RESEARCH ARTICLE

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Bark water storage capacity influences epiphytic orchid preference for host trees

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PREMISE: Of all orchid species described, 70% live on phorophytes. Trees offer a vital space with characteristics that influence the successful establishment and life cycle of orchids. Field inventory and distribution analysis suggest that phorophyte selection is biased to certain tree species that would serve as better hosts. Phorophyte bark is known as an important factor that influences this preference, but the chemical and physical properties of bark that contribute to creating a favorable space for orchids are still poorly understood. In this work, the effect of bark physical characteristics on phorophyte preference of tropical orchids was studied.

METHODS: Orchids and their phorophytes were counted and identified along transects inside two natural reserves in Southeast Mexico. A rhytidome classification was used to describe the bark decoration patterns of the phorophytes. To quantify bark fissuring, we developed a new protocol based on image processing of light micrographs using free-access software. Bark topology characterization was complemented with scanning electronic microscopy. Maximum and minimum water content was also determined.

RESULTS: Analyses of bark decorations and bark fissuring were not enough to explain the preference found for some tropical trees. In contrast, a positive relationship was found among water-storage capacity, bark porosity, and phorophyte preference. The host trees preferred by most orchids have bark with higher pore density and higher water retention after draining.

CONCLUSIONS: Unexpectedly, the phorophytes preferred by orchids are not those with more fissured bark but those with a higher ability to retain minimum water content after draining, which is a bark property positively correlated with higher pore density. Our data indicate that the bark microenvironment, determined by topology and water storage capacity, has a pivotal role in phorophyte specificity, a key factor that affects orchid diversity and distribution in the world.

KEY WORDS bark; orchid; Orchidaceae; phorophyte specificity; porosity; water content.

Orchids are probably the most well-known and admired flowers throughout the world (Nurfadilah, 2015). They comprise the Orchidaceae family, one of the largest plant groups in the plant kingdom with about 25,000 species (Givnish et al., 2015); they are mostly restricted to subtropical and tropical regions, which constitute the most complex terrestrial ecosystems (Benzing, 2018). Epiphytic orchids use mostly trees as hosts (phorophytes) and constitute a dominant group of vascular plants (Adhikari and Fischer, 2012; Nurfadilah, 2015). Many studies suggest that phorophyte specificity may influence the distribution and abundance of epiphyte populations (McCormick and Jacquemyn, 2014; Wagner et al., 2015). However, the factors that determine such specificity are still poorly understood. This knowledge is a fundamental for understanding the ecology of tropical ecosystems and designing and managing conservation programs (Wagner et al., 2015). Various studies suggest that the phorophyte's bark could be a factor contributing to such specificity, especially by modulating the microenvironment. Also supporting this idea are probability interaction models that analyze factors that explain epiphyte–host networks and have shown that epiphytes are more common on trees with denser wood or rougher bark (Sáyago et al., 2013).

Phorophyte selection has been suggested to be an indirect process during the early phases of an orchid's life (Rasmussen et al., 2015). Because orchid seeds lack an endosperm, there seems to be a bias toward trees with bark with suitable physical characteristics to host mycorrhizal fungi, which promote orchid seed germination, allow orchid roots to anchor, help plants establish, and thus might influence the distribution and size of orchid populations (Mújica et al., 2009; Gowland et al., 2011). In lichens, bark variables such as pH, structure, shedding grade, relative humidity, presence/absence of lenticels, and milky sap affect the richness of lichen species in tropical forests in Costa Rica, Colombia, and Brazil (Caceres et al., 2007; Soto-Medina et al., 2012). In a study in a dry tropical forest in Costa Rica, *Laelia rubescens*, a neotropical species, was found mostly on phorophytes with rough, fissured or shaggy bark, and exceptionally on smooth-barked species (Trapnell and Hamrick, 2006). Rough-barked trees have been suggested to be preferred because orchid seeds may become more easily lodged and retain moisture for longer (Otero et al., 2007; Adhikari and Fischer, 2012; Timsina et al., 2016). Methods to measure bark roughness in orchid phorophytes are based on subjective visual assessments (smooth or rough) or indices calculated using a cotton thread to trace fissures in the bark (Otero et al., 2007; Adhikari and Fischer, 2012; de la Rosa-Manzano et al., 2014). In the present study, we developed a new approach that uses light microscopy micrographs and open source image processing software to determine the fissuring index. This new method and scanning electronic microscopy (SEM) were used to characterize in detail the bark topology of host trees from two contrasting sites from a tropical forest in southern Mexico and determine its influence on phorophyte specificity. Our results highlight the determining roles that physical characteristics and water storage properties of phorophyte bark have in orchid preference.

MATERIALS AND METHODS

Study sites

The two tropical forests analyzed in this study are located in the Yucatan Peninsula, Mexico: (1) Petenes Biosphere Reserve, a low-flood forest; and (2) Constitución, a medium subperennial forest adjacent to the Calakmul Biosphere Reserve (Appendix S1). The low-flood forest site is at sea level, with a mean annual rainfall of 1100 mm (Zamora-Crescencio et al., 2015) and mean annual temperature of 27°C. Constitución is 300 m above sea level, with a mean annual rainfall of 1076 mm (Martinez and Galindo-Leal, 2002) and mean annual temperature of 31.14°C. The low-flood forest region is a big coastal wetland that combines large portions of semi-deciduous tropical forest isolated from each other by fragments of mangrove forest and marshland (Rojas-Soto and Bocanegra, 2002). In contrast, Constitución is immersed in a region considered as medium subperennial forest (Martinez and Galindo-Leal, 2002). Both sites were chosen because they are nature reserves free of human activity.

