

HOST SUITABILITY FOR GERMINATION DIFFERS FROM THAT OF LATER STAGES OF DEVELOPMENT IN A RARE EPIPHYTIC ORCHID

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ABSTRACT. We explore phorophyte suitability for germination and establishment of the epiphytic orchid, *Psychilis kraenzlinii*. We found that the orchid grows on a subset of the available tree species and shows preference for the endemic *Machaonia portoricensis* (Rubiaceae). The orchid preferred trees with smoother bark, high water holding capacity and low water retention capacity. Microclimatic conditions under which embryos began pre-germination stages mirrored that of the adult orchid, but germination did not, suggesting that suitable germination sites are not necessarily the best sites for later stages of development.

RESUMEN. Exploramos la utilización de árboles y los patrones de germinación de la orquídea epífita, *Psychilis kraenzlinii*. Encontramos que *P. kraenzlinii* crece en un subconjunto de las especies de árboles disponibles y muestra preferencia por la endémica *Machaonia portoricensis*. La orquídea prefiere árboles con corteza lisa y alta capacidad de sostener agua y baja capacidad de retención de agua. Las condiciones microclimáticas bajo las cuáles los embriones empiezan etapas pre-germinación, reflejan los de la orquídea adulta, pero las condiciones bajo las cuáles los embriones llegan a etapas de germinación no. Lo que sugiere que los sitios de germinación adecuados no son necesariamente los lugares donde mejor se producirá el desarrollo a etapas más avanzadas de la germinación.

KEYWORDS / PALABRAS CLAVE: community dynamics, dinámica comunitaria, dinámica poblacional, epiphytes, epífitas, forófitos, germinación, germination, phorophytes, population dynamics, Orchidaceae

Introduction. Orchids are generally characterized by small, scattered populations (Ackerman 1986, Tremblay 1997), making many species vulnerable to deforestation, habitat fragmentation, and illegal collection (Adhikari & Fischer 2011). Factors of paramount importance that limit orchid abundance and distribution are believed to be pollinator availability and its influence on seed production (Ackerman *et al.* 1996), and OMF availability, which may be microsite-limited (Izuddin *et al.* 2019a, 2019b, Otero & Flanagan 2006). Because of their highly variable and important symbiotic relationships, orchid conservation and management strategies might need to be developed individually for genera or even species and include the entire communities in which they occur (Fay 2018, Phillips *et al.* 2020, Rasmussen *et al.* 2015).

Approximately 70% of orchid species are epiphytes, accounting for approximately 72% of epiphyte species in the world (Gentry & Dodson 1987, Graven-deel *et al.* 2004). Epiphytic orchid conservation and management techniques may include the protection of suitable and existing phorophytes, as well as planting new ones (Adhikari & Fischer 2011). While our knowledge of the relationship between epiphytes and phorophytes has advanced, relatively few epiphytic species have been studied in detail (e.g., Benzing 1990, Gowland *et al.* 2011, Sáyo *et al.* 2013, Zotz *et al.* 2021) yet we do know that phorophyte specificity is rare. Still, some degree of preference is commonly found within sites (Gowland *et al.* 2011, Laube & Zotz 2006, Migenis & Ackerman 1993, Sulist 1950, 1953, Trapnell & Hamrick 2006, Tremblay *et al.* 1998, Wagner *et al.*

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2015). On the other hand, among sites, Hietz & Hietz-Seifert (1995) found epiphyte community composition was more closely associated with elevation rather than the availability of particular phorophyte species.

The epiphytic environment is in constant change, as host trees grow and age. Changes in the crown of the tree, for example, affect radiation, temperature, and humidity along the entire tree (Benzing 1979, 2004, Rasmussen & Rasmussen 2018). Physical and chemical characteristics of the bark can also affect the presence of mycorrhizal fungi, probability of seed attachment, germination and/or establishment (Frei & Dodson 1972, Sáyago *et al.* 2013, Siaz-Torres *et al.* 2021). Bark traits that may affect the presence of epiphytes include rugosity (which might be affected by age), water storage capacity (that could be affected by bark rugosity), pH, and secondary metabolites (Adhikari & Fisher 2011, Frei 1973, Frei & Dodson 1972, Migenis & Ackerman 1993, Sáyago *et al.* 2013, Siaz-Torres *et al.* 2021, Timsina *et al.* 2016). Here we study an epiphytic orchid endemic to Puerto Rico, *Psychilis kraenzlinii* (Bello) Sauleda. The genus *Psychilis* is composed of 15 epiphytic species that are distributed among Hispaniola, Puerto Rico, the US and British Virgin Islands, and Northern Lesser Antilles (Ackerman & Collaborators 2014, Sauleda 1988). The genus is severely understudied, lacking conservation and management strategies for most species. The present study uses a population of *P. kraenzlinii* in the Susúa State Forest as a model to explore the relationship of orchids with their phorophytes (González-Orellana *et al.* 2022).

First, we asked if *P. kraenzlinii* shows phorophyte preferences and whether these preferences correspond to where germination occurs most frequently. Like the closely related species, *Psychilis monensis* Sauleda (Otero *et al.* 2007), *Psychilis krugii* (Bello) Sauleda (Ackerman *et al.* 1989), and *Psychilis truncata* (Cabrera-García *et al.* 2023), we expected *P. kraenzlinii* will not be host-specific and would instead show a preference for a subset of the available phorophyte species. Using different taxa as phorophytes could be advantageous for epiphytes as the epiphytic habitat is a stressful one and constantly changing (Benzing 1979, Trapnell & Hamrick 2006, Tremblay *et al.* 2006). We also hypothesized that seed germination would mirror phorophyte associations of established epiphytic

orchids since one may assume that, like terrestrial orchids, the presence of an established orchid can be an indicator of suitable environmental conditions and OMF availability (Jacquemyn *et al.* 2007, McCormick *et al.* 2016, Petrolli *et al.* 2021).

Secondly, we explored other factors that may affect germination and establishment of *P. kraenzlinii* in the Susúa State Forest. We measured Water Storage Capacity (WSC) and bark roughness of phorophytes to determine whether these traits differed among phorophyte species and between trees with and without the orchid. Epiphytes are prone to be water stressed (Benzing 2004). Rough-barked trees are generally colonized more frequently by epiphytes (Callaway *et al.* 2002) perhaps due to better water retention capacity or because seeds more readily attach to them (Adhikari & Fisher 2011, Timsina *et al.* 2016). Consequently, we expected to find higher seed germination rates and more orchids on phorophytes with high roughness and water retention capacity.

Finally, we used the germination stages of seeds as a proxy for the presence of orchid mycorrhizal fungi (OMF) on phorophytes. Orchid seed imbibition must occur before mycorrhizal infection (Bidartondo 2005, Rasmussen 1995). Imbibition is indicated when the embryo swells and breaks the seed testa (Brandner 2005). Afterwards, fungal infection can occur, which leads to the uptake of nutrients by the plant making cell division and growth possible (Arditti 1992, Rasmussen 1995). Hence, we assumed that seeds that reached germination were infected by their OMF. If orchids and their OMF share similar niche requirements (Izuddin *et al.* 2019a, 2019b), then we expect that protocorm formation will be more likely on phorophyte species that have a higher occurrence of established orchids.

Materials and methods. *Study system*—*Psychilis kraenzlinii* is a rewardless, self-incompatible epiphyte that produces long, erect peduncles topped by racemes of sequentially produced red-carmine flowers (Ackerman & Collaborators 2014). Populations flower and set fruit throughout the year, but studies done on the closely related species *P. krugii* and *P. monensis* (Ackerman *et al.* 1989, Aragón & Ackerman 2004, Otero *et al.* 2007) suggest that peak flowering occurs from April through July. *Psychilis kraenzlinii* resides in the limestone hills and margins of mangrove swamps on

the north side of the island, and in tropical moist forest regions on the southern slopes of the Cordillera Central. Although it is widely distributed across the island of Puerto Rico, many populations are now believed to be extinct due to habitat destruction through anthropogenic activities such as deforestation, limestone mining and urbanization. Populations have also been severely affected by legal and illegal collection. There are no published ecological studies on this species, but it was classified as vulnerable by Miller *et al.* (2013).

