

Replicated radiations in the South American Marsh Pitcher genus *Heliamphora* (Sarraceniaceae) lead to convergent carnivorous trap morphologies

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Abstract

The evolution of carnivorous pitcher traps across multiple angiosperm lineages represents a classic example of morphological convergence. Nevertheless, no comparative study to-date has examined pitcher evolution from a quantitative morphometric perspective. In the present study, we used comparative morphometric approaches to quantify the shape space occupied by *Heliamphora* pitchers and to trace evolutionary trajectories through this space to examine patterns of divergence and convergence within the genus. We also investigated pitcher development, and in particular, how the packing of pitchers is affected by crowding, a common condition in their natural environments. Our results showed that *Heliamphora* pitchers have diverged along three main axes in morphospace: pitcher curvature, nectar spoon elaboration, and pitcher stoutness. Both curvature and stoutness were correlated with pitcher size, suggesting structural constraints in pitcher morphological evolution. Among these four traits (curvature, spoon elaboration, stoutness and size), all but curvature lacked phylogenetic and showed marked convergence across the phylogeny. We also observed tighter packing of pitchers in crowded conditions, and this effect was most pronounced in curved, slender pitchers. Overall, our study demonstrates that diversification and convergent evolution of carnivory-related traits extends to finer evolutionary timescales, reinforcing the notion that ecological specialization may not necessarily be an evolutionary dead end.

摘要 Abstract (Simplified Chinese)

食虫植物的瓶状捕虫器在不同被子植物谱系中的演化是形态趋同进化的经典案例。然而，迄今为止还没有研究使用系统发育比较分析法与计量方法来研究瓶状捕虫器的演化。本研究使用了上述手段来研究南美沼泽瓶子草 (*Heliamphora*) 的瓶状捕虫器是如何占据形态空间，并在捕虫器的形态空间内追踪它们的演化轨迹，以研究沼泽瓶子草属捕虫器的趋异与趋同的演化模式。同时，本研究还探讨了沼泽瓶子草属植株（捕虫瓶）的发育模式，特别是在沼泽瓶子草在原生地的发育是如何受到高种群密度（拥挤）的影响。本研究的结果表明，沼泽瓶子草属捕虫瓶在形态空间里沿着三个主要形态轴分化：捕虫瓶的弯曲度、蜜匙的精细程度和捕虫瓶的粗壮程度。捕虫瓶的弯曲度和粗壮度均与瓶子的大小相关，表明捕虫瓶在形态进化中存在结构约束。在这四个形态特征中（捕虫瓶曲率、蜜匙精细程度、

捕虫瓶粗壮度与大小)，除了捕虫瓶曲率，其他的形态特征都没有显示系统发育信号。在南美沼泽瓶子草属的进化中，蜜匙精细程度、捕虫瓶粗壮度与大小都显示了趋同演化。本研究还阐明了瓶子草植株在拥挤条件下，捕虫瓶会更加紧密地排列，而且这种效应在有着弯曲、细长的捕虫瓶的物种中最为显著。总体来说，本研究显示与食肉有关的特征的趋异和趋同演化发生在更微观的时间尺度上，也表明了生态专化或许并不是生物进化的死胡同。

Keywords: Sarraceniaceae, *Heliamphora*, Carnivorous Plants, Morphological Evolution, Phylogenetic Comparative Methods

Introduction

Pitcher plants are one of the most dramatic examples of convergent evolution in carnivorous plants, with at least 6 independent origins in angiosperms (Fleischmann et al. 2018). The pitchers are specialized leaves that form cavities filled with an often viscous liquid containing a suite of digestive enzymes (Adlassnig, Peroutka, and Lendl 2011). Pitcher plants utilize these pitchers to attract, drown, and consume small animal prey in order to obtain nutrients not available from the abiotic environment (Juniper, Robins, and Joel 1989; Givnish et al. 2018).

Among the independently evolved pitcher plant lineages, *Nepenthes* (Caryophyllales), Sarraceniaceae (Ericales), and *Cephalotus follicularis* (Oxalidales) are commonly considered as the true pitcher plants due to their highly specialized carnivorous pitchers. Moreover, the true pitcher plants are considerably older (all emerged during the Cretaceous) as compared to the more recently evolved (< 3 mya) pitcher lineages in Poales (e.g. *Brocchinia reducta* and *Paepalanthus bromelioides*), which have structurally simpler pitchers morphologically similar to their tank forming relatives (Magallón et al. 2015; Cross et al. 2018). With the exception of the monotypic Australian pitcher plant *C. follicularis*, both *Nepenthes* (the tropical pitcher plants) and Sarraceniaceae (the American pitcher plants) are true pitcher lineages with high species diversity, together comprising more than 98% of all extant pitcher plant taxa (McPherson et al. 2011; McPherson and Schnell 2011; Fleischmann et al. 2018; Murphy et al. 2020).

In addition to dispersal and vicariance (Ellison et al. 2012; Naczi 2018; Murphy et al. 2020), adaptive radiation played an important role in the diversification of *Nepenthes* and Sarraceniaceae (Pavlovic 2012; Clarke et al. 2018; Thorogood, Bauer, and Hiscock 2018). The emergence of pitcher traps has likely facilitated the diversification in *Nepenthes* and Sarraceniaceae by allowing them to adapt to new ecological niches, resulting in dramatic morphological variations in pitcher traps (Pavlovic 2012; Clarke et al. 2018; Thorogood, Bauer, and Hiscock 2018). Many *Nepenthes* species have diversified and further modified their pitcher traps to specialize in novel symbiotic relationships (e.g. ant-mutualism in *N. bicalcarata*) or novel nutrient acquisition strategies (e.g. detritus-feeding in *N. ampullaria*, fecal-feeding in *N. lowii*, and subterranean-feeding in *N. pudica*) (Thornham et al. 2012; Moran et al. 2012; Gilbert et al. 2022; Dančák et al. 2022). A similar radiation has occurred across Sarraceniaceae lineages,

giving rise to many species with novel trap types (e.g. lobster traps of *Darlingtonia californica* and *Sarracenia psittacina*), trapping mechanisms (e.g. superhydrophilic hairy surface of *Heliophora nutans*), symbiotic relationship (e.g. *S. purpurea* and commensal *Wyeomyia smithii*), and nutrient specializations (Jaffe et al. 1992; Peterson et al. 2008; McPherson et al. 2011; Bauer et al. 2013; Naczi 2018). Pitcher morphology in Sarraceniaceae and *Nepenthes* are also plastic to varying degree, affected by both biotic (e.g. crowding or competition from neighboring plants) and abiotic factors (e.g. extreme microclimate) (Brewer 1999; McPherson et al. 2011; McPherson and Schnell 2011; Clarke et al. 2018).

Despite the wide variety of morphologies associated with pitcher traps, few comparative studies have focused on the morphological evolution of carnivorous traps, and none with quantitative methods. Morphometric analyses allow us to define the spaces occupied by phenotypic combinations as well as those that are empty (Stayton 2019; Segall et al. 2020; Jardine et al. 2022) and, in the context of a phylogeny, identify instances of morphological convergence (Papadopoulos et al. 2013; Smith and Kriebel 2018). This quantitative shape information also sets the stage for rigorous hypothesis testing (Smith and Kriebel 2018; Dellinger et al. 2019; Kriebel et al. 2022).

Here we focus on the evolution of pitcher morphologies in the South American marsh pitcher plants *Heliophora* (Sarraceniaceae). This genus is endemic to the Guiana Highlands, and is the most species-rich genus in the family, with 23 extant species and several yet to be described (McPherson et al. 2011). The major clades are thought to have emerged through both vicariance and dispersal during the Miocene with more recent diversification in each clade driven by vertical displacement during the Pleistocene glacial-interglacial cycles (Liu and Smith 2021). The adult pitchers varying dramatically in pitcher shape, size, nectar spoon structure and many other taxonomically important characters across species (Figure 1) (McPherson et al. 2011). It has been suggested these diverse morphologies may relate to differences in microclimate (Nerz 2004), prey attraction strategies (Wistuba, Harbarth, and Carow 2001; Wistuba, Carow, and Harbarth 2002), and/or shifts in prey composition (Jaffe et al. 1992). Furthermore, structural stability of the pitcher could pose constraints on the morphological evolution of *Heliophora* pitchers, limiting available pitcher morphospace.

In the present study, we used comparative morphometric approaches to quantify the shape space occupied by *Heliophora* pitchers and to trace evolutionary trajectories through this space. Building on previous phylogenetic work in the genus (Liu and Smith 2021), we also tested for phylogenetic signal in pitcher shape and investigated how pitcher shape evolution may be related to pitcher size. In particular, we hypothesize that aspects of shape evolution may be tightly correlated with height given the structural constraints imposed by supporting high volume of pitcher fluid. In light of the dense packing in many natural populations (McPherson et al. 2011), we also considered how pitcher development responds to crowding and tested the hypothesis that pitcher angle (the angle between two most recently developed pitchers) shrinks in crowded conditions to allow for tighter packing. In interpreting these results, we discuss the potential

ecological significance of *Heliamphora*'s diverse pitcher morphologies and ability to modify pitcher development in response to varying conditions.

