



Experimental reintroduction and host preference of the microendemic and endangered orchid *Barkeria whartonia* in a Mexican Tropical Dry Forest

Alí Segovia-Rivas, Jorge A. Meave, Edgar J. González, Eduardo A. Pérez-García*

Departamento de Ecología y Recursos Naturales, Facultad de Ciencias, Universidad Nacional Autónoma de México, Circuito Exterior s/n, Ciudad Universitaria, Coyoacán, 04510 Ciudad de México, Mexico



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ABSTRACT

Barkeria whartonia is an epiphytic, microendemic orchid of southern Mexico. This species exclusively inhabits limestone outcrops within a tropical dry forest in Oaxaca State, and its current population size is very small. The goals of this study were to characterize its phorophyte preference, and to assess the success of the experimental reintroduction of young individuals into its habitat. In an area of 15 km² we censused all individuals of this orchid and estimated its area of occupancy. The occurrence frequency on different phorophytes was compared with the estimated densities of potential phorophytes. In July 2014, 76 orchid plants obtained through *in vitro* cultivation were reintroduced by attaching them on the trunks of two phorophyte species, one with rugose bark (*Comocladia engleriana*) and the other with smooth bark (*Plumeria rubra*). In the estimated area of occupancy of this orchid (0.016 km²), we only recorded 254 individuals, 42.4% of which were located on *C. engleriana*, suggesting a strong preference for this phorophyte. In October 2016, 13 reintroduced plants (ca. 17%) were still alive, almost all of which had successfully established. Initial plant size (stem length) emerged as an important driver of future survival. The artificial reintroduction of orchids facing extinction risk into their habitats may represent an efficient way to skip two critical phases in their life cycles, namely seed dispersal and establishment.

1. Introduction

Numerous orchid species are listed under various extinction risk categories (e.g., IUCN, 2017; Semarnat, 2010). Like in many other tropical countries, the uncontrolled extraction of specimens from the wild and the increasing destruction of their habitats are bringing about the decline of the wild populations in many of the 1254 orchid species of Mexico, 40% of which are endemic to this country (Soto-Arenas, Solano Gómez, & Hágsater, 2007). However, less studied and thus little understood endogenous factors may also affect their vulnerability (Dixon & Phillips, 2007), particularly those limiting population size, for example slow growth rates, long life cycles and highly random seed dispersal (Mondragón, Valverde, & Hernández-Apolinar, 2015). Likewise, these plants strongly depend on their biotic interactions, particularly on their pollinators and mycorrhizal fungi, with which they often develop highly specific interactions (Dearnaley, Martos, & Selosse, 2012; Tremblay, Ackerman, Zimmerman, & Calvo, 2005).

Despite the cosmopolitan distribution of Orchidaceae, epiphytic orchids strongly concentrate in tropical and subtropical regions (Benzing, 2008; Cribb, Kell, Dixon, & Barrett, 2003). Besides, their

presence and abundance is also related to the abundance and distribution of their host plants (phorophytes), as in many cases orchids display strong specificity (Migenis & Ackerman, 1993; Otero, Aragón, & Ackerman, 2007; Vergara-Torres, Pacheco-Álvarez, & Flores-Palacios, 2010; Wagner, Mendieta-Leiva, & Zotz, 2015). The establishment probability of epiphytic orchids on a given tree species is related to morphological or chemical traits of the latter, such as bark rugosity, presence of exfoliating bark, pH, nutrient availability, as well as other factors such as tree height, and crown architecture (ter Steege & Cornelissen, 1989; Zimmerman & Olmsted, 1992).

The epiphytic environment offers certain advantages, such as higher light availability, improved exposure to pollinators and reduced herbivory; yet, some disadvantages are also obvious, particularly nutrient and water limitations (Benzing, 2008). Thus, orchids have acquired diverse physiological, morphological, and phenological adaptations to their habitats, which allow them to maximize water acquisition and conservation (Lüttge, 2004; Silvera, Santiago, Cushman, & Winter, 2009). In humid environments (e.g., cloud forests) there are no strong water limitations and thus orchids abound in them, whereas in drier environments orchid abundance and diversity is considerably reduced

Abbreviations: TDF, Tropical Dry Forest; TDFr, Tropical Dry Forest on rock; TDFs, Tropical Dry Forest on soil; XS, Xerophytic Scrub

* Corresponding author.

E-mail addresses: ali_seg@ciencias.unam.mx (A. Segovia-Rivas), jorge.meave@ciencias.unam.mx (J.A. Meave), edgarjgonzalez@ciencias.unam.mx (E.J. González), garcia@ciencias.unam.mx (E.A. Pérez-García).

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(Zimmerman & Olmsted, 1992). Particularly, seasonally dry tropical deciduous forests can be considered marginal environments for orchids, in consistency with the scarcity and patchy distribution of orchids in these habitats.

Germination is one of the most vulnerable phases in an orchid's life cycle (Mondragón et al., 2015). To be successful, a seed must be transported by wind to an adequate microsite and colonized by mycorrhizal fungi. The seedling phase that follows is also challenging because seedlings grow very slowly and they need to display a functional root system to ensure their survival (Rasmussen, Dixon, Jersáková, & Těšitelová, 2015). Seasonally dry tropical ecosystems are characterized by prolonged periods of water shortage, which renders these phases of the life cycle even more difficult. The scanty endosperm in orchid seeds exacerbates this situation, so these plants must necessarily develop mycorrhizal symbioses quickly (Dearnaley et al., 2012). Given the non-directional dispersal of orchid seeds and the complexity of the mycorrhizal interaction, among the thousands of seeds produced by a single capsule only a few seedlings become established. Thus, the artificial reintroduction (sensu Reiter et al., 2016) of young orchid specimens to the field, which implies skipping these critical phases of the life cycle, makes it possible to substantially increase population size. Such goal would be highly desirable for those species facing extinction risk, or for those that require assisted migration due to their vulnerability to global climate change (Dixon & Phillips, 2007; Reina-Rodríguez, Rubiano Mejía, Castro Llanos, & Otero, 2016; Reina-Rodríguez, Rubiano Mejía, Castro Llanos, & Soriano, 2017; Reiter et al., 2016; Yam, Tay, Ang, & Soh, 2011).

