

Leaf water relations in epiphytic ferns are driven by drought avoidance rather than tolerance mechanisms

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Funding: This material is based upon work supported by the National Science Foundation grant #IOS-1656876.

Conflicts of interest: (None)

Abstract

Opportunistic diversification has allowed ferns to radiate into epiphytic niches in angiosperm dominated landscapes. However, our understanding of how ecophysiological function allowed establishment in the canopy and the potential transitional role of the hemi-epiphytic life form remain unclear. Here, we surveyed 39 fern species in Costa Rican tropical forests to explore epiphytic trait divergence in a phylogenetic context. We examined leaf responses to water deficits in terrestrial, hemi-epiphytic, and epiphytic ferns and related these findings to functional traits that regulate leaf water status. Epiphytic ferns had reduced xylem area (-63%), shorter stipe lengths (-56%), thicker laminae (+41%), and reduced stomatal density (-46%) compared to terrestrial ferns. Epiphytic ferns exhibited similar turgor loss points, higher osmotic potential at saturation, and lower tissue capacitance after turgor loss than terrestrial ferns. Overall, hemi-epiphytic ferns exhibited traits that share characteristics of both terrestrial and epiphytic species. Our findings clearly demonstrate the prevalence of water conservatism in both epiphytic and hemi-epiphytic ferns, via selection for anatomical and structural traits that avoid leaf water stress. Even with likely canalized physiological function, adaptations for drought avoidance have allowed epiphytic ferns to successfully endure the stresses of the canopy habitat.

Key Words: xylem, fern, functional traits, hemi-epiphyte, stomata, turgor loss, water stress

Acknowledgments: We would like to thank Juliette Bechard, Kathleen Bynon, Luke Calderaro, Christopher Krieg and Alexandra Russell for their hard work in the field and in the lab. We would like to thank Rodolfo Quiros Flores and Bernal Matarrita Carranza for their organizational support at each OTS field station. We would also like to thank Caroline Fromont for her advice on building phylogenetic trees.

52 **Introduction**

53 A major radiation led to the proliferation of numerous modern fern lineages in the Cenozoic,
54 shortly after the rise of the angiosperms (Schuettpelz & Pryer 2009; Testo & Sundue 2016).
55 Comprised of over 12,000 species found across the globe, ferns thrive and serve important
56 ecological roles in shaded understory environments, often acting as filters during seedling
57 establishment (Bazzaz & Pickett 1980; George & Bazzaz 1999; Coomes *et al.* 2005). However,
58 part of the large Cenozoic radiation event included opportunistic diversification into more
59 complex niches provided by emergent angiosperm canopies in tropical forests (Schneider *et al.*
60 2004). Consequently, one large clade of ferns (eupolypods I) has radiated extensively in
61 epiphytic niches, while its sister clade (eupolypods II) mostly remained and diversified on the
62 forest floor in tropical ecosystems (Watkins & Cardelús 2012; Sundue & Rothfels 2014). Along
63 with orchids, extant ferns are now the predominant tropical vascular epiphytes (Gentry &
64 Dodson 1987a; Cardelús *et al.* 2006; Zotz 2013a, 2016). Because epiphytes reside at the
65 potentially water-limited interface between vegetation and the atmosphere, they may be
66 especially vulnerable to global change; however, evolutionary selection for tolerance
67 mechanisms in epiphytic species may dampen this projection (Colwell *et al.* 2008; Lüttge 2012a;
68 Gotsch *et al.* 2015).

69
70 Epiphytic tropical ferns grow across a continuum of canopy positions, from heavily shaded low
71 trunk positions to exposed upper canopy tree branches (Watkins & Cardelús 2012). A hemi-
72 epiphytic life form also exists, with germination and establishment as epiphytic gametophytes
73 and young sporophytes leading eventually to a terrestrial root-soil connection (Moffett 2000;
74 Zotz 2013b). Although limited examples of fern hemi-epiphytism are documented, the growth of
75 feeder roots to establish a connection to the ground may represent a role in habitat transition
76 during the radiation of ferns (Testo & Sundue 2014; Fawcett & Sundue 2016; Watts *et al.* 2019).
77 The ecophysiology of all epiphytic ferns (and likely to some degree hemi-epiphytes) is
78 moderated by the inconsistent availability of irradiance, minerals, and water (Lüttge 2012b). For
79 example: vapor pressure deficit, light exposure, and wind speed fluctuate with canopy position
80 and size of tropical trees (Cardelús *et al.* 2006; Watkins *et al.* 2010; Lowman & Schowalter
81 2012; Woods *et al.* 2015); nitrogen and phosphorus availability can be limited to foliar leaching

and highly variable canopy soil (Cardelús & Mack 2010); and water supply can be negatively impacted by the lack of soil connections, even with ample daily precipitation (Gotsch *et al.* 2015). The resulting micro-habitats of epiphytic fern species are undoubtedly distinct from habitats that terrestrial ferns inhabit. Therefore, evolutionary selection for physiological and anatomical traits to adapt to these variable environmental conditions should have coincided with the opportunistic diversification of ferns into new epiphytic niches. Indeed, Nitta *et al.* (2020) found that, at the community level, epiphytic species were significantly more diverse functionally than terrestrial taxa.

The ecological stresses of the epiphytic habitat have likely selected for traits to accommodate water deficit in all residing plant lineages. For example, epiphytic angiosperms exhibit high adaptive capacity to maintain a positive leaf water balance, including both drought tolerance and avoidance mechanisms, tank water storage (in bromeliads), foliar water uptake, and higher leaf capacitance (Benzing & Burt 1970; Gotsch *et al.* 2015; Zotz 2016; North *et al.* 2019). Greater morphological diversity (i.e., total size, leaf thickness and leaf dissection) in epiphytic ferns compared to terrestrial ferns is another adaptive outcome for tolerance of the drier and more variable canopy habitat (Nitta *et al.* 2020). While leaf morphology appears to have responded to water deficit, there is less evidence for significant physiological adaptation to drought stress, and several studies have argued that ferns are in fact, physiologically canalized. For example, passive stomatal control has been reported several times in ferns (Brodribb & McAdam 2011; Ruszala *et al.* 2011; McAdam & Brodribb 2012; Cardoso *et al.* 2019), suggesting that they possess a diminished capacity to optimize water-use efficiency (Brodribb *et al.* 2009). A suite of studies also reveals how anatomical traits directly influence fern water relations and gas exchange in a manner that is functionally different from seed plants (Woodhouse & Nobel 1982; Brodribb *et al.* 2005; Zhang *et al.* 2009; Pittermann *et al.* 2011; Brodersen *et al.* 2012; Baer *et al.* 2016). Taken together, the weight of the evidence suggests that fern ecophysiology is inherently constrained, leaving means by which a third of all tropical fern species adapted and diversified into canopy habitats an open question (Schuettepelz & Pryer 2009; Zotz 2013a).

Compared to terrestrial ferns with more constant water supply, ferns in epiphytic habitats should

exhibit variation in anatomical and biomechanical traits to cope with water stress (e.g., high light, high VPD, and variable daily access to water; Saldaña *et al.* 2005; Kessler *et al.* 2007; Watkins *et al.* 2007a; Nishida & Hanba 2017). Water transport in ferns occurs exclusively through hydraulically efficient primary vascular tissue, with xylem that exhibits a broad range of cavitation resistance (Watkins *et al.* 2010; Pittermann *et al.* 2011; Holmlund *et al.* 2016). Ferns have also been shown to have higher hydraulic safety margins than angiosperms, resulting from reduced hydraulic conductivity and rapid stomatal closure (Brodribb & Holbrook 2004). In epiphytic ferns, decreased conductivity in the petiolar xylem of stipes limits the amount of water available to a given leaf area; however, this reduction in hydraulic capacity may reduce leaf water loss (Watkins *et al.* 2010). Additionally, thicker lamina (Watkins *et al.* 2007b) and shorter stipes (Watkins *et al.* 2010) in epiphytic ferns are likely adaptations to minimize water stress. These findings suggest that the evolution of leaf functional traits in ferns are derived from ancestral states of water conservatism (Sperry 2004), and the more recent diversification of epiphytic ferns allows exploration of the degree to which drought tolerance or avoidance mechanisms have developed.

