Czech Science Foundation - Part C1 - Project Description

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Name of the project: Mycorrhizas – drivers of orchid paucity in disjunct islands?

1. Present state of knowledge (relevance to hypotheses tested indicated by boldfaced hypothesis number in brackets)

The frequency and diversity of interactions between mycorrhizal fungi and plants are now becoming a hot issue in ecology, as documented by the comprehensive review that has just appeared in Science (Tedersoo *et al.* 2020). Tedersoo *et al.* (2020) demonstrated that mycorrhizal interactions have a major impact on plant-plant interactions and ecosystem processes, including plant dispersal and establishment. They further demonstrated that the characteristics of the mycorrhizal interactions vary among the four dominant types of mycorrhiza: arbuscular mycorrhiza (AM), ectomycorrhiza (EcM), ericoid mycorrhiza (ErM) and orchid mycorrhiza (OM). The consequences of mycorrhizal interactions are important for all, but especially important for the distribution and success of the Orchidaceae because of the specialized nutritional interactions that have evolved between orchids and fungi. Orchids primarily interact with saprotrophic fungi of the phylogenetically heterogeneous 'rhizoctonia' group (McCormick, Jacquemyn 2014), EcM simultaneously interacting with other plants (Bidartondo *et al.* 2004; Barrett *et al.* 2014; Rock-Blake *et al.* 2017) and wood- or litter-decomposing saprotrophic fungi (Ogura-Tsujita *et al.* 2009; Lee *et al.* 2015).

Orchids interact with mycorrhizal fungi at all life history stages (Smith, Read 2008; Swarts *et al.* 2010; Phillips *et al.* 2011; Davis *et al.* 2015) and their survival in any ecosystem depends on the distribution and abundance of mycorrhizal fungi (McCormick *et al.* 2012, 2018; Těšitelová *et al.* 2015). Orchid dependence on these fungi is especially important at critical life history stages. Orchid seeds in nature are unable to germinate and the embryos cannot grow to the protocorm stage without obtaining carbon and other essential resources from mycorrhizal fungi (Rasmussen *et al.* 2015), and seedlings in nature that are experimentally deprived of their mycorrhizal associates often have lower survival rates (Bayman *et al.* 2002). There is also mounting evidence that the vast majority of orchids are partially mycoheterotrophic (Bidartondo *et al.* 2004; Hynson *et al.* 2013; Gebauer *et al.* 2016; Schiebold *et al.* 2018) and obtain carbon from mycorrhiza at all life history stages. The importance of orchid dependence on mycorrhiza as a carbon source is demonstrated by the number of species that have evolved to become fully mycoheterotrophic (Barrett *et al.* 2014), far more than any other plant family. The ability to utilize mycorrhizal fungi to obtain carbon has also enabled many orchid species to survive long periods of vegetative dormancy, i.e., not producing any aboveground biomass (Tremblay *et al.* 2009) compared to any other family of flowering plants (Shefferson *et al.* 2018).

Tedersoo *et al.* (2020) concluded that "mycorrhiza-dependent dispersal limitation tends to be relatively more important in determining the establishment success and population dynamics of OM and EcM plants because of their generally high partner specificity". Simonsen *et al.* (2017) and Harrison *et al.* (2018) also found evidence for the importance of mycorrhiza for dispersal in legumes where species with general rhizobia associations were more likely to become established outside their native ranges and have larger introduced ranges than species requiring specific rhizobia. Orchids and their mycorrhizal fungi are an ideal system to examine global dispersal patterns because, with very few exceptions (Suetsugu *et al.* 2015), orchids have wind-dispersed seeds and Correia *et al.* (2018) suggested that OM spores are wind-dispersed. EcM, in contrast, have more limited dispersal capabilities (Peay *et al.* 2010; Sato *et al.* 2012; Horton 2017).

To date, there are no clear patterns of the relationship between the distribution of different groups of orchid mycorrhizal fungi (OMF) and the rarity and distributions of orchids (**H1, H2**). Published research has produced contrasting results and it is still unclear whether the rarity or commonness and distribution of orchids is associated with the distribution and abundance of specific mycorrhizal fungi (e.g. Swarts *et al.* 2010; Bailarote *et al.* 2012; McCormick, Jacquemyn 2014). The global distribution of orchid mycorrhizal fungi on islands remains poorly understood and has yet to be compared with mainland diversity and distribution (**H1, H2**). McCormick *et al.* (Melissa McCormick, personal communication) have, for example, recently found that many of the OMF on Palau, a remote disjunct island,

are not represented elsewhere in the world based on available molecular data. Varying degrees of orchid-OMF specificity have been reported on islands, even between closely related species (Jacquemyn *et al.* 2017). A high degree of specificity was found on the most remote archipelago, the Hawaiian Islands, where only three cosmopolitan fungal OTUs were isolated from the roots of the endemic terrestrial orchid *Anoectochilus sandvicensis* on Hawai'i, Kaua'i, Maui, and O'ahu (Swift *et al.* 2018). A high degree of specificity has recently been found on Palau between OMF and endemic orchids compared to OMF isolated and identified from orchids that have a wider range of distribution across the Pacific (Melissa McCormick, personal communication). In contrast to these examples of specificity, Otero *et al.* (2002) found a range of specificities in epiphytic orchids of Puerto Rico and an analysis of 77 orchid species on La Réunion found a total of 95 Rhizoctonia Operational Taxonomic Units (OTUs) and low mycorrhizal specialization (Martos *et al.* 2012).

In addition to issues related to the specificity or commonality of the relationship between orchids and OMF on islands, orchids have been shown to be under-represented on most of the world's islands (Taylor *et al.* 2019). Interestingly, this under-representation in the flora did not change with increasing island isolation, suggesting that dispersal is not the principle limiting factor structuring orchid assemblages on islands. Ferns, on the other hand, form mycorrhizal associations in only about 60% of the species studied and these mostly involve common and widespread arbuscular mycorrhizal fungi (Lehnert, Kessler 2016). Perhaps as a result, ferns were significantly over-represented on islands and their proportional representation significantly increased with increasing island isolation, suggesting that constraints unrelated to dispersal acting on colonization were much greater for orchids than for ferns (Taylor *et al.* 2019). Moreover, after correcting for island area, orchid species richness on disjunct islands reached just 10% of that on continental islands, although their proportional representation of the vascular flora is nearly identical (Taylor *et al.* 2019). Terrestrial orchids critically rely at least partially on OMF as a carbon source at all life history stages (Schiebold *et al.* 2018), and all orchids require OMF for seed germination and seedling establishment (Rasmussen 1995; Smith, Read 2008), but the degree of adult dependence varies and this may affect how mycorrhizal fungi contribute to orchid distribution (**H4**). It can therefore be hypothesized that mycorrhizal fungi play a major role in orchid colonization and rarity on islands (**H1-H4**).