MATERIALS AND METHODS

Taxon sampling and phylogenetic framework

We sampled 23 described and 2 additional yet to be described taxa of *Heliamphora* for this study (Supplementary Table 1). Data for adult pitcher maximum pitcher size were obtained from the monograph of *Heliamphora* (McPherson et al. 2011). We used the recently published phylogeny of 24 of the 25 *Heliamphora* taxa (Liu and Smith 2021) to reconstruct the evolution of adult pitcher shape, maximum adult pitcher size and pitcher angle. This phylogeny lacks *H. macdonaldae*, which is thought to be sister to *H. tatei* based on similarities in pitcher morphology and distributional range (Maguire 1978; McPherson et al. 2011; Liu and Smith 2021). We manually included this species in the tree (Figure 1) by placing it sister to *H. tatei* and setting their split to 0.75 mya, which is the average node depth of other sister pairs in the W lineage, i.e., (*H. ceracea*, *H. hispida*) and (*H. neblinae*, *H. parva*).

Morphometric analysis of pitcher shapes

Adult pitcher shape was characterized using images of fully opened and matured pitchers taken from living individuals. For each taxon, a single adult pitcher from the individual sampled for the phylogeny was photographed in lateral view, providing a two-dimensional representation of the adult pitcher shape along the proximal-distal axis. The lateral view captured shape variation among major structural components involved in carnivorous functions, such as the shapes of nectar spoon and pitcher body associated with prey attraction and prey capture/retention, respectively (Supplementary Figure 1). These images were converted into silhouettes in Affinity Designer 1.10.5, which were then transformed into outlines using the R package Momocs (Bonhomme et al. 2014). Also using Momocs, the outlines were converted into lists of two-dimensional coordinates describing the polygons and then centered. Six landmarks were placed on each outline to delineate pitcher functional zones according to McPherson et. al. (2011) and avoid twisting of the outlines (Supplementary Figure 1). Out of the six landmarks, one was placed at the bottom of the pitcher, and two were placed at the constriction of the pitcher body, close to the water line of the pitcher fluid and the drainage structure (zone 3), together defining the mid-lower pitcher body digestive zone (zone 4) where nutrients from digested prey would be absorbed. Morphologically, *Heliamphora* pitchers are similar to those of *Sarracenia* and *Darlingtonia*, except that they lack a covering at the apex and instead possess a drainage structure that allows rainwater to escape. Two landmarks were placed at the base of the nectar spoon, which separated the mid-upper pitcher prey capture zone (zone 2) from the nectar spoon structure (zone 1) responsible for prey attraction. Finally, an additional landmark was placed at the tip of the nectar spoon. Using the landmarks, shape variation of adult pitchers was quantified using elliptical Fourier analysis (EFA) that decomposed the outlines into 21 harmonics, each with four coefficients. After aligning the outlines using Full Generalized Procrustes alignment in Momocs, a principal component analysis (PCA) was used to summarize the 84 coefficients

resulting from the EFA. Taxa were plotted along the PC axes to illustrate their distribution in morphospace. Blomberg's K (Blomberg, Jr, and Ives 2003) for the PCs were estimated to infer phylogenetic signals in pitcher shapes by using 1000 randomizations of the data across the phylogeny to test if the value was significantly different from $K=0$ (no phylogenetic signal). To estimate the ancestral pitcher morphology, we computed the ancestral states of shape PCs (PC1, PC2, and PC3) and maximum pitcher size using the 'fastAnc' function in R package phytools (Revell 2012), with each trait individually estimated.

Effects of crowding on pitcher angle

We used living collections of *Heliamphora* species to characterize the angle of pitcher emergence and examine the effect of crowding on this angle. Depending on availability of material, we sampled 3 to 63 individuals per taxa for all 25 taxa. For each sampled individual, images were taken directly above each growing point, i.e., where new pitchers were constantly produced (Supplementary Figure 2). For each growing point, a line was drawn from the growing point (rosette center) to the tip of the nectar spoon of the youngest (the most recently developed) pitcher. Similarly, a second line was drawn for the second youngest pitcher. Pitcher angle (PA) was measured as the angle between the two straight lines using the software ImageJ (Schneider, Rasband, and Eliceiri 2012). We replicated these measurements for both juvenile and adult pitchers, since pitchers are dimorphic in *Heliamphora* (McPherson et al. 2011) and may respond differently to crowding. To test whether PA is affected by the presence of neighboring individuals (i.e., if crowding reduces PA), phylogenetic paired t-tests were performed for both adult and juvenile pitchers using the 'phyl.pairedttest' function in R package phytools (Revell 2012). Similarly, the effect of developmental stage on PA was also tested with phylogenetic paired t-tests. In addition to phylogenetic t-tests, regular t-tests were also conducted for each comparison. An additional chi-squared test was performed to test whether the drastic bending observed in juvenile pitchers was associated with the presence of neighbors. Similar to the PCs, we computed Blomberg's K for PA in adult and juvenile individuals growing with and without neighbors, and then tested if the value was significantly different from zero.

Model testing for correlations among morphological traits

We used phylogenetic generalized linear models (PGLM) (Paradis and Claude 2002) to test the relationship between shape variation and maximum pitcher size in adult pitchers. For some combinations of shape PCs and size, we predicted a non-linear relationship. Thus, we compared linear and quadratic models for each pair and present results for the best fitting model (that with the lowest AIC score). We fit these models using the R package 'phylolm' with 1000 bootstraps (Ho and Ane 2014). We assumed a correlation structure based on the Brownian motion (BM) process, which models stochastic evolution in those traits. Similarly, the relationships between shape variation and pitcher angle were also tested using PGLM in adult pitchers growing singly and ones growing with neighbors present. Assuming BM process, the PCs were regressed against pitcher angle with PGLM. Taxa with incomplete pitcher angle data due to sampling limitations were dropped from the analysis.

RESULTS

Morphospace of *Heliamphora* Pitchers

The PCA of harmonic coefficients from the elliptic Fourier analysis captured 79% of shape variation (SV) of adult pitchers in the first three PCs (Figure 2). PC1, accounting for 33.4% of SV, describes ventral pitcher body curvature or concavity. While the negative and positive PC1 values described concave and convex pitcher shapes, respectively, PC1 values closer to zero represented pitchers with straighter ventral bodies. PC2, comprising 29.5% of SV, describes the elaboration (shape, relative size, and position) of nectar spoon with negative and positive values, respectively, representing attached (to the dorsal pitcher body) and highly elaborated structures. PC3, explaining 16.1% of SV, is related to pitcher stoutness, or the general pitcher body length-to-width ratio. On the PC3 axis, negative values described more slender pitcher body shapes whereas positive values corresponded to bulkier body shapes.

We found that all of the measured pitcher traits, except for curvature, lacked significant phylogenetic signal. Specifically, we found Blomberg's K was not significantly different from zero for nectar spoon elaboration (PC2, $K = 0.06$, $p = 0.886$), pitcher stoutness (PC3, $K = 0.09$, $p = 0.391$), or maximum pitcher size ($K = 0.12$, $p = 0.198$), consistent with the frequent patterns of convergence across the phylogeny (Figure 3). By contrast, significant phylogenetic signal was detected in pitcher curvature (PC1, $K = 0.18$, $p = 0.018$) (Figure 3). Looking at the phylogeny, pitcher curvature appears conserved in W+E1 and E2a clades but not in other lineages. In W+E1 clade, all species were associated with convex pitcher body to varying degrees ($PC1 > 0.02$), with the exception of *H. chimantensis*, which was associated with a straighter pitcher body ($PC1 = -0.00321$). In E2a, the adult pitchers are all associated with very concave pitcher body shapes ($PC1 < -0.04$).

Relationships between Pitcher Shape and Size

Model comparisons indicated a strong quadratic relationship between pitcher curvature (PC1) and maximum pitcher size (Supplementary Table 2). As pitchers become straighter (i.e., less concave or convex), the maximum pitcher size generally increases (Figure 4), consistent with the notion that more erect pitchers can accommodate more pitcher fluid (see discussion). A strong linear relationship was found between pitcher stoutness (PC3) and maximum pitcher size, with larger pitchers being stouter (Figure 4). No relationship was found between nectar spoon elaboration (PC2) and maximum pitcher size (Figure 4).