Adaptations of morphological, anatomical, and physiological traits that define plant water relations are essential to the ecophysiological success of any epiphytic plant, yet our knowledge of how epiphytic fern species maintain plant water balance with inconsistent daily water supply is currently underdeveloped. This study tests the hypothesis that the evolution of leaf traits related to enduring water stress (drought tolerance) promoted the radiation of tropical ferns from largely moist, terrestrial habitats to far more unpredictable canopy habitats. We examined leaf responses to water deficit across field-sampled terrestrial, hemi-epiphytic, and epiphytic ferns in Costa Rican tropical forests and related these data to a suite of anatomical and morphological traits that modulate leaf water status. We focused on a large representation of species from two major fern lineages, eupolypods I and II, to explore epiphytic functional trait divergence in a phylogenetic context. These two sister clades include nearly 6000 species and are distinguished by morphological character states of petiole vasculature, soral shape and placement, indusial presence and shape, and leaf indument (Schneider *et al.* 2004; Smith *et al.* 2006; Rothfels *et al.* 2012; Sundue & Rothfels 2014; Regalado *et al.* 2018). We also sought to elucidate the

143 transitional role the hemi-epiphytic life form serves, if any, in the evolution of the epiphytic
144 habit.

Materials and methods

Study site and species selection

The sites used for this study included two wet tropical forest locations in Costa Rica: La Selva Biological Research Station in Heredia (10°25'53" N, 84°00'24" W) and Las Cruces Research Station in San Vito (8°47'4" N, 82°57'31" W). The La Selva site is a low elevation (ca 50 m) tropical forest, with a moderate dry season. The Las Cruces site is a premontane tropical forest located at a higher elevation (ca 1200 m). Both sites receive approximately 4000mm of annual rainfall (Holdridge 1967; Gentry 1993). Measuring campaigns occurred in successive summer rainy seasons, during the month of July.

A suite of morphological, stoichiometric, anatomical, and leaf water relation parameters were determined for individuals ($n = 6-8$) from 39 fern species representing three fundamentally distinct life forms (Table S1). Across the two sites, 20 terrestrial (including climbing species), 15 epiphytic, and 4 hemi-epiphytic species were collected and measured. In this study, terrestrial life forms were all collected from the shaded forest floor in closed canopy understories. Epiphytic life forms were sampled from trunks (minimum 1 m height) or on branches within dominant tree canopies, depending on the species, using single-rope climbing techniques when necessary. Hemi-epiphytic species were collected along lower sections of tree trunks (1–3 m). Importantly, all sampled hemi-epiphytic species had established root connections to forest floor soils. Individuals of species were collected across multiple populations but within similar micro-habitat conditions. All sampled fern species were restricted to the eupolypods I and II clades. Vouchers for each species were deposited at the respective site of collection at either the La Selva (LSCR) or Las Cruces (LCCR) herbarium.

Plant material

Two complete fronds from sampled individuals were field collected in the early morning (6–7:30 am). One frond from each individual was utilized for pressure volume curves, while the other was sampled for structural morphology, lamina stoichiometry, and anatomical traits. Fronds were cut at the base of the stipe and cut ends were wrapped in wet paper towels and transported to the lab in black plastic bags. Fronds for pressure volume curves were re-cut under water and re-

174 hydrated for 1–6 hours. Due to the difficulty in sampling some high canopy species, whole
175 epiphytic individuals were carefully removed, maintained overnight in well-watered conditions
176 in an ambient air laboratory and sampled the following day.

177 ***Leaf morphometric traits***

178 Stipe length (cm) and lamina length (cm) were measured from one sampled frond per individual.
179 Total frond length was calculated as the sum of stipe and lamina lengths. Total lamina area for
180 each frond was measured with a Li-3100 leaf area meter (LiCor Biosciences, Lincoln, NE,
181 USA). Leaf mass per unit area (LMA, g cm⁻²) was calculated from biopsy punch sub-samples.
182 For each individual, ten lamina punches (5 mm²) were dried to a constant mass and LMA was
183 calculated as the total dry mass divided by the total area of all leaf punches.

184 ***Anatomical traits***

185 Stomatal density (SD) was measured by directly counting stomata on the abaxial leaf surface
186 under 40x magnification. Three leaf punches (4 mm² diameter) were sampled across different
187 locations on different pinnae from each individual. The number of stomata in each field of view
188 were counted in three regions on each of three leaf punches. The stomatal density (# mm⁻²) for
189 each individual is presented as the mean across all 9 sampled regions. Individual images of
190 stomata were directly photographed under 40x magnification across all three leaf punches per
191 individual. Stomatal length (mm) and width (mm) of both guard cells were calculated for 9
192 stomata for each individual using ImageJ (National Institutes of Health, Bethesda, ML, USA).
193 Stomatal size (SS, mm²) was calculated as guard cell length multiplied by the combined width of
194 each guard cell pair, as in Franks & Beerling (2009).

195

196 Stipes were transversely sectioned by razor blade mid-stipe. Sections were stained in toluidine
197 blue and then mounted in glycerine on microscope slides. Each section was photographed using
198 a light microscope mounted with a digital camera (Amscope FMA050). Section photographs
199 were anatomically analyzed using ImageJ. Total xylem vascular area (mm²) was calculated as the
200 sum of the conduit lumen area and conduit wall area for each cross section.

201 ***Foliar chemistry***

Sub-samples of foliage tissue, used for lamina area calculation, were collected across multiple locations on each pinna for every individual. These sub-samples were dried to a constant mass and ground using a Wig-L-Bug (Sigma-Aldrich Co, St. Louis, MO, USA). Nitrogen content and $\delta^{13}\text{C}$ were measured using a Delta V isotope ratio mass spectrometer interfaced to a NC2500 elemental analyzer (Thermo Scientific, Waltham, MA, USA) and corrected by comparison with certified standards. Foliar nitrogen content represents both a structural component (LMA) and an investment into photosynthesis. Foliar $\delta^{13}\text{C}$ represents an index of relative stomatal limitation of photosynthesis and less negative $\delta^{13}\text{C}$ values are often tied to increased intrinsic plant water use efficiency.

Lamina chlorophyll content was determined on three different pinnae for each individual. Single point measurements of chlorophyll content (mg m^{-2}) were measured within a 3 mm diameter circle with the CCM-300 chlorophyll content meter (Opti-Sciences, Hudson, NH, USA). Lamina chlorophyll content is expressed as the mean of point measurements across the entire frond. Foliar chlorophyll content represents investment into light harvesting in photosynthesis, which may be impacted by nitrogen supply.