The focus of this proposal is to investigate questions related to the role of mycorrhizas as drivers of plant distribution, addressing orchid paucity on remote islands through a global study of orchid-mycorrhizal relationships on mainland areas compared to islands in different climatic regions. In addition to addressing unresolved issues related to orchid-mycorrhizal relationships, the proposed research will address ecological issues that respond to the global need for information in support of orchid conservation. It is becoming increasingly clear that efforts to conserve and restore native orchids require an understanding of orchid-OMF interactions (Swarts, Dixon 2017, **H3**). The proposed research will also address some of the conclusions reached by Tedersoo *et al.* (2020) by globally evaluating spatial issues to more fully understand the importance of mycorrhiza in the establishment and maintenance of plant species diversity and distributions.

2. Objectives and hypotheses

The major aim of this study is to test the hypothesis that the observed underrepresentation of orchids on disjunct islands is mainly due to high mycorrhizal specificity and/or a lack of compatible fungi. We predict that:

(H1) The number of mycorrhizal OTUs is lower on disjunct islands than on the mainland and land-bridge islands, restricting the number of orchid species able to colonize an island.

Substantiation:

Because of their high degree of isolation and small size, disjunct islands will harbor lower OMF diversity than mainland areas or land-bridge islands as a result of dispersal limitation. Due to their wind-dispersed spores, fungi have long been assumed not to be limited by dispersal, but recent studies have indicated that mycorrhizal fungi may show limited capacities for long distance dispersal. For example, the initial failure of *Pinus* plantations to establish in the southern hemisphere was due to the lack of their mycorrhizal partners, indicating that spore dispersal via wind at a global scale is unlikely (Pringle *et al.* 2009). This is certainly the case on a regional scale as well: *Pinus caribaea* is common on the Caribbean island of Hispaniola, but just 120 km east is Puerto Rico, without pines or their EcM fungi until both

were introduced by foresters (Rivera *et al.* 2015). Similarly, Peay *et al.* (2012) showed that spore deposition of EcM fungi and root colonization of bait seedlings decreased with increasing distance from forest patches. Vannette *et al.* (2016) showed that Glomeromycete community composition in the roots of the tree *Metrosideros polymorpha* was significantly affected by distance to nearest neighbor in an extremely fragmented lava-landscape. Comparing AMF communities of a newly constructed island with those of a neighboring natural island, Nielsen *et al.* (2016) further showed that AMF communities of the new island were a non-random subset of those of the neighboring island and consisted of typical early-successional species. Although data on dispersal of OMF are scarce and virtually nothing is known about the effect of island isolation on OMF communities and diversity, it is reasonable to assume that most OMF have limited dispersal capabilities and that isolated islands therefore have impoverished mycorrhizal communities compared to well-connected islands. We therefore predict that orchid-fungus interactions will act as a strong colonization filter for orchids on remote islands. Similarly, island size can be expected to have a pronounced impact on OMF diversity and community structure. Previous research has, for example, shown a strong species-area relationship for ectomycorrhizal fungal communities, with increasing diversity with island size and with communities from small islands nested within those of larger islands. This nested pattern, where species-poor communities of small habitat patches consisting of common species are a subsample of the species-rich communities of larger habitat patches, suggests that especially rare species are susceptible to variation in island size (Peay *et al.* 2007).

(H2) There will be fewer orchid mycorrhizal fungus OTUs per orchid species on disjunct islands, compared to continental sites (including continental islands).

Substantiation:

Because small and isolated islands can be expected to support fewer OMF than mainland areas or land-bridge islands, assemblages of OMF associating with orchids on islands will differ relative to assemblages found associating with orchids growing in mainland or islands in closer proximity to mainland regions. In particular, due to the limited availability of OMF on disjunct islands and the assumed nested structure of OMF assemblages, we hypothesize that orchids with more generalized interactions will be more frequently observed on islands than in mainland regions. Because of the limited availability of OMF on islands, we further predict that among-population differentiation (beta-diversity) in OMF communities will be significantly larger on mainland areas than on islands.

(H3) Irrespective of island type, orchid species that are geographically wide-ranging will associate with a larger number of OTUs than species with a limited geographic distribution, even within a single site, and species with a limited geographic distribution will be limited by the specific fungi in the habitats they rely on.

Substantiation:

Widely distributed species may have spread due to a greater likelihood of encountering at least one suitable fungal partner when colonizing new environments, whereas endemic orchids may have evolved to utilize the most beneficial and/or common fungal symbionts in a given area. For example, legumes that associated with specific rhizobia had much smaller introduced ranges than those that could use a wider range of rhizobia (Simonsen *et al.* 2017; Harrison *et al.* 2018). Conversely, it is also possible that successful colonists of new areas (e.g., islands) are those species that can use a single widespread fungus (Davis *et al.* 2015), but are unable to associate with fungi that are unique to the area, while endemics may have evolved to use the full range of fungi that occur in limited areas such as islands or, indeed, are themselves endemics. Consequently, it is also possible that island endemics may disproportionately be mycorrhizal generalists (Martos *et al.* 2012).

To test these hypotheses, we will molecularly compare mycorrhizal communities associating with terrestrial, epiphytic and lithophytic (growing in or on rocks) orchids on both mainland and disjunct islands. In addition, high-throughput sequencing of environmental (bark, soil) samples will allow us to distinguish mycorrhizal availability from orchid specificity and to test whether it differs between disjunct islands and mainland sites.

(H4) Irrespective of temperate or tropical region, fully and partially mycoheterotrophic carbon gain by orchids is highest in continental forest understories and less in (1) disjunct islands, (2) non-forest habitats, and (3) in the tropics in epiphytic, compared with terrestrial, orchids.