Relationship of pitcher angle to growth conditions and pitcher shape

Our results show that *Heliamphora* growing in crowded conditions tend to put out new pitchers at a tighter angle than those in isolated conditions. A significant difference in pitcher angle (PA) was detected between adult pitchers growing in isolated vs. crowded conditions with both

phylogenetic and non-phylogenetic t-tests (phylogenetic mean difference [PMD] = 13.64°, $p = 0.002$; $p < 0.0001$ for regular t-test, Figure 5). In juveniles, a significant difference was detected in regular t-test ($p < 0.0001$) but not in the phylogenetic t-test (PMD = 6.36°, $p = 0.199$). Moreover, we found juvenile pitchers tend to bend unnaturally when their growth was obstructed by neighboring pitchers (chi-squared test, $p < 0.001$), possibly due to the more slender and parallel to ground body shape. No significant difference in PA was found between juvenile and adult pitchers within each condition (crowded or not) using either t-test (Figure 5). Raw measurements for all taxa, stages and conditions are shown in Supplementary Figure 3. No phylogenetic signal was detected in PA across developmental stages and crowding conditions ($K = 0.12$, $p = 0.481$ [juvenile, not crowded]; $K = 0.11$, $p = 0.353$ [juvenile, crowded]; $K = 0.10$, $p = 0.494$ [adult, not crowded], $K = 0.09$, $p = 0.597$ [adult, crowded]).

To investigate the association between pitcher shape and PA, similar PGLM analyses were performed for each crowding condition using data for adult pitchers. The analyses indicated a strong quadratic relationship between pitcher curvature and pitcher angle only in crowded conditions (Supplementary Table 2). When crowded, species with straighter pitcher bodies generally put out new pitchers at a wider angle than those with curvier bodies (Supplementary Figure 4). Similarly, a strong linear relationship between pitcher stoutness and pitcher angle was only found in crowded condition. No relationship was found between nectar spoon elaboration (PC2) and pitcher angle in either condition, indicating that, in crowded conditions, the degree of pitcher angle reduction depends on the morphology of the pitcher body rather than the morphology of nectar spoon.

DISCUSSION

Divergent and convergent evolution of pitcher morphologies

Our morphospace analysis revealed three main shape axes across which *Heliamphora* pitchers have diversified, namely in curvature (PC1), nectar spoon elaboration (PC2), and pitcher stoutness (PC3). In accordance with the generally low phylogenetic signal in these traits, we observed that most clades contain a diversity of shapes, e.g. curved and straight, with and without elaborate spoons (Figure 1). *Heliamphora* pitchers also vary widely in size (almost five-fold difference across the genus), even between closely related species (e.g., the sympatric *H. chimantensis* at 35cm and *H. pulchella* at 10cm). Across these axes, *Heliamphora* species are clustered in distinct regions of morphospace, e.g., curved with elaborate spoons or straight and slender (Figure 2, Supplementary Figure 5, Supplementary Animations 1 and 2).

Accompanying these patterns of divergence in shape and size among close relatives, we also documented frequent convergence across the phylogeny. For example, small pitchers (< 20 cm) evolved convergently in three lineages (Figure 3). Interestingly, these endemic small pitcher species can be found across all major areas of distribution but none of them occur sympatrically (e.g. *H. hispida*, Neblina Massif; *H. puchella*, Chimantá Massif; *H. minor*, Auyán Tepui; *H.*

nutans and *H. arenicola*, different parts of Eastern Tepui Chain). Such widespread convergence is often associated with adaptation to a common ecological niche (e.g. Donoghue et al. 2022), and previous authors suggest that small pitchers may be favored in habitats with suboptimal growth conditions, e.g., shallow or infertile substrates, heavy shade, and periodic drought or heat stress (Wistuba, Carow, and Harbarth 2002; Givnish et al. 2018). Small pitchers impose lower construction costs (Karagatzides and Ellison 2009; McPherson et al. 2011) and are more drought and heat tolerant due to the reduced transpiration from the pitcher and evaporation from the pitcher fluid (Adlassnig, Pranjic, et al. 2010). Overall, the diversity of habitats provided by the topologically complex Pantepui region combined with the geological isolation between tepuis may account for the repeated diversification of pitcher size in each clade and the resulting convergence across the genus.

We observed similarly marked convergence in aspects of pitcher shape, although the possible drivers are less clear. The degree of elaboration in the nectar spoon may relate the available prey and the reward needed to attract them (Jaffé et al. 1995; Plachno, Swiatek, and Wistuba 2007). Differences in shape have been related to the surrounding vegetation, for example, with *Heliamphora* growing through dense vegetation tending to have straight pitchers to protrude above the rest to capture prey (Brewer-Carias 1972; Jaffe et al. 1992; McPherson et al. 2011). The stoutness of the pitcher may be more directly related to the overall size of the pitcher and may represent a developmental constraint (see below).

Gaps in pitcher morphospace due to evolutionary constraints

Unoccupied regions in the space of possible phenotypes are often attributed to constraints on evolution, be they structural, ecological or developmental (e.g. (Vernescu and Ryser 2009; Stayton 2019; Chitwood and Mullins 2022)). We observed several gaps in pitcher space represented the absence of certain trait combinations, such as large and curvy pitchers or small and slender pitchers. The strong correlations between size and shape, in particular curvature and stoutness (Figure 4), are consistent with structural constraints, i.e., that stout and/or curved pitchers are limited in height while straight, slender pitchers can remain erect at greater heights. We postulate that, compared to species with straight body shapes, taxa with curvy shapes are less structurally stable due to the center of their pitcher mass being further away from the growing point on the rosette which provides structural support.

This trade-off between pitcher size and shape may allow some conservation of pitcher volume, as pitchers can achieve similar volume by expanding laterally or vertically. Pitcher volume is expected to be under strong selection as maintaining a high level of fluid is crucial to carnivory in *Heliamphora* (Jaffe et al. 1992; Nerz 2004; Bauer et al. 2013). At a high level, the pitcher fluid is able to effectively rise along the pubescent inner pitcher wall up to the rim by capillary action, forming superhydrophilic (slippery) trapping surface that facilitates prey capture (Jaffe et al. 1992; Bauer et al. 2013). The regulation and maintenance of the pitcher fluid level might be important in other ecological functions, such as thermal regulation and water reservoir during hot and dry periods, and microhabitat for commensal microbiomes to facilitate digestion (Jaffe et al. 1992; Nerz 2004; Adlassnig, Pranjic, et al. 2010). We expect that there are also lower limits on

pitcher fluid level, bounded by the minimum volume needed to allow for carnivorous function. In *Heliamphora*, the maximum pitcher fluid level is enforced by the drainage structures (slits or holes) that prevent the pitchers from becoming overfilled and unstable (Bauer et al. 2013).

Plasticity in pitcher development

Like all leaves, the pitcher development is plastic and can be affected by many environmental factors (Brewer 1999; Ellison and Gotelli 2002; Fukushima et al. 2021). This is the first study to examine plasticity in *Heliamphora* pitcher rosette development, and we focused on a condition that is common in nature — crowding due to the presence of neighboring plants. In nature, *Heliamphora* populations are often crowded and restricted to habitats with suitable growth conditions, such as small depressions on tepui summits and open clearings in the montane forests (McPherson et al. 2011). Our study found that rosettes in such crowded conditions put out new pitchers at tighter angle while those in growing without neighbors grow out at wider angle. This effect is apparent in both juvenile and adult pitchers (Figure 5). In adult pitchers, the effect is dependent on pitcher curvature and pitcher stoutness (Supplementary Table 2, Supplemental Figure 4), suggesting the degree of angle reductions is further constrained by pitcher body shape.

In order to visualize how the plastic differences in pitcher angle would translate to spacing of pitchers and rosettes, we created two sets of diagrams based on two angles of emergence (140° and 125°, Figure 6, Supplementary Figure 6). The wider angle, roughly the mean estimated in uncrowded conditions (Figure 5), leads to evenly spaced pitchers in the mature rosette (Figure 6 A & B). Mature rosettes typically comprise five active pitchers (e.g. (Butschi, Huber, and Ammann 1989; Wistuba et al. 2005; Wrazidlo 2019; Golos 2020)), and we added an sixth pitcher to show how a new pitcher will overlap an older inactive pitcher (Figure 6 B). Next, we can imagine how a clonal cluster of rosettes, emerging from the rhizomes of a single plant could fill a constrained space, such as a depression in the rock substrate (Figure 6 C). When we model the same developmental process with the narrower pitcher angle observed under crowded conditions (Figure 5), we see that the individual pitchers on each rosette will have greater overlap (Figure 6 D & E), but more new rosettes can be packed into the constrained space (Figure 6 F). While these visual models are only hypothetical, they illustrate how pitcher angle will have direct consequences for the packing of pitchers into confined spaces and likely affect fitness through access to light, rainwater, and prey resources.

While we chose to focus on the effects of crowding, other biotic and abiotic factors may influence pitcher development and represent adaptive plasticity. For example, *Heliamphora* grown under drought stress produce smaller pitchers, a response which may serve to minimize transpiration (McPherson et al. 2011). Pitchers growing in heavy shade typically lose carnivorous activity and function primarily as photosynthetic structures, as indicated by the elongated pitcher bodies, and the absence of pitcher coloration, nectar spoon structure, or attractants (McPherson et al. 2011). Similar plastic responses are found in *Darlingtonia*, *Sarracenia* and all other pitcher plants, suggesting pitcher plasticity commonly associated with trade-offs between carnivory and photosynthesis (Givnish et al. 1984; Ellison and Gotelli 2002;

Ellison and Farnsworth 2005; Pavlovič and Saganová 2015; Givnish et al. 2018; Fukushima et al. 2021).

