

Research Article

Inter- and intraspecific adaptations of pteridophyte leaf traits in limestone and non-limestone forests of monsoon tropical regions of southwest China

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Abstract

Plant species often show different taxonomic and functional characteristics between limestone forests (LFs) and non-limestone forests (NLFs) in tropical regions. Pteridophyte species are one of the major components in tropical rainforests; however, the morphological and physiological characteristics of pteridophytes occurring in LFs are poorly understood. We evaluated the differences in seven leaf functional traits between pteridophyte species in LFs and NLFs in southwest China. We measured leaf water content, morphological traits including leaf size, leaf thickness, stomatal length and stomatal density (SD), and physiological traits including stomatal conductance and photosynthetic rate from a total of 25 species. We found that pteridophytes had thicker and smaller leaves with lower SD and stomatal conductance in LFs compared with NLFs, probably reflecting their adaptations in water use strategies. These differences, however, became non-significant when phylogenetic relationships were taken into account, suggesting that phylogenetic conservatism shapes trait differences and ultimately species composition in LFs and NLFs. Some species that were commonly found in both LFs and NLFs exhibited intraspecific variation between forest types, with lower SD in LFs. Our findings suggest that only a handful of pteridophyte species can adapt to their water use strategies in both LFs and NLFs, and thus adaptive radiation is unlikely to occur.

Keywords drought, fern, limestone forest, pteridophytes, leaf functional traits, Xishuangbanna

中国西南热带季风区石灰岩和非石灰岩森林蕨类植物叶片性状的种间和种内适应性

摘要：在热带地区，石灰山森林(LFs)和非石灰山森林(NLFs)之间的植物通常表现出不同的分类学和功能型特征。蕨类植物是热带雨林的主要组成成分之一。然而，我们对LFs中分布的蕨类植物的形态和生理特征了解甚少。在本项研究中，我们评估了中国西南热带地区LFs和NLFs中的蕨类植物物种在7个叶片功能性状上的差异。我们测量了25种蕨类植物的叶片水分含量、形态特征(包括叶片大小、叶片厚度、气孔长度和气孔密度)，以及生理特征(包括气孔导度和光合速率)。研究结果表明，与NLFs相比，LFs中蕨类植物的叶片更厚、更小，气孔密度和气孔导度更低，这可能反映了它们的水

分利用策略。然而，当考虑系统发育关系时，这些差异变得不显著了，这表明系统发育保守性影响了LFs和NLFs的性状差异并最终影响了物种组成。在LFs和NLFs中共同出现的一些物种具有一定的种内性状变异，在LFs中气孔密度更低。这些发现表明，只有少数蕨类植物的水分利用策略能够同时适应LFs和NLFs，因此蕨类植物不太可能发生适应性辐射。

关键词：干旱，蕨类，石灰山森林，蕨类植物，叶片功能性状，西双版纳

INTRODUCTION

Pteridophyte species have existed on the Earth since the middle of the Devonian more than 300 million years ago (Raven *et al.* 1999; Saldaña *et al.* 2007; Sharpe *et al.* 2010), and now have over 11 000 extant species worldwide (PPG I 2016). Their long evolutionary history suggests that pteridophytes have successfully adapted to various environments with different life forms (Mehltreter *et al.* 2010). A majority of pteridophytes are found in shady and moist environments such as rainforests, but some of them are found in dry, rocky habitats (Aldasoro *et al.* 2004; Karst *et al.* 2005; Page 2002; Saldaña *et al.* 2007). Abiotic and biotic factors are known to mediate the growth and abundance of pteridophytes species (Sharpe *et al.* 2010), and the ecological characteristics of individual species are related to their locally available resources and climatic conditions (Lu *et al.* 2016).

Changes in pteridophyte functional traits across different habitat conditions are important indicators of ecological adaptation, which may explain how pteridophyte species distribute across different forest types (Kluge and Kessler 2007). Such differences in functional traits are attributable to evolutionary history through which species, genera or families adapted to a given habitat (Watkins and Cardelús 2009). Pteridophyte species distributed in drier habitats generally display consistent responses in functional traits related to their water use strategies, such as lower leaf size (LS), higher leaf thickness (LT), higher leaf water contents (LWCs), and lower stomatal density (SD) (Hietz and Briones 1998; Kessler *et al.* 2007).

Limestone forests (LFs) are commonly found in southwest China (Tang *et al.* 2011) and represent isolated habitats surrounded by lowland and mountain tropical forests. These habitats are distinctive and contain numerous calcicolous species unique to LFs (Clements *et al.* 2006). Compared with non-limestone forests (NLFs), LFs have thin soil with low water holding capacity, high Ca and high pH (Cao *et al.* 2006; Li *et al.* 2008). LFs harbor

different and unique sets of pteridophyte species compared with NLFs, though there are some species that occur in both LFs and NLFs (Han *et al.* 2018; Huang *et al.* 2007; Phouthavong *et al.* 2019). Species turnover of local habitats is higher in LFs than NLFs (Phouthavong *et al.* 2019), suggesting that the distributions of pteridophyte species are relatively patchy and spatially restricted in LFs.

Recent plant functional traits studies suggest that LF tree species have higher photosynthetic capacity and water use efficiency and greater adaxial and abaxial epidermal thickness than NLF tree species (Fu *et al.* 2019; Zhu *et al.* 2017). Unlike trees, pteridophytes have primitive vascular networks, with smaller vessel and tracheid diameters, and maintain lower stomatal densities (Carlquist and Schneider 2001; Wang *et al.* 2013). We therefore expect similar or probably more distinctive differences in pteridophyte functional traits between LFs and NLFs. In wet and shaded tropical rainforests, pteridophytes generally display lower water transport capacity, lower maximum photosynthetic rates (A_{\max}) (Wang *et al.* 2013; Watkins and Cardelús 2009), higher SD and larger and thinner leaves than those occurring in dry environments (Kessler *et al.* 2007; Page 2002; Salehi *et al.* 2020). However, we know little about the functional traits of pteridophytes in LFs.

Phylogenetically related species are generally distributed across similar habitats and display similar leaf traits (Flynn *et al.* 2011; Sun *et al.* 2014). Phylogenetic diversity (PD) may explain the evolutionary history of the relatedness among species occurring in different habitats (Bagousse-Pinguet *et al.* 2019; Flynn *et al.* 2011). The relationships between community phylogenetic structure and community trait structure depend on whether the traits display phylogenetic signals (Srivastava *et al.* 2012). A recent study found strong phylogenetic signals in pteridophytes species on the island of Moorea, French Polynesia (Nitta *et al.* 2020). In contrast, another study found that epiphytic pteridophytes in Costa Rica are phenotypically plastic (i.e. traits are phylogenetically convergent), and character

displacement and adaptive radiation are likely to occur (Kluge and Kessler 2011). Thus, differences in traits among pteridophytes species across different habitats may or may not reflect their phylogenetic relatedness.

