Hydraulics link leaf shape and environmental niche in terrestrial bromeliads

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ABSTRACT

Terrestrial species of the megadiverse bromeliad family display a wide variety of leaf shapes, many of which have evolved convergently in different lineages. Here, I examine the links between leaf shape, venation architecture, hydraulic function, and bioclimatic relations in two bromeliad groups displaying diverse leaf shapes, the genus *Pitcairnia* (Pitcairnioideae) and the terrestrial grade of the Bromelioideae subfamily. Leaf shapes with broader leaf blades, notably petiolate and lanceolate morphologies, tend to show wider vein spacing, which is associated with reduced hydraulic capacity and higher hydraulic vulnerability. In turn, these leaf shapes tend to occur in species restricted to moist, aseasonal environments, suggesting that hydraulic function is an important mediator of the relationship between leaf shape and bromeliad environmental niches. This network of trait–trait and trait–environment relationships may have been of profound important in the ecological and evolutionary diversification of the bromeliads. Similar structure–function principles are likely to apply in other tropical herbaceous monocots, which are of great ecological importance but generally neglected in plant hydraulic research.

Key words: bioclimatic envelopes; Bromeliaceae; leaf hydraulics; leaf shape; morphology; Neotropics.

THE BROMELIACEAE (POALES) IS A HIGHLY DIVERSE MONOCOT FAMILY DISTRIBUTED ACROSS THE NEOTROPICS AND ADJACENT TEMPERATE ZONES (Benzing 2000). A series of nested adaptive radiations have taken place in the bromeliads, spurred by serial invasions of contrasting environments and origins of distinctive morphological and physiological key innovations including absorptive foliar trichomes, epiphytism, Crassulacean acid metabolism (CAM), and the 'tank' growth form (Givnish *et al.* 2014). The bromeliads utilize a range of water-use strategies, with different species occupying a spectrum running from full dependence on roots for water uptake to reliance on foliar trichomes only (Pittendrigh 1948, Benzing 2000, Males 2016). Many aspects of plant morphology vary in tandem with this spectrum, including root:shoot ratios, rosette architecture, and leaf succulence (Benzing 2000, Males 2016). The coordinated evolution of this array of functional traits represents a strong example of concerted convergence (*sensu* Patterson & Givnish 2002) and has produced striking cases of homoplastic form and function. For example, the tank-epiphyte functional type has evolved independently in three bromeliad subfamilies (Brocchinioideae, Tillandsioideae, and Bromelioideae), each instance involving concerted changes in trichome structure and function, rosette architecture, and leaf physiology (Benzing 2000, Males & Griffiths 2017).

A trait of profound ecophysiological significance which shows interesting patterning across the Bromeliaceae is leaf shape. While leaf shape is of direct relevance to many aspects of angiosperm leaf function (Nicotra *et al.* 2011) and has been considered in general terms in treatments of the evolution of bromeliad radiations such as the genus *Brocchinia* (Givnish *et al.* 1997), it

has rarely been included as a factor in quantitative investigations of bromeliad ecophysiology. Given the centrality of water relations to bromeliad ecophysiological strategies (Males 2016), possible connections between leaf shape and leaf hydraulics could be of particular significance. There is mixed evidence regarding the interactions between leaf shape and hydraulic function in other plant groups (Sack *et al.* 2003, 2012, Sack & Frole 2006, Niinemets *et al.* 2007, Nicotra *et al.* 2011, Scoffoni *et al.* 2011), although it is notable that tropical monocots are poorly represented in the literature. Fundamental to the hypothetical link between leaf shape and leaf hydraulics is the assumption that leaves of different shapes will display venation networks of divergent structural properties (Dengler & Kang 2001, Niinemets *et al.* 2007). These differences in venation architecture could underlie differential capacity to supply water to the mesophyll for hydration and to the stomata for transpiration.

The plesiomorphic leaf shape for the bromeliad clade was probably narrowly linear, a morphology that is typical for the Poales and occurs in the sister group to the bromeliads, the Typhaceae (Benzing 2000, Bouchenak-Khelladi *et al.* 2014). While this leaf shape is retained in some of the earlier-diverging bromeliad lineages (*e.g.*, certain *Brocchinia* spp.), in other lineages it has undergone substantial evolutionary modification (see examples in Fig. S1). Some bromeliad groups show a particularly high level of diversity in leaf shape and are, therefore, good targets for testing hypotheses linking leaf shape with physiological functions and ecological specialization. Among these groups are the genus *Pitcairnia* L'Her. (Pitcairnioideae) and the terrestrial grade at the base of the Bromelioideae subfamily. This investigation sought to test the hypothesis that divergences in leaf shape among species in these two groups are associated with differences in venation architecture and hydraulic function, and that this can in turn

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explain an important proportion of interspecific variation in bioclimatic habitat occupancy.

METHODS

Taxon sampling and leaf shape categorization.—The genus *Pitcairnia* includes approximately 395 species (The Plant List, 2016), among which are some of the most mesomorphic bromeliads. *Pitcairnia* species occur in a wide range of habitats across northern South America, Central America, Mexico, and the Caribbean. Most are terrestrial, but some species are hemiepiphytic or facultative epiphytes (Benzing 2000). All appear to use C_3 photosynthesis (Crayn *et al.* 2015). Among the range of *Pitcairnia* species considered here, the following leaf shapes could be assigned through examination of online living collection images (Florida Council of Bromeliad Societies Photo Index Database) and herbarium specimens (Global Plants, JSTOR): linear (with reduced and broad subtypes), lanceolate (including a reduced subtype), linear-spinose, reduced subulate, tabuliform, and petiolate (Fig. 1A). The terrestrial Bromelioideae include 235 species (The Plant List, 2013), among which are both C_3 genera (*e.g.*, *Fascicularia* Mez) and CAM genera (*e.g.*, *Bromelia* L. Crayn *et al.* 2015). They are distributed across approximately the same overall geographical range in the Neotropics as *Pitcairnia*. Five major leaf shapes can be identified in the terrestrial Bromelioideae: linear entire, linear/subulate-spinose, lanceolate-undulate, spathulate, and petiolate (Fig. 1B).

Phylogenetic distribution of leaf shapes.—To evaluate the phylogenetic distribution of leaf shapes in the genus *Pitcairnia* and the terrestrial Bromelioideae, approximate phylogenies were constructed for either group based on previously published phylogenetic analyses. For *Pitcairnia*, the tree used here was based on the morphological phylogeny of Saraiva *et al.* (2015) and included 56 terminal taxa. For the terrestrial Bromelioideae, a composite tree was produced based on molecular phylogenetic analyses, using information from Givnish *et al.* (2011) and Silvestro *et al.* (2014) for intergeneric relationships, from Louzada *et al.* (2014) for relationships within *Orthophytum*, Monteiro *et al.* (2015) for relationships within *Bromelia*, and Cruz *et al.* (2017) for relationships within *Cryptanthus*. It included 111 terminal taxa including a tip for the Core Bromelioideae. Some weakly supported nodes in source trees and cases of taxonomic confusion were collapsed to polytomies. Where all species within a genus were deemed to display the same leaf shape, the genus was represented by a single tip.