CONCLUSION

Both divergent and convergent evolution of *Heliamphora* pitcher morphologies was likely a result of adaptive radiation across the dynamic Pantepui landscape over the last 20 million years (Liu and Smith 2021). We observed repeated diversification of pitcher shape and size within geographically structured clades as well as frequent convergence in form across the entire genus. This convergence upon similar combinations of shapes and sizes suggest that these suites of traits may be adaptive, as has been proposed for convergently evolved pitcher trait combinations in other carnivorous plant lineages (Clarke et al. 2018; Thorogood, Bauer, and Hiscock 2018). Many traits beyond shape and size contribute to pitcher function (e.g., volatiles, pigmentation, external nectaries, pubescence), and we predict that these traits may show similar patterns of convergence and correlated evolution. While many studies have demonstrated phenotypic and even genomic convergence associated with carnivory across angiosperms (e.g. (Fukushima et al. 2017; Bittleston et al. 2018; Clarke et al. 2018)), our study demonstrate that diversification and convergent evolution of carnivory-related traits extends to finer evolutionary timescales, reinforcing the notion that ecological specialization may not necessarily be an evolutionary dead end (Tripp and Manos 2008; Wim A. Ozinga 2012; Day, Hua, and Bromham 2016; Thorogood, Bauer, and Hiscock 2018).

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Author Contributions

Sukuan Liu: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Validation, Visualization, Writing - original draft, Writing - review & editing. **Stacey Smith:**

Conceptualization, Formal analysis, Investigation, Methodology, Project administration, Resources, Supervision, Writing - review & editing.

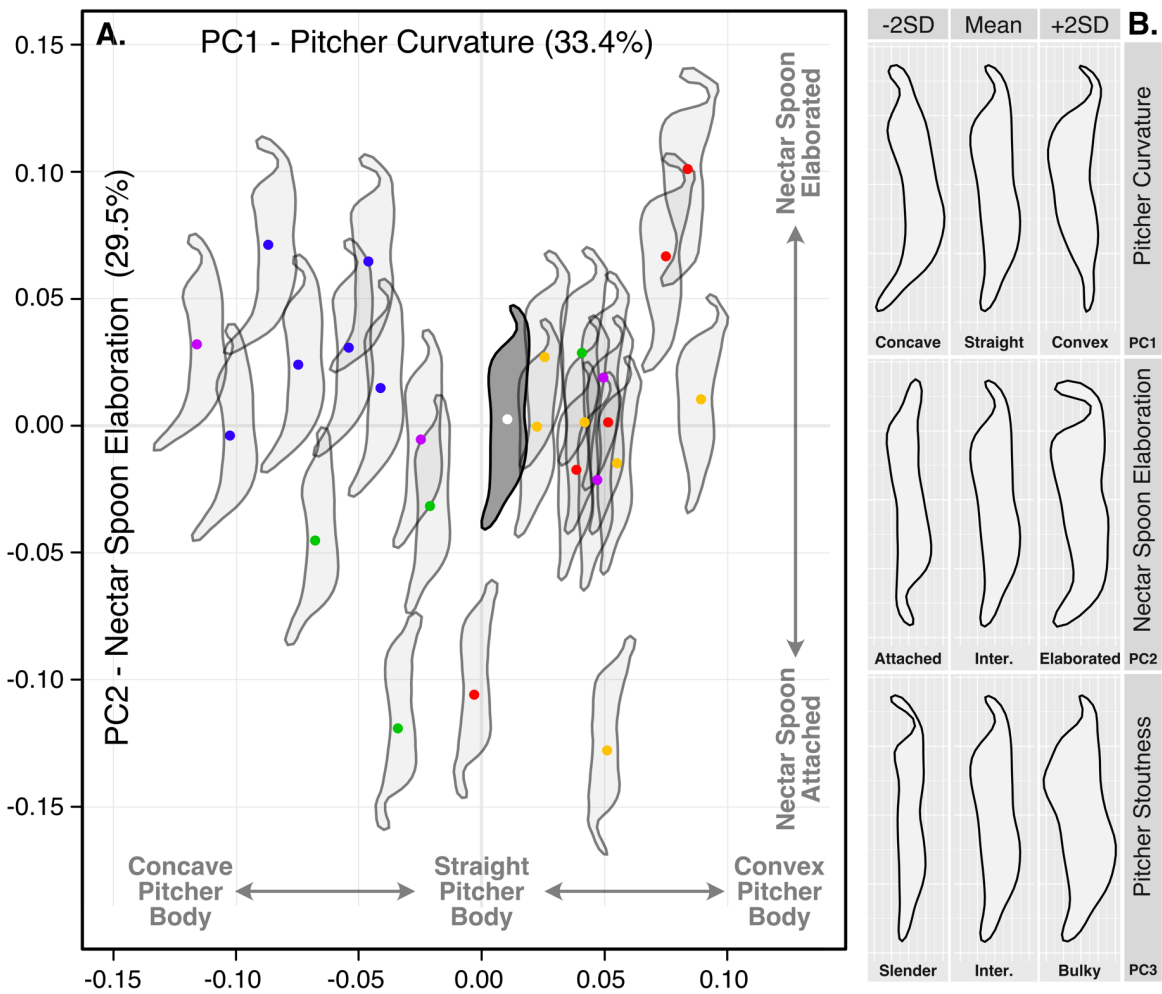
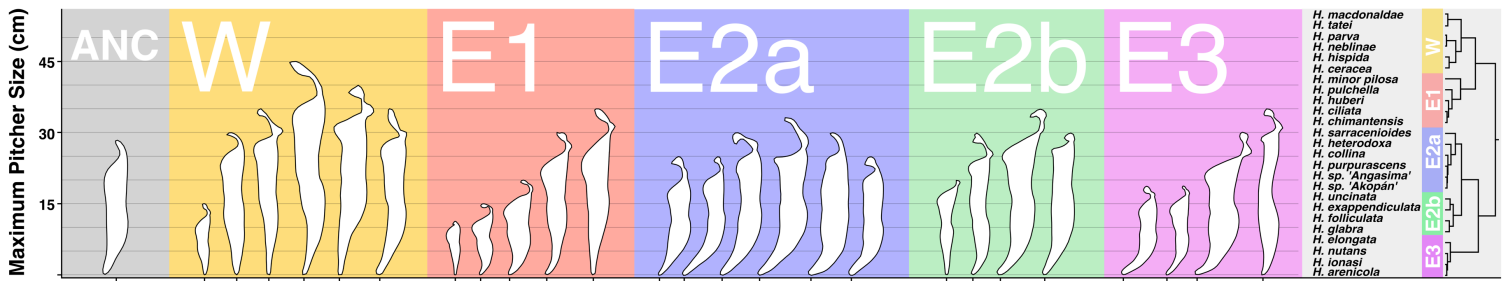


Figure 2. A. Adult pitcher morphospace of pitcher curvature (PC1) and nectar spoon elaboration (PC2) with outlines of each taxa and the reconstructed ancestral *Heliamphora* adult pitcher (darker gray). Taxa are colored by clades: W (orange), E1 (red), E2a (blue), E2b (green), E3 (purple). **B. Share variation along PC axes.** For each of the first three PCs, the mean is shown along with shapes corresponding to ± 2 standard deviation (SD). +2 SD was chosen to better visualize the morphological variation along shape PC axes. The abbreviation "Inter." stands for "Intermediary".

