

## ARTICLE

## Vegetation Ecology

# Untangling the effects of water availability on flower size: Insights from the largest tropical wetland

Layla M. A. Rodrigues<sup>1</sup> | Thais B. Zanata<sup>2</sup> | Camila Aoki<sup>3</sup> | Alberto L. Teixeira<sup>2</sup>

<sup>1</sup>Programa de Pós-Graduação em Biologia Vegetal, Instituto de Biociências, Universidade Federal de Mato Grosso, Cuiabá, Mato Grosso, Brazil

<sup>2</sup>Departamento de Botânica e Ecologia, Instituto de Biociências, Universidade Federal de Mato Grosso, Cuiabá, Mato Grosso, Brazil

<sup>3</sup>Programa de Pós Graduação em Recursos Naturais, Faculdade de Engenharias, Arquitetura e Urbanismo e Geografia, Universidade Federal de Mato Grosso do Sul, Campo Grande, Mato Grosso do Sul, Brazil

**Correspondence**

Alberto L. Teixeira  
Email: [al Teixeido@ucm.es](mailto:al Teixeido@ucm.es)

**Present address**

Alberto L. Teixeira, Departamento de Biodiversidad, Ecología y Evolución, Facultad de Ciencias Biológicas, Universidad Complutense de Madrid, Madrid, Spain.

**Funding information**

Coordenação de Aperfeiçoamento de Pessoal de Nível Superior; CAPES, Brazil; Universidade Federal de Mato Grosso do Sul (UFMS/MEC)

**Handling Editor:** Jaime

Madrigal-González

**Abstract**

Flower size plays a crucial role in the reproduction of animal-pollinated plants. Larger flowers usually increase pollinator visitation and reproductive success, but they are also associated with higher water investment for production and maintenance. Thus, water availability is a relevant factor in determining flower size, which may be consequently reduced in hot and dry environments. However, the phylogenetic relationships across plants may limit the variation in flower size, a strongly conserved trait among closely related species. We tested this hypothesis across 97 animal-pollinated species in the Pantanal, the world's largest tropical wetland. To determine how phylogeny and water availability affect community-level flower size, we quantified the variation of this trait between seasons (dry and rainy), substrates (terrestrial and aquatic), and species (phylogenetic signal). We observed a high variability in flower size, which showed a significant phylogenetic signal. Conversely, we did not detect a significant influence of season and substrate on flower size, although we observed a trend toward larger flowers in aquatic species, especially during the rainy season. Our results demonstrate that phylogeny mostly determines flower size variation in the Pantanal. Our findings also suggest that water availability may still exert evolutionary pressures on flower size, even in a context of phylogenetic conservatism. This study highlights the need to investigate the multiple interacting factors shaping flower size and contributes to a deeper understanding of its variation in response to environmental factors and phylogeny.

**KEYWORDS**

aquatic plants, dry season, Pantanal, phylogenetic signal, rainy season, substrate, terrestrial plants

**INTRODUCTION**

Flower size is a relevant trait in the reproductive ecology of animal-pollinated plants, with larger flowers receiving

more pollinator visits and thus increasing reproductive success (Bell, 1985; Galen, 1999; Teixeira et al., 2016). Pollinators have long been considered the most important selective pressure on flower size, favoring larger-flowered

This is an open access article under the terms of the [Creative Commons Attribution](https://creativecommons.org/licenses/by/4.0/) License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

© 2025 The Author(s). *Ecosphere* published by Wiley Periodicals LLC on behalf of The Ecological Society of America.

individuals (Gervasi & Schiestl, 2017; Schemske & Bradshaw, 1999; Stanton et al., 1986). However, variation in flower size persists within populations. Galen (1999) suggested that both biotic factors (pollen limitation) and abiotic factors (resource limitation) influence flower size. Subsequent studies have shown that larger-flowered species require more resources, such as higher water allocation, to produce and maintain flowers physiologically active (Patiño & Grace, 2002; Phillips et al., 2018; Teixido & Valladares, 2014). Recent literature indicates that smaller flowers are favored in hot and dry environments due to water limitation and high physiological maintenance costs related to elevated transpiration rates (Kuppler & Kotowska, 2021). Environmental conditions can thus mitigate pollinator-mediated selection on flower size depending on the intensity of pollen and water limitation (Caruso et al., 2019). Beyond intrapopulation variation, abiotic factors may also determine flower size in animal-pollinated plant communities within ecosystems exposed to drought periods (Phillips et al., 2018).