Substantiation:

An essential component determining how the availability of mycorrhizal fungi affects orchid ability to colonize islands is the extent to which they depend on their fungi. Due to their tiny dust-like seeds, all orchids require nutritional support by a species-specific suited set of fungi for their germination (Merckx 2013). This initially mycoheterotrophic ontogenetic stage predisposes orchids to an exploitation of their mycorrhizal fungi at adulthood. In fact, about 1% of all orchid species are achlorophyllous and rely completely on the nutritional support from their host fungi (Leake 1994). Fungal hosts of these fully mycoheterotrophic orchids are either fungi simultaneously forming EcM with forest trees (Bidartondo *et al.* 2004; Hynson *et al.* 2013) or saprotrophic wood- or litter-decomposing fungi (Ogura-Tsujita *et al.* 2009; Lee *et al.* 2015). Thus, these orchids are mostly limited to living on forest grounds hosting suited tree species or providing suited dead wood or litter. Currently there is only one report for an achlorophyllous albino variant of an orchid species living outside of forests and mycorrhizal with fungi of the rhizoctonia group (Suetsugu *et al.* 2019). Due to the complete nutritional support by their host fungi, fully mycoheterotrophic and initially mycoheterotrophic orchids are enriched in heavy isotopes of the elements C, N and H (Gebauer, Meyer 2003; Trudell *et al.* 2003; Hynson *et al.* 2013; Stöckel *et al.* 2014; Gebauer *et al.* 2016; Schweiger *et al.* 2018), while partially mycoheterotrophic orchids are positioned between fully mycoheterotrophic orchids and accompanying fully autotrophic non-orchids in their stable isotope pattern (Gebauer, Meyer 2003; Hynson *et al.* 2013). This isotopic positioning of partially mycoheterotrophic orchids between two end points allows quantifying their proportional C gains from two sources, their own photosynthesis and associated fungi (Hynson *et al.* 2013). The success in proportional C gains from fungal hosts appears to be low for 'rhizoctonia'-mycorrhizal green orchids from grassland sites and specifically high for EcM-associated green orchids from dark forest understories (Preiss *et al.* 2010; Gebauer *et al.* 2016; Schiebold *et al.* 2018).

To test this hypothesis, we will conduct stable isotope abundance analyses of orchids, compared with the already identified OMF (H1, H2), to investigate whether mycorrhizal availability and C gain from associated fungi differs between disjunct islands and mainland sites.

3. Methods

3.1. Study sites – including justification for their selection

The sites were selected in tropical and temperate regions, mainland regions and islands of different sizes and degrees of isolation. Sites were also selected to include dominant orchid habitats in each selected site. In each ***region*** – the combination of ***mainland/island***, ***tropical/temperate*** and ***continent*** (3 continents selected for each of the previous 4 combinations) we selected 3 ***sites*** to represent the diversity of environment and orchids of the region. Feasibility of access to sampling the sites was also considered, as was the availability of individuals with knowledge of the orchids in each region (see Table 1 for the list and responsibilities). The approach that was used to select sites assures that there are sufficient numbers of orchid individuals and orchid species (at least 10) to sample. Species names are available on request, but not shown here due to lack of space.

In the tropics, there are few sufficiently orchid-rich islands close to South America outside of the Caribbean, while the tropical part of the mainland of this continent is extremely rich in orchids. To the contrary, in mainland Africa, data accessibility is problematically difficult. However, there are several orchid-rich islands close to Africa. Therefore, we considered only mainland South America and islands close to Africa, to maintain the mainland/island and tropical/temperate balance in number of continents considered. The 36 sites that have been selected are listed in Table 1.

Table 1. Study sites. Scientists responsible for sampling in red. Coll. – date(s) of collecting, months in Roman characters. The laboratory, where the molecular analyses will be performed, is indicated by MA_{SERC} for SERC or by MA_{KUL} for KU Leuven. I_x means that invoice for fieldwork will be issued by institution X, where X = SERC for SERC, KUL for KU Leuven, UT for Univ. Tasmania, AF for ANALiTICA Foundation; absence of this indicator means that people from CzechGlobe will do the sampling and therefore no invoice will be issued – CzechGlobe will cover these expenses directly.

A. Temperate regions

	Island	Mainland
North America	***Kodiak Island (Aleutian Islands, Alaska)*** – temperate rainforest, wetlands, tundra. Coll.: VII, IX. Elevation: 0-700 m. Location: 57°29'2.15"N 153°28'41.70"W. Whigham, MA_{SERC}, I_{SERC} ***Adak (Aleutian Islands, Alaska)*** **–** tundra, shrublands, wetlands. Coll.: VII, IX. Elevation: 0-300 m. Location: 51°44'51.87"N 176°40'15.42"W. Whigham, MA_{SERC}, I_{SERC} ***Unalaska (Aleutian Islands, Alaska)*** **–** tundra, shrublands, wetlands. Coll.: VII, IX. Elevation: 0-1000 m. Location: 53°53'3.98"N 166°31'59.68"W. Whigham, MA_{SERC}, I_{SERC}	***Chugach National Forest (Alaska)*** – temperate rainforest, wetlands, tundra. Coll.: VII, IX. Elevation: 0-1000 m. Location: 60°10'2.27"N 149°23'42.48"W. Whigham, MA_{SERC}, I_{SERC} ***Kenai National Wildlife Refuge (Alaska)*** – temperate rainforest, wetlands, tundra. Coll.: VII, IX. Elevation: 0-2000 m. Location: 60°27'52.63"N 151° 4'24.04"W. Whigham, MA_{SERC}, I_{SERC} ***Kachemak Bay State Park (Alaska)*** – temperate rainforest, wetlands, tundra. Coll.: VII, IX. Elevation: 0-1200 m. Location: 59°36'37.69"N 151° 9'55.14"W. Whigham, MA_{SERC}, I_{SERC}
Australia	***Dudley Peninsula (Kangaroo Island, South Australia)*** – Sclerophyll (mallee) woodland, Coll.: V, X. Elevation: 30-150 m. Location: 35°49'53.86"S 137°52'46.28"E. Faast, MA_{KUL}, I_{UT} ***Tom Gibson Reserve, Powranna (Tasmania)*** – cool/temp, dry sclerophyll woodland, heathlands, grasslands. Coll.: III, X. Elevation: 20-1300 m. Location: 41°46'26.8"S 147°19'23.7"E. Swarts, MA_{KUL}, I_{UT} ***Darling Range Conservation Area (Flinders Island, Tasmania)*** – tall sclerophyll forest, woodland, coastal heathlands scrub. Coll.: III, X. Elevation: 20-500 m. Location: 40°05'43.7"S 148° 07'21.8"E. Swarts, MA_{KUL}, I_{UT}	***Mt Lofty Ranges (South Australia)*** – cool temperate forest, heathy woodlands & shrublands. Coll.: V, X. Elevation: 50-500 m. Location: 34°59'06.5"S 138°42'14.2"E. Faast, MA_{KUL}, I_{UT} ***Grampians NP (Victoria)*** – sclerophyll forest & woodland, shrublands, grasslands. Coll.: V, X. Elevation: 200-700 m. Location: 37°10'21.29"S 142°25'45.72"E. Faast, MA_{KUL}, I_{UT} ***Cape Arid NP (West Australia)*** – biodiversity hotspot, dry sclerophyll forest, shrublands. Coll.: IX. Elevation: 50-350 m. Location: 33°49'13.9"S 123°04'22.9"E. Swarts, MA_{KUL}, I_{UT}
Europe	***Samos (Greece)*** – Sparse olive grove with scattered pine trees. Coll.: IV. Elevation: 50-100 m. Location: 37°41'59.7"N 26°37'25.7"E. Tsiftsis, MA_{KUL} ***Lesvos (Greece)*** – olive groves. Coll.: IV,V. Elevation: 20-30 m. Location: 39°05'07.5"N 26°31'42.3"E. Tsiftsis, MA_{KUL} ***Vatos village (Crete)*** – phrygana. Coll.: III, IV. Elevation: 650-700 m. Location: 35°10'37.8"N 24°32'26"E. Tsiftsis, MA_{KUL}	***Mt. Falakron (Greece)*** – subalpine grassland with sparse *Pinus nigra* trees. Coll.: V. Elevation: 1300-1350 m. Location: 41°17'31"N 24°01'16.2"E. Tsiftsis, MA_{KUL} ***Foothills of Mt. Olympus (Greece)*** – grassland with sparse shrubs. Coll.: IV. Elevation: c. 50-70 m. Location: 40°07'45.6"N 22°32'25.6"E. Tsiftsis, MA_{KUL} ***Manthyrea (Arkadia, Greece)*** – grassland with sparse oak and cypress trees. Coll.: IV, V. Elevation: 700-750 m. Location: 37°24'42.4"N 22°23'25.5"E. Tsiftsis, MA_{KUL}