Study site—. Susúa State Forest is a Natural Reserve under the jurisdiction of the Department of Natural and Environmental Resources of Puerto Rico. The forest occupies about 13 km² across the municipalities of Yauco and Sabana Grande (18°04'14.6" N 66°54'23.4" W), on the southwestern slope of the Cordillera Central (Departamento de Recursos Naturales y Ambientales 2015). This moist forest is characterized by serpentine and volcanic soils, and has 157 tree species, 16 of which are classified as rare or endangered. Average annual precipitation is 1413 mm and average temperature is 23.9°C. Before the establishment of the State Forest in 1935, the area was almost completely deforested for agriculture, wood products, and minerals (DRNA 2015). The combination of secondary growth and nutrient-poor ultramafic soils has resulted in a mostly evergreen forest comprised of slender trees averaging 12 m tall, with a light canopy (Miller & Lugo 2009).

Phorophyte Specificity Assessment—. The study site consisted of a single population in one area of the forest. To cover as much of the area as possible, we established four 15 × 5 m plots at approximately 5 m from each other. Trees and shrubs inside the plots were identified, DBH was measured, and we noted if they had *P. kraenzlinii*. All *P. kraenzlinii* plants inside plots were tagged. Given the small sample size, for the analysis we filtered the data and kept only the tree species that had a frequency higher than 3%. We applied a Fisher's Exact Test to see if there was a relationship between each tree species and the presence of the orchid.

Phorophyte Physical Characteristics—. The two physical characteristics of the bark that we considered were Water Storage Capacity (WSC) and Fissuring Index (FI), both of which influenced orchid host tree prefer-

ences in Mexico (Zarate-García *et al.* 2020). The tree species from which we collected bark data were chosen based on the Phorophyte Specificity Assessment described previously to create a gradient from positive to negative relationship as follows: *Machaonia portoricensis* Baill. (Rubiaceae), *Phyllanthus cuneifolius* (Britton) Croizat (Phyllanthaceae), *Ouratea littoralis* Urb. (Ochnaceae), *Rondeletia inermis* (Spreng.) Krug & Urb. (Rubiaceae), *Tabebuia haemantha* (Bertol. Ex Spreng.) DC. (Bignoniaceae), *Swietenia mahagoni* (L.) Jacq. (Meliaceae) and *Coccoloba microstachya* Willd. (Polygonaceae). Because most orchids grew attached to phorophytes at a height below 0.75 m, we collected bark samples no higher than that. When possible, half the samples were collected near the roots of adult orchids, and half from trees where the orchid was absent.

Water Storage Capacity Assessment (WSC)—. To measure WSC we adapted the methodologies described by Callaway *et al.* (2002) and Zarate-García *et al.* (2020). In the laboratory, samples were cut to approximately 1 cm² and dried in an oven at 40°C. Drying time fluctuated between 24 h and 72 h for each species, since bark rugosity and thickness of the bark varies among species. After drying, samples were weighed to obtain *dry mass* and their length, width, and thickness was measured with a caliper to calculate volume. We then submerged the samples in water treated with Triton X-100 for 30 min, allowed to drip for a minute and weighed to obtain *wet mass*. Finally, they were left to air dry for 24 h, after which they were weighed again to obtain *held mass*. Water Holding Capacity (WHC) and Water Retention Capacity (WRC) were calculated per volume of the sample as defined by Callaway *et al.* (2002):

$$WHC = \frac{\text{wet mass (g)} - \text{dry mass (g)}}{\text{volume (mm}^3\text{)}}$$

$$WRC = \frac{\text{held mass (g)} - \text{dry mass (g)}}{\text{volume (mm}^3\text{)}}$$

Where WHC refers to how much water adheres to the bark immediately after it becomes wet (cohesion), while WRC refers to how much water adheres and remains within the bark after 24h of becoming wet.

We collected 187 samples of bark from which 74 were from trees with *P. kraenzlinii*. Samples from *S. mahagoni* were collected only from trees without the orchid because it rarely served as a host to *P. kraenzlinii* in our study site. The number of samples per species is described in Appendix 1. We applied a Kruskal-Wallis Test to determine if WSC was different among species, and a Mann-Whitney U to evaluate if differences in indices between trees with and without *P. kraenzlinii* where significantly different. If significant differences were found, then a Conover-Iman Pair-Wise Comparison was applied to detect which species had a significant effect. Intraspecific differences between trees with and without *P. kraenzlinii* could only be evaluated by removing *S. mahagoni*, since no data for trees with the orchid was surveyed. The following trees species were excluded when evaluating the effect of WRC because no differences was observed between trees with and without orchids and all values were 0.0 g/mm³ (*M. portoricensis*, *R. aculeata*, and *R. inermis*).

Fissuring Index Assessment— We used two methods for preparing bark to calculate a Fissuring Index (FI). In the first method, the bark samples were dried and cleaned carefully with alcohol (Zarate-Gracia *et al.* 2020). The second procedure was to use untreated, fresh bark samples. To test which was the better method, we took 3 samples from 3 trees of 3 species growing on campus of University of Puerto Rico, Río Piedras. We selected flaky bark from *S. mahagoni*, smooth bark from *Ficus macrocarpa* L.f. (Moraceae), and rough bark from *Tabebuia heterophylla* (DC.) Britton (Bignoniaceae). All samples were photographed, and photos were cropped to cover 1 cm². Photos were uploaded into R where they were transformed into gray scale and then into binary (black and white) images using the package *imager* v.45.2 (Bartelme *et al.* 2023). We counted the number of black (fissured bark) and white (non-fissured bark) pixels, and with these data calculated the fissuring index of Zarate-García *et al.* (2020):

$$FI = \frac{\text{white pixels}}{\text{black pixels}} = \frac{\text{nonfissured bark}}{\text{fissured bark}}$$

We compared the FI measured by each method using a Mann-Whitney U Test that revealed no significant difference ($p > 0.05$) between the two meth-

odologies. Since there was no significant difference, we decided to use fresh samples for the *P. kraenzlinii* work. The fissuring index is a measure of the texture of the surface of the bark (fissures, bumps, and irregularities) (See Zarate-García *et al.* 2020). The higher the fissuring index, the less rough or irregular the surface of the bark.

We collected 194 samples of bark of which 83 were from trees with *P. kraenzlinii*. The number of samples per species is described in Appendix 2. Samples from *R. aculeata* and *S. mahagoni* were only from trees without the orchid, since finding the orchid growing on these species was rare. A Kruskal-Wallis Test was applied to see if FI was different among species. If a significant difference was found, a Conover-Iman Pair-Wise Comparison was applied to know which species had a significant effect. Mann-Whitney U test was used to detect significant differences between trees with and without the orchid, both in general and within each species of phorophytes.

In situ seed germination— Seed packets were built by sewing 3 × 5 cm nylon plankton netting fabric with mesh size 45 μ (an adaptation of Zi *et al.* 2014). A sample of the seeds from each fruit was tested for viability with tetrazolium chloride (TTC). Once viability was confirmed, 200–230 seeds were placed inside packets which were then secured to tree bark with gutter mesh (Khamchatra *et al.* 2016). On each of six phorophyte species selected, we placed one packet on 20 trees, and on *Coccoloba microstachya* and *Machaonia portoricensis*, we placed one packet on 30 trees in May and June 2021. After 7 months we collected the packets and examined them under a dissecting microscope in the laboratory. We then created a developmental stage classification system for *P. kraenzlinii* based on Stenberg & Kaine (1998) and Brandner (2005) (Table 1).

We recovered 174 seed packets from the forest of which 51 were on trees with *P. kraenzlinii*. (The imbalance between numbers of trees with and without *P. kraenzlinii* in *C. microstachya*, *R. aculeata*, *R. inermis* and *S. mahagoni* is because *P. kraenzlinii* rarely grew on them.) Packets on *C. microstachya* and *R. aculeata* were only placed on trees without the orchid. Only 6 packets were placed on trees with the orchid on *R. inermis*, and only 2 packets were placed on trees with the orchid on *S. mahagoni* (Appendix 3).

We investigated the influence of the presence of *P. kraenzlinii* and/or the phorophyte species on the number of packets with developing seeds by applying a Generalized Linear Model (GLM) with a binomial distribution. Odds ratios were calculated to measure the association between the presence of the orchid or the species of phorophyte and the number of packets with developing seeds. Odds ratio is used to measure the strength of an association between an observation and an outcome, where an odds ratio equal to 1 suggests no association, odds ratio greater than 1 suggests positive association, and odds ratio less than 1 suggests a negative association (see Szumilas 2010). To detect an association between the presence of *P. kraenzlinii* and orchid developing seeds, we excluded data from *R. aculeata* and *C. microstachya* because no trees with the orchid were available to place packets for comparisons. Packets placed on *R. inermis* were removed from all analyses related to embryo development because there was no development on *R. inermis*.