In addition to the influence of biotic (plant–pollinator: pollen limitation) and abiotic factors (plant–environment: water limitation), the evolutionary history of species may also explain variation in flower size in animal-pollinated plants. Phylogenetic analyses are relevant to determine whether a particular trait in closely related species shows high similarity due to a common evolutionary history with a significant phylogenetic signal or high divergence due to diversification into contrasting ecological niches (Ackerly, 2009; Blomberg & Garland, 2002). Although flower size may be prone to convergences among distantly related species during adaptation to similar environmental conditions, this floral trait has been reported to be strongly conserved across the phylogeny as flowers are directly involved in the first stages of reproduction that determine fitness (Armbruster et al., 1999; Teixido et al., 2017). A phylogenetic approach using multiple species is required to understand flower size evolution and diversity beyond microclimatic conditions and a potential divergent adaptation across species flowering in contrasting environments.

Seasonal tropical ecosystems are hot environments with highly variable water availability throughout the year, which acts as a limiting factor for plant reproduction (Morellato et al., 2013; Oliveira et al., 2021). Climatic seasonality and the resulting variation in water availability may influence flower size, favoring smaller flowers during the dry season and larger ones during the rainy season (Machado & Lopes, 2004; Teixido et al., 2019). However, more comprehensive studies involving a broader range of species are required for more robust conclusions. Flower size variation in response to water availability may be particularly important in seasonal

wetlands with a regime of periodic flooding and differences between dry and rainy seasons. The type of substrate (aquatic or terrestrial) where plants grow may also be relevant in determining flower size. Evidence of a higher occurrence of larger-flowered species during the rainy season and in aquatic substrates, and smaller-flowered species during the dry season and on terrestrial substrates, would support water investment-based arguments, explaining the dominance of smaller flower sizes under hot and dry conditions (Galen, 1999; Kuppler & Kotowska, 2021; Teixido et al., 2016). However, variation in this trait, which is crucial for the ecology and evolution of animal-pollinated plants, remains unknown across plant communities in seasonally variable wetlands.

The Pantanal, one of the world's largest tropical wetlands, is characterized by pronounced seasonal variations in precipitation between dry and rainy seasons and different regimes of periodic flooding throughout the year (Junk et al., 2006). This ecosystem covers approximately 160,000 km<sup>2</sup>, with most of it (140,000 km<sup>2</sup>) located in Brazil, and portions extending into Bolivia (15,000 km<sup>2</sup>) and Paraguay (5000 km<sup>2</sup>) (Junk & da Nunes Cunha, 2012). The seasonality and flood pulse in this wetland lead to an alternation between aquatic and terrestrial phases, causing changes in the structure, composition, dynamics, and phenology of vegetation (Catian et al., 2019; da Nunes Cunha et al., 2010). Environmental heterogeneity in the Pantanal defines various plant communities, including forests, shrublands, savannas, and grasslands (da Nunes Cunha et al., 2023). The Pantanal is ideal for studying the effects of seasonality and phylogenetic relationships on flower size due to its seasonal variations in rainfall and flooding, combined with high plant diversity and biological forms. It includes elements from the Amazon rainforest and the Cerrado savanna-like ecosystem, with significant diversity in terrestrial, aquatic, and amphibious plants (Pott & Pott, 2021). These changes may influence the evolution of flower size, which is mediated by selective pressures from abiotic factors (e.g., microclimatic conditions and water availability) and plant–pollinator interactions.