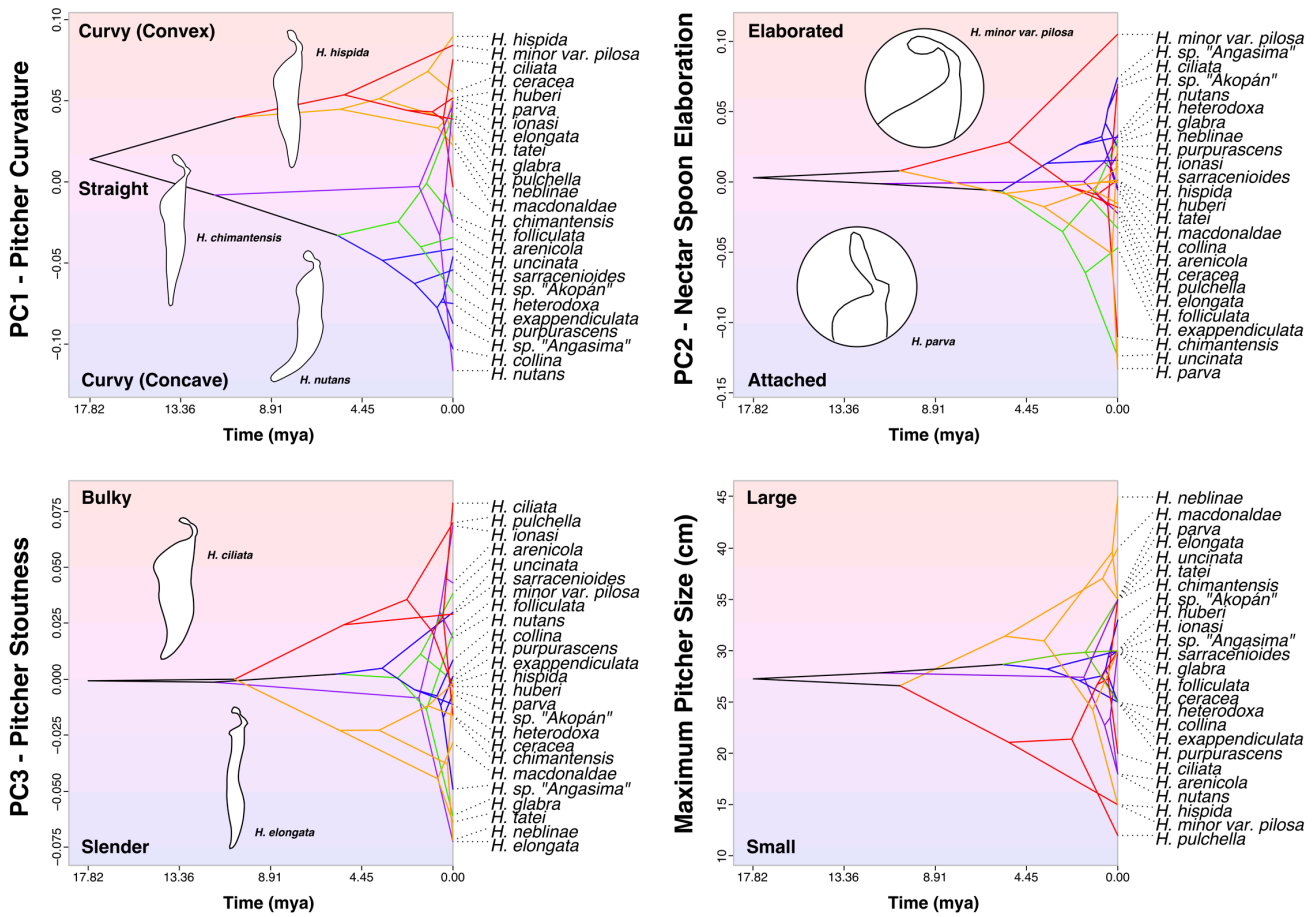


Figure 3. Traitgrams of pitcher curvature (PC1), nectar spoon elaboration (PC2), pitcher stoutness (PC3), and maximum pitcher size. Branches are colored by clades. Examples of pitcher and nectar spoon morphologies are shown in the traitgrams.

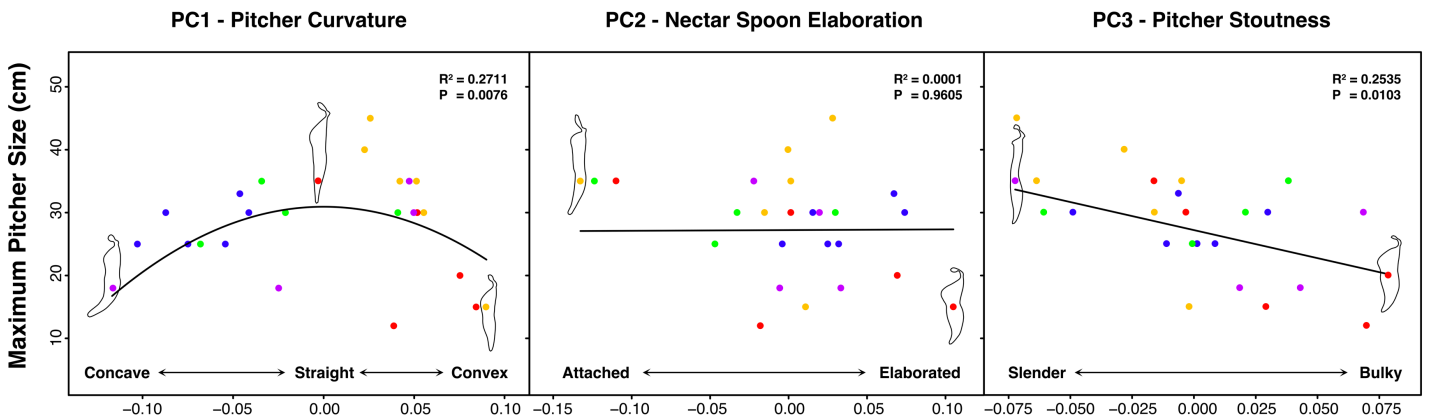


Figure 4. Associations between adult pitcher shape variations and maximum pitcher size in pitcher morphospace. Taxa are represented by solid circles and colored by clades. Regression lines were drawn based on the best fit phylogenetic generalized linear models. Example pitcher shapes (corresponding to the taxa encircled) are shown in each morphospace.

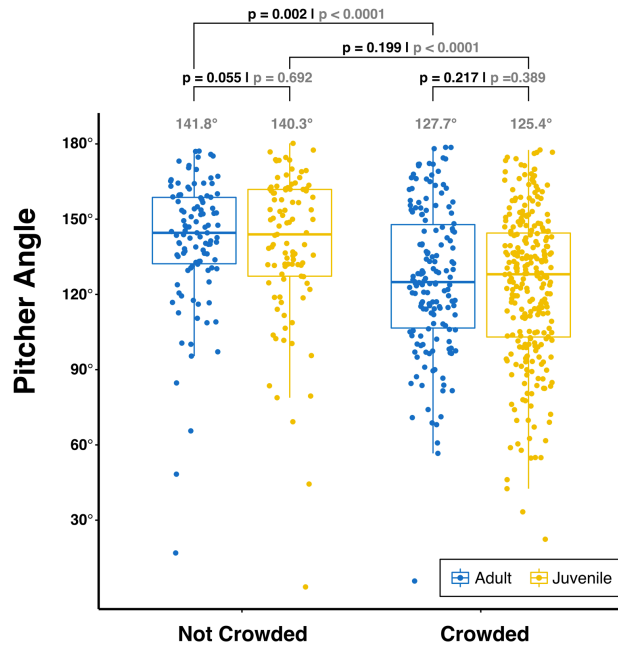


Figure 5. Effect of crowdedness on pitcher angle across developmental stages. Both phylogenetic and simple unpaired t-tests were conducted for each comparison with test statistics shown in black and gray, respectively. Note the angles shown above each condition were averages pooled from all samples, with potential bias toward species that were over sampled.

