University of Puerto Rico, Rio Piedras Campus
Department of Biology, Faculty of Natural Sciences

# Microsite limitation, distribution, and germination patterns of the endemic epiphyte *Psychilis kraenzlinii* (Orchidaceae)

Master's Thesis

Normandie González-Orellana

Noviembre 16, 2023

Thesis Advisor:

James D. Ackerman, PhD.

Thesis Committee:

Paul Bayman, PhD

Raymond L. Tremblay, PhD.

Elvira Cuevas, PhD.

This thesis has been accepted by the faculty of the:
DEPARTMENT OF BIOLOGY
FACULTY OF NATURAL SCIENCES
UNIVERSITY OF PUERTO RICO
RIO PIEDRAS CAMPUS


In partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE IN BIOLOGY


Thesis committee:

_____
James D. Ackerman, PhD.                Advisor


_____
Paul Bayman, PhD.


_____
Raymond L. Tremblay, PhD.


_____
Elvira Cuevas, PhD.


November 16, 2023

©Normandie González-Orellana

## Dedication

During my short career, I have already been called bossy, hysterical and, my favorite one yet, opinionated, multiple times. This is for the women in my life, mami (Norma I. Orellana Mangual), abuela (María M. Navarro Roldán), and Mamasusin (Jesusa Mangual Rojas), whom with their unconditional love and example, taught me to be strong, independent, and undomesticated.

This is also yours.

ABSTRACT- I explore the distribution and germination patterns of the epiphytic orchid, *Psychilis kraenzlinii* in the Susua State Forest, Puerto Rico. The orchid grows on a subset of the available tree species and shows preference for the endemic *Machaonia portorricensis* (Rubiaceae). The orchid preferred trees with smoother bark with 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 –Exploré la distribución y los patrones de germinación de la orquídea epífita, *Psychilis kraenzlinii* en el Bosque Estatal de Susúa. *Psychilis kraenzlinii* crece en un subconjunto de las especies de árboles disponibles y muestra preferencia por la endémica *Machaonia portorricensis*. La orquídea prefiere árboles con cortezas lisas y alta capacidad de sostener agua y baja capacidad de retención de agua. Las condiciones microclimáticas bajo las cuáles los embriones entran a etapas pre-germinacíon 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.

**Introduction.** Vascular epiphytes are important for global plant diversity since they account for 10% of all vascular plant species (Kress 1986) and may represent as many as 35% of the species in a tropical rainforest (Gentry & Dodson 1987a). Plants are epiphytic if they use trees and/or shrubs as a substrate without directly harming them (Adhikari & Fisher 2011) and they remain in the epiphytic environment throughout their entire life cycle (Zotz, Hietz & Einzmann 2021). Epiphytes provide important ecosystem services by accumulating biomass, providing food for other organisms or substrates for other epiphytes, serving as shelter and even as biofilters (Hietz 1998, Adhikari & Fischer 2011, Tay, Zotz & Einzmann 2023). The highest diversity of epiphytes is found in the Neotropics (Sáyago *et al*. 2013), and their distribution might be limited not only by host-tree (phorophyte) availability but also seed dispersal (Migenis & Ackerman 1994, Ackerman, Sabat & Zimmerman 1996). Phorophyte preference could be driven by physical and chemical characteristics of the bark that affect the presence of mycorrhizal fungi, seed 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 roughness (which might be affected by age), water storage capacity (that could be affected by bark roughness), pH, and secondary metabolites (Frei & Dodson 1972, Frei 1973, Migenis & Ackerman 1993, Adhikari & Fisher 2011, Sáyago *et al*. 2013, Timsina *et al*. 2016, Siaz-Torres *et al*. 2021).

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, Sáyago *et. al*. 2013, Zotz, Hietz & Einzmann 2021) yet we do know that phorophyte specificity is rare. Still, some degree of preference is commonly found within sites (Sulit 1950, Sulit 1953, Migenis & Ackerman 1993, Tremblay *et al.* 1998, Laube & Zotz 2006, Trapnell & Hamrick 2006, Gowland *et al*. 2011, Wagner, Mendieta-Leiva & Zotz 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.

Approximately 70% of orchid species are epiphytes, accounting for approximately 72% of epiphyte species in the world (Gentry & Dodson 1987b, Gravendeel et al. 2004). Orchids can be considered bioindicators of overall diversity and health of an ecosystem because of their complex relationships with other organisms, particularly pollinators, orchid mycorrhizal fungi (OMF) and, in the case of epiphytes, phorophytes (Adhikari & Fischer 2011, Pierce & Belotti 2011).

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, Sabat & Zimmerman 1996), and OMF availability which may be microsite-limited (Otero & Flanagan 2006, Izuddin *et al*. 2019, Izuddin, Yam & Webb 2019).

Epiphytic orchid conservation and management techniques may include the protection of suitable and existing phorophytes, as well as planting new ones (Adhikari & Fischer 2011). 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 (Rasmussen *et* al. 2015, Fay 2018, Phillips, Reiter & Peakall 2020) and include the entire communities in which they occur.

Here I 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 (Sauleda

1989, Ackerman and Collaborators 2014). 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, I asked if *P. kraenzlinii* shows phorophyte preferences and whether the distribution of established orchids corresponds to where germination occurs most frequently. Like the closely related species, *Psychilis monensis* Sauleda (Otero, Aragón, & Ackerman 2007), *Psychilis krugii* (Bello) Sauleda (Ackerman *et al.* 1989), and *Psychilis truncata* (Cabrera-García *et al.* 2023), I expected *P. kraenzlinii* will neither be host-specific nor randomly distributed but 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 an extreme one constantly changing (Benzing, 1979, Tremblay, Meléndez-Ackerman & Kapan 2006, Trapnell & Hamrick 2006).  I also hypothesized that seed germination would mirror the distribution of established orchids since one may assume that, like terrestrial orchids, the presence of an established epiphytic orchid is a beacon for suitable environmental conditions and OMF availability.

Secondly, I explored other factors that may affect germination and establishment of *P. kraenzlinii* in the Susúa State Forest. I measured Water Storage Capacity (WSC) and bark roughness of phorophytes to see 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 may more readily attach to them (Timsina *et al.* 2016, Adhikari & Fisher 2011). Consequently, I expected to find higher seed

germination rates and more orchids on phorophytes with high roughness and water retention capacity.

Finally, I 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, I assumed that seeds that reached germination were infected by their OMF. If orchids and their OMF share similar niche requirements (Izuddin *et al*. 2019, Izuddin, Yam & Webb 2019), then I expect that protocorm formation will be more likely on phorophyte species that have a higher occurrence of established orchids.

**Methods.** *Study System*. *Psychilis kraenzlinii* is an orchid endemic to Puerto Rico. It is a rewardless, self-incompatible epiphyte that produces long, erect inflorescences with 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, Montalvo & Vera 1989, Otero, Aragón, & Ackerman 2007) suggest that peak flowering occurs from April through July (Ackerman, Montalvo & Vera 1989, Aragón & Ackerman 2004). *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 Environmental and Natural Resources of Puerto Rico. The forest occupies about 13 km$^2$ 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 (DRNA 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. Prior to 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 forest comprised of slender trees averaging 12 m tall, with a light canopy (Miller & Lugo 2009).

*Phorophyte Specificity Assessment.* Four 15 × 5 m plots were established in the study area. Trees and shrubs inside the plots were identified, DBH was measured, and I noted if they had *P. kraenzlinii*. All *P. kraenzlinii* plants inside plots were tagged and the cardinal orientation of orchids growing on phorophytes as well as the height at which orchids were attached to their phorophytes was measured.

For the analysis, I filtered the data and kept only the data where the tree species on which the orchid was found growing had a frequency higher than 3%. I applied a Fisher's Exact Test to see if there was a relationship between each tree species and the presence of the orchid. To determine if the orchid was growing more often on a particular cardinal direction of the tree trunk, I treated cardinal orientation as a categorical variable with 8 categories (North, Northeast, East,

Southeast, South, Southwest, West, and Northwest) and performed a Chi-squared test of independence.

*Phorophyte Physical Characteristics.* The two physical characteristics of the bark that I considered were Water Storage Capacity (WSC) and Fissuring Index (FI), both of which influenced orchid host tree preferences in Mexico (Zarate-Garcia *et al*. 2020). The tree species from which bark data were collected were chosen based on the Phorophyte Specificity Assessment described previously to create a gradient from positive to negative relationship as follows: *Machaonia portorricensis* 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, I 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 I adapted the methodologies described by Callaway *et al*. (2002) and Zarate-Garcia *et al.* (2020). In the laboratory, samples were cut to approximately 1 cm$^2$ 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. I 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):

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

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

Where WHC refers to how much water sticks to the bark right after it becomes wet (cohesion), while WRC refers to how much water adheres and stays within the bark after 24h of getting wet.

I 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 A. I applied a Kruskal-Wallis Test to determine if WSC was different among species, and a Mann-Whitney U to evaluate 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. portorricensis*, *R. aculeata*, and *R. inermis*).

*Fissuring Index Assessment.* I 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-Gracía *et al.* 2020). The second procedure was to use untreated, fresh bark samples. To test which was the better method, I took 3 samples from 3 trees of 3 species at the University of Puerto Rico,

Río Piedras. I 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 (Barthelme *et al*. 2022). I counted the number of black (fissured bark) and white (non-fissured bark) pixels, and with these data calculated the fissuring index of Zarate-Garcia *et al*. (2020):

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

I compared the FI measured by each method using a Mann-Whitney U Test that revealed no significant difference (p > 0.05) between the two methodologies. Since there was no significant difference, I used 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.

I collected 194 samples of bark from which 83 were from trees with *P. kraenzlinii*. The number of samples per species is described in Appendix B. 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 the eight phorophyte species selected, I placed one packet on 20 trees. Packets were placed in May and June 2021. Packets were collected after 7 months and observed under a dissecting microscope in the laboratory. I then developed our own developmental stage classification system for *P. kraenzlinii* based on Stenberg & Kaine (1998) and Brandner (2005) (Figure 1).

I 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 C).