Pressure-volume relations

Tissue-water relations were determined with pressure-volume analysis (Tyree & Hammel 1972) on fully expanded fronds with a Scholander pressure bomb (PMS Instruments Co., Albany, OR, USA). For each pressure-volume (PV) curve, we sampled intact frond tips (approx. 15 cm length) after full rehydration of the whole frond. We generated pressure-volume curves by taking sequential water potential measurements (ψ_{leaf}) as fronds air dried, first in closed plastic bags (0–3 hours) and then in open air. The fresh mass was recorded immediately before and after each ψ determination. Following each PV curve, foliar samples were dried to a constant mass to calculate relative water content. For each PV curve, we graphed the relationship between $1/\psi_{\text{leaf}}$ and leaf relative water content to estimate parameters related to leaf turgor and bulk tissue water relations. We then calculated leaf water potential at turgor loss (ψ_{tp}), the osmotic potential at saturation (ψ_{o}), the bulk modulus of tissue elasticity (ϵ), and tissue capacitance between saturation and turgor loss (C_{R}) and after turgor loss (C_{TLF}) according to Sack *et al.* (2011).

Statistical analysis

Linear mixed-effect models were used to test responses of functional traits to categorical fixed effects of life form and collection site, as well as to test for bivariate relationships among key functional traits. The interaction between life form and collection site was tested to confirm any potential environmental or climate influence on functional trait patterns. A collection site effect was only detected for lamina $\delta^{13}\text{C}$. Thus, we used AIC model selection to choose the best-fit model among full models and models with life form and collection site as only main effects. To test for differences among life forms, individual species were treated as random effects in each model. Tukey's post-hoc tests were performed in conjunction with ANOVA to determine which mean values of functional traits were different among fixed effect treatments with the 'multcomp' package (Hothorn *et al.* 2008). We utilized a type 3 ANOVA due to an unbalanced design with the limited number of hemi-epiphytes species available. If interactions were present, we conducted pairwise comparisons with the 'emmeans' package (Lenth 2018) to investigate interactions between trait relationships and life form. For mixed-effect models, the conditional and marginal R^2 values were calculated as per Nakagawa & Schielzeth (2013). Tests of differences in slopes and elevations of bivariate relationships between functional traits were implemented using standardized major axis regression in the 'smatr' package in R (Warton *et al.* 2012).

A phylogenetic tree for the 39 sampled fern species was constructed using Bayesian inference. For each taxon, previously published sequences were obtained from GenBank (Table S1). In eight instances, it was necessary to use sequences from a closely-related species in the same genus as sequences from the focal species were unavailable. The resulting tree was rooted with *Dennstaedtia dissecta* (Dennstaedtiaceae), a representative of the sister lineage to eupolypods. We first selected the best model of nucleotide substitution with jModelTest2 (default parameters) using both Akaike and Bayesian information criteria (AIC and BIC). The selected model was GTR+G+I. We used MrBayes to analyze sequence data (Huelsenbeck & Ronquist 2001; Ronquist & Huelsenbeck 2003). Two runs, each with four Markov Chain Monte Carlo (MCMC) chains (3 "heated" and 1 "cold") were run in parallel in MrBayes for 1×10^6 generations and sampled every 5000 generations. Tracer (shape of the trace + effective sample size (ESS) > 200) (Rambaut *et al.* 2018) as well as the standard deviation of split frequencies (<0.01) were used to assess stationarity of the Markov chains.

263

264 The phylogenetic signal (K-statistic) based on Brownian motion-based metrics was calculated
265 for each continuous trait using the ‘picante’ package in R (Kembel *et al.* 2010). Values of ‘K’ for
266 a given trait that are below 1 mean that relatives resemble one another less than we would expect
267 for Brownian motion (lower phylogenetic signal), and values of ‘K’ above 1 indicate strong
268 phylogenetic signal and trait conservatism (Blomberg *et al.* 2003). Significant *P* values for ‘K’
269 indicate that close relatives are more similar than random species pairs for the given trait.
270 Additionally, bivariate relationships among mean species values of continuous traits were
271 analyzed with phylogenetically independent contrasts (PIC) in the ‘ape’ package in R (Paradis &
272 Schliep 2019) to correct for possible non-independence resulting from phylogeny (Felsenstein
273 1985). All PIC analyses were directly compared with the results of conventional ANOVA
274 (described above) to better understand the functional and evolutionary relationships between
275 traits. All tests of statistical significance were conducted at an α level of 0.05 and means \pm SE are
276 reported for data not presented in figures. All analyses were performed with R 4.0.0 (Team
277 2013).

278 Results

279 *Fronde morphology and anatomy*

280 Total frond length was reduced by 29 % in epiphytic (59 ± 3.3 cm) compared to terrestrial (85 ± 2.6
281 cm) species, with frond length of hemi-epiphytes statistically similar to both groups ($P = 0.009$).
282 The reduction in total frond length was driven by a large reduction (-56 %) in stipe length in
283 epiphytic compared to terrestrial species ($P < 0.001$, Figure 1A). The majority of epiphytic and
284 hemi-epiphytic ferns had stipes restricted to less than 20 cm (80 % quantile), while maximum
285 stipe length of terrestrial ferns from the same probability distribution was 41.2 cm. Allometric
286 relationships in stipe length and lamina area were functionally different between epiphytic
287 species compared to terrestrial and hemi-epiphytic species (Figure 1B, stipe length x life form; P
288 = 0.005). Log relationships of stipe length and lamina area were positively correlated for each
289 life form, however, pair-wise differences were detected in both the slopes ($P < 0.001$) and
290 elevation ($P < 0.001$) of this structural relationship for epiphytic species. Phylogenetic
291 independent contrasts support the positive relationship between stipe length and lamina area for
292 measured species ($P < 0.001$, $R^2 = 0.46$).

293

294 Total xylem area was 63 % smaller in epiphytic compared to terrestrial species, with xylem area
295 of hemi-epiphytes again intermediate ($P < 0.001$). The standardized leaf xylem fraction,
296 calculated as the ratio of xylem area to lamina area, was smaller in epiphytic and hemi-epiphytes
297 compared to terrestrial species ($P < 0.023$, Figure 2A). Increases in stipe length were positively
298 correlated with increases in total xylem area across all life forms ($P = 0.002$). Pair-wise
299 differences were detected for the slopes ($P < 0.001$) of this positive relationship between stipe
300 length and xylem area across life forms, while elevations were similar (Figure 2B). Phylogenetic
301 independent contrasts support the positive relationship between xylem area and stipe length for
302 measured species ($P < 0.001$, $R^2 = 0.26$).

303

304 No statistical differences were detected in total lamina area between any of the life form groups,
305 due to the large amount of variation in lamina area across species (R^2 marginal = 0.17 and R^2
306 conditional = 0.89). However, leaf mass per unit area (LMA) was 41 % higher in epiphytic

compared to terrestrial species, with hemi-epiphytic species intermediates between both life forms ($P = 0.002$, Figure 3A). Broadly, LMA of terrestrial and hemi-epiphytic ferns was constrained to less than 300 g m^{-2} , while epiphytic species reached much higher LMA values ($\sim 500 \text{ g m}^{-2}$).

Foliar chemistry

Lamina nitrogen content (N) was 30 % lower in epiphytic ferns compared to terrestrial, with hemi-epiphytes intermediate ($P = 0.008$). On a mass basis, N decreased with increases in LMA for terrestrial and hemi-epiphytic species, but not for epiphytic species (LMA x life form $P < 0.001$). Slopes and elevations of the significant relationship between N and LMA varied across life forms (Figure 3C). Phylogenetic independent contrasts, however, did not detect a relationship between N and LMA. Additionally, lamina $\delta^{13}\text{C}$ for terrestrial and hemi-epiphytic species was more negative than for epiphytic species ($P = 0.005$, Figure 3B). Lamina $\delta^{13}\text{C}$ for fern species at the higher elevation Las Cruces site was also less negative ($-33.0 \pm 0.26 \text{ ‰}$) than for fern species at the low elevation La Selva site ($34.0 \pm 0.15 \text{ ‰}$, $P = 0.015$). Total chlorophyll content was similar between terrestrial and epiphytic species, although epiphytic species had a lower possible range of chlorophyll content (Figure S1).