To analyze the influence of the presence of *P. kraenzlinii* and/or the phorophyte species on the percentage of developing seeds, we applied a Generalized Linear Model (GLM) using a nonbinomial distribution. Odds ratios were calculated to measure the association between the presence of the orchid or the species of the phorophyte and the percentage of developing seeds (Szumilas 2010). Then we asked whether some phorophyte species had a higher percentage of seeds at each developmental stage and used a Kruskal-Wallis test for each germination stage among the different phorophyte species, and a Mann-Whitney test to compare trees with and without an established *P. kraenzlinii*. If a difference was detected when using the Kruskal-Wallis, then a Conover-Iman test was applied to identify which species were significantly different. This would suggest that some tree species were a better substrate for seeds to develop than others. Finally, we explored if the presence of *P. kraenzlinii* or the species of the phorophyte could predict the presence of OMF by using the germination stages as a proxy for the presence of OMF on a GLM with binomial distribution. Odds ratio for the association between the presence of *P. kraenzlinii* or the phorophyte species and the presence of the OMF were calculated (Szumilas 2010).

TABLE 1. Description of embryo development stages of *Psychilis kraenzlinii* grown in situ developed by the authors based on Stenberg & Kaine (1998) and Brandner (2005). Stages 1 and 2 are early development, whereas Stages 3 and 4 are considered the first germination stages, since the protocorm is formed.

Stage	Description
Stage 1	Seed has imbibed, embryo has swollen and become green, still covered by testa.
Stage 2	The embryo has grown to the point of breaking testa.
Stage 3	Testa is almost or entirely gone, protocorm is formed with a pointed shoot apex.
Stage 4	Disc-like or elongated protocorm.

Results.

Phorophyte Utilization Assessment—Size of the trees: The plots had 568 trees belonging to at least 27 species. Most trees in our plots had a DBH less than 3.0 cm (\bar{x} = 2.5 cm, $Q_{0.25}$ = 1.3 cm, $Q_{0.5}$ = 1.9 cm, $Q_{0.75}$ = 2.9 cm), and trees with *P. kraenzlinii* growing on them had a larger mean (\bar{x} = 2.7 cm) than the median ($Q_{0.5}$ = 2.0 cm). We tagged 117 *P. kraenzlinii* growing on 13 (48%) tree species (Table 2). Most orchids grew less than 0.75 m above ground (\bar{x} = 0.42 m, $Q_{0.25}$ = 0.22 m, $Q_{0.5}$ = 0.36 m, $Q_{0.75}$ = 0.58 m).

Phorophyte and orchid association: There was a significant association between the presence of *Psychilis kraenzlinii* and the species of tree (Fisher Exact Test, $p < 0.01$, Monte Carlo Simulation = 2000). A Fisher’s pairwise comparison of pooled plot data revealed significant differences between *Machaonia portoricensis* and *Coccoloba microstachya* ($p < 0.005$, Fig. 1), where the former has a higher number of orchids than the latter. There were more orchids growing on *M. portoricensis* than expected if the presence of the orchid among phorophyte species was random. Conversely, there were fewer orchids growing on *C. microstachya* than expected.

Water Storage Capacity—Water Holding Capacity: We found WHC to be significantly different among phorophyte species (WHC, Kruskal-Wallis test: χ^2 = 82.62, $df = 7$, $p < 0.005$), but not between trees with or without the orchid (Mann-Whitney: $U = 4074.5$, $p = 0.77$). The Conover-Iman Pairwise test for the WHC (Appendix 4) showed that *M. portoricensis* has

TABLE 2. Distribution of established *Psychilis kraenzlinii* among phorophytes surveyed in 30 m.

Family	Species	Number of Trees	Number of <i>P. kraenzlinii</i>	Number of Trees Occupied	Percent of Trees Occupied
Primulaceae	<i>Bonellia umbellata</i>	2	4	2	100
Rubiaceae	<i>Machaonia portoricensis</i>	20	10	7	35
Rubiaceae	<i>Rondeletia inermis</i>	14	3	3	21
Phyllanthaceae	<i>Phyllanthus cuneifolius</i>	35	12	7	20
Rubiaceae	<i>Guettarda scabra</i>	112	36	21	19
	Dead tree	26	7	5	19
Ochnaceae	<i>Ouratea littoralis</i>	28	7	5	18
	Unidentified tree	12	2	2	17
Anacardiaceae	<i>Comocladia dodonaea</i>	8	2	1	13
Malpighiaceae	<i>Byrsonima lucida</i>	24	5	3	13
Myrtaceae	<i>Myrcia citrifolia</i>	25	3	3	12
Bignoniaceae	<i>Tabebuia haemantha</i>	133	19	13	10
Myrtaceae	<i>Pimenta racemosa</i>	13	2	1	8
Meliaceae	<i>Swietenia mahagoni</i>	12	1	1	8
Polygonaceae	<i>Coccoloba microstachya</i>	65	4	3	5
Apocynaceae	<i>Plumeria krugii</i>	1	0	0	0
Asteraceae	<i>Lepidaploa sericea</i>	1	0	0	0
Boraginaceae	<i>Varronia lima</i>	2	0	0	0
Celastraceae	<i>Gyminda latifolia</i>	2	0	0	0
Ehretiaceae	<i>Bourreria succulenta</i>	1	0	0	0
Ehretiaceae	<i>Bourreria virgata</i>	1	0	0	0
Fabaceae	<i>Poitea punicea</i>	5	0	0	0
Malpighiaceae	<i>Stigmaphyllon floribundum</i>	5	0	0	0
Polygalaceae	<i>Badiera penaea</i>	1	0	0	0
Primulaceae	<i>Ardisia elliptica</i>	3	0	0	0
Rubiaceae	<i>Randia aculeata</i>	10	0	0	0
Schoepfiaceae	<i>Schoepfia obovata</i>	1	0	0	0
Solanaceae	<i>Cestrum citrifolium</i>	4	0	0	0
Theaceae	<i>Ternstroemia stahlII</i>	1	0	0	0

the highest WHC, being significantly different from all species except *R. inermis*. The lowest WHC is that of *O. littoralis*, which was significantly different from all species but *S. mahagoni*. Among trees occupied by *P. kraenzlinii*, *T. haemantha* had a significantly higher WHC, whereas *Coccoloba microstachya* and *M. portoricensis* had a significantly lower WHC (Appendix 5).

Water Retention Capacity: We discovered differences in WRC among phorophyte species (Kruskal-Wallis test: $X^2 = 43.22$, $df = 7$, $p < 0.005$), but not between trees with or without the orchid (Mann-Whitney: $U = 4560.5$, $p = 0.22$). The Conover-Iman Pairwise Comparison test for WRC (Appendix 4) showed that the highest WRC was that of *S. mahagoni*, and it was significantly different from all other species. *Randia*