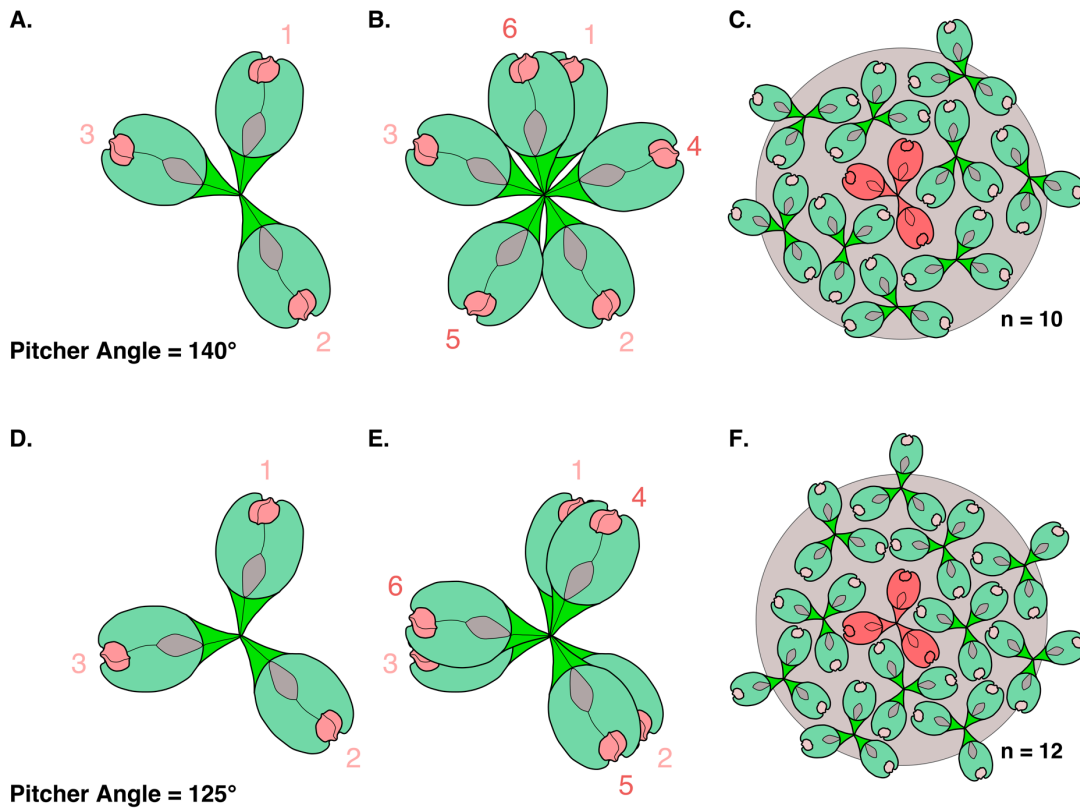
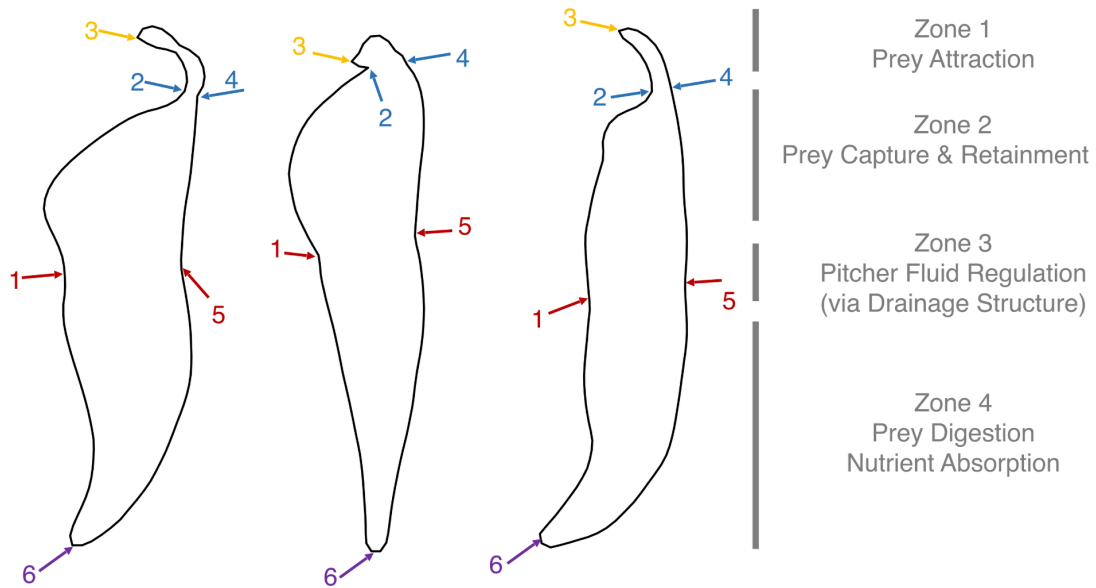
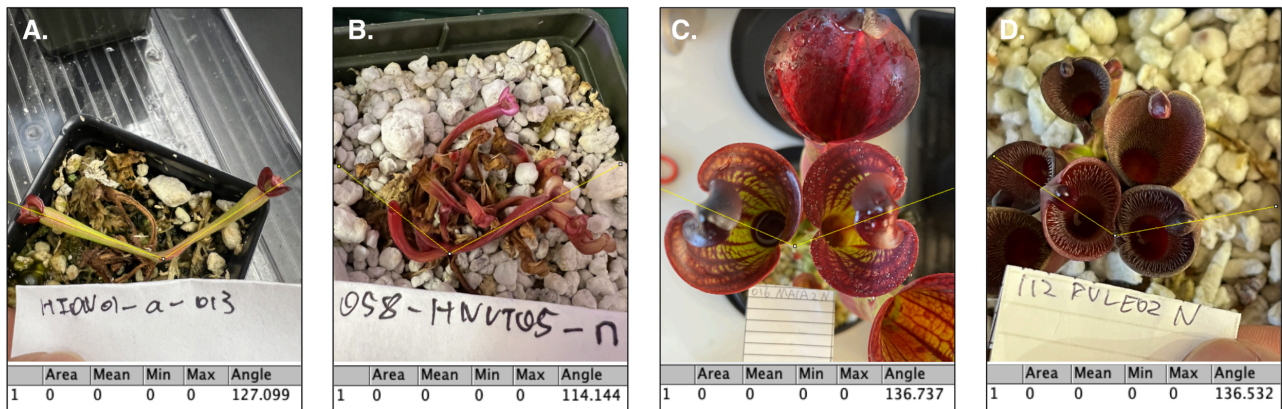


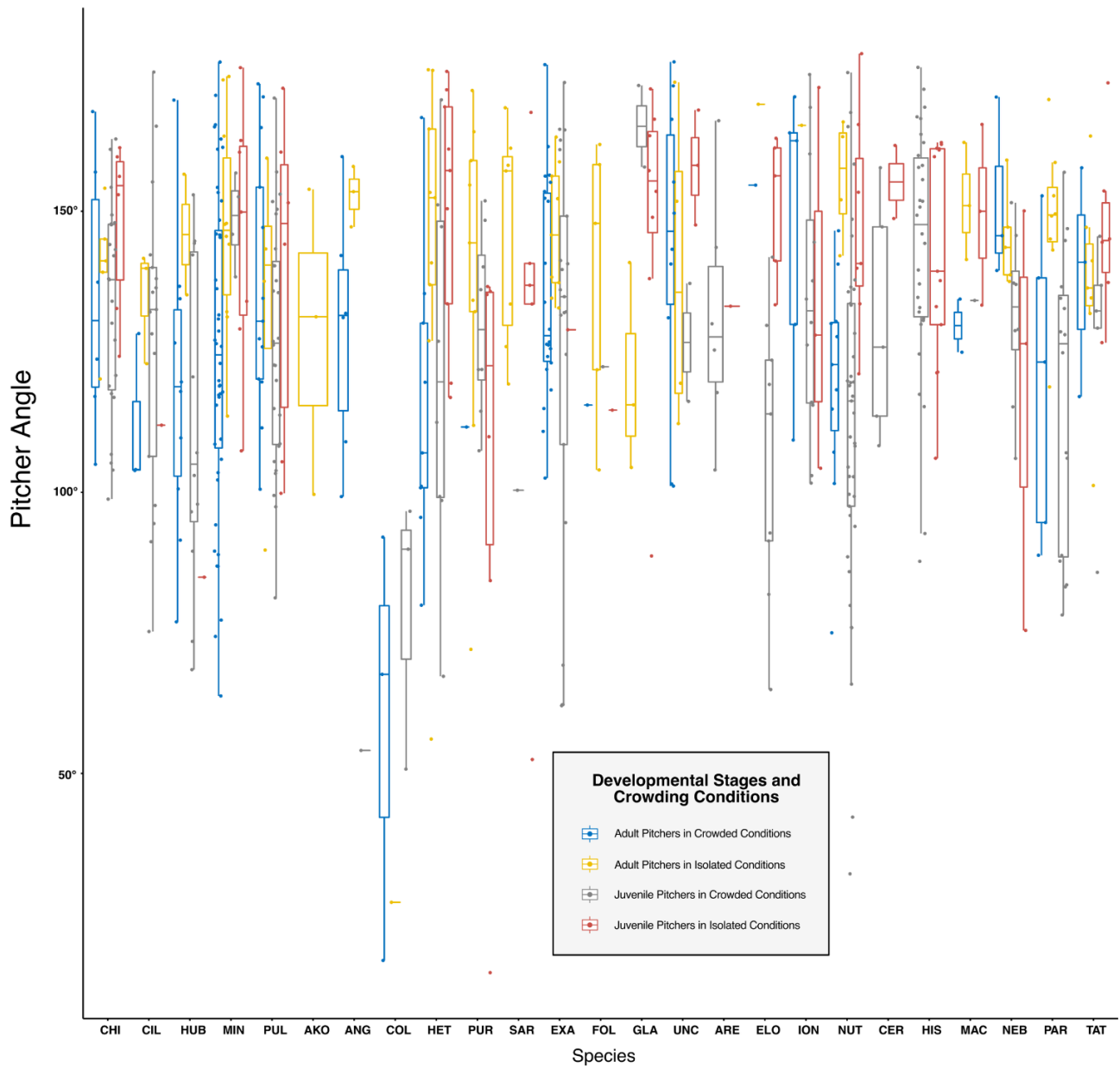
Figure 6. Morphologies of *Heliamphora* rosettes associated with wider (A, B) and sharper pitcher angles (D, E). For each pitcher angle, rosettes are illustrated with three (A, D) to six (B, E) to demonstrate the effect of shading from overlapping pitchers. In each rosette, pitchers are numbered increasingly from the oldest to the youngest pitcher (most active pitcher). **Hypothetical scenario illustrating the effect of wider (C) and sharper pitcher angles (F) on optimal packing in crowded conditions.** In this hypothetical scenario, the wider and sharper pitcher angles, respectively, allowed the populations to fit in up to 10 and 12 rosettes when space is limited. The sharper pitcher angle allowed *Heliamphora* population to occupy available space more efficiently, with an increase of 20% more active pitchers.



