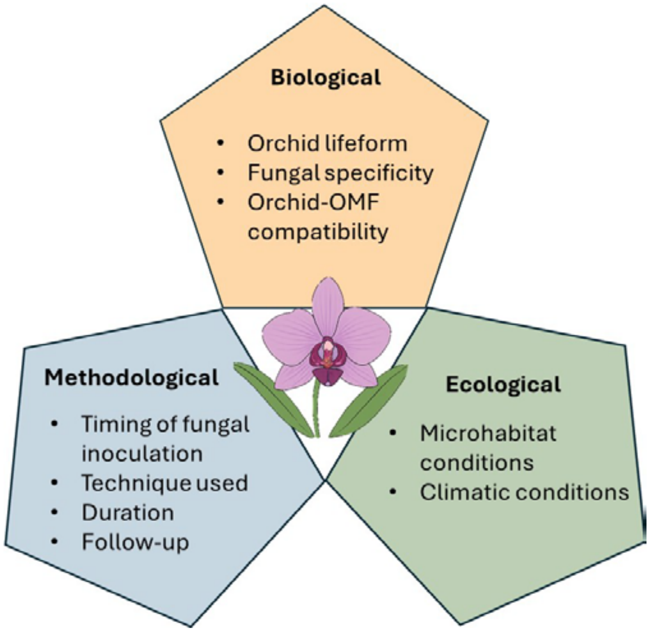

Fig. 3. Key factors influencing the success of orchid reintroduction with mycorrhizal fungi. Source: Authors.

Ecologically, site-level variables such as moisture, soil texture, pH, and shading strongly affect both fungal viability and orchid growth. For example, *Caladenia colorata* and *Schomburgkia crispa* responded favorably to microhabitats aligned with their natural niches (Reiter & Menz, 2022; Soares et al., 2020). These examples underscore the importance of ecological filtering and detailed habitat assessment for matching orchid–fungus compatibility.

Methodologically, timing and technique of inoculation matter. Early-stage fungal integration promotes stronger compatibility and resilience, whereas late inoculation often results in weak colonization (Emeterio-Lara & Damon, 2024). In vitro symbiotic propagation offers high control but requires infrastructure, while seed–fungus packets and soil inoculation offer accessible alternatives when site conditions are favorable (Yang et al., 2020). Long-term monitoring remains limited, but extended studies like *Dactylorhiza incarnata* demonstrate the value of tracking both plant and fungal persistence (Sarasan et al., 2021).

Logistically, outcomes are often shaped by institutional infrastructure and data transparency. Programs in North America and Australia benefit from centralized networks, training, and culture libraries (Krupnick et al., 2013; Reiter et al., 2016), while under-resourced regions in Africa and South America face constraints (Oliveira et al., 2014). The lack of standardized metadata across studies hampers reproducibility and knowledge exchange (Xing et al., 2019).

6. Challenges and Gaps

Despite major advancements in orchid reintroduction using mycorrhizal fungi, several key challenges remain that limit the scalability, reproducibility, and long-term success of these efforts. These challenges range from technical barriers to institutional and geographic disparities (Fig. 4).

Among these, the most critical challenge is the difficulty in isolating and maintaining compatible fungal partners. Many orchid-associated fungi are highly specific and difficult to culture in vitro, especially those from tropical or mycoheterotrophic species. Studies such as those involving *Paphiopedilum spicerianum*, *Gastrodia elata*, and *Aerangis ellisii* relied on one or two fungal isolates due to difficulties in recovering viable strains (Yang et al., 2020; Liu et al., 2010; Kendon et al., 2020). Moreover, even when successfully isolated, fungal performance may decline with repeated subculturing, leading to reduced colonization and seedling support during reintroduction (Shao et al., 2024).

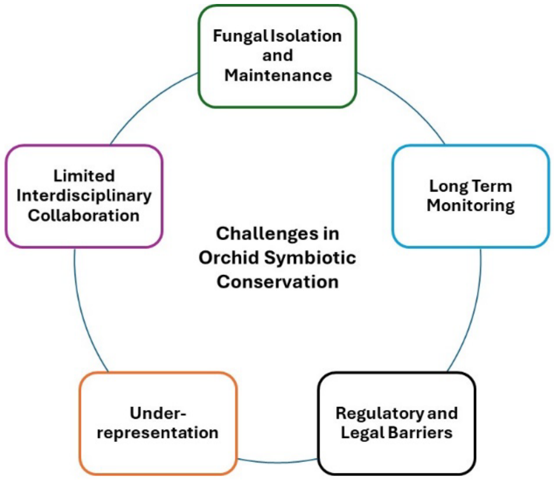

Fig. 4. Major challenges in orchid symbiotic conservation. Source: Authors.