B. Tropical regions

	Island	*Mainland*
Central America	***Sierra del Rosario (Cuba)*** **–** mixed broadleaf and pine forest. Coll.: II, V. Elevation 200-500 m. Location: 22°49'16.4"N 83°14'55.2"W Ackerman, MA_{SERC}, I_{AF} ***St. Andrew Parish (Dominica)*** – tropical rain forest. Coll: III, V. Elevation: 300-800 m. Location: 15°31'21.4"N 61°20'44.9"W. Ackerman, MA_{SERC}, I_{AF} ***Sierra de Luquillo (Puerto Rico)*** – tropical rain forest. Coll: II, V. Elevation: 300-800 m. Location: 18°19'15.0"N 65°49'14.0"W. Ackerman, MA_{SERC}, I_{AF}	***Tapanti NP (Costa Rica)*** – lower montane rainforest and pre-montane rainforest. Coll.: III, X. Elevation: 1200-1700 m. Location: 9°45'32.5"N 83°47'01.5"W. Pupulin, MA_{SERC} ***Manuel Antonio NP (Costa Rica)*** – lowland tropical forest. Coll.: III, X. Elevation: 0-120 m. Location 9°23'09.1"N 84°08'41.5"W. Pupulin, MA_{SERC} ***Corcovado NP (Costa Rica)*** – largest primary tropical forest on the American Pacific coastline. Coll.: III, X. Elevation: 0-300 m. Location: 8°36'04.3"N 83°39'05.8"W. Pupulin, MA_{SERC}
South America/Africa	***Forêt de Bébour (Reunion)*** – moist cloud forest. Coll. III, X. Elevation: 1100-3000 m. Location: 21°06'24.0"S 55°32'53.3"E. Roberts, MA_{SERC} ***Le Pétrin (Black River Gorges NP, Mauritius)*** – upland marsh and heathland. Coll. III, X. Elevation: 669 m. Location: 20°24'30.8"S 57°28'18.4"E. Roberts, MA_{SERC} ***Solitude (Rodrigues)*** – lowland dry forest. Coll. III, X. Elevation: 0-398 m. Location: 19°41'24.5"S 63°26'24.8"E. Roberts, MA_{SERC}	***La Palma (Putumayo, Colombia)*** – humid montane forest. Coll.: VIII, XI. Elevation: 2500-2800 m. Location: 1°10'25.9"N 76°51'23.9"W. Kolanowska, MA_{KUL} ***Páramo de Bordoncillo (Nariño/Putumayo, Colombia)*** – páramo. Coll.: IV, VIII. Elevation: 3200-3300 m. Location: 1°9'15.0"N 77°5'57.9"W. Kolanowska, MA_{KUL} ***Zabaletas (Valle del Cauca, Colombia)*** – lowland wet forest. Coll.: III, X. Elevation: 100 m. Location: 3°44'28.8"N 76°57'52.4"W. Kolanowska, MA_{KUL}
South-East Asia	***Ngardok Nature Reserve (Rep. of Palau)*** – humid tropical lowland forest. Coll.: IV, IX. Elevation: 75 m. Location: 7°31'43.5"N 134°34'59.1"E. Crain, MA_{SERC}, I_{SERC} ***National Wildlife Refuge Yigo (Guam)*** – humid tropical lowland forest. Coll.: IV, IX. Elevation: 25 m. Location: 13°39'04.6"N 144°52'00.1"E. Crain, MA_{SERC}, I_{SERC} ***Nahnalaud Mountain and Nanmeir Valley (Pohnpei, Salapwuk, Federated States of Micronesia)*** – humid tropical lowland/upland forest. Coll.: IV, IX. Elevation: 200 m. Location: 6°52'26.6"N 158°13'42.7"E. Crain, MA_{SERC}, I_{SERC}	***Xishuangbanna (Mengla County, China)*** – tropical rainforest. Coll.: I, VII. Elevation: 1500 m. Location: 21°36'42.1"N 101°34'26.4"E. Jacquemyn, MA_{KUL}, I_{KUL} ***Bidoup-Nui Ba National Park (Lac Duong District, Vietnam)*** – tropical evergreen broad-leaved forest mixed with conifers. Coll.: II, VIII. Elevation: 1550 m. Location: 12°08'09.9"N 108°31'52.5"E. Jacquemyn, MA_{KUL}, I_{KUL} ***Khao Chong (Thailand)*** – tropical rainforest. Coll.: III, IX. Elevation: 1000 m. Location: 7°32'59.1"N 99°47'12.5"E. Jacquemyn, MA_{KUL}, I_{KUL}

3.1. Sampling methods and analyses

3.2.1. Field sampling. Sites listed in Table 1 will be sampled either once or twice during the project: (i) Sites with long growing seasons (i.e., spring-summer based on temperature, wet-dry season based on precipitation) will be visited twice. Orchids will be sampled here at the beginning and near the end of the growing season. (ii) At sites with very short growing seasons or where orchid flowering is synchronous, orchids will be sampled once during the project.