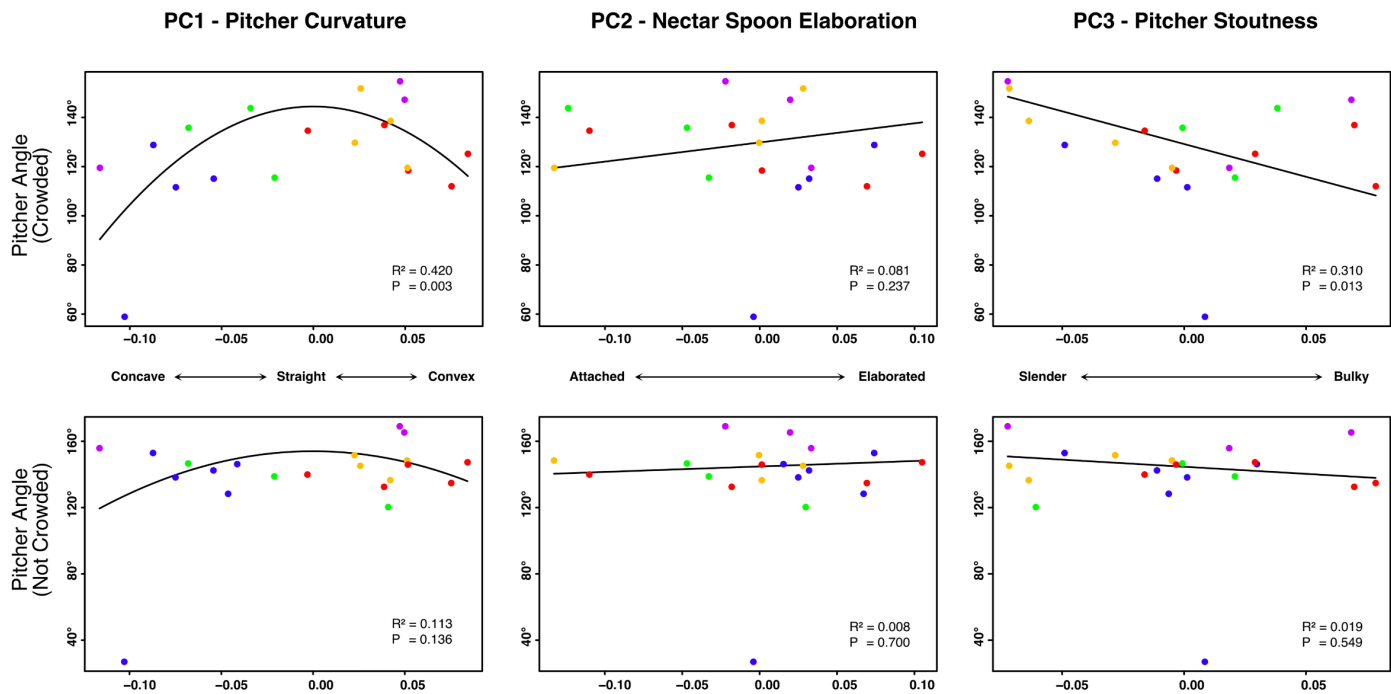
Supplementary Figure 1. Landmarking Examples of three *Heliophora* species (from left to right: *H. ciliata*, *H. pulchella* and *H. sarracenioides*). The adult pitcher functional zones (McPherson et al. 2011) were delineated by using six landmarks. Zone 1 (landmarks 2-4) comprises the nectar spoon and is responsible for prey attraction. Zone 2 (landmarks 1, 2, 4, 5) comprises the upper section of the pitcher above the narrow band and is responsible for prey capture and retainment. Zone 3 (landmarks 1, 5) consists of a narrow restriction of the pitcher interior close to the waterline of the pitcher fluid. The position of drainage structure (indicated by landmark 1) determines the maximal level of pitcher fluid in the pitcher. Zone 4 (landmarks 1, 5, 6) consists the bottom part of the pitchers and involves in prey digestion as well as nutrient absorption.



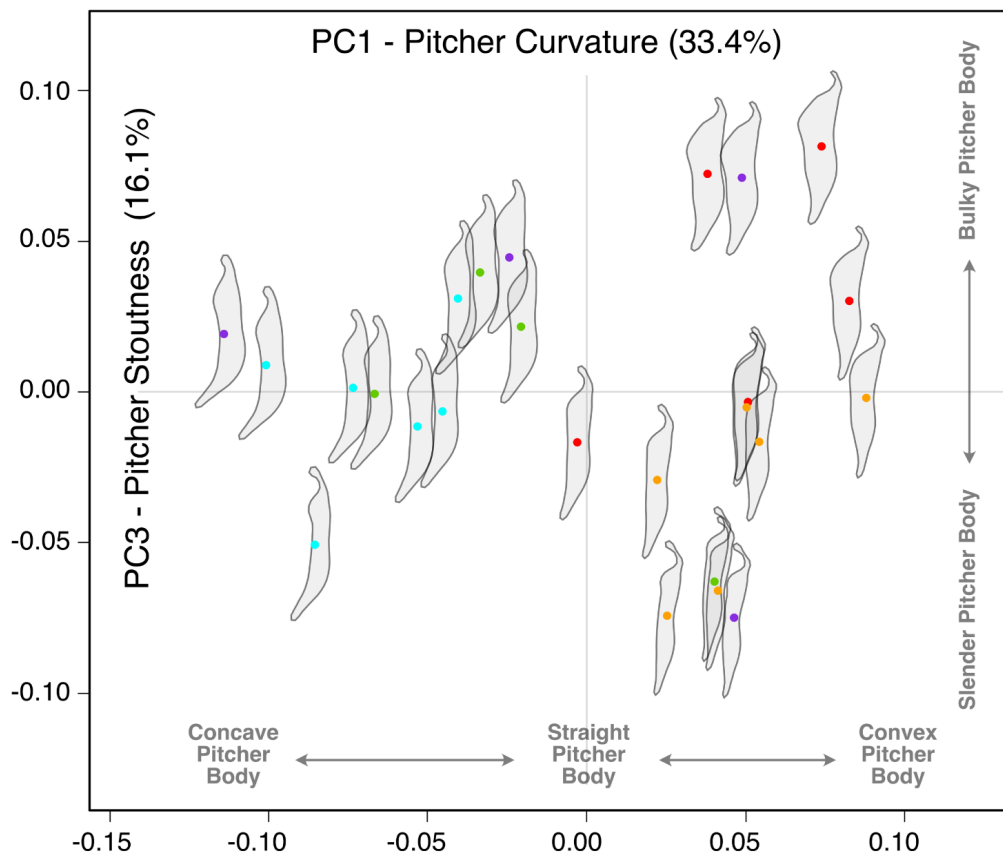
Supplementary Figure 2. Pitcher Angle Measurements. **A.** *H. ionasi* juvenile growing in isolated condition (growing singly). **B.** *H. nutans* juvenile in crowded condition (neighbors present). Note the juvenile pitcher being measured here does not have a linear pitcher body and curves significantly away from the straight line drawn for pitcher angle measurement. **C.** *H. macdonaldae* adult growing singly. **D.** *H. pulchella* adult in crowded condition.



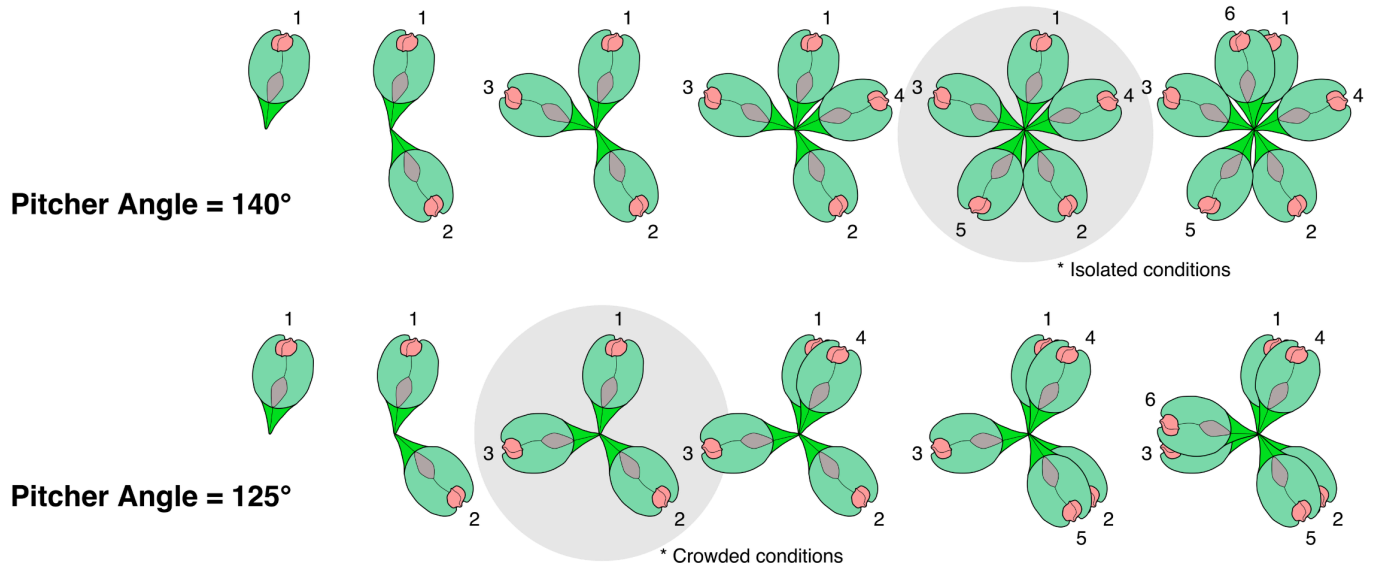
Supplementary Figure 3. Pitcher angle by taxa across developmental stages and crowding conditions (box plot). Each measurement is indicated by the solid circle. Taxa names are abbreviated to the first three letters (e.g. CHI = *H. chimantensis* & AKO = *H. sp.* "Akopán").



Supplementary Figure 4. Associations between adult pitcher shape variations and pitcher angle in different crowding conditions. Taxa are represented by solid circles and colored by clades. Regression lines were drawn based on the best fit phylogenetic generalized linear models.



Supplementary Figure 5. Adult pitcher morphospace of pitcher curvature (PC1) and stoutness (PC3) with outlines of each taxa colored by clades: W (orange), E1 (red), E2a (blue), E2b (green), E3 (purple).



Supplementary Figure 6. Developmental timeline of *Heliamphora* adult pitcher rosettes associated with wider and sharper pitcher angles. Rosette morphologies frequently found in isolated and crowded conditions are indicated with grey circles.

Supplementary Table 1. Sampling Information

Species	Location Sampled	Original Source	Accession Numbers
<i>Heliampora chimantensis</i>	GP	Wistuba - Exotische Pflanzen	MN428593, MN428558, MN428612
<i>Heliampora ciliata</i>	UCB	Wistuba - Exotische Pflanzen	MN428592, MN428557, MN428613
<i>Heliampora huberi</i>	GP	Wistuba - Exotische Pflanzen	MN428585, MN428556, MN428622
<i>Heliampora minor var. pilosa</i>	UCB	Wistuba - Exotische Pflanzen	MN428583, MN428555, MN428624
<i>Heliampora pulchella</i>	GP	Wistuba - Exotische Pflanzen	MN428579, MN428554, MN428605
<i>Heliampora sp. "Akopán"</i>	UCB	Wistuba - Exotische Pflanzen	MN428597, MN428567, MN428608
<i>Heliampora sp. "Angasima"</i>	UCB	Wistuba - Exotische Pflanzen	MN428596, MN428566, MN428609
<i>Heliampora collina</i>	UCB	Wistuba - Exotische Pflanzen	MN428591, MN428565, MN428614
<i>Heliampora heterodoxa</i>	UCB	Wistuba - Exotische Pflanzen	MN965850, MN428561, MN428620
<i>Heliampora purpurascens</i>	UCB	Wistuba - Exotische Pflanzen	MN428578, MN428560, MN428604
<i>Heliampora sarracenioides</i>	GP	Wistuba - Exotische Pflanzen	MN428577, MN428559, MN428603
<i>Heliampora exappendiculata</i>	UCB	Wistuba - Exotische Pflanzen	MN428589, MN428564, MN428616
<i>Heliampora folliculata</i>	UCB	Wistuba - Exotische Pflanzen	MN428588, MN428563, MN428617
<i>Heliampora glabra</i>	GP	Wistuba - Exotische Pflanzen	MN428587, MN428562, MN428619
<i>Heliampora uncinata</i>	GP	Wistuba - Exotische Pflanzen	MN428575, MN428569, MN428601
<i>Heliampora arenicola</i>	GP	Wistuba - Exotische Pflanzen	MN428595, MN428573, MN428610
<i>Heliampora elongata</i>	GP	Wistuba - Exotische Pflanzen	MN428590, MN428572, MN428615
<i>Heliampora ionasi</i>	GP	Wistuba - Exotische Pflanzen	MN428584, MN428571, MN428623
<i>Heliampora nutans</i>	GP	Wistuba - Exotische Pflanzen	MN428581, MN428570, MN428618
<i>Heliampora ceracea</i>	UCB	Wistuba - Exotische Pflanzen	MN428594, MN428553, MN428611
<i>Heliampora hispida</i>	UCB	Wistuba - Exotische Pflanzen	MN428586, MN428552, MN428621
<i>Heliampora macdonaldae</i>	GP	Wistuba - Exotische Pflanzen	—
<i>Heliampora neblinae</i>	GP	Wistuba - Exotische Pflanzen	MN428582, MN428551, MN428625
<i>Heliampora parva</i>	GP	Wistuba - Exotische Pflanzen	MN428580, MN428550, MN428606
<i>Heliampora tatei</i>	GP	Wistuba - Exotische Pflanzen	MN428576, MN428594, MN428602
	* grown in growth chambers under controlled environmental conditions similar to their native habitats (photoperiod 15hr per day; temperature range 12–24 °C; relative humidity > 80%)		* ITS, 26S, and PHYC sequences used for phylogenetic inferences (Liu and Smith, 2021)
	* UCB = University of Colorado Boulder (Boulder, Colorado)		
	* GP = Collection from Grep Pipes (Aurora, Colorado)		

Supplementary Table 2. Model Fit Results

Models	Coefficients	Estimate	Standard Error	P	R Squared	Log Likelihood	AIC
MPS ~ PC1	Intercept	27.06487	15.73888	0.09893			
	PC1	14.93703	33.66331	0.66139			
					0.008488	-96.58	199.17
MPS ~ PC1 ²	Intercept	30.9794	13.5475	0.031745			
	PC1 ²	-1035.0922	353.8593	0.007613			
					0.2711	-92.74	191.47
MPS ~ PC2	Intercept	27.26932	15.79838	0.09774			
	PC2	1.09369	21.82126	0.96046			
					0.0001092	-96.69	199.38
MPS ~ PC2 ²	Intercept	27.68341	15.72479	0.09162			
	PC2 ²	-129.27288	246.25393	0.60464			
					0.01184	-96.54	199.08
MPS ~ PC3	Intercept	27.2155	13.6505	0.05817			
	PC2	-89.133	31.8931	0.01029			
					0.2535	-93.04	192.07
MPS ~ PC3 ²	Intercept	26.28749	15.58319	0.1051			
	PC3 ²	499.10168	570.3424	0.3906			
					0.03222	-96.28	198.56
PA ~ PC1 (Crowded Condition)	Intercept	129.23588	46.76209	0.01328			
	PC1	58.70529	108.85249				
					0.01682	-95.11	196.22
PA ~ PC1 ² (Crowded Condition)	Intercept	144.451	36.1699	0.00094			
	PC1 ²	-3993.7726	1139.4111	0.002714			
					0.4195	-90.11	186.21
PA ~ PC2 (Crowded Condition)	Intercept	129.8277	45.1972	0.01056			
	PC2	77.9747	63.6177	0.23703			
					0.08119	-94.47	194.94
PA ~ PC2 ² (Crowded Condition)	Intercept	132.6357	45.7973	0.01004			
	PC2 ²	-750.7669	719.7467	0.31151			
					0.06015	-94.68	195.37
PA ~ PC3 (Crowded Condition)	Intercept	129.1813	39.171	0.00425			
	PC3	-266.5068	96.4594	0.0133			
					0.3099	-91.75	189.5
PA ~ PC3 ² (Crowded Condition)	Intercept	122.658	42.3171	0.009993			
	PC3 ²	3325.0145	1613.6892	0.054994			
					0.1998	-93.16	192.31
PA ~ PC1 (Isolated Condition)	Intercept	144.0131	59.49266	0.02567			
	PC1	79.95094	130.58697	0.54763			
					0.01935	-109.6	225.2
PA ~ PC1 ² (Isolated Condition)	Intercept	153.9686	56.8551	0.01394			
	PC1 ²	-2551.2459	1639.1573	0.1361			
					0.1131	-108.5	223.1
PA ~ PC2 (Isolated Condition)	Intercept	144.77842	59.81938	0.0257			
	PC2	32.98884	84.58356	0.7009			
					0.007942	-109.7	225.4
PA ~ PC2 ² (Isolated Condition)	Intercept	142.83018	59.43009	0.02662			
	PC2 ²	662.55658	980.06487	0.50717			
					0.02349	-109.6	225.1
PA ~ PC3 (Isolated Condition)	Intercept	144.61047	59.47953	0.02511			
	PC3	-85.7028	140.47022	0.54901			
					0.01922	-109.6	225.2
PA ~ PC3 ² (Isolated Condition)	Intercept	144.57	60.25	0.02683			
	PC3 ²	171.51	2246.2	0.93993			
					0.0003068	-109.8	225.6
* Best fit model colored in red							

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