Plant material.—Empirical measurements of leaf structure and function were made on representative species of contrasting leaf shapes. Eight *Pitcairnia* species were selected, including two with linear leaves (*P. integrifolia* Ker Gawl. and *P. poortmanii* André), two with broad linear leaves (*P. imbricata* (Brongn.) Regel and *P. sceptrigera* Mez), one with reduced linear leaves (*P. fuertesii* Mez), two with lanceolate leaves (*P. atrorubens* (Beer) Baker and *P. maidifolia* (C.Morren) Decne. ex Planch), and one with petiolate leaves (*P. undulata* Scheidw). Six terrestrial Bromelioideae species were used: one with linear leaves (*Cryptanthus maritimus* L.B.Sm.), two with linear-spinose leaves (*Ananas bracteatus* (Lindl.) Schult. & Schult.f. and *Bromelia humilis* Jacq.), two with lanceolate-undulate leaves (*C. pickelii* L.B.Sm. and *C. zonatus* (Vis.) Beer), and one with petiolate leaves (*C. beuckeri* E.Morren).

All plant material for the experimental species used in this investigation was obtained from the living collections at Cambridge University Botanic Garden and RBG Kew (see Table S1 for provenance by species). At either site, plants were grown under tropical glasshouse conditions, with daytime temperature between 25 and 30 °C and relative humidity between 80 and 100 percent, night-time temperature between 16 and 22 °C and relative humidity between 85 and 100 percent, and a photosynthetic photon flux density of at least 300 μmol/m^2/s achieved through a combination of natural and artificial illumination.

Venation architecture and leaf area.—For each experimental species, venation architecture traits were quantified by inspection of hand-cut transverse sections from the central portion of the leaf blade under a light microscope. Twelve sections were examined from each of five leaves sampled from at least three individuals. Interveinal distance (IVD) was quantified as the straight-line horizontal distance between the centers of adjacent vascular bundles. Vein-epidermis distance (VED) was measured as the straight-line vertical distance between the center of a vascular bundle and the stomatiferous abaxial epidermis. The ratio of these two parameters (IVD: VED) was also calculated.

Leaf area was measured using an AM350 leaf area meter (ADC Bioscientific Ltd, Hoddesdon, UK) to test how this parameter was related to leaf shape and venation architecture. At
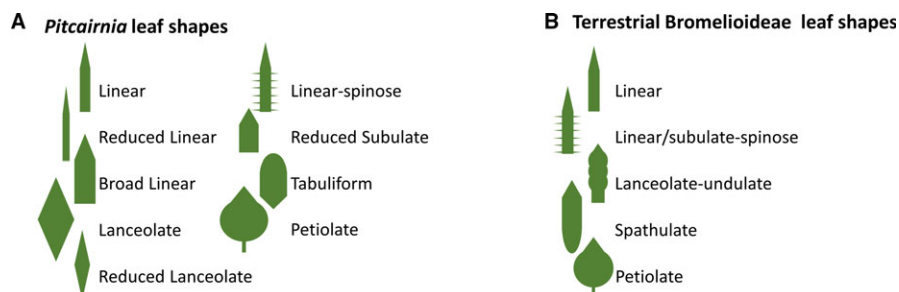

FIGURE 1. (A) Leaf shapes in *Pitcairnia*. (B) Leaf shapes in terrestrial Bromelioideae.

least six fully expanded leaves were measured per species, sampled from at least three individuals.

LEAF HYDRAULIC CONDUCTANCE AND VULNERABILITY.—For each of the 14 experimental species, at least six replicate measurements of maximal leaf hydraulic conductance ($K_{leaf,max}$, mmol/m^2 s MPa) were made with fully hydrated leaves detached at dawn. Measurements were made on leaves incubated in a custom-built, climate-controlled glass cuvette under standardized conditions (25 °C, 80 percent relative humidity, 300 μmol photons/m^2/s for C$_3$ species and 0 μmol photons/m^2/s for CAM species). The evaporative flux method was used to measure $K_{leaf,max}$ (Sack & Scoffoni 2012). Transpiring leaves were connected via PVC tubing to an isothermal (25 °C) reservoir of 15 mM KCl in degassed reverse-osmosis H$_2$O. The internal diameter of the tubing was adjusted according to species morphology. Across a known time interval, the volume of KCl solution taken up by the leaf (and assumed to be transpired) was recorded and divided by the product of leaf area, time elapsed, and the change in leaf water potential (Ψ_{leaf}) during the transpiration period to provide $K_{leaf,max}$. For non-petiolate species, the connection of leaves to the tubing leading to the KCl reservoir was made directly to the leaf base, cut as low as possible. Laboratory film, adhesive putty, and Hoffman clamps were used to make this junction air-tight without damaging the leaf tissue. A PMS 1505D pressure chamber (PMS Instrument Company, Albany, OR, USA) was used to measure leaf water potential before and after a transpiration period of at least 30 min after 20-min acclimation. The same methodology was used to measure K_{leaf} in leaves that had been allowed to dehydrate on the bench to different levels of Ψ_{leaf}. This enabled the construction of leaf hydraulic vulnerability curves (percentage loss of leaf hydraulic conductance, PLC, vs. Ψ_{leaf}). Sigmoid curves were fitted by nonlinear least squares in R (R Development Core Team, 2008), and the value of Ψ_{leaf} at which K_{leaf} was reduced by 50 percent relative to $K_{leaf,max}$ (P50) was calculated.

ESTIMATION OF SPECIES BIOCLIMATIC RELATIONS.—To test for differences in bioclimatic distributions among leaf shapes, present data for the experimental taxa and all other species in the genus *Pitcairnia* and the terrestrial Bromelioideae grade were downloaded from the Global Biodiversity Information Facility (GBIF) and subjected to manual quality-control (*i.e.*, removal of obvious geographic outliers and records with insufficient metadata). For species with at least two reliable presence points (N = 223 for *Pitcairnia*; N = 123 for terrestrial Bromelioideae), bioclimatic data from the Bioclim (Hijmans *et al.* 2005) and CGIAR-CSI (Zomer *et al.* 2007, 2008) data bases were accessed using a custom script in R for the 30 arc-second resolution grid-square in which each point was located. Moisture-related bioclimatic indices were selected on the basis of the acknowledged pre-eminence of water-use strategies in bromeliad ecophysiology (Males 2016) and included mean annual precipitation (MAP, mm), the ratio of actual to potential evapotranspiration (AET/PET, mm/mm), the aridity index (MAP/PET, mm/mm), and precipitation seasonality (coefficient of variation, %, in monthly mean precipitation).

RESULTS

PHYLOGENETIC DISTRIBUTION OF LEAF SHAPES.—The phylogenetic distribution of leaf shapes across the genus *Pitcairnia* is shown in Fig. S2. On the basis of these phylogenetic topology assumed here (Saraiva *et al.* 2015), early-diverging species tended to show linear-spinose leaves, while through the remainder of the genus, there appear to have been repeated transitions between linear, reduced linear, broad linear, lanceolate, and petiolate leaf shapes.