Prior to sampling a site, 10 target orchid species will be chosen from the known orchid flora and, as much as can be determined from existing data on orchid distribution, the species will be equally distributed between species with limited geographic distributions and species with broad geographic distributions.

At all sites, orchids will be sampled in 19.6 m^2 (= 5 m diameter) randomly located circles. At some sites, where the orchids will be patchily distributed and each patch will contain only few individuals, it will be necessary to search for orchids in several locations (= sub-sites). The starting point for establishing sampling circles at each location will be based on guidance from a local orchid expert who knows where the desired species can be found. At each location the starting point for the circles will be randomly chosen. The location of the first circle (C_1) will be determined by choosing a pair of random numbers; the first representing the direction (0-360º) and the second the distance (5-25 m) from the starting location. The location of all subsequent sampling circles will be determined using the same procedures for direction (0-360º) and distance (5-25 m). Random numbers will be chosen from an EXCEL uniform random number generator (360*RAND() and 20*RAND()+5). In each sampling circle, one individual of each target orchid species will be sampled, if present. Sampling circles will be repeated, until either 5 individuals of each of the 10 target species in long-season sites, or 10 individuals of each of the 10 target species in short-season sites, have been collected.

Table 1 provides information on the months when the sites will be sampled. Each orchid sample will consist of a root for molecular analysis and a leaf for isotope determination (sampling protocols described below). When a leaf or leaves of target orchids are collected from within a circle, leaves of 3 non-orchid plants growing under identical micro-climate, substrate and light conditions will be collected for reference isotopic analyses (See 3.2.3 for description of methods). Triplicate substrate samples (e.g., soil for terrestrial and lithophytic orchids, bark for epiphytes) will be collected within 5 cm of each orchid sampled and mixed to get one composite sample. This is because OMF abundance is greatest within 5 cm of an orchid and the variability of OMF abundance is less when a composite substrate sample is obtained (McCormick *et al.* 2012, 2018; Waud *et al.* 2017).

Freshly collected orchid roots will be preserved in CTAB Buffer and sent to SERC or Leuven for analysis of OMF using methods described below. Composited substrate samples will be preserved in CTAB and sent to SERC or Leuven for analysis of OMF using methods described below. Freshly collected samples of orchid leaves and leaves of non-orchid species will be placed in fresh silica gel for drying and sent to Bayreuth for isotope analysis.

3.2.2. Molecular analysis of orchid roots and soils. We will examine the roots under a dissecting microscope to identify sections with fungal pelotons (fungal structures indicative of functional mycorrhizal associations). We will extract DNA from two peloton-containing root sections from each orchid using Biosprint96 Plant DNA extraction kits. The ITS region of OMF in each orchid root sample will be PCR amplified using ITS1-OF/ITS4-OF and OMF identified using Sanger and Illumina sequencing.

To determine the distribution of OMF on trees and in soils, we will isolate DNA from each substrate sample using DNEasy ultra clean soil DNA kits (Qiagen). We will compare the DNA sequences from the fungi we identify from roots to those found in substrate samples to determine how many OMF OTUs are available in different sites and sample locations, how widespread OMF OTUs are within and among sites, and whether orchids are forming mycorrhiza with a subset or with all of the available OMF OTUs.

3.2.3. Isotope analysis. The isotope analysis will enable us to determine if the orchid species are autotrophic, partially mycoheterotrophic or fully mycoheterotrophic. For partially mycoheterotrophic orchids, furthermore, we can estimate the proportional C gain from the fungal source. For isotope abundance analyses, orchid and reference plant tissues collected from each sampling circle will be oven-dried at 105°C, ground to fine powder and stored in desiccators. Carbon and N isotope abundances and concentrations will be analyzed simultaneously by coupling of an elemental analyzer to an isotope ratio mass spectrometer (EA-IRMS) as described by Bidartondo *et al.* (2004).

Oxygen and hydrogen isotope abundances and concentrations of the same sample materials will be analyzed separately by coupling of a thermal conversion unit to an isotope ratio mass spectrometer (TC-IRMS) as described by Gebauer *et al.* (2016). Hydrogen isotope abundances will be measured four times consecutively to avoid memory effects. Sample gases (CO_2 for carbon, N_2 for nitrogen, CO for oxygen, and H_2 for hydrogen isotope abundances) will be measured versus standard gases. The standard gases will be calibrated using international standard substances obtained from the International Atomic Energy Agency (IAEA, Vienna). According to international convention isotope abundances are primarily presented in the δ notation as $\delta x = (R_{sample} / R_{standard} - 1) \times 1000$ [‰], where x represents the heavy isotopes ^{2}H, ^{13}C, ^{15}N or ^{18}O, respectively, and R_{sample} and $R_{standard}$ represent the ratios of heavy isotope to light isotope of the sample and the respective standard.

3.3. Statistical analysis of the data

Statistical approaches to analyses of the hypotheses will depend on the experimental design and distribution of the response variables (H1: number of OTUs, H2: relative measures of OTU specialization, H3: indices of number of OTUs or bivariate or ordinal relative measure of orchid OTU fungal specialization and orchid specialization status, and H4: indices of carbon gain from mycoheterotrophic orchids). Assumptions of the statistical approach will be evaluated, and statistical methods will be adjusted; some approaches easily implemented include: GLM, GAM, Monte Carlo Simulation, Robust Estimation (Wilcox 2017) and Bayesian statistics (McElreath 2016). Comparison of models will be evaluated using AIC (Akaike Information Criterion). *A priori*, we expect to evaluate the following hypotheses using the following approaches.

H1: This hypothesis will test the probability of the number of OTUs as a function of the type of habitat (mainland, bridge and island), environment (temperate vs. tropical), with the distance from the mainland as a covariate. The number of OTUs per orchid species is expected to be between 1 and 10, where the response variable will follow a Poisson distribution.

H2: There are multiple indices of orchid specialization to fungal OTUs. A common approach is to use the number of OTUs per species of orchid divided by the sampling effort (number of roots). This index follows an index from close to zero to infinity (theoretically), where small number (close to zero) are specialized orchids with only one species of OMF to very large numbers where infinite number of OMF are found in the roots of the orchids (theoretically). This response variable follows a gamma distribution and can be evaluated as suggested by Chen *et al.* (2018) and Christidis *et al.* (2020). However, this can be controversial in that some OTUs may be circumstantial and have no relationship to orchid health or survivorship. Alternatively, the specialization to OMF of OTUs found in an orchid can be categorized as specialized, not based or based on an *a priori* index of the frequency of specific orchids OMF in the sampled roots (Shefferson *et al.* 2019). This approach can be evaluated using either a binomial response, which would be evaluated using logistic regression or as relative measure of specialization (specialized, intermediate or generalized) and the response can be evaluated using an ordinal response regression (Agresti 2010; Gutierrez *et al.* 2016).