Stomatal anatomy

Epiphytic and hemi-epiphytic species had 46 % lower stomatal density compared to terrestrial species (Figure 4A, $P < 0.001$). The majority of epiphytic and hemi-epiphytic ferns had stomatal density restricted to fewer than 55 per mm^2 (80 % quantile), while stomatal density of terrestrial ferns from the same probability distribution was 103 per mm^2 . Stomata of hemi-epiphytic species were 19.2 % larger compared to terrestrial species, with epiphytes intermediates (Figure 4B, $P = 0.037$). A linear mixed model of the relationship between stomatal density and stomatal size was not significant ($P = 0.091$), largely due to the large amount of variation present in the random effect of species. Increases in stomatal density were negatively correlated with stomatal size for terrestrial and epiphytic ferns when assessed individually (Figure S2). Phylogenetic independent contrasts did support the negative relationship between stomatal density and stomatal size for measured species ($P = 0.004$, $R^2 = 0.19$).

Leaf pressure-volume traits

Minimal differences in leaf pressure-volume parameters were detected among the three life forms (Figure 5A). The turgor loss point (Ψ_{tlp}) marginally varied by life form ($P = 0.051$) and post-hoc comparisons did not detect differences in Ψ_{tlp} across terrestrial, hemi-epiphytic or epiphytic ferns. Broadly, terrestrial and hemi-epiphytic fern species had trended toward slightly lower Ψ_{tlp} than epiphytic species (Figure 5B). The osmotic potential at saturation (Ψ_o) was also lower in terrestrial compared to epiphytic species ($P = 0.009$, Figure 5C), while the modulus of elasticity (ϵ) was similar across all life forms. The relative water content at turgor loss point did not vary between fern life forms. The tissue capacitance between saturation and turgor loss (C_{fi}) was not different between life forms, however; the tissue capacitance after turgor loss (C_{TLP}) was 54 % lower in epiphytic compared to terrestrial species ($P = 0.009$, Figure 5D insert). Additionally, C_{fi} declined quickly with increasing ϵ for all species (Figure 5D).

Functional trait evolution

The degree to which a phylogenetic signal (K-statistic) was expressed in the functional traits for these tropical ferns species was generally low (Table 1). A significant phylogenetic signal was detected for stomatal traits, but was less apparent in morphological, anatomical, chemical or isotopic traits. Broadly, the trait phenotypes of surveyed species had less phylogenetic signal than expected under Brownian motion evolution.

Ancestral state reconstructions elucidated how tropical fern character states derived from terrestrial origins were adapted under selection pressures from new and distinct epiphytic environments within the eupolypod clades. The selection for shorter stipes can be seen in divergence of both epiphytic and hemi-epiphytic species on the phylogeny (Figure 6). The selection for fewer stomata is also readily apparent in epiphytic species divergence (Figure 7). Additionally, the emergence of thick leaves (higher LMA) and the reduction of total xylem area is almost entirely constrained to the diversification of epiphytic species examined in this study (Figure S3 & S4).

Discussion

This study sought to provide new insight on the mechanisms by which ferns, with potentially limited plasticity in ecophysiological function, successfully diversified into epiphytic habitats. Our results suggest that adaptations of functional traits related to the avoidance, rather than tolerance, of water stress (e.g., higher LMA and reduced stomatal density) allowed the radiation of ferns into canopy habitats. Although the role of hemi-epiphytic species in this radiation remains uncertain, we provide additional evidence to help understand the evolutionary origins of this life form. Here, we examine a suite of traits and biomechanical relationships linked to leaf water relations that likely underpin reductions in size seen in epiphytic ferns.

Anatomical and biomechanical traits regulate how epiphytic ferns thrive in sub-optimal resource environments

Epiphytic ferns routinely face environmental conditions that are distinct from the forest floor (Lüttge 2012b). Despite large annual precipitation in the tropics, epiphytic ferns without a root-soil connection face selection pressures from a transient water supply on a daily basis (Gotsch *et al.* 2015). In conjunction with limited access to soil born nutrients, epiphytic ferns must acquire functional traits to optimize both nutrient retention and efficient hydraulic transport. The evolution of such traits would have been essential to maintain a positive carbon balance as ferns radiated into canopy niches. Here, evidence of the selection for both reduced xylem area and reduced stipe length for water transport is evident across epiphytes and hemi-epiphytes. Additionally, the selection for a thick lamina (higher LMA) is readily apparent in the epiphytic species. Evaluating why these structural traits evolved requires unpacking the role of each trait in the biomechanics of form and function in epiphytic ferns.

Here, we detected key differences in allometric relationships related to structural form and frond hydraulic function. First, many epiphytes supported equally large lamina as terrestrial species with shorter or minimal stipes. Reduced stipes increase mechanical resistance, via sterome attributes, in ferns (Mahley *et al.* 2018), providing an advantage in exposed canopy habitats that are more susceptible to mechanical damage. Second, distinct positive relationships between xylem area and stipe length were also evident in epiphytic ferns due to large reductions in both

traits. Lower water availability in the canopy habitat likely selected for reduced xylem tissues to prevent embolism, as well as fewer stomata to lower leaf water loss. As ferns rely entirely on primary xylem due to lack of secondary growth and limited leaf venation to supply lamina tissues, reductions in xylem area could constrain frond morphology and leaf hydraulic function (Brodribb *et al.* 2007; Pittermann *et al.* 2011, 2013, 2015; Nitta *et al.* 2020). Shorter stipes, however, appear to compensate for selection of reduced xylem tissue by decreasing hydraulic path length. This coordination allows support of large lamina with reduced total frond length (smaller size) in epiphytic ferns with less danger of embolism, a finding supported by earlier work on fern hydraulics (Watkins *et al.* 2010). Relationships between petiole width and LMA have also been shown to differ between epiphytic and terrestrial ferns (Peppe *et al.* 2014), further suggesting that trait coordination may shift roles between support and hydraulic function across fern life forms. These shifts in frond structure and anatomy represent adaptations to prevent water stress and are realized by a greater water use efficiency (less negative lamina $\delta^{13}\text{C}$) detected in epiphytic ferns.

Plant species adapted to low resource supplies tend to invest in higher LMA and to maintain leaves longer (Wright *et al.* 2004). The radiation of ferns into tropical canopy niches included the adaptation of thick lamina, which may have been exacerbated in ferns compared to other epiphytic groups. For example, leaf dry matter content (a component of LMA) was found to be twice as high in epiphytic ferns compared to epiphytes from other angiosperm taxonomic groups (Petter *et al.* 2016). The benefits of higher LMA for tropical epiphytes likely centers on improved water retention (Watkins *et al.* 2007b; Zhang *et al.* 2015) and leaf persistence (Reich *et al.* 1991) but may come at the consequence of resource utilization and photosynthetic capacity. Globally, mass-based *N* investment decreases in plants as LMA increases (Wright *et al.* 2004). A similar relationship between nitrogen and LMA can be inferred for tropical understory ferns from several studies (Karst & Lechowicz 2007; Campany *et al.* 2019) and from the data presented here. This relationship appears to become uncoupled in epiphytic ferns, however, largely due to the extremely high values of LMA produced in many species of epiphytic ferns (Figure 3C). This decoupling may help explain the comparatively low rates of photosynthesis reported in epiphytic ferns and provide further support for the evolution of leaf traits adapted

more for water conservation (Zhang *et al.* 2009, 2014). Here, higher intrinsic WUE likely confirms this trade-off against CO₂ uptake because *N* (area-based) was not higher in epiphytes.

Does canalized physiological function underpin drought avoidance?