Very few studies have attempted to assess reintroduction success for an epiphytic orchid in highly marginal environments such as the tropical dry forest (Parthibhan, Kumar, & Rao, 2015). Such deficiency is critical for the conservation of endangered epiphytic orchids in these habitats, among which *Barkeria whartonian* (C. Schweinf.) Soto-Arenas is a prime example. This attractive micro-endemic species is a long-lived (> 30 years), sympodial epiphytic orchid with slightly thickened stems, up to 70 cm long (Fig. 1A). Its succulent roots are covered with a whitish velamen. Shoot initiation occurs in the early rainy season, and flowering takes place in the transition from the rainy to the dry season. Flowers are relatively small (19–36 mm), with pale pink to lilac color, and they develop successively along the inflorescence (Fig. 1B). Even though its specific pollinator is unknown, the production of capsules and the recruitment of a few young individuals have been recorded.

Barkeria whartonian has a very narrow geographical range, as it is restricted to the southern portion of the Isthmus of Tehuantepec, Oaxaca State, southern Mexico. The species has been only encountered on a few isolated limestone outcrops of the Cordón Range, distributed within a polygon approximately measuring 26 km² in area (this area includes other hills with different geology and miscellaneous vegetation types). Further, this species is noteworthy for its habitat specificity, as it uniquely occurs on the xerophytic vegetation associated to these isolated limestone outcrops. Although the species was initially reported as being mostly rupicolous and seldom epiphytic (Soto-Arenas, 1993), recent observations indicate that it is predominantly epiphytic, frequently growing on *Comocladia engleriana* Loes. (Anacardiaceae) and *Neobuxbaumia scoparia* (Poselg.) Backeb. (Cactaceae) (Pérez-García, 2013). Yet, a formal characterization of preferred host trees for *B. whartonian* is lacking, and neither is it known whether there is a clear preference for a given host plant, or whether its occurrence on certain phorophytes is rather a function of the abundance of the latter.

Through an exhaustive survey of individuals established naturally, in this study we first examined whether *Barkeria whartonian* has a significant preference for a given phorophyte. On the basis of this information, we experimentally assessed survival and establishment success among young plants reintroduced on two phorophyte species contrasting in terms of their bark rugosity: *Comocladia engleriana* Loes. and *Plumeria rubra* L. (Apocynaceae), with rugose and smooth bark, respectively. Because *B. whartonian* occurs naturally more often on *C.*

engleriana, and based on the premise that a rugose bark would potentially retain more water and nutrients, we predicted that reintroduced plants would survive and become established more successfully on this phorophyte than on the host tree with smooth bark.

2. Methods

2.1. Study site

We conducted this study in the surroundings of Nizanda, a village located in the Isthmus of Tehuantepec, Oaxaca state, Mexico (16° 39' N, 95° 00' W; Fig. 2A, B). The climate is tropical sub-humid, with an average total annual precipitation of 902.6 mm, highly concentrated from June to October, and a mean annual temperature of 27.6 °C (<http://clicom-mex.cicese.mx>). Tropical Dry Forest is the most extensive plant formation in the region, although other more localized plant communities also occur (Pérez-García, Meave, Villaseñor, Gallardo-Cruz, & Lebrija-Trejos, 2010). The regional landscape consists mainly of rolling hills with elevations between 100 and 250 m above sea level, with some isolated limestone peaks reaching 520 m (Mount Cerro Verde). Prevailing geology is a metamorphic complex of volcaniclastic and siliciclastic phyllite, which is intermingled with sporadic outcrops of light gray limestone (Pérez-Gutiérrez, Solari, Gómez-Tuena, & Valencia, 2009).

On the hilltops and escarpments of the limestone outcrops, especially in places where limestone is totally exposed, rupicolous xerophytic vegetation occurs that is distinctly different from other local plant communities and hosts a floristic set with a high degree of endemism (Pérez-García & Meave, 2004). This vegetation was subdivided into two types based on the degree of tree cover: Tropical Dry Forest on exposed rock (TDFr) is a community with tree cover > 100%, and Xerophytic Scrub (XS), which has a tree cover < 100% (Fig. 2C). *B. whartonian* is more frequent in TDFr than in XS.

2.2. Estimation of habitat size and population assessment of *Barkeria whartonian*

Although the entire range of *B. whartonian* is circumscribed to a small polygon of ca. 26 km² in area, we were able to thoroughly inspect a heterogeneous polygon of ca. 15 km² around the village of Nizanda; only 1.3 km² of this area correspond to limestone outcrops, whereas the remaining parts are mostly occupied by phyllite hills (Pérez-Gutiérrez et al., 2009). The size of the distribution areas were calculated from the panchromatic band of a GeoEye image acquired on November 10, 2010, which was ortho-corrected with a 0.5 m × 0.5 m resolution with ArcGIS© 10.3.

At Nizanda, *Barkeria whartonian* can be confounded with no other orchid species, thus individuals of this species can be correctly identified even in a non-reproductive state. Because generally these plants do not attain large sizes, are very scarce, and do not display an extensive clonal growth, each clump of stems encountered was considered a different individual. Each individual was classified as either seedling, juvenile or adult, according to its developmental stage, based on the following criteria: (1) seedlings, well-developed individuals with no more than two leaves; (2) juveniles, individuals with stem length > 5 cm, with well-differentiated pseudobulbs, but lacking inflorescences or signs of having produced them; and (3) adults, individuals generally > 15 cm long, and with unambiguous evidence of having produced inflorescences at least once.