In the terrestrial Bromelioideae, linear/subulate-spinose leaves are plesiomorphic and common throughout almost all genera (Fig. S3). However, there have been independent origins of petiolate or spathulate leaves in *Bromelia*, *Disteganthus*, and *Cryptanthus*, of lanceolate-undulate leaves in *Cryptanthus* and *Orthophytum*, and of linear leaves in *Cryptanthus* and *Fernseea*. Moreover, within the genus *Cryptanthus*, there appear to have been multiple transitions between different leaf morphologies.

VENATION ARCHITECTURE AND LEAF AREA.—Interspecific variation in interveinal distance (IVD), vein-epidermis distance (VED), IVD: VED, and leaf area are displayed in Table 1. Among *Pitcairnia* species, IVD ranged between 0.155 mm in the reduced species *P. fuertesii* and 0.687 mm in the petiolate species *P. undulata*, while VED varied between 0.077 mm in the broad linear species *P. imbricata* and 0.189 mm in *P. undulata*. Meanwhile IVD: VED ranged from 1.42 in *P. fuertesii* to 6.67 in the lanceolate *P. maidifolia*. Among the early-diverging Bromelioideae (N = 6), IVD ranged from 0.085 mm in the linear/subulate-spinose species *Cryptanthus maritimus* to 0.662 mm in the petiolate species *C. beuckeri*, while VED varied between 0.104 mm in *C. maritimus* and 0.622 mm in *A. bracteatus*. The ratio of IVD:VED was lowest in *A. bracteatus* (0.30) and highest in *C. beuckeri* (2.18). Figure 2 illustrates how interspecific variation in IVD:VED corresponds to differential overinvestment or underinvestment in veins *sensu* Zwieniecki and Boyce (2014).

Among the terrestrial Bromelioideae, leaf area ranged between 27.8 cm^2 in the lanceolate-undulate species *Cryptanthus pickelii* and 82.1 cm^2 in the petiolate *C. beuckeri*. In the *Pitcairnia* species, leaf area varied between 26.1 cm^2 in the reduced linear species *P. fuertesii* and 148.2 cm^2 in the lanceolate *P. maidifolia*. Across all experimental *Pitcairnia* and terrestrial Bromelioideae species (N = 14), linear regression showed that there was a strong positive correlation between IVD and leaf area (R^2 = 0.64, P < 0.001). This suggests that vein spacing and leaf area are evolutionarily coordinated, perhaps through developmental constraints.

LEAF HYDRAULIC CONDUCTANCE AND VULNERABILITY.—Values of area-specific $K_{leaf,max}$ and of P50 for all species are shown in Table 2, and summary hydraulic vulnerability curves showing mean data for all species are displayed in Fig. 3. $K_{leaf,max}$ values were comparable with those reported for related *Pitcairnia* and terrestrial Bromelioideae species in Males and Griffiths (2017). Statistical comparison of hydraulic traits among species was not possible due to the low number of species representative of each

TABLE 1. *Mean and standard error for vascular anatomical traits of six early-diverging Bromelioideae and eight* Pitcairnia *species of contrasting leaf shape, based on measurements made on at least three individuals per species.*

Group	Leaf shape	Species	IVD (mm) $\pm$ SE	VED (mm) $\pm$ SE	IVD: VED	Leaf area (cm^2) $\pm$ SE
Pitcairnia	Reduced	*Pitcairnia fuertesii*	0.155 $\pm$ 0.031	0.109 $\pm$ 0.008	1.42	26.1 $\pm$ 0.9
	Linear	*Pitcairnia poortmanii*	0.235 $\pm$ 0.036	0.105 $\pm$ 0.015	2.24	40.5 $\pm$ 2.6
	Linear	*Pitcairnia integrifolia*	0.214 $\pm$ 0.030	0.131 $\pm$ 0.011	1.63	49.2 $\pm$ 3.8
	Broad Linear	*Pitcairnia sceptrigera*	0.395 $\pm$ 0.042	0.092 $\pm$ 0.006	4.29	119.8 $\pm$ 5.5
	Broad Linear	*Pitcairnia imbricata*	0.364 $\pm$ 0.046	0.077 $\pm$ 0.006	4.73	105.6 $\pm$ 4.7
	Lanceolate	*Pitcairnia maidifolia*	0.567 $\pm$ 0.051	0.085 $\pm$ 0.009	6.67	148.2 $\pm$ 6.2
	Lanceolate	*Pitcairnia atrorubens*	0.618 $\pm$ 0.044	0.101 $\pm$ 0.006	6.12	115.7 $\pm$ 5.1
	Petiolate	*Pitcairnia undulata*	0.687 $\pm$ 0.047	0.189 $\pm$ 0.014	3.63	110.0 $\pm$ 4.1
Terrestrial Bromelioideae	Petiolate	*Cryptanthus beuckeri*	0.662 $\pm$ 0.038	0.304 $\pm$ 0.012	2.18	82.1 $\pm$ 2.4
	Lanceolate-Undulate	*Cryptanthus pickelii*	0.141 $\pm$ 0.027	0.250 $\pm$ 0.011	0.56	27.8 $\pm$ 0.4
	Lanceolate-Undulate	*Cryptanthus zonatus*	0.145 $\pm$ 0.022	0.202 $\pm$ 0.012	0.72	63.1 $\pm$ 2.8
	Linear/subulate-spinose	*Bromelia humilis*	0.212 $\pm$ 0.020	0.317 $\pm$ 0.023	0.67	44.6 $\pm$ 3.7
	Linear/subulate-spinose	*Cryptanthus maritimus*	0.085 $\pm$ 0.019	0.104 $\pm$ 0.018	0.82	29.0 $\pm$ 0.4
	Linear/subulate-spinose	*Ananas bracteatus*	0.189 $\pm$ 0.028	0.622 $\pm$ 0.041	0.30	36.7 $\pm$ 1.6

IVD = interveinal distance; VED = vein-epidermis distance.

leaf shape. However, qualitative examination of the data showed that in *Pitcairnia* species, the mean value of $K_{leaf,max}$ followed the sequence: reduced linear > linear > broad linear > lanceolate > petiolate. Similarly, the value of P50 followed the sequence: petiolate > lanceolate > broad linear > linear > linear. For the six terrestrial Bromelioideae, the patterns were similar. $K_{leaf,max}$ declined
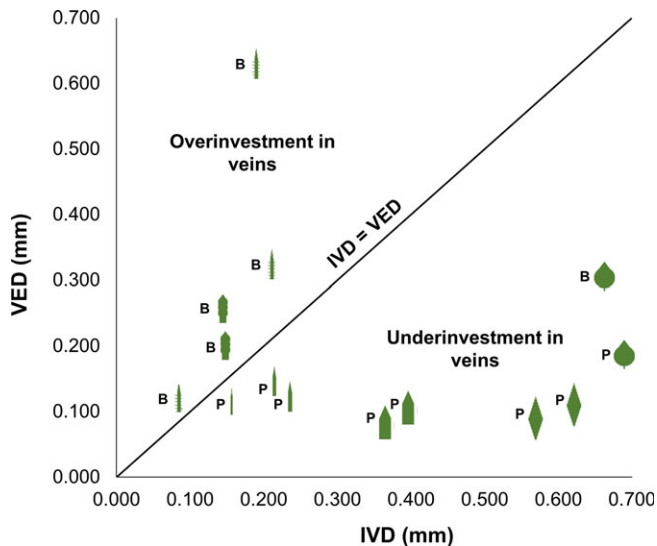

FIGURE 2. Variation in the ratio of interveinal distance (IVD) to vein–epidermis distance (VED) in the bromeliads of different taxonomic groups and leaf shapes used in the experimental part of this investigation ($N = 14$). Occupation of the region of the morphospace where IVD > VED is consistent with underinvestment in veins, while IVD < VED indicates overinvestment in veins (Zwieniecki & Boyce 2014). B = terrestrial Bromelioideae; P = *Pitcairnia* spp. Leaf shape symbols follow the scheme laid out in Fig. 2.

across leaf shapes following the sequence: linear > linear-spinose > lanceolate-undulate > petiolate. The sequence was equivalent for P50.