We detected surprisingly few intrinsic differences in leaf water relations between terrestrial and epiphytic ferns. More negative leaf water potentials at turgor loss point (Ψ_{TLP}) were not detected in epiphytic ferns compared to terrestrial ferns. More negative Ψ_{TLP} is a classic indicator of drought tolerance across a wide range of plants and ecosystems (Bartlett *et al.* 2012). The components of tissue water potential; osmotic potential at saturation (Ψ_o), cell wall elasticity (ϵ) and water storage capacity after turgor loss (C_{TLP}), also did not exhibit aspects of drought tolerance in epiphytic ferns. Our evidence suggests that epiphytic ferns have not evolved traits to tolerate water stress, in contrast to neighboring angiosperms species. For example, tropical epiphytic angiosperms were shown to adjust osmotic potentials and sap flow to seasonal climate (Gotsch *et al.* 2018), having more negative Ψ_{TLP} in both wet and dry seasons (approx. -1.25—3.5 MPa) than the epiphytic fern species in this study. This is confirmed by Martin *et al.* (2004), who also detected relatively high osmotic potentials (~1.0 MPa) in epiphytic ferns and lycophytes.

Evidence now strongly suggests that passive stomatal function persists in ferns, compared to the development of active stomata control in seed plants (Brodribb & McAdam 2011; Ruzsala *et al.* 2011; McAdam & Brodribb 2012; Cardoso *et al.* 2019), limiting the leaf water potential margin between stomatal closure and leaf death via water stress (Zhang *et al.* 2014). Therefore, we evaluated whether plasticity in stomatal traits could explain the water conservation strategies observed in epiphytic ferns (i.e., the lack of apparent drought tolerance). Stomatal anatomical traits have been shown to exhibit a wide range of inter-specific variability among tropical understory ferns (Campany *et al.* 2019) and epiphytes (Hietz & Briones 1998) and between epiphytic and terrestrial ferns in a tropical common garden (Zhang *et al.* 2014). Synthesizing findings from these studies with our data reveals a broad pattern of decreased stomatal density (approx. 2.5-fold) in epiphytic compared to terrestrial tropical ferns. Patterns in stomatal size are less clear but do suggest slightly larger stomatal size in epiphytes. Fern stomata have been shown to close rapidly with changes in vapor pressure difference, despite hydro-passive regulation, compared to angiosperms (McAdam & Brodribb 2015) and ferns close their stomata at very low

levels of dehydration (Brodribb & Holbrook 2004; McAdam & Brodribb 2013). To persist in epiphytic niches characterized by inconsistent water supply, reduced stomatal density with passive stomatal behavior likely allows ferns to minimize exposure to daily water loss. Although this water conservatism reduces photosynthetic potential (McElwain 2011), it likely represents an evolutionary drought avoidance pathway that allowed the impressive diversification of epiphytic ferns. Overall, the derived nature of functional traits seen in the radiation of epiphytic ferns (lower stomatal density, reduced xylem area with shorter stipe lengths and higher LMA) reveal strategies for avoidance of low leaf water potentials.

The role of hemi-epiphytic life-forms in the evolution of epiphytism in ferns

Comparative ecological and functional surveys have consistently demonstrated that epiphytic and terrestrial ferns maintain a suite of unique character traits (Watkins & Cardelús 2009, 2012), which can be seen in many of our modern tropical floras where there is limited reciprocal establishment of epiphytic and terrestrial species (Kluge *et al.* 2006; Watkins *et al.* 2006; Watkins & Cardelús 2009). Radiation into the canopy clearly required retooling of both gametophyte and sporophyte generations, resulting in radical changes of form and function, biochemistry and demography (Watkins *et al.* 2007a, 2007c; Mahley *et al.* 2018). How did this literal leap occur and could hemi-epiphytic ferns have provided a bridge? Unfortunately, understanding the evolutionary role of hemi-epiphytic ferns in the radiation into epiphytic niches is complicated by the limited total species diversity (Putz & Holbrook 1986; Gentry & Dodson 1987b; Testo & Sundue 2014) and by conflicting evidence in papers utilizing phylogenetic approaches (e.g., Testo & Sundue 2014; Watkins & Moran 2019; Watts *et al.* 2019). Our constructed phylogenies offer conflicting evidence in support of this bridge hypothesis as both terrestrial and epiphytic lineages are positioned as ancestors for the surveyed hemi-epiphytic species. In this study, hemi-epiphytic species share characteristics of both terrestrial and epiphytic life forms. The morphological construction of fronds in hemi-epiphytic species resemble those of terrestrial ferns. Similarity in structural allometry (stipe - lamina) and LMA in hemi-epiphytic and terrestrial ferns suggests that hemi-epiphytic species optimize functional traits for a low-light environment with an established root-soil connection. This terrestrial-centric trait optimization is also apparent in the comparable foliar *N* status and lamina water-use efficiency ($\delta^{13}\text{C}$) of hemi-epiphytic and terrestrial fern species. Overall, these similarities should

not be surprising if the root-soil connection in hemi-epiphytic species is established early in their sporophytic life history. However, if these morphological and stoichiometric traits are optimized differently in life stages of hemi-epiphytic fern species with or without a root-soil connection is still unknown. As such, the plasticity in trait function as hemi-epiphytic fern sporophytes first establish and then develop soil connections should be investigated further.

Alternatively, hemi-epiphytic ferns express traits associated with avoiding water stress that more closely resemble epiphytic ferns. Reductions of both stipe length and total xylem area in hemi-epiphytic ferns suggest that adaptations to reduced water availability in their early establishment as epiphytes are likely conserved throughout their life history. Similarly, decreases in stomatal density for hemi-epiphytes mirror adaptations for water conservatism detected in epiphytic ferns. The evolutionary origins of hemi-epiphytic species remain unclear, but our data do suggest that ‘epiphytic-like’ traits optimized to avoid desiccation are likely maintained even when hemi-epiphytic ferns establish a permanent connection with a terrestrial water supply. This could suggest an holoeiphytic origin of extant hemi-epiphytic ferns, agreeing with Testo & Sundue (2014), which could be further elucidated when the plasticity of functional traits in early establishing sporophytes (as epiphytes) are better understood. It is likely that such complicated and competing selective pressures on a plant to live in both worlds limits the evolution of these species, obscuring our ability to solve the riddle of the role of hemi-epiphytic ferns in the evolution of epiphytic ferns.

Conclusions

The variation in growing sites and the different types of inhabited vegetation reveal that there is not a simple definition of the ‘epiphytic habitat’ nor of the environmental conditions faced by epiphytic species (Zotz & Hietz 2001). However, scarcity of water is likely the most impactful constraint on epiphytic plants, as most epiphytes receive ‘pulse supplied’ water without access to soil (Benzing 1990; Zotz & Hietz 2001). The adaptability of functional traits between epiphytic and terrestrial ferns and the reconstructions of trait evolution presented here reveal how tropical ferns were likely able to opportunistically diversify into the canopy habitat. Although research comparing the ecophysiology of different epiphytic plant lineages, such as orchids and bromeliads would add to our understanding of the evolution of epiphytism, it appears that ferns

511 and angiosperms living side by side on canopy branches likely utilize distinct strategies to deal
512 with water stress.

513 **Tables**

514 Table 1. Phylogenetic signal for 15 functional traits of tropical ferns sampled across the
 515 eupolypods I & II clades. Measured species (n=39) encompass terrestrial, hemi-epiphytic and
 516 epiphytic habitats across two Costa Rican forest sites.