2.3. Assessment of phorophyte preference

We determined the species identity of each individual woody plant hosting *Barkeria whartonian* plants (active phorophyte). Also, for each active phorophyte we recorded the number of *B. whartonian* individuals on its trunk and branches, along with their developmental

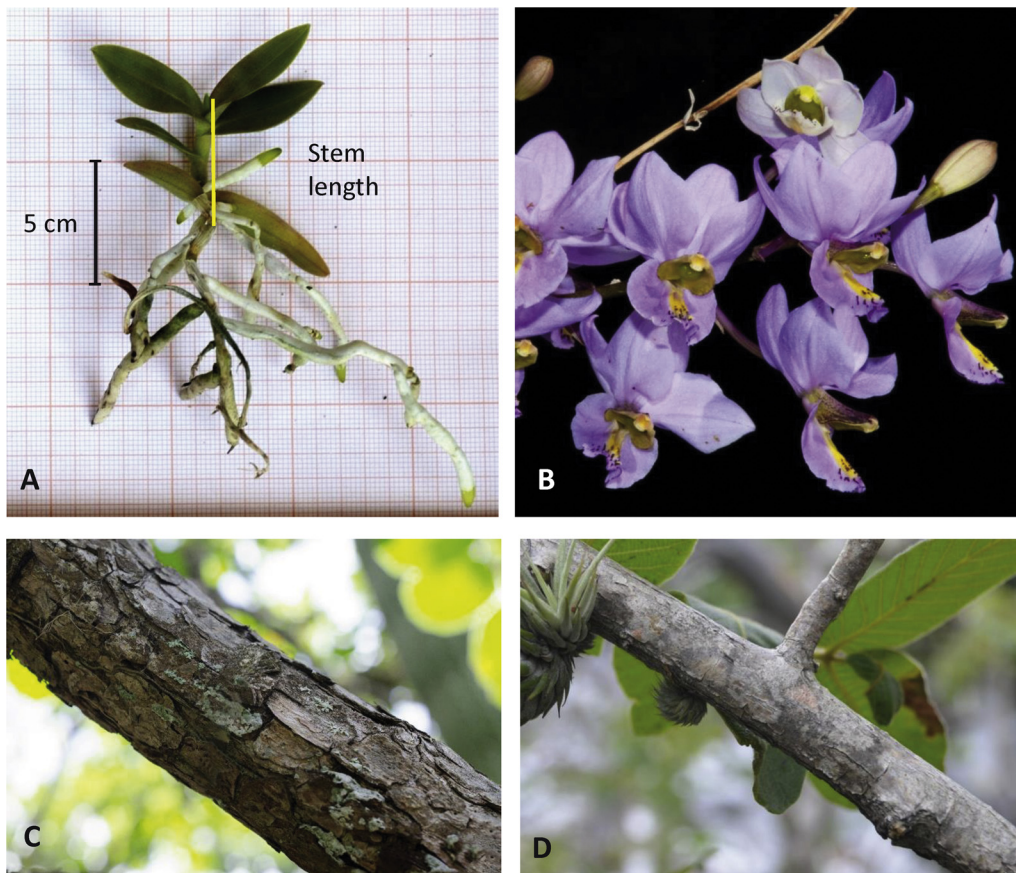


Fig. 1. Morphology of *Barkeria whartonian* and bark of the two experimental phorophytes. (A) Measurement of stem length. (B) Detail of the inflorescence. (C) Rugose bark of *Comocladia engleriana*. (D) Smooth bark of *Plumeria rubra*.

stage. To discriminate between true host specificity for *B. whartonian* from a presence of this orchid unrelated to host identity (i.e., presence probability driven by local host abundance), we used a Spearman rank correlation between the densities of woody species occurring in the TDFr and the number of phorophytes actually occupied by *B. whartonian*. Potential number of phorophytes in this orchid's habitat was estimated from available information on TDFr vegetation structure, gathered in nine 10 m × 10 m-sampling plots (Pérez-García & Meave, 2004). Density of woody species with two or more individuals recorded in such sampling area (900 m²) was extrapolated to the total area in which *B. whartonian* was found (0.016 km²).

2.4. Survival experiment

A total of 76 young plants of *Barkeria whartonian* were reintroduced, all of which shared a provenance from a single capsule from Nizanda. Seeds were germinated at the same time *in vitro* in Murashige-Skoog (MS, 1962) medium, to which we added glycine (2 mg/l), inositol (100 mg/l), and sucrose (30 g/l); pH was adjusted to 5.7 and then gerlite was added (3.7 g/l). Seedlings were grown in the Laboratory of Plant Tissue Cultivation of the Botanical Garden, Instituto de Biología, Universidad Nacional Autónoma de México; they were kept in the flasks until they produced leaves and roots (Villafuerte, 2013). Next, individuals were removed from the flasks and hardened during one year and a half in a greenhouse in Mexico City. Prior to reintroduction, for each individual we measured its stem length (the distance from the base of the pseudobulb to the apical meristem or the point where the growth of the most recent leaf began). Moreover, we took photographs for each individual placed on scale paper for later reference of size and shape (Fig. 1A). Each plant was individually labeled with numbers used for the random assignment to the experimental treatments.

We reintroduced young plants in pairs on the surface of two phorophytes with contrasting barks. Each pair included one plant placed on *Comocladia engleriana* (rugose bark; Fig. 1C) and another one on *Plumeria rubra* (smooth bark; Fig. 1D); the plants of one such pair were placed on trees that were very close to each other (< 1.5 m), on branches of similar size, at the same height, and with the same orientation. Reintroduced plants were attached to their foster phorophytes with plastic-covered twist wire, and these twist wires were removed after two years when the orchids were established. Additionally, we attached a finger pinch of moist *Sphagnum* moss at the base of each plant with thin hemp thread. We used the same *Sphagnum* on which *B. whartonian* grew in the greenhouse, in order to keep the roots moist and to maintain the pH of their former substrate. We watered the plants only once immediately after reintroduction; thereafter they only received rainwater. Within a single limestone hill, we selected three almost contiguous areas for the reintroduction experiment, with 30 individuals in Site A, 18 in Site B, and 28 in Site C.

