

Correlations between leaf economics, mechanical resistance and drought tolerance across 41 cycad species

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- **Background and Aims** We conducted a comprehensive analysis of the functional traits of leaves (leaflets) of cycads. The aim of this study was to clarify the functional divergence between the earlier origin Cycadaceae and the later differentiated Zamiaceae, and the differences in trait associations between cycads and angiosperms.
- **Methods** We selected 20 Cycadaceae species and 21 Zamiaceae species from the same cycad garden in South China, and measured their leaf structure, economic traits, mechanical resistance (F_p) and leaf water potential at the turgor loss point (π_{tp}). In addition, we compiled a dataset of geographical distribution along with climatic variables for these cycad species, and some leaf traits of tropical–sub-tropical angiosperm woody species from the literature for comparison.
- **Key Results** The results showed significantly contrasting leaf trait syndromes between the two families, with Zamiaceae species exhibiting thicker leaves, higher carbon investments and greater F_p than Cycadaceae species. Leaf thickness (LT) and π_{tp} were correlated with mean climatic variables in their native distribution ranges, indicating their evolutionary adaptation to environmental conditions. Compared with the leaves of angiosperms, the cycad leaves were thicker and tougher, and more tolerant to desiccation. Greater F_p was associated with a higher structural investment in both angiosperms and cycads; however, cycads showed lower F_p at a given leaf mass per area or LT than angiosperms. Enhancement of F_p led to more negative π_{tp} in angiosperms, but the opposite trend was observed in cycads.
- **Conclusions** Our results reveal that variations in leaf traits of cycads are mainly influenced by taxonomy and the environment of their native range. We also demonstrate similar leaf functional associations in terms of economics, but different relationships with regard to mechanics and drought tolerance between cycads and angiosperms. This study expands our understanding of the ecological strategies and likely responses of cycads to future climate change.

Key words: Angiosperm, biogeography, Cycadaceae, functional traits, leaf anatomy, mechanical resistance, nutrient, photosynthesis, phylogeny, turgor loss point, Zamiaceae.

INTRODUCTION

Cycads originated about 300 million years ago, flourished and spread widely in the Jurassic–Cretaceous era, and today are regarded as living fossils of seed plants (Brenner *et al.*, 2003; Nagalingum *et al.*, 2011; Feng *et al.*, 2017). The extant cycads are largely confined to tropical–sub-tropical warm environments (Jones, 2002; Renner, 2011). According to the World List of Cycads (www.cycadlist.org), there are a total of 360 cycad species belonging to ten genera, with the majority of cycad species (307) included in the IUCN Red List of Endangered Species (www.iucnredlist.org). The endangered status of so many cycads is caused by environmental change, poor competitive ability, and illegal human collection (Alvarez-Yepiz *et al.*, 2019). Cycads have great ornamental and medicinal value (Hill *et al.*, 2004; Bamigboye and Tshisikhawe, 2020), and have received much attention in their evolution (Brenner

et al., 2003; Nagalingum *et al.*, 2011), conservation (Gutierrez-Ortega *et al.*, 2018; Zheng *et al.*, 2017) and biology (Zhang *et al.*, 2015; Tomlinson *et al.*, 2018).

Several studies support the division of cycads into two families, the Cycadaceae, comprising the single genus *Cycas*, and the Zamiaceae, comprising the remaining nine genera (Hill *et al.*, 2003; Wang and Ran, 2014; Chang *et al.*, 2020). Fossils and phylogenetic analysis have revealed that *Cycas* is the oldest genus of cycads, which separated very early (Jurassic) from other genera as a distinct line of evolution (Jones, 2002). The genus *Cycas* originated from humid Indo-China and spread southward to Australia, eastern Africa and the Pacific Islands (Hill, 2004; Mankga *et al.*, 2020). However, most Zamiaceae genera are currently confined to a single continent (e.g. *Macrozamia* in Australia) or two continents (e.g. *Zamia* in the tropical areas of North and South America; Norstog and Nicholls, 1997; Nagalingum *et al.*, 2011). In addition, a small proportion of

Zamiaceae species are distributed in relatively dry habitats, such as tropical savannas, shrublands and rocky regions (Norstog and Nicholls, 1997; Segalla *et al.*, 2019). Recently, Gutierrez-Ortega *et al.* (2018) proposed that climate aridification is a major driver of the diversification of cycad species (i.e. *Dioon*). Hence, we anticipate a functional divergence between Cycadaceae and Zamiaceae, which is related to their geographical distribution and evolution (Marler and Krishnapillai, 2018).

Plant functional traits, including carbon acquisition, water transportation and nutrient use efficiency, represent their ecological strategies and adaptation to native environments (Violle *et al.*, 2007; Perez-Harguindeguy *et al.*, 2013). Many previous studies have shown that leaf functional traits are good predictors of species performance, e.g. species with the ‘fast-return strategy’ are associated with high photosynthetic rates (A_{\max}) and nutrient concentrations, and low leaf mass per area (LMA) and construction costs, whereas species with the ‘slow-return strategy’ exhibit the opposite traits (Wright *et al.*, 2004; Adler *et al.*, 2014; Reich, 2014). Leaf mechanical resistance reflects the ability of plants to resist physical and insect damage (Onoda *et al.*, 2011), which relates to leaf structure and affects A_{\max} (Kitajima and Poorter, 2010; He *et al.*, 2019). Leaf water potential at the turgor loss point (π_{up}) is a key trait for estimating plant physiological drought tolerance and is correlated to plant distribution to environments with different moisture levels (Bartlett *et al.*, 2012a). A more negative π_{up} indicates that cell turgor and stomatal opening are maintained under lower water availability (Bartlett *et al.*, 2012a). In addition, π_{up} is found to be significantly correlated with leaf economic traits (Zhu *et al.*, 2018). The trait-based approach has been used to clarify the eco-physiological differences between angiosperms and gymnosperms; however, most of the relevant studies have mainly concentrated on conifers (McElwain *et al.*, 2016; Xiong and Flexas, 2020; Laughlin *et al.*, 2020). As an ancient group of gymnosperm, relevant functional analyses of cycads are rare, except for some studies on leaf economics (Krieg *et al.*, 2017; Zhang *et al.*, 2015, 2017). The further characterization of leaf economics, mechanics and drought tolerance of cycads could improve our understanding of the ecological adaptations of this ancient lineage (Prado *et al.*, 2014) and facilitate the management and conservation of these threatened species (Romermann *et al.*, 2008; Lauterbach *et al.*, 2013).