The use of phylogenetic signals in trait-based studies can be misleading if there are differences in ecological traits within a species, as conventional phylogenetic tests do not capture such intraspecific variations (Wiens *et al.* 2010). Plants often display adaptive intraspecific variation in functional traits through phenotypic plasticity and heritable genetic variation (O'Dell and Rajakaruna 2011). Previous studies have suggested that intraspecific variation is an important source of variation in functional traits (Messier *et al.* 2010; Siefert *et al.* 2015).

This study aims to understand how pteridophyte species adapted to LFs and NLFs by investigating their relevant morphological and physiological traits. We test the differences in functional traits with and without incorporating phylogenetic relationships to quantify the importance of phylogenetic relationships in determining pteridophyte distributions. We also included five pteridophyte species that were commonly found in both LFs and NLFs to investigate the intraspecific adaptations to different habitat types. We hypothesized that (i) the morphological characters of LS and SD are lower, and leaves are thicker, in LFs compared with NLFs, due to differences in water availability; (ii) the physiological characters, namely A_{\max} and stomatal conductance, are greater in LFs than NLFs due to lower soil humidity in LFs; (iii) trait variations between species are smaller in LFs due to environmental filtering (water stress); (iv) such differences, however, diminish after controlling for their phylogenetic relationships due to phylogenetic conservatism and (v) despite phylogenetic conservatism, some generalist species that can occur in both LFs and NLFs display intraspecific variation in functional traits across forest types.

MATERIALS AND METHODS

Study sites

The study sites were selected in LFs and NLFs of Xishuangbanna, southwestern Yunnan Province, China. The area is located in the transition zone of temperate and tropical Asia and is recognized as the northern edge of tropical southeast Asia (Cao and Zhang 1997; Zhu *et al.* 2006). The local climate is characterized by a monsoon climate with two distinct

seasons: wet season (May–October) and dry season (November–April). The average annual temperature is 21.7 °C, average annual precipitation is 1500 mm, and average annual relative humidity is 87% (Cao *et al.* 2006; Lü *et al.* 2010). LFs included limestone outcrop and limestone understory on a slope with thin soil, and NLFs included forest understory and riparian forest.

Focal species

We selected species that were commonly found in local LFs and NLFs of Xishuangbanna based on the data in Phouthavong *et al.* (2019). In each habitat type, we included 10 species unique to this habitat type with different life forms and additional five species (*viz.* *Hymenasplenium apogamum*, *Tectaria impressa*, *Tectaria subtriphylloides*, *Tectaria polymorpha* and *Leptochilus pothifolius*) that were found in both habitat types. We therefore selected a total of 25 species representing 7 families (Table 1). These species also well represent different phylogenetic positions of the pteridophyte species found in LFs and NLFs of Xishuangbanna (Fig. 1).

Leaf functional traits measurements

We measured the relevant morphological and physiological traits, namely LS, LT, LWC, SD, stomatal lengths (SLs), stomatal conductance (g_s), and maximum net photosynthesis rate per unit leaf area (A_{\max}). At least five leaves from five individuals belonging to each of the 25 pteridophytes species were used to measure the traits. For the five species occurring in both habitats, we sampled five individuals separately from both habitats. The morphological and physiological measurements were conducted in the late rainy season (September–October 2017), when the pteridophytes were fully grown. LS was measured on digital photographs using Sigma-Scan Pro5 software (SPSS Inc., Chicago, IL, USA). The measurements included the entire leaves, leaflets, or compound leaves, representing adaxial frond surface (upper side) (Cornelissen *et al.* 2003; Saldaña *et al.* 2007). LT was measured using a digital caliper (0.01 mm sensitivity; Mitutoyo, Kanagawa, Japan). LT was measured at the base, middle, and apex of the leaves and averaged over the three values for each individual. Leaf mass was recorded before and after drying at 70 °C to constant weight. LWCs were then calculated by subtracting leaf dry mass from leaf fresh mass and dividing by leaf fresh mass.

SD and SL were measured using clear nail polish imprints (Voleníková and Tichá 2001). Clear nail

Table 1: A list of the species used to measure functional traits

Species name	Family	Habitats	Microhabitats	Life forms
<i>Adiantum edgeworthii</i>	Pteridaceae	LFs	Semi-shaded upslope	Terrestrial
<i>Allantodia dilatata</i>	Athyriaceae	NLFs	Shaded riparian or upslope	Terrestrial
<i>Allantodia viridissima</i>	Athyriaceae	NLFs	Shaded riparian or upslope	Terrestrial
<i>Bolbitis heteroclita</i>	Dryopteridaceae	NLFs	Shaded riparian or upslope	Epilithic, epiphytic or terrestrial
<i>Cyclosorus paralatipinnus</i>	Thelypteridaceae	NLFs	Semi-shaded riparian or upslope	Terrestrial
<i>Drynaria quercifolia</i>	Polypodiaceae	LFs	Semi-shaded upslope	Epilithic, epiphytic or terrestrial
<i>Hymenasplenium apogamum</i>	Aspleniaceae	LFs and NLFs	Shaded riparian or valley	Terrestrial
<i>Lemmaphyllum carnosum</i>	Polypodiaceae	LFs	Semi-shaded upslope	Epilithic or epiphytic
<i>Lepisorus contortus</i>	Polypodiaceae	LFs	Shaded upslope	Epilithic or epiphytic
<i>Leptochilus decurrens</i>	Polypodiaceae	NLFs	Shaded riparian	Epilithic, epiphytic or terrestrial
<i>Leptochilus ellipticus</i> sbp. <i>potifolius</i>	Polypodiaceae	LFs	Lightly shaded upslope	Epilithic
<i>Leptochilus pothifolius</i>	Polypodiaceae	LFs and NLFs	Shaded riparian or valley	Epilithic, epiphytic or terrestrial
<i>Lomagramma matthewii</i>	Dryopteridaceae	NLFs	Shaded riparian	Epilithic, epiphytic or terrestrial
<i>Microsorium insignis</i>	Polypodiaceae	NLFs	Shaded riparian	Epilithic or epiphytic
<i>Microsorium membranaceum</i>	Polypodiaceae	LFs	Lightly shaded upslope	Epilithic or epiphytic
<i>Phymatosorus cuspidatus</i>	Polypodiaceae	LFs	Semi-shaded upslope	Epilithic or epiphytic
<i>Pronephrium grymnopteridifrons</i>	Thelypteridaceae	NLFs	Shaded riparian or upslope	Terrestrial
<i>Pteridrys cnemidaria</i>	Tectariaceae	NLFs	Shaded riparian or upslope	Terrestrial
<i>Pteris ensiformis</i>	Pteridaceae	LFs	Semi-shaded upslope	Terrestrial
<i>Pteris semipinnata</i>	Pteridaceae	NLFs	Shaded riparian or upslope	Terrestrial
<i>Pyrrosia calvata</i>	Polypodiaceae	LFs	Semi-shaded upslope	Epilithic or epiphytic
<i>Pyrrosia nummularifolia</i>	Polypodiaceae	LFs	Semi-shaded upslope	Epilithic or epiphytic
<i>Tectaria impressa</i>	Tectariaceae	LFs and NLFs	Shaded riparian or upslope	Terrestrial
<i>Tectaria polymorpha</i>	Tectariaceae	LFs and NLFs	Shaded riparian or upslope	Terrestrial
<i>Tectaria subtriphyllo</i>	Tectariaceae	LFs and NLFs	Shaded riparian or upslope	Terrestrial

These species are commonly found in LFs and/or NLFs of Xishuangbanna (Phouthavong *et al.* 2019). The five species with bold font were found in both LFs and NLFs. Microhabitats were defined by occurring locations and visual estimations of light conditions.