Reintroduction of *Barkeria whartonian* plants took place in late July 2014, at the onset of the rainy season in that particularly dry year. Thereafter, plants were monitored in September (mid-rainy season) and December of the same year, and in March (dry season) and August (the subsequent rainy season) 2015. The final census was done one year later, in October 2016. A plant was considered established when its roots were attached to the phorophyte. To tally a plant as a survival case, at least some part of the stem needed to be alive and a part of the root system needed to be turgid.

2.5. Statistical analyses

During the length of the experiment we assessed survival and establishment success of the reintroduced plants. For each of these

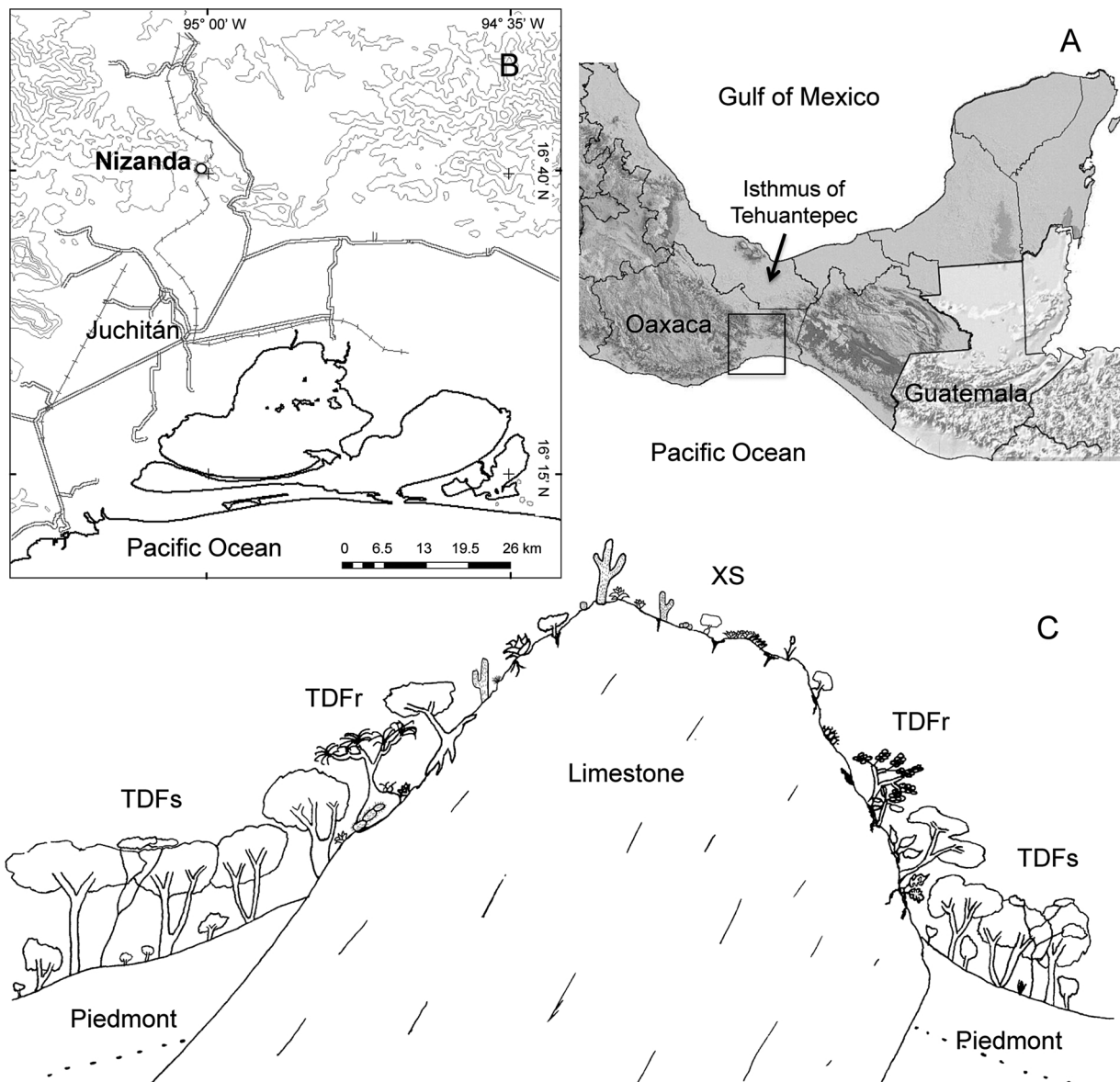


Fig. 2. Location of study site. (A) Oaxaca state in southern Mexico. (B) Nizanda in the Isthmus of Tehuantepec region. (C) Diagrammatic profile of *Barkeria whartonianiana*'s habitat in the Tropical Deciduous Forest on rock (TDFr), amidst the Tropical Deciduous Forest on soil (TDFs) and the Xerophytic Scrub (XS); modified from Pérez-García and Meave (2004).

processes we constructed four generalized linear mixed models (GLMMs, with binomial error, logit link function, and Site [A–C] and plant pair as nested random effects): Model 0, null model with no explanatory variable included; Model 1, with phorophyte identity (*Plumeria rubra* or *Comocladia engleriana*) as explanatory variable; Model 2, with stem length as explanatory variable; and Model 3, including these two latter variables. Akaike Information Criterion (AIC) was used for selecting among these competing models; models having the lowest AIC were considered the best-supported ones, and these were used as reference for calculation of ΔAIC . Curves depicting survival and establishment along time were constructed through FMM (Forsythe, Malcolm, & Moler, 1977) and natural spline interpolation, respectively. All analyses were conducted in R (R Core Team, 2017), using the lme4 package (Bates, Maechler, Bolker, & Walker, 2015).