In the present study, we selected 41 cycad species belonging to Cycadaceae and Zamiaceae from the same cycad garden in South China. The common garden approach excludes the environmental influence on leaf functional traits, and allows the study of the adaptive genetic variation among plant species (Ramírez-Valiente *et al.*, 2020; De Villemereuil *et al.*, 2021). We measured a series of leaf functional traits, including morphological and structural traits, mechanical resistance, nitrogen and phosphorus concentrations, construction and maintenance costs, photosynthetic characteristics and π_{up} . In addition, we compiled a dataset of geographical distributions along with climatic variables in the native range for these cycad species, and some leaf functional traits of tropical–sub-tropical woody plants from the literature for comparison. By exploring trait associations and trait–environment relationships, our main objective was to elucidate the differences in leaf ecological strategies between the earlier origin Cycadaceae and the later differentiated Zamiaceae, and between cycads and angiosperms.

MATERIALS AND METHODS

Study site and plant materials

This study was conducted in the cycad garden (Supplementary data Fig. S1) in the Nanning Botanical Garden, southern China (22°47′12.93″W, 108°23′3.30″E, 190 m a.s.l.). The cycad garden was built in 1998, and covers an area of 6.7 ha. As one of China’s cycad conservation bases, it is home to >10 000 cycad plant individuals belonging to 78 species, eight genera and two families (Cycadaceae and Zamiaceae). The mean annual precipitation and temperature are approx. 1290 mm and 21.8 °C, respectively. These cycad plants grow on latosolic soil with a pH of 3.91–4.78 and organic matter content of 1.5–2.2 %.

A total of 41 cycad species (Supplementary data Table S1) were selected from the cycad garden for the measurement of functional traits, of which 20 species belong to Cycadaceae and the rest belong to Zamiaceae. Three to five healthy and mature individuals from each species were used for the leaf trait measurements. As cycads have large compound leaves, their leaflets were used in our study which are comparable with the leaves of angiosperms. All measurements were performed during the wet season of 2020.

Leaf anatomy and mechanical resistance

We sampled three mature and healthy leaflets from each individual per species. Leaflets were kept in sealed bags with wet tissues and were brought back to the laboratory within 30 min of sampling. Cross-sections from the middle of the leaflet avoiding the midrib vein were made using a razor blade, and we randomly selected three fields to take photographs with a light microscope (DM2500, Leica, Wetzlar, Germany) at $\times 10$ magnification. Leaf thickness (LT, mm) was measured using ImageJ software (National Institutes of Health, Bethesda, MD, USA). Stomatal density (SD, no. mm^{−2}) on the abaxial epidermis of the middle of the leaflet was determined using the nail polish impression method. The leaf force to punch (F_p , kN m^{−1}) was defined as the maximum force (F) per fraction circumference along the leaf lamina surface (Onoda *et al.*, 2011), and was measured using a puncture strength tester (precision 0.001 N, ZQ-990A-9, Dongguan, China). The diameter of the flat-end punch rod (dpr) was 0.5 mm and the test speed was 80 mm min^{−1}. The F_p measurements were taken in the middle of the leaflet laminas, avoiding the midribs, and calculated as $F_p = F/(\pi \times \text{dpr})$.

Leaf water potential at the turgor loss point

Leaf water potential at the turgor loss point (π_{up} , MPa) was determined using the osmometer method (Bartlett *et al.*, 2012b). Leaves were sampled in the early morning, placed in black bags with wet paper towels and immediately transferred to the laboratory. One disc was obtained from each leaflet centrally between the midrib and the margin (avoiding the leaf veins) using an 8 mm diameter punch. The discs were wrapped in foil and placed in liquid nitrogen for at least 2 min, after which they were immediately punctured 10–15 times with a needle and sealed in

a vapour pressure osmometer chamber (VAPRO 5600; Wescor, Logan, UT, USA). Then, π_{osm} (osmometer) was calculated as: $\pi_{\text{osm}} = 2.5 \times c_0/1000$, where the equilibrium solute concentration value c_0 (mmol kg⁻¹) was recorded from the osmometer when the difference between consecutive 2 min measurements fell below 5 mmol kg⁻¹. The π_{lp} value was estimated from π_{osm} using the following equation (Bartlett *et al.*, 2012b): $\pi_{\text{lp}} = 0.832 \times \pi_{\text{osm}} - 0.631$. Since angiosperms and cycads differ in leaf traits, it is unknown whether the equation could be applied to cycads. To test this, we randomly selected five cycad species, and determined their π_{lp} by using both a traditional pressure–volume technique (S.-D. Zhu *et al.*, unpubl. data) and the above equation (osmometer method), and found that the two methods produce similar π_{lp} values (Supplementary data Table S2). In addition, we pooled predicted π_{lp} and the actual π_{lp} of the five cycad species into the original angiosperm calibration dataset (Bartlett *et al.*, 2012b), and found that the cycad species did not deviate from the 1:1 regression line (Supplementary data Fig. S2).