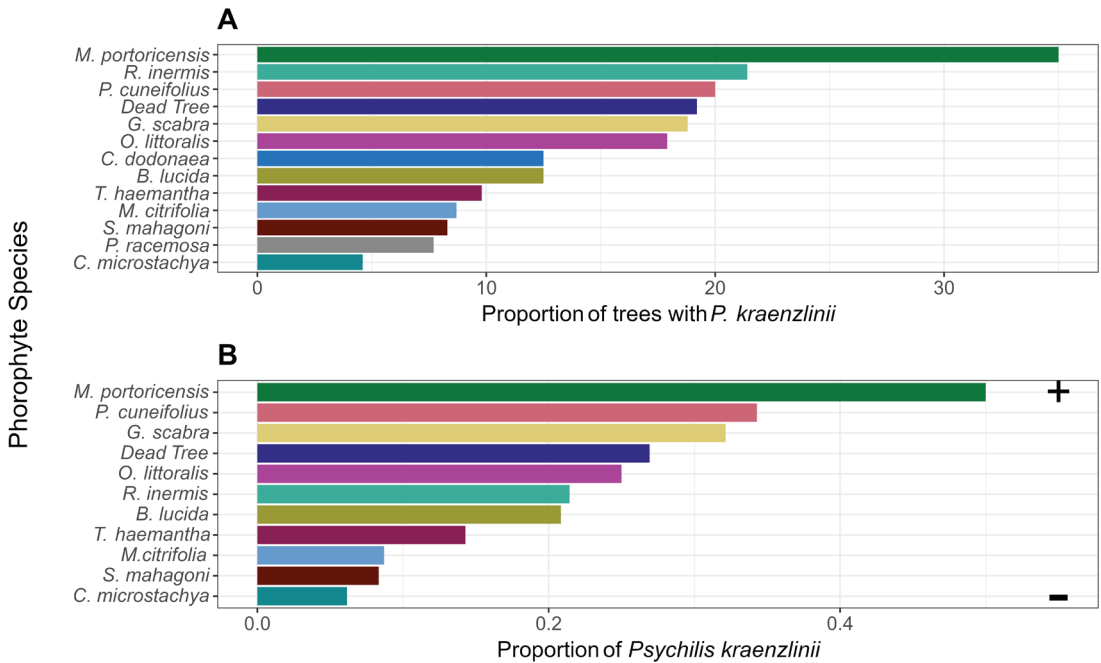


FIGURE 1. **A.** Proportion of trees of each phorophyte species harboring *Psychilis kraenzlinii* (unidentified phorophytes not included). **B.** Average number of *P. kraenzlinii* growing on the most common phorophyte species (Plus sign (+) marks a positive and significant association, whereas minus sign (-) marks a negative significant association. Associations based on residuals from Fisher Exact Test with Monte Carlo Simulation ($p < 0.05$, simulations = 2000).

aculeata, *R. inermis* and *M. portoricensis* have WRC of $<0.01 \text{ g/mm}^3$ which was significantly lower than *S. mahagoni*, *T. haemantha*, and *O. littoralis*. *Phyllanthus cuneifolius* has a WRC significantly higher than *M. portoricensis* and *R. inermis*, and although higher than *R. aculeata*, this last difference is not significant. When comparing trees of each species with and without the orchid, we also found no statistically significant differences (Appendix 5).

Fissuring Index Assessment— Phorophyte species differed significantly in FI (Kruskal-Wallis: $X^2 = 15.07$, $df = 7$, $p = 0.04$). *Phyllanthus cuneifolius* had the higher FI, but the difference was only significant when compared to *C. microstachya*, *O. littoralis*, *R. inermis*, or *T. haemantha*. We found that *P. cuneifolius* and *R. inermis* had the greatest variation in FI among species, but in general *P. cuneifolius* had a higher FI while *R. inermis* had the lowest (Appendix 4, Fig. 2A). We observed that 3 out of the top 4 species with higher fissuring index

also had high WHC, while 3 out of the 4 species with lower FI had higher WRC.

Significant differences exist in the fissuring index between trees with and without the orchid (Mann-Whitney: $U = 5.29$, $df = 1$, $p = 0.02$). Trees with *P. kraenzlinii* had a significantly lower FI. When evaluating this relationship for each species, the trend repeated within most, but it was only significant for *M. portoricensis* (Mann-Whitney: $U = 208$, $p < 0.005$, Fig. 2B).

In situ seed germinations— Of the 174 recovered packets, only 37 (21%) contained developing seeds. Of the 37 packets with developing seeds, 20 (54%) were near an established *P. kraenzlinii*. Of the 118 packets without developing seeds, 90 (76%) were on trees without an established orchid. A chi-square revealed that the number of packets with developing seeds near an established orchid is not significantly higher than if an established orchid was not present ($X^2 = 0.24$, $df = 1$, $p = 0.62$). The best model to explain the number

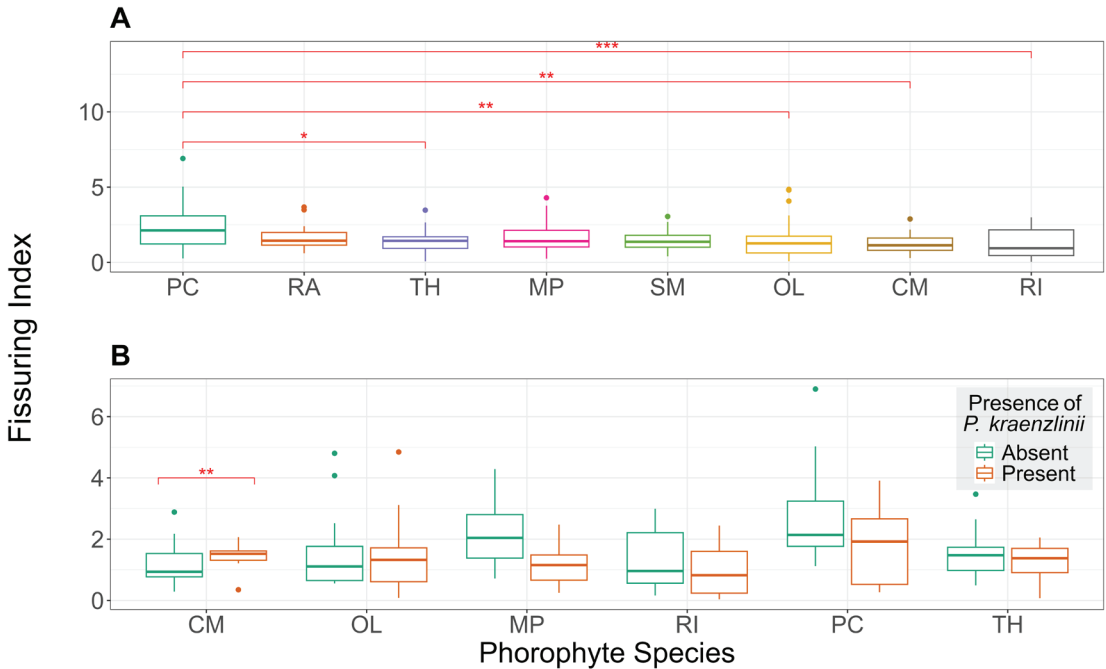


FIGURE 2. **A.** Box plots of fissuring index of the bark among phorophyte species. **B.** Box plots of the fissuring index of the bark of trees with and without *Psychilis kraenzlinii* among phorophyte species. *Randia aculeata* and *Swietenia mahagoni* not shown because data of trees with a *P. kraenzlinii* was not available. Red brackets with asterisks mark significant differences where: * = $p \leq 0.05$; ** = $p \leq 0.01$; *** = $p \leq 0.001$.

of packets with and without developing seeds was a binomial one where *C. microstachya* was placed as the intercept (Model A in Table 3). The odds of a packet with developing seeds were higher near an established orchid (OR = 1.4) and if it was located on *T. haemantha* (OR = 2.2) or *M. portoricensis* (OR = 1.9, Model A in Table 4, Fig. 3).

We observed 228 developing seeds among all packets, 121 of which were in packets near a *P. kraenzlinii*. The proportion of developing seeds per packet near an established orchid was significantly higher than that of packets in trees without the orchid (Mann-Whitney test, $U = 2049.5$, $p = 0.02$). The development stage of the seeds was evaluated according to our classification scheme (Table 1). We found 85 (40%) seeds in Stage 1, 76 (35%) in Stage 2, 35 (16%) in Stage 3, and 7 (3%) in Stage 4. No germination was noted from packets on *R. inermis*. The distribution of developing seeds in different stages among phorophytes is shown in Figure 4.

The best model to explain the percentage of developing seeds was a negative binomial GLM with the

phorophyte species as predictor variable (Model B in Table 3). The presence of an established *P. kraenzlinii* did not have a significant effect on the percentage of such seeds. Among all phorophytes, *M. portoricensis* is the only species with a significant and positive effect on the percentage of developing seeds, while *C. microstachya* is the intercept with a significant and negative effect. The odds of *P. kraenzlinii* developing on *C. microstachya* are near zero, while the odds for developing on *M. portoricensis* are 5.22. Other species with high odds of *P. kraenzlinii* seeds developing are *S. mahagoni* (OR = 4.18), *T. haemantha* (OR = 3.76), and *P. cuneifolius* (OR = 3.10, Model B in Table 4).