H3: Under the same concept of OTUs of the previous hypothesis, we will evaluate the frequency of unique and widely dispersed OTUs, as these relate to the distribution of the orchids. The relationship can be evaluated using categorical analysis, specifically a log-linear model for contingency tables and counts or proportions (Agresti 2019) of the specialized OTUs as a function of the distribution of the orchid. The distribution of the orchid will be evaluated using two alternative methods, as a continuous variable (area range of physical distribution, or – more specifically – the number of polygons of specific size) or as a categorical variable (orchid specialization status: endemic, locally abundant, widely distributed and a colonizing species). The log-linear model considers an $r \times c$ contingency table that cross-classifies on two categorical response variables (Menard 2002) – for a simple example, the OTUs special or not and the orchid specialization status. In this case, the probabilities are parameters of the multinomial distribution. This type of model treats the parameters as cell count from a Poisson distribution and uses a log-link function. Alternatively, when the predictor variable is continuous, we can use a multivariate logistic regression (Menard 2002). In addition, we will add mainland vs. island as a co-variate.

H4: In order to circumvent the site-dependency of stable isotope abundance data and to allow a direct comparison of isotope abundances of the orchid samples collected at our various sampling sites, δ values will be converted into

enrichment factors (ε values) according to $\varepsilon_{Sx} = \delta_{Sx} - \delta_{REF}$. Here S is a single value of a sample from an autotrophic, partially mycoheterotrophic or fully mycoheterotrophic plant, x is a specific sampling plot within each of the study sites and REF is the mean value of all autotrophic reference plants – see section 3.2.1 for details (Preiss & Gebauer 2008).

If fully mycoheterotrophic plants are present at a study site, three groups of normalized enrichment factors will result from the conversion: $\varepsilon_R = \varepsilon$ of the autotrophic reference plants (whereas the mean ε_R of all reference plants from a site is always 0 ‰), $\varepsilon_{PMH} = \varepsilon$ of the partially mycoheterotrophic plants and $\varepsilon_{MH} = \varepsilon$ of the fully mycoheterotrophic plants. The percentage nutrient gain from fungi ($\%x_{df}$ with x = C or H) can then be calculated from the proportion between ε_{PMH} and ε_{MH} via a linear two-source mixing model, i.e. $\%x_{df} = (\varepsilon_{PMH} / \varepsilon_{MH}) \times 100$.

Significant differences from 0 % (no nutrient gain from fungi) and from 100 % (nutrients exclusively derived from OMF) are verified by statistical tests (Kruskal-Wallis *H* test, followed by Mann-Whitney *U* test with Bonferroni-Holm correction) between ε_{PMH} and ε_R or ε_{PMH} and ε_{MH}, respectively, since the enrichment factors already reflect the nutritional mode and the percentage C and H gain is just a more convenient form of data presentation.

4. Time schedule and milestones

Sampling schedule will be coordinated among the teams, so that the data will be collected at the average speed of nine sites per year during the first four years of the project, in order not to overburden the labs performing the molecular and isotope analyses. Also, each collection must be preceded by getting corresponding sampling permits, which takes time in many of these remote areas. In addition, two samples must be done per season at some sites. This means that scientist responsible for the region will not be able to organize sampling more than one or two sites per year.

Molecular and isotope analyses of each site will be done in the year of collection or in the subsequent year, so that the time spent on the analyses will be uniformly spread through years 1-4 of the project. Years 3-4 and mainly year 5 will be devoted to statistical analyses of the results and preparing publications. The corresponding time schedule is shown in Table 2 and the milestones in Table 3.

Table 2. Time schedule - see Table 3. for explanation of the codes in the first column.

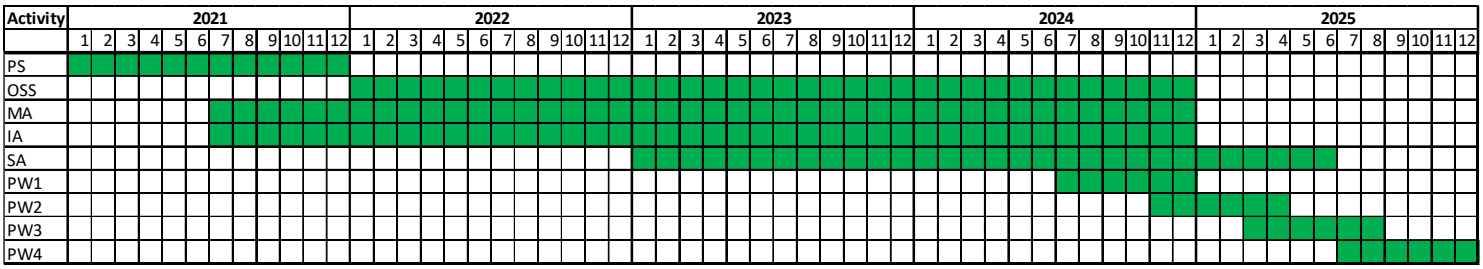

Table 3. Milestones List

Code	Title	Duration of effort (months)	Means of Verification
PS	Pilot sampling	1-12	First 9 sites sampled, method modified if needed
OSS	Orchid and soil sampling	13-36	Remaining samples collected – see 3.2.1.
MA	Mol. analyses	7-48	Molecular analyses performed
IA	Isotope analyses	7-48	Isotope analyses performed
SA	Statistical analyses	25-54	Statistical analyses performed
PW1	Tests of (H1) published	48	Publication(s) in a journal with IF>3
PW2	Tests of (H2) published	52	Publication(s) in a journal with IF>3
PW3	Tests of (H3) published	56	Publication(s) in a journal with IF>3
PW4	Tests of (H4) published	60	Publication(s) in a journal with IF>3

5. Research teams and collaborating institutions

(P = lifetime number of SCI publications, C = lifetime number of citations, excluding self-citations according to WoS, P5 = number of SCI publications during the last 5 years, H = H-index, key persons and their bibliography boldfaced, books = only books published with prestigious publishers).