Field data collection and methods

Field expeditions were conducted from spring to autumn of 2018. In each study area, seven sampling plots of 400 m^2 were established to record the diversity of phorophytes and epiphytic orchids. These transects were marked using a geographic geopositioning system unit (eTrex 20, Garmin, Olathe, KS, USA) (Appendices S1 and S2). All trees (phorophytes and nonphorophytes) and epiphytic orchids in each transect were recorded. For taxonomical classification, phorophytes and orchids were sampled and identified by comparison against the collections deposited at the regional CICY herbarium (Centro de Investigación Científica de Yucatán, Mérida, México). Phorophyte trees were visually divided into thirds so that the height of the lowermost orchid in the lowermost third could be determined (Mendes-Marcusso et al., 2019). Tree diameter at breast height (DBH) was also calculated as a relative estimate of tree age by measuring trunk circumference (C) 1.3 m above ground ($n = 10$) in accordance with international practices (Zhao et al., 2015) and used in the formula DBH = C/π (Callaway et al., 2002; García-González et al., 2016). Plant growth is affected by sunlight exposure, especially for epiphyte species growing in tropical forests where competition for sunlight is constant (Krause et al., 2001). In this study, cardinal positions of orchid individuals growing on phorophytes were registered using a lensatic compass.

Species composition and ecological distribution analysis

To estimate the levels of similarity of species composition in the selected forests, Jaccard's index [J (A,B) = $|A \cap B| \ / \ |A \cup B|$] was used. An ecological distribution analysis of orchid species was performed using a neighbor-joining clustering method (unrooted tree, bootstrap = 1000 replicates) using the free statistical package PAST (Øyvind Hammer, Natural History Museum, University of Oslo, Oslo, Norway; http://folk.uio.no/ohammer/past/).

Bark stability analysis

Because bark stability has been reported as a factor that could affect epiphyte colonization (Callaway et al., 2002), we determined bark stability as previously described (Callaway et al., 2002). For each phorophyte species, 10 trees were randomly selected, and 20 oil paint dots per tree (2.5 cm diameter) were marked in the bottom third of the phorophyte trunks (200 dots per phorophyte species). After 10 months, permanency of each dot was verified.

Bark topology and roughness

Scanning electron microscopy (SEM) was used to record surface topology of bark sampled adjacent to the roots of the lowest orchids. Small pieces of these samples (1 cm^2, $n = 3$) were dried at 65°C for 24 h in a drying oven, then sputter-coated with a gold layer using a Denton Vacuum DESK II sputter coater (Moorestown, NJ, USA). Each bark sample was mounted on adhesive tape and placed on a sample-holder plate. Micrographs were obtained using a JEOL JSM-6360LV Scanning Electron Microscope (Tokyo, Japan), equipped with an integrated digital image acquisition system. SEM micrographs were used to classify phorophyte rhytidome texture according to the scheme of Coder (2014). A new approach was designed to calculate a fissuring index as a bark roughness variable for recorded phorophytes. Dried bark samples (4 cm^2, $n = 10$) were imaged with a stereomicroscope (Nikon, SMZ800, Japan) equipped with an LED ring light and a camera (Moticam 5, ShiftCapture software, Hong Kong, China). Afterward, the obtained images were converted to vector graphics using GIMP 2.8 free software (GIMP Development Team). The black and white graphics were then analyzed with the UTHSCSA Image tool version 1.25 software (https://uthscsa-imagetool.software.informer.com/) to quantify fissured (black) and smooth (white) areas. A step-by-step guide of this protocol, from sample washing to the fissuring index calculation, is described in detail in Appendix S3.