Functional Trait	K-statistic	<i>P</i>
Stomatal Density	0.7370624	0.002
Stomatal Size	0.3913156	0.013
Xylem Area	0.3338348	0.124
Turgor Loss Point	0.3139500	0.073
Osmotic Potential	0.3874653	0.023
Elasticity	0.1674148	0.556
Capacitance _{full turgor}	0.1621564	0.63
Capacitance _{turgor loss}	0.1922907	0.629
Frond Length	0.2596125	0.218
Stipe Length	0.3175584	0.145
Lamina Area	0.2908901	0.105
LMA	0.2690727	0.319
Lamina ¹³ C	0.2704244	0.207
Foliar Nitrogen	0.3447475	0.034
Chlorophyll Content	0.2024845	0.373

Figures

Figure 1. Structural shifts in path length alter how tropical fern life forms build conductive structures. (A) Box plots of stipe length across tropical fern life forms. (B) Lamina area in tropical ferns is positively related to stipe length (log-based) and this relationship varies by life form. Box plot widths are proportional to the number of measured species in each life form. Significant log-linear fits for each life form are shown with dashed lines. The conditional and marginal R^2 for the overall allometric relationship are reported.

Figure 2. Conductive hydraulic supply, via total xylem area, constrains stipe length in tropical fern species. (A) Box plots of xylem fraction (total xylem area (mm^2): lamina area (mm^2)) across tropical fern life forms. (B) The capacity for greater total xylem area in terrestrial tropical ferns supports the construction of large stipes. Box plot widths are proportional to the number of measured species in each life form. Significant linear relationships for each life form are shown with dashed lines representing model fits and gray shaded areas as 95 % confidence intervals for the mean. The conditional and marginal R^2 for the overall linear relationship are reported.

Figure 3. Lamina thickness (LMA) and lamina chemistry differ between tropical fern life forms. (A) Box plots of leaf mass per unit area (LMA) and (B) lamina $\delta^{13}\text{C}$ across tropical fern life forms (higher values indicate higher water-use efficiency) across tropical fern life forms. (C) Negative relationships (log-based) between mass-based lamina nitrogen content and LMA for ferns in this study compared to the GLOPNET data set (Wright *et al.* 2004). Box plot widths are proportional to the number of measured species in each life form. Significant log-linear fits for each life form are shown with dashed lines. The conditional and marginal R^2 for the overall allometric relationship are reported.

Figure 4. Stomatal traits differ between tropical fern life forms. (A) Box plots of stomatal density and (B) stomatal size across tropical fern life forms. Box plot widths are proportional to the number of measured species in each life form.

Figure 5. Epiphytic tropical ferns are not more drought tolerant than terrestrial or hemi-epiphytic tropical ferns. (A) Raw results from 211 pressure volume curves from 38 tropical fern species. Box plots of turgor loss point (B) and osmotic potential at saturation (C) across tropical fern life forms. Relationship between tissue capacitance at full turgor and the bulk modulus of elasticity for tropical ferns (D) and box plots of the tissue capacitance after turgor loss (inset). Fits for panels A and D for each fern life form uses loess regression for visualization purposes. Box plot widths are proportional to the number of measured species in each life form.

Figure 6. Ancestral state reconstruction for stipe length mapped onto the phylogeny of the selected ferns examined in this study. A posterior probability is indicated at each node. Eupolypods I (EI) and II (E2) clades are marked on basal nodes, respectively.

Figure 7. Ancestral state reconstruction for stomatal density mapped onto the phylogeny of the selected ferns examined in this study. A posterior probability is indicated at each node. Eupolypods I (EI) and II (E2) clades are marked on basal nodes, respectively.

561 **Data Availability Statement**

562 The data that support the findings of this study are openly available in Dryad. DOI and reference
563 number will be provided once the article is accepted for publication.

Bibliography

- Baer A., Wheeler J.K. & Pittermann J. (2016) Not dead yet: The seasonal water relations of two perennial ferns during California's exceptional drought. *New Phytologist* **210**, 122–132.
- Bartlett M.K., Scoffoni C. & Sack L. (2012) The determinants of leaf turgor loss point and prediction of drought tolerance of species and biomes: A global meta-analysis. *Ecology Letters* **15**, 393–405.
- Bazzaz F.A. & Pickett S.T.A. (1980) Physiological ecology of tropical succession: A comparative review. *Annual Review of Ecology and Systematics* **11**, 287–310.
- Benzing D.H. (1990) *Vascular epiphytes: General biology and related biota*. Cambridge University Press.
- Benzing D.H. & Burt K.M. (1970) Foliar Permeability Among Twenty Species of the Bromeliaceae. *Bulletin of the Torrey Botanical Club* **97**, 269–279.
- Blomberg S.P., Garland T. & Ives A.R. (2003) Testing for phylogenetic signal in comparative data: Behavioral traits are more labile. *Evolution* **57**, 717–745.
- Brodersen C.R., Roark L.C. & Pittermann J. (2012) The physiological implications of primary xylem organization in two ferns. *Plant, Cell & Environment* **35**, 1898–1911.
- Brodribb T.J. & Holbrook N.M. (2004) Stomatal protection against hydraulic failure: A comparison of coexisting ferns and angiosperms. *New Phytologist* **162**, 663–670.
- Brodribb T.J. & McAdam S.A.M. (2011) Passive origins of stomatal control in vascular plants. *Science* **331**, 582–585.

- Brodribb T.J., Holbrook N.M., Zwieniecki M.A. & Palma B. (2005) Leaf hydraulic capacity in ferns, conifers and angiosperms: Impacts on photosynthetic maxima. *New Phytologist* **165**, 839–846.
- Brodribb T.J., Feild T.S. & Jordan G.J. (2007) Leaf maximum photosynthetic rate and venation are linked by hydraulics. *Plant Physiology* **144**, 1890–1898.
- Brodribb T.J., McAdam S.A.M., Jordan G.J. & Feild T.S. (2009) Evolution of stomatal responsiveness to CO₂ and optimization of water-use efficiency among land plants. *New Phytologist* **183**, 839–847.
- Campany C.E., Martin L. & Watkins J.E. (2019) Convergence of ecophysiological traits drives floristic composition of early lineage vascular plants in a tropical forest floor. *Annals of Botany* **123**, 793–803.
- Cardelús C.L. & Mack M.C. (2010) The nutrient status of epiphytes and their host trees along an elevational gradient in Costa Rica. *Plant Ecology* **207**, 25–37.
- Cardelús C.L., Colwell R.K. & Watkins J.E. (2006) Vascular epiphyte distribution patterns: Explaining the mid-elevation richness peak. *Journal of Ecology* **94**, 144–156.
- Cardoso A.A., Randall J.M. & McAdam S.A.M. (2019) Hydraulics regulate stomatal responses to changes in leaf water status in the fern *Athyrium filix-femina*. *Plant Physiology* **179**, 533–543.
- Colwell R.K., Brehm G., Cardelus C.L., Gilman A.C. & Longino J.T. (2008) Global Warming, Elevational Range Shifts, and Lowland Biotic Attrition in the Wet Tropics. *Science* **322**, 258–261.

- Coomes D.A., Allen R.B., Bentley W.A., Burrows L.E., Canham C.D., Fagan L., ... Wright E.F. (2005) The hare, the tortoise and the crocodile: The ecology of angiosperm dominance, conifer persistence and fern filtering. *Journal of Ecology* **93**, 918–935.
- Fawcett S. & Sundue M. (2016) Evidence of primary hemiepiphytism in *Pleopeltis bradeorum* (Polypodiaceae). *Brittonia* **68**, 187–194.
- Felsenstein J. (1985) Phylogenies and the Comparative Method. *The American Naturalist* **125**, 1–15.
- Franks P.J. & Beerling D.J. (2009) Maximum leaf conductance driven by CO₂ effects on stomatal size and density over geologic time. *Proceedings of the National Academy of Sciences* **106**, 10343–10347.
- Gentry A.H. (1993) *Four Neotropical Rainforests*. Yale University Press.
- Gentry A.H. & Dodson C.H. (1987a) Diversity and biogeography of neotropical vascular epiphytes. *Annals of the Missouri Botanical Garden* **74**, 205–233.
- Gentry A.H. & Dodson C. (1987b) Contribution of nontrees to species richness of a tropical rain forest. *Biotropica* **19**, 149–156.
- George L.O. & Bazzaz F.A. (1999) The fern understory as an ecological filter: Growth and survival of canopy-tree seedlings. *Ecology* **80**, 846–856.