Successful orchid research requires a multidisciplinary approach because of the multi-dimensional interactions between orchids, mycorrhizal fungi and pollinators. All teams involved in the project have a long history of collaboration and mutual exchange of scientific knowledge. The scientists are all part of a core group that organized workshops on orchid biology approximately every three years starting in 1990. In 2015 this core group founded the European Orchid Conservation Centre (EOCC), a sister organization of the already existing North American Orchid Conservation Center (NAOCC). Close collaborations between the multi-disciplinary groups that will participate in the proposed project is essential to assure success. Thus, the research will be conducted by interaction between 8 teams, complementary in their specialization (field research, laboratory DNA analyses, isotope analyses, statistical analysis) and spatial position relative to the study sites. Partitioning of the field work is detailed in Table 1 and therefore not mentioned in the sections devoted to individual teams. All teams are well equipped by all field equipment necessary for the field study both in temperate and in the tropical regions. They will cooperate on writing the publications. Members of all teams specialize on orchid research for their whole scientific life. Specific tasks of each RT are below.

5.1. Research team RT1 – CzechGlobe, Czech Republic, head: P. Kindlmann

The Department of Biodiversity Research (DBR), Global Change Research Institute ("CzechGlobe") of the Czech Academy of Sciences is located in Ceske Budejovice, Czech Rep. Research: evolution of life history strategies, population dynamics and stability of ecological communities. Results of DBR research are applied to biodiversity protection and nature conservation. Terrestrial orchids are the focus of the major DBR research group. The team is well equipped by all infrastructure for performing complicated computer simulations.

Role in the project: Project coordination, data collection, statistical analyses, modelling.

Personnel participating in the project:

Pavel Kindlmann, Head of DBR (**P = 141, P5 = 28, C = 2516, H = 27, 7 books**), Principal Investigator (PI) of the proposed project, founding director of the EOCC. Expert in mathematical modelling of biological processes, population dynamics and life history strategies, with particular reference to orchids. Head of the Biodiversity Research Center, 2006-2011. Member of the Orchid Specialist Group of the IUCN. Organizer of 2 international orchid conferences and of the 4th International Orchid Conservation Congress. (Coordinating) Lead Author of Chapter 4 of the Global Assessment organized by the Intergovernmental Panel on Biodiversity and Ecosystem Services (IPBES) under the auspices of the United Nations. Will be responsible for coordination of the project and for the statistical analyses and modelling.

Dr. Marta Kolanowska (P = 166, P5 = 85, C = 131, H = 7, 9 books), discovered about 250 new orchid species and **Zuzana Štípková (P = 7, P5 = 6, C = 29, H = 3),** now PhD student. Both named are working on determinants of orchid species diversity in the world, have a good training in fieldwork both in Europe and in Greece, Ecuador, Colombia etc.

Dr. Spyros Tsiftsis (P = 18, P5 = 12, C = 127, H = 5, 2 books), long time experience in orchids of Greek mainland and islands. External collaborator, main employer: Int. Hellenic Univ., Drama, Greece.

Lenka Atexingerová, technician. She will (i) perform the simple and time-consuming runs of the models, (ii) retrieve, create and maintain databases of reprints and empirical data, (iii) type in the data collected into the PC.

5.2. Research team RT2 – Smithsonian Institution, USA, head: D. Whigham

The Smithsonian Institution is the largest education/research museum complex in the world. One of its research centers (Smithsonian Environmental Research Center - SERC) will participate in the project. SERC is located near Annapolis, Maryland and consists of modern laboratory facilities that are home to world-class research. SERC has the Smithsonian's first LEED Platinum laboratory building, which has up-to-date equipment and world-class facilities ranging from molecular ecology to trace metal chemistry. This laboratory will serve for the laboratory part of the project. SERC is the home of the North American Orchid Conservation Center (NAOCC).

Role in the project: data collection, DNA analyses of root and substrate samples (see Table 1 for list of their sources).

Personnel participating in the project:

Dennis Whigham (**P = 154, P5 = 28, C = 6428, H = 46**), Senior Botanist, conducted orchid-based research for decades. Founding Director of the NAOCC. Co-author of a technique to conduct field-based studies of orchid seed germination, recognized as the world standard. His laboratory houses the largest living collection of orchid-mycorrhizal in the world. He has been the leader of several major interdisciplinary projects funded by EPA, NOAA and the US Forest Service. He co-designed and co-supervised a course for international students that was based at the SERC and was conducted on five separate occasions. Regularly mentors undergraduate interns and graduate students and post-doctoral fellows.

Melissa McCormick (**P = 56, P5 = 19, C = 1848, H = 24**), Senior Scientist and Lead Scientist of the Molecular Ecology Laboratory at SERC. Leader of molecular aspects of orchid-mycorrhizal fungi. Pioneered many of the techniques for joint assessment of orchid mycorrhizal diversity and OMF distribution in soils. In the Palau Orchid Conservation Initiative, she identified all collected fungi and synthesized that information to understand which fungi are needed to support orchids and how they vary spatially and among species and habitats.

Lawrence W. Zettler (**P = 41, P5 = 8, C = 517, H = 14**), Professor of Biology at Illinois College. Specializes in isolation of orchid endophytes and symbiotic seed germination. Has experience with epiphytic, terrestrial and lithophytic orchids in the tropics (e.g., Palau, Madagascar, Hawaii), subtropics (e.g., Florida), and temperate regions (e.g., North America).

Benjamin J. Crain (P = 12, P5 = 4, C = 59, H = 5), Research Associate with NAOCC, SERC, and the National Museum of Natural History (Smithsonian). Leading the Palau Orchid Conservation Initiative. Research: orchid diversity assessments, demographic monitoring, population viability estimation, species diversity, habitat distribution GIS models, assessments of orchid community interactions, environmental change, orchid extinction risk.

5.3. Research team RT3 – BayCEER Bayreuth, Germany, head: G. Gebauer

The Bayreuth Center of Ecology and Environmental Research (BayCEER) is engaged in bio and geosciences at the University of Bayreuth. The group conducts global change, biodiversity, and ecosystem research and is involved in projects related to the environmental protection of nature. The BayCEER Laboratory of Isotope Biogeochemistry is a leading scientific technical facility equipped with five state-of-the-art isotope ratio mass spectrometers online coupled to a suite of peripheries allowing high-precision C, N, O and H isotope abundance analysis from a broad range of solid, liquid and gaseous samples. The laboratory has long-term expertise in preparation and isotope abundance analysis of plant, fungi, soil and trace gas samples.

Role in the project: isotope analyses of the samples from all sites.