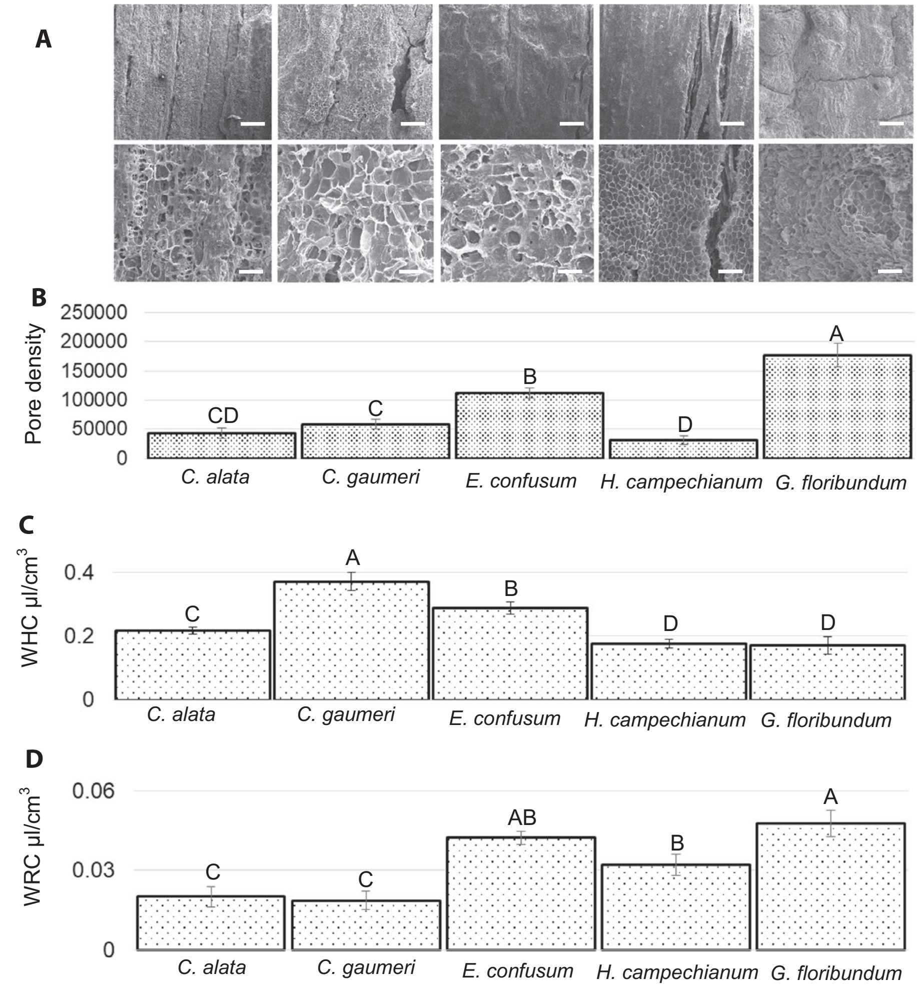

FIGURE 1. Characterization of two populations of orchids and phorophytes from Mexican tropical forests. (A) Tree and (B) orchid densities were evaluated in two contrasting tropical forests. In A and B, bars represent means ± SE ($n = 60$). Frequency for all species found is shown for (C) phorophytes and (D) orchids. Full names of all species are listed in Table 1. Different letters indicate significant differences in trees or orchid density determined by Tukey's HSD test ($P \leq 0.05$). Statistical models and post hoc results can be found in the Appendix S6.

Water-holding capacity and water-retention capacity

Water-holding capacity of phorophyte bark was estimated as reported previously (Callaway et al., 2002; Einzmann et al., 2015). For water-holding capacity at saturation (WHC), 10 random samples (4 cm^2) per phorophyte species were chiseled from the bark adjacent to orchid roots. At the laboratory, each bark sample was re-sized to 1 cm^2 and 3 ± 1 mm thickness using a scalpel and dried at 60°C for 48 h. Dry samples were weighed and placed in 24-well plates and submerged in 2 mL of ultrapure water (Millipore Simplicity 185, SimPak 1, Merck, Darmstadt, Germany) treated with 0.05% v/v Triton X-100, for 30 min at room temperature. After this treatment, samples were placed in Petri dishes for 1 min, then weighed again to estimate WHC values. Finally, wet samples (30 min in ultrapure water treated with 0.05% Triton X-100) were kept at 22°C for 8 h, then weighed every hour for 8 h to estimate water-retention capacity (WRC).

Statistical analyses and image processing

All data for bark stability, DBH, fissuring index, porosity, WRC, WHC, and porosity were analyzed with the free InfoStat software, version 2018 (FCA-UNC, Cordóba, Argentina) in one-way independent ANOVAs to test for significant differences among treatments analyzed. In all analyses, we considered the factors as fixed effects that fitted into a linear mixed-effect model, assuming that the dependent variable is continuous, unbounded, and measured on an interval or ratio scale, and that residuals are normally distributed. If differences were significant, we performed Tukey's honestly significant difference (HSD) tests to determine which treatment means (compared with each other) are different ($P \leq 0.05$). All images were processed using Microsoft Office Publisher (Microsoft, USA) and GIMP 2.10.12 free software (GIMP Development Team, USA).

RESULTS

Structure and aggregation patterns of epiphytic orchid–phorophyte communities in two tropical forests

Both tropical forests differed significantly in tree and orchid density. In the low-flood forest (Petenes), tree density was lower (0.8 ± 0.045 trees per m^2) than that in the medium subperennial forest (Calakmul) (0.19 ± 0.05 trees per m^2) (Fig. 1A; Appendix S6). The higher tree density in the medium subperennial forest was reflected in a higher phorophyte frequency compared to that in the low-flood forest. However, the low-flood forest had a higher diversity of phorophyte species in contrast to the medium subperennial forest (Fig. 1C). Four phorophyte species were identified in the low-flood forest (Table 1): *Haematoxylum campechianum* (Fabaceae), *Crescentia alata* (Bignoniaceae), *Gymnopodium floribundum* (Polygonaceae), and *Caesalpinia gaumeri* (Fabaceae). The most abundant phorophyte was *H. campechianum* (17.94%) and the least was *G. floribundum* (8.9%; Fig. 1C). In the medium subperennial forest, only two species were phorophytes: *G. floribundum* and *E. confusum* (Erythroxylaceae). When the orchid populations (juveniles and adults) were registered in both sites, a higher orchid density was calculated for the low-flood forest compared to the medium subperennial forest (Fig. 1B). Interestingly, orchid diversity was higher in the medium subperennial forest: 14 species compared to 5 in the low-flood forest (Table 1, Fig. 1D; Appendix S4). No new orchid species were recorded for either of the sites. Jaccard's index

TABLE 1. Main species of orchids and naturally associated hosts.