Here, we aim to determine the influence of water availability related to seasonal climatic variation (seasons and substrate) on flower size of animal-pollinated species in the Pantanal. Specifically, our objectives are (1) to assess whether community-level flower size differs between species flowering during the dry and rainy seasons and (2) to examine whether community-level flower size varies between species flowering in aquatic versus terrestrial substrates. We expect, respectively, flower size to be larger in species sampled during the rainy season and in species growing on aquatic substrates. In brief, we

predict that water availability is relevant in determining flower size in the Pantanal. Additionally, we conducted phylogenetic analyses to ascertain interspecific variation in flower size among related species. By investigating the variation in flower size across different seasons, substrates, and species, we aim to gain insights into the effects of microclimate, environment, and evolutionary history on the evolution of this important trait for animal-pollinated plants. This information may contribute to understanding flower size variation in seasonal ecosystems and other wetlands worldwide.

## MATERIALS AND METHODS

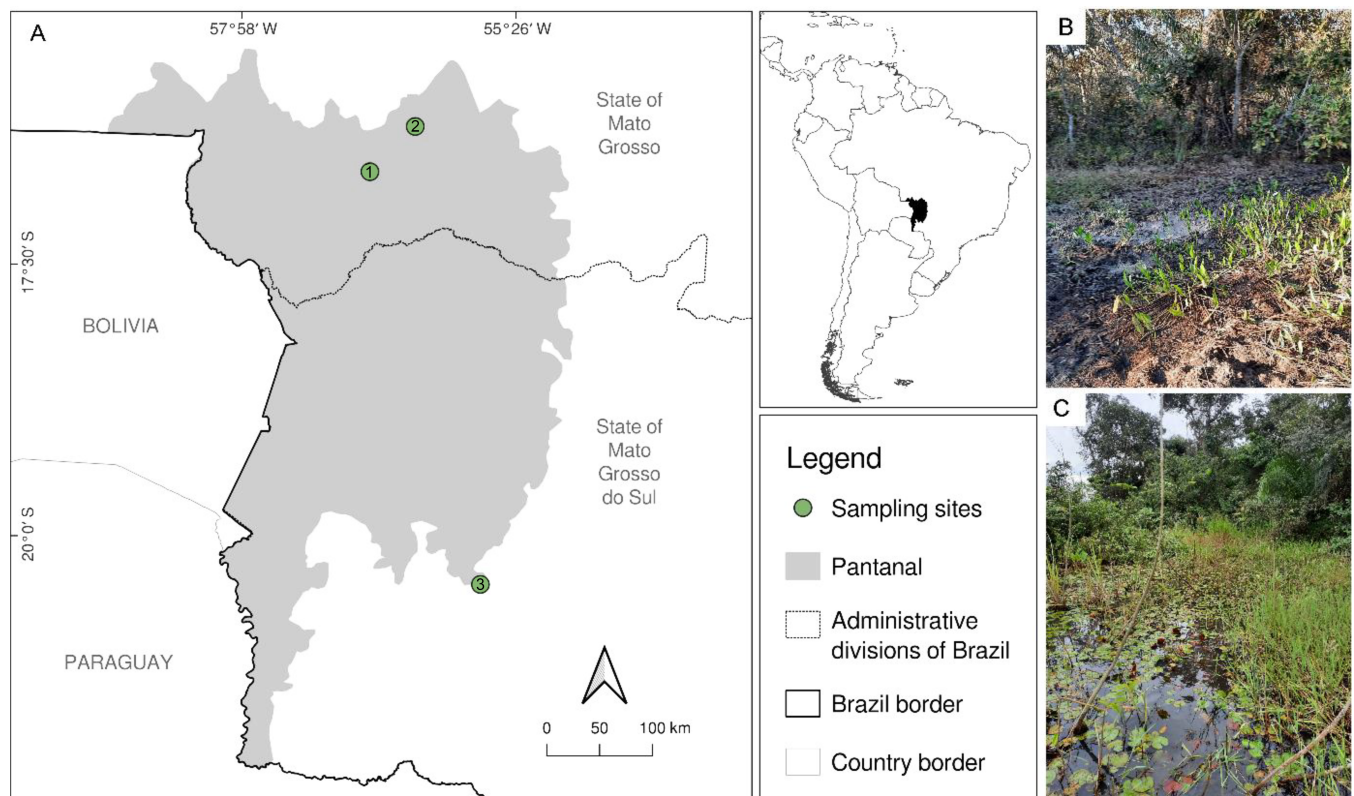
### Study area

Seasonality in the Pantanal is characterized by a rainy period (from October to April) and a dry period (from May to September), with two highly variable hydrological cycles that determine the flood regime and the predominance of terrestrial and aquatic substrates (Junk et al., 2006). The mean annual temperature is 25°C, reaching over 40°C in some months of the year, and the mean annual precipitation is 1300 mm (Junk et al.,

2006). To compare annual temperature and precipitation with historical data, we obtained the records for mean annual precipitation and temperature from the weather station in Cuiabá, Mato Grosso, Brazil, from the Instituto Nacional de Meteorologia (INMET-Brazil; <https://portal.inmet.gov.br>). Historical data show the mean variation between 2002 and 2022, whereas data for monthly precipitation and mean temperature show the variation during the study period, between January 2021 and December 2022 (Appendix S1: Figure S1).