Across the *Pitcairnia* species ($N = 8$), linear regression identified a strong negative relationship between $K_{leaf,max}$ and P50 ($R^2 = 0.93$, $P < 0.001$). Among the terrestrial Bromelioideae ($N = 6$), there was an equivalent negative correlation between $K_{leaf,max}$ and P50 ($R^2 = 0.67$, $P = 0.028$). These results appear to contravene the hydraulic safety versus efficiency hypothesis, according to which there should be a positive correlation between $K_{leaf,max}$ and P50.

Among *Pitcairnia* species ($N = 8$), linear regression showed that there was a strong negative relationship between $K_{leaf,max}$ and IVD ($R^2 = 0.89$, $P < 0.001$), whereas $K_{leaf,max}$ and VED were not correlated ($R^2 = 0.21$, $P = 0.139$). Although not statistically significant, there was a weak negative correlation between $K_{leaf,max}$ and leaf area ($R^2 = 0.38$, $P = 0.062$). P50 was strongly positively correlated with IVD ($R^2 = 0.96$, $P < 0.001$), but not with VED ($R^2 = -0.01$, $P = 0.372$), and there was a significant positive correlation between P50 and leaf area ($R^2 = 0.61$, $P = 0.013$).

In the terrestrial Bromelioideae ($N = 6$), $K_{leaf,max}$ and IVD showed an equivalent negative correlation ($R^2 = 0.35$, $P = 0.001$), while $K_{leaf,max}$ was not correlated with VED ($R^2 = -0.25$, $P = 0.988$) or leaf area ($R^2 = 0.31$, $P = 0.148$). Among these species, P50 was not correlated with IVD ($R^2 = 0.43$, $P = 0.094$), VED ($R^2 = -0.12$, $P = 0.529$), or leaf area ($R^2 = 0.42$, $P = 0.097$).

BIOCLIMATIC RELATIONS.—Species showed extensive variation in mean scores for bioclimatic indices (full data in Table S2). In *Pitcairnia* ($N = 223$), leaf shape was a significant factor for species' bioclimatic distributions in terms of MAP ($F = 11.68$, $P < 0.001$; Fig. 4A), AI ($F = 13.80$, $P < 0.001$; Fig. 4B), AET/PET

TABLE 2. *Mean and standard error for leaf hydraulic traits of six early-diverging Bromelioideae and eight* Pitcairnia *species of contrasting leaf shape, based on at least six measurements per species.*

Group	Leaf shape	Species	$K_{leaf,max}$ (mmol/m^2 s MPa) $\pm$ SE	P50 (MPa) $\pm$ SE
Pitcairnia	Reduced	*Pitcairnia fuertesii*	5.31 $\pm$ 0.178	$-$1.89 $\pm$ 0.06
	Linear	*Pitcairnia poortmanii*	5.01 $\pm$ 0.139	$-$1.80 $\pm$ 0.04
	Linear	*Pitcairnia integrifolia*	4.85 $\pm$ 0.172	$-$1.73 $\pm$ 0.05
	Broad Linear	*Pitcairnia sceptrigera*	4.57 $\pm$ 0.194	$-$1.61 $\pm$ 0.06
	Broad Linear	*Pitcairnia imbricata*	4.50 $\pm$ 0.233	$-$1.56 $\pm$ 0.04
	Lanceolate	*Pitcairnia maidifolia*	4.04 $\pm$ 0.144	$-$1.41 $\pm$ 0.05
	Lanceolate	*Pitcairnia atrorubens*	3.89 $\pm$ 0.128	$-$1.37 $\pm$ 0.04
	Petiolate	*Pitcairnia undulata*	2.84 $\pm$ 0.178	$-$1.21 $\pm$ 0.03
Terrestrial Bromelioideae	Petiolate	*Cryptanthus beuckeri*	0.04 $\pm$ 0.001	$-$1.11 $\pm$ 0.03
	Lanceolate-Undulate	*Cryptanthus pickelii*	0.05 $\pm$ 0.002	$-$1.24 $\pm$ 0.03
	Lanceolate-Undulate	*Cryptanthus zonatus*	0.05 $\pm$ 0.003	$-$1.26 $\pm$ 0.02
	Linear/subulate-spinose	*Bromelia humilis*	0.07 $\pm$ 0.002	$-$1.29 $\pm$ 0.03
	Linear/subulate-spinose	*Cryptanthus maritimus*	0.08 $\pm$ 0.005	$-$1.39 $\pm$ 0.04
	Linear/subulate-spinose	*Ananas bracteatus*	0.07 $\pm$ 0.002	$-$1.44 $\pm$ 0.02

$K_{leaf,max}$ = maximal leaf hydraulic conductance; P50 = leaf water potential at 50 percent loss of maximal leaf hydraulic conductance.

(F = 11.90, P < 0.001; Fig. 4C), and P$_{seas}$ (F = 9.30, P < 0.001; Fig. 4D). The leaf shapes associated with high rainfall, aseasonal environments were lanceolate (N = 46), broad linear (N = 15), and petiolate (N = 25), as well as the unique tabuliform leaves of *P. tabuliformis* Linden. Mean values for bioclimatic indices were intermediate for species with reduced lanceolate (N = 9), bromelioid (N = 4), and linear (N = 78) leaves, although the range for linear-leaved species was very high in all indices. Variation was also high among species with reduced linear (N = 43) and reduced subulate (N = 2) leaves, but on average, these types tended to be associated with the most arid and seasonal environments.

In the terrestrial Bromelioideae (N = 123), similar but slightly weaker effects were observed (Fig. 5). Leaf shape was a significant factor for MAP (F = 7.05, P < 0.001; Fig. 5A), AI (F = 4.03, P = 0.004; Fig. 5B), AET/PET (F = 4.33, P = 0.003; Fig. 5C), and P$_{seas}$ (F = 5.15, P < 0.001; Fig. 5D). Lanceolate-undulate (N = 8) and linear/subulate-spinose leaves (N = 103) were associated with drier, more seasonal environments, while petiolate (N = 5) and spathulate (N = 3) leaves were associated with wetter, less seasonal environments. Linear leaves (N = 4) occurred in habitats of intermediate moisture levels and relatively strong seasonality. Thus broader, spathulate, or petiolate leaf morphology was linked with less arid, less seasonal environments in both *Pitcairnia* and the terrestrial Bromelioideae.