When comparing the percentage of seeds in each germination development stage among phorophytes, *O. littoralis*, *C. microstachya* and *R. aculeata* had significantly more seeds that did not develop (stage 0) than *T. haemantha*; *O. littoralis* and *R. aculeata* also had more seeds in stage 0 than *P. cuneifolius* (Conover-Iman: $p < 0.05$) (Appendix 6). Conversely, *T. haemantha* had significantly more seeds that went through imbibition

TABLE 3. Factors associated with the effect of phorophyte species on seed germination. Analyses are based on coefficients generated by Generalized Linear Models. **Model A:** Negative Binomial Model for the effect of phorophyte species and the presence of an established *Psychilis kraenzlinii* on the number of packets with germinated seeds. **Model B:** Negative Binomial Model for the effect of phorophyte species on the percent developing seeds. **Model C:** Effect of phorophyte species and presence of established *Psychilis kraenzlinii* over the presence of Orchid Mycorrhizal Fungi (OMF) when using embryo development stages as a proxy for OMF presence. SE = Std. Error.

Phorophyte Species	Model A				Model B				Model C			
	Estimate	SE	z	p	Estimate	SE	z	p	Estimate	SE	z	p
Intercept (<i>Coccoloba microstachya</i>)	-1.65	0.49	-3.38	<0.01*	-1.49	0.61	-2.43	0.02*	-3.43	1.02	-3.38	<0.01*
<i>Machaonia portoricensis</i>	0.63	0.68	0.92	0.36	1.65	0.81	2.03	0.04*	-0.56	1.6	-0.35	0.73
<i>Ouratea littoralis</i>	-1.82	1.19	-1.53	0.13	0.28	0.95	0.3	0.76	-0.22	1.43	-0.16	0.88
<i>Randia aculeata</i>	-0.43	0.9	-0.48	0.63	0.21	0.99	0.21	0.83	0.54	1.44	0.38	0.71
<i>Phyllanthus cuneifolius</i>	0.56	0.74	0.76	0.45	1.13	0.9	1.26	0.21	1.31	1.23	1.07	0.29
<i>Swietenia mahagoni</i>	0.28	0.76	0.37	0.72	1.43	0.91	1.57	0.12	-0.62	1.45	0.04	0.97
<i>Tabebuia haemantha</i>	0.78	0.72	1.08	0.28	1.33	0.89	1.49	0.14	0.06	1.45	0.04	0.97
<i>P. kraenzlinii</i> Present	0.88	0.48	1.83	0.07	NA	NA	NA	NA	1.55	0.86	1.79	0.07
	Null deviance: 170.37 on 154 df				Null deviance: 80.77 on 154 df				Null deviance: 80.96 on 165 df			
	Residual deviance: 152.9 on 147 df				Residual deviance: 152.9 on 147 df				Residual deviance: 74.5 on 158 df			

(stage 1) than *C. microstachya*, *O. littoralis*, and *R. aculeata* (Conover-Iman: $p < 0.05$) (Appendix 6). *Tabebuia haemantha* and *P. cuneifolius* had significantly more seeds whose embryo swelled to the point of breaking the testa (stage 2) than *C. microstachya* and *O. littoralis*; *T. haemantha* also had more seeds in stage 2 than *M. portoricensis*, and *P. cuneifolius* had more than *R. aculeata* (Conover-Iman: $p < 0.05$, Appendix 6). There was no difference in the occurrence of stage 3 (Kruskal-Wallis: $X^2 = 4.16$, $df = 6$, $p = 0.66$) and 4 (Kruskal-Wallis: $X^2 = 8.36$, $df = 6$, $p = 0.21$) among phorophytes. Trees with an established orchid had significantly fewer non-germinated seeds (Mann-Whitney: $U = 2997.5$, $p < 0.005$), and significantly more seeds in stage 1 (Mann-Whitney: $U = 2048.5$, $p = 0.006$), stage 2 (Mann-Whitney: $U = 1891.5$, $p < 0.005$), and stage 3 (Mann-Whitney: $U = 2228.5$, $p = 0.04$). There was no difference in the percentage of seeds on stage 4 between trees with and without an established *P. kraenzlinii*.

The best model to predict if the OMF was present or not was a GLM with binomial distribution that had both the phorophyte species and the presence of an established orchid as predictor variables. *Coccoloba microstachya* had a significant and negative effect over the presence of the OMF (Model C in Table 3). The OMF was 4.7 (OR) times more likely to be found near an established *P. kraenzlinii* and 3.7 (OR) times more likely to be found on *S. mahagoni*, irrelevant of whether there was an established orchid or not, according to the odds ratio (Model C in Table 4).

Discussion. We evaluated the phorophyte preferences in a population of *Psychilis kraenzlinii* and found that they are not randomly distributed among the available tree species in our study population. Furthermore, the best phorophytes for germination are not necessarily the same as those for adults. The best phorophytes for *P. kraenzlinii* are either rare or endemic species. None-

TABLE 4. Association among phorophyte species, presence of adult *Psychilis kraenzlinii*, and germination success based on Odds Ratios (OR) calculated for each Generalized Linear Model with 95% Confidence Intervals (CI). **Model A:** Negative Binomial Model for the effect of phorophyte species and the presence of an established *Psychilis kraenzlinii* on the number of packets with germinated seeds. **Model B:** Negative Binomial Model for the effect of phorophyte species on the percent developing seeds. **Model C:** effect of phorophyte species and presence of established *Psychilis kraenzlinii* over the presence of Orchid Mycorrhizal Fungi (OMF) when using embryo development stages as a proxy for OMF presence.

Phorophyte Species	Model A			Model B			Model C		
	OR	95% CI		OR	95% CI		OR	95% CI	
Intercept (<i>Coccoloba microstachya</i>)	0.19	0.07	0.46	0.23	0.07	0.82	0.03	0	0.15
<i>Machaonia portoricensis</i>	1.87	0.50	7.50	5.22	1.05	27.44	0.80	0.05	21.54
<i>Ouratea littoralis</i>	0.16	0.01	1.24	1.33	0.21	9.43	0.57	0.02	21.54
<i>Randia aculeata</i>	0.65	0.09	3.42	1.23	0.18	9.57	1.72	0.07	45.36
<i>Phyllanthus cuneifolius</i>	1.75	0.41	7.69	3.10	0.55	20.38	0.54	0.02	17.32
<i>Swietenia mahagoni</i>	1.32	0.28	5.87	4.18	0.73	29.24	3.70	0.39	80.85
<i>Tabebuia haemantha</i>	2.19	0.53	9.47	3.76	0.68	24.48	1.06	0.06	29.2
<i>P. kraenzlinii</i> Present	2.41	0.95	6.30	NA	NA	NA	4.70	0.97	31.63

theless, the population is healthy and with recruitment, which underscores the importance of continued protection of the forest.

Phorophyte Specificity Assessment—. Migenis & Ackerman (1993) suggested that host preference rather than specificity is common in Puerto Rico and the Neotropics. As for closely related species *P. monensis* and *P. krugii* of Puerto Rico (Ackerman *et al.* 1989, Otero *et al.* 2007), and *P. truncata* in the Dominican Republic (Cabrera-García *et al.* 2023), *P. kraenzlinii* only grows on a subset of available phorophytes. While it shows highest preference for *Machaonia portoricensis*, *Coccoloba microstachya* is the least preferred phorophyte given the abundance of this tree species in the study area. Contrastingly, Otero *et al.* (2007) found that *C. microstachya* is a common phorophyte of *P. monensis* on Mona Island, Puerto Rico. Sanford (1974) suggested that the different usage of phorophytes by an orchid species in different geographical areas was indicative of the importance of the whole habitat instead of only a few factors such as phorophyte species and their characteristics. Thus, the ability of an epiphyte to germinate and develop on a certain tree species, not only depends on climate, habitat, forest structure and characteristics of phorophytes, but also on microsite conditions such as temperature, humidity, microbial symbionts, etc. Otero *et al.* (2007) noted that the rela-

tionship of *P. monensis* with its phorophyte species is site-dependent and they suggested this was due to water relations. Data for phorophyte usage of *P. kraenzlinii* in other regions of Puerto Rico are not available, but phorophyte preferences of this species, like that of other epiphytes, might change according to environmental stressors (Sanford 1974, Timsina *et al.* 2016).