3. Results

3.1. Distribution and population size

Our field survey showed that in the Nizanda region, *Barkeria whartonianiana*'s range is highly restricted; in the entire area covered by the limestone outcrops (1.3 km²), we only found individuals in seven clusters that together occupied an effective area of 0.016 km² (defined as *B. whartonianiana*'s realized habitat). Also, we confirmed the extreme scarcity of this species, as we only recorded 254 individuals growing in the wild. A conservative estimate of the total population size for this species is < 1000 individuals in the wild. A large proportion of recorded individuals (242 individuals, 95.3%) grew on phorophytes (six tree species and three columnar cactus species), while only 12 individuals grew directly on the substrate (nine on limestone rock covered with a thin soil layer, and three on exposed limestone).

We estimated a total number of ca. 5570 woody arborescent plants (potential phorophytes) in *Barkeria whartonianiana*'s realized habitat (0.016 km²), a figure that is equivalent to a phorophyte density of 3481

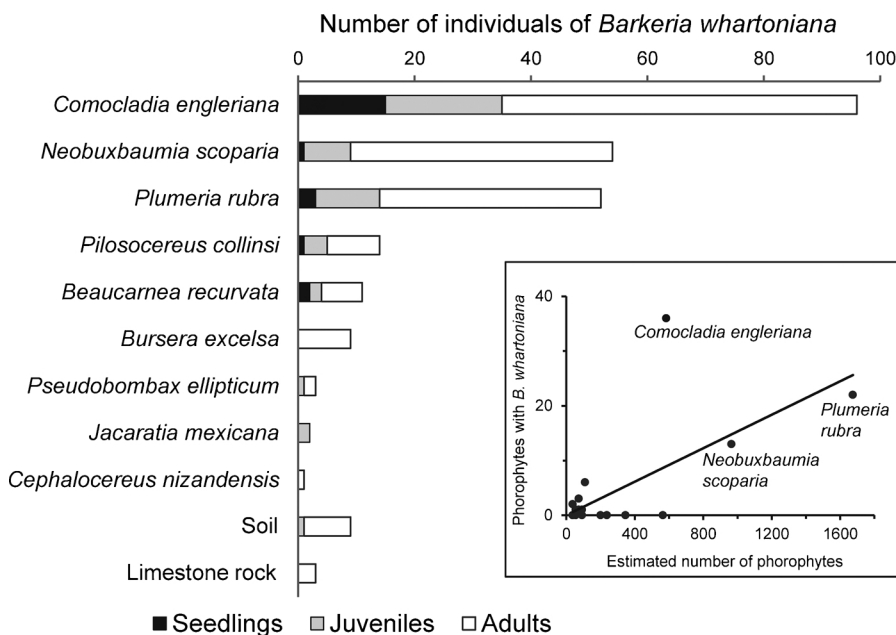


Fig. 3. Stage structure of *Barkeria whartonian* individuals on its different phorophytes and abiotic substrates. The correlation between the number of trees per species hosting *B. whartonian* individuals and the estimated number of trees in the area covered by Tropical Dry Forest on rock (inset) shows the disproportionate number of *Comocladia engleriana* trees hosting this orchid.

ind./ha, but we only recorded individuals of this orchid on 85 phorophytes, 36 of which were *Comocladia engleriana* (42.4%) and 22 *Plumeria rubra* (25.9%; Fig. 3). The mean (\pm SD) number of *B. whartonian*'s individuals per phorophyte was 2.9 ± 1.04 (range, 1–13 plants per phorophyte); only about 15% of all recorded individuals grew alone in their host tree.

3.2. Phorophyte preference

The assessment of phorophyte preference in *Barkeria whartonian* was based on the 242 individuals that grew as epiphytes. Around 40% of these plants occurred on *Comocladia engleriana* trees, almost two-fold the fractions recorded for the two other important phorophytes, namely *Plumeria rubra* (22.3%) and *Neobuxbaumia scoparia* (21.5%) (Fig. 3). These three most common phorophytes of *B. whartonian*, both according to the orchid's frequency and abundance, were the most common arborescent species on the limestone outcrops; however, their ranking according to their contribution to community structure is not the same as phorophytes, as in this forest there are almost three times as many individuals of *P. rubra* than of *C. engleriana* (Fig. 3, inset; Appendix 1). Other relatively frequent tree species in the TDFr did not host any *B. whartonian* (e.g., *Bursera* spp., *Pseudosmodium multifolium*, and *Gyrocarpus jatrophifolius*). The estimated tree density in the limestone outcrops was significantly correlated with the number of phorophytes of each species actually occupied by *B. whartonian* ($r_s = 0.477$, $P = 0.0159$; Fig. 3, inset); yet, the number of individuals of this orchid growing on *C. engleriana* trees seems to be larger than expected at random.

Population structure of *Barkeria whartonian* ($N =$ all 254 individuals) was as follows: 8.6% (22 individuals) were classified as seedlings, 19.3% (49) as juveniles, and the remaining 72% (183) as adults (Fig. 3). In spite of the few *B. whartonian* plants observed growing on rock or soil, we failed to record a single orchid seedling on these substrates. Both in absolute and relative numbers, there were more seedlings on *Comocladia engleriana* (15.6% of all individuals hosted by this species were seedlings), compared to *Plumeria rubra* and *Neobuxbaumia scoparia*, which hosted 5.7% and 1.8% of *B. whartonian* seedlings, respectively.