Leaf mass per area, construction and maintenance costs

Leaf samples were scanned with a scanner (Epson Perfection V800 photo, Seiko Epson CORR, Indonesia), and their areas were calculated using ImageJ (<https://imagej.nih.gov/ij>, USA). The leaves were then oven-dried at 70 °C for 48 h to determine their dry mass. The LMA (g m⁻²) was calculated by dividing the leaf dry mass by the leaf area. The total nitrogen concentration (N_m, mg g⁻¹) was determined by Kjeldahl analysis and the total phosphorus concentration (P_m, mg g⁻¹) was determined using atomic absorption spectrum photometry. Area-based nitrogen (N_a, g m⁻²) and phosphorus concentrations (P_a, g m⁻²) were converted using LMA. The ash concentration was determined after combustion for 4 h at 500 °C in a muffle furnace. The heat of combustion was quantified using an oxygen bomb calorimeter (Model 6400; Parr, Moline, IL, USA), and the ash-free combustion was calculated by converting the heat of combustion on a total dry mass basis to the corresponding ash-free mass. Leaf construction cost (CC_m, g glu g⁻¹) is defined as the amount of glucose required to produce 1 g of biomass from glucose and minerals, and it was calculated using the following equation (Williams *et al.*, 1987):

$$\text{CC}_m = \{(0.06968 \times \text{Hc} - 0.065) \times (1 - \text{Ash}) + 7.5 \times (k \times \text{N}/14.0067)\}/0.89,$$

where Hc is ash-free heat of combustion (kJ g⁻¹), Ash is ash concentration (g g⁻¹), N is nitrogen concentration (g g⁻¹) and k is the oxidation state of the nitrogen source (+5 for nitrate or -3 for ammonium). In the present study, we used $k = 5$ because nitrate was the main source of soil N at the study site. Leaf maintenance cost (MC_m, mg glucose g⁻¹ d⁻¹) is defined as requiring energy, such as the maintenance of ion gradients across lipid membranes and the turnover of organic compounds, but does not directly result in a net increase in dry matter. This parameter was calculated according to the following equation (Merino *et al.*, 1984; Cavatte *et al.*, 2012):

$$\text{MC}_m = 0.0425 \times \text{Lipid} + 0.0405 \times \text{Protein} + 0.008 \times \text{Ash},$$

where Lipid is lipid content (g g⁻¹), Protein is protein concentration (g g⁻¹) and Ash is ash concentration (g g⁻¹). Lipid content

was extracted from a mixture of chloroform and methanol. Protein concentration was estimated by multiplying the nitrogen concentration by 6.25. Area-based construction cost (CC_a, g glucose m⁻²) and maintenance cost (MC_a, g glucose m⁻² d⁻¹) were calculated using LMA.

Photosynthetic capacity

Maximum CO₂ assimilation rate (A_{max} , μmol m⁻² s⁻¹) and stomatal conductance (g_s , mol m⁻² s⁻¹) were determined using a portable photosynthesis system (Li-6800, Li-Cor, Lincoln, NE, USA) between 8.30 and 11.30 h on consecutive sunny days during the wet season. Photosynthetic photon flux density was set at 1500 μmol m⁻² s⁻¹ (Zhang *et al.*, 2015), an ambient temperature of 36–42 °C and a CO₂ concentration of 400 ppm. Two mature and healthy leaflets were measured from each individual. Photosynthetic nitrogen (PNUE, μmol mol⁻¹ s⁻¹) and phosphorus use efficiency (PPUE, mmol mol⁻¹ s⁻¹) were calculated as the ratios of A_{max} to N_a and P_a, respectively. Intrinsic water use efficiency (WUE, μmol mol⁻¹) was calculated as $\text{WUE} = A_{\text{max}}/g_s$. The cost–benefit ratio (CC/A) was calculated as $\text{CC}_a/A_{\text{max}}$.

Phylogenetic tree, climate variables of native distribution and dataset of angiosperms

The phylogenetic trees of the sampled species were combined from two previous studies; the backbone and part of Cycadaceae were derived from Forest *et al.* (2018), while a part of Zamiaceae was derived from the GBOTB.extend tree from Smith and Brown (2018). Limited by the data, we only matched the data of 33 of the 41 cycad species in this study. Method nnls of function force. ultrametric of the package phytools was used to rescale the combined tree to be ultrametric (Revell, 2012). Phylogenetic signals (Pagel's lambda, Pagel, 1999) were calculated using the physisig function in phytools (Revell, 2012). To verify the robustness of the results, phylogenetic signals were calculated using both the Forest tree and the GBOTB.extend tree, and compared with the combined tree (Supplementary Data Table S3).

The geographical distribution data of the 41 cycad species were obtained from the Global Biodiversity Information Facility (GBIF; <https://www.gbif.org>), the Chinese Virtual Herbarium (<https://www.cvh.ac.cn/index.php>), the *Cycads of the World* (Jones, 2002) and field survey data. Outliers, isolated extreme points or duplicated points of the geographic distribution were manually removed to eliminate errors. Only species with at least five distribution points were identified. A total of 19 bioclimatic variables of each distribution point were extracted from the WorldClim database (<https://www.worldclim.org>, with 30 s resolution). In this study, three variables highly correlated to leaf traits were presented in the main results: (1) mean diurnal range (MDR), calculated as the mean of monthly (maximum temperature – minimum temperature); (2) isothermality, calculated as (mean diurnal range/temperature annual range) × 100; and (3) precipitation of the warmest quarter (PWQ). For each species, we used the average value of each bioclimate variable in all distribution records.

For comparison, we compiled a dataset of leaf functional traits for angiosperm woody species from tropical and

sub-tropical forests in southern China, including F_p , LMA, LT and π_{tlp} that were from published papers (Zhu et al., 2018; He et al., 2019; Wang et al., 2021) and our unpublished data.

Statistical analysis

Principal component analysis (PCA) was conducted to analyse the 18 leaf functional traits of 41 cycad species using \log_{10} -transformed average values in the FactoMineR package (Le et al., 2008). Relationships among leaf traits were analysed using the cor.test function in the package stats. To compare the differences in the relationships of traits between cycads and angiosperms, standardized major axis (SMA) analysis was used with the sma function in the smart package (Warton et al., 2012). All analyses and figures were conducted in R 4.0.2 (R Core Team, 2020).

RESULTS

Trait variations and principal component analysis

Leaf photosynthetic traits varied significantly across the 41 cycad species: A_{max} varied 7.2-fold, g_s varied 10.0-fold, PNUE

varied 13.0-fold and PPUE varied 17.9-fold. Leaf nutrient concentrations, construction and maintenance costs, and structural traits showed moderate variations across species: N varied 3.1-fold, P varied 5.5-fold, LT varied 3.7-fold, LMA varied 4.4-fold, SD varied 4.3-fold, CC_a varied 4.3-fold and MC_a varied 4.7-fold. Leaf mechanical resistance ranged from 0.28 to 1.40 kN m^{-1} , and π_{tlp} ranged from -2.10 to -1.57 MPa (Supplementary data Table S4).

The PCA results showed that the first axis accounted for 43 % of the total variance of 18 leaf traits, which was associated with leaf construction cost and mechanical resistance (e.g. CC_a , MC_a , F_p and LT). The second PCA axis accounted for 18 % of the total variance, which was associated with leaf photosynthetic capacity (PPUE, PNUE, A_{max} and g_s). The Cycadaceae and Zamiaceae species were separated into two distinct groups along the first axis, with the Zamiaceae species showing higher LT, F_p , P_a , CC_a , MC, and CC/A (Fig. 1; Supplementary data Table S5). Area-based leaf traits were consistently used in this study. However, there are concerns about the expression of leaf traits on an area vs. mass basis in data analyses (Lloyd et al., 2013; Osnas et al., 2013). We found PCA results on mass-based and area-based traits were mostly similar, except that leaf construction and maintenance cost could not distinguish the two

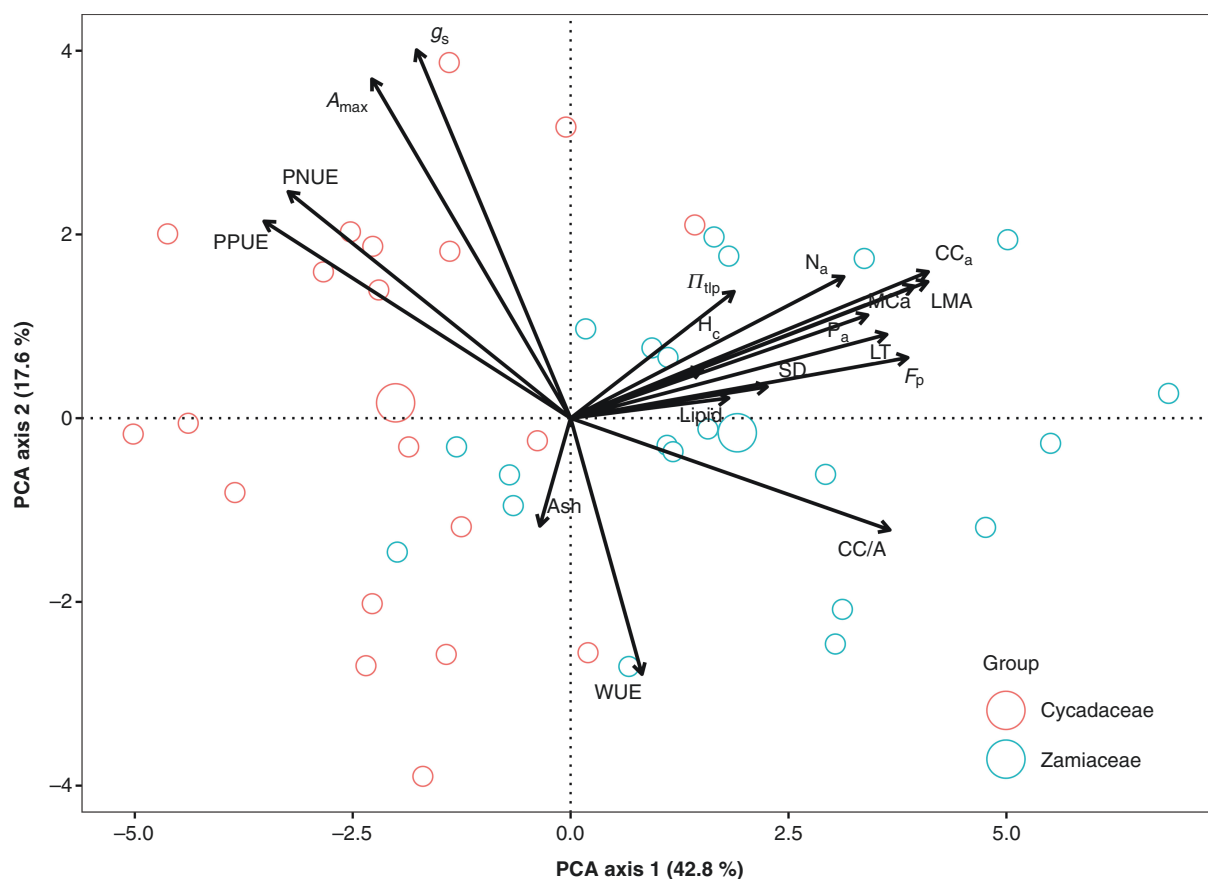


FIG. 1. Principal component analysis for (A) 18 leaf traits and (B) 41 cycad species loadings on the first and second axes. Trait abbreviations: LMA, leaf mass per area; π_{tlp} , leaf water potential at the turgor loss point; LT, leaf thickness; SD, stomatal density; F_p , force to punch; Ash, ash content; N_a , nitrogen content per unit area; P_a , phosphorus content per unit area; Lipid, lipid content; H_c , ash free of heat combustion; CC_a , leaf construction cost per unit area; MC_a , leaf maintenance cost per unit area; A_{max} , maximum CO_2 assimilation rate; g_s , stomatal conductance; PNUE, photosynthetic N-use efficiency; PPUE, photosynthetic P-use efficiency; WUE, photosynthetic water-use efficiency; CC/A, cost to benefit ratio.

families when expressed on a mass basis (Fig. 1; Supplementary data Fig. S3).

Influence of phylogeny and climate on leaf traits

According to the phylogenetic relationship of the 33 cycad species, Cycadaceae and Zamiaceae species were also clustered into two major branches, indicating different evolutionary directions (Fig. 2). Among the measured traits, LT, P_a and π_{tip} showed strong phylogenetic signals (Supplementary data Table S3, $K > 0.6$), indicating that these traits were restricted by phylogeny. The two cycad families significantly differed in MDR and PWQ ($P < 0.01$; Fig. 2), with Cycadaceae species distributed in relatively wet environments.

There was a significantly positive relationship between LT and MDR, and a significantly negative relationship between LT and PWQ (Fig. 3). In addition, π_{tip} was positively related to isothermality, and negatively related to PWQ (Fig. 3), which was partially driven by the strong correlation between π_{tip} and LT (Supplementary data Table S6). These results indicated that cycad species with distribution ranges in less isothermal and drier environments tended to have thicker leaves but less negative π_{tip} .

Comparison of trait correlations between cycads and angiosperms

We found that F_p was positively correlated with LMA and LT in both cycads and tropical–sub-tropical angiosperm woody species; however, the intercepts of the regression lines differed significantly between the two plant groups. At a given LMA or LT, angiosperms had greater F_p than cycads (Fig. 4). There was a non-significant correlation between π_{tip} and LT in angiosperms, but a significant and positive correlation in cycads. It was found that with increasing F_p and CC_a , π_{tip} significantly decreased (became more negative) in angiosperms but significantly increased in cycads (Fig. 5).

DISCUSSION

Our results showed that the two cycad families grown in the common garden differed significantly in terms of leaf economic, mechanical and drought tolerance traits. In general, the Zamiaceae showed higher structural investments (e.g. higher leaf thickness and construction cost) and greater mechanical resistance, but higher resistance to desiccation compared with Cycadaceae (Fig. 1; Supplementary data Fig. S4). This leaf

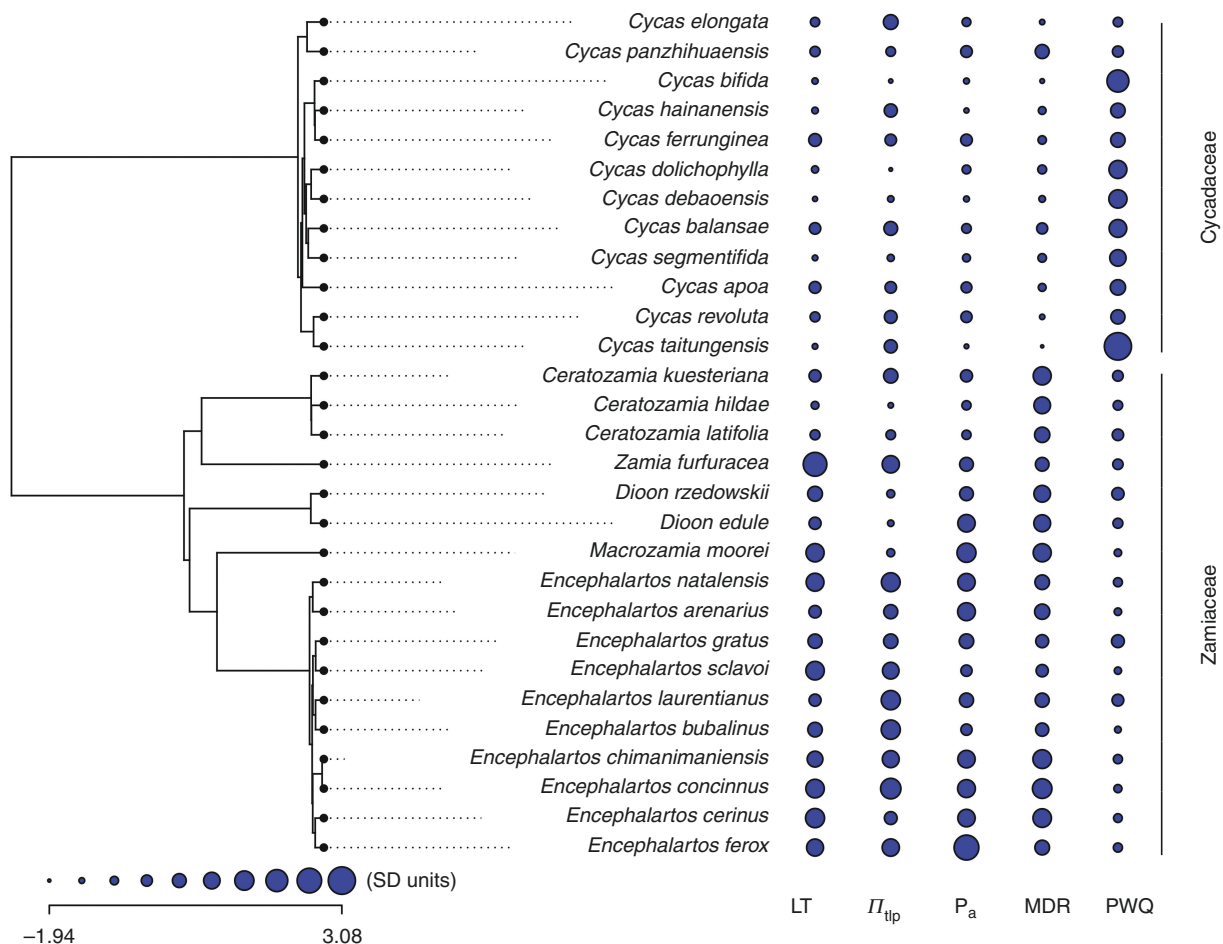


FIG. 2. Phylogeny, leaf traits and main climate factors of original distribution ranges for the cycad species. Trait values in each column are in proportion to the size of circles for each species, where larger circles indicate higher (less negative) values. MDR, mean diurnal range of temperature; PWQ, precipitation of warmest quarter. The bioclimatic variables were extracted from WorldClim database (<https://www.worldclim.org/>). Trait abbreviations are shown in Fig. 1.

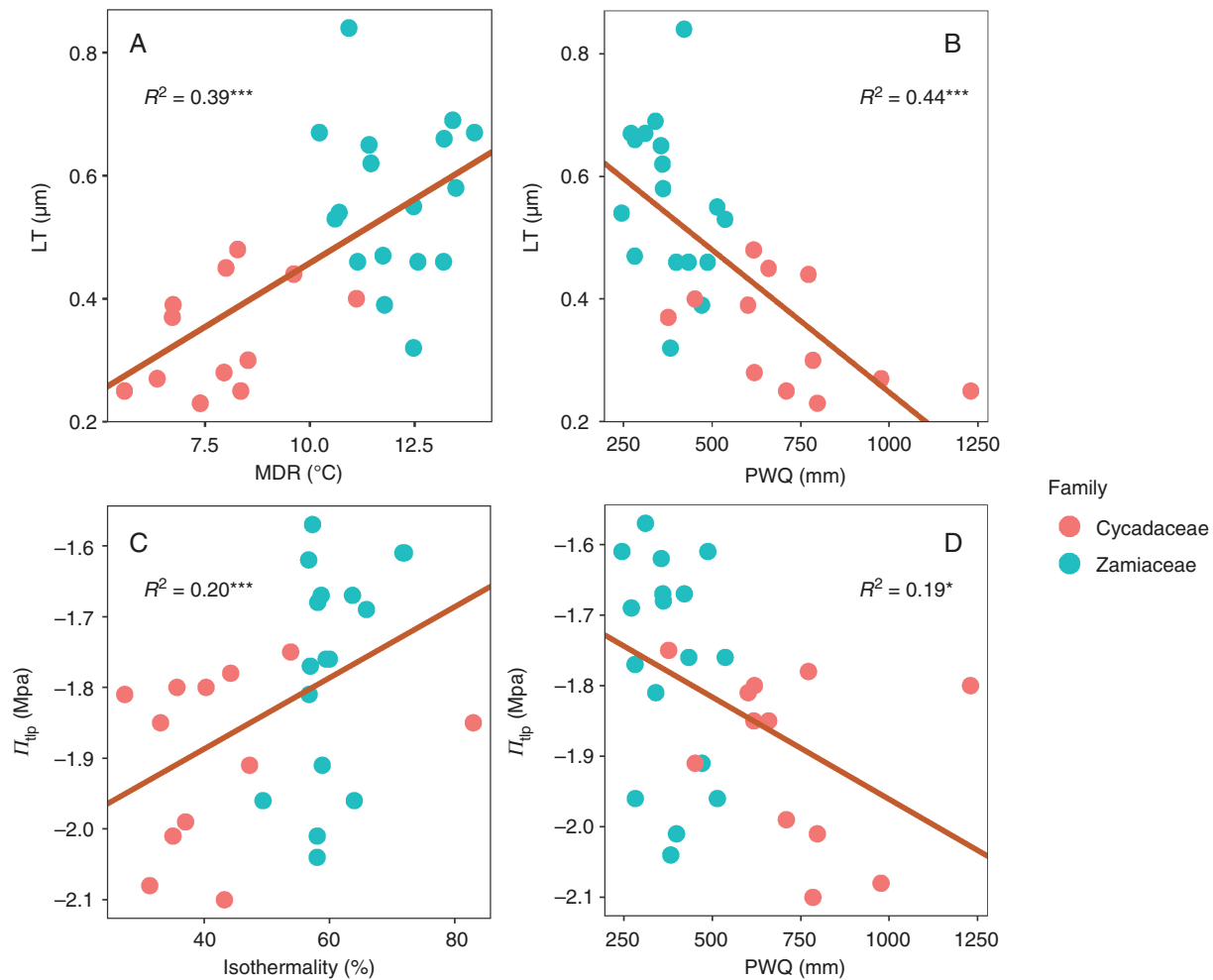


FIG. 3. Relationship between leaf traits and climatic variables across Cycadaceae (red) and Zamiaceae (blue). Abbreviations: LT, leaf thickness; π_{tip} , leaf water potential at the turgor loss point; MDR, mean diurnal range of temperature; PWQ, precipitation of the warmest quarter; Isothermality (mean diurnal range/temperature annual range) \times 100.

functional differentiation between the two families was influenced by both phylogenetic conservatism and environmental adaptation (Figs 2 and 3). It is thought that the Zamiaceae and Cycadaceae diverged in the Mesozoic (approx. 171.5 million years ago; Magallón *et al.*, 2013) or even earlier (Salas-Leiva *et al.*, 2013; Condamine *et al.*, 2015). The long history of divergence has caused the two families to possess different leaf traits. However, Nagalingum *et al.* (2011) revealed that the extant cycads mostly diversified relatively recently and over a short time (approximately <12 million years). This indicates that the leaf traits of cycads might have been shaped under the Cenozoic climate change, i.e. from the warm humid to cool and dry transition, after the middle Miocene (Westerhold *et al.*, 2020). Indeed, our results showed that the leaf traits of cycads were correlated with the climatic conditions (e.g. MDR, PWQ and isothermality) of their original habitats (Fig. 3). Unlike the Zamiaceae species, the Cycadaceae species tended to be distributed in relatively wet or more isothermal habitats (Fig. 2; Supplementary data Table S1). Therefore, contrasting combinations of leaf functional traits indicated the eco-physiological adaptation of two cycad families to their different native

environments. Similarly, Pittermann *et al.* (2012) also reported that Cenozoic climate change shaped the modern ecophysiology and biogeography of the Cupressaceae conifers.

Unexpectedly, we found that π_{tip} was less negative in low-precipitation habitats in cycads (Fig. 3), which was in contrast to angiosperms (Bartlett *et al.*, 2012a; Zhu *et al.*, 2018). In particular, the Zamiaceae species evolved thicker leaves to avoid water loss under drought conditions rather than lower π_{tip} . In addition, grown in the common garden the Cycadaceae species had significantly lower leaf phosphorus contents than Zamiaceae species (Fig. 2), indicating an adaptation to the soil P deficiency in their native habitats in southern China and Indo-China peninsula regions (Du *et al.*, 2020).

This study reported the F_p values of 41 cycad species, with the mean value (0.65 kN m^{-1} , Supplementary data Table S2) being approx. 2-fold higher than that of angiosperm species (0.32 kN m^{-1} , Onoda *et al.*, 2011). Higher leaf mechanical resistance was associated with higher LMA and higher construction and maintenance costs (Supplementary data Table S7), providing great mechanical strength against herbivores and physical injury for cycad species (Prado *et al.*, 2014). However, at a given LMA

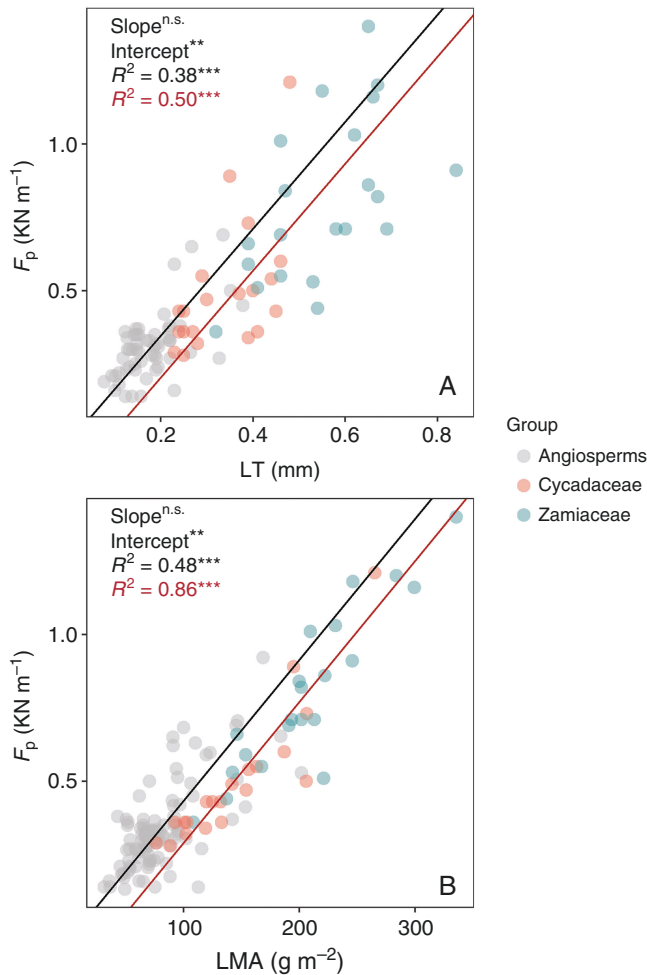


FIG. 4. Relationship between leaf force to punch (F_p) and (A) leaf mass per area (LMA), and (B) leaf thickness (LT) across angiosperms (black) and cycads (Cycadaceae, red; Zamiaceae, blue). The linear regression lines of the standardized major axis are fitted for angiosperms (black) and cycads (red), respectively. Data sources: cycads, 41 species of the present study; angiosperms, 57 sub-tropical woody species (He *et al.*, 2019) and 43 tropical-sub-tropical woody species (S.-D. Zhu *et al.*, unpubl. data). Differences in slopes and intercepts of the regression line between cycads and angiosperms are tested by analysis of covariance. n.s., not significant; ** $P < 0.01$; *** $P < 0.001$.

or LT, angiosperm tree species showed higher F_p than cycad species (Fig. 4). This may be partly because cycad species have lower vein density than angiosperms (Zhang *et al.*, 2015), which is a key structural property contributing to leaf biomechanical resistance (Hua *et al.*, 2020). In addition, this might also be because many cycad species, particularly in Zamiaceae, have succulent leaves (Ogburn and Edwards, 2010). Thicker leaves of cycads were associated with a greater cross-sectional area of accessory transfusion tissues within the mesophyll (Supplementary data Fig. S5). This conspicuous transfusion tissues function as water storage and translocation (Hu and Yao, 1981; Zhang *et al.*, 2014), and are thus not structurally strong. In contrast to a study on sub-tropical forest woody plants (He *et al.*, 2019), our results showed that F_p was negatively correlated with PNUE and PPUE and positively correlated with CC/A in cycads (Supplementary data Table S6), thus confirming a trade-off between leaf mechanics and economics.

We found that the π_{ip} of cycads was generally more negative than that of trees of humid sub-tropical forest (e.g. Zhu *et al.*, 2018) but less negative than that of conifer species (e.g. Bartlett *et al.*, 2012a). Our results indicated that in sub-tropical woody plant species, greater leaf drought tolerance was associated with higher mechanical resistance and carbon investment (Fig. 5). An explanation is that π_{ip} is mainly determined by the osmotic potential at full turgor (solute concentration in cells; Bartlett *et al.*, 2012a), and higher osmotic concentrations require greater cell wall reinforcement (Bartlett *et al.*, 2012b). This covariation between drought tolerance and mechanical traits has also been reported from the semi-arid grasslands (Blumenthal *et al.*, 2020) and sub-tropical evergreen broadleaved forest (Wang *et al.*, 2021), thus facilitating the prediction of plant responses to increasing drought- and insect-related stress under climate change. However, opposite patterns of π_{ip} - F_p and π_{ip} -CC_a correlations were observed in cycad species. The high F_p and CC_a of cycad species could be primarily due to their thick leaves (Supplementary data Table S7), particularly the thickening of cuticular and epidermal cells (Onoda *et al.*, 2012; Griffith *et al.*, 2014). Meanwhile, the larger area of transfusion tissues in tougher (thicker) leaves of cycads might be a ‘dilution’ effect on solute concentration, thus resulting in less negative π_{ip} . Similar to this, Ogburn and Edwards (2012) found that across 25 succulent species of Caryophyllales, higher LT was significantly associated with greater cross-sectional area of water storage cells, higher saturated leaf water content and less negative π_{ip} . These results thus provide an explanation for the less negative π_{ip} in Zamiaceae species from low-precipitation regions (discussed above), because leaf succulence (e.g. high water storage in thick leaves) is also a mechanism to deal with drought.

Cycad species under high or low threat could not be distinguished based on their functional traits (Supplementary data Fig. S6). According to the IUCN criteria, threatened categories are mainly assessed by geographic distribution and population size (Marler and Marler, 2015), which might not be fully reflected in the structural and physiological characteristics of their leaves. A recent study suggested that a combination of trait-based and traditional approaches (i.e. population dynamics, habitat suitability and genetic structure) could be a promising way to estimate extinction risks and promote conservation strategies in cycads (Alvarez-Yepiz *et al.*, 2019). It should be noted that all the cycad species investigated in this study were grown in the same cycad garden with favourable resource availability, which may prevent the detection of phenotypic variation in their native environmental gradients. In addition, Marler and Krishnapillai (2018) found that leaf nutrient concentrations decreased linearly with an increased stem height of an arborescent cycad species, indicating that plant size may influence the functional traits of cycads. Moreover, Krieg *et al.* (2017) found several patterns of physiological and morphological differences between female and male individuals of the same cycad species. These factors should be considered in future studies on the functional analysis of cycad species.

Conclusion

We suggest that the Cycadaceae and Zamiaceae exhibit contrasting leaf functional traits with different evolutionary

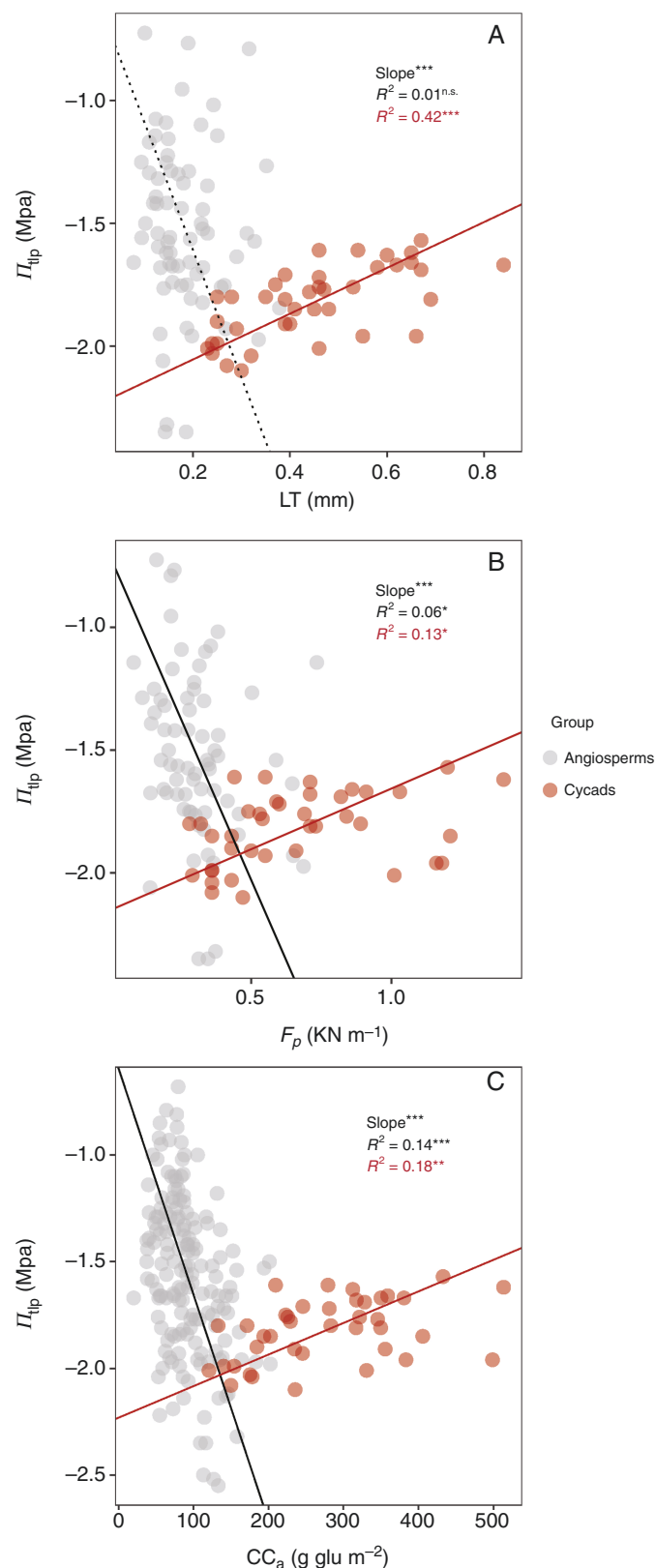


FIG. 5. Relationship between leaf water potential turgor loss point (π_{tp}) and (A) leaf thickness (LT) and (B) leaf force to punch (F_p), and (C) leaf construction cost on an area basis (CC_a) across the cycads (red) and angiosperms (grey). Data sources: cycads, 41 species of the present study; angiosperms, 70 woody

histories and environmental conditions. Compared with Zamiaceae species, most Cycadaceae species are distributed in humid and warm habitats, and have high photosynthetic capacity and low structural investments. The analyses of trait correlations support similar leaf economics design principles and economics–mechanics correlations between cycads and angiosperms (Supplementary data Fig. S7; Table S7). However, our results reveal that the correlations between leaf drought tolerance, construction cost and mechanical resistance in cycads are opposite to those in angiosperms. Substantial structural investments in the leaves of cycad species result in greater mechanical resistance but lower physiological drought tolerance. This study on cycad species will help expand our understanding of the ecological strategies of cycads and their eco-physiological adaptations to environmental changes.

SUPPLEMENTARY DATA

Supplementary data are available online at <https://academic.oup.com/aob/article/130/3/345/6454392> and consist of the following. Figure S1: photos of the cycad garden and several studied cycad species. Figure S2: relationship between predicted and measured π_{tp} across cycads and angiosperms. Figure S3: principal component analysis for mass-based leaf traits. Figure S4: trait comparison between Cycadaceae and Zamiaceae. Figure S5: relationship between leaf thickness and cross-sectional accessory transfusion tissues. Figure S6: principal component analysis for the 41 cycad species differing in threatened status. Figure S7: relationships between leaf economics traits in cycads and angiosperms. Table S1: lists of the 41 cycad species. Table S2: mean π_{tp} determined from the pressure–volume method and osmometer method for five cycad species. Table S3: the phylogenetic signal of leaf functional traits. Table S4: mean values of leaf functional traits. Table S5: factor loading, eigenvalues, the percentage of variance explained and independent t -tests of the first two principal components. Table S6: the linear model for π_{tp} and LT using single or multiple predictors. Table S7: Pearson's correlations between each pair of traits.

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species in (A and B) and 163 woody species in (C) are compiled from published papers (Zhu *et al.*, 2018; He *et al.*, 2019; Wang *et al.*, 2021) and our own data (S.-D. Zhu *et al.*, unpubl. data).

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