Personnel participating in the project:

Gerhard Gebauer (**P = 115, P5 = 23, C = 4535, H = 41**), conducting research on pedosphere-biosphere-atmosphere interactions for 30 years with a major focus on matter fluxes between ecosystem compartments; biogeochemical and plant ecophysiological processes; interactions between organisms that influence nutrient fluxes in nutrient-limited environments (e.g. carnivory, nitrogen fixation, parasitism, and mycoheterotrophic nutrition of orchids). He established facilities at the University of Bayreuth for stable isotope abundance analysis as a unique and powerful tool to measure matter fluxes between organisms and ecosystem compartments and underlying biogeochemical processes.

Technical Assistant (half-time for 4 years). Technical assistance is required for sample grinding, weighing, data management and help with isotope abundance analysis.

5.4. Research team RT4 – KU Leuven, Belgium, head: H. Jacquemyn

The Laboratory of Plant Conservation and Population Biology, Department of Biology at the Katholieke University of Leuven (Belgium) is part of the Ecology, Evolution and Biodiversity Conservation (EEBC) section, which brings ecology and evolution together. The EEBC combines the fields of ecology, molecular biology, genetics, evolution and statistical modelling. The Plant Conservation and Population Biology laboratory has strong expertise in plant ecology and conservation biology, with current focus on patterns of biodiversity.

The group has all the necessary equipment to perform next-generation sequencing analyses of fungal mycorrhizal communities. This involves a FastPrep-24™ 5G Instrument, Qubit® 3.0 Fluorometer, NanoDrop 2000 UV-Vis Spectrophotometer, several PCR machines and centrifuges.

Role in the project: data collection, DNA analyses of root and substrate samples (see Table 1 for list of their sources).

Personnel participating in the project:

Hans Jacquemyn (**P = 287, P5 = 122, C = 6066, H = 45**), Research Professor at KU Leuven; below- and above-ground biotic interactions in plants, including orchids; effect of mycorrhizal fungi on the distribution, abundance and population dynamics of orchids. He combines the latest developments in high-throughput sequencing with population dynamics models to investigate how variation in mycorrhizal abundance and diversity below the ground structures orchid populations above the ground. He was awarded an ERC Starting grant in 2010 to study specificity in orchid-mycorrhiza interactions and its consequences for orchid fitness.

5.5. Research team RT5 – Univ. Puerto Rico, Humacao and Rio Piedras, Puerto Rico, USA, head: J. Ackerman

Role in the project: data collection, statistical analysis of empirical data, modelling.

Personnel participating in the project:

James D. Ackerman (**P = 95, P5 = 12, C = 2973, H = 30**), Professor and Director of the UPRRP herbarium and zoology museum. He works primarily in floristics, plant reproductive ecology, biogeography and invasive species biology. He specializes on orchids, but also on the biology of mycorrhizae, vegetation characterization, floral fragrance analyses, and taxa other than orchids. World-best expert on Caribbean orchids.

Raymond L. Tremblay (**P = 57, P5 = 15, C = 1223, H = 17**), Professor at the University of Puerto Rico and member of the Graduate Faculty of the sister campus at the University of Puerto Rico at Rio Piedras; population ecology and evolutionary processes, with present emphasis on modeling, data analysis and data visualization; extensive experience working with epiphytic orchids in the Neotropics. Was awarded multiple large grants from USA agencies.

5.6. Research team RT6 – Univ. Tasmania, Australia, head: Nigel Swarts

Role in the project: data collection.

Personnel participating in the project:

Nigel Swarts (**P = 24, P5 = 12, C = 595, H = 11**), Senior Research Fellow in the Tasmanian Institute of Agriculture at the University of Tasmania. He works primarily on horticultural production and physiology of cool climate tree fruit crops. He maintains his research interest in orchid biology and ecology researching the mycorrhizal associations of terrestrial orchids across Australia with a strong focus on conservation and reintroduction.

Renate Faast (**P = 24, P5 = 1, C = 1150, H = 14**), Research Fellow in the School of Biological Sciences at the University of Adelaide. She has over 14 years of ecological research experience including long-term monitoring of orchid population dynamics, vegetation characterization, seed viability and fire ecology.

5.7. Research team RT7 – Lancaster Botanical Garden, San Jose, Costa Rica, head: Franco Pupulin

Role in the project: data collection.

Personnel participating in the project:

Franco Pupulin (**P = 92, P5 = 25, C = 897, H = 11**), Head of Research at the Lankester Botanical Garden Experimental Station (JBL-UCR). He currently coordinates cooperation programs with the University of Florida, USA, Museum of Natural History, UCR, The Universidad della Tuscia, Italy, the Herbariums of Harvard University and the Royal Botanical Garden of Kew, among others. Pupulin received the 2003-2005 Robert Lee Dressler Award from the Charles H. Lankester Foundation. He is Editor-in-Chief of the specialized orchid journal Lankesteriana, world-best expert on Central American mainland orchids.

5.8. Research team RT8 – School of Anthropology and Conservation, Durrell Institute of Conservation Ecology, Univ. Kent, UK, head: Dave Roberts

Personnel participating in the project:

David L. Roberts (**P = 39, P5 = 12, C = 734, H = 16**) Reader in Biodiversity Conservation, Deputy Head of School, Academic Head Conservation Biology. Prior to moving to the University of Kent in 2010, he spent over eight years at the Royal Botanic Gardens, Kew as a senior scientist in the orchid section. During this time, he conducted extensive fieldwork in Africa, Madagascar and the Western Indian Ocean islands. Much of his initial work focused on taxonomy and the uses of museum specimens in relation to conservation, including modelling extinction, phenological responses to climate change and conservation status. During this time, he also held the Hrdy Fellowship in Conservation Biology

at Harvard University. Since moving to the University of Kent, David has continued within these areas of research, as well as moving into areas investigating the wildlife trade and the psychology of species identification.

6. Risk analysis

The risks associated with the project can be divided into four categories:

(1) Unexpectedly low numbers of plants in the study areas, which would prevent statistical analyses. This risk is minimized by using plots, where basic research has already been done, which enables us to focus on sites where there will be an adequate number of individuals to perform the statistical analyses. In addition, if sample sizes are low, we can use Monte Carlo simulations, Robust Estimation (Wilcox 2017) or Bayesian approaches to evaluate our models (McElreath 2016).

(2) Problems with permissions of export of the plant material (especially from the tropical plots) to the labs. This may cause delays in sampling and/or delivering the samples for analyses. Therefore year 4 is considered here as a reserve for such cases.

(3) Problems with mycorrhiza determination. These have been minimized by the choice of laboratories – those three involved in the project have the largest mycorrhizal fungus collections in the world.

(4) Coronavirus, which may prevent between-teams travelling and sampling. This will be prevented by accruing local people for data collections and teleconferencing among individual teams.

7. Literature

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