3.3. Survival and establishment of reintroduced individuals

Of the 76 *Barkeria whartonian* individuals reintroduced to their habitat, only 15 (20%) had survived by the end of the first year, ten of them on *Plumeria rubra* and five on *Comocladia engleriana*. By October 2016, two years after the initial reintroduction, this number decreased to 13 individuals in total (17%), eight on *P. rubra* ($8/38 = 21.1\%$) and five on *C. engleriana* ($5/38 = 13.2\%$; Fig. 4A, B). Mortality decreased from 80% in the first year to 14% in the second. The highest mortality occurred during the first three months of the experiment, a period in which 70% of the reintroduced set of plants died. Interestingly, practically all individuals that survived by the end of the experiment had established successfully at this time (Fig. 4C, D).

At the beginning of the experiment, the mean (\pm SD) length of the main stem in reintroduced plants of *Barkeria whartonian* was 34.5 ± 18.20 mm, whereas plants that survived until the end of the experiment had a mean (\pm SD) initial size of 47.3 ± 18.14 mm. In accordance with this observation, those GLMMs that included plant's initial size (i.e. stem length) performed better in explaining *B. whartonian*'s survival and establishment success (Model 2) than those that excluded this variable (Table 1).

4. Discussion

Micro-endemic species with very small populations pose enormous challenges for conservation biologists. Such challenges become more arduous when focal species are insufficiently known and when they display additional complications in their life cycles, as is the case of the orchid studied by us. *Barkeria whartonian* is entirely constrained to a few limestone outcrops occurring across a very small area, with an epiphytic habit on a few phorophytes, and grows in an environment as marginal for the epiphytic plant life as the Tropical Dry Forest. Moreover, the exploitation of limestone rock and the increasing deforestation in the area represent potential threats for this orchid. In this context, our study is pioneer for an orchid species under these circumstances, which additionally combines observational and experimental approaches.

4.1. Phorophyte preference

Considering that 95% of the population of this species has an

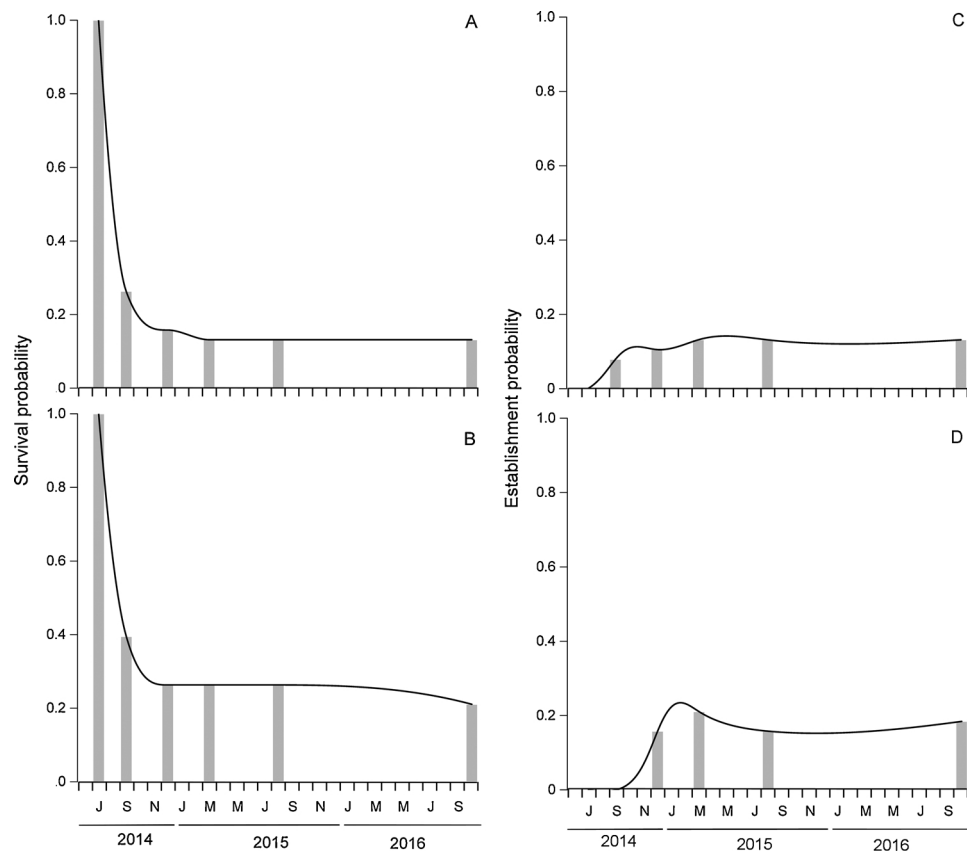


Fig. 4. Survival (A, B) and establishment (C, D) probabilities of *Barkeria whartonianiana* over the 2-yr monitoring period on two phorophytes: *Comocladia engleriana* (A, C); *Plumeria rubra* (B, D). Bars are observed probabilities and lines are spline-interpolated probabilities.

Table 1
Selection procedure among the four competing models constructed for survival and establishment probabilities of experimentally reintroduced *Barkeria whartonianiana* individuals on two phorophytes (*Plumeria rubra* and *Comocladia engleriana*). The analysis was based on the data recorded at the end of the 2-yr experiment (October 2016). (Site/Pair) refers to the specific site and pair of plants introduced close to each other, forming an experimental unit (see text for details).