To explore associations between the presence of an established orchid or the phorophyte species and whether a packet had seeds developing or not, I applied a Chi-Square test for Independence for each. To detect an association between the presence of *P. kraenzlinii* and orchid embryo development, I 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*. Furthermore, I investigated the influence of the presence of *P. kraenzlinii* and/or the phorophyte species on the number of packets with developing seeds, I applied a Generalized Linear
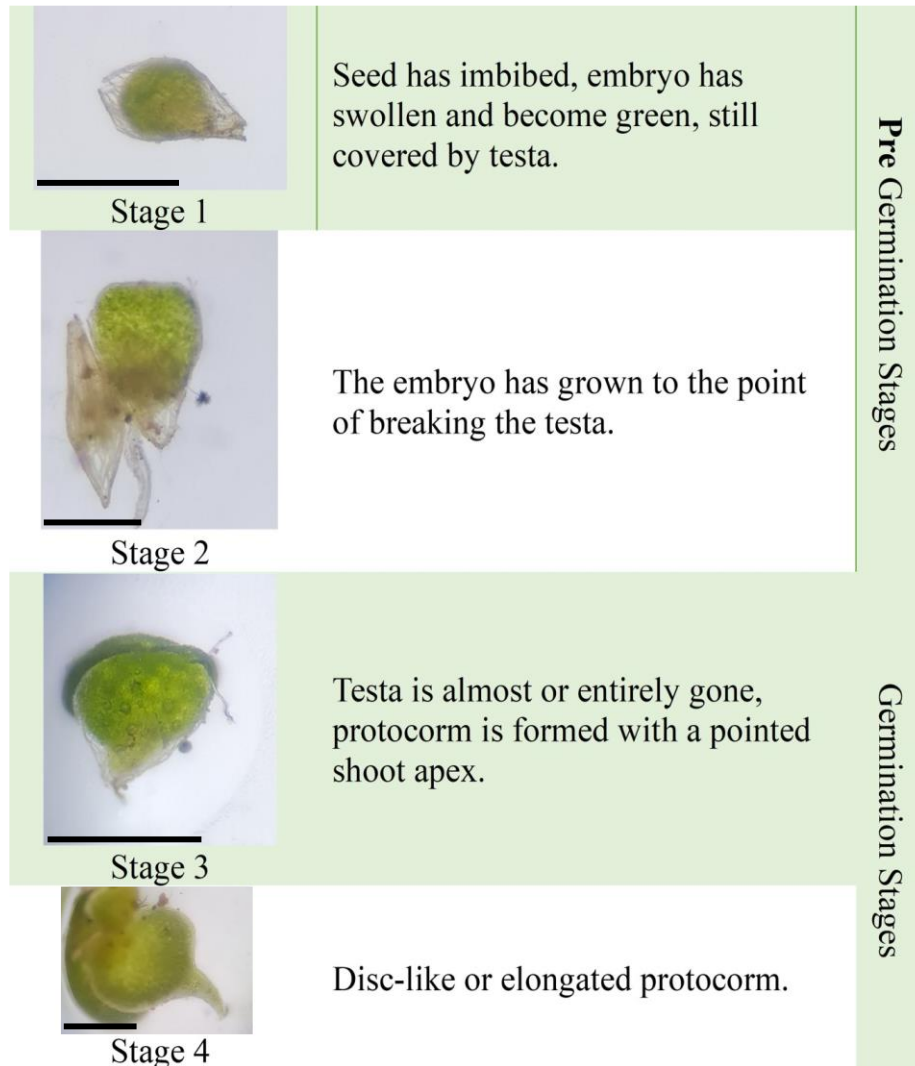
**Figure 1.** Description of embryo development stages *of Psychilis kraenzlinii* grown in situ developed by the authors based on Stenberg & Kaine (1998) and Brandner (2005) (Scale bars = 0.5 mm). Stages 1 and 2 are early development, whereas Stages 3 and 4 are considered the first germination stages, since the protocorm is formed.

Model (GLM) using a binomial distribution and the response variable being whether seeds were developing or not. 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 suggest positive association, and odds ratio smaller than 1 suggest a negative association (see Szumilas 2010).

To analyze the influence of the presence of *P. kraenzlinii* and/or the phorophyte species on the percentage of developing seeds, I 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 I asked whether some phorophyte species had a higher percentage of seeds at each developmental stage and applied a Kruskal-Wallis test for each germination stage among the different phorophyte species, and a Mann-Whitney to compare between 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, I explored if the presence of *P. kraenzlinii* or the species of the phorophyte could predict the presence of OMF by using the germination stages of the development 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).
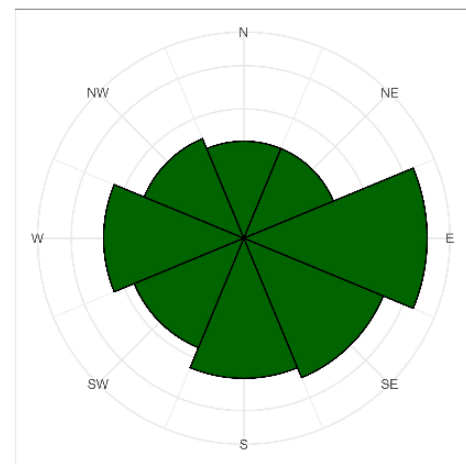


**Figure 2:** Cardinal Orientation to which *P. kraenzlinii* is growing inside plots in Susúa State Forest ($N = 100$).

**Results.** *Phorophyte Utilization Assessment*. 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 than the median ($\bar{x} = 2.7$ cm, $Q_{0.25} = 1.4$ cm, $Q_{0.5} = 2.0$ cm, $Q_{0.75} = 3.0$ cm). I tagged 117 *P. kraenzlinii*

growing on 13 (48%) tree species (Table 1). Most orchids grew less than 0.75 m above ground ($\bar{x}$ = .42 m, $Q_{0.25}$= 0.22 m, $Q_{0.5}$ = 0.36 m, $Q_{0.75}$ = .58 m).

**Table 1.** Distribution of established *Psychilis kraenzlinii* among phorophytes surveyed in 30 m.

| Family | Species | Number of Trees | Number of *P. kraenzlinii* | Number of Trees Occupied | Percent Trees Occupied |
|---|---|---|---|---|---|
| Primulaceae | *Bonellia umbellata* | 2 | 4 | 2 | 100 |
| Rubiaceae | *Machaonia portorricensis* | 20 | 10 | 7 | 35 |
| Rubiaceae | *Rondeletia inermis* | 14 | 3 | 3 | 21 |
| Phyllanthaceae | *Phyllanthus cuneifolius* | 35 | 12 | 7 | 20 |
| Rubiaceae | *Guettarda scabra* | 112 | 36 | 21 | 19 |
| | Dead tree | 26 | 7 | 5 | 19 |
| Ochnaceae | *Ouratea littoralis* | 28 | 7 | 5 | 18 |
| | Unidentified tree | 12 | 2 | 2 | 17 |
| Anacardiaceae | *Comocladia dodonaea* | 8 | 2 | 1 | 13 |
| Malpighiaceae | *Byrsonima lucida* | 24 | 5 | 3 | 13 |
| Myrtaceae | *Myrcia citrifolia* | 25 | 3 | 3 | 12 |
| Bignoniaceae | *Tabebuia haemantha* | 133 | 19 | 13 | 10 |
| Myrtaceae | *Pimenta racemosa* | 13 | 2 | 1 | 8 |
| Meliaceae | *Swietenia mahagoni* | 12 | 1 | 1 | 8 |
| Polygonaceae | *Coccoloba microstachya* | 65 | 4 | 3 | 5 |
| Apocynaceae | *Plumeria krugii* | 1 | 0 | 0 | 0 |
| Asteraceae | *Lepidaploa sericea* | 1 | 0 | 0 | 0 |

| | | | | | |
|---|---|---|---|---|---|
| Boraginaceae | *Varronia lima* | 2 | 0 | 0 | 0 |
| Celastraceae | *Gyminda latifolia* | 2 | 0 | 0 | 0 |
| Ehretiaceae | *Bourreria succulenta* | 1 | 0 | 0 | 0 |
| Ehretiaceae | *Bourreria virgata* | 1 | 0 | 0 | 0 |
| Fabaceae | *Poitea punicea* | 5 | 0 | 0 | 0 |
| Malpighiaceae | *Stigmaphyllon floribundum* | 5 | 0 | 0 | 0 |
| Polygalaceae | *Badiera penaea* | 1 | 0 | 0 | 0 |
| Primulaceae | *Ardisia elliptica* | 3 | 0 | 0 | 0 |
| Rubiaceae | *Randia aculeata* | 10 | 0 | 0 | 0 |
| Schoepfiaceae | *Schoepfia obovata* | 1 | 0 | 0 | 0 |
| Solanaceae | *Cestrum citrifolium* | 4 | 0 | 0 | 0 |
| Theaceae | *Ternstroemia stahlii* | 1 | 0 | 0 | 0 |

*Psychilis kraenzlinii* are not randomly distributed around the trunk of trees. I found a significant association between orchids and their cardinal position ($X^2$ = 18.4, df = 7, p = 0.01). The number of orchids growing on the east side of the trunk was 2.6 times higher than expected by the standardized residuals. Orchids growing on the northeast and southwest side were 2.6 and 2.0 times less than expected by the standardized residuals, respectively (Figure 2).

*Psychilis kraenzlinii* significantly associates with certain tree species over others (Fisher Exact Test, p < 0.01, *Monte Carlo Simulation* = 2000). A Fisher's pairwise comparison of pooled plot data revealed significant differences between *Machaonia portorricensis* and *Coccoloba microstachya* (p < 0.005, Figure 3), where the former has a higher number of orchids than the latter. There were more orchids growing on *M. portoricensis* than expected if the orchid

distribution among phorophyte species was random. Conversely, there were less orchids growing

on *C. microstachya* than expected if the distribution of the orchid among phorophyte species was
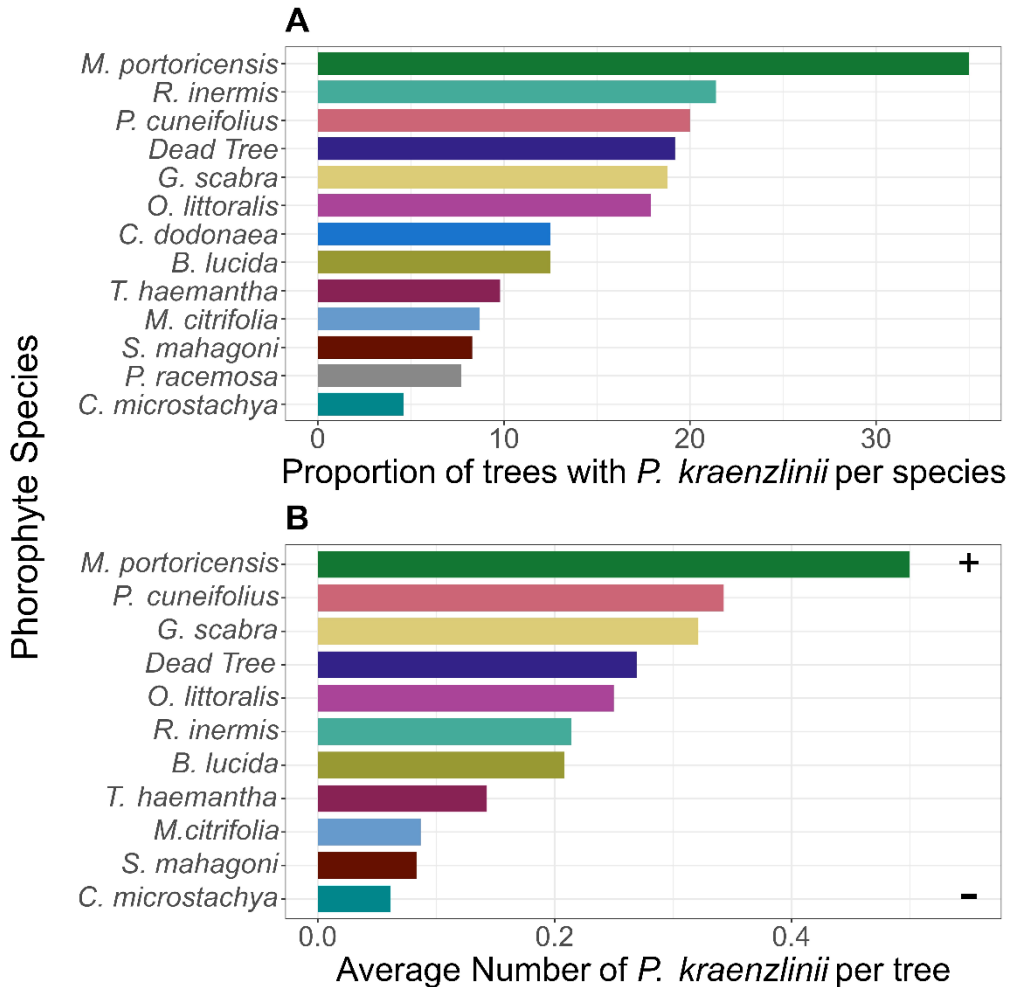
random.