Phorophytes	Orchid species	Forest type
Caesalpinia gaumeri Greenm. (Fabaceae Lindl.)	*Encyclia alata* (Bateman) Schltr.	Low flood
	Encyclia nematocaulon (A.Rich.) Acuña	
	Brassavola nodosa (L.) Lindl. var. Nodosa	
Crescentia alata Kunth (Bignoniaceae Juss.)	*Encyclia alata* (Bateman) Schltr.	Low flood
	Encyclia nematocaulon (A.Rich.) Acuña	
	Laelia rubescens Lindl.	
Gymnopodium floribundum Rolfe (Polygonaceae Juss.)	*Catasetum integerrimum* Hook.	Low flood
	Encyclia nematocaulon (A.Rich.) Acuña	
Haematoxylum campechianum L. (Fabaceae Lindl.)	*Encyclia alata* (Bateman) Schltr.	Low flood
	Encyclia nematocaulon (A.Rich.) Acuña	
	Brassavola nodosa (L.) Lindl. var. Nodosa	
Erythroxylum confusum Britton (Erythroxylaceae Kunth)	*Rhetinantha friedrichsthalii* (Rchb.F.) MA Blanco	Medium subperennial
	Encyclia alata (Bateman) Schltr.	
	Nemaconia striata (Lindl.)	
	Epidendrum nocturnum Jacq.	
	Specklinia grobyi (Bateman ex Lindl.) F.Barros	
	Rhyncholaelia digbyana (Lindl.) Schltr.	
	Polystachyacaracasana Rchb.F.	
Gymnopodium floribundum Rolfe (Polygonaceae Juss.)	*Cohniella ascendens* (Lindl.) Christenson	Medium subperennial
	Lophiaris lindenii (Brongn.) Braem	
	Encyclia nematocaulon (A.Rich.) Acuña	
	Oncidium sphacelatum Lindl.	
	*Laelia rubescen*s Lindl.	
	Myrmecophila christinae Carnevali & Gómez-Juárez	
	Epidendrum cardiophorum Schltr.	

analysis revealed the orchid and phorophyte similarity between the low-flood forest and the medium subperennial forest. Both sites share 20% similarity of phorophyte vegetation, with only *G. floribundum* common to both forests. Regarding orchid vegetation, Jaccard's index revealed 18% similarity for both sites, with *E. alata* and *E. nematocaulon* the only two species shared. A tendency for growing on the northwest and southwest sides of the phorophytes was observed when the cardinal orientation of orchids was analyzed (Appendix S5A). These cardinal points received lower sun radiation levels during the hottest hours of the day, in the seasons of the year when the study was conducted.

Data of presence/absence were used in the analysis of the ecological distribution that showed a bias of orchid species among the phorophyte species identified. Figure 2 shows the top clade clusters of orchid species that use only *E. confusum* as phorophyte. In the middle clade, it is possible to identify a group that inhabits only *G. floribundum,* except *L. rubescens* that also uses *C. alata* as a phorophyte. The three orchid species in the bottom clade of the dendrogram apparently do not have any phorophyte preference (Fig. 2).

General phorophyte characteristics

The occurrence analysis of phorophytes and epiphytic orchids showed that a small number of phorophytes host a higher orchid diversity. To assess whether the physical characteristics of bark in phorophytes could be influencing this apparent specificity, we first evaluated phorophyte size as an age marker. Significant differences were observed in phorophyte size. Most host trees found in the low-flood forest had similar values (10–20 cm) for DBH, except for *G. floribundum* (Appendix S5B). In contrast, the only two phorophyte species recorded in the medium subperennial forest were notably different: *E. confusum* presented a larger diameter compared to *G. floribundum*, which showed a slightly smaller growth in the low-flood forest region (Appendix S5B). No correlation between phorophyte frequency and DBH was found (Fig. 1C; Appendix S5B). When the height of the lowermost orchid on a phorophyte (stem or branch) was analyzed, orchid individuals were found at similar heights from the ground level (about 1.5 m), except on *E. confusum* and *H. campechianum,* which were colonized at higher points (Appendix S5C). These data suggest that phorophyte size was not a limiting factor for orchid establishment, nor positively associated with phorophyte preference in the tropical forests studied.

Bark physical characteristics: stability and roughness and their relationship to water-holding capacity

Analysis of bark stability showed no significant differences in phorophytes (Appendix S5D). All phorophytes in this study had fissured bark according to the qualitative classifications (Otero et al., 2007). However, large differences in bark topology and four bark textures were revealed by the SEM ultrastructural analysis: lined for *C. guameri* and *H. campechianum*, flaky for *C. alata*, fissured for *G. floribundum*, and plated for *E. confusum* (Fig. 3A). Analyses indicated that bark decorations and orchid frequency were not correlated. The host trees preferred by orchids had fewer decorations, as observed for *H. campechianum* and *G. floribundum* (Fig. 3A). When the bark-fissuring grade was calculated using our new protocol, the fissuring index (FI) was found to differ significantly among all phorophyte barks analyzed ($P < 0.05$) (Fig. 3B). *Caesalpinia gaumeri*, *C. alata*, and *H. campechianum* had the highest FI, whereas *E. confusum* had an intermediate value. Interestingly, *G. floribundum*, the phorophyte with the highest orchid frequency, had the lowest FI. Similar to bark decorations results, FI data showed no clear correlation between bark roughness and orchid preference for host trees. In an analysis of higher resolution SEM images to evaluate the