Among the experimental *Pitcairnia* species (N = 8), there were surprisingly strong negative correlations between $K_{leaf,max}$ and MAP (R^2 = 0.89, P < 0.001) and AI (R^2 = 0.78, P < 0.001), but not with AET/PET or P$_{seas}$ (P > 0.05). In these species, P50 was also strongly positively correlated with MAP (R^2 = 0.91, P < 0.001) and AI (R^2 = 0.87, P < 0.001). Among the experimental terrestrial Bromelioideae (N = 5, no bioclimatic data available for *Bromelia humilis*), there were no significant correlations

between $K_{leaf,max}$ or P50 and bioclimatic variables (P > 0.05), possibly due to the small sample size.

DISCUSSION

Through a combination of empirical characterization of the links between leaf morphology, venation architecture, and hydraulic function, and wide-ranging assessment of the correlations between leaf shape and bioclimatic relations, this investigation provides timely insights into the evolutionary diversification of an ecologically important family of Neotropical herbaceous angiosperms. By shedding light on the fundamental relationships between leaf structural-functional traits and climatic niche differentiation, the results presented here are of broad relevance to plant evolutionary ecology and highlight several key challenges for future research.

VARIATION IN LEAF SHAPE IN BROMELIAD GROUPS.—The diversity in leaf shape in the Bromeliaceae has long been recognized, but rarely investigated in terms of functional significance. Even just across the two lineages considered in this study, there is extensive variation in leaf shape, ranging from narrow linear forms, through subulate spinose forms, to broader lanceolate or petiolate blades. Moreover, different leaf shapes have clearly evolved convergently in distinct bromeliad lineages, including within genera. For example, lanceolate leaves may have arisen on multiple occasions within *Pitcairnia*. More comprehensive phylogenetic and biogeographical analyses for this important genus making use of wider taxonomic sampling and molecular data are on the way (Schutz *et al.* 2016). This will provide greater clarity regarding the evolutionary history and significance of leaf shape in *Pitcairnia*. Resolution of uncertainties regarding the phylogenetic relationships among the terrestrial Bromelioideae will also be critical to pursuing the same issues in that group.
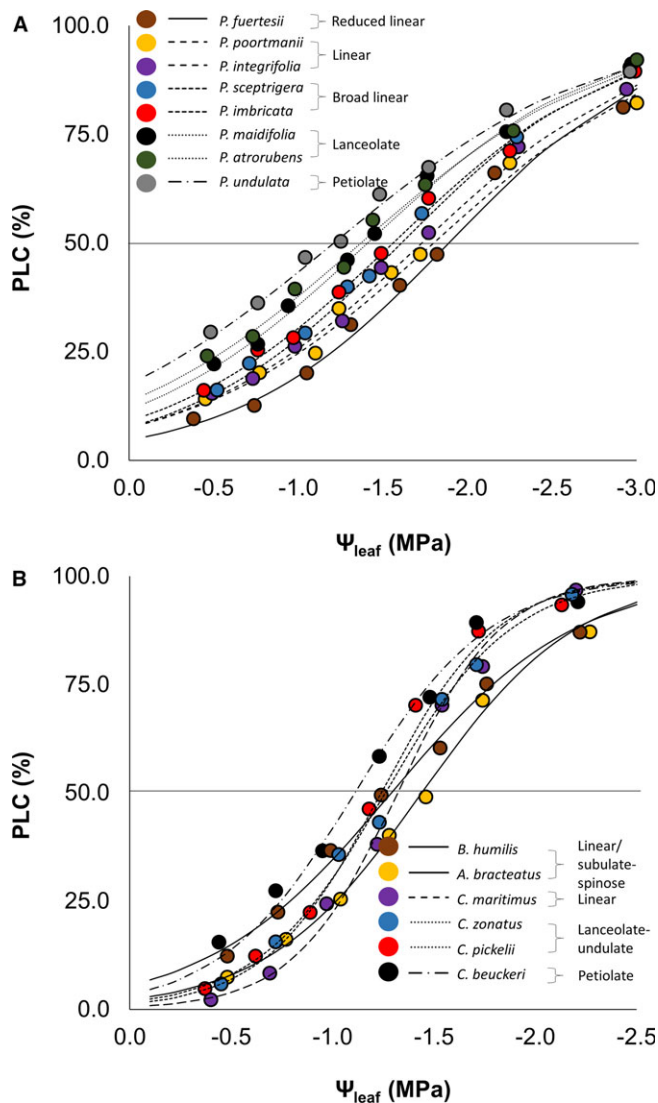
FIGURE 3. Hydraulic vulnerability curves for all bromeliad species used in the experimental part of this investigation. (A) *Pitcairnia* species ($N = 8$); (B) terrestrial Bromelioideae species ($N = 6$). PLC = percentage loss of maximal leaf hydraulic conductance.

Many of the leaf shapes that occur in *Pitcairnia* and the terrestrial Bromelioideae are also found in other bromeliad groups. For example, petiolate leaves are displayed by species in the epiphytic genera *Aechmea* and *Ronnbergia* (Bromelioideae), which adds to the compelling case for adaptive convergence, possibly underlain by a shared developmental basis. Similar patterns of recurrent evolution of leaf morphology have been identified in some other monocot groups (*e.g.*, Rudall 1990, 1994), while equivalent effects have been observed in leaf anatomy (*e.g.*, Horn *et al.* 2009). Recurrent evolution could arise from developmental canalization (Brakefield 2006), a phenomenon which could represent an important line of enquiry in the emerging field of bromeliad molecular genetics (Ming *et al.* 2015). Understanding the extent to which convergence in leaf form is associated with the same or different adaptive advantages requires consideration of the structure–function relationships linking leaf shape with environmental tolerances.

LINKAGES BETWEEN LEAF SHAPE AND VENATION ARCHITECTURE.—A key hypothesis tested in this investigation was that there should be evolutionary coordination between leaf shape and venation architecture (Niinemets *et al.* 2007, Nicotra *et al.* 2011, Sack *et al.* 2012). In particular, leaves with broader blades were expected to display higher values of IVD, and this was indeed borne out by the data. In the bromeliads, greater horizontal expansion of the leaf blade is associated with wider vein spacing, but not with a consistent increase in VED. This leads to apparent underinvestment in veins (*sensu* Zwieniecki & Boyce 2014) in species with petiolate or lanceolate broad-bladed leaves. Overinvestment in veins also occurs in some representatives of the terrestrial Bromelioideae, driven by particularly high VED value. However, variation in VED appears not to be closely related to leaf shape, suggesting there is no strong developmentally constrained coupling of chlorenchyma thickness and horizontal leaf expansion. These results represent an important contribution to knowledge of the relationships between leaf allometry and venation in the monocots, which (perhaps due to the perceived simplicity of monocot venation patterns) have received less attention than the dicots by investigators of plant vascular anatomy and hydraulics.

The strong correlation between leaf shape and size in the Bromeliaceae makes it difficult to account for leaf size as a confounding factor, and while this was not a primary objective of this investigation, it should be examined more closely in future work. Comparisons of the venation architecture and hydraulic properties of species of the same leaf shape but differing in leaf size would be fruitful in this respect.