**A**



**B**



**Figure 3: A.** Proportion of trees of each phorophyte species harboring *P. 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).

*Water Storage Capacity.* I found water holding capacity (WHC) to be significantly different among

phorophyte species (WHC, Kruskal-Wallis test: $X^2 = 82.62$, df = 7, p < 0.005, Figure 4A), 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 D) showed that *M. portorricensis* has the highest
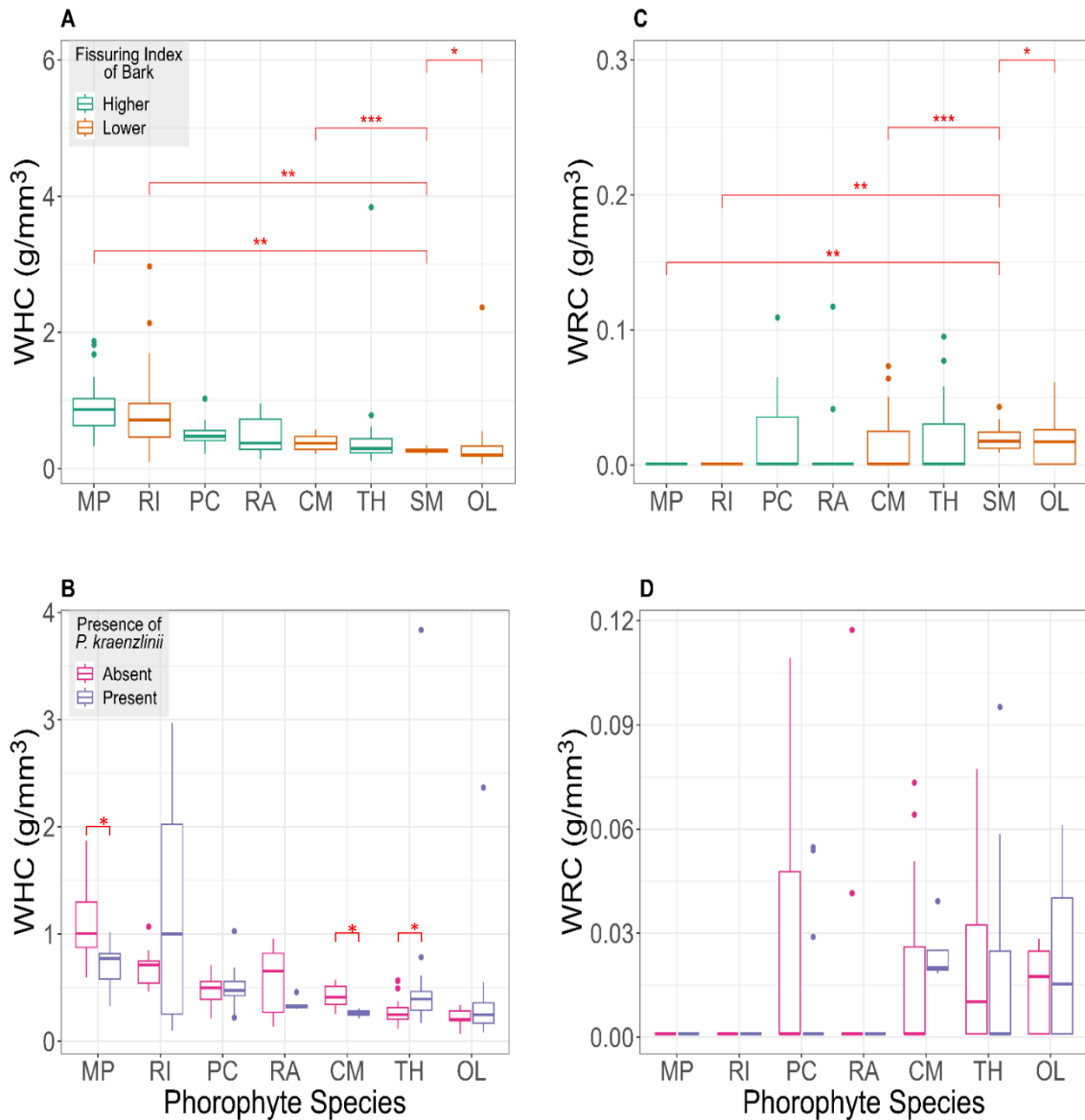
**Figure 4. A.** Box plots of Water holding capacity (WHC) among phorophyte species with different fissuring index levels. (Red brackets represent equal distributions, differences among species ot marked with brackets is statistically significant, p < 0.05). **B.** Box plots of Water holding capacity (WHC) among trees with and without *P. kraenzlinii* among phorophyte species. *Swietenia mahagoni* not shown because data of trees with a *P. kraenzlinii* was not available. (Orange asterisk marks significant differences, p < 0.05). **C.** Box plots of Water Retention Capacity (WRC) among phorophyte species with different fissuring index levels. (Orange asterisk marks significant differences, p < 0.05). **D.** Box plots of Water Retention Capacity (WRC) among trees with and without *P. kraenzlinii* among phorophyte species.

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*. When looking at

trees with and without the orchid within each species, I found that trees with the orchid had a significantly higher WHC if the species was *T. haemantha*, but significantly lower if the species was *Coccoloba microstachya* or *M. portorricensis* (Appendix E, Figure 4B).

Water retention capacity (WRC) was found to be different among phorophyte species (Kruskal-Wallis test: $X^2 = 43.22$, df = 7, p < 0.005, Figure 4C), but not between trees with or without the orchid in general (Mann-Whitney: U = 4560.5, p = 0.22). The Conover-Iman Pairwise Comparison test for WRC (Appendix D) showed that the highest WRC was that of *S. mahagoni*, and it was significantly different from all other species. *Randia aculeata*, *R. inermis* and *M. portorricensis* have WRC of 0.0 g/mm$^3$ or nearly so, no difference among them, but significantly lower than *S. mahagoni*, *T. haemantha*, and *O. littoralis*. *Phyllanthus cuneifolius* has a WRC significantly higher than *M. portorricensis* 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, I also found no statistically significant differences (Appendix E, Figure 4D).

*Fissuring Index Assessment.* Fissuring index differed significantly among phorophyte species (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*. I 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. Still, *R. inermis* was only significantly lower than *M. portorricensis* (Appendix D, Figure 5A). I 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 (Figure 4A and 4C).

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. portorricensis* (Mann-Whitney: U = 208, p < 0.005, Figure 5B).
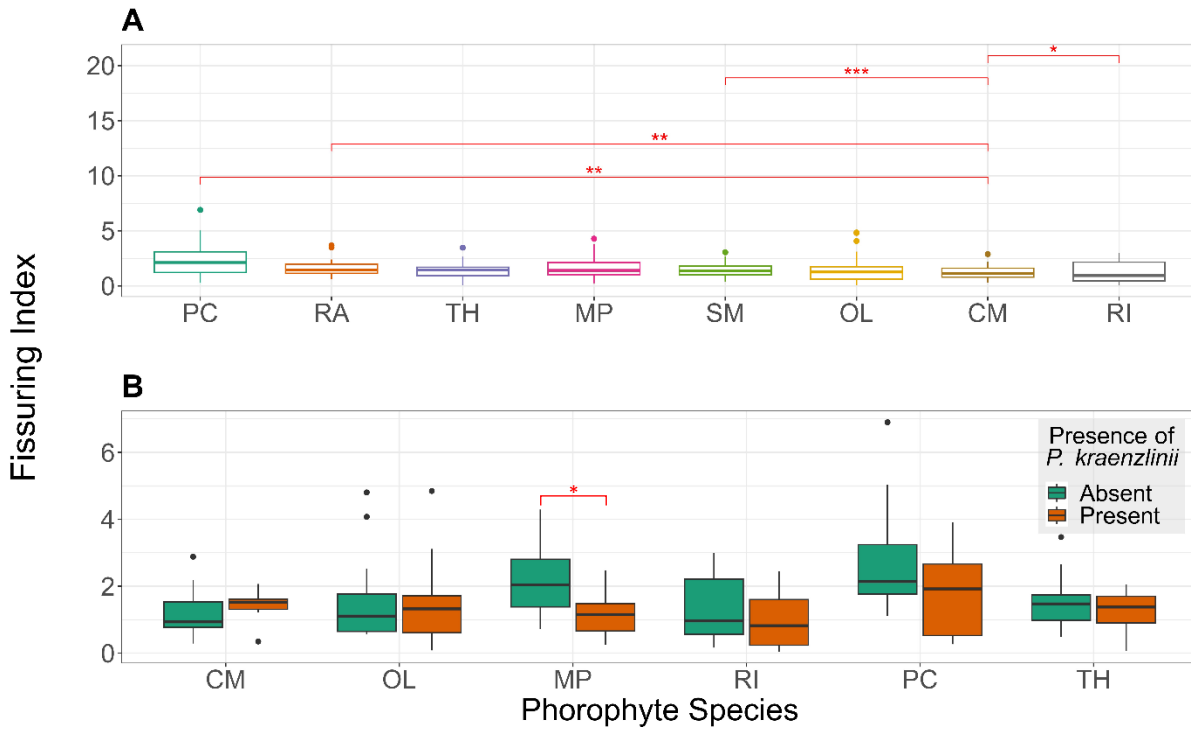


**Figure 5. A.** Box plots of fissuring index of the bark among phorophyte species. **B.** Box plots of the fissuring index of the bark of trees with and without *P. kraenzlinii* among phorophyte species. *Randia aculeata* and *Swietenia mahagoni* not shown because data of trees with a *P. kraenzlinii* was not available. Red brackets with asterisks mark significant differences where: * = p ≤ 0.05; ** = p ≤ 0.01; *** = p ≤ 0.001.