Model	Explanatory variables	Survival ΔAIC	Establishment ΔAIC
0	1 + (Site/Pair)	4.79	6.79
1	Phorophyte + (Site/Pair)	5.80	8.29
2	Stem length + (Site/Pair)	0	0
3	Phorophyte + Stem length + (Site/Pair)	1.52	1.90

epiphytic growth habit, and that no single seedling has been observed growing on the soil (Fig. 3), assessing phorophyte preference is crucial, as this may help explain the causes of its narrow endemic character (Migenis & Ackerman, 1993; Vergara-Torres et al., 2010). The majority (80%) of *Barkeria whartonianiana* individuals concentrate on three phorophyte species, namely *Comocladia engleriana*, *Neobuxbaumia scoparia*, and *Plumeria rubra*. The former two species are very abundant in, and practically exclusive of, limestone outcrops, whereas *P. rubra* is also frequent in the TDF on phyllite that makes up the landscape matrix in Nizanda (Pérez-García & Meave, 2004). Moreover, some *B. whartonianiana* individuals were observed growing on *Pilosocereus collinsii*, *Bursera excelsa* and *Jacaratia mexicana*; although these species are also abundant in the TDF on phyllite, no single individual of these species growing on this parental rock was ever observed to host *B. whartonianiana* specimens (and the same is true for *P. rubra*). In fact, we only encountered

individuals of this orchid within the limestone outcrops but never on trees growing beyond areas of exposed rock growing on the same hill, a few meters away. These results point to parental rock as a key factor in the spatial distribution of this orchid species, although the precise causes determining such specificity are far from understood.

If parent material were the only driver of the spatial distribution of *Barkeria whartonianiana*, one could reasonably expect a strong correlation between the abundance of phorophytes growing on the proper substrate and the abundance of *B. whartonianiana*. While we actually found such positive correlation, it was not very strong ($r_s = 0.477$). A likely explanation for this weak correlation is the high abundance of several very common tree species in the TDFr, such as *Bursera simaruba*, *B. ovalifolia*, *Pseudosmodium multifolium*, and *Gyrocarpus jatrophiifolius*, none of which hosted a single individual of *B. whartonianiana*. Apparently, this correlation was largely determined by the relationship between the estimated abundances of *P. rubra* and *Neobuxbaumia scoparia*, and the number of trees of those species that hosted *B. whartonianiana* plants, as well as by the clear over-representation of *B. whartonianiana* on *Comocladia engleriana*. That the number of *B. whartonianiana* plants recorded on *C. engleriana* was larger than expected at random suggests a clear preference of this orchid for this phorophyte. Notably, *C. engleriana* trees hosted the largest numbers of *B. whartonianiana* seedlings and juveniles; interestingly, these two categories were also well represented on *Beaucarnea recurvata* (Asparagaceae), an arborescent plant that also has a very rugose and scaly bark. Therefore, it seems that rugged barks favour seed adherence compared to smooth barks (Benzing, 2008).

Phorophyte preference is a recurrent topic of debate in the field of epiphytic orchid ecology. Various studies have reported a lack of specificity for a particular phorophyte (Migenis & Ackerman, 1993; Wagner et al., 2015; Zimmerman & Olmsted, 1992), and there is unquestionable evidence that some epiphytic orchids can survive even on introduced (alien) tree species and in secondary vegetation stands

where pioneer trees dominate (Sosa & Platas, 1998). In strong contrast, our study showed that despite the lack of a one-to-one specificity, the effective number of host species for *B. whartonian* is indeed very limited. Such host specificity, along with its extremely narrow habitat specificity, may explain the restricted distribution of this species. Admittedly, however, other factors are also likely to influence this species' narrow spatial and ecological distribution, such as facilitation and dispersal limitations, as > 60% of all individuals shared their host tree with at least one more conspecific plant.

4.2. Reintroduction experiment

One of the most striking results of the reintroduction experiment was the very high mortality (almost 80%) concentrated in the first two months of the experiment on both phorophytes. This early high mortality was surprising, considering the fact that the reintroduction of young plants (but well beyond the seedling stage) results in the evasion of strong demographic filters like seed dispersal, seed germination, and seedling establishment, where mortality is highly concentrated in epiphytic plants (Ávila-Díaz, Oyama, Gómez-Alonso, & Salgado-Garciglia, 2009; Mondragón et al., 2015). Our result could be due to insufficient hardening of the plants, as they grew in a greenhouse in Mexico City (2200 m above sea level), not in Nizanda (< 200 m above sea level), although a more likely explanation is that 2014 was particularly dry, with a late onset of the rainy season. In addition to the marked rainfall seasonality, in the study region there is a large inter-annual variation in total precipitation, which is largely unpredictable (data from Ciudad Ixtepec for the 1948–2014 period; <http://clicom-mex.cicese.mx>). Future reintroduction efforts in this type of seasonal ecosystem will necessarily have to deal with such uncertainty. Interestingly, the dry season did not seem to have an important negative effect on the subsequent survival of the remaining specimens, as plants that were alive at the end of the rainy season continued to be alive at the beginning of the next wet season.

In this experiment, our ultimate intention was to successfully reintroduce a number as large as possible of *Barkeria whartonian* individuals into its habitat, with the aim of contributing to the conservation of the species. To this end, for the reintroduction experiment we selected two phorophytes on which this orchid seemed to be quite successful. Although bark rugosity was the most obvious difference between them, other factors may also be involved, such as differences in bark's pH and physico-chemical properties, radiation level through their crowns, throughfall/stemflow ratio, and even in the presence of mycorrhizal fungi (Hirata, Kamijo, & Saito, 2009; Yam et al., 2011; Zotz & Winkler, 2013). Contrary to our initial expectations, the experiment failed to show a better performance of our focal orchid on *Comocladia engleriana*, while it revealed that both the establishment and survival of this orchid are mostly driven by vigour of reintroduced plants (i.e., initial plant size, Model 2). An implication of this result is that increasing survival of reintroduced plants may be possible through the production of bigger plants with better-developed roots (Raventós, González, Mújica, & Doak, 2015; Reiter et al., 2016; Yam et al., 2011). Yet, in a seasonally dry tropical environment this strategy would imply waiting at least one more year due to the short duration of the rainy period, with the concomitant costs of plant maintenance in a greenhouse.