Closely tied to this issue are regulatory barriers that restrict access to ideal fungal strains. Legal and biosecurity regulations often prohibit the transport of living microbial cultures across borders, even for conservation purposes. In North America, these restrictions have led programs to rely on locally available fungi rather than ecologically optimal partners (Zettler & Corey, 2018). In Madagascar, similar challenges were encountered during attempts to propagate *Aerangis ellisii* using native fungal strains (Kendon et al., 2020).

A third major limitation is the lack of long-term monitoring. While early survival and flowering are commonly reported, few studies extend beyond 1–2 years, and even fewer confirm fungal persistence in roots or track reproductive recruitment. The UK reintroduction of *Dactylorhiza incarnata* subsp. *ochroleuca* is one of the few examples that included five years of follow-up with fungal verification (Sarasan et al., 2021).

Collaboration gaps between fungal biologists and conservation practitioners further limit the integration of symbiotic knowledge into practical restoration. Studies from India, for example, identified mycorrhizal communities in native orchids but did not extend these findings into propagation or reintroduction trials (Aggarwal & Zettler, 2010). Conversely, some conservation programs have used fungi without clear taxonomic or functional confirmation, leading to uncertain results.

Finally, underrepresentation in tropical and developing regions poses a substantial equity gap in orchid conservation. Despite hosting the world's highest orchid diversity, some countries have conducted few full-scale reintroductions involving fungal symbionts. This underrepresentation is largely due to limited infrastructure, local fungal culture capacity, and long-term funding (Kendon et al., 2020; Wei et al., 2025). Addressing these challenges will require coordinated global action.

7. Recommendations

To ensure successful and sustainable orchid reintroduction, we recommend an integrated approach that combines ecological precision, symbiotic propagation, and inclusive collaboration. The following actions are proposed as adaptable guidelines for conservation practitioners, researchers, and policymakers.

1. Incorporate mycorrhizal compatibility testing into early planning, using locally sourced fungal strains and germination trials to validate functional symbiosis before large-scale propagation.

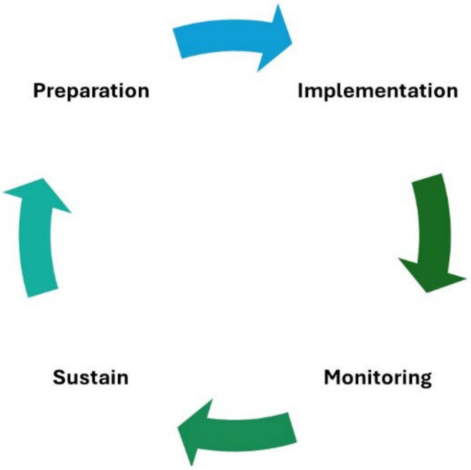

Fig. 5. Adaptive cycle of orchid–fungus reintroduction programs. Source: Authors.

2. Adopt symbiotic propagation methods as the standard practice where feasible, ensuring that orchids are pre-colonized with effective fungi prior to reintroduction.

3. Tailor fungal inoculation techniques to species ecology and field logistics, selecting among in vitro symbiotic seedlings, seed–fungus packets, or plug inoculation based on habitat conditions and infrastructure.

4. Match reintroduction sites to both orchid and fungal habitat requirements, guided by microhabitat assessments of soil, moisture, shade, and organic matter.

5. Establish long-term monitoring frameworks that include both plant performance and fungal colonization, using low-cost tools such as microscopy and photographic records where molecular tools are not available.

6. Develop and maintain regional fungal culture banks, equipped with metadata on host origin, compatibility, and ecological context to ensure continued access to viable inoculum.

7. Strengthen interdisciplinary collaboration and community involvement, engaging local knowledge holders in site selection, planting, and stewardship to enhance ecological and social outcomes.

8. Institutionalize OMF integration into national policies and conservation strategies, ensuring that orchid reintroduction protocols, funding mechanisms, and restoration targets formally include fungal components.

8. Conclusion

Successful orchid reintroduction depends on more than planting propagated seedlings, it requires restoring the symbiotic relationships essential to orchid survival. This review highlights that integrating mycorrhizal fungi improves germination, establishment, and long-term persistence, especially when fungal partners are species-specific and ecologically matched. Despite encouraging progress, challenges such as fungal culturing difficulties, regulatory barriers, and limited long-term monitoring remain widespread. Moving forward, a more integrated approach, combining scientific, institutional, and community efforts is essential. With better coordination, localized capacity, and sustained support, orchid–fungus symbiosis can become a reliable foundation for conservation and restoration worldwide.

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