In situ *seed germinations.* Of the 174 recovered packages, only 37 (21%) contained developing seeds. Of the 37 packets with developing seeds, 20 (54%) were near an established *P. kraenzlinii*. A chi-squared test revealed that there is an association between the presence of the orchid and whether there were developing seeds in a packet or not ($X^2$ = 5.7, df = 1, p = 0.01). When revising the standard residual, I found that there are 1.9 times more packets with developing

seeds near established orchids than expected. I also found an association between the phorophyte species and whether there were developing seeds in the packet or not ($X^2 = 12.97$, df = 6, p = 0.04). The best model to explain the number of packets with and without developing seeds was a binomial one where *C. microstachya* was placed as the intercept (Model A in Table 2). 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. portorricensis* (OR = 1.9, Model A in Table 3, Figure 6).
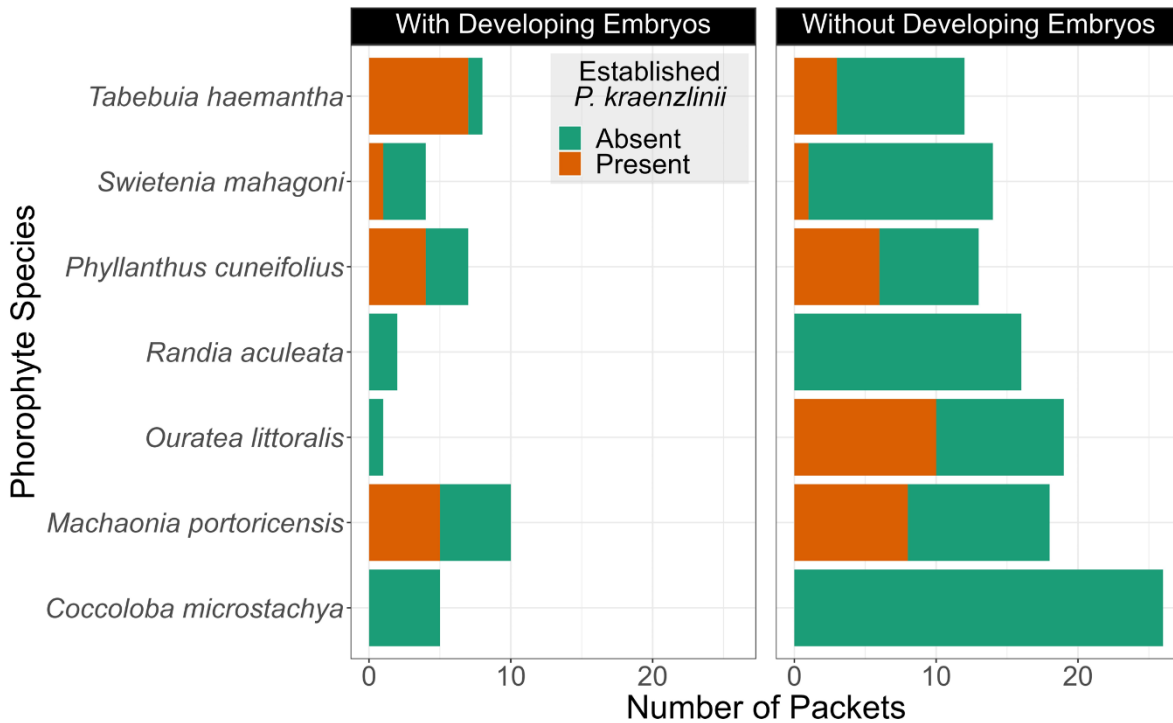


**Figure 6.** The number of packets with and without developing seeds on each phorophyte species and whether they were placed near an established *P. kraenzlinii* or not.

I observed 228 developing seeds among all packets, 121 of which were in packets near a *P. kraenzlinii*. The proportion of developing seeds in packets 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 (Figure 1). I found 85 (40%) seeds in Stage 1, 76 (35%) in Stage 2, 35 (16%) in Stage 3,
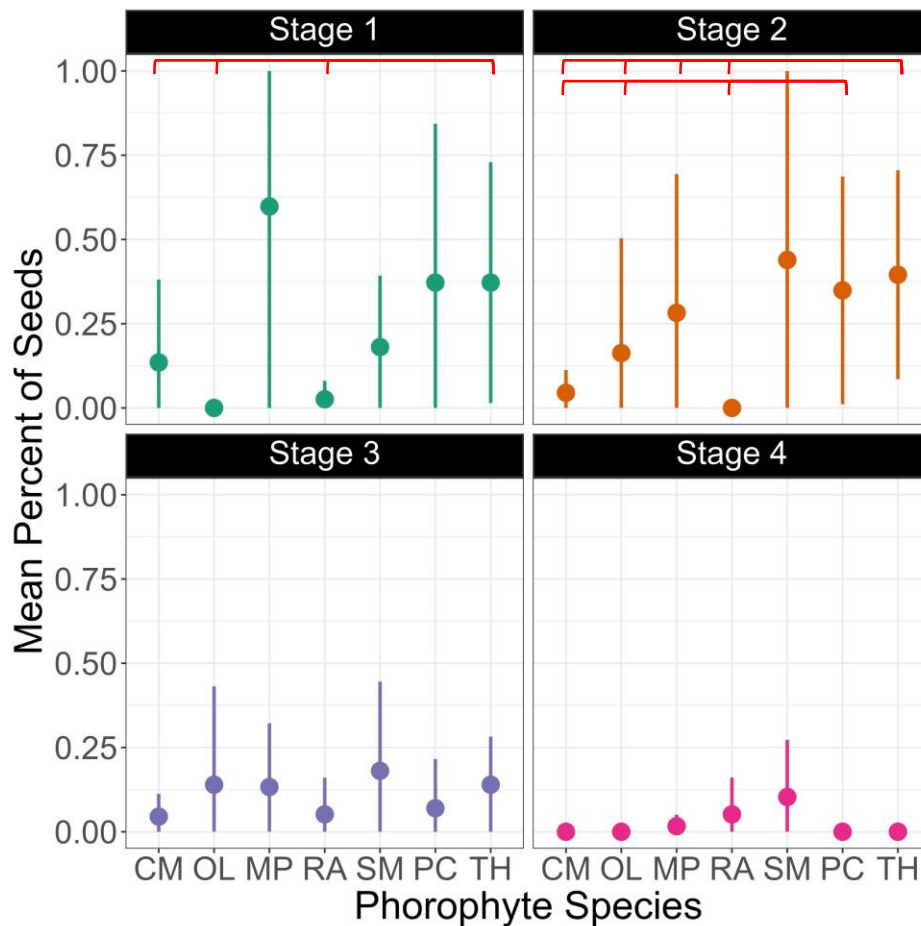
**Figure 7.** Mean percent of developing seeds per packet and their development stage on phorophytes with and without an established *P. kraenzlinii*. Where Stage 1 refers to seeds with swollen embryos, Stage 2 are seeds whose embryo have swelled to the point of breaking the testa, Stage 3 the testa is gone and the protocorm is formed, and Stage 4 the protocorm has elongated. For *O. littoralis*, no seeds developed near an established *P. kraenzlinii*, whereas in the case of *C. microstachya* and *R. aculeata*, no packets were placed near and established orchid. Red lines indicate significant differences according to Conover-Iman Pairwise Comparisons (p<0.05).

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 7.

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 2). The presence of an established

*P. kraenzlinii* does not have a significant effect over the percentage of developing seeds. Among

all phorophytes, *M. portorricensis* 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 over the percentage of developing seeds. The odds of *P. kraenzlinii* developing on *C. microstachya* are near zero, while the odds for developing on *M. portorricensis* 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 3).

When comparing the percentage of seeds in each 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 F). Conversely, *T. haemantha* had significantly more seeds that went through imbibition (stage 1) than *C. microstachya, O. littoralis*, and *R. aculeata* (Conover-Iman: p < 0.05) (Appendix F)*. 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. portorricensis*, and *P. cuneifolius* had more than *R. aculeata* (Conover-Iman: p < 0.05, Appendix F). 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 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

**Table 2.** Factors associated with the effect of phorophyte species on seed germination. Analyses are based on coefficients generated by Generalized Linear Models. Model A: Negative Bionomial 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 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Estimate | Std. Error | *z* | *p* | Estimate | Std. Error | *z* | *p* | Estimate | Std. Error | *z* | *p* |
| Intercept *(Coccoloba microstachya)* | -1.65 | 0.49 | -3.38 | **<0.01\*** | -1.49 | 0.61 | -2.43 | **0.02\*** | -3.43 | 1.02 | -3.38 | **<0.01\*** |
| *Machaonia portorricensis* | 0.63 | 0.68 | 0.92 | 0.36 | 1.65 | 0.81 | 2.03 | **0.04\*** | -0.56 | 1.6 | -0.35 | 0.73 |
| *Ouratea littoralis* | -1.82 | 1.19 | -1.53 | 0.13 | 0.28 | 0.95 | 0.30 | 0.76 | -0.22 | 1.43 | -0.16 | 0.88 |
| *Randia aculeata* | -0.43 | 0.90 | -0.48 | 0.63 | 0.21 | 0.99 | 0.21 | 0.83 | 0.54 | 1.44 | 0.38 | 0.71 |
| *Phyllanthus cuneifolius* | 0.56 | 0.74 | 0.76 | 0.45 | 1.13 | 0.90 | 1.26 | 0.21 | 1.31 | 1.23 | 1.07 | 0.29 |
| *Swietenia mahagoni* | 0.28 | 0.76 | 0.37 | 0.72 | 1.43 | 0.91 | 1.57 | 0.12 | -0.62 | 1.45 | 0.04 | 0.97 |
| *Tabebuia haemantha* | 0.78 | 0.72 | 1.08 | 0.28 | 1.33 | 0.89 | 1.49 | 0.14 | 0.06 | 1.45 | 0.04 | 0.97 |
| *P. kraenzlinii* 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 | | | |
| | Reidual deviance: 152.86 on 147 df | | | | Residual deviance: 152.86 on 147 df | | | | Residual deviance: 74.46 on 158 df | | | |

**Table 3**. 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 Bionomial 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 (*Coccoloba microstachya*) | 0.19 | 0.07 | 0.46 | 0.23 | 0.07 | 0.82 | 0.03 | 0 | 0.15 |
| *Machaonia portorricensis* | 1.87 | 0.50 | 7.50 | 5.22 | 1.05 | 27.44 | 0.80 | 0.05 | 21.54 |
| *Ouratea littoralis* | 0.16 | 0.01 | 1.24 | 1.33 | 0.21 | 9.43 | 0.57 | 0.02 | 21.54 |
| *Randia aculeata* | 0.65 | 0.09 | 3.42 | 1.23 | 0.18 | 9.57 | 1.72 | 0.07 | 45.36 |
| *Phyllanthus cuneifolius* | 1.75 | 0.41 | 7.69 | 3.10 | 0.55 | 20.38 | 0.54 | 0.02 | 17.32 |
| *Swietenia mahagoni* | 1.32 | 0.28 | 5.87 | 4.18 | 0.73 | 29.24 | 3.70 | 0.39 | 80.85 |
| *Tabebuia haemantha* | 2.19 | 0.53 | 9.47 | 3.76 | 0.68 | 24.48 | 1.06 | 0.06 | 29.2 |
| *P. kraenzlinii* Present | 2.41 | 0.95 | 6.30 | NA | NA | NA | 4.70 | 0.97 | 31.63 |

OMF (Model C in Table 2). 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 3).

**Discussion.** *Phorophyte Specificity Assessment*. Migenis and Ackerman (1993) suggested that host preference rather than specificity is common in Puerto Rico and the Neotropics. As for closely related species *P. monensis* (Otero, Aragón & Ackerman 2007) and *P. krugii* (Ackerman *et al*. 1989), 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 portorricensis, Coccoloba microstachya* is the least preferred phorophyte given the availability of this tree species in the study area. Contrastingly, Otero, Aragón & Ackerman (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 characteristics of phorophyte, but also on microsite conditions such as temperature, humidity, competition and cardinal direction, etc. Otero, Aragón & Ackerman (2007) noted that the relationship 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).