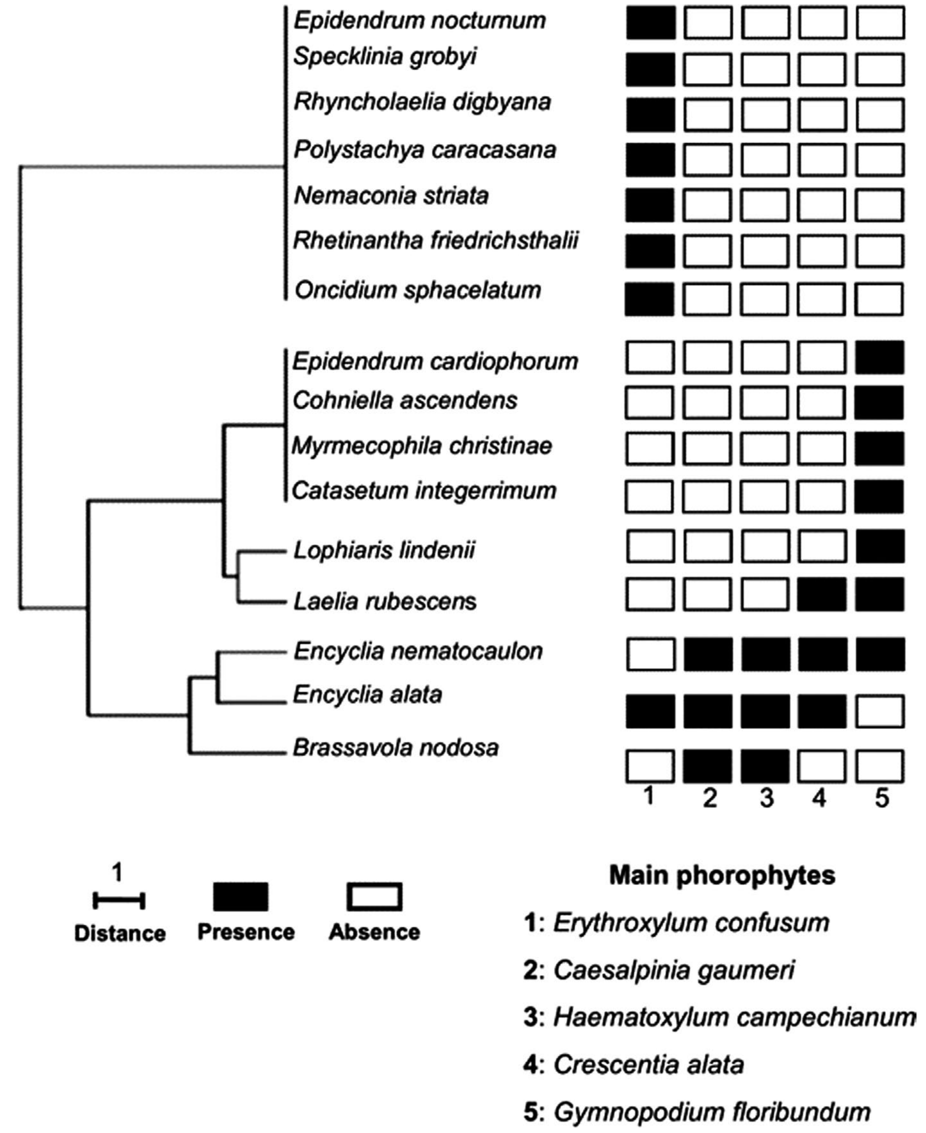

FIGURE 2. Phorophyte preference by orchid species recorded. Neighbor-joining clustering in PAST 3.24 software was used to determine the distribution of the orchid species among the phorophytes found. On the right, presence (full box) or absence of orchid species (empty box) for each phorophyte species is indicated.

porosity in the bark microrelief more precisely, visual differences were found in the microrelief (Fig. 4A). The bark of *G. floribundum*, the tree most preferred by orchids, had a high number of small and regular-shaped pores. In contrast, *C. alata* and *C. gaumeri* had bark with big, irregular-shaped pores (Fig. 4A). Confirming these observations, statistical analysis of pore density showed significant differences in among the different phorophyte species. Indeed, except for *H. campechianum*, the preferred phorophytes had a high pore density (Fig. 4B). Because porosity features directly affect the bark water-hold capacity at saturation (WHC), WHC and water-retention capacity (WRC) were determined. Interestingly, our analysis revealed that *H. campechianum* and *G. floribundum* had the lowest WHC values, while *C. gaumeri* and *E. confusum* had the highest (Fig. 4C). With the exception of C. *alata*, with an intermediate WHC, the orchid phorophyte preference seemed to be inversely correlated to WHC. In contrast, WRC data showed that phorophytes with higher orchid frequency had bark that retained more water after draining (Fig. 4D). All the physical characterization data obtained by ultra-resolution microscopy and quantitative analyses indicate that water-retention capacity determined by bark microrelief traits directly influenced orchid preference for their host trees.