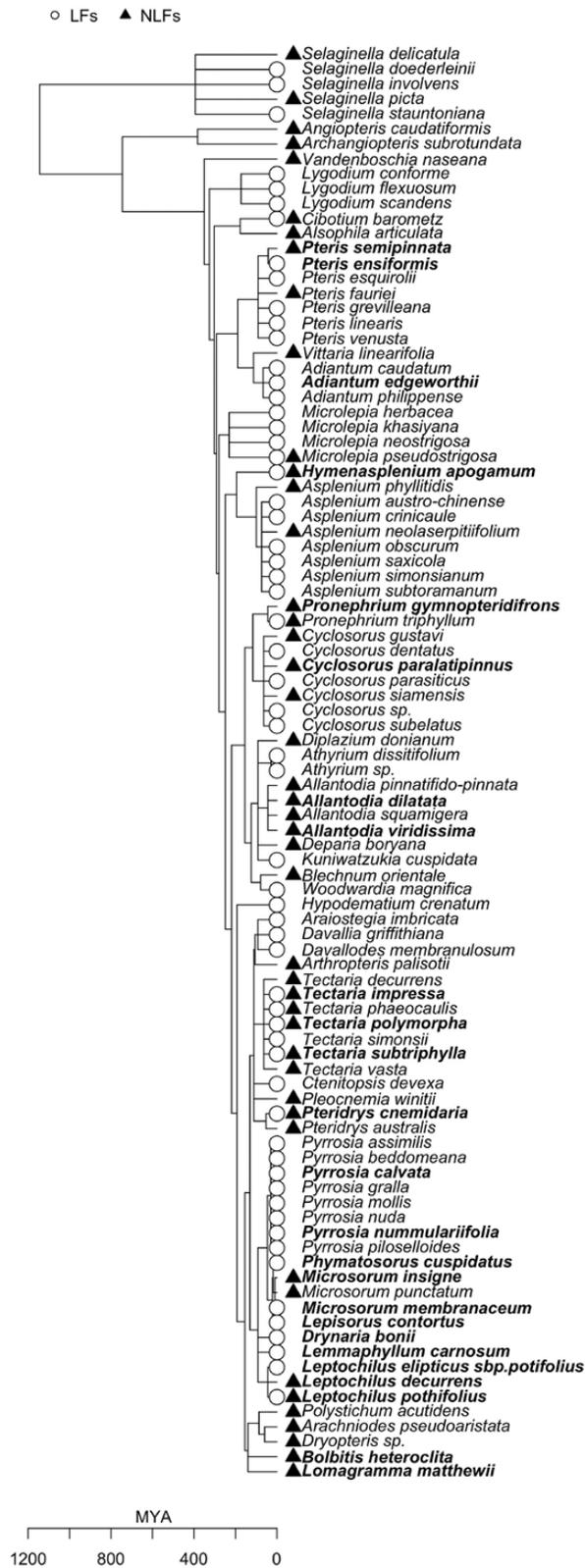


Figure 1: A phylogenetic tree of the local pteridophyte species found in Xishuangbanna, China. Circles and triangles indicate species that were found in LFs and NLFs, respectively. Species that were used for trait measurements in this study are highlighted in bold. The scale in the bottom shows millions of years ago (MYA).

polish was applied to the lower leaf surface at the middle portion, avoiding major veins, and then dry nail polish with the leaf impression was removed from the leaves. Digital images of dry nail polish with leaf impressions were captured using a stage microscope (Leica DM500, Leica Microsystems, Wetzlar, Germany) (Zhang *et al.* 2014), and the number and length of stomata were measured using ImageJ software (Rueden *et al.* 2017).

A_{\max} and g_s were measured *in situ* in January 2023. We used Li-Cor 6800 portable photosynthesis system attached with a 6800-01A multiphase flash fluorometer chamber which can choose a 6- or 2-cm² leaf area by swapping interchangeable apertures (Li-Cor Inc., Lincoln, NE, USA). The A_{\max} and the daily maximum g_s were measured in the morning between 9:30 am and 11:30 am. Before the measurements, each leaf was exposed to a light intensity of 300 $\mu\text{mol m}^{-2} \text{s}^{-1}$ for 30 min to induce the maximum stomatal opening. This light level was confirmed as the saturation point for photosynthesis of pteridophytes in preliminary experiments. During the measurement period, the CO₂ concentration in the chamber was set to 400 $\mu\text{mol mol}^{-1}$, with leaf temperature at 25 °C, light intensity at 300 $\mu\text{mol m}^{-2} \text{s}^{-1}$, flow rate at 500 mol s^{-1} and leaf-to-air vapor pressure deficit at 0.1–1.0 kPa. A 6-cm² leaf area was set for species with big leaf area and 2-cm² leaf area was set for species with small leaf area (Brodrribb *et al.* 2007; Zhang *et al.* 2014). To avoid any interference with g_s measurements were conducted under a clear sky with no wind (Zhang *et al.* 2013). LF species (*Microsorium membranaceum*) was not included for g_s and A_{\max} due to the leaves not being available during our recollection time.

Statistical analyses

We analyzed each trait separately to find the differences in leaf functional traits between the two forest types using generalized least squares (GLS) and phylogenetic GLS (PGLS) (Symonds and Blomberg 2014). The pairwise *t*-test was used to compare intraspecific differences in species that occurred in both LFs and NLFs. A phylogenetic tree for LF and NLF pteridophyte species listed in Phouthavong *et al.* (2019) was reconstructed by pruning mega-phylogeny of vascular plants (Smith and Brown 2018) in the *V.PhyloMaker* package (Jin and Qian 2019). We assigned branch lengths to the phylogenetic tree using the *chronos* function in the R package ape (Paradis *et al.* 2004). Family ages were based on the data from Testo and Sundue (2016).