The position occupied on trees was not random. More orchids were found growing on the east side of the trunk, and no higher than 0.74 m from the ground. This distribution pattern in the forest might be explained by radiation and wind patterns, as well as humidity and nutrient availability alongside the trunk (see Petter *et al*. 2016), but I lack the microenvironmental data to test it.

*Phorophyte Physical Characteristics.* Variation in WHC and WRC between trees with and without the orchid irrespective of their species, was not significant. However, I found that *Machaonia portorricensis*, the most preferred phorophyte species, compared to the other tree 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 relative to the other tree species evaluated. 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. portorricensis*, *P. cuneifolius*, *R. inermis*), *O. littoralis* has the second lowest WHC and an intermediate WRC. Nonetheless, in general, preferred phorophytes tend to have high WHC and low WRC. I 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, 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, Mendieta-Leiva & Zotz (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 WSC (Migenis & Ackerman 1993, Otero, Aragón & Ackerman 2007, Zarate-García *et al.* 2020). I 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. portorricensis*, where more orchids were growing on trees with rougher bark (lower FI). This relationship is also present as a non-significant 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 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 Guanica, Puerto Rico, and Otero, Aragón & Ackerman (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 I studied is not uniformly colored, which may affect the FI results (Figure 8). 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 I 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
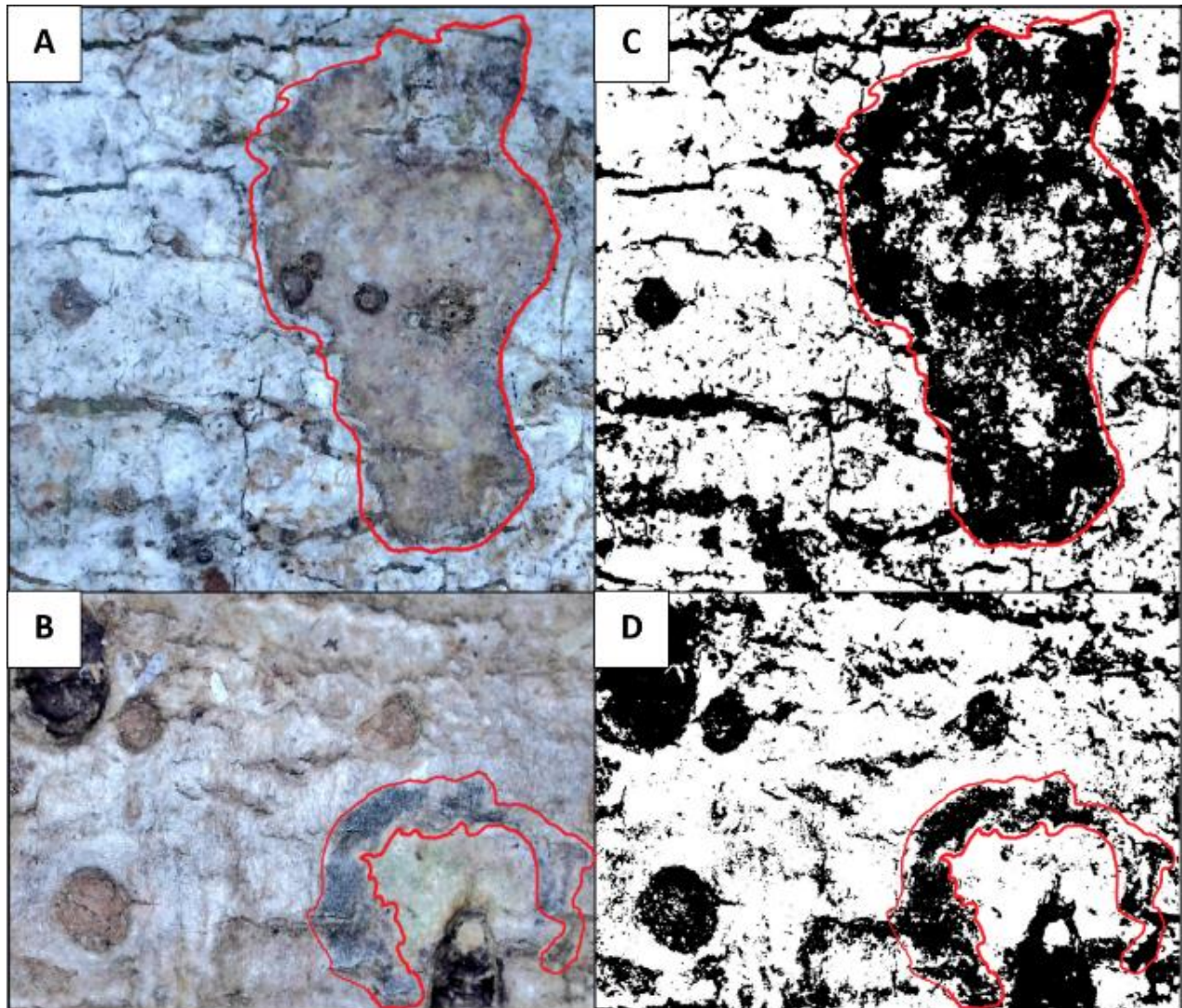
**Figure 8.** Effect of bark pigmentation on the fissuring index. **A:** Pictures of the bark surface of *Coccoloba microstachya* taken with a camera coupled to a dissecting microscope and a ring light. **B:** Pictures of the bark surface of *Phyllanthus cuneifolius* taken with a camera coupled to a dissecting microscope and a ring light. **C:** Photos converted to black and white (binary) images of *Coccoloba microstachya*. **D:** Photos converted to black and white (binary) images of *Phyllanthus cuneifolius*. Enclosed in red is the area where roughness interpretation could be affected by bark pigmentation. Photos by N. González-Orellana.

seasonality as well as by the method of attachment used in each stage of the life history of an epiphytic species (Tay, Zotz & Einzmann 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, Aragón & Ackerman (2007) found no clear relationship for either *P. monensis* nor *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, Zotz & Einzmann 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 previous studies Kartzinel, Trapnell & Shefferson (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, Kaur & Sharma (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 developing seeds 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. I hypothesized that germination would be higher near established orchids because of a higher propagule pressure, higher probability of OMF availability and appropriate microsite conditions. Results suggest that embryo development 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 developing seeds *P. kraenzlinii* that are ready to undergo germination tend to mirror that of the distribution of established orchids in the Susúa State Forest. A higher probability for developing seeds than expected was found on *S. mahagoni* and *T. haemantha*, species on which it is rare to find established orchids. In Guánica State Forest, *P. krugii* did not grow on *Tabebuia heterophylla*—a closely related species to *T. haemantha* (Gross & Olmstead 2007)—, although authors did not discuss this further because of the lack of information on the species, both the phorophyte and epiphyte (Ackerman, Montalvo & Vera 1989). In one of the sites on Mona Island, *P. monensis* was found growing on 60% of the available *T. haemantha* (Otero, Aragón & Ackerman 2007). Moreover, unlike for *P. kraenzlinii* in Susua State Forest, *C. microstachya* was a good host for *P. monensis*. Although the authors do not discuss the occurrence of *P. monensis* on *T. haemantha* directly, they mention that differences in the occurrence of *P. monensis* on different phorophytes

between the two sites in Mona Island could be explained by microsite limitations, specifically water relations, and phorophyte species composition (Otero, Aragón & Ackerman 2007).

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, I 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 my plots, but no embryo development was observed on this phorophyte species. Thus, best sites for germination are not necessarily the same as sites where plants can develop and survive, as observed by Crain, Sánchez-Cuervo, & Deren (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 Jersáková & Malinová 2007 and Gowland *et al*. 2011). Moreover, Otero, Aragón & Ackerman (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 along 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 development (Otero, Bayman & Ackerman 2005, Bidartondo & Read 2008, Meng *et al.* 2019a, 2019b, Fernández, Kaur, & Sharma 2023).

**Conclusions and Recommendadtions.**

- *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 in Puerto Rico, so comparative studies of different populations might reveal how environmental conditions affect phorophyte preferences.

- Since populations of *P. kraenzlinii* have been affected by deforestation and habitat fragmentation, preservation of the orchid might benefit from reintroducing the species to previously known areas as well as introduction to new areas with characteristics that could harbor the species. To reintroduce and/or introduce the orchid successfully, a comprehensive understanding of the dynamics of the orchid with its environment is necessary (See Swarts & Dixon 2017).

- 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.

- In the case that the OMF has not been identified, once the proper sites for introduction and reintroduction of the orchid have been scrupulously identified, I would suggest germinating the seeds *in situ* in an established population where the OMF can be found. Once seedlings develop, they can be translocated to the desired sites. For this exercise, fruit production might need to be stimulated by manual cross pollinations to ensure: (1) the production of viable seeds, and (2) promote genetic variability.

- 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 ecosystem (Pierce & Belotti 2011).

- This is a population near the edge of the Forest Reserve and should be monitored for any incursions and adjacent development which may affect ecosystem function.

- 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 (Phillips, Reiter & Peakall 2020, Fay 2018, Myers *et al*. 2000).

**References**

Ackerman, J. D. (1986). Coping with the epiphytic existence: pollination strategies. *Selbyana, 9*(1), 52-60.

Ackerman, J. D., Montalvo, A.M., and Vera, A.M. (1989). Epiphyte host specificity of *Encyclia krugii*, a Puerto Rican endemic orchid. *Lindleyana*, *4*(2), 74-77.

Ackerman, J. D. and Collaborators. (2014). *Orchid flora of the Greater Antilles*. Memoirs of the New York Botanical Garden, *105*.

Ackerman, J. D., Sabat, A., & Zimmerman, J. K. (1996). Seedling establishment in an epiphytic orchid: an experimental study of seed limitation. *Oecologia*, *106*(2), 192-198. doi: 10.1007/BF00328598

Adhikari, Y. P., & Fischer, A. (2011). Distribution pattern of the epiphytic orchid

    *Rhynchostylis retusa* under strong human influence in Kathmandu valley, Nepal.

    *Botanica Orientalis: Journal of Plant Science, 8*, 90-99. doi: 10.3126/botor.v8i0.5956

Aragón, S. & Ackerman, J. D. (2004). Does flower color variation matter in deception pollinated

    *Psychilis monensis* (Orchidaceae)?. *Oecologia, 138*(3), 405-413. doi: 10.1007/s00442-

    003-1443-9

Arditti, J. (1992). *Fundamentals of orchid biology*. Wiley. 595.

Axelrod, F. S. (2011). *A systematic vandemecum to the vascular plants of Puerto Rico*. Brit

    Press, 34.

Barthelme, S. (2023). imager: Image processing library based on 'CImg' (R package version

    0.45.2). https://CRAN.R-project.org/package=imager

Benzing, D. H. (1979). Alternative interpretations for the evidence that certain orchids and

    bromeliads act as shoot parasites. *Selbyana 5*(2), 135-144.