657 Gotsch S.G., Nadkarni N., Darby A., Glunk A., Dix M., Davidson K. & Dawson T.E. (2015)
658 Life
659 in the treetops: Ecophysiological strategies of canopy epiphytes in a tropical montane
660 cloud forest. *Ecological Monographs* **85**, 393–412.
661

662 Gotsch S.G., Dawson T.E. & Draguljić D. (2018) Variation in the resilience of cloud forest
663 vascular epiphytes to severe drought. *New Phytologist* **219**, 900–913.

664 Hietz P. & Briones O. (1998) Correlation between water relations and within-canopy distribution
665 of epiphytic ferns in a Mexican cloud forest. *Oecologia* **114**, 305–316.
666

667 Holdridge L.R. (1967) Life zone ecology. *Life zone ecology*.
668

669 Holmlund H.I., Lekson V.M., Gillespie B.M., Nakamatsu N.A., Burns A.M., Sauer K.E., ...
670 Davis
671 S.D. (2016) Seasonal changes in tissue-water relations for eight species of ferns during
672 historic drought in California. *American Journal of Botany* **103**, 1607–1617.
673

674 Hothorn T., Bretz F. & Westfall P. (2008) Simultaneous inference in general parametric models.
675 *Biometrical Journal* **50**, 346–363.
676

677 Huelsenbeck J.P. & Ronquist F. (2001) MRBAYES: Bayesian inference of phylogenetic trees.
678 *Bioinformatics* **17**, 754–755.
679

680 Karst A.L. & Lechowicz M.J. (2007) Are correlations among foliar traits in ferns consistent with
681 those in the seed plants? *New Phytologist* **173**, 306–312.
682

683 Kembel S.W., Cowan P.D., Helmus M.R., Cornwell W.K., Morlon H., Ackerly D.D., ... Webb
684 C.O. (2010) Picante: R tools for integrating phylogenies and ecology. *Bioinformatics* **26**,
685 1463–1464.
686

687 Kessler M., Siorak Y., Wunderlich M. & Wegner C. (2007) Patterns of morphological leaf traits

688 among pteridophytes along humidity and temperature gradients in the Bolivian Andes.
689 *Functional Plant Biology* **34**, 963–971.
690

691 Kluge J., Kessler M. & Dunn R.R. (2006) What drives elevational patterns of diversity? A test of
692 geometric constraints, climate and species pool effects for pteridophytes on an elevational
693 gradient in Costa Rica. *Global Ecology and Biogeography* **15**, 358–371.
694

695 Lenth R. (2018) Emmeans: Estimated marginal means, aka least-squares means.

696 Lowman M.D. & Schowalter T.D. (2012) Plant science in forest canopies – the first 30 years of
697 advances and challenges (1980–2010). *New Phytologist* **194**, 12–27.
698

699 Lüttge U. (2012a) Epiphytic Plants in a Changing World-Global: Change Effects on Vascular
700 and
701 Non-Vascular Epiphytes. Springer Science & Business Media, Berlin, Heidelberg.
702

703 Lüttge U. (2012b) *Vascular Plants as Epiphytes: Evolution and Ecophysiology*. Springer Science
704 & Business Media.
705

706 Mahley J.N., Pittermann J., Rowe N., Baer A., Watkins J.E., Schuettpelz E., ... Beck J. (2018)
707 Geometry, allometry and biomechanics of fern leaf petioles: Their significance for the
708 evolution of functional and ecological diversity within the Pteridaceae. *Frontiers in Plant*
709 *Science* **9**.
710

711 Martin C.E., Lin T.-C., Lin K.-C., Hsu C.-C. & Chiou W.-L. (2004) Causes and consequences of
712 high osmotic potentials in epiphytic higher plants. *Journal of Plant Physiology* **161**,
713 1119–1124.
714

715 McAdam S.A.M. & Brodribb T.J. (2012) Fern and lycophyte guard cells do not respond to
716 endogenous abscisic acid. *The Plant Cell* **24**, 1510–1521.
717

718 McAdam S.A.M. & Brodribb T.J. (2013) Ancestral stomatal control results in a canalization of

- fern and lycophyte adaptation to drought. *New Phytologist* **198**, 429–441.
- McAdam S.A.M. & Brodribb T.J. (2015) The evolution of mechanisms driving the stomatal response to vapor pressure deficit. *Plant Physiology* **167**, 833–843.
- McElwain J.C. (2011) Ferns: A xylem success story: Commentary. *New Phytologist* **192**, 307–310.
- Moffett M.W. (2000) What’s “up”? A critical look at the basic terms of canopy biology. *Biotropica* **32**, 569–596.
- Nakagawa S. & Schielzeth H. (2013) A general and simple method for obtaining R² from generalized linear mixed-effects models. *Methods in Ecology and Evolution* **4**, 133–142.
- Nishida K. & Hanba Y.T. (2017) Photosynthetic response of four fern species from different habitats to drought stress: Relationship between morpho-anatomical and physiological traits. *Photosynthetica* **55**, 689–697.
- Nitta J.H., Watkins J.E. & Davis C.C. (2020) Life in the canopy: Community trait assessments reveal substantial functional diversity among fern epiphytes. *New Phytologist*.
- North G.B., Brinton E.K., Browne M.G., Gillman M.G., Roddy A.B., Kho T.L., ... Brodersen C.R. (2019) Hydraulic conductance, resistance, and resilience: How leaves of a tropical epiphyte respond to drought. *American Journal of Botany* **106**, 943–957.
- Paradis E. & Schliep K. (2019) Ape 5.0: An environment for modern phylogenetics and evolutionary analyses in R. *Bioinformatics* **35**, 526–528.

750 Peppe D.J., Lemons C.R., Royer D.L., Wing S.L., Wright I.J., Lusk C.H. & Rhoden C.H. (2014)
751 Biomechanical and leaf–climate relationships: A comparison of ferns and seed plants.
752 *American Journal of Botany* **101**, 338–347.
753

754 Petter G., Wagner K., Wanek W., Delgado E.J.S., Zotz G., Cabral J.S. & Kreft H. (2016)
755 Functional leaf traits of vascular epiphytes: Vertical trends within the forest, intra- and
756 interspecific trait variability, and taxonomic signals. *Functional Ecology* **30**, 188–198.
757

758 Pittermann J., Limm E., Rico C. & Christman M.A. (2011) Structure-function constraints of
759 tracheid-based xylem: A comparison of conifers and ferns. *New Phytologist* **192**, 449–
760 461.
761

762 Pittermann J., Brodersen C. & Watkins J.E. (2013) The physiological resilience of fern
763 sporophytes and gametophytes: Advances in water relations offer new insights into an old
764 lineage. *Frontiers in Plant Science* **4**.
765

766 Pittermann J., Watkins J.E., Cary K.L., Schuettpelz E., Brodersen C., Smith A.R. & Baer A.
767 (2015)
768 The structure and function of xylem in seed-free vascular plants: An evolutionary
769 perspective. In: *Functional and Ecological Xylem Anatomy* (ed Hacke U), pp. 1–37.
770 Springer International Publishing, Cham.
771