Phorophyte Physical Characteristics—. Variation in WHC and WRC between trees with and without the orchid irrespective of their species, was not significant. However, we found that *Machaonia portoricensis*, the most preferred phorophyte species, has the highest WHC, but no WRC. Other species on which the orchid was commonly found, *Phyllanthus cuneifolius* and *Rondeletia inermis*, also had a high WHC and no WRC. Conversely, *C. microstachya*, the least preferred phorophyte species, has the second lowest WHC, and a higher WRC. Intermediate conditions do exist. *Psychilis kraenzlinii* is frequently found on *Ouratea littoralis*, but unlike other preferred phorophytes (*M. portoricensis*, *P. cuneifolius*, *R. inermis*), it has the second lowest WHC and an intermediate WRC. Nonetheless, in general, preferred phorophytes tend to have high WHC and low WRC. We hypothesize that when it rains, the preferred phorophytes have the capacity to hold more water, giving the opportunity for seeds to go through the imbibition process rapidly. Then,

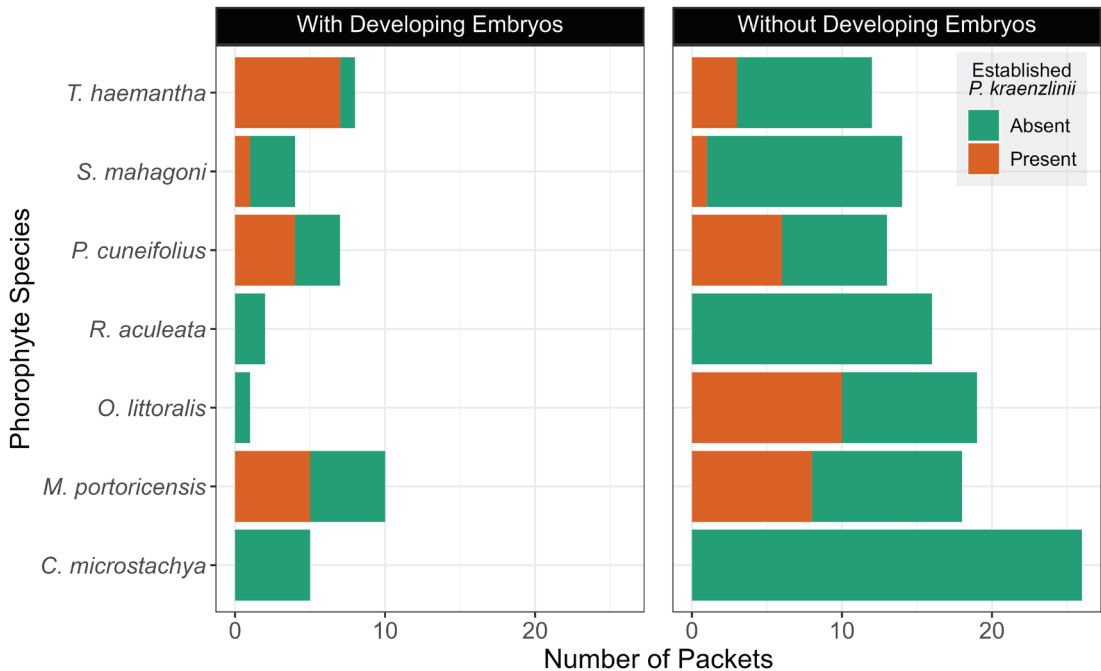


FIGURE 3. The number of packets with and without developing seeds on each phorophyte species and whether they were placed near an established *Psychilis kraenzlinii* or not.

these phorophytes quickly lose water (low WRC), preventing seeds from becoming waterlogged. As the imbibition process must occur before the infection of the OMF (Rasmussen, 1995), preferred phorophytes with high WHC promote rapid imbibition, resulting in ready-to-infect seeds faster than those phorophytes that have low WHC. Seeds growing on phorophytes with low WHC, but high WRC, might take longer to go through the imbibition process and become infected by their OMF, resulting in longer exposure to adverse environmental conditions, pathogens, or grazers. Although *O. littoralis* seems to share characteristics with the less common phorophytes, it has the lowest WHC. The fact that the orchid is commonly found growing on this species might be explained by the low WHC preventing the seeds from waterlogging, and its high WRC giving the seeds time to go through the imbibition process without desiccating. Wagner *et al.* (2015) mention that a low WRC might be suitable for epiphytes on a mesic habitat. Hence, in the moist forest of Susúa, low WRC might render smooth barked species good phorophytes for *P. kraenzlinii*, since water relations may be balanced.

Bark roughness may be associated with water storage capacity (Migenis & Ackerman 1993, Otero *et al.* 2007, Zarate-García *et al.* 2020). We observed a trend where species with higher WHC had lower FI (smoother bark), while those species with higher WRC had higher FI (rougher bark). Nonetheless, this association could not be statistically tested with our data. Bark roughness may also help seeds attach to the trunk of trees (Callaway *et al.* 2002, Siaz-Torres *et al.* 2020). Hence, it might explain why those phorophytes with low WHC, but high WRC, like *O. littoralis*, still harbor the orchid. Their roughness promotes attachment, and the crevices might serve as protection to give time for seeds to develop under a low but time-continuous water supplement. This hypothesis is supported by the fact that irrespective of the phorophyte species, trees on which *P. kraenzlinii* was growing had significantly rougher bark (lower FI) than those trees lacking the orchid. Furthermore, intraspecific differences in FI between trees with and without the orchid was only significant in *M. portoricensis*, where more orchids were growing on trees with rougher bark (lower FI). This relationship is also present as a non-significant

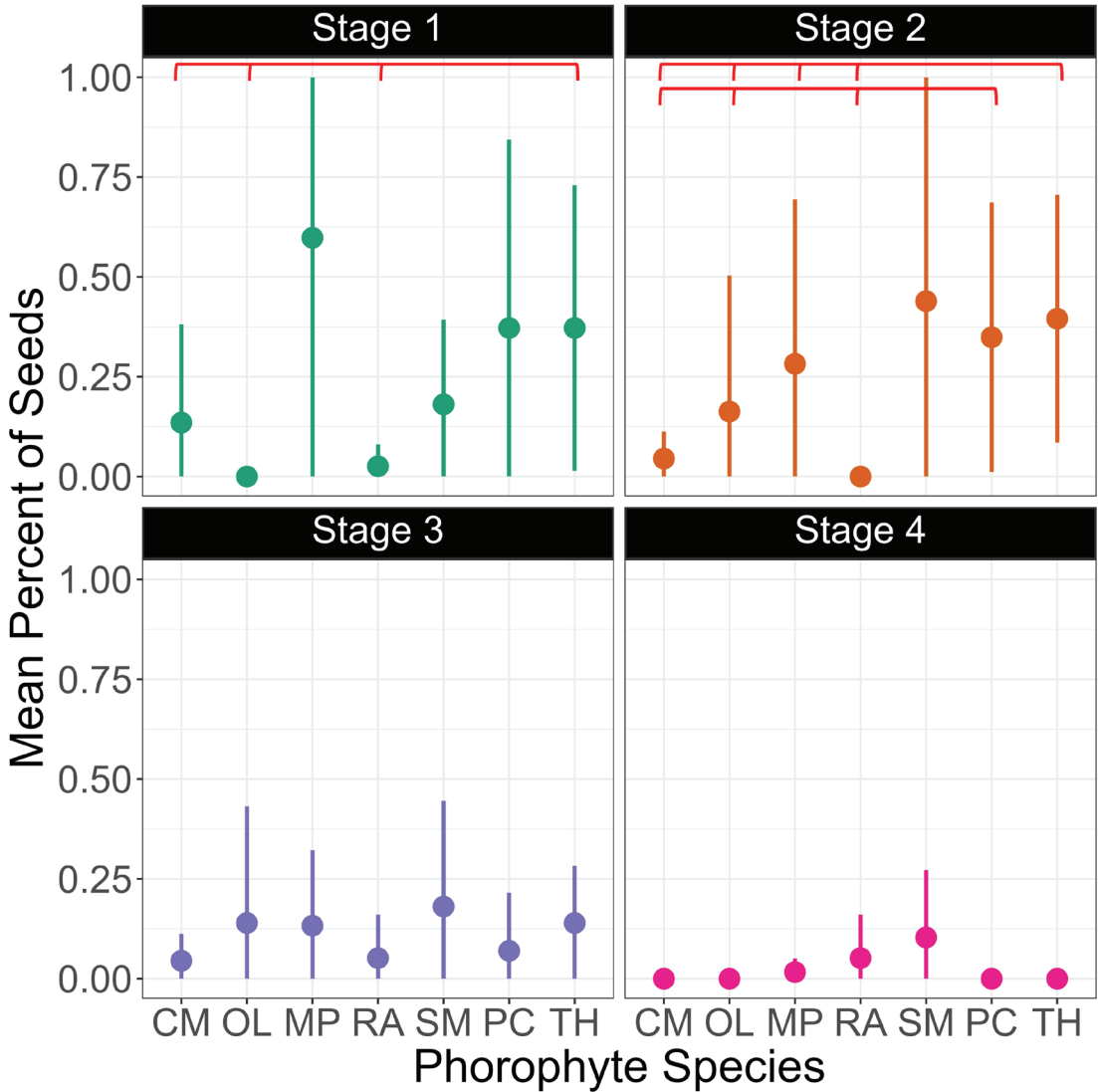


FIGURE 4. Mean percent of developing seeds per packet and their development stage on phorophytes with and without an established *Psychilis kraenzlinii*. Where Stage 1 refers to seeds with swollen embryos, Stage 2 are seeds whose embryo have swelled to the point of breaking the testa, Stage 3 the testa is gone and the protocorm is formed, and Stage 4 the protocorm has elongated. For *Ouratea littoralis*, no seeds developed near an established *P. kraenzlinii*, whereas in the case of *Coccoloba microstachya* and *Randia aculeata*, no packets were placed near and established orchid. Red lines indicate significant differences according to Conover-Iman Pairwise Comparisons ($p < 0.05$).