Benzing, D. H. (1990). *Vascular epiphytes. General biology and related biota*. Cambridge

    University Press.

Benzing, D. H. (2004). Vascular epiphytes. In: D. M. Lowman & H. B. Rinker (2nd Ed.), *Forest

    canopies* (2nd Ed.) (pp. 175-211). Elsevier.

Bidartondo, M. I. (2005). The evolutionary ecology of myco-heterotrophy. *New Phytologist*,

    *167*(2), 335-352. doi: 10.1111/j.1469-8137.2005.01429.x

Bidartondo, M. I., & Read, D. J. (2008). Fungal specificity bottlenecks during orchid germination and development. *Molecular Ecology*, *17*(16), 3707-3716. doi: 10.1111/j.1365-294X.2008.03848.x

Brandner, S. (2005). An assessment of the fungal specificity of the orchid sub-tribe Pterostylis. *Centre for Australian National Biodiversity Research and Australian National Herbarium*. Retrieved from https://www.cpbr.gov.au/cpbr/summer-scholarship/2005-projects/brandner-susan-orchids/index.html

Cabrera-García, B., Guerrero, A., Folgado, R., Jimenez, F. & Serra, C. (2023) Biología reproductiva de *Psychilis truncata* (Orchidaceae) en Arroyo Corral, Partido, Dajabón, República Dominicana. *Moscosoa*, *21*, 99-114

Callaway, R. M., Reinhart, K. O., Moore, G. W., Moore, D. J., & Pennings, S. C. (2002). Epiphyte host preferences and host traits: mechanisms for species-specific interactions. *Oecologia*, *132*(2), 221-230. doi: 10.1007/s00442-002-0943-3

Crain, B. J., Sánchez-Cuervo, A. M., & Deren, V. (2022). Mixed evidence of a commensal relationship between a rare epiphytic orchid and cohabiting bryophytes. *Botanical Journal of the Linnean Society*, *201*(4). doi: https://doi.org/10.1093/botlinnean/boac049

Departamento de Recursos Naturales. (2015). El Bosque Estatal de Susua. Retrieved from http://drna.pr.gov/wp-content/uploads/2015/04/El-Bosque-Estatal-de-Susúa.pdf

Diez, J. M. (2007). Hierarchical patterns of symbiotic orchid germination linked to adult

proximity and environmental gradients. *Journal of Ecology*, *95*(1), 159-170.

10.1111/j.1365-2745.2006.01194.x

Fay, M. F. (2018). Orchid conservation: how can we meet the challenges in the twenty-

first century?. *Botanical Studies*, *59*(16), 1-6. doi: 10.1186/s40529-018-0232-z

Fernández, M., Kaur, J., & Sharma, J. (2023). Co-occurring epiphytic orchids have

specialized mycorrhizal fungal niches that are also linked to ontogeny.

*Mycorrhiza*, *33*(1-2), 1-19. doi: 10.1007/s00572-022-01099-w

Frei, S. J. K. (1973). Effect of bark substrate on germination and early growth of

*Encyclia tampensis* seeds. *American Orchid Society Bulletin*, *42*(8), 701-708.

Frei, S. J. K. & Dodson, C. H. (1972). The chemical effect of certain bark substrates on

the germination and early growth of epiphytic orchids. *Bulletin of the Torrey

Botanical Club*, *99*(6), 301-307. doi: https://doi.org/10.2307/2997072

Gentry, A.H. & Dodson, C. H. (1987a). Contributions of nontrees to species richness of a tropical

rain forest. *Biotropica*, *19*(1), 149-156. doi: https://doi.org/10.2307/2388737

Gentry, A.H. & Dodson, C. H. (1987b). Diversity and biogeography of neotropical

vascular epiphytes. *Annual Missouri Botanical Garden, 74*(2), 205–233. doi:

https://doi.org/10.2307/2399395

González-Orellana, N., Salazar-Mendoza, A., Numan, Y. & Ackeman, J. D. (2022).

Understanding orchid conservation, one species at a time. *Orchids*, *91*(12),

914-919.

Gowland, K. M., Wood, J., Clements, M. A., & Nicotra, A. B. (2011). Significant phorophyte

(substrate) bias is not explained by fitness benefits in three epiphytic orchid species.

*American Journal of Botany*, *98*(2), 197-206. doi: 10.3732/ajb.1000241

Gravendeel, B., Smithson, A., Slik, F. J. W., & Schuiteman, A. (2004). Epiphytism and pollinator

specialization: drivers for orchid diversity? *Philosophical Transactions of the Royal

Society London B*, *359*, 1523-1535.

Hietz, P. (1998). Diversity and conservation of epiphytes in a changing environment. *International

Union of Pure and Applied Chemistry, 70*(11).

Hietz, P., & Hietz-Seifert, U. (1995). Composition and ecology of vascular epiphyte

communities along an altitudinal gradient in central Veracruz, Mexico. *Journal of

Vegetation Science*, *6*(4), 487-498. doi: https://doi.org/10.2307/3236347

Izuddin, M., Srivathsan, A., Lee, A. L., Yam, T. W., & Webb, E. L. (2019). Availability of

orchid mycorrhizal fungi on roadside trees in a tropical urban landscape. *Scientific Reports,

9*(1), 1-12. doi: 10.1038/s41598-019-56049-y

Izuddin, M., Yam, T. W., & Webb, E. L. (2019). Germination niches and seed

persistence of tropical epiphytic orchids in an urban landscape. *Journal of Plant

Research, 132*(3), 383-394. doi: 10.1007/s10265-019-01110-0

Jacquemyn, H., Brys, R., Vandepitte, K., Honnay, O., Roldán-Ruiz, I., & Wiegand, T.

(2007). A spatially explicit analysis of seedling recruitment in the terrestrial orchid

*Orchis purpurea. New Phytologist, 176*(2), 448-459. doi: 10.1111/j.1469-8137.2007.02179.x

Jersáková, J., & Malinová, T. (2007). Spatial aspects of seed dispersal and seedling

recruitment in orchids. *New Phytologist 176*(2), 237-241. doi: 10.1111/j.1469-8137.2007.02223.x

Khamchatra, N. M., Dixon, K., Chayamarit, K., Apisitwanich, S., & Tantiwiwat, S. (2016).

Using in situ seed baiting technique to isolate and identify endophytic and

mycorrhizal fungi from seeds of a threatened epiphytic orchid, *Dendrobium*

*friedericksianum* Rchb. f.(Orchidaceae). *Agriculture and Natural Resources,*

*50*(1), 8-13. doi: https://doi.org/10.1016/j.anres.2016.01.002

Kartzinel, T. R., Trapnell, D. W., & Shefferson, R. P. (2013). Highly diverse and spatially

heterogeneous mycorrhizal symbiosis in a rare epiphyte is unrelated to broad

biogeographic or environmental features. *Molecular Ecology, 22*(23), 5949-5961. doi:

10.1111/mec.12536

Kress, W. J. (1986). The systematic distribution of vascular epiphytes: an update. *Selbyana, 9*(1),

2-22.

Laube, S., & Zotz, G. (2006). Neither host-specific nor random: vascular epiphytes on

three tree species in a Panamanian lowland forest. *Annals of Botany, 97*(6),

1103-1114. doi: 10.1093/aob/mcl067

McCormick, M. K., Taylor, D. L., Whigham, D. F., & Burnett Jr, R. K. (2016). Germination patterns in three terrestrial orchids relate to abundance of mycorrhizal fungi. *Journal of Ecology, 104*(3), 744-754. doi: https://doi.org/10.1111/1365-2745.12556

Meng, Y. Y., Fan, X. L., Zhou, L. R., Shao, S. C., Liu, Q., Selosse, M. A., & Gao, J. Y. (2019a). Symbiotic fungi undergo a taxonomic and functional bottleneck during orchid seeds germination: a case study on *Dendrobium moniliforme*. *Symbiosis, 79*, 205-212. doi: 10.1007/s13199-019-00647-x

Meng, Y. Y., Zhang, W. L., Selosse, M. A., & Gao, J. Y. (2019b). Are fungi from adult orchid roots the best symbionts at germination? A case study. *Mycorrhiza, 29*(5), 541-547. doi: 10.1007/s00572-019-00907-0

Migenis, L. E., & Ackerman, J. D. (1993). Orchid—phorophyte relationships in a forest watershed in Puerto Rico. *Journal of Tropical Ecology, 9*(2), 231-240. doi: 10.1017/S0266467400007227

Miller, J. S., Krupnick, G. A., Stevens, H., Porter-Morgan, H., Boom, B., Acevedo-Rodríguez, P., Ackerman, J. D., Kolterman, D., Santiago, E., Torres, C. and Velez, J. (2013). Toward Target 2 of the Global Strategy for Plant Conservation: An Expert Analysis of the Puerto Rican Flora to Validate New Streamlined Methods for Assessing

Conservation Status1. *Annals of the Missouri Botanical Garden, 99*(2), 199-205. doi:

https://doi.org/10.3417/2011121

Miller, G., & Lugo, A. E. (2009). *Guide to the ecological systems of Puerto Rico*. USDA, IITF-

GTR-35.

Myers, N., Mittermeier, R. A., Mittermeier, C. G., Da Fonseca, G. A., & Kent, J. (2000).

Biodiversity hotspots for conservation priorities. *Nature, 403*(6772), 853-858. doi:

https://doi.org/10.1038/35002501

Otero, J. T., Aragón, S., & Ackerman, J. D. (2007). Site variation in spatial aggregation

and phorophyte preference in *Psychilis monensis* (Orchidaceae). *Biotropica*,

*39*(2), 227-231. doi: 10.1111/j.1744-7429.2006.00258.x

Otero, J. T., Bayman, P., & Ackerman, J. D. (2005). Variation in mycorrhizal performance

in the epiphytic orchid *Tolumnia variegata* in vitro: the potential for natural

selection. *Evolutionary Ecology, 19*, 29-43. doi: 10.1007/s10682-004-5441-0

Otero, J. T., Flanagan, N. S. (2006). Orchid diversity – beyond deception. *Trends in Ecology and

Evolution, 21*(2), 64-65. doi: https://doi.org/10.1016/j.tree.2005.11.016

Petrolli, R., Augusto Vieira, C., Jakalski, M., Bocayuva, M. F., Vallé, C., Cruz, E. D. S., Selosse,

M., Martos, F., and Kasuya, M. C. M. (2021). A fine-scale spatial analysis of fungal

communities on tropical tree bark unveils the epiphytic rhizosphere in orchids. *New

Phytologist, 231*(5), 2002-2014. doi: 10.1111/nph.17459

Petrolli, R., Zinger, L., Perez-Lamarque, B., Collobert, G., Griveau, C., Pailler, T., Selosse, M. and Martos, F. (2022). Spatial turnover of fungi and partner choice shape mycorrhizal networks in epiphytic orchids. *Journal of Ecology, 110*(11), 2568-2584. doi: https://doi.org/10.1111/1365-2745.13986

Petter, G., Wagner, K., Wanek, W., Sánchez Delgado, E. J., Zotz, G., Cabral, J. S., & Kreft, H. (2016). Functional leaf traits of vascular epiphytes: vertical trends within the forest, intra-and interspecific trait variability, and taxonomic signals. *Functional Ecology, 30*(2), 188-198. doi: https://doi.org/10.1111/1365-2435.12490

Phillips, R. D., Reiter, N., & Peakall, R. (2020). Orchid conservation: from theory to practice. *Annals of Botany, 126*(3), 345-362. doi: https://doi.org/10.1093/aob/mcaa093

Pierce, S. & Belotti, J. (2011). *The Conservation of Terrestrial Orchids from the Alps to the Po Plain Lombardy*. Parco delle Orobie Bergamasche and Centro Flora Autoctona della Regione Lombardia. Galbiate LC, Italy.