STRUCTURAL DETERMINANTS OF LEAF HYDRAULICS.—Among the terrestrial bromeliads examined here, divergences in venation architecture are closely related to differences in leaf hydraulic properties. The strong negative correlations between IVD and $K_{leaf,max}$ across both the experimental *Pitcairnia* species and terrestrial Bromelioideae species suggest that venation density is an important determinant of hydraulic supply in leaves of these plants. This is consistent with a large corpus of evidence for many plant groups (Brodribb *et al.* 2007, Boyce *et al.* 2009, Brodribb & Feild 2010, Scoffoni *et al.* 2016), and could be a key mechanism underpinning the reduced hydraulic capacity of species with broader leaf blades. The positive correlation between IVD and P50 and negative correlation between $K_{leaf,max}$ and P50 in *Pitcairnia* species are notable, since they appear to contradict the hydraulic safety versus efficiency hypothesis. In many comparisons within and between plant groups, species with higher hydraulic capacity are found to be more vulnerable to hydraulic dysfunction, although the strength of this phenomenon is acknowledged to be highly dependent on which plants are analyzed, which organ-specific conductances are quantified, and whether whole-organ or xylem-specific conductance is considered (Gleason *et al.* 2016). The fact that the relationship between $K_{leaf,max}$ and P50 in *Pitcairnia* species takes the opposite sign in to
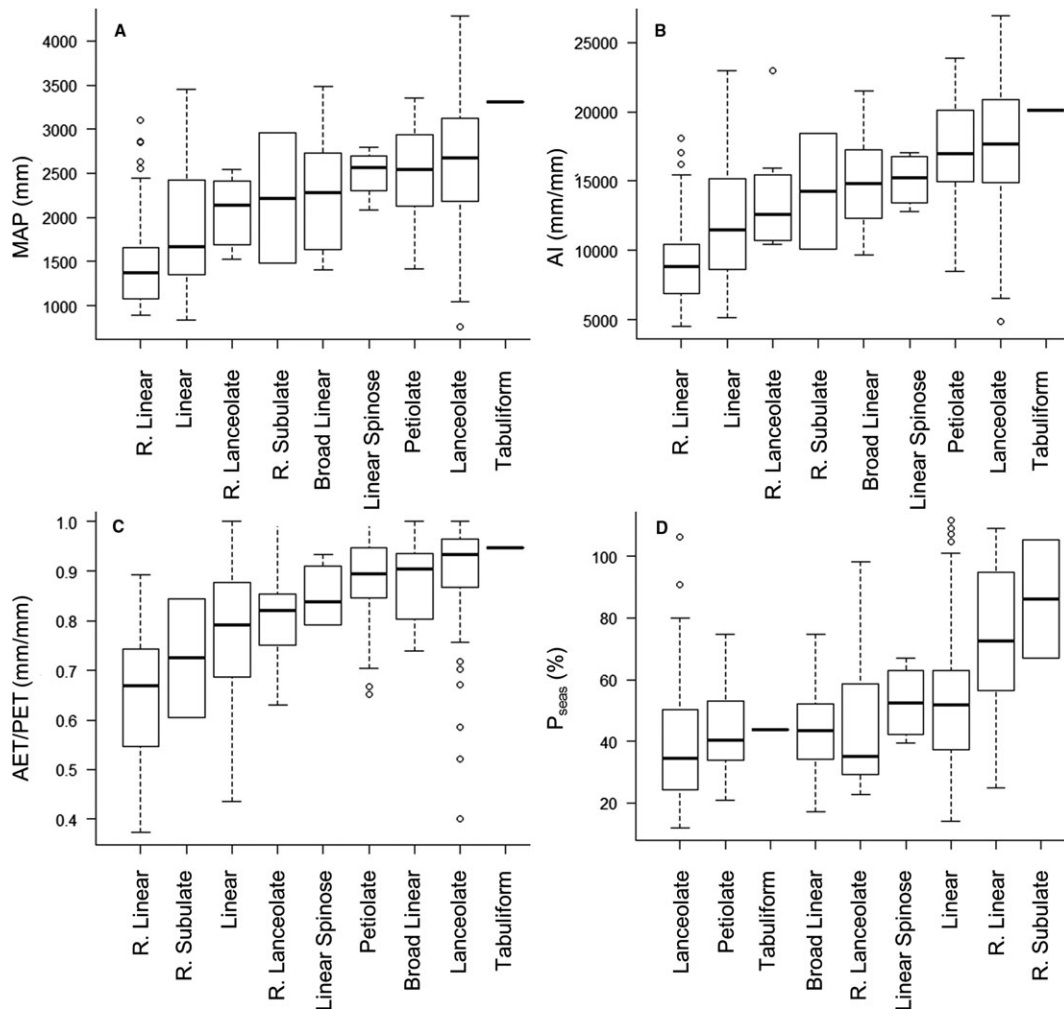
FIGURE 4. Variation in mean scores for bioclimatic variables among *Pitcairnia* species (*n* = 223) of contrasting leaf shape: (A) mean annual precipitation (MAP); (B) aridity index (AI); (C) ratio of actual to potential evapotranspiration (AET/PET); (D) precipitation seasonality (P_{seas}). Leaf shape abbreviations: R. Linear = reduced linear; R. Lanceolate = reduced lanceolate; R. Subulate = reduced subulate.

that predicted by the safety versus efficiency hypothesis could either be the result of concerted convergence or could suggest that P50 is governed by factors different from those controlling $K_{leaf,max}$. With respect to the latter possibility, recent insights into the importance of the extraxylary compartment as a determinant of hydraulic vulnerability could be relevant (Scoffoni et al. 2017). Very little is known regarding the role of aquaporins in the regulation of bromeliad leaf hydraulics, beyond their apparent significance in trichome-mediated rehydration (Ohrui et al. 2007). Further research will be needed to explore whether the spatiotemporal expression dynamics of different aquaporin isoforms can explain water distribution and remobilization processes in bromeliad leaves in response to changing water status (Hachez et al. 2008, Sade et al. 2014). Meanwhile, the absence of any statistically significant relationships among venation architecture metrics and P50 in the terrestrial Bromelioideae could be due to the low sample size for this group, or again point to non-vascular factors as being more important determinants of P50.