trend among species with smoother bark (*R. inermis* and *P. cuneifolius*). *Rondeletia inermis* superficially appears to have smooth bark, but microscopically the bark appears rough with numerous crevices. Nonetheless, *R. inermis* behaves as a smooth bark species (high WHC, no WRC) because its bark is thin, unlike other rough-barked species (*T. haemantha*, *O. littoralis*, *C.*

microstachya and *S. mahagoni*) which all have thick spongy bark. The hypothesis that *P. kraenzlinii* prefers phorophytes with rough bark and high WRC is not supported. It appears that the contrary is true.

In fact, most *P. kraenzlinii* were found growing at the base of the tree—no higher than 0.75 m from the ground—where humidity is higher and light exposure

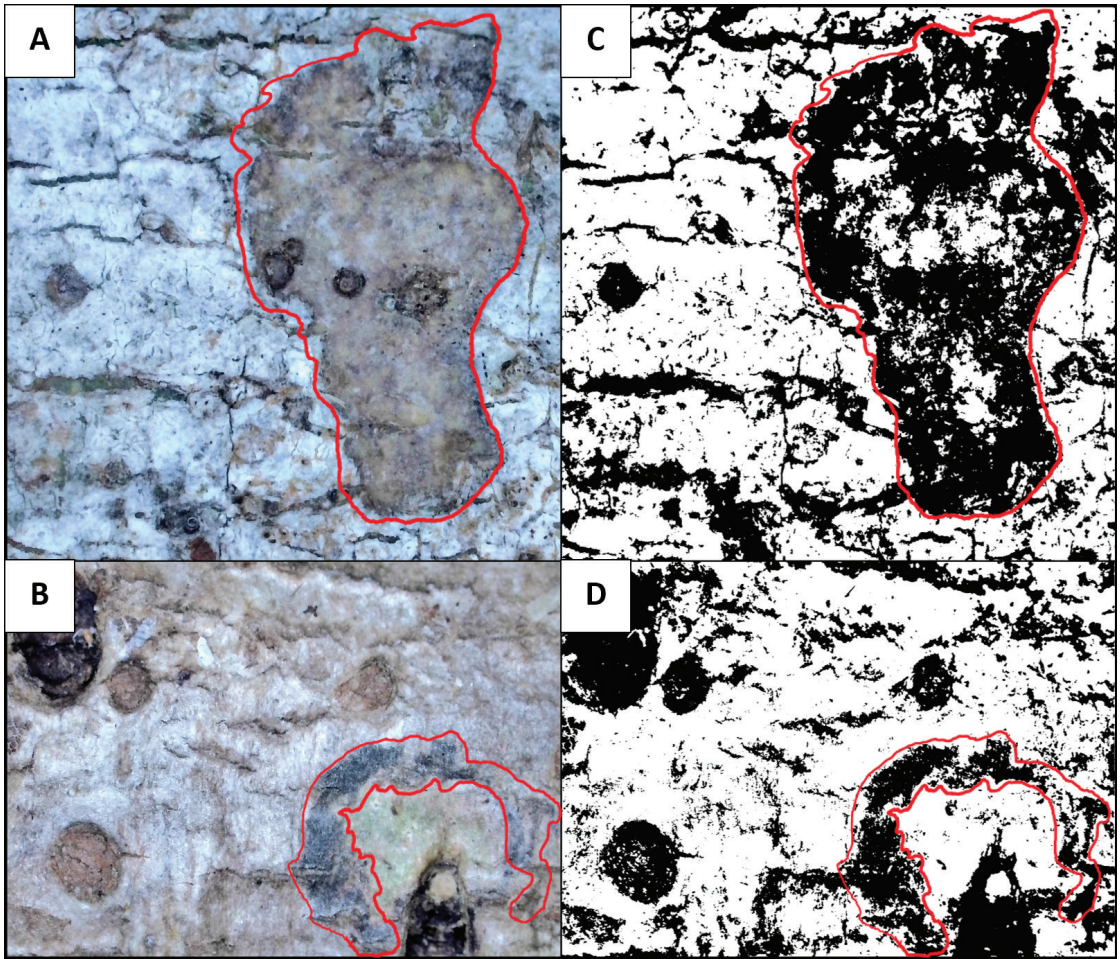


FIGURE 5. Effect of bark pigmentation on the fissuring index. **A.** Pictures of the bark surface of *Coccoloba microstachya* taken with a camera coupled to a dissecting microscope and a ring light. **B.** Pictures of the bark surface of *Phyllanthus cuneifolius* taken with a camera coupled to a dissecting microscope and a ring light. **C.** Photos converted to black and white (binary) images of *C. microstachya*. **D.** Photos converted to black and white (binary) images of *P. cuneifolius*. Enclosed in red is the area where roughness interpretation could be affected by bark pigmentation. Photos by N. González-Orellana.

is lower Petter *et al.* (2016), likely meaning more water availability. Phorophyte preferences of two dry-forest *Psychilis* species have also been studied using subjective assessments of bark roughness. Ackerman *et al.* (1989) found no preference for rough-barked species by *P. krugii* in Guánica, Puerto Rico, and Otero *et al.* (2007) discovered that *P. monensis* on Mona Island was very common on rough-barked *Phyllanthus epiphyllanthus*, but when they eliminated that phorophyte from their analysis they found no preference for other rough-barked phorophyte species.

Since trees tend to have different pigmentation patterns on their bark, the FI results must be interpreted cautiously. The bark of tree species we studied is not uniformly colored, which may affect the FI results (Fig. 5). Sections of the bark with dark colors such as green and brown could be interpreted by the algorithm as roughness, while light pigmentation like white and pink could be interpreted as smoothness. While we do not yet know whether this is a problem, staining the bark surface to cover such pigmentation may be advisable.

Psychilis kraenzlinii prefers trees with smoother bark (high FI), high WHC and a low WRC. Conversely, Zarate-García *et al.* (2020) found no clear correlation between FI of phorophytes and the presence of orchids. Furthermore, they did find phorophyte preference was inversely correlated with WHC, while positively correlated with WRC. Bark roughness preferences might be influenced by microsite conditions such as radiation exposure, humidity, and seasonality as well as by the method of attachment used in each stage of the life history of an epiphytic species (Tay *et al.* 2023). The study sites in Zarate-García *et al.* (2020), were low coastal forests in the Yucatan Peninsula, Mexico, where mean annual temperature is higher and mean annual rainfall is lower than our study site in Susúa State Forest, Puerto Rico. Environmental conditions and phorophyte phenology at these sites might affect phorophyte preferences of the orchid species studied (Zarate-García *et al.* 2020). Similarly, Ackerman, Montalvo & Vera (1989) and Otero *et al.* (2007) found no clear relationship for either *P. monensis* or *P. krugii* between phorophyte preference and bark roughness. However, subjective assessments of bark topography, such as the one used by those authors could be misleading (Tay *et al.* 2023). Guánica State Forest and Mona Island are dry environments with low, open canopies where *P. krugii* and *P. monensis* might be exposed to direct sunlight and drought. Phorophyte preferences might be governed by factors such as light exposure, rather than only by phorophyte characteristics. In contrast, the Susúa State Forest is a moist forest with a dense canopy cover that protects orchids against radiation and water evaporation. Hence, orchid germination might be influenced by higher WHC rather than WRC because it promotes rapid germination, reducing the probability of experiencing adverse conditions during early stages of development.