Rasmussen, H. N. (1995). *Terrestrial orchids: from seed to mycotrophic plant*. United Kingdom: Cambridge University Press.

Rasmussen, H. N., Dixon, K.W., Jersáková, J. and Těšitelová. (2015). Germination and seedling establishment in orchids: a complex of requirements. *Annals of Botany, 116*(3), 391-402. doi: 10.1093/aob/mcv087

Sanford, W. W. (1974) The Ecology of Orchids. In: C. L. Withner (1st Ed.), *The orchids scientific*

*studies*.

Sauleda, R. P. (1988). A revision of the genus *Psychilis* Rafinesque (Orchidaceae).

*Phytologia, 65,* 1-33.

Sáyago, R., Lopezaraiza-Mikel, M., Quesada, M., Álvarez-Añorve, M. Y., Cascante-

Marín, A., & Bastida, J. M. (2013). Evaluating factors that predict the structure of

a commensalistic epiphyte–phorophyte network. *Proceedings of the Royal*

*Society B, 280*(1756), 20122821. doi:

https://doi.org/10.1098/rspb.2012.2821

Siaz-Torres, S. S., Mora-Olivo, A., Arellano-Méndez, L. U., Vanoye-Eligio, V.,

Flores-Rivas, J., & de la Rosa-Manzano, E. (2021). Contribution of peeling host

for epiphyte abundance in two tropical dry forests in the "El Cielo Biosphere

Reserve", Mexico. *Plant Species Biology, 36*(2), 269-283. doi:

https://doi.org/10.1111/1442-1984.12317

Stenberg, M. L., & Kane, M. E. (1998). In vitro seed germination and greenhouse

cultivation of *Encyclia boothiana* var. erythronioides, an endangered Florida

orchid. *Lindleyana, 13*(2), 101-112. doi: https://doi.org/10.1016/j.anres.2016.01.002

Sulit, M. D. (1950). Field observations on tree hosts of orchids in the Philippines. *Philippines*

*Orchid Review 3*, 3-8.

Sulit, M. D. (1953). Field observations on tree hosts of orchids in Palawan. *Philippines Orchid*

   *Review 5*, 16.

Swarts, N.D. & Dixon, K.W. (2017). *Conservation methods for terrestrial orchids*. Florida, USA:

   J. Ross Publishing.

Szumilas, M. (2010). Explaining odds ratios. *Journal of the Canadian Academy of Child*

   *and Adolescent Psychiatry, 19*(3), 227-229.

Tay, J. Y., Zotz, G., & Einzmann, H. J. (2023). Smoothing out the misconceptions of the

   role of bark roughness in vascular epiphyte attachment. *New Phytologist, 238*(3), 983-

   994. doi: https://doi.org/10.1111/nph.18811

Timsina, B., Rokaya, M. B., Münzbergová, Z., Kindlmann, P., Shrestha, B., Bhattarai, B.,

   & Raskoti, B. B. (2016). Diversity, distribution and host-species associations of

   epiphytic orchids in Nepal. *Biodiversity and Conservation, 25*(13), 2803-2819. doi:

   10.1007/s10531-016-1205-8

Trapnell, D. W., & Hamrick, J. L. (2006). Variety of phorophyte species colonized by the

   neotropical epiphyte, *Laelia rubescens* (Orchidaceae). *Selbyana*, *27*(1), 60-64. doi:

   10.2307/41760261

Tremblay, R. L. (1997). Distribution and Dispersion Patterns of Individuals in Nine Species of

Lepanfhes (Orchidaceae). *Biotropica, 29*(1), 38-45.

Tremblay, R. L., Meléndez-Ackerman, E. J., & Kapan, D. (2006). Do epiphytic orchids behave as metapopulations? Evidence from colonization, extinction rates and asynchronous population dynamics. *Biological Conservation, 129*(1), 70-81. doi: 10.1016/j.biocon.2005.11.017

Tremblay, R. L., Zimmerman, J. K., Lebrón, L., Bayman, P., Sastre, I., Axelrod, F., & Alers-García, J. (1998). Host specificity and low reproductive success in the rare endemic Puerto Rican orchid Lepanthes caritensis. *Biological Conservation, 85*(3), 297-304.

Wagner, K., Mendieta-Leiva, G., & Zotz, G. (2015). Host specificity in vascular epiphytes: a review of methodology, empirical evidence and potential mechanisms. *AoB plants, 7*, plu092. doi: https://doi.org/10.1093/aobpla/plu092

Whitman, M., & Ackerman, J. D. (2015). Terrestrial orchids in a tropical forest: best sites for abundance differ from those for reproduction. *Ecology, 96*(3), 693-704. doi: https://doi.org/10.1890/14-0104.1

Zarate-García, A. M., Noguera-Savelli, E., Andrade-Canto, S. B., Zavaleta-Mancera, H. A., Gauthier, A. and Alatorre-Cobos, F. (2020). Bark water storage capacity influences epiphytic orchid preference for host trees. *American Journal of Botany, 107*(5), 1–9. doi: 10.1002/ajb2.1470

Zi, X. M., Sheng, C. L., Goodale, U. M., Shao, S. C., & Gao, J. Y. (2014). In situ seed

baiting to isolate germination-enhancing fungi for an epiphytic orchid,

*Dendrobium aphyllum* (Orchidaceae). *Mycorrhiza, 24*(7), 487-499. doi: 10.1007/s00572-014-0565-8

Zotz, G., Hietz, P., & Einzmann, R. (2021). Functional ecology of vascular epiphytes. *Annual Plant Reviews, 4*(4), 869-906. doi: 10.1002/9781119312994.apr0777

**Appendix A**. Number of samples per tree for the assessment of water storage capacity.

| Tree Species | Tree ID | *Psychilis kraenzlinii* | |
| --- | --- | --- | --- |
| | | **Absent** | **Present** |
| *Coccoloba microstachya* | 1 | 4 | 4 |
| *Coccoloba microstachya* | 2 | 4 | 0 |
| *Coccoloba microstachya* | 3 | 4 | 0 |
| *Coccoloba microstachya* | 4 | 4 | 0 |
| *Machaonia portorricensis* | 1 | 3 | 2 |
| *Machaonia portorricensis* | 2 | 4 | 2 |
| *Machaonia portorricensis* | 3 | 3 | 4 |
| *Machaonia portorricensis* | 4 | 4 | 3 |
| *Ouratea littoralis* | 1 | 3 | 4 |
| *Ouratea littoralis* | 2 | 4 | 4 |
| *Ouratea littoralis* | 3 | 4 | 4 |
| *Ouratea littoralis* | 4 | 4 | 4 |
| *Randia aculeata* | 1 | 2 | 3 |
| *Randia aculeata* | 2 | 3 | 2 |
| *Randia aculeata* | 3 | 4 | 0 |

| | | | |
|---|---|---|---|
| *Randia aculeata* | 4 | 4 | 0 |
| *Rondeletia inermis* | 1 | 4 | 3 |
| *Rondeletia inermis* | 2 | 1 | 3 |
| *Rondeletia inermis* | 3 | 4 | 0 |
| *Phyllanthus cuneifolius* | 1 | 4 | 4 |
| *Phyllanthus cuneifolius* | 2 | 4 | 4 |
| *Phyllanthus cuneifolius* | 3 | 3 | 4 |
| *Phyllanthus cuneifolius* | 4 | 4 | 4 |
| *Swietenia mahagoni* | 1 | 3 | 0 |
| *Swietenia mahagoni* | 2 | 4 | 0 |
| *Swietenia mahagoni* | 3 | 4 | 0 |
| *Swietenia mahagoni* | 4 | 4 | 0 |
| *Tabebuia haemantha* | 1 | 4 | 4 |
| *Tabebuia haemantha* | 2 | 4 | 4 |
| *Tabebuia haemantha* | 3 | 4 | 4 |
| *Tabebuia haemantha* | 4 | 4 | 4 |

**Appendix B.** Number of samples per tree for the fissuring index.

| Tree Species | Tree ID | *Psychilis kraenzlinii* | |
| --- | --- | --- | --- |
| | | **Absent** | **Present** |
| *Coccoloba microstachya* | 1 | 4 | 4 |
| *Coccoloba microstachya* | 2 | 4 | 4 |
| *Coccoloba microstachya* | 3 | 4 | 0 |
| *Coccoloba microstachya* | 4 | 4 | 0 |
| *Ouratea littoralis* | 1 | 4 | 4 |
| *Ouratea littoralis* | 2 | 3 | 4 |
| *Ouratea littoralis* | 3 | 4 | 4 |
| *Ouratea littoralis* | 4 | 4 | 4 |
| *Machaonia portorricensis* | 1 | 4 | 4 |
| *Machaonia portorricensis* | 2 | 4 | 4 |
| *Machaonia portorricensis* | 3 | 4 | 4 |
| *Machaonia portorricensis* | 4 | 4 | 4 |
| *Randia aculeata* | 1 | 3 | 0 |
| *Rondeletia inermis* | 1 | 1 | 4 |
| *Rondeletia inermis* | 2 | 4 | 4 |

| | | | |
|---|---|---|---|
| *Rondeletia inermis* | 3 | 4 | 4 |
| *Rondeletia inermis* | 4 | 4 | 0 |
| *Swietenia mahagoni* | 1 | 4 | 0 |
| *Swietenia mahagoni* | 2 | 4 | 0 |
| *Swietenia mahagoni* | 3 | 4 | 0 |
| *Swietenia mahagoni* | 4 | 4 | 0 |
| *Phyllanthus cuneifolius* | 1 | 4 | 3 |
| *Phyllanthus cuneifolius* | 2 | 4 | 0 |
| *Phyllanthus cuneifolius* | 3 | 4 | 4 |
| *Phyllanthus cuneifolius* | 4 | 4 | 4 |
| *Phyllanthus cuneifolius* | 5 | 0 | 4 |
| *Tabebuia haemantha* | 1 | 4 | 4 |
| *Tabebuia haemantha* | 2 | 4 | 4 |
| *Tabebuia haemantha* | 3 | 4 | 4 |
| *Tabebuia haemantha* | 4 | 4 | 4 |

**Appendix C.** Number of seed packets placed *in situ* per phorophyte species.

| Phorophyte Species | Seed Packets | |
|---|---|---|
| | On trees orchid-free | Near established orchid |
| *Coccoloba microstachya* | 31 | 0 |
| *Machaonia portorricensis* | 15 | 13 |
| *Ouratea littoralis* | 10 | 10 |
| *Randia aculeata* | 18 | 0 |
| *Rondeletia inermis* | 13 | 6 |
| *Phyllanthus cuneifolius* | 10 | 10 |
| *Swietenia mahagoni* | 16 | 2 |
| *Tabebuia haemantha* | 10 | 10 |