Considering IVD and VED together, it is clear that many bromeliads depart from the comparatively tight 1:1 coordination that has been proposed to provide optimal hydraulic function and has been an important feature of angiosperm evolution (Noblin et al. 2008, Zwieniecki & Boyce 2014). This has important consequences for diversity of hydraulic design in bromeliad leaves and may involve critical interactions with other leaf anatomical traits. Underinvestment in veins (IVD > VED) has been suggested to run the risk of heterogeneous Ψ_{leaf} developing across the lamina, which could be physiologically damaging (Zwieniecki & Boyce 2014). However, in the leaves of many bromeliad species that appear to be underinvesting in veins, including *Pitcairnia* spp., a considerable proportion of the space between veins may be occupied by aerenchyma rather than mesophyll cells. While the adaptive significance of these air channels is probably manifold and difficult to disentangle (Males 2016), the upshot in terms of hydraulic design is that the field of mesophyll cells irrigated by each leaf vein is reduced, effectively resetting the
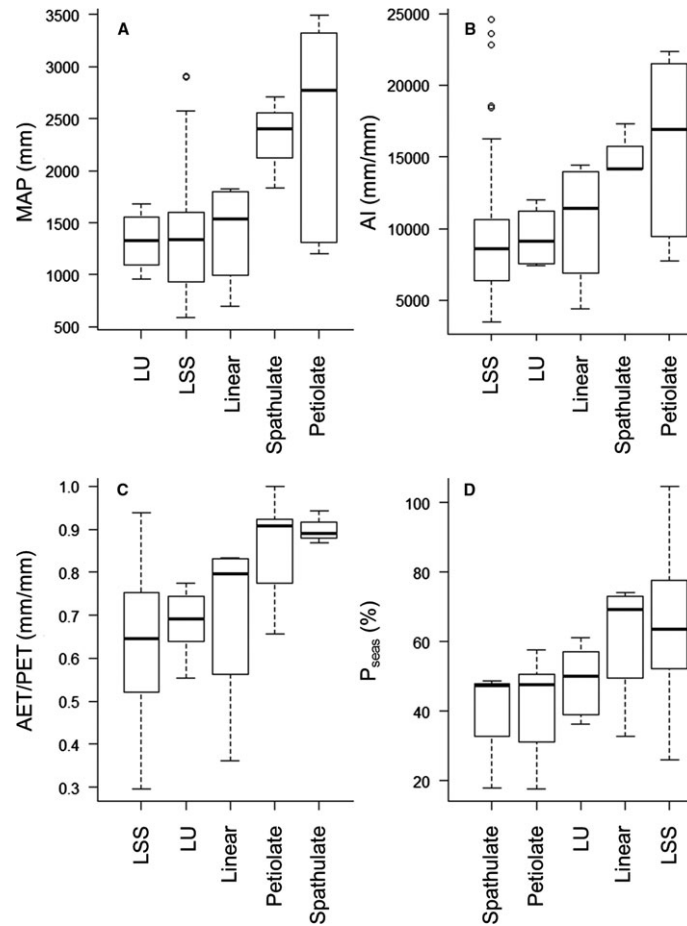
FIGURE 5. Variation in mean scores for bioclimatic variables among terrestrial Bromelioideae species ($N = 123$) of contrasting leaf shape: (A) mean annual precipitation (MAP); (B) aridity index (AI); (C) ratio of actual to potential evapotranspiration (AET/PET); (D) precipitation seasonality (P_{seas}). Leaf shape abbreviations: LSS = linear/subulate-spinose; LU = lanceolate-undulate.

balance between IVD and VED. Meanwhile overinvestment in veins (IVD < VED) in succulent bromeliads, including some of the terrestrial Bromelioideae sampled for empirical study in this investigation, could relate to adaptation for hydraulic recharge of leaf water-storage tissue (Males 2017, Males, in review). In some terrestrial bromeliad species (*e.g.*, *Puya* spp.), the hydraulic significance of overinvestment in veins could be somewhat mitigated by the presence of bundle sheath extensions, which can provide a low-resistance pathway for extraxylary water transport to the epidermis (Buckley *et al*. 2011, 2015, Zsogon *et al*. 2015, Males 2016).

Besides variation in the fundamental venation architecture metrics IVD and VED, several other aspects of vascular anatomy are likely to influence the hydraulic properties of bromeliad leaves. First, the differentiation of vein orders could affect both hydraulic capacity and resilience to xylem embolism (McKown *et al*. 2010). Various hierarchical venation patterns occur in the Bromeliaceae, and research is currently underway to determine the functional significance of this structural diversity. In the context of the bromeliad groups considered in this study, the presence of midribs in

most *Pitcairnia* species could be of special significance if they make a disproportionate contribution to hydraulic supply in addition to their probable mechanical role. At the other end of the scale, the involvement in bromeliad leaf hydraulics of minor commissural veins, which run perpendicular to the larger parallel veins, is unknown (Males 2016). In some species (*e.g.*, *Vriesea* spp.), the photosynthetic chlorenchyma is concentrated around these commissural veins, which may be suggestive of a specialized role in assimilate distribution (Altus & Canny 1982, Fritz *et al*. 1989, Benzing 2000). However, the degree of reticulation afforded by commissural venation could be of hydraulic significance, by providing alternative routings for water transport that could facilitate the bypassing of embolisms (Altus & Canny 1985, Sperry 1986, Sack *et al*. 2004, 2008). Further work will be needed to explore this possibility. Finally, the anatomy of the vascular bundles and individual xylem conduits could be an important source of variation in hydraulic function between species. Leaf xylem vessels are highly reduced or absent in some bromeliad lineages (Tomlinson 1969), but no research has yet explored the functional significance of this observation. Similarly, variation in bundle sheath suberization in

the Bromeliaceae is of potentially profound physiological importance (Tomlinson 1969, Males 2016), but has not been systematically investigated.

FROM WATER DISTRIBUTION TO SPECIES DISTRIBUTIONS.—Spatial data portals such as GBIF represent a valuable resource for ecologists, but data obtained from them must be interpreted carefully due to spatiotemporal sampling biases and incomplete metadata (*e.g.*, Beck *et al*. 2014). When utilized prudently and not overinterpreted, these data can provide valuable insights into questions in plant biogeography and evolutionary ecology (*e.g.*, Spalink *et al*. 2016).

The statistically significant relationships between leaf shape and bioclimatic distributions found in this investigation among *Pitcairnia* species and terrestrial Bromelioideae species can be at least partly explained by divergences in venation architecture and hydraulic function between leaf shapes. In these terrestrial bromeliad groups, certain leaf shapes clearly tend to be associated with specific regions of bioclimatic space. Notably, petiolate leaves and other morphologies involving broad leaf blades are generally restricted to environments with higher levels of moisture and lower seasonality. In these environments, low hydraulic conductance and relatively high hydraulic vulnerability may be permissible, whereas in more exposed and arid habitats, this trait combination would be highly disadvantageous (Males & Griffiths 2017). Because wider leaf blades have evolved independently in multiple lineages which are all restricted to more mesic habitats, there is natural replication to support this line of argument. As discussed above, further resolution of phylogenetic relationships will help add to this picture in future.