In Situ Seed Germination—Germination of terrestrial orchids is higher near established plants, which may serve as a beacon of suitable conditions and/or a reservoir of mycorrhizal fungi (Diez 2007, McCormick *et al.* 2016). In situ germination studies of epiphytic orchids are limited, contrary to the studies of terrestrial orchids, Kartzinel *et al.* (2013) found that *Epidendrum firmum* Rchb.f. was dependent on the microclimates of large trees and closed canopies, rather than proximity

of conspecific adults. Conversely, Petrolli *et al.* (2021) found a correlation between OMF community composition with epiphyte root proximity, suggesting that the bark near established orchids likely harbor their OMF. Further evidence of spatial structure was revealed when Petrolli *et al.* (2022) and Fernández *et al.* (2023) discovered that epiphytic orchid communities formed modular networks with their OMF. In addition, studies for both terrestrial (Whitman & Ackerman 2015, Jacquemyn *et al.* 2007, Jersáková & Malinová 2007) and epiphytic species have suggested that spatial distribution of orchids may be dependent on propagule pressure which is strongest near seed sources (Ackerman *et al.* 1996).

A higher frequency of seeds in process of germinating was obtained near established orchids. Still, the model (negative binomial GLM) that best explains the data did not include the variable of presence of an established *P. kraenzlinii* as a predictor. We hypothesized that germination would be higher near established orchids because of a higher propagule pressure, higher probability of OMF availability and appropriate microsite conditions. Our results suggest that germination is more probable near established orchids, supporting our hypothesis, but that the phorophyte species has a stronger effect on the percentage of developing seeds. According to the model, *P. kraenzlinii* has a significantly higher probability of developing on *M. portoricensis*, and a significantly lower probability of developing on *C. microstachya*. Hence, the patterns of *P. kraenzlinii* seeds that are ready to undergo germination tend to mirror that of the distribution of established orchids in the study site.

Germination development stages reached by seeds in packets on different phorophyte species varied. The percentage of non-germinated seeds (Stage 0) was highest on *O. littoralis* and *R. aculeata*. Those that reached Stages 1 and 2 were more common on *T. haemantha* and *P. cuneifolius*. Nonetheless, the later stages (Stage 3 and 4) showed no difference among phorophyte species so that early-stage success is not necessarily indicative of success in reaching later stages. In fact, we found no significant difference among phorophyte species in the presence of OMF. Remarkably, the highest probability of having OMF (as evidenced by germination to at least stage 4) was *S. mahagoni*, a species where established orchids are rare to

find. Furthermore, *R. aculeata*, a species largely unoccupied by *P. kraenzlinii* was one of the few species where seeds reached protocorm stages during *in situ* germination experiments. On the contrary, *P. kraenzlinii* grew on 21% of the *R. inermis* within our plots, but no embryo development was observed on this phorophyte species. Thus, population dynamics of orchids can be context dependent where best sites for one life history stage are not necessarily best for another stage. Indeed, we found that best sites for germination are not always the same as sites where plants can develop and survive, as observed by Crain *et al.* (2022) for epiphytic *Lepanthes caritensis* in the Carite State Forest in Puerto Rico, Whitman & Ackerman (2015) for terrestrial *Prescottia stachyodes* in El Yunque, Puerto Rico, and by Jacquemyn *et al.* (2007) for *Orchis purpurea* in Belgium (see also Gowland *et al.* 2011 and Jersáková & Malinová 2007). Moreover, Otero *et al.* (2007) found that the best sites for germination of *P. monensis* are different from those sites with high pollination, suggesting that the major production of seeds may occur far from suitable germination sites. Which is why, when developing conservation strategies for orchids, the environmental conditions in which an established population exists should not be assumed to be good for germination and establishment, unless recruitment is observed (Rasmussen *et al.* 2015). Another factor that needs to be considered is that of OMF usage throughout the life cycle of an orchid. Ontogenic turnover of OMF species exists in some orchids, suggesting that the OMF that trigger seed germination is not necessarily the best for later development (Otero *et al.* 2005, Bidartondo & Read 2008, Meng *et al.* 2019a, 2019b, Fernández *et al.* 2023).

Conclusion. *Psychilis kraenzlinii* was shown to prefer a subset of available phorophytes as well as higher probability of developing near established orchids. The orchid was found more often on substrates with a high WHC and lower FI (smoother bark). These results are not entirely consistent with similar studies of other orchids done under different climatic regimes and vegetation types, including closely related *P. monensis*, suggesting that preferences for certain substrate conditions may be context dependent. *Psychilis kraenzlinii* is the most widespread member of the genus, so comparative studies of different populations might reveal

how environmental conditions affect phorophyte preferences. The results described here lay the foundation to develop informed conservation and management strategies for *P. kraenzlinii* and other species of the genus. However, various unknowns must be clarified: (1) pollinator identity and visitation frequency; (2) the OMF that triggers seed germination and development; (3) distribution of the orchid and how it relates to the distribution of its pollinators and OMF; (4) abiotic factors affecting the distribution of this orchid and its symbionts. Nonetheless, the population studied here is unusually large and apparently robust having evidence of fruit production and germination success which may be viewed generally as an indicator of a healthy population (Pierce & Belotti 2011). Still, this is a population near the edge of the Forest Reserve and should be monitored for any incursions and adjacent development which may affect critical ecosystem functions. The phorophyte that *P. kraenzlinii* prefers in the Susúa State Forest, *M. portoricensis*, is an endemic and rare shrub found in the southwest of Puerto Rico (Axelrod 2011). The protection and monitoring of this tree species might also be beneficial for *P. kraenzlinii*. It cannot be overstated, orchid conservation needs to target whole ecosystems, particularly in biodiversity hotspots of which the Caribbean is one (Fay 2018, Myers *et al.* 2000, Phillips *et al.* 2020).

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APPENDIX 7. Do not bother...

As part of this project other experiments took place without much success. We tried isolating the orchid mycorrhizal fungi (OMF) that triggers the germination of *Psychilis kraenzlinii*. We tried isolating the OMF from both adult roots and protocorms. To isolate the OMF from the roots we first confirmed the presence of pelotons and then put both a thin cross-sectional slice of the root and the peloton already isolated, in cultivation media. We used water agar (WA) and Potato Dextrose Agar (PDA); the media was both poured over the tissue and already set on the plate. Although several strains grew, none were Rhizoctonia-like fungi. To isolate the OMF from protocorms that resulted from the *in-situ* germination experiment we used WA and PDA, both poured over the pelotons and already set. Again, several strains grew, none of them Rhizoctonia-like. Although we paid some attention to two strains that were likely to be *Fusarium* and *Xylaria*. With these strains we did germination assays to see if either would promote germination. Although the seeds swelled, some to the point of breaking the seed coat (testa), there was no further development. It is worth noting that Otero *et al.* (2002) attempted OMF isolation from *Psychilis monensis* using PDA and did not find an obvious OMF strain. We would suggest that, if the reader intends to conduct research on the OMF of *P. kraenzlinii* or a *Psychilis* spp., then they should try other fungi cultivation media.

As part of the phorophyte characterization phase of my research we followed the methodology described by Callaway *et al.* (2002) to measure bark stability. This methodology consists of painting dots with oil paint on the bark of phorophytes and checking them after a pre-determined time (in my case a year) to see if the dots have disappeared or changed. Changes on the dots suggest that the bark is shedding, and it can be used as a proxy for stability. The study site for Callaway *et al.* (2002) was the subtropical Sapelo Island in Georgia (USA), and phorophyte composition was mostly pines and oaks which shed their bark in pieces. A much different scenario than where we did our work: a secondary forest of the tropical moist Susúa State Forest in Puerto Rico, where the oil dots stayed through the length of this study (2 years). We also followed the methodology described by Zarate-Garcia *et al.* (2020) for rhytidome texture characterization. Here, one uses scanning electron microscopy (SEM) to closely look at the bark texture and porosity. After looking at the images closely, the methodology appeared too subjective. The rhytidome classification was too variable and without patterns among phorophyte species. As for the pores, we are not convinced that the so-called pores are, in fact, pores. They seem to be cells. Hence, the data was archived and not used for publication.

With this section our hope is not to avert the reader to conduct research on these topics, but rather to give some input so they can develop a methodology with a higher probability of being successful.

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