772 Putz F.E. & Holbrook N.M. (1986) Notes on the natural history of hemiepiphytes. *Selbyana* **9**,
773 61–
774 69.
775

776 Rambaut A., Drummond A.J., Xie D., Baele G. & Suchard M.A. (2018) Posterior summarization
777 in Bayesian phylogenetics using Tracer 1.7. *Systematic Biology* **67**, 901–904.
778

779 Regalado L., Schmidt A.R., Krings M., Bechteler J., Schneider H. & Heinrichs J. (2018) Fossil

- evidence of eupolypod ferns in the mid-Cretaceous of Myanmar. *Plant Systematics and Evolution* **304**, 1–13.
- Reich P.B., Uhl C., Walters M.B. & Ellsworth D.S. (1991) Leaf lifespan as a determinant of leaf structure and function among 23 Amazonian tree species. *Oecologia* **86**, 16–24.
- Ronquist F. & Huelsenbeck J.P. (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19**, 1572–1574.
- Rothfels C.J., Sundue M.A., Kuo L.-Y., Larsson A., Kato M., Schuettpelz E. & Pryer K.M. (2012) A revised family-level classification for eupolypod II ferns (Polypodiidae: Polypodiales). *TAXON* **61**, 515–533.
- Ruszala E.M., Beerling D.J., Franks P.J., Chater C., Casson S.A., Gray J.E. & Hetherington A.M. (2011) Land plants acquired active stomatal control early in their evolutionary history. *Current Biology* **21**, 1030–1035.
- Sack L., Pasquet-Kok J. & Contributors P. (2011) PrometheusWiki Leaf pressure-volume curve parameters.
- Saldaña A., Gianoli E. & Lusk C.H. (2005) Ecophysiological responses to light availability in three *Blechnum* species (Pteridophyta, Blechnaceae) of different ecological breadth. *Oecologia* **145**, 251.
- Schneider H., Schuettpelz E., Pryer K.M., Cranfill R., Magallón S. & Lupia R. (2004) Ferns diversified in the shadow of angiosperms. *Nature* **428**, 553–557.

- Schuettpelz E. & Pryer K.M. (2009) Evidence for a Cenozoic radiation of ferns in an angiosperm-dominated canopy. *Proceedings of the National Academy of Sciences* **106**, 11200–11205.
- Smith A.R., Pryer K.M., Schuettpelz E., Korall P., Schneider H. & Wolf P.G. (2006) A classification for extant ferns. *TAXON* **55**, 705–731.
- Sperry J.S. (2004) Coordinating stomatal and xylem functioning – an evolutionary perspective. *New Phytologist* **162**, 568–570.
- Sundue M.A. & Rothfels C.J. (2014) Stasis and convergence characterize morphological evolution in eupolypod II ferns. *Annals of Botany* **113**, 35–54.
- Team R.C. (2013) R: A language and environment for statistical computing.
- Testo W. & Sundue M. (2014) Primary hemiepiphytism in *Colysis ampla* (Polypodiaceae) provides new insight into the evolution of growth habit in ferns. *International Journal of Plant Sciences* **175**, 526–536.
- Testo W. & Sundue M. (2016) A 4000-species dataset provides new insight into the evolution of ferns. *Molecular Phylogenetics and Evolution* **105**, 200–211.
- Tyree M.T. & Hammel H.T. (1972) The measurement of the turgor pressure and the water relations of plants by the pressure-bomb technique. *Journal of Experimental Botany* **23**, 267–282.
- Warton D.I., Duursma R.A., Falster D.S. & Taskinen S. (2012) Smatr 3- an R package for estimation and inference about allometric lines: The smatr 3 - an R package. *Methods in Ecology and Evolution* **3**, 257–259.

- Watkins J.E. & Cardelús C. (2009) Habitat differentiation of ferns in a lowland tropical rain forest. *American Fern Journal* **99**, 162–175.
- Watkins J.E. & Cardelús C.L. (2012) Ferns in an angiosperm world: Cretaceous radiation into the epiphytic niche and diversification on the forest floor. *International Journal of Plant Sciences* **173**, 695–710.
- Watkins J.E. & Moran R.C. (2019) Gametophytes of the fern genera *Dracoglossum* and *Lomariopsis* (Lomariopsidaceae) and their phylogenetic significance. *International Journal of Plant Sciences* **180**, 1004–1015.
- Watkins J.E., Cardelús C., Colwell R.K. & Moran R.C. (2006) Species richness and distribution of ferns along an elevational gradient in Costa Rica. *American Journal of Botany* **93**, 73–83.
- Watkins J.E., Mack M.C., Sinclair T.R. & Mulkey S.S. (2007a) Ecological and evolutionary consequences of desiccation tolerance in tropical fern gametophytes. *New Phytologist* **176**, 708–717.
- Watkins J.E., Rundel P.W. & Cardelús C.L. (2007b) The influence of life form on carbon and nitrogen relationships in tropical rainforest ferns. *Oecologia* **153**, 225.
- Watkins J.E., Mack M.K. & Mulkey S.S. (2007c) Gametophyte ecology and demography of epiphytic and terrestrial tropical ferns. *American Journal of Botany* **94**, 701–708.
- Watkins J.E., Holbrook N.M. & Zwieniecki M.A. (2010) Hydraulic properties of fern sporophytes: Consequences for ecological and evolutionary diversification. *American Journal of Botany* **97**, 2007–2019.

- Watts J.L., Moran R.C. & Watkins J.E. (2019) *Hymenasplenium volubile*: Documentation of its gametophytes and the first record of a hemiepiphyte in the Aspleniaceae. *Annals of Botany* **124**, 829–835.
- Woodhouse R.M. & Nobel P.S. (1982) Stipe anatomy, water potentials, and xylem conductances in seven species of ferns (Filicopsida). *American Journal of Botany* **69**, 135–140.
- Woods C.L., Cardelús C.L. & DeWalt S.J. (2015) Microhabitat associations of vascular epiphytes in a wet tropical forest canopy. *Journal of Ecology* **103**, 421–430.
- Wright I.J., Reich P.B., Westoby M., Ackerly D.D., Baruch Z., Bongers F., ... Villar R. (2004) The worldwide leaf economics spectrum. *Nature* **428**, 821–827.
- Zhang Q., Chen J.-W., Li B.-G. & Cao K.-F. (2009) Epiphytes and hemiepiphytes have slower photosynthetic response to lightflecks than terrestrial plants: Evidence from ferns and figs. *Journal of Tropical Ecology* **25**, 465–472.
- Zhang S.-B., Sun M., Cao K.-F., Hu H. & Zhang J.-L. (2014) Leaf photosynthetic rate of tropical ferns is evolutionarily linked to water transport capacity. *PLOS ONE* **9**, e84682.
- Zhang S.-B., Dai Y., Hao G.-Y., Li J.-W., Fu X.-W. & Zhang J.-L. (2015) Differentiation of water-related traits in terrestrial and epiphytic *Cymbidium* species. *Frontiers in Plant Science* **6**.
- Zotz G. (2013a) The systematic distribution of vascular epiphytes – a critical update. *Botanical Journal of the Linnean Society* **171**, 453–481.
- Zotz G. (2013b) “Hemiepiphyte”: A confusing term and its history. *Annals of Botany* **111**, 1015–1020.

904

905 Zotz G. (2016) *Plants on plants: The biology of vascular epiphytes*. Springer.

906

907 Zotz G. & Hietz P. (2001) The physiological ecology of vascular epiphytes: Current knowledge,
908 open questions. *Journal of Experimental Botany* **52**, 2067–2078.