The relevance of variation in leaf hydraulic conductance and vulnerability to plant–environment relations is intimately linked with stomatal behavior (Bartlett *et al*. 2016, Sperry *et al*. 2016). The stomatal morphology of bromeliads is highly variable, particularly in terms of stomatal density and the micromorphology of the stomatal complex (Tomlinson 1969, Males 2016). No reports have been published on the degree of coordination between stomatal density, IVD, and leaf area in the bromeliads, but in other plant groups, this coordination is strong and considered to be of great physiological and evolutionary significance (Zhang *et al*. 2012, Brodribb *et al*. 2013). The developmental link between vein and stomatal density maintains a balance between water supply to the leaf and atmospheric demand (Carins Murphy *et al*. 2012). Evolution of stomatal size and maximal conductance has been an important component of ecological diversification in the Bromeliaceae and is associated with adaptive variation in stomatal sensitivity and kinetics in response to environmental stimuli (Males & Griffiths, in review). Another important aspect of stomatal biology that could modify the interactions between leaf hydraulic capacity and species bioclimatic relations is photosynthetic pathway. Whereas all *Pitcairnia* species are C_3 plants and therefore display stomatal opening exclusively during the light period, the terrestrial Bromelioideae include a mixture of C_3 and CAM lineages (Crayn *et al*. 2015). Since CAM involves primarily nocturnal stomatal opening, the leaf-air vapor pressure deficit (VPD), which acts as the driving force for transpiration, is generally lower for

CAM species than for C_3 species (Osmond 1978). This enables CAM bromeliads to achieve high levels of water-use efficiency, and presumably reduces the selective pressure for high leaf/plant hydraulic conductance in arid environments (Males & Griffiths 2017). Given the apparent links between $K_{\text{leaf,max}}$, IVD, and leaf width, origins of CAM might therefore allow the evolution of wider leaves at no physiological cost in terms of a mismatch between hydraulic supply and demand. This could explain the apparently weaker relationships between leaf shape, hydraulic function, and bioclimatic relations in the terrestrial Bromelioideae, where CAM is frequent, compared with C_3 *Pitcairnia*.

A related issue is the hydraulic capacitance of leaves, another factor which could confound the relationship between leaf morphology and water use. Depending on anatomical and biochemical control of leaf hydraulic design (Blackman & Brodribb 2010), the presence of significant levels of leaf water storage could provide a symplastic reservoir for transpiration, effectively decoupling gas exchange from at least the vascular component of $K_{\text{leaf,max}}$. In this connection, it is interesting to note that within the genus *Bromelia*, abaxial water-storage tissue occurs only in the small clade which includes the species with petiolate and spathulate leaves (Monteiro *et al*. 2011). Since the stomata are exclusively abaxial, if $K_{\text{leaf,max}}$ is limited by the venation architecture associated with these leaf shapes, abaxial water-storage tissue could provide an important reservoir to buffer against rapid changes in leaf water status following any sudden increase in evaporative demand. Meanwhile, the combination of low cellular drought tolerance with the use of stored water to buffer chlorenchyma water potential during drought is a key characteristic of bromeliad ecophysiology (Nowak & Martin 1997, Males & Griffiths 2017, in review). Internal water remobilization could be important in partially mitigating the impact of leaf shape and venation architecture on hydraulic limitations of gas exchange in the succulent terrestrial Bromelioideae.

Beyond the leaves, the hydraulic properties of the roots and stems of terrestrial bromeliads remain almost entirely unexplored (Males 2016). Tubers and rhizomes occur in many species, potentially conferring a high resistance to water transport. Conversely, the evolution of root xylem vessels with simple perforation plates in *Pitcairnia* could strongly enhance root hydraulic conductance in these species (Tomlinson 1969).

Another important consideration is the connection between leaf morphology and the expression of water-capturing tanks. Species with non-petiolate leaves of various morphologies may be able to form at least rudimentary axillary tanks, and in some cases, this can make a significant contribution to the overall water budget of the plant (*e.g.*, Sakai & Sanford 1980). By contrast, petiolate species by definition cannot form axillary tanks and must, therefore, be more strongly dependent on root-mediated water uptake. One possible ecological ramification is that this could in principle tend to restrict petiolate species to soils with higher water-retention capacity.

Future work could seek to establish the generality of the leaf structure–function–climate relationships identified in *Pitcairnia* species and terrestrial Bromelioideae species across other

bromeliad lineages with diverse leaf shapes (*e.g.*, *Brocchinia*, *Tillandsia*). In addition, the potential relevance of interactions between leaf morphology, anatomy, and hydraulics in the convergent origins of major adaptive syndromes such as epiphytism and terrestrial xerophytism warrants further investigation.

INTERACTIONS WITH OTHER ASPECTS OF LEAF ECOPHYSIOLOGY.—Leaf hydraulics is not the only mechanism by which leaf shape can interact with species' bioclimatic relations. Other physiological processes that interact with leaf shape include the boundary layer resistance to gas exchange and leaf temperature relations (*e.g.*, Nicotra *et al.* 2008). For instance, the boundary layer resistance will tend to be higher in broader leaves (Schuepp 1993), which translates to the petiolate and lanceolate morphologies seen in the Bromeliaceae. By reducing the maximum potential rate of transpirational water loss, this effect could partially offset the hydraulic limitations imposed on petiolate/lanceolate species by the reduction in $K_{leaf,max}$ associated with wider vein spacing.

The light relations of bromeliads are closely linked with leaf morphology (Benzing 2000), and while hydraulic limitations may be crucial in restricting species with broader leaf blades to more humid environments, the selective pressures that favor broad leaf blades in those environments are probably associated primarily with light capture. Particularly in shaded forest-floor habitats where, for example, many petiolate *Pitcairnia* species occur, broad leaf blades and petioles of flexible length and angle could help to maximize the interception of diffuse light and of sunflecks (Chazdon & Pearcy 1991). However, at present, relatively little is known of the photosynthetic ecology of these and other understorey and forest-floor bromeliads of specialized leaf morphology.

While the measurements presented here were made on plants grown in standardized conditions, there are reports of acclimatory modification of leaf shape in some bromeliad species (*e.g.*, Scarano *et al.* 2002, Cavallero *et al.* 2009). This could add an important additional dimension to the story. However, to date, there has been no systematic analysis of plasticity in leaf form across the bromeliads. New research into the extent of foliar morphological and anatomical plasticity in these plants should include an integrated physiological component in order to determine the relevance of this plasticity for environmental relations. Further work is also needed to establish the physiological significance of developmental changes in morphology (Zotz *et al.* 2004, Meisner *et al.* 2013), and the interaction between leaf shape and leaf/plant size (Schmidt & Zotz 2001, Zotz *et al.* 2001).

CONCLUSIONS

Relationships links between morphology, anatomy, physiology, and bioclimatic ecology are fundamental to explaining the evolution of diversity in major plant groups such as the Neotropical bromeliads. Strong developmental coupling of leaf shape and venation architecture determines leaf hydraulic properties, which are associated with the divergent and convergent evolution of environmental niche in two important groups of terrestrial bromeliads. The hydraulic limitations of petiolate and lanceolate morphologies tend to restrict such phenotypes to moist, aseasonal zones. Further exploration of the relationships reported here both in the bromeliads and in other groups of tropical monocots, making use of the increasing availability of new technologies, empirical methodologies, and physiological modeling approaches will advance our understanding of the biology of these ecologically valuable but underappreciated plants.

ACKNOWLEDGMENTS

DATA AVAILABILITY

All data used in this study are reported in the article and Supporting Information.

SUPPORTING INFORMATION

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

FIGURE S1. Examples of variation in leaf form in the focal groups of bromeliads used in this investigation.

FIGURE S2. Phylogenetic distribution of leaf shapes across 56 species in the genus Pitcairnia.

FIGURE S3. Phylogenetic distribution of leaf shapes across 110 species in the terrestrial Bromelioideae.

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