DISCUSSION

Based on observational and quantitative data, this study showed that the recorded orchids in both forests were limited to few phorophyte species, contributing to the idea of host specificity, widely described for epiphytic orchids (Callaway et al., 2002; Otero et al., 2007; Nurfadilah, 2015; Timsina et al., 2016; Naranjo et al., 2019). In our study, orchid and phorophyte diversity seems to be determined by climatic features and vegetation type. Both forests had a similar mean annual rainfall but a different mean annual temperature throughout the year. Temperature and precipitation variations have an impact on orchid seedling recruitment, directly on germination or indirectly on mycobiont availability (Rasmussen et al., 2015). In the low-flood forest, long periods of flooding might reduce the environmental temperature by increasing air humidity (Appendix S1) and promote dew formation. Air humidity is the most important abiotic determinant of epiphyte diversity in tropical environments (Ding et al., 2016). In tropical deciduous forests, air humidity boosts dew deposition on orchid leaves, which in turn positively influences the survival and development of orchids, as reported for *E. nematocaulon* populations (de la Rosa-Manzano et al., 2014). On the other hand, regular floods in the low-flood forest could be a constraining factor for tree species sensitive to hypoxic conditions. For example, *G. floribundum*, the preferred phorophyte for orchids, was less frequent in the low-flood forest than in the medium subperennial forest. The orchid preference bias for this tree species could also be affected by leaf phenology. For example, *G. floribundum* keeps up to 65% of its leaves during the dry season compared to *C. gaumeri*, which loses all its leaves (Interián-Ku et al., 2018). Sunlight exposure, modulated by canopy or leaf phenology, could influence orchid population size by affecting germination or juvenile development (Rasmussen et al., 2015). Or it could decrease orchid diversity by promoting more sunlight-tolerant species. Therefore, environmental conditions and phorophyte phenology could explain the low diversity of orchid species in the low-flood forest compared to the medium subperennial forest and the clear orchid preference bias for some phorophyte species.

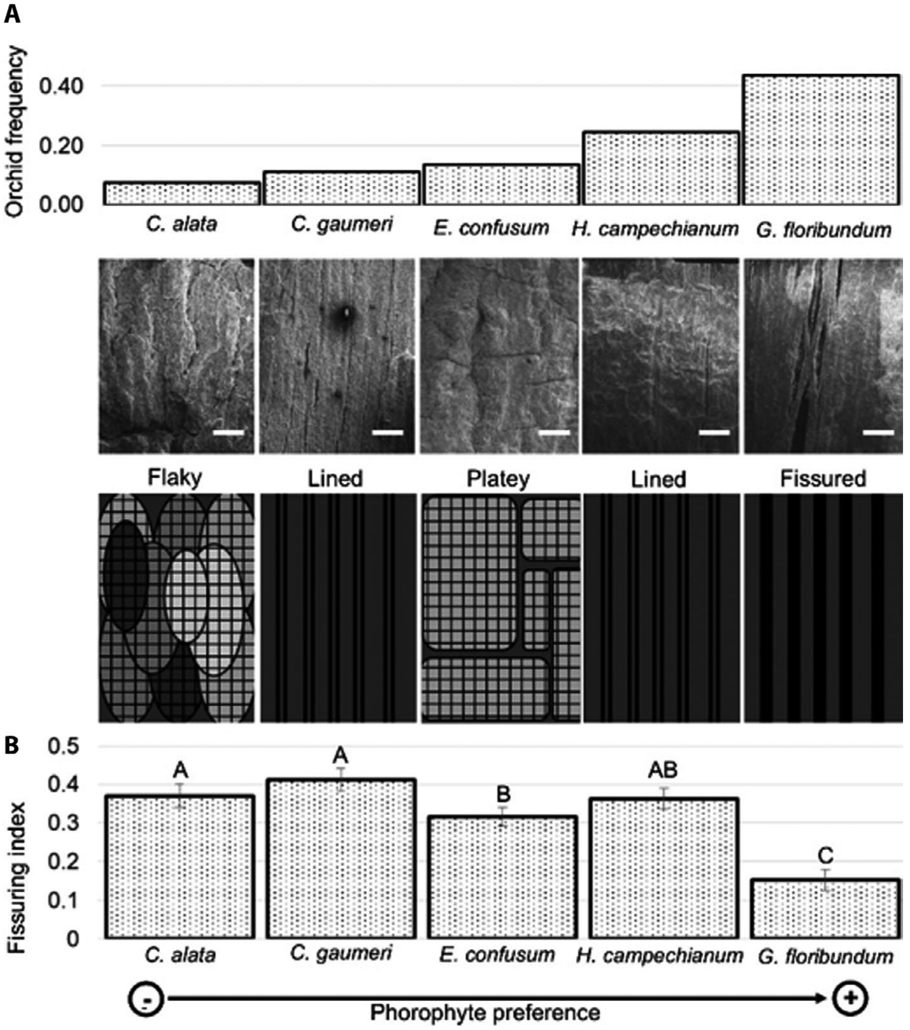

FIGURE 3. Phorophyte specificity is partially influenced by the bark roughness trait. (A) High orchid frequency is not directly associated with high-decorated bark. Top: Orchid frequency in all phorophyte species recorded. Middle: Bark topology classification according to rhytidome nomenclature. SEM micrographs, bar = 1 mm ($n = 3$). Bark decoration illustrations were re-drawn from Coder, 2014. (B) Fissuring index (FI) for each phorophyte species. FI values were calculated using the novel protocol developed here; $n = 10$. Bars represent means ± SE. Different letters indicate significant differences determined by Tukey's HSD test ($P \leq 0.05$) between the values calculated for all phorophyte species. Statistical models and post hoc results can be found in Appendix S6. Full names of all species are listed in Table 1.

One of the most frequently discussed mechanisms explaining phorophyte specificity is based on physicochemical bark properties of host trees. Compelling evidence shows that bark stability, texture, and water-holding capacity influence vascular and nonvascular epiphyte colonization (Wagner et al., 2015). Bark stability has likely been an underrated physical influence on orchid–phorophyte relation, perhaps because it is intrinsically linked to bark texture. Most studies have determined bark stability by evaluating bark-shedding rate over a certain duration. High peeling rates impact epiphyte survival negatively by offering an unstable substrate for colonization (Mondragon and Elliott, 2013; Wagner et al., 2015). Our results show that the phorophytes analyzed had stable barks with similar peeling rates. Due to host-tree preference by orchids, bark stability does not seem to be a critical factor that contributes to the apparent specificity observed. More studies evaluating bark stability along trunk and branches harboring orchids, combined with a vertical distribution analysis, would provide more information about the role of bark stability. Like texture and water-holding capacity, this feature depends on tree ontogeny and plant anatomy (Wagner et al., 2015).

Bark texture, specifically fissuring grade or roughness, is most likely the main physical factor that explains how bark influences orchid preference for host trees (Adhikari et al., 2016). Paradoxically, there are no precise methods to evaluate bark texture in detail. The most common method used to evaluate bark roughness is based on the use of a cotton string (Callaway et al., 2002; García-González et al., 2016). A more precise protocol has not been developed to quantify bark fissures in orchid phorophytes. In this study, we developed a new quantitative method for a detailed fissuring grade analysis and explored bark topology at an ultra-resolution by SEM. Our method calculates the fissuring index and is based on digital processing of bark images. In this sense, it is similar to the one used for bark analysis in lichen phorophytes (McDonald et al., 2017); however, it may be more accurate, as light micrographs offer more detail than graphite rubbing. Using 3D vision systems in a new protocol to characterize bark microrelief, Sioma et al. (2018) found that automated image processing allows the calculation of the water interception potential and fissure depth by analyzing a reference plane and the bark surface. Although this protocol allows reliable analysis, 3D vision systems are not available to all researchers, so our method provides a good alternative for analyzing phorophyte barks.

Our data showed there is no clear correlation between fissuring index and phorophyte preference. However, in several studies, epiphytes preferred fissured or rough barks to smooth barks in neotropical or template forests (Callaway et al., 2002; Boelter et al., 2014; Lamit et al., 2015; Adhikari et al., 2016; Segovia-Rivas et al., 2018), but when collected data are analyzed statistically, weak or non-existent correlations are usually found (Callaway et al., 2002; Boelter et al., 2014; Adhikari et al., 2016; McDonald et al., 2017), which supports our results. It has been suggested other bark factors should be analyzed to understand the role of bark in phorophyte specificity (Boelter et al., 2014). Here, we analyzed bark microrelief properties and their effects on water-holding and water-retention capacities. The nomenclature for rhytidome classification used here was useful to describe phorophyte bark more consistently and it is an alternative to replace traditional and subjective classifications and to homogenize data collection in future work. Furthermore, SEM

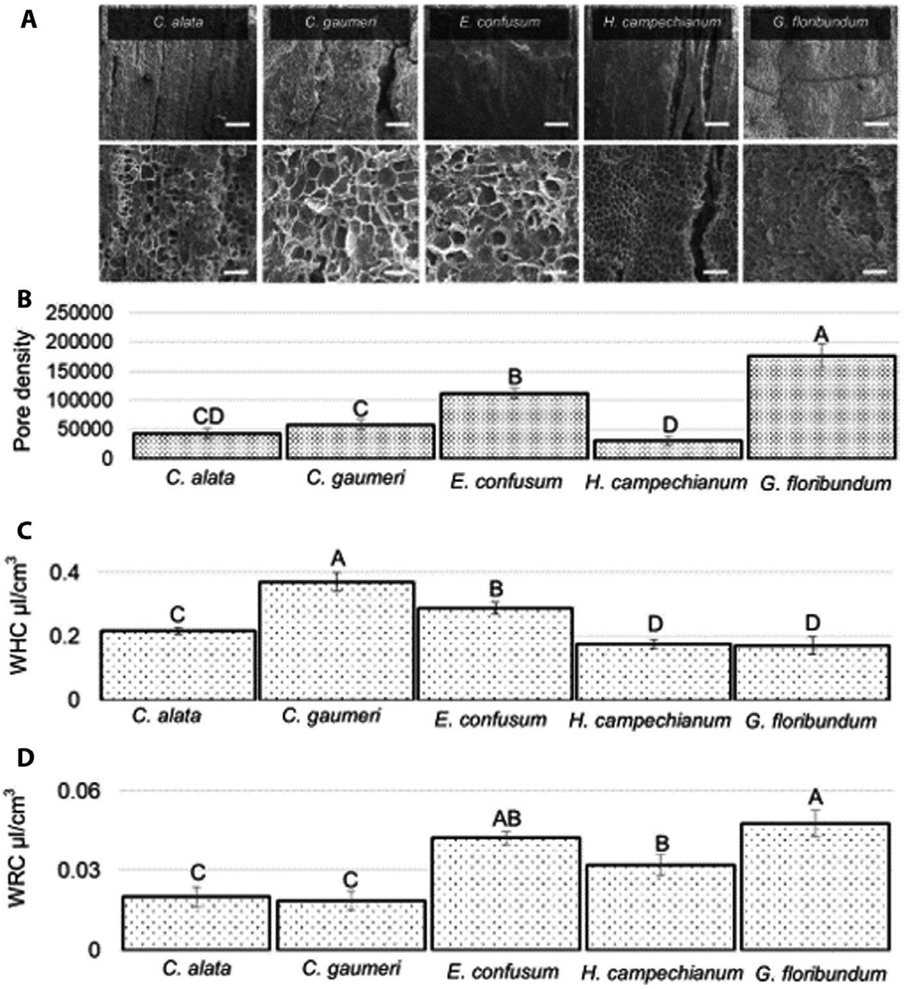

FIGURE 4. Bark microenvironment is closely linked to phorophyte specificity. (A) Ultra-morphology of phorophyte bark revealed by SEM analysis (*n* = 3). Top: bark (bar = 500 µm). Bottom: pore morphology (bar = 100 µm). (B) Bark pore density revealed by SEM micrographs (bar = 100 µm) (*n* = 3). (C) Water-holding capacity (WHC) and (D) water-retention capacity (WRC) were determined for each bark (*n* = 10). In B–D, bars represent means ± SE. Different letters indicate significant statistical differences determined by Tukey's HSD test ($P \leq 0.05$) between the values calculated for all phorophyte species. Statistical models and post hoc results can be found in Appendix S6. Full names of all species are listed in Table 1.

ultra-resolution allowed us to describe bark topology and to reveal orchid preference for phorophytes with lined decorated rhytidomes over those with flaky or plated bark. This observation was consistent with results reported for lichens, which prefer maple trees with shallow, vertical-line fissures to pine trees with pleated and flaky bark (McDonald et al., 2017). Line-shaped fissures might improve stem flow of water and therefore modify chemical properties of bark such as pH, which influences seed germination and orchid distribution (Frei and Dodson, 1972; Adhikari et al., 2012). Our analysis also found a close association between microrelief characteristics and bark water storage; species preferred by orchids had higher water-retention capacity, consistent with a previous study (Callaway et al., 2002). Water-storage capacity, determined by bark porosity, affects bark hygroscopicity, which is a significant component of water balance in forest ecosystems, especially for epiphytes (Johansson, 1974; Ilek et al., 2016). Barks with high porosity and WRC can be found in a wide range of phorophyte species. In this sense, phorophyte specificity might not be biased to taxonomic groups but to suitable bark features, in line with recent results on epiphyte–phorophyte interactions (Naranjo et al., 2019).

CONCLUSIONS

In summary, our SEM and quantitative analyses of the different physical properties of phorophyte bark indicate that bark microrelief and bark water storage largely modulate epiphytic orchid performance. This study was limited to two tropical semideciduous forests, so research focusing on bark properties of phorophytes from different ecosystems is needed to generalize our results.

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AUTHOR CONTRIBUTIONS

A.M.Z.G. aided in designing the experiments, conducted the experiments, collected and analyzed data, and helped write the manuscript. E.N.S. contributed to the experimental design and provided feedback on the manuscript. S.B.A.C. collected data for SEM analysis and discussed the results. H.A.Z.M. discussed data. A.G. wrote and provided feedback on the manuscript. F.A.C. formulated the idea, designed all experiments, discussed data, and wrote and revised the manuscript.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

APPENDIX S1. Location and climatic conditions of the study areas in southern Mexico. (A) Location of the two forests in the state of Campeche. Elevation map created using GFP data Q gis 3.4.2. The orange circle in the map in the upper left is the state of Campeche in Mexico. Transects for each forest are on the right. (B) Mean annual temperature and mean annual rainfall at each site. Graphs were obtained using data from two meteorological stations in the study areas.

APPENDIX S2. GPS coordinates of all transects sampled in this study.

APPENDIX S3. Protocol for new method to calculate the fissuring index.

APPENDIX S4. Orchids found in this study (bar = 1 cm).

APPENDIX S5. Phorophyte morphometrics and cardinal distribution of orchids. (A) Cardinal orientation frequency of orchids in the two forests analyzed. Cardinal orientation of orchid individuals was determined by their cardinal position ton stem or branches. (B) Diameter at breast height (1.3 m above the ground) as indicator of phorophyte age. (C) Height of lowest orchid. (D) Stability of bark of phorophyte species ($n = 10$). In all cases, means ± SE are shown. Different letters indicate significant differences determined by Tukey's HSD test ($P \leq 0.05$). Statistical models and post hoc results can be found in Appendix S6. Full names of all species are listed in Table 1.

APPENDIX S6. ANOVA and Tukey's HSD results for data on tree and orchid density, DBH, stability bark of phorophyte species, dissuring index, bark pore density, water-holding capacity (WHC) and water-retention capacity (WRC).

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