Because our phylogeny was not fully resolved, Pagel's lambda (λ) (Freckleton *et al.* 2002) was used to assess phylogenetic signals, which is robust to polytomies (Molina-Venegas and Rodríguez 2017). The value λ varies from 0 to 1, with values close to 0 indicating no phylogenetic signal and values close to 1 indicating trait evolution according to Brownian motion. Since λ is a descriptive statistic, we randomly shuffled the trait values across the tip of the phylogenetic tree 999 times and generated a null distribution of λ values, which were used to calculate *P*-values.

Using assemblage data obtained from Phouthavong *et al.* (2019), we calculated PD (Faith 1992) in LFs and NLFs. PD is calculated as the sum of the total phylogenetic branch length for all of the species that occur in local communities. Because PD is not independent from species richness, we standardized PD values by controlling for the effects of species richness using the *ses.pd* function in the R package *picante* (Webb *et al.* 2008). Standardized effect size of PD (SES.PD) was calculated by comparing the observed PD against a null distribution of PD values generated by the same null model described above (i.e. shuffling trait values across tips). We subsequently used a *t*-test to determine whether mean values of SES were significantly different from zero among all local communities.

Finally, we conducted principal component analysis (PCA) and phylogenetic PCA (PPCA) (Polly *et al.* 2013) to analyze the covariance structure in leaf traits among species. We pooled all the species in LFs and NLFs with different life forms in these analyses. Note that *M. membranaceum* was excluded from PCA and PPCA because of the missing trait values but kept in the rest of the analyses. Data were log-transformed when necessary to achieve normality. All the analyses were implemented in R (R Core Team 2022).

RESULTS

Pteridophyte species used for this study covered a wide range of phylogenetic positions represented by the species found in local LFs and NLFs of Xishuangbanna (Fig. 1). PD ($P < 0.001$) and SES.PD ($P < 0.0001$) were lower in LFs compared with NLFs (Fig. 2). The confidence interval of the mean SES.PD in NLFs (0.36 to 1.09) was greater than zero suggesting phylogenetic overdispersion, whereas the mean SES.PD in LF was not significantly different from zero (confidence interval: -0.98 to 0.38),

suggesting weak phylogenetic clustering to random dispersion.

The results of the GLS analyses showed that pteridophyte species in LFs tended to have significantly smaller LS, thicker LT, lower SD, and lower g_s , while LWC, SL, and A_{\max} were not significantly different between LFs and NLFs (Table 2). The four traits that were significantly different between LFs and NLFs also exhibited phylogenetic signal: the Pagel's lambda values showed a significant phylogenetic signal for LS, g_s and relatively strong (but non-significant) phylogenetic signals for LT and SD, and the other traits like A_{\max} , LWC, and SL exhibited no phylogenetic signal (Table 2). After accounting for the phylogenetic signals using PGLS, differences in these four traits (LS, LT, SD, and g_s) became non-significant (Table 2).

PCA and PPCA showed trait composition between LF and NLF species were overlapping (Fig. 3), but trait composition in LFs showed greater variance, suggesting that the LF species had more differences in their trait values than the NLF species. SD and LT were important explanatory variables accounting for (P)PCA axis 1, and those two traits were in the opposite direction. SL and g_s were positively associated along (P)PCA axis 2 and were approximately orthogonal to SD and LT. After incorporating the life forms, PCA and PPCA showed trait composition of species with different life forms in both LFs and NLFs were overlapping, but LFs showed greater variance (Supplementary Fig. S1).

The pairwise *t*-tests for the traits of five species commonly found in both LFs and NLFs found significant differences in SD (Fig. 4). SD was lower in LFs than those of the same species found in NLFs (Fig. 4).

DISCUSSION

The adaptation of pteridophyte species is known to be affected by soil substrate and water availability (Huang *et al.* 2023; Lu *et al.* 2016). NLF species, such as *Bolbitis heteroclite*, *Lomagramma matthewii*, *Leptochilus decurrens*, *Microsorium insigne*, and *Pteris semipinnata*, are typically associated with riparian habitats near streams or found in dense forests (Lin *et al.* 2013), clearly reflecting their dependence on water resources. In contrast, LFs are characterized by having thin soil with low water holding capacity compared with NLFs (Cao *et al.* 2006), and some species, such as *M. membranaceum* and *Phymatosorus cuspidatus*, are typically associated with LFs. Despite

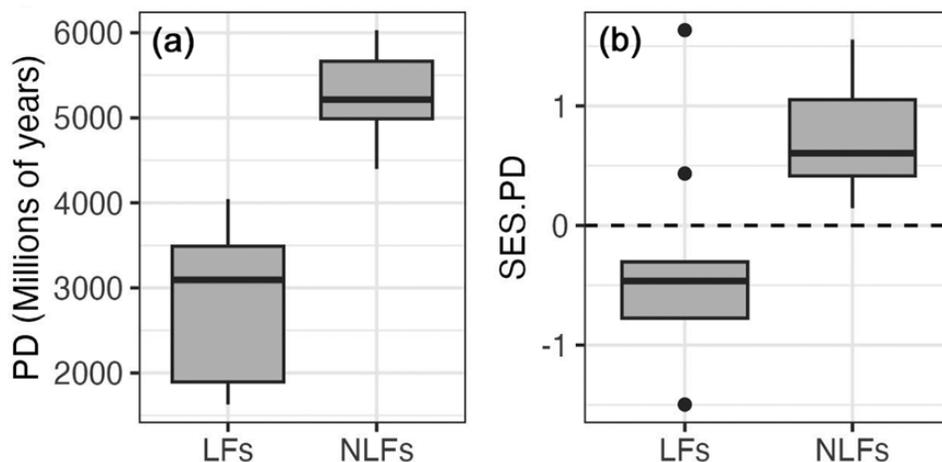


Figure 2: (a) PD and (b) SES.PD of pteridophyte species found in local LFs and NLFs of Xishuangbanna. The *t*-tests of PD and SES.PD both showed significant differences between the habitats ($P < 0.05$).

Table 2: Mean (\pm SE) values and phylogenetic signals (Pagel's λ) of the seven leaf functional traits in LF and NLF pteridophyte species with corresponding results from GLS and PGLS analyses

Trait	Mean (\pm SE)		Phylogenetic signal		GLS		PGLS	
	LFs	NLFs	λ	<i>P</i>	<i>t</i> -Value	<i>P</i>	<i>t</i> -Value	<i>P</i>
LS (cm ²)	383.6 \pm 106.3	1395.2 \pm 338.4	0.862	0.005	3.081	0.005	0.615	0.543
LT (mm)	0.296 \pm 0.075	0.139 \pm 0.007	0.580	0.089	-2.424	0.022	-0.824	0.417
LWC (%)	77.99 \pm 3.06	79.06 \pm 1.51	<0.001	1	0.494	0.625	0.010	0.922
SL (μ m)	99.2 \pm 5.9	87.6 \pm 4.7	0.191	1	-1.353	0.187	-0.400	0.692
SD (no. mm ⁻²)	0.071 \pm 0.006	0.116 \pm 0.015	0.642	0.087	2.081	0.047	0.571	0.572
Stomatal conductance (μ mol m ⁻² s ⁻¹)	0.024 \pm 0.003	0.041 \pm 0.004	0.828	0.003	2.559	0.016	1.685	0.103
A_{\max} (μ mol m ⁻² s ⁻¹)	4.13 \pm 0.56	4.75 \pm 0.25	<0.001	1	1.305	0.203	0.689	0.496

Significant phylogenetic signals or trait differences ($P < 0.05$) are in bold.

contrasting differences in microenvironmental characteristics, inter- and intraspecific differences in leaf traits of pteridophyte species have not been well studied in LFs.

We found that species in LFs had smaller, thicker leaves with less SD and lower stomatal conductance, results consistent with many previous studies using pteridophytes as well as trees and grasses in drier habitats (Ke *et al.* 2022; Liu *et al.* 2018; Lu *et al.* 2016; Song *et al.* 2013; Zhang *et al.* 2014). Small surface areas, less SD, and low stomatal conductance of leaves help to reduce water loss through transpiration (Farquhar and Sharkey 1982; Lin *et al.* 2021). Thick leaves often have more cuticle, which insulate the leaves to reduce water loss and store more water to

maintain hydraulic function during drought periods (Zhang *et al.* 2014). Such trait variabilities could be explained by the differences in the dominant life forms between the two habitats, as LFs included more epilithic or epiphytic fern species whereas NLFs included more terrestrial species (Table 1). Previous studies found that epiphytic ferns generally show lower SD and photosynthetic capacity but higher LT compared with terrestrial ferns (Zhang *et al.* 2014). However, different life forms did not appear to explain the trait variability between LF and NLF species (Supplementary Fig. S1).

Unlike LS, LT, SD, and stomatal conductance, photosynthetic rate (A_{\max}) was not different between LFs and NLFs. A_{\max} changes in response to light

availability, hence species found in open forests generally have greater A_{\max} than those found in closed forest understory (Saldaña *et al.* 2010; Zhang *et al.* 2014). However, our previous study found that light availability did not change between LFs and NLFs (Phouthavong *et al.* 2019), and thus A_{\max} did not change. Our study suggests that interspecific differences in pteridophyte leaf traits between LFs and NLFs are mainly due to their responses to water stress rather than differences in light availability.

Although phylogenetic signals were generally weak except for LS and stomatal conductance, interspecific trait differences between LFs and NLFs became non-significant after accounting for the phylogenetic relationships (Table 2). This suggests that the evolution of the measured traits of pteridophyte species in this study is not strongly conserved, but their phylogenetic relationships may play some roles in determining their habitat preferences. Because of these weak phylogenetic signals, it is complicated to infer functional or ecological similarities of species in local communities from the community phylogenetic structure (Cavender-Bares *et al.* 2004; Swenson 2013).

We found phylogenetic overdispersion in NLFs and phylogenetic clustering to random dispersion in LFs (Fig. 2). Cavender-Bares *et al.* (2004) suggested that the weak phylogenetic signals (convergent trait evolution) together with clustered or random phylogeny should result in more overdispersed

trait distributions. Indeed, the results of our (P)PCA showed more dispersed traits in LFs compared with those in NLFs. This is in contrast to previous regional-scale, tree-based studies that found that strong phylogenetic signals (conserved trait evolution) and phylogenetic clustering resulted in trait clustering in drier habitats (Bose *et al.* 2019; Muscarella *et al.* 2016). Greater trait variations in LFs were contrary to our hypothesis in which we expected that strong environmental filtering (water stress) selected species with certain traits (Li *et al.* 2018), resulting in lower trait variation among LF species. This may be explained by the fact that habitat conditions were more variable in LFs than NLFs. The data obtained from Phouthavong *et al.* (2019) showed that the coefficients of variation in slope, elevation, and canopy openness were all more variable in LFs (CV values for slope, elevation, and canopy openness were 36.6, 37.1, and 27.8, respectively) than NLFs (18.3, 15.1 and 13.1). This was also reflected in the species composition, where beta diversity was greater in LFs than NLFs (Figure 5 in Phouthavong *et al.* 2019).

Five species (*H. apogamum*, *L. pothifolius*, *T. impressa*, *T. polymorpha*, and *T. subtriphyllo*) were found in both LFs and NLFs. These five species are known as generalist species, and they are well adapted to different habitats with wide distributions in tropical regions (Cheng and Murakami 1998; Lin *et al.* 2013; Zhang *et al.* 2019). Leaves of these

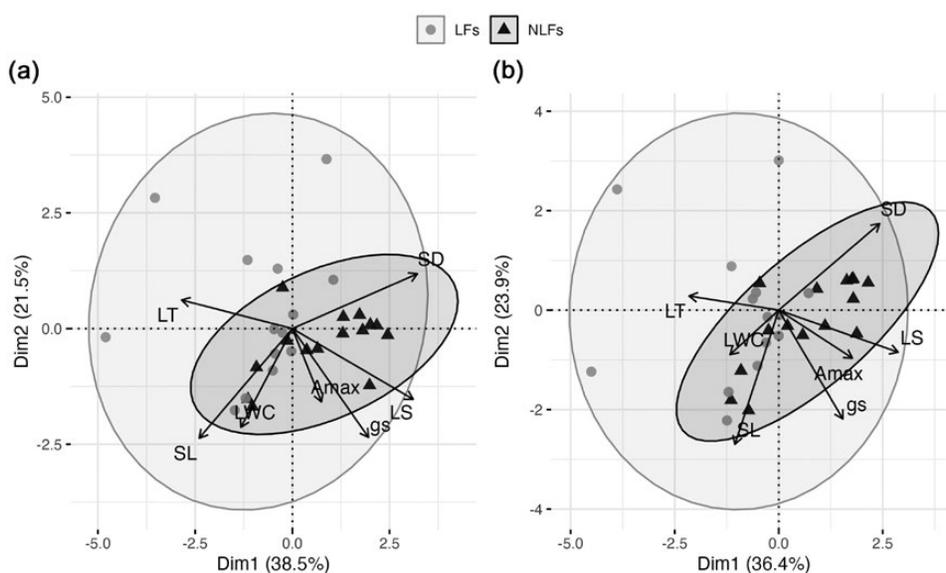


Figure 3: (a) PCA and (b) PPCA of the pteridophyte species commonly found in LFs (14 species, gray circles) and NLFs (15 species, black triangles) based on the seven leaf functional traits. Gray and dark gray ellipses represent the 95% confidence intervals of the centroids of (P)PCA scores of LFs and NLFs, respectively.

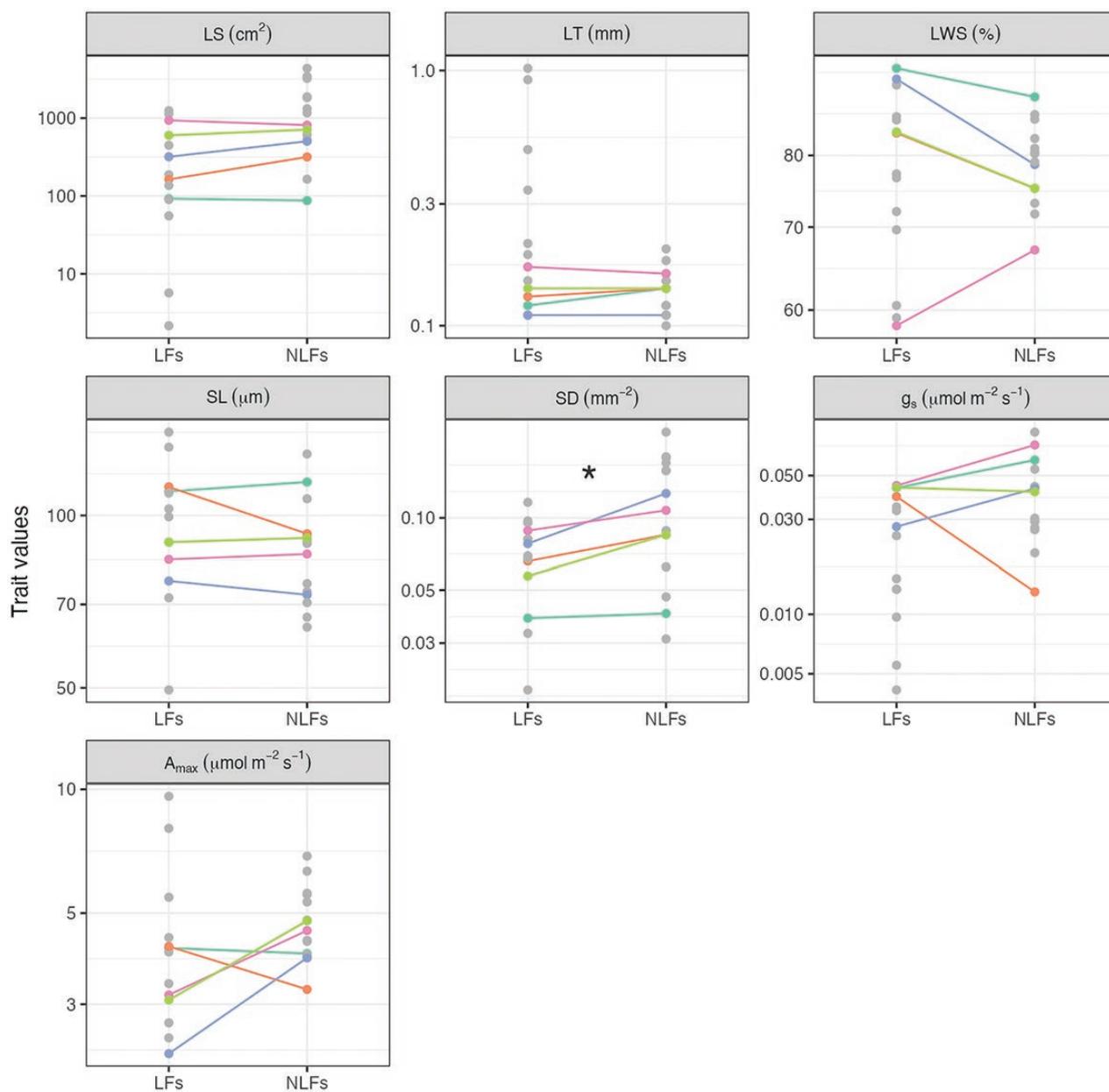


Figure 4: Inter- and intraspecific differences in leaf functional traits between LFs and NLFs. Species that occurred in both the LFs and NLFs are plotted in the same color, with lines connecting the trait values of the same species. Species found in either LFs or NLFs are shown in grey. The asterisks indicate significant differences (pairwise *t*-test; $P < 0.05$).

species in LFs had lower SD than those of the same species in NLFs (Fig. 4). SD increases within species experiencing increasing temperature and aridity to enhance daytime cooling via transpiration (Carlson *et al.* 2016; Chieppa *et al.* 2020). Our study found decreased SD in LFs, as the pteridophytes are likely to have adapted to using less water (Burton *et al.* 2017). In both LFs and NLFs, the forest canopy was well closed (Phouthavong *et al.* 2019) and, unlike other studies mentioned above, significant cooling by transpiration was unlikely. The results of our

study are consistent with a previous tree-based study which also showed that tree species occurring in both LFs and NLFs differed between forest types based on intraspecific variation (Fu *et al.* 2019).

Although we found a number of generalist species with intraspecific leaf trait variations between LFs and NLFs, many other species were restricted to either LFs or NLFs, and their morphological and physiological leaf traits were adapted to their local habitat conditions, especially in relation to water availability. These interspecific trait variabilities were

related to phylogenetic relationships, suggesting that these specialist species are unlikely to flexibly adapt to changing habitat conditions resulting from habitat disturbances and global climate change events. It is therefore imperative that we protect forests on different substrate types (limestone and non-limestone in our study) with different types of microhabitats to maintain diverse traits and, consequently, greater taxonomic beta diversity. Further studies should focus on pteridophyte functional and taxonomic diversity in LFs and NLFs at various spatial scales, namely among different latitudes, elevational strata, and microhabitats, so that we can improve our understanding of pteridophyte biogeography and develop effective conservation strategies at different spatial scales.

Supplementary Material

Supplementary material is available at *Journal of Plant Ecology* online.

Figure S1: Principal component analysis (PCA) (a) and phylogenetic PCA (PPCA) (b) of the pteridophyte species commonly found in limestone forests (LFs, 14 species, gray circles) and non-limestone forests (NLFs, 15 species, black circles) based on the seven leaf functional traits.

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REFERENCES

- Aldasoro JJ, Cabezas F, Aedo C (2004) Diversity and distribution of ferns in sub-Saharan Africa, Madagascar and some islands of the South Atlantic. *J Biogeogr* **31**:1579–1604.
- Bagousse-Pinguet YL, Soliveres S, Gross N, *et al.* (2019) Phylogenetic, functional, and taxonomic richness have both positive and negative effects on ecosystem multifunctionality. *Proc Natl Acad Sci U S A* **116**:8419–8424.
- Bose R, Ramesh BR, Pélissier R, *et al.* (2019) Phylogenetic diversity in the Western Ghats biodiversity hotspot reflects environmental filtering and past niche diversification of trees. *J Biogeogr* **46**:145–157.
- Brodribb TJ, Feild TS, Jordan GJ (2007) Leaf maximum photosynthetic rate and venation are linked by hydraulics. *Plant Physiol* **144**:1890–1898.
- Burton JI, Perakis SS, McKenzie SC, *et al.* (2017) Intraspecific variability and reaction norms of forest understorey plant species traits. *Funct Ecol* **31**:1881–1893.
- Cao M, Zhang JH (1997) Tree species diversity of tropical forest vegetation in Xishuangbanna, SW China. *Biodivers Conserv* **6**:995–1006.
- Cao M, Zou XM, Warren M, *et al.* (2006) Tropical forests of Xishuangbanna, China. *Biotropica* **38**:306–309.
- Carlquist S, Schneider EL (2001) Vessels in ferns: structural, ecological, and evolutionary significance. *Am J Bot* **88**:1–13.
- Carlson JE, Adams CA, Holsinger KE (2016) Intraspecific variation in stomatal traits, leaf traits and physiology reflects adaptation along aridity gradients in a South African shrub. *Ann Bot* **117**:195–207.
- Cavender-Bares J, Ackerly DD, Baum DA, *et al.* (2004) Phylogenetic overdispersion in Floridian oak communities. *Am Nat* **163**:823–843.
- Cheng X, Murakami N (1998) Cytotaxonomic study of genus *Hymenasplenium* (Aspleniaceae) in Xishuangbanna, southwestern China. *J Plant Res* **111**:495–500.
- Chieppa J, Brown T, Giresi P, *et al.* (2020) Climate and stomatal traits drive covariation in nighttime stomatal conductance and daytime gas exchange rates in a widespread C₄ grass. *New Phytol* **229**:2020–2034.
- Clements R, Sodhi NS, Schilthuizen M, *et al.* (2006) Limestone karsts of southeast Asia: imperiled arks of biodiversity. *BioScience* **56**:733–742.
- Cornelissen JHC, Lavorel S, Garnier E, *et al.* (2003) A handbook of protocols for standardised and easy measurement of plant functional traits worldwide. *Aust J Bot* **51**:335–380.
- Faith DP (1992) Conservation evaluation and phylogenetic diversity. *Biol Conserv* **61**:1–10.
- Farquhar GD, Sharkey TD (1982) Stomatal conductance and photosynthesis. *Annu Rev Plant Physiol* **33**:317–345.
- Flynn DFB, Mirotnick N, Jain M, *et al.* (2011) Functional and phylogenetic diversity as predictors of biodiversity—ecosystem-function relationships. *Ecology* **92**:1573–1581.
- Freckleton RP, Harvey PH, Pagel M (2002) Phylogenetic analysis and comparative data: a test and review of evidence. *Am Nat* **160**:712–726.
- Fu PL, Zhu SD, Zhang JL, *et al.* (2019) The contrasting leaf functional traits between a karst forest and a nearby

- non-karst forest in south-west China. *Funct Plant Biol* **46**:907–915.
- Han MQ, Liu Y, Zhang LB (2018) Eight new species of *Polystichum* (subg. *Haplopolystichum*; Dryopteridaceae) from limestone caves in Guangdong and Yunnan, China, with reference to species diversity in the karst terrains at high elevations in subtropical areas. *Phytotaxa* **365**:145–168.
- Hietz P, Briones O (1998) Correlation between water relations and within-canopy distribution of epiphytic ferns in a Mexican cloud forest. *Oecologia* **114**:305–316.
- Huang Y, Li H, Xie Y, *et al.* (2007) A preliminary study on the ecological benefit of fern in limestone area of Guangxi. *J Guangxi Agric Biol Sci* **58**:52–58.
- Huang C, Zhang M, Zhang Y, *et al.* (2023) Response of pteridophyte richness to water-energy dynamic hypothesis under climate change in China. *Plant Ecol* **224**:1–12.
- Jin Y, Qian H (2019) VPhyloMaker: an R package that can generate very large phylogenies for vascular plants. *Ecography (Cop)* **42**:1353–1359.
- Karst J, Gilbert B, Lechowicz MJ (2005) Fern community assembly: the roles of chance and the environment at local and intermediate scales. *Ecology* **86**:2473–2486.
- Ke X, Kang H, Tang Y (2022) Reduction in leaf size at higher altitudes across 39 broad-leaved herbaceous species on the northeastern Qinghai-Tibetan Plateau. *J Plant Ecol* **15**:1227–1240.
- Kessler M, Siorak Y, Wunderlich M, *et al.* (2007) Patterns of morphological leaf traits among pteridophytes along humidity and temperature gradients in the Bolivian Andes. *Funct Plant Biol* **34**:963–971.
- Kluge J, Kessler M (2007) Morphological characteristics of fern assemblages along an elevational gradient: patterns and causes. *Ecotropica* **13**:27–43.
- Kluge J, Kessler M (2011) Phylogenetic diversity, trait diversity and niches: species assembly of ferns along a tropical elevational gradient. *J Biogeogr* **38**:394–405.
- Li Q, Cao J, Yu L (2008) Biogeochemical effect of karst soil on the element contents in the leaves of two species of *Flos Lonicerae*. *Plant Soil Environ* **54**:486–492.
- Li Y, Shipley B, Price JN, *et al.* (2018) Habitat filtering determines the functional niche occupancy of plant communities worldwide. *J Ecol* **106**:1001–1009.
- Lin Y, Kuang L, Tang S, *et al.* (2021) Leaf traits from stomata to morphology are associated with climatic and edaphic variables for dominant tropical forest evergreen oaks. *J Plant Ecol* **14**:1115–1127.
- Lin YX, Zhang LB, Zhang XC, *et al.* (2013) *Pteridophytes (Lycophytes and Ferns)*. *FOC Vol. 2–3*. http://efloras.org/florataxon.aspx?flora_id=2&taxon_id=51 (1 February 2023, date last accessed).
- Liu C, He N, Zhang J, *et al.* (2018) Variation of stomatal traits from cold temperate to tropical forests and association with water use efficiency. *Funct Ecol* **32**:20–28.
- Lu HZ, Song L, Liu WY, *et al.* (2016) Survival and growth of epiphytic ferns depend on resource sharing. *Front Plant Sci* **7**:416.
- Lü XT, Yin JX, Tang JW (2010) Structure, tree species diversity and composition of tropical seasonal rain forest in Xishuangbanna, south-west China. *J Trop For Sci* **22**:260–270.
- Mehlreter K, Walker LR, Sharpe JM (2010) *Fern Ecology*. New York: Cambridge University Press.
- Messier J, McGill BJ, Lechowicz MJ (2010) How do traits vary across ecological scales? A case for trait-based ecology. *Ecol Lett* **13**:838–848.
- Molina-Venegas R, Rodríguez M (2017) Revisiting phylogenetic signal; strong or negligible impacts of polytomies and branch length information? *BMC Evol Biol* **17**:53.
- Muscarella R, Uriarte M, Erickson DL, *et al.* (2016) Variation of tropical forest assembly processes across regional environmental gradients. *Perspect Plant Ecol Evol Syst* **23**:52–62.
- Nitta JH, Watkins JE Jr, Davis CC (2020) Life in the canopy: community trait assessments reveal substantial functional diversity among fern epiphytes. *New Phytol* **227**:1885–1899.
- O'Dell RE, Rajakaruna N (2011) Intraspecific variation, adaptation, and evolution. In Rajakaruna N, Harrison S (eds). *Serpentine*, 1st edn. Berkeley, CA: University of California Press, 97–138.
- Page CN (2002) Ecological strategies in fern evolution: a neopteridological overview. *Rev Palaeobot Palynol* **119**:1–33.
- Paradis E, Claude J, Strimmer K (2004) APE: analyses of phylogenetics and evolution in R language. *Bioinformatics* **20**:289–290.
- Phouthavong K, Nakamura A, Cheng X, *et al.* (2019) Differences in pteridophyte diversity between limestone forests and non-limestone forests in the monsoonal tropics of southwestern China. *Plant Ecol* **220**:917–934.
- Polly PD, Lawing AM, Fabre A-C, *et al.* (2013) Phylogenetic principal components analysis and geometric morphometrics. *Hystrix Ital J Mammal* **24**:33–41.
- PPG I (2016) A community-derived classification for extant lycophytes and ferns. *J Syst Evol* **54**:563–603.
- R Core Team (2022) *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing. <https://www.r-project.org/> (29 June 2023, date last accessed).
- Raven PH, Evert RF, Eichhorn SE (1999) *Biology of Plants*, 6th edn. New York: W H Free Company.
- Rueden CT, Schindelin J, Hiner MC, *et al.* (2017) ImageJ2: ImageJ for the next generation of scientific image data. *BMC Bioinformatics* **18**:529.
- Saldaña AO, Hernández C, Coopman RE, *et al.* (2010) Differences in light usage among three fern species of genus *Blechnum* of contrasting ecological breadth in a forest light gradient. *Ecol Res* **25**:273–281.
- Saldaña A, Lusk CH, Gonzáles WL, *et al.* (2007) Natural selection on ecophysiological traits of a fern species in a temperate rain forest. *Evol Ecol* **21**:651–662.
- Salehi M, Walthert L, Zimmermann S, *et al.* (2020) Leaf morphological traits and leaf nutrient concentrations of European beech across a water availability gradient in Switzerland. *Front For Glob Change* **3**:19.
- Sharpe JM, Mehlreter K, Walker LR (2010) Ecological importance of ferns. In Mehlreter K, Walker LR, Sharpe

- JM (eds). *Fern Ecology*. New York: Cambridge University Press, 1–18.
- Siefert A, Violle C, Chalmandrier L, *et al.* (2015) A global meta-analysis of the relative extent of intraspecific trait variation in plant communities. *Ecol Lett* **18**:1406–1419.
- Smith SA, Brown JW (2018) Constructing a broadly inclusive seed plant phylogeny. *Am J Bot* **105**:302–314.
- Song J, Li RH, Zhu SD, *et al.* (2013) Leaf functional traits of ferns from different habitats in monsoon evergreen broad-leaved forest in Dinghushan Mountain. *J Trop Subtrop Bot* **21**:489–495.
- Srivastava DS, Cadotte MW, MacDonald AAM, *et al.* (2012) Phylogenetic diversity and the functioning of ecosystems. *Ecol Lett* **15**:637–648.
- Sun M, Yang SJ, Zhang JL, *et al.* (2014) Correlated evolution in traits influencing leaf water balance in *Dendrobium* (Orchidaceae). *Plant Ecol* **215**:1255–1267.
- Swenson NG (2013) The assembly of tropical tree communities—the advances and shortcomings of phylogenetic and functional trait analyses. *Ecography (Cop)* **36**:264–276.
- Symonds MRE, Blomberg SP (2014) A primer on phylogenetic generalised least squares. In Garamszegi LZ (ed). *Modern Phylogenetic Comparative Methods and Their Application in Evolutionary Biology: Concepts and Practice*. Berlin, Heidelberg: Springer Berlin Heidelberg, 105–130.
- Tang JW, Lü XT, Yin JX, *et al.* (2011) Diversity, composition and physical structure of tropical forest over limestone in Xishuangbanna, south-west China. *J Trop For Sci* **23**:425–433.
- Testo W, Sundue M (2016) A 4000-species dataset provides new insight into the evolution of ferns. *Mol Phylogenet Evol* **105**:200–211.
- Voleníková M, Tichá I (2001) Insertion profiles in stomatal density and sizes in *Nicotiana tabacum* L. plantlets. *Biol Plant* **44**:161–165.
- Wang JH, Li SC, Sun M, *et al.* (2013) Differences in the stimulation of cyclic electron flow in two tropical ferns under water stress are related to leaf anatomy. *Physiol Plant* **147**:283–295.
- Watkins JE, Cardelús C (2009) Habitat differentiation of ferns in a lowland tropical rain forest. *Am Fern J* **99**:162–175.
- Webb CO, Ackerly DD, Kembel SW (2008) Phylocom: software for the analysis of phylogenetic community structure and trait evolution. *Bioinformatics* **24**:2098–2100.
- Wiens JJ, Ackerly DD, Allen AP, *et al.* (2010) Niche conservatism as an emerging principle in ecology and conservation biology. *Ecol Lett* **13**:1310–1324.
- Zhang L, Lu NT, Zhou XM, *et al.* (2019) A plastid phylogeny of the old world fern genus *Leptochilus* (Polypodiaceae): implications for cryptic speciation and progressive colonization from lower to higher latitudes. *Mol Phylogenet Evol* **134**:311–322.
- Zhang YJ, Meinzer FC, Qi JH, *et al.* (2013) Midday stomatal conductance is more related to stem rather than leaf water status in subtropical deciduous and evergreen broadleaf trees. *Plant Cell Environ* **36**:149–158.
- Zhang SB, Sun M, Cao KF, *et al.* (2014) Leaf photosynthetic rate of tropical ferns is evolutionarily linked to water transport capacity. *PLoS One* **9**:e84682.
- Zhu H, Cao M, Hu H (2006) Geological history, flora, and vegetation of Xishuangbanna, southern Yunnan, China. *Biotropica* **38**:310–317.
- Zhu SD, Chen YJ, Fu PL, *et al.* (2017) Different hydraulic traits of woody plants from tropical forests with contrasting soil water availability. *Tree Physiol* **37**:1469–1477.