**Appendix D.** Conover-Iman Pairwise Comparisons among phorophyte species for Water Holding

Capacity (WHC), Water Retention Capacity (WRC) and Fissuring Index (FI)

| Comparison | WHC | | WRC | | FI | |
|---|---|---|---|---|---|---|
| | *Z* | *P* | *Z* | *P* | *Z* | *P* |
| *Coccoloba microstachya - Ouratea littoralis* | 3.56 | **<0.01*** | -0.51 | 0.6 | -0.25 | 0.8 |
| *Coccoloba microstachya - Machaonia portorricensis* | -5.55 | **<0.01*** | 3.52 | **<0.01*** | -1.39 | 0.17 |
| *Coccoloba microstachya - Randia aculeata* | -0.95 | 0.34 | 2.3 | **0.02*** | -1.22 | 0.22 |
| *Coccoloba microstachya - Rondeletia inermis* | -2.99 | **<0.01*** | 3.09 | **<0.01*** | 0.47 | 0.64 |
| *Coccoloba microstachya - Swietenia mahagoni* | 2.8 | **0.01*** | -2.35 | **0.02*** | -0.76 | 0.45 |
| *Coccoloba microstachya - Phyllanthus cuneifolius* | -1.87 | 0.06 | 0.94 | 0.35 | -2.8 | **0.01*** |
| *Coccoloba microstachya - Tabebuia haemantha* | 1.24 | 0.21 | 0.21 | 0.84 | -0.64 | 0.53 |
| *Ouratea littoralis - Machaonia portorricensis* | -9.99 | **<0.01*** | 4.47 | **<0.01*** | -1.21 | 0.23 |
| *Ouratea littoralis - Randia aculeata* | -4.49 | **<0.01*** | 3.01 | **<0.01*** | -1.06 | 0.29 |
| *Ouratea littoralis - Rondeletia inermis* | -6.5 | **<0.01*** | 3.82 | **<0.01*** | 0.77 | 0.45 |
| *Ouratea littoralis - Swietenia mahagoni* | -0.2 | 0.84 | -2.08 | **0.04*** | -0.57 | 0.57 |
| *Ouratea littoralis - Phyllanthus cuneifolius* | -6.13 | **<0.01*** | 1.64 | 0.11 | -2.73 | **0.01*** |
| *Ouratea littoralis - Tabebuia haemantha* | -2.64 | **0.01*** | 0.82 | 0.42 | -0.41 | 0.68 |
| *Machaonia portorricensis - Randia aculeata* | 4.38 | **<0.01*** | -1.18 | 0.24 | 0.09 | 0.92 |

| | | | | | | |
|---|---|---|---|---|---|---|
| *Machaonia portorricensis - Rondeletia inermis* | 1.96 | 0.05 | 0.18 | 085 | 1.95 | 0.05 |
| *Machaonia portorricensis - Swietenia mahagoni* | 8.03 | **<0.01\*** | -5.68 | **<0.01\*** | 0.42 | 0.67 |
| *Machaonia portorricensis - Phyllanthus cuneifolius* | 4.19 | **<0.01\*** | -2.92 | **<0.01\*** | -1.54 | 0.13 |
| *Machaonia portorricensis - Tabebuia haemantha* | 7.56 | **<0.01\*** | -3.73 | **<0.01\*** | 0.81 | 0.42 |
| *Randia aculeata - Rondeletia inermis* | -2.04 | **0.04\*** | 0.88 | 0.38 | 1.67 | 0.10 |
| *Randia aculeata - Swietenia mahagoni* | 3.63 | **<0.01\*** | -4.43 | **<0.01\*** | 0.44 | 0.66 |
| *Randia aculeata - Phyllanthus cuneifolius* | -0.77 | 0.44 | -1.61 | 0.11 | -1.14 | 0.25 |
| *Randia aculeata - Tabebuia haemantha* | 2.25 | **0.03\*** | -2.33 | **0.02\*** | 0.74 | 0.46 |
| *Rondeletia inermis - Swietenia mahagoni* | 5.42 | **<0.01\*** | -5.08 | **<0.01\*** | -1.2 | 0.23 |
| *Rondeletia inermis - Phyllanthus cuneifolius* | 1.55 | 0.12 | -2.5 | **0.01\*** | -3.42 | **<0.01\*** |
| *Rondeletia inermis - Tabebuia haemantha* | 4.4 | **<0.01\*** | -3.18 | **<0.01\*** | -1.17 | 0.24 |
| *Swietenia mahagoni - Phyllanthus cuneifolius* | -4.75 | **<0.01\*** | 3.4 | **<0.01\*** | -1.68 | 0.10 |
| *Swietenia mahagoni - Tabebuia haemantha* | -1.93 | 0.05 | 2.75 | **<0.01\*** | 0.24 | 0.81 |
| *Phyllanthus cuneifolius - Tabebuia haemantha* | 3.53 | **<0.01\*** | -0.84 | 0.41 | 2.34 | **0.02\*** |

**Appendix E:** Intraspecific differences in water holding capacity (WHC) and water retention capacity (WRC) between trees with and without *Psychilis kraenzlinii*. Mann-Whitney U test.

| Phorophyte Species | WHC | | WRC | |
|---|---|---|---|---|
| | *U* | *p* | *U* | *p* |
| *Coccoloba microstachya* | 59 | **0.01** | 17 | 0.13 |
| *Machaonia portorricensis* | 129 | **<0.01** | NA | NA |
| *Ouratea littoralis* | 105 | 0.57 | 101.5 | 0.46 |
| *Randia aculeata* | 39 | 0.57 | 37.5 | 0.42 |
| *Rondeletia inermis* | 27 | 1 | NA | NA |
| *Phyllanthus cuneifolius* | 116 | 0.89 | 148.5 | 0.17 |
| *Tabebuia haemantha* | 71 | **0.03** | 149 | 0.39 |

**Appendix F:** Conover-Iman Pairwise Comparisons for the percentage of seeds at Stage 0, 1 and 2 among phorophyte species. Values in bold indicate significant results.

| Comparison | Stage 0 | | Stage 1 | | Stage 2 | |
|---|---|---|---|---|---|---|
| | Z | P | Z | P | Z | P |
| *Coccoloba microstachya - Ouratea littoralis* | -0.55 | 0.29 | 0.66 | 0.26 | 0.06 | 0.48 |
| *Coccoloba microstachya - Machaonia portorricensis* | 1.48 | 0.07 | -1.4 | 0.08 | -1.2 | 0.12 |
| *Coccoloba microstachya - Randia aculeata* | -0.53 | 0.3 | 0.15 | 0.44 | 0.59 | 0.29 |
| *Coccoloba microstachya - Swietenia mahagoni* | 1.02 | 0.15 | -1 | 0.16 | -1.52 | 0.07 |
| *Coccoloba microstachya - Phyllanthus cuneifolius* | 1.83 | **0.03*** | -1.41 | 0.08 | -2.29 | **0.01*** |
| *Coccoloba microstachya - Tabebuia haemantha* | 2.44 | **0.01*** | -2.77 | **<0.01*** | -3.22 | **<0.01*** |
| *Ouratea littoralis - Machaonia portorricensis* | 1.85 | **0.03*** | -1.89 | **0.03*** | -1.13 | 0.13 |
| *Ouratea littoralis - Randia aculeata* | 0 | 0.5 | -0.45 | 0.33 | 0.48 | 0.31 |
| *Ouratea littoralis - Swietenia mahagoni* | 1.42 | 0.08 | -1.5 | 0.07 | -1.44 | 0.08 |
| *Ouratea littoralis - Phyllanthus cuneifolius* | 2.16 | **0.02*** | -1.9 | **0.03*** | -2.13 | **0.02*** |
| *Ouratea littoralis - Tabebuia haemantha* | 2.72 | **<0.01*** | -3.11 | **<0.01*** | -2.97 | **<0.01*** |
| *Machaonia portorricensis - Randia aculeata* | -1.8 | **0.04*** | 1.35 | 0.09 | 1.62 | 0.05 |
| *Machaonia portorricensis - Swietenia mahagoni* | -0.27 | 0.39 | 0.22 | 0.41 | -0.45 | 0.33 |
| *Machaonia portorricensis - Phyllanthus cuneifolius* | 0.48 | 0.32 | -0.14 | 0.45 | -1.17 | 0.12 |

| | | | | | | |
|---|---|---|---|---|---|---|
| *Machaonia portorricensis - Tabebuia haemantha* | 1.08 | 0.14 | -1.47 | 0.07 | -2.08 | **0.02*** |
| *Randia aculeata - Swietenia mahagoni* | 1.38 | 0.08 | 1.02 | 0.15 | -1.87 | **0.03*** |
| *Randia aculeata - Phyllanthus cuneifolius* | 2.1 | **0.02*** | -1.38 | 0.09 | -2.56 | **0.01*** |
| *Randia aculeata - Tabebuia haemantha* | 2.64 | **<0.01*** | -2.58 | **0.01*** | -3.38 | **<0.01*** |
| *Swietenia mahagoni - Phyllanthus cuneifolius* | 0.68 | 0.25 | -0.33 | 0.38 | -0.64 | 0.26 |
| *Swietenia mahagoni - Tabebuia haemantha* | 1.22 | 0.11 | -1.53 | 0.06 | -1.45 | 0.07 |
| *Phyllanthus cuneifolius - Tabebuia haemantha* | 0.55 | 0.29 | -1.23 | 0.11 | -0.84 | 0.2 |

**Appendix G**: Do not bother…

As part of this project other experiments took place without much success. I tried isolating the orchid mycorrhizal fungi (OMF) that triggers the germination of *Psychilis kraenzlinii*. I tried isolating the OMF from both adult roots and protocorms. To isolate the OMF from the roots I 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. I 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 I used WA and PDA, both poured over the pelotons and already set. Again, several strains grew, none of them *Rhizoctonia*-like. Although I paid some attention to two strains that were likely to be *Fusarium* and *Xylaria*. With these strains I 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. I 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 I 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 I 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). I 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, I am not convinced that the so-called pores are, in fact, pores. They seem to be cells. Hence, the data was archived and not used.

With this section my 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.

**Literature Cited**

Callaway, R. M., Reinhart, K. O., Moore, G. W., Moore, D. J., & Pennings, S. C. (2002). Epiphyte

host preferences and host traits: mechanisms for species-specific interactions. *Oecologia,*

*132*(2), 221-230. doi: 10.1007/s00442-002-0943-3

Otero, J. T., Ackerman, J. D., & Bayman, P. (2002). Diversity and host specificity of endophytic

Rhizoctonia-like fungi from tropical orchids. *American Journal of Botany*, *89*(11), 1852-

1858. doi: 10.3732/ajb.89.11.1852

Zarate-García, A. M., Noguera-Savelli, E., Andrade-Canto, S. B., Zavaleta-

Mancera, H. A., Gauthier, A. and Alatorre-Cobos, F. (2020). Bark water storage capacity

influences epiphytic orchid preference for host trees. *American Journal of Botany,*

*107*(5), 1–9. doi: 10.1002/ajb2.1470