At first glance, these results are in contradiction with the clear phorophyte preference observed in the wild. However, these two conclusions can be reconciled. On the basis of our results, we can argue that the initial attachment of the seed to the phorophyte's bark, but more importantly, the establishment of recently-germinated plants, are the most critical phases for this orchid species (Rasmussen et al., 2015); once a given specimen overcomes them, its survival probability increases substantially. Therefore, it is possible that these two phases are

less critical on *Comocladia engleriana*, an issue not resolved by our experiment and which would explain the observed preference for this host in the field, and that the artificial attachment of the young plants to their hosts is not the same as the natural establishment of the plants.

Based on the experience gained in this experiment, we are now able to make the following recommendations that may increase the rate of success in future efforts of orchid reintroduction in tropical dry forests: (1) use plants as big as possible and with well developed root systems (in orchids lacking true pseudobulbs like our study species, care must be exerted to avoid root damage while handling the specimens); (2) increase the hardening time under local environmental conditions before placing the plants at its final location; (3) perform the reintroduction only after the onset of the rains, and if possible water the plants artificially if rain is scarce; (4) if possible, place the reintroduced plants in the vicinity of naturally established individuals, as the latter provide evidence of suitable micro-environmental conditions at the location; and (5) spread the reintroduction of the plants over several years in order to better cope with the inter-annual stochastic variation in precipitation, which is typical of these systems (Murphy & Lugo, 1995).

4.3. Final remarks

Two years after the reintroduction of *Barkeria whartonian* plants into their habitat, only 17% of them had survived. A review on the success of reintroduced young plants revealed that, on average, survival after the first year is ca. 66% (Reiter et al., 2016), and in multi-year projects survival values as high as 80% have been recorded, mainly in humid forests (Yam et al., 2011). However, it is likely that survival in dry forests could be much lower; for example, a survival of only 12% was obtained for plants of *Dendrobium aqueum* in a forest with a three-month drought period in Kolli Hills, India (Parthibhan et al., 2015). Thus, the survival found in this study of 20% after two years is not so low. Further, if we take into account that the total number of plants of this species recorded by us in the region is 254, the successful reintroduction of 13 new individuals in a first attempt is not negligible, as it represents a considerable increase (5.1%) in the size of the extant population. Admittedly, longer assessment periods are needed to draw sounder conclusions on the success of reintroduction efforts (at least 4 years; Godefroid et al., 2011).

The reduction of natural ecosystems derived from increasing deforestation and the associated isolation of vulnerable species makes them face increased extinction risks (Sosa & Platas, 1998). The prospect is even more sombre when the expected effects of global change are added to this problem. Thus it is necessary to develop better protocols that ensure successful reintroductions of plant species into their natural habitats; these protocols will probably also prove very useful in efforts of assisted migration (Hoegh-Guldberg et al., 2008). Undoubtedly, these strategies are always associated with some risks; yet, they may be the only way to guarantee the future persistence of those species that represent the most rare components of biodiversity.

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Appendix 1. Checklist of the most common species in the Tropical Dry Forest on limestone rock in the surroundings of Nizanda, Oaxaca, Mexico. The numbers are their recorded abundances in nine 100-m² sampling plots (Pérez-García & Meave, 2004) (N_{obs}); their estimated abundances in the area of occupancy (0.016 km²) of *Barkeria whartonian* (N_{est}); and the observed number of trees hosting *B. whartonian* plants (NT_{Bw}).

Species	Family	N _{obs}	N _{est}	NT _{Bw}
<i>Plumeria rubra</i> L.	Apocynaceae	92	1674	22
<i>Neobuxbaumia scoparia</i> (Poselg.) Backeb.	Cactaceae	53	964	13
<i>Comocladia engleriana</i> Loes.	Anacardiaceae	32	582	36
<i>Bursera</i> spp. *	Burseraceae	31	564	0
<i>Pseudosmodium multifolium</i> Rose	Anacardiaceae	19	346	0
<i>Gyrocarpus jatrophifolius</i> Domin	Hernandiaceae	13	237	0
<i>Cnidococcus</i> sp.	Euphorbiaceae	11	200	0
<i>Pilosocereus collinsii</i> (Britton & Rose) Byles & G.D. Rowley	Cactaceae	6	109	6
<i>Bursera schlechtendalii</i> Engl.	Burseraceae	5	91	0
<i>Pseudobombax ellipticum</i> (Kunth) Dugand	Malvaceae	5	91	1
<i>Beaucarnea recurvata</i> Lem.	Asparagaceae	4	73	3
<i>Jacaratia mexicana</i> A.DC.	Caricaceae	4	73	1
<i>Cephalocereus nizandensis</i> (Bravo et T.MacDoug.) Buxb.	Cactaceae	3	55	1
<i>Ficus petiolaris</i> Kunth	Moraceae	3	55	0
<i>Hippomane mancinella</i> L.	Euphorbiaceae	3	55	0
<i>Lysiloma microphyllum</i> Benth.	Fabaceae	3	55	0
<i>Spondias purpurea</i> L.	Anacardiaceae	3	55	0
<i>Bunchosia canescens</i> (Aiton) DC.	Malpighiaceae	2	36	0
<i>Bursera excelsa</i> (Kunth) Engl.	Burseraceae	2	36	2
<i>Casearia tremula</i> (Griseb.) Griseb. ex C. Wright	Salicaceae	2	36	0
<i>Ceiba aesculifolia</i> (Kunth) Britten & Baker f.	Malvaceae	2	36	0
Leguminosae sp.	Leguminosae	2	36	0
<i>Pachycereus pecten-aboriginum</i> (Engelm. ex S.Watson) Britton & Rose	Cactaceae	2	36	0
<i>Euphorbia schlechtendalii</i> Boiss.	Euphorbiaceae	2	36	0
<i>Roldana eriophylla</i> (Greenm.) H.Rob. & Brettell	Asteraceae	2	36	0

**Bursera* spp. includes *B. simaruba* (L.) Sarg. and *B. ovalifolia* (Schltdl.) Engl., which could not be differentiated in the field.

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