

Coordination of intertracheid pit traits and climate effects among cycads

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Abstract

Interconduit pit membranes, which are permeable regions in the primary cell wall that connect to adjacent conduits, play a crucial role in water relations and the movement of nutrients between xylem conduits. However, how pit membrane characteristics might influence water-carbon coupling remains poorly investigated in cycads. We examined pit characteristics, the anatomical and photosynthetic traits of 13 cycads from a common garden, to determine if pit traits and their coordination are related to water relations and carbon economy. We found that the pit traits of cycads were highly variable and that cycads exhibited a similar tradeoff between pit density and pit area as other plant lineages. Unlike other plant lineages (1) pit membranes, pit apertures, and pit shapes of cycads were not coordinated as in angiosperms; (2) cycads exhibited larger pit membrane areas but lower pit densities relative to ferns and angiosperms, but smaller and similar pit membrane densities to non-cycad gymnosperms; (3) cycad pit membrane areas and densities were partially coordinated with anatomical traits, with hydraulic supply of the rachis positively coordinated with photosynthesis, whereas pit aperture areas and fractions were negatively coordinated with photosynthetic traits; (4) cycad pit traits reflected adaptation to wetter habitats for Cycadaceae and drier habitats for Zamiaceae. The large variation in pit traits, the unique pit membrane size and density, and the partial coordination of pit traits with anatomical and physiological traits of the rachis and pinna among cycads may have facilitated their dominance in a variety of ecosystems from the Mesozoic to modern times.

1 | INTRODUCTION

The origin of the cycads can be dated to the late Paleozoic, and they later dominated and prospered with dinosaurs in the Mesozoic

(Mamay, 1969, Norstog & Nicholls, 1998). Cycads are considered living fossils because extant cycads are morphologically similar to their fossil ancestors (Brenner, 2003, Mamay, 1969), although all modern cycads seemed to have recently and rapidly diversified during the

Neogene, 12 million years ago (Nagalingum et al., 2011). Cycads are now distributed in tropical and subtropical regions across a range of habitats, from very moist tropical rainforests to drylands and from lowlands to high elevations (Hill et al., 2004, Norstog & Nicholls, 1998, Zhang et al., 2015). Ten genera and 367 species are currently accepted (Calonje et al., 2022), with nearly 70% of these species listed on the IUCN red list (IUCN, 2022). Given the current threats of climate change and human disturbance (Álvarez-Yépez et al., 2019), there exists increasing urgency for the conservation of this relict plant lineage.

Two families of cycads are currently accepted—Cycadaceae and Zamiaceae (Chang et al., 2020, Christenhusz et al., 2011, Jiang et al., 2016). Cycadaceae have diversified earlier than Zamiaceae and are distributed in colder and higher precipitation habitats than Zamiaceae (Meng et al., 2021). The breadth of climates occupied by each family and the differences in climate between families suggest that there may be differences in functional anatomy that influence cycad leaf water balance. Like other gymnosperms, cycads possess xylem tracheids for water transport, although vessels have also been reported in some species (Huang et al., 2010, Huang & Zhang, 1999, Jacobsen, 2021, Xu et al., 2005). The xylem organization in the rachis of cycads is typically shaped like an “omega,” resulting in the distal leaflets being irrigated by abaxial bundles, thus guaranteeing uniform water supply along the entire leaf length (Tomlinson et al., 2018). Cycad leaflets are highly diverse, with Cycadaceae having a single primary vein (midvein) and Zamiaceae exhibiting multiple primary veins (Stevenson et al., 1996). Furthermore, Zamiaceae leaflets have been shown to be thicker and tougher than Cycadaceae leaflets (Meng et al., 2021). Although hydraulic coordination between stem and leaf has been well studied in angiosperms (Brodribb & Feild, 2000, Hao et al., 2011, Jiang et al., 2017, Pivovarov et al., 2014), it remains largely unknown in cycads. Some leaf functional traits of cycads generally align with the global leaf economic spectrum, though in contrast to other vascular plants, leaf hydraulic conductance has been shown to be unrelated to the photosynthetic rate in cycads (Zhang et al., 2015). Additionally, in contrast to other major groups of vascular plants, the stomatal density of some cycad leaves does not change with elevated atmospheric CO₂, with the exception of the stomatal number in *Zamia furfuracea*, which increased with higher atmospheric CO₂, further suggesting that cycad leaves are diverse and may have species-dependent physiological responses to changing climate (Haworth et al., 2011, Steinhorsdottir et al., 2021). These differences between cycads and other extant vascular plants suggest that xylem structure–function relationships in cycads may also differ from those observed in other vascular plants.

Plant species with low water transport capacity may also exhibit reduced gas exchange through their stomata because water loss from the leaves is likely also limited by stem hydraulic supply and hydraulic architecture (Brodribb & Feild, 2000, Santiago et al., 2004). Thus, the structure and water transport properties of the vascular system affect the balance between water supply and total transpiring leaf area (Cardoso et al., 2020, Jiang et al., 2022, Sorek et al., 2021). Previous studies have suggested that a larger interconduit pit aperture

diameter may confer higher rates of mass-based photosynthesis in conifers (Song, Sterck, et al., 2022). In contrast, pit membrane diameter was reported to be negatively correlated with mass-based photosynthetic rate among congeneric angiosperms (Liet et al., 2019). These two contrasting results suggest that the effect of interconduit pit trait variation on transpiration and photosynthesis may differ among plant lineages (Li et al., 2019, Song, Sterck, et al., 2022).

Among plant lineages, pit membranes fall into one of two categories: the torus-margo type found in most gymnosperms and the homogenous pit membranes characteristic of angiosperms (Choat et al., 2008, Pittermann et al., 2005), ferns (Carlquist & Schneider, 2007), and several cycad species (Jacobsen, 2021). More permeable pit membranes are found in basal tracheophytes, as well as in vesselless angiosperms and conifers, possibly to partially compensate for the higher hydraulic resistances imposed by a tracheid-based vascular system (Brodersen et al., 2014, Hacke et al., 2007, Pittermann et al., 2005). This suggests that there could be a tradeoff among pit membrane, tracheid, and vessel characteristics across different plant lineages. Because pit membranes have the potential to act as the nexus of both cavitation safety and hydraulic transport efficiency, pit anatomical traits may influence both safety and efficiency. Pit traits include the thickness and size of pit membranes, the size and arrangement of pit membrane pores (Lens et al., 2011), and the number of interconduit pits per vessel (Hargrave et al., 1994). For example, lower pit density, larger pit membrane area, larger pit aperture area, and higher aperture fraction (aperture area per pit membrane area) may be associated with higher sapwood hydraulic conductivity and decreased embolism resistance (Brodersen et al., 2014, Jacobsen et al., 2016, Lens et al., 2011, Pittermann et al., 2010). Additionally, pit aperture shape is also correlated with embolism resistance, with more cavitation-resistant species exhibiting narrower and more elliptical pit apertures (Lens et al., 2011, Scholz et al., 2013, Zhang et al., 2021).

From the hydraulic efficiency perspective, larger diameter conduits are more vulnerable to embolism because of their intertracheid pits. Larger conduits may have more pit membranes that allow for higher hydraulic efficiency. This higher total pit membrane area would increase the chance of possessing a large membrane pore through which air may seed to an adjacent vessel. This idea is known as the rare-pit hypothesis (Hargrave et al., 1994, Lens et al., 2011, Wheeler et al., 2005). Consequently, angiosperms adapted to drier habitats might be expected to exhibit smaller vessel diameters, thicker, denser, and more elliptical pit apertures, and smaller pit membranes than plants inhabiting wetter environments (Hacke et al., 2007, Jansen et al., 2009, Lens et al., 2011, Scholz et al., 2013, Wheeler et al., 2005). However, results demonstrating vessel diameter is the mechanistic link to hydraulic safety are lacking, suggesting a more nuanced role of interconduit pit anatomy in influencing both hydraulic safety and efficiency (Lens et al., 2022). However, the connection between interconduit pit anatomy among cycads and their physiological and habitat affinities remains unclear. Although several cycad species have been shown to possess homogenous pit membranes similar to angiosperms (Jacobsen, 2021), these studies have been limited to just a few species without analyzing the possible connections

TABLE 1 Definition of all traits for the study.

Symbol	Definition	Unit
Pit traits of rachis from scanning electron microscopy		
D_{pms}	Diameter of the outer pit membrane along the shorter axis	μm
D_{pml}	Diameter of the outer pit membrane along the longer axis	μm
D_{pas}	Diameter of the outer pit aperture along the shorter axis	μm
D_{pal}	Diameter of the outer pit aperture along the longer axis	μm
A_{pit}	Intertracheid pit surface area or Intertracheid pit membrane surface area	μm^2
A_{pa}	Intertracheid pit aperture surface area	μm^2
F_{pf}	Pit membrane fraction = proportion of pit membrane area per tracheid wall area	%
F_{pa}	Pit aperture fraction = pit aperture surface area/pit membrane surface area	%
R_{pit}	Pit shape = ratio of the longest axis of outer pit membrane to the shortest axis	—
R_{pa}	Pit aperture shape = ratio of the longest axis of outer pit aperture to the shortest axis	—
D_p	Pit density = number of intertracheid pits per tracheid wall area	No. μm^2
Anatomical traits of rachis from light microscopy		
A_l	Tracheid lumen area = total tracheid lumen area of a cross section for rachis	mm^2
A_w	Tracheid wall area = total wall area of all tracheids of a cross section for rachis	mm^2
A_{phl}	Tracheid phloem area = total phloem area of a cross section for rachis	mm^2
A_x	Tracheid xylem area = total xylem area of a cross section for rachis	mm^2
D	Tracheid diameter	μm
D_h	Hydraulically-weighted tracheid diameter	μm
F_x	Fraction of xylem = proportion of xylem area to the cross-section area for rachis	%
F_{phl}	Fraction of phloem = proportion of phloem area to the cross-section area for rachis	%
N	Number of tracheid = total tracheid number of a cross section for rachis	—
R_x	Ratio of lumen area to xylem area of rachis = A_l/A_x	—
T_d	Tracheid density = total tracheid number of a cross section for rachis/area of a cross section for rachis	No. mm^2
T_w	Double tracheid wall thickness	μm
Anatomical traits leaf from light microscopy		
D_s	Stomatal density	No. cm^2
D_v	Vein density	mm mm^2
S_s	Stomatal size	μm^2
Physiological functional traits		
A_a	Light-saturated photosynthetic rate per area	$\mu\text{mol m}^2 \text{ s}^{-1}$
A_m	Light-saturated photosynthetic rate per mass	$\text{nmol g}^{-1} \text{ s}^{-1}$
C_i	Intercellular CO_2 concentration	$\mu\text{mol mol}^{-1}$
G_s	Stomatal conductance	$\text{mol m}^{-2} \text{ s}^{-1}$
K_{th}	Theoretical hydraulic conductivity	$\text{kg m}^{-1} \text{ MPa}^{-1} \text{ s}^{-1}$

between anatomical and physiological traits (Jacobsen, 2021). Comparative studies have revealed large intraspecific and interspecific variations in pit membrane and pit aperture size, shape, density, and the ratio of total pit aperture area to vessel wall area, likely reflecting differences in environmental adaptation and community filtering processes (Ellmore et al., 2006, Lens et al., 2011, Schmitz et al., 2007, Zhang et al., 2017). For example, polyploid *Betula* species have more but smaller pits, possibly giving them a greater ability to withstand harsh climate conditions relative to their diploid counterparts (Zhang et al., 2017). Given the broad ecological habitats of cycads, as well as their unique evolutionary history (Meng et al., 2021, Zhang

et al., 2015), they make an ideal plant group to study pit trait variation.

In this study, we examined intertracheid pit traits of eight Cycada-ceae and five Zamiaceae species in individuals growing in a common garden. Scanning electron microscopy (SEM) was used to quantify pit traits, and light microscopy (LM) was used to quantify rachis and leaf traits. We tested for bivariate correlations among these anatomical and functional traits (e.g., photosynthetic traits, theoretical hydraulic conductivity) of the rachis and leaf (Table 1). By combining LM and SEM anatomical traits and photosynthetic measurements, we addressed the following questions: (1) How do pit traits vary among

TABLE 2 Pit traits in 13 cycad species. Abbreviations are as defined in Table 1.

Family	Habitat	Species	D_{pms} (μm)	D_{pmi} (μm)	A_{pit} (μm^2)	D_{pas} (μm)	D_{pas} (μm)	A_{pa} (μm^2)	F_{pa} (%)	R_{pa}	R_{pit}	F_{pf} (%)	D_p (no. μm^2)
Cycadeaceae	Wet	<i>Cycas debaoensis</i>	7.62 ± 0.33	9.20 ± 0.93	55.67 ± 6.50	1.58 ± 0.28	5.89 ± 0.62	5.64 ± 0.97	10.39 ± 0.44	4.66 ± 1.07	1.23 ± 0.11	55.93 ± 6.90	0.011 ± 0.0027
	Dry	<i>Cycas elongata</i>	7.91 ± 0.45	7.04 ± 0.38	44.95 ± 4.50	2.10 ± 0.12	4.18 ± 0.01	4.09 ± 0.26	9.14 ± 0.92	2.26 ± 0.06	0.91 ± 0.03	55.80 ± 3.20	0.016 ± 0.0012
	Dry	<i>Cycas ferruginea</i>	6.92 ± 0.44	7.79 ± 0.07	42.33 ± 2.90	1.37 ± 0.22	5.05 ± 1.15	5.84 ± 1.99	13.96 ± 4.82	3.75 ± 0.45	1.13 ± 0.07	58.09 ± 5.20	0.012 ± 0.0009
	Wet	<i>Cycas guizhouensis</i>	7.70 ± 0.56	8.22 ± 0.71	51.46 ± 7.46	1.12 ± 0.17	4.85 ± 0.52	4.38 ± 0.94	7.98 ± 1.66	4.65 ± 0.56	1.06 ± 0.03	56.65 ± 4.24	0.013 ± 0.0025
	Wet	<i>Cycas hainanensis</i>	9.39 ± 0.29	9.39 ± 0.80	70.27 ± 7.98	2.16 ± 0.08	5.92 ± 0.20	9.96 ± 0.12	14.88 ± 1.47	2.89 ± 0.16	1.02 ± 0.08	54.74 ± 5.24	0.008 ± 0.0016
	Wet	<i>Cycas parvula</i>	7.45 ± 0.42	8.65 ± 0.52	53.08 ± 6.53	2.40 ± 0.12	6.71 ± 0.23	12.92 ± 1.08	20.39 ± 2.24	2.83 ± 0.15	1.18 ± 0.01	51.60 ± 4.93	0.010 ± 0.0013
	Wet	<i>Cycas revoluta</i>	7.70 ± 0.63	8.64 ± 0.61	53.55 ± 8.32	1.72 ± 0.16	4.49 ± 0.14	6.12 ± 0.50	11.74 ± 1.26	2.76 ± 0.33	1.14 ± 0.05	46.61 ± 8.54	0.009 ± 0.0015
	Wet	<i>Cycas segmentifida</i>	7.77 ± 0.35	8.27 ± 0.32	52.90 ± 3.36	1.46 ± 0.04	4.93 ± 0.81	5.74 ± 0.89	13.48 ± 0.74	3.56 ± 0.71	1.07 ± 0.03	51.96 ± 5.95	0.010 ± 0.0007
	Wet	<i>Ceratozamia latifolia</i>	7.04 ± 0.29	8.50 ± 0.06	47.41 ± 1.72	1.60 ± 0.13	5.30 ± 0.05	6.20 ± 0.71	14.35 ± 1.53	3.49 ± 0.34	1.10 ± 0.06	54.84 ± 1.55	0.012 ± 0.0004
	Dry	<i>Encephalartos laurentianus</i>	6.52 ± 0.57	8.50 ± 0.86	44.49 ± 7.90	1.20 ± 0.14	6.59 ± 0.77	6.38 ± 1.44	14.12 ± 0.87	5.65 ± 0.38	1.16 ± 0.10	64.68 ± 1.32	0.015 ± 0.0027
Zamiaceae	Dry	<i>Encephalartos tegulaneus</i>	7.95 ± 0.42	8.61 ± 0.22	54.23 ± 3.11	1.50 ± 0.11	6.11 ± 0.40	7.20 ± 0.60	13.43 ± 0.62	4.20 ± 0.44	1.15 ± 0.04	62.47 ± 2.75	0.012 ± 0.0012
	Dry	<i>Macrozamia moorei</i>	6.31 ± 0.53	8.00 ± 0.43	40.50 ± 5.38	1.49 ± 0.10	5.37 ± 0.17	6.48 ± 0.64	17.21 ± 1.69	3.68 ± 0.16	1.29 ± 0.06	47.28 ± 2.65	0.012 ± 0.0017
	Dry	<i>Zamia furfuracea</i>	5.42 ± 0.41	6.67 ± 0.79	29.13 ± 3.56	1.67 ± 0.08	6.30 ± 0.52	8.79 ± 0.90	18.63 ± 3.58	3.83 ± 0.43	1.35 ± 0.19	33.39 ± 3.01	0.014 ± 0.0027

cycads and differ from other plant lineages (angiosperms, ferns, and noncycad gymnosperms)? (2) Are pit traits correlated with other anatomical and physiological traits across cycad species, such as theoretical hydraulic conductivity of the rachis and photosynthetic capacity of the pinna? (3) Are cycad pit traits linked to variation in habitat? We hypothesized that pit traits would exhibit high variation based on the observation that cycads are highly diverse in their overall morphology, habitats (Whitelock, 2003), and leaf physiology and structure (Coiro et al., 2020, Coiro et al., 2021, Glos et al., 2022, Zhang et al., 2015, Zhang et al., 2017). We also hypothesized that pit traits and the coordination of anatomical and physiological traits of the rachis and leaf would be different for cycads compared with other plant lineages, and that the strength of this coordination would depend on the relationships between leaf hydraulics and photosynthesis (Zhang et al., 2015).

2 | MATERIALS AND METHODS

2.1 | Study site and plant material

The studied cycad species (Table 2) included eight Cycadaceae species (*Cycas segmentifida*, *Cycas guizhouensis*, *Cycas hainanensis*, *Cycas revoluta*, *Cycas ferruginea*, *Cycas parvula*, *Cycas debaoensis*, and *Cycas elongata*) and five Zamiaceae species (*Z. furfuracea*, *Macrozamia moorei*, *Ceratozamia latifolia*, *Encephalartos laurentianus*, and *Encephalartos tegulaneus*), representing 5 of the 10 genera of cycads. All plants were sampled at the Nanning Botanical Garden, southern China (for more information, please see Meng et al., 2021). The native habitats of the species range from wet to dry zones (Table S1). Three to five randomly selected individuals per species were selected for sampling. On each plant, a sun-exposed rachis with pinna was cut and put in a sealed black plastic bag with wet tissues in the evening or early dawn, then transported back to the laboratory within half an hour.

2.2 | Estimation of pit characteristics by SEM

The collected rachises were cut into 1–3 cm long pieces and placed in 100 mL of 5% FAA fixative (90:5:5 ratio of 70% ethanol, acetic acid, formaldehyde) at room temperature (25 °C) to prevent expansion or shrinkage. A 1-cm segment of the rachis was cut and longitudinal sections of the segments were made with a sliding microtome (RM225, Leica Inc.) at a thickness of 2–3 mm. Then the sections were fixed to aluminum sample holders with carbon double-sided tape (NISSHIN EM Co., Ltd.), air-dried for 12 h at room temperature, and coated with gold using a sputter coater (Cressington 108Auto) for 40 s at 0.08 mA to get a 20-nm thick gold layer, under an argon atmosphere. A conventional SEM (FEI Quattro S) with a voltage of 2 kV was used to visualize intertracheid pit parameters according to standard protocols (Jansen et al., 2009, Lens et al., 2011, Zhang et al., 2021).

ImageJ (Rueden et al., 2017) was used to determine the following pit characteristics: intertracheid pit aperture surface area (A_{pa}),

intertracheid pit surface area or intertracheid pit membrane surface area (A_{pit}), pit aperture fraction (F_{pa}), pit membrane fraction (F_{pm}), pit aperture longest diameter (D_{pal}), pit aperture shortest diameter (D_{pas}), pit aperture shape (R_{pa}), pit membrane longest diameter (D_{pml}), pit membrane shortest diameter (D_{pms}), pit shape or pit membrane shape (R_{pit}), and pit density (D_p) (Table 1). Mean values for intertracheid pit aperture surface area (A_a), area or intertracheid pit membrane surface area (A_{pit}), pit aperture longest diameter (D_{pal}), pit aperture shortest diameter (D_{pas}), pit aperture shape (R_{pa}), pit membrane longest diameter (D_{pml}), pit membrane shortest diameter (D_{pms}), pit shape or pit membrane shape (R_{pit}) were based on at least 50 measurements from SEM images of various intertracheid walls per individuals.

2.3 | LM of anatomical traits

All measurements were made on a fully expanded, healthy, sun-exposed rachis with pinna for each individual. Cross-sections of the rachis were made using a sliding microtome (RM225, Leica Inc.) at a thickness of 40–60 μm . Sections were bleached for 10 min, then rinsed in water, and then stained with Safranin O (0.5% wt/vol in water) for 5 min and with Alcian Blue (1% wt/vol in 3% acetic acid) for 20 s to 1 min, and then mounted on glass slides.

Pinna samples, avoiding the margin and midrib, were cut into sections of about 120 mm^2 and incubated at 70 $^\circ\text{C}$ in a 1:1 solution of H_2O_2 (30%) and CH_3COOH (100%) until all pigments had been removed. The sections were removed from this solution and placed in a petri dish with clear water for 3 min, then the epidermises were separated with forceps from the mesophyll and veins, allowing these three layers (upper epidermis, lower epidermis, and mesophyll with veins) to be stained and mounted separately. To increase contrast, all samples were stained with Safranin O (0.5% wt/vol in water) for 5 min and Alcian Blue (1% wt/vol in 3% acetic acid) for 10 s to 20 min, then washed in water and mounted on microscope slides. In the following analysis, we used only the abaxial (bottom surface) to estimate stomatal density.

Images were taken at 5, 10, and 20, with fields of view of 3.99 mm^2 , 0.89 mm^2 and 0.22 mm^2 , respectively, using a compound microscope outfitted with a digital camera (DM3000, Leica Inc.). For the rachis cross-sections, 50–80 images were taken for each rachis sample and stitched together using Leica Application Suite (v.4.11; Switzerland). The following traits from the stitched images were measured with ImageJ (Rueden et al., 2017): phloem area (A_{ph}), tracheid lumen area (A), tracheid wall area (A_w), xylem tracheid area (A), number of tracheids (N), tracheid density (T_d), long/short diameter of tracheids, and double tracheid wall thickness (\bar{w}).

From images of paradermal sections of pinna, vein density (D_v) was measured as the total length of leaf vascular tissue per mm^2 of leaf area, stomatal density (D_s) was measured by counting the number of stomata in the image and dividing by the area of the field of view, stomatal area (S) was manually measured as the 2D-projected surface area of each of at least five stomata per image. Partial stomata and epidermal cells were included in the density counts if they were

bisected by the top and left borders of the image and ignored if they were bisected by the bottom and right borders of the image.

Mean hydraulically weighted tracheid diameter (D_h), was calculated as Tyree and Zimmermann (2013):

$$D_h = \frac{\sum P_i D_i^{0.25}}{N}$$

where D is the diameter of a circle with the equivalent area of tracheids whose area was calculated from perpendicular long and short diameters, and N is the number of tracheids measured.

According to the Hagen–Poiseuille law, theoretical hydraulic conductivity was calculated as:

$$K_{th} = \frac{\pi \rho_w T_d D_h^4}{128 \eta}$$

where T_d is tracheid density (no. mm^2), ρ_w is water density (998.2 kg m^{-3} at 20 $^\circ\text{C}$), η is water viscosity (1.002 $\times 10^{-9}$ MPa s^{-1} at 20 $^\circ\text{C}$).

2.4 | Photosynthesis measurements

During July–August 2020, we measured mature and healthy pinnae from three different individual plants of each species using a portable gas exchange system (LI-6800, LI-COR). Leaves were measured on sunny days between 08:30 and 11:30 h solar time, at a photosynthetic photon flux density of 1500 $\mu\text{mol m}^{-2} \text{s}^{-1}$, ambient temperature (30 $^\circ\text{C}$ –36 $^\circ\text{C}$), and CO_2 concentration of 400 ppm, allowing VPD to range from 1.2 to 1.7 kPa. For species with pinna area smaller than the cuvette (6 cm^2), we normalized the measurements by the leaf area in the chamber, which was measured afterwards using a LI-3000A leaf area meter (Li-Cor). Pinnae were then oven dried at 70 $^\circ\text{C}$ to constant mass and weighed. Photosynthetic traits, including light-saturated photosynthetic rate per leaf area (A_a), light-saturated photosynthetic rate per mass (A_m), stomatal conductance (G_s), and intercellular CO_2 concentration (C_i) were obtained.

2.5 | Statistical analysis

All statistical analyses were performed using R (version 4.1.3, R Development Core Team). We used linear regression and standard major axis regression (R package “smatr”) to determine the relationships between traits (Warton et al., 2012), and the statistical tests were considered significant at $p < 0.05$. Principal component analysis (PCA) was carried out with the “vegan” package. Mean values were z-scaled and centered prior to calculating principal components, and we removed strongly correlated traits with similar functional implications to prevent having more traits than the number of taxa. Differences among cycads and other plant lineages were analyzed using one-way

ANOVA, followed by Tukey post hoc tests. Paired t-tests were used to determine differences in PC scores between Cycadaceae and Zamiaceae, and between wet and dry habitat species. Published data for intertracheid pit membrane surface area (A_{pit}) and pit density (D_p) were obtained from the literature listed in Table S2.

To account for the statistical non-independence of relations between the species sampled, we incorporated phylogenetic covariance into our regression analyses. We used the phylogenetic tree from (Nagalingum et al. 2011) by keeping the 13 species in this study. We calculated phylogenetically corrected generalized least squares regressions (Symonds & Blomberg, 2014) for pairwise trait combinations using the R packages “ape” (Paradis & Schliep, 2018) and “caper” (Orme et al., 2012), “geiger” for analysis of evolutionary diversification with test statistics reported in the Supplementary Data S1.

3 | RESULTS

3.1 | Variation of pit traits among cycad species

SEM images were used to examine intertracheid pits in Cycadaceae (Figure 1A–C) and Zamiaceae (Figure 1D–F). Pit membranes were typically homogeneous within species. Pit apertures were generally oval to elliptical in shape and sealed with a pit membrane (Figure 1). We observed more than two columns of pits on the tracheid wall surfaces, and scalariform perforation plate-like structures were observed for some species (Figure 1).

There was wide variation in many intertracheid pit traits: D_{pms} (1.73-fold), D_{pml} (1.41-fold), A_{pit} (2.41-fold), D_{pas} (1.50-fold), D_{pal} (2.15-fold), A_{pa} (3.16-fold), F_{pf} (1.94-fold), F_{pa} (2.55-fold), R_{pit} (1.48-fold), R_{pa} (2.5-fold), and D_p (1.87-fold; Table 2), with only D_{pms} being significantly larger in Cycadaceae than in Zamiaceae (Table S3). D_{pas} varied about 2.2-fold (from $1.12 \pm 0.12 \mu\text{m}^2$ in *C. guizhouensis* to $2.4 \pm 0.17 \mu\text{m}^2$ in *C. parvula*), whereas A_{pa} varied 3.2-fold (from $4.09 \pm 0.26 \mu\text{m}^2$ in *C. elongata* to $12.92 \pm 1.08 \mu\text{m}^2$ in *C. parvula*). F_{pa} also varied about 2.6-fold (from $7.98\% \pm 1.66\%$ in *C. guizhouensis* to $20.39\% \pm 2.24\%$ in *C. parvula*), and R_{pa} varied about 2.1-fold (from 2.26 ± 0.06 in *C. elongata* to 4.66 ± 1.07 in *C. debaoensis*) among Cycadaceae species. In addition, D_{pas} varied about 1.39-fold (from $1.2 \pm 0.14 \mu\text{m}$ in *E. tegulaneus* to $1.67 \pm 0.08 \mu\text{m}$ in *Z. furfuracea*), A_{pa} varied about 1.42-fold (from $6.2 \pm 0.71 \mu\text{m}^2$ in *C. latifolia* to $8.79 \pm 0.9 \mu\text{m}^2$ in *Z. furfuracea*), F_{pa} varied about 1.39-fold (from $13.43\% \pm 0.62\%$ in *E. laurentianus* to $18.63\% \pm 3.58\%$ in *Z. furfuracea*), and R_{pa} varied about 1.6-fold (from 3.49 ± 0.34 in *C. latifolia* to 5.65 ± 0.38 in *E. tegulaneus*) among Zamiaceae species, indicating that intertracheid pit traits were more diverse in Cycadaceae.

3.2 | Relationships between interconduit pit traits

Pit density D_p was negatively related to D_{pms} , D_{pml} , A_{pit} (Figure 2A–C). No correlations were found between A_{pit} and A_{pa}

(Figure 2D), R_{pa} and R_{pit} (Figure 2E), or between D_p and pit aperture traits D_{pas} , D_{pal} , and A_{pa} (Figure S1). Interestingly, F_{pa} was positively correlated with R_{pit} (Figure 2F). Almost all of these correlations remained the same after accounting for shared evolutionary history, except for D_p and A_{pa} which became significantly negatively correlated (Table S6).

In order to compare pit packing density and pit area among the plant lineages (ferns, cycads from this study, non-cycad gymnosperms, and angiosperms) A_{pit} and D_p data were collected from the published references (Table S2). Results showed that A_{pit} was significantly negatively correlated with D_p among the four plant groups (Figure 3), with ferns, angiosperms, and cycads being closer to the theoretical packing limit ($1/A_{\text{pit}}$) than non-cycad gymnosperms (Figure 3). Pit area A_{pit} of cycads was significantly larger than angiosperms and ferns, but significantly smaller than non-cycad gymnosperms ($p < 0.05$, Table 3). In contrast, the pit density D_p of cycads was smaller than for angiosperms and ferns, but similar to non-cycad gymnosperms ($p < 0.05$; Table 3).

3.3 | Correlations among pit density, membrane area, and anatomical and physiological traits of the rachis and pinna

Pit density D_p was found to be positively correlated with tracheid lumen area A_l (Figure 4A) and phloem area A_{phl} (Figure 4B) of the rachis, as well as with vein density D (Figure 4E) in leaves, suggesting that larger rachis lumen area was associated with more numerous pits (Figure 4E). No relationship was found between D_p and anatomical trait of xylem area A_x of the rachis (Figure 4C), stomatal density D_s (Figure 4F), nor with physiological traits such as theoretical hydraulic conductivity K_{th} (Figure 4D), leaf area-based photosynthetic rate A_a , or stomatal conductance G (Figure 4G,H).

Pit shape was positively correlated with tracheid thickness (T_w ; Figure S2) and the fraction of xylem (F_x ; Figure S2). In contrast, pit shape was negatively correlated with the ratio of lumen area to xylem area (R_x ; Figure S2) in the rachis, indicating that more elliptically shaped pits were associated with thicker tracheid walls.

Pit membrane area (A_{pit} ; Figure 5A) was not correlated with K_{th} among cycads, though A_{pit} was negatively correlated with K_{th} only among the Zamiaceae species (Figure 5A). No other correlations were found between pit membrane traits and anatomical or physiological traits of the rachis among the 13 cycad species (Table S4). While A_{pit} was negatively correlated with D_l and D_s (Figure 5B,C), it was not correlated with other functional traits related to gas exchange, for example, G_s , A_a , and C_i (Table S4). It is worth noting that the correlation between A_{pit} and D_v was driven largely by differences in both traits between the two families (Figure 5B). Thus, while rachis pit membrane traits may be coordinated with some leaf anatomical traits, these pit traits may not be linked to leaf function. All of these correlations remained the same after accounting for shared evolutionary history, and only the significant negative relationships of R_{pit} and R_x became marginally significant ($p = 0.0525$; Table S6).

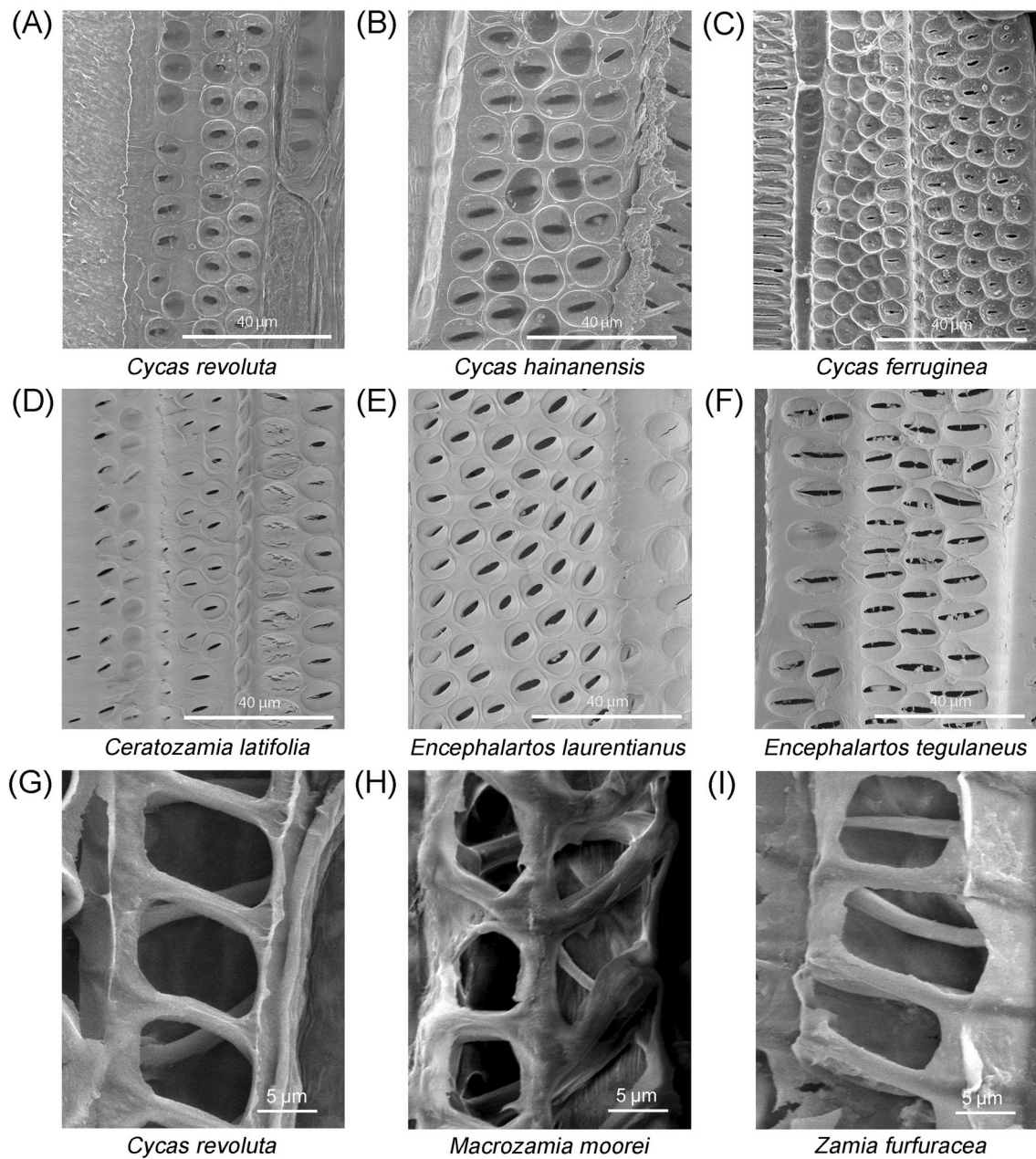


FIGURE 1 Scanning electron micrographs of intertracheid pits of species from Cycadaceae (A, *Cycas hainanensis*; B, *Cycas revoluta*; C, *Cycas ferruginea*) and Zamiaceae (D, *C. latifolia* Miq; E, *E. laurentianus*; F, *E. tegulaneus*). Scalariform perforation plate-like structures are shown in (G–I) for which species were reported with primitive vessels (Huang et al., 2010; Huang & Zhang, 1999; Jacobsen, 2021; Xu et al., 2005). No torus-margo structures were found in pits of the studied cycad species.

3.4 | Correlations among pit aperture and anatomical and physiological traits of the rachis and leaf

The functional trait K_{th} was positively correlated with the light-saturated photosynthetic rate per mass A_m (Figure 6A). Because tracheid diameter D_h is the major driver of K_{th} (Table S4), this pattern suggests that water supply from the rachis was positively coordinated with pinna photosynthesis. Like pit membrane traits, pit

aperture traits were found to be independent of anatomical and physiological traits of the rachis (Table S4). Only pit aperture area (A_{pa} ; Figure 6B) and fraction (F_{pa} ; Figure 6C) were positively correlated with K_{th} in Zamiaceae, suggesting that pit aperture size influences theoretical hydraulic conductivity in Zamiaceae, but not in Cycadaceae.

Although pit aperture was not correlated with leaf anatomical traits (Table S4), significant negative correlations were found between F_{pa} and A_m (Figure 6D), D_{pal} (Figure 6E) and stomatal conductance \mathcal{G}

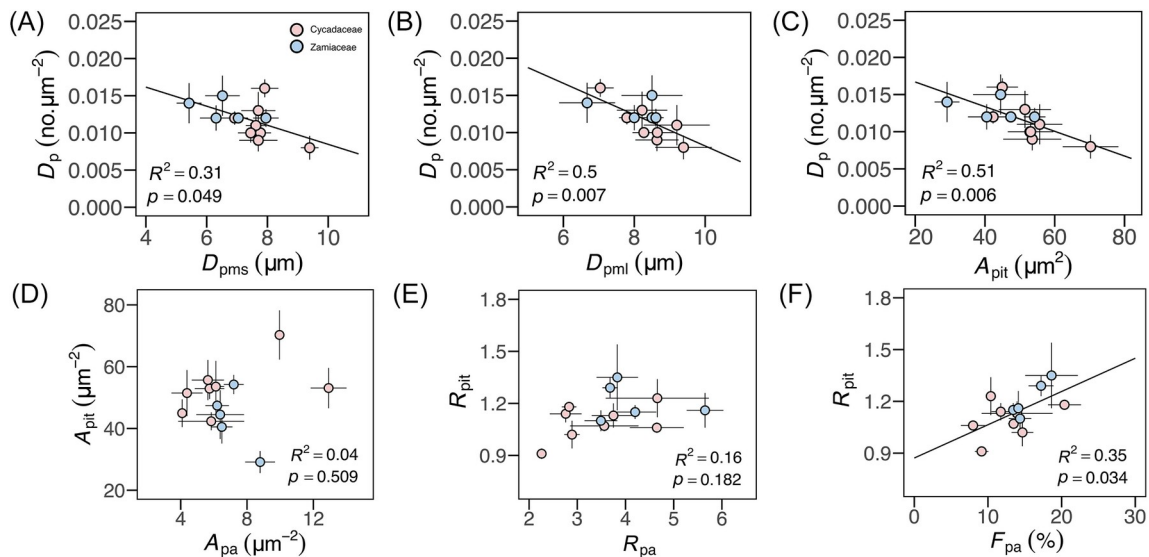


FIGURE 2 Relationships within pit traits. Pit density (D_p) was negatively correlated with pit membrane shortest diameter (D_{pms} , A), pit membrane longest diameter (D_{pml} , B), and intertracheid pit membrane surface area (A_{pit} , C). No correlations were found between A_{pit} and pit aperture area (A_{pa} , D), pit shape (R_{pit}), and pit aperture shape (R_{pa} , E). R_{pit} was positively correlated with pit aperture fraction (F_{pa} , F). Points and error bars indicate mean \pm SE ($n = 3$ individual plants, at least 50 pits measured in each). Zamiaceae are shown in blue dots, whereas pink dots indicate Cycadaceae.

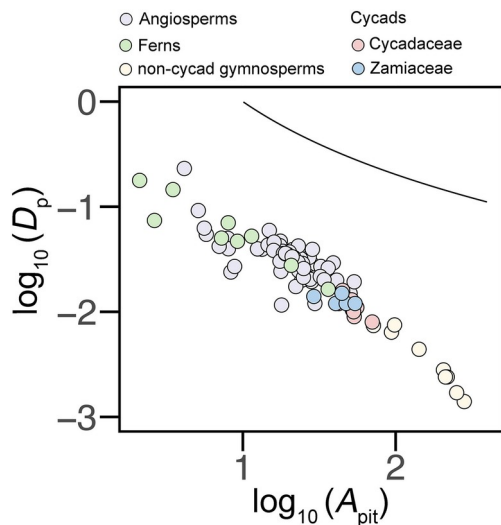


FIGURE 3 Packing relationship between pit membrane surface area (A_{pit}) and pit density (D_p) among major plant lineages. The solid line represents the maximum packing limit of pit density (D_p) where $D_p = 1/A_{pit}$ at a given pit membrane surface area (A_{pit}). The light purple circles represent angiosperms, the light green circles represent ferns, the blue and pink circles represent Zamiaceae and Cycadaceae for cycads, yellow circles represent non-cycad gymnosperms. Additional information for pit traits of ferns, angiosperms, and non-cycad gymnosperms is listed in Table S2. Each data point was a mean value ($n = 3-6$ individual plants, at least 50 pits measured in each).

as well as A_{pa} (Figure 6F) and G_s , indicating that gas exchange rates may decrease with increasing pit aperture size. All the correlations mentioned above remained the same after accounting for shared evolutionary history (Table S6).

TABLE 3 Statistical differences of D_p and A_{pit} among angiosperms, ferns, cycads, and gymnosperms.

Groups	A_{pit} (μm^2)	D_p (no. μm^{-2})
Fern	11.30 \pm 3.65a	0.0734 \pm 0.0179a
Angiosperm	22.53 \pm 1.36a	0.0351 \pm 0.0034b
Cycad	49.23 \pm 2.69b	0.0118 \pm 0.0006c
Gymnosperm	174.74 \pm 25.09c	0.0040 \pm 0.0008c

Note: Pit membrane surface area (A) and pit density (D_p) among four groups ($p < 0.05$; one-way ANOVA). Different letters indicate significant differences between groups. Data for angiosperms, gymnosperms, and ferns were collected from published references (Table S2).

3.5 | Correlations of cycad intertracheid pit traits and their habitats

Although pit traits were overall very similar among both Cycadaceae and Zamiaceae except for the difference in pit membrane short diameter (Table S3), PCA using species-scaled and centered trait values of pit revealed that the first two components explained 36.99% and 32.97% of the total variation, respectively. The first PC was most strongly associated with A_{pit} , F_{pa} , R_{pit} , F_{pf} , and A_{pit} (Figure 7). The second PC was driven mainly by A_{pit} , A_{pa} , R_{pa} , R_{pit} , and D_p (Figure 7). Eigenvalues and loadings are listed in Table S7. Among species, *Z. furfuracea* had the most elliptical pit shape, whereas the pit aperture area of *C. parvula*, and the pit diameter of *C. hainanensis* were significantly larger than those of the other species. Furthermore, the seven wet habitat species and the six dry habitat species were separated mainly along the second PC (Figure 7). Wet species had significantly larger A_{pit} and significantly fewer pits (D_p) than dry habitat species (t-test, $p < 0.05$; Table S8).

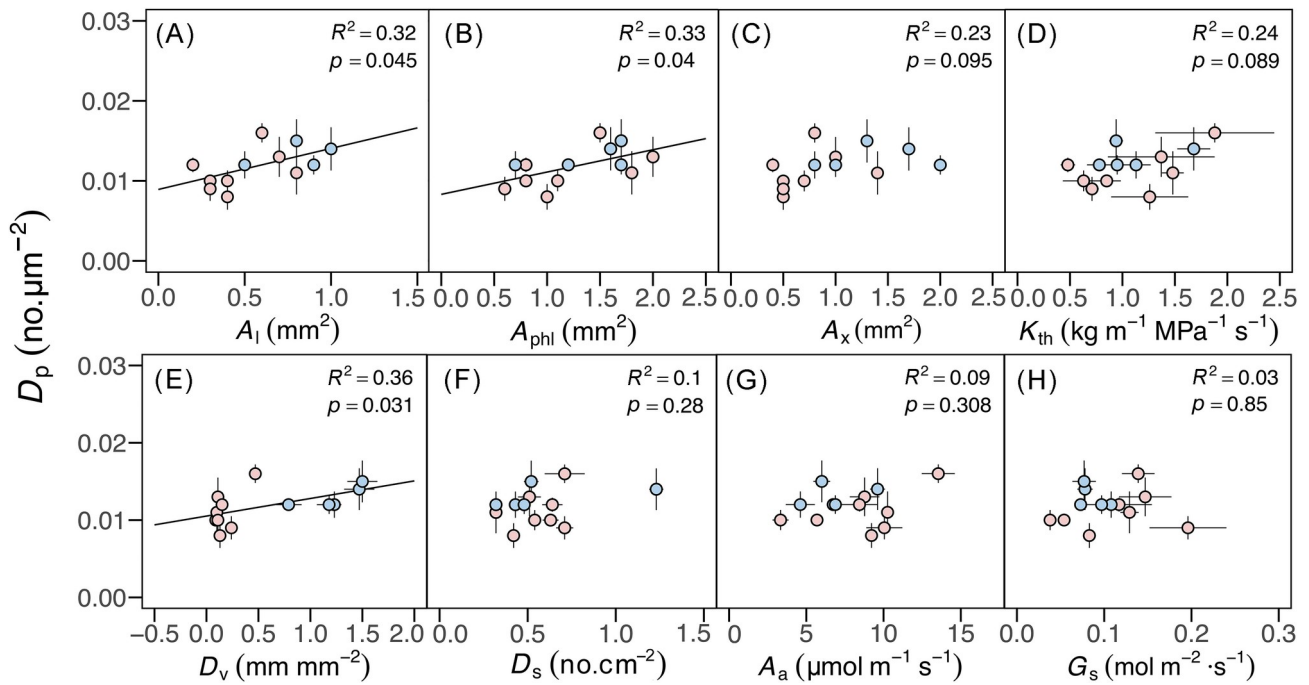


FIGURE 4 Relationships between pit density (D_p) and anatomical traits of rachis xylem tracheid lumen area (A_l , A), phloem area (A_{phl} , B), tracheid xylem area (A_x , C), vein density (D_v , E), and stomatal density (D_s , F); functional traits of theoretical hydraulic conductivity of rachis xylem tracheid (K_{th} , D), light-saturated photosynthetic rate per area (A_a , G), and stomatal conductance (G_s , H). Points and error bars indicate mean \pm SE ($n = 3$ –6 individual plants, at least 50 pits measured in each). Zamiaeceae are shown in blue dots, whereas pink dots indicate Cycadaceae.

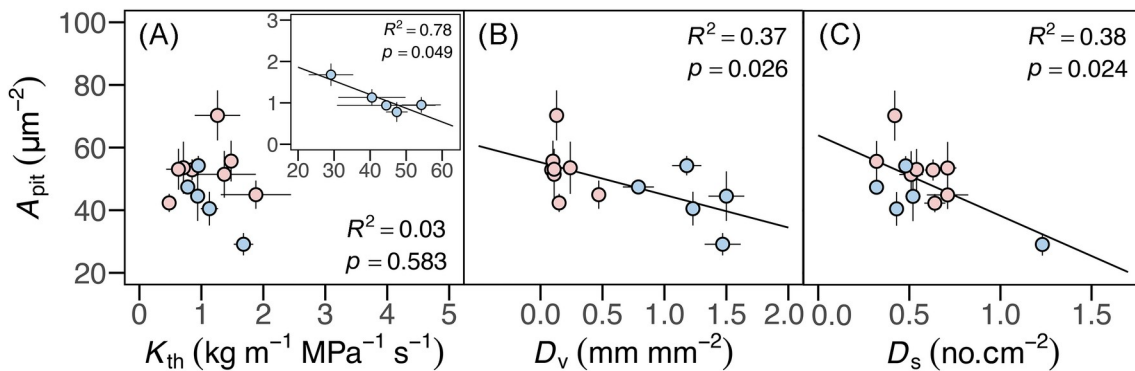


FIGURE 5 Relationships between pit membrane area (A_{pit}) and functional traits of theoretical hydraulic conductivity of rachis xylem (K_{th} , A), leaf vein density (D_v , B), and stomatal density (D_s , C) among cycad species. Points and error bars indicate mean \pm SE ($n = 3$ –6 individual plants, at least 50 pits measured in each). Zamiaeceae are shown in blue dots, whereas pink dots indicate Cycadaceae.

4 | DISCUSSION

Cycads are highly diverse in morphology, leaf structure, and physiological traits (Whitelock, 2003; Zhang et al., 2015), with nearly 70% of living cycads listed as endangered (IUCN, 2022). Threats to cycad survival and mortality may depend on their physiological capacity to withstand future climate change, such as prolonged droughts. Anatomical and physiological traits associated with both xylem hydraulic efficiency and xylem hydraulic safety may be important traits that determine the thresholds of plant mortality to drought-induced hydraulic failure (Brodersen et al., 2014; Kaack et al., 2021; Lens

et al., 2011; Pittermann et al., 2010). Here, we surveyed 13 species of cycads to determine (1) how traits vary among cycad families and compare them to other plant lineages, (2) the relationships between xylem anatomical traits and leaf physiological traits, and (3) whether these xylem anatomical traits are linked to differences in habitat affinities.

We found that intertracheid pit traits of the rachis varied markedly among cycads (Table 2). Further, cycads differed from other lineages in pit membrane area and density, and we report, for the first time, a clear tradeoff between these traits across lineages. Although cycads deviate in relationships among pit traits and between pit traits

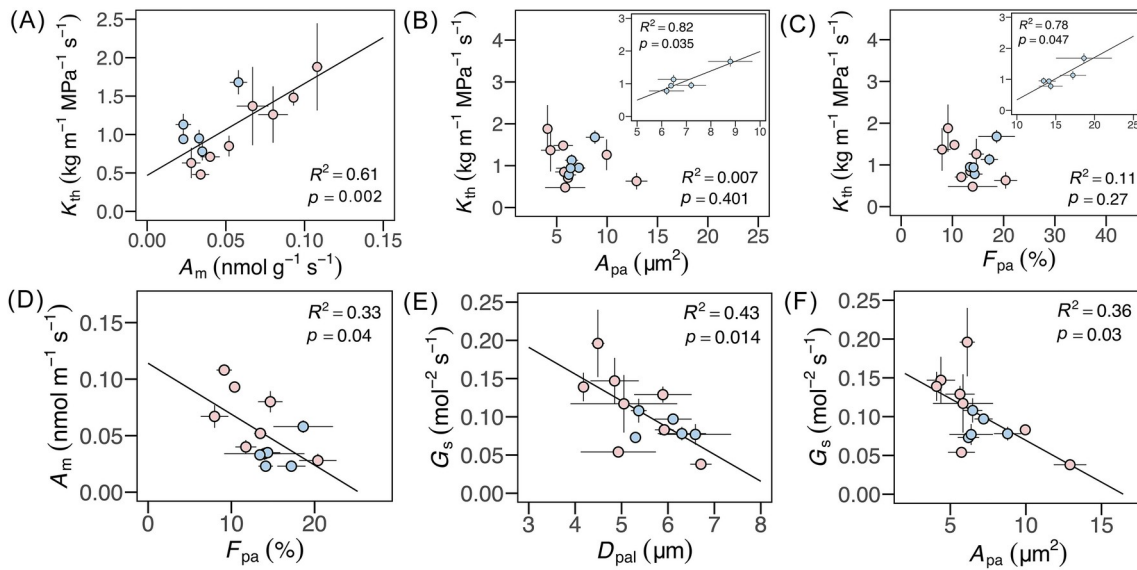


FIGURE 6 Relationships among theoretical hydraulic conductivity (K) and light-saturated photosynthetic rate per mass (A_m), pit aperture area (A_{pa}), pit aperture fraction (F_{pa}), and relationships of pit aperture fraction (F_{pa}) and (A_m), pit aperture longest diameter (D_{pal}), and stomatal conductance (G_s), and A_{pa} to (G_s), among cycads. Points and error bars indicate mean \pm SE ($n = 3$ individual plants, at least 50 pits measured in each). Zamiaceae are shown in blue dots, whereas pink dots indicate Cycadaceae.

and other anatomical and physiological traits, there are some pit characteristics relationships across cycad species that are similar to relationships reported among angiosperms (Jacobsen et al., 2016; Li et al., 2019).

4.1 | Variation of pit traits and comparisons with other lineages

We observed wide variation in intertracheid pit characteristics of the 13 cycad species we studied (Table 2) which was consistent with our hypothesis that pit traits would be species-dependent. There is generally a tradeoff between pit membrane area and density across vascular species such that pit aperture area and shape are coordinated with pit membrane traits (Hacke et al., 2004; Jacobsen et al., 2016; Li et al., 2019; Orians et al., 2004). However, we found that cycads only partially supported this trend, with pit membrane area being correlated with pit density (Figure 2), while pit aperture remained independent of pit membrane traits concerning size and shape. Importantly, the large variation in pit traits we report here is from species growing in a common garden, indicating that this variation in traits is due to genetic differences among species that may result in different habitat preferences (Monson, 1996).

Pit size and density also differed between cycads and other major clades. Cycads had larger A_{pit} than ferns and angiosperms but smaller than non-cycad gymnosperms (Figure 3). Cycad pit density D_p was also much lower among cycads than among ferns and angiosperms, but higher than among non-cycad gymnosperms. The smaller, more densely packed pits of ferns and angiosperms bring them closer to the maximum packing limit, while cycads and other gymnosperms tend to

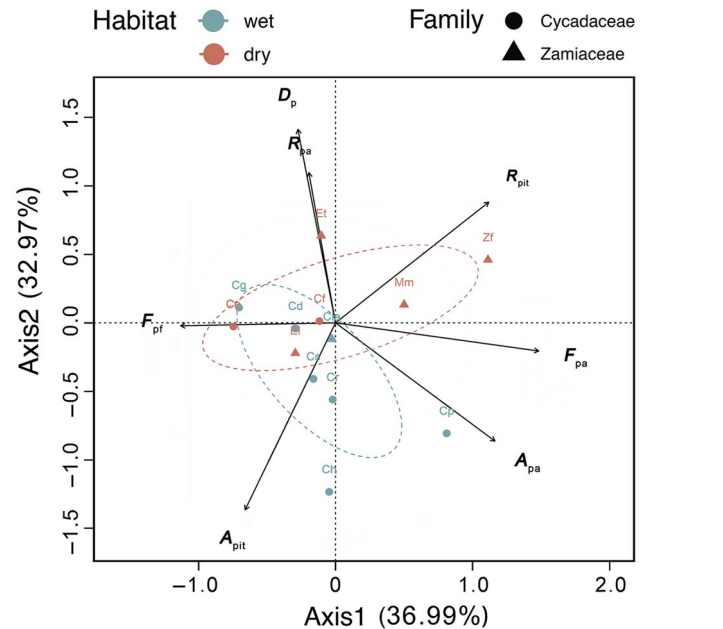


FIGURE 7 The first two principal components of 7 pit traits measured on 13 cycad species. Green circles represent wet habitat, orange circles represent dry habitat, and the regions encircled by dashed lines indicate the volume of trait space occupied by wet (green) and dry (orange) habitat species. Species PC scores are plotted as colored points with adjacent abbreviations for each species, where Cs, *Cycas segmentifida*; Cg, *Cycas guizhouensis*; Ch, *Cycas hainanensis*; Cr, *Cycas revoluta*; Cf, *Cycas ferruginea*; Cp, *Cycas parvula*; Cd, *Cycas debaensis*; Ce, *Cycas elongate*; Zf, *Zamia furfuracea*; Mm, *Macrozamia moorei*; Cla, *Ceratozamia latifolia*; Et, *Encephalartos laurentianus*; Et, *Encephalartos tegulaneus*.

be farther away from the maximum packing limit (Figure 3; Table 3). Larger but fewer homogenous pits have been reported to confer higher hydraulic efficiency (Brodersen et al., 2014; Hacke et al., 2007; Pittermann et al., 2005), which may provide a potential anatomical explanation for the dominance of cycads across a variety of terrestrial habitats in the Mesozoic. On the other hand, cycads have short tracheids, similar to non-cycad gymnosperms, which should result in low xylem-specific conductivity. However, non-cycad gymnosperms have more permeable torus-margo pits (Pittermann et al., 2005), which would likely give non-cycad gymnosperms a hydraulic advantage over cycads, all else being equal. Although ferns generally have more permeable pit membranes (Brodersen et al., 2014), they are usually smaller compared with cycad pits and may therefore result in relatively lower hydraulic conductance. Thus, different xylem anatomical features of pits and xylem conduits may either reinforce or counteract each other to influence hydraulic conductivity and vulnerability to embolism. Without more data on hydraulic conductivity, it is difficult to resolve the effects of each individual trait alone.

4.2 | Correlations with anatomical and physiological traits

Pit characteristics are frequently related to other xylem anatomical and physiological traits (Lens et al., 2011; Li et al., 2019; Song, Sterck, et al., 2022). For example, fewer and larger pits or pit apertures provide greater hydraulic efficiency (Lens et al., 2011; Mrad et al., 2018; Zhang et al., 2022), whereas smaller surface area and higher density of intervessel pits increase embolism resistance and lower hydraulic efficiency (Choat et al., 2005; Ellmore et al., 2006; Hacke et al., 2006; Wheeler et al., 2005). Larger diameter and longer conduits increase hydraulic efficiency, whereas more numerous pits on the conduit wall increase hydraulic safety (Lens et al., 2011). While angiosperm shrubs tend to have lower pit density and greater hydraulic efficiency (Jacobsen et al., 2016), in some cases, higher pit density can confer greater hydraulic efficiency and higher rates of gas exchange; for example, in nonhemiepiphytic compared with hemiepiphytic *Ficus* species (Liet et al., 2019). These patterns suggest that pit traits are, in some cases, linked to species-habitat associations. For the cycad species studied here, pit density (D_p) and area (A_{pit}) were not correlated with tracheid diameter or theoretical hydraulic efficiency (K_{th}), or with other physiological traits (Table S4). The one exception was that pit membrane area was negatively correlated with K_{th} in Zamiaceae (Figure 5A). Pit membrane area was only coordinated with a few anatomical traits of the rachis and pinna (Figure 5B,C and Table S4). One possible reason why pit membrane area was not coordinated with other anatomical traits may be because cycads universally have relatively low pit density and large pit membranes. However, pit density (D_p) increased with pinna vein density (Figure 4E), suggesting that D_p might be associated with adaptation to warmer and drier habitats among the Zamiaceae (Table S8; Qin et al., 2022). Consistent with this possible effect of habitat, Zamiaceae also had relatively larger xylem fractions (Brenner, 2003) and more elliptical pits (R_{pit} ; Table 2).

Additionally, among all Zamiaceae, R_{pit} was positively correlated with tracheid double-wall thickness, which is thought to confer higher hydraulic safety (Figure S2; Lens et al., 2011; Scholz et al., 2013; Zhang et al., 2021).

A negative correlation between pit membrane area A_{pit} and K_{th} in Zamiaceae (Figure 5A) is likely associated with compensatory changes in other pit traits, for example, larger pit apertures were associated with higher K_{th} in Zamiaceae (Figure 6B,C). Pit aperture area in cycads was relatively larger than in angiosperm species (Li et al., 2019), which may contribute to higher K_{th} in Zamiaceae. In other plant lineages, the pit aperture diameter represents a smaller and constant fraction of the pit membrane diameter, with a pit aperture of around 10% of the pit membrane area (Lens et al., 2011; Scholz et al., 2013). However, the higher fraction (F_{pa}) among cycads was negatively correlated with mass-based photosynthesis (A_m ; Figure 6D), and, among all pit traits, only pit aperture area and fraction were correlated with the physiological traits (but not to K_{th}) in cycads, consistent with previous results that sapwood hydraulic conductivity is not correlated with pit traits among conifers (Song, Poorter, et al., 2022).

Some pit traits were coordinated with physiological traits among cycads, particularly among the Zamiaceae. The negative relationship between F_{pa} and both A_a and A_m (Table S4) is consistent with *Ficus* species (Li et al., 2019) but opposite a previous report showing that a larger pit aperture was positively associated with carbon assimilation and increased hydraulic vulnerability among conifers (Song, Sterck, et al., 2022). Although A_{pa} and F_{pa} were not correlated with K_{th} across all 13 cycad species, these traits were correlated among only the Zamiaceae species, with increasing K_{th} being associated with higher mass-based photosynthesis (A_m ; Figure 6A). These results are consistent with the coordination between stem hydraulic capacity and leaf photosynthetic rates (Brodrribb & Feild, 2000; Santiago et al., 2004) and suggest that this coordination was achieved at least in part by adjusting the pit aperture area. However, in contrast to both theory and prior results, area-based leaf hydraulic conductance was reported to be unrelated to A_a among cycads (Zhang et al., 2015). Though some pit traits exhibit similar relationships among cycads as among other lineages, other relationships deviate, suggesting that further work on cycads could help elucidate important mechanistic links between traits that might not be possible by examining only non-cycad lineages.

4.3 | Physiological and ecological implications of intertracheid pits

The native habitats of the cycad species studied here vary greatly in precipitation, mean annual temperature, and habitat (Table S1). These habitat differences may impact vulnerability to hydraulic failure and traits associated with drought-induced embolism. While there have been numerous reports about different water availability influencing vulnerability to drought among closely related species (Brodrribb et al., 2014; Larter et al., 2017; Skelton et al., 2021), there are few measurements of embolism vulnerability among cycads. Because pit anatomical traits can influence embolism vulnerability (Brodersen

et al., 2014; Jacobsen et al., 2016; Lens et al., 2011; Pittermann et al., 2010), relationships between pit traits and climate would suggest that cycad species may differ in their vulnerability to embolism. Indeed, our previous data showed that the embolism vulnerability of three cycads was in the range reported for noncycad gymnosperms; however, *M. moorei* had a negative hydraulic safety margin, whereas *C. revoluta* and *C. elongata* had a positive hydraulic safety margin (Qin et al., 2022). In our dataset, Cycadaceae tended to be from cooler and wetter habitats than Zamiaceae (Meng et al., 2021), and the short diameters of pit membranes were longer in Cycadaceae than in Zamiaceae (Table S3). This was further evidenced by the fact that cycad species from wet habitats tend to have larger but fewer pit membranes (Table S8). This pattern is consistent with other data showing that species from drier environments often have more elliptical pits (Lens et al., 2011, Scholz et al., 2013, Zhang et al., 2021).

Plants from drier habitats typically have smaller vessels and tracheids, thicker and more densely packed intervessel pits, more elliptical pits and pit apertures, and smaller pit membranes than plants from moist environments (Hacke et al., 2007; Jacobsen et al., 2016; Lens et al., 2011; Scholz et al., 2013). Six of the eight Cycadaceae species (all except *C. ferruginea* and *C. elongata*) are found in humid habitats, whereas five of the six Zamiaceae species (all except *C. latifolia*) are distributed in arid habitats. These wet-habitat and dry-habitat species differed in their pit membrane traits (D_{pms} , D_p , and A_{pit}), pit aperture size (A_{pa} , R_{pa} , and D_{pas}), and pit shape (R_{pit}). Species from wet habitats tended to have larger pit membranes and pit apertures and more circular pit apertures (*C. hainanensis* and *C. parvula*; Table 2), whereas Zamiaceae species from drier habitats tended to have more elliptical pits and pit apertures, and higher pit densities (*Z. furfuracea*; Table 8, S8, and Figure 7). However, these habitat differences in pit traits were driven largely by differences among the two families. Comparing congeneric species that differed in wet-habitat versus dry-habitat affinities revealed smaller differences due to habitat, for example, *C. elongata* and *C. guizhouensis* differ in habitat but had similar traits in multivariate space (Figure 7). More data on a broader set of cycad species would help to elucidate the effects of climate on these traits and to what extent pit traits influence xylem hydraulic safety and efficiency.

Cycads first arose ca 250 Myr ago but extant species diversified only very recently (Nagalingum et al., 2011) during a period of declining atmospheric CO₂ and increasing aridity (Beerling & Royer, 2011; Steinthorsdottir et al., 2021; Tanner et al., 2020; Trayler et al., 2020). Despite this rapid diversification during the Neogene, extinct and extant cycads are morphologically very similar, and some anatomical traits in cycads do not respond plastically to differences in atmospheric CO₂, suggesting that the trait relationships reported here may reflect the traits of extinct cycads from long-extinct ecosystems (Haworth et al., 2011; Zhang et al., 2015). The fossils of extant Cycadales, however, are limited to the Cenozoic (Coiro & Pott, 2017) when angiosperms started to prosper (Condamine et al., 2015; Simonin & Roddy, 2018). Cycadales have morphologies similar to the Bennettitales and Nilssoniales, two dominant plant groups of the mid-Mesozoic (Coiro & Pott, 2017; Harris, 1961). Extant cycads still grow

successfully in a wide range of habitats, from very wet tropical rainforests to dry habitats (Calonje et al., 2022; Norstog & Nicholls, 1998; Whitelock, 2003). However, the lack of plasticity in cycad anatomy suggests that climate-driven changes in traits may occur only during speciation and that modern cycads have limited capacity to acclimate to current and future climate change and must rely instead on adaptive responses.

5 | CONCLUSION

Our results highlight that studying cycads can reveal important information about the coordination and evolution of anatomical and physiological traits among vascular plants. Cycads exhibit a wide variation in intertracheid pit characteristics and a similar tradeoff between pit density and pit area, as has been reported for other plant lineages, despite deviating in relationships among pit traits and between pit traits and other anatomical and physiological traits. The larger pit membrane area but lower density of pits compared with ferns and angiosperms may provide higher hydraulic efficiency for cycads. On the other hand, the homogeneous but smaller pit membrane area and similar low pit density of non-cycad gymnosperms suggest that the xylem hydraulic capacity of cycads may be low, potentially leading to lower competitive fitness among cycads in present-day terrestrial ecosystems. That pit trait variation among cycad lineages was associated with differences in habitat affinities suggests that selection on hydraulic traits may have driven pit trait evolution among cycads. Whether the pit traits, including pit membrane thickness and pit resistivity, play crucial roles in the hydraulic system and confer success in different environments for cycads deserves further investigation. Furthermore, the position of cycads as sister to all other gymnosperms except *Gnetum* and their very recent and rapid radiation suggest that understanding the role of these anatomical traits on cycad physiology could reveal important clues to the evolution of vascular plant physiology more generally.

AUTHOR CONTRIBUTIONS

Guo-Feng Jiang conceived the ideas and designed the study. Yu-Kun Pang, Lan-Li Qin, Jin-Yan Lei, and Guo-Feng Jiang collected the data. Yu-Kun Pang, Lan-Li Qin, Tian-Hao Zhang, and Guo-Feng Jiang analyzed the data. Guo-Feng Jiang and Yu-Kun Pang wrote the article, and all authors reviewed each draft before giving approval for submission of the final version.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interest.

DATA AVAILABILITY STATEMENT

All data supporting the findings of this study are available within the paper and within its supplementary data published online. Reuse of the data is permitted after obtaining permission from the corresponding author.

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