The hydrological cycle and seasonality are environmental determinants that influence biodiversity and maintain the ecological functioning of this wetland. According to Pott and Pott (1994a, 2021), the Pantanal harbors 144 families of flowering plants, of which 104 are exclusively terrestrial, 21 are exclusively aquatic, and 19 include both aquatic and terrestrial species, among them 213 amphibious species. The total richness reaches around 2000 species, including a diversity of herbaceous (climbers, epiphytes, parasites, and, especially, grasses) and woody plants (subshrubs, shrubs, trees, vines, and palms) (Pott & Pott, 2021).

Sampling was conducted at three different locations in the Pantanal (Figure 1). The first sampling site was located in the SESC Pantanal-Baía das Pedras



**FIGURE 1** (A) Location of the three sampling sites used (point 1, SESC Baía das Pedras; point 2, Pirizal; and point 3, Aquidauana) between January 2021 and December 2022 in the Pantanal. The pictures show the change of the sampling sites in the study area during (B) the dry season and (C) the rainy season (including a flooded area). Photo credit: Camila Aoki.

(Poconé, Mato Grosso, 16°39' S, 56°47' W; point 1 in Figure 1), which constitutes the Advanced Research Base of the Pantanal (Federal University of Mato Grosso-UFMT). The second site was in the Pirizal district (Poconé, Mato Grosso, 16°14' S, 56°22' W; point 2 in Figure 1), which is located between the right bank of the Cuiabá River and the left bank of the Bento Gomes River. The third sampling site was located in the Municipal Park of Lagoa Comprida (Aquidauana, Mato Grosso do Sul, 20°27' S, 55°46' W; point 3 in Figure 1), a peri-urban Conservation Unit comprising 74 ha (Ximenes et al., 2017). In all study locations, shrublands and savanna areas on sandy and clay soils are dominated by mesophytic and xerophytic species during the dry season. In contrast, hydrophytic species become more prevalent in flooded areas during the rainy season (da Nunes Cunha et al., 2023).

## Data collection

The effects of season (dry or rainy) and substrate (aquatic or terrestrial) on flower size were evaluated in 97 species from 32 families by monthly field samplings from January 2021 to December 2022 (Rodrigues et al., 2025; Appendix S1: Table S1). Every month, between one and four species with at least 10 flowering individuals were sampled, and mean flower size, sampling season, and substrate were recorded for each species. Trees were not considered in this study because we focused our assessment of the potential variation in flower size on aquatic and terrestrial substrates of plants, since aquatic trees are not present in the Pantanal (Pott & Pott, 1994b), and not on the differences between the form of plant growth (e.g., herbs, lianas, shrubs, trees). Species were identified using field guides of plant identification (Pott & Pott, 1994a, 1994b): the Reflora—Virtual Herbarium site (<https://reflora.jbrj.gov.br/reflora/herbarioVirtual/>), the Missouri Botanical Garden Virtual Herbarium (<https://tropicos.org>), and contacts to experts in flora of the Pantanal (see *Acknowledgments*).

Across the 97 studied species, 54 were sampled during the rainy season and 43 during the dry season, while 74 species were sampled on terrestrial substrate and 23 on aquatic substrate. Ten of the species we recorded are considered amphibious: *Aeschynomene americana*, *Bacopa myriophylloides*, *Helanthisium tenellum*, *Ipomoea carnea*, *Justicia laevilinguis*, *Ludwigia decurrens*, *Ludwigia lagunae*, *Ludwigia leptocarpa*, *Ludwigia tomentosa*, and *Melochia arenosa* (Pott & Pott, 2021). We have also occasionally observed some species blooming during the dry and the rainy seasons (*B. myriophylloides*, *Duguetia furfuracea*, *H. tenellum*, *L. lagunae*, *L. leptocarpa*,

*L. tomentosa*, and *Richardia grandiflora*). Within the community of aquatic plants, frequency, coverage, and flowering of species show considerable seasonal fluctuations, with several species showing a significant correlation between such phenological variation and water levels (Catian et al., 2019; Gomes & Aoki, 2015).

To estimate mean flower size, we measured 5 to 10 individuals per species and 1 to 10 flowers per individual. We included only individuals with fully developed flowers and complete anthesis. The full corolla of gamopetalous flowers (with fused petals) and all free petals of dialypetalous flowers were carefully removed to avoid any damage during manipulation. The corolla of gamopetalous flowers were gently unfolded with razor blades, while free petals were separated. No species with petaloid perianth flowered during the field samplings. Each corolla and set of free petals were placed horizontally between two glass plates on a background contrasting with corolla color (e.g., black paper) and a small scale of 1 cm<sup>2</sup> using a millimeter graph paper. Subsequently, digital photographs of the corollas or petals were taken from each composition. Flower size per flower, individual, and species was calculated using the software ImageJ (Schneider et al., 2012). Flower size was assessed in terms of area (in square centimeters) to provide a more accurate estimate of its variability, avoiding underestimation or overestimation of different floral morphologies (e.g., bell-shaped, lipped, radial, tubular flowers; Ortiz et al., 2023). In this regard, interspecific variation in flower size has been mostly considered in terms of corolla height or width (e.g., diameter; Teixido et al., 2018), dry biomass (Herrera, 2009), or even as a categorical variable by size classes (e.g., small, intermediate, large; Machado & Lopes, 2004), losing much information about the actual corolla size among different species within plant communities.

Additionally, the number of open flowers per individual and the resulting mean number of open flowers per species at the time of sampling (hereafter, flower number) were considered to more accurately estimate any potential trade-off between flower size and number, that is, whether larger-flowered species produce fewer flowers than smaller-flowered species (e.g., Goodwillie et al., 2010). The absence of a correlation between both traits associated with water allocation to flowers reduces a confounding effect of flower number and accounts for the estimates of variation in flower size depending on water availability. This correlation was conducted for 78 out of the 97 species considered in this study. Data for the 19 species sampled in the Municipal Park of Lagoa Comprida during 2022 could not be included due to logistical challenges during sampling, which resulted

in an incomplete observation and recording of flower number for these species.

## Data analysis

To examine the importance of season, substrate, species, and individuals as sources of variation in flower size, we determined the percentage of variation in this trait. Thus, the percentage of variation was calculated between seasons (rainy and dry), substrates (aquatic and terrestrial), and among species and individuals within species for each factor (season and substrate). We assessed the percentage of variation following the model for two-level nested ANOVA with unequal sample sizes (Sokal & Rohlf, 1995).

To test the effects of season and substrate on community-level flower size, we considered the phylogenetic relatedness among plants. We generated a phylogeny with the studied species using as a backbone the most updated and largest molecular phylogeny available for seed plants (Smith & Brown, 2018). The phylogenetic tree was constructed using the “phylo.maker” function from the package V.Phylomaker v0.1.0 (Jin & Qian, 2019) in the software R (R Core Team, 2022). The phylogenetic tree was generated with 97 tips representing species and 87 internal nodes. Among the 97 sampled species, 36 were present in the backbone phylogeny and 61 were successfully bound to this phylogeny (Appendix S1: Table S2). The binding processes added new genera and species to the branches of families or genera in the phylogeny, respectively, using approaches implemented in Phylomatic and BLADJ (details can be found in Jin & Qian, 2019). This procedure has been proven to be as effective as a fully resolved phylogeny in describing the phylogenetic relatedness among species. The phylogenetic tree was graphically generated using the FigTree program v1.4.4 (<http://tree.bio.ed.ac.uk/software/figtree/>).

To determine the influence of phylogenetic relatedness on mean flower size, we tested the phylogenetic signal for this trait using the “phylosig” function in the phytools package v1.5-1 (Revell, 2012) in R. We used the lambda index (Pagel, 1999) as it tends to provide a more robust effect size than other indices when calculating phylogenetic signals (Münkemüller et al., 2012). This index ranges from 0 to 1, with 0 (or values close to 0) indicating no phylogenetic signal, that is, the trait does not vary in association with phylogeny, and 1 (or close to 1) indicating a linear relationship between trait variation and phylogenetic distances among species, following a pattern typically approximated by a Brownian motion of the character across the phylogeny (i.e., presence of

phylogenetic signal; Pagel, 1999). To visualize the flower size distribution across the phylogeny, we used the “plotTree.barplot” function of the phytools package v1.5-1 (Revell, 2012).

We fitted a phylogenetic generalized linear mixed model (PGLMM; Pearse et al., 2014) to test the effects of season and substrate on flower size considering the phylogeny. To normalize the data, flower size was log-transformed as a suitable phylogenetic model was not found for quasi-Poisson error distribution with overdispersed data. In the PGLMM, season, substrate, and the interaction season  $\times$  substrate were included as fixed factors, whereas species nested within the interaction season  $\times$  substrate and individual nested within species were used as random factors.

We also tested the effects of season and/or substrate on flower size between sister species pairs or groups (i.e., three or more species) across the phylogeny. Specifically, we compared cases where one or more species bloom during the dry season, while the other(s) bloom during the rainy season, as well as cases where one or more species grow in aquatic substrates and the other(s) in terrestrial substrates. We fitted generalized linear mixed models (GLMMs). For sister species pairs, species was included as a fixed factor and individual nested within species as a random factor. For sister species groups, season or substrate was included as a fixed factor, whereas species nested within season or substrate and individual nested within species were used as random factors in the model. As flower size did not follow a normal distribution and due to overdispersion of data, we considered a quasi-Poisson error distribution with a log link function using the MASS package v7.3-60 (Venables & Ripley, 2013) in R.

## RESULTS

Flower size and number of flowers per species were not correlated (Spearman's rank correlation coefficient =  $-0.069$ ,  $p = 0.540$ ;  $N = 78$ ). Flower size was highly variable among species ( $8.54 \pm 21.79$  cm<sup>2</sup> [mean  $\pm$  SE], range: 0.09–140.29 cm<sup>2</sup>; Appendix S1: Figure S2). The high interspecific variation in flower size remained consistent for both seasons and substrates (Table 1). On average, flowers were 1.5 times larger during the rainy season than during the dry season ( $10.13 \pm 16.96$  and  $6.48 \pm 8.13$  cm<sup>2</sup>, respectively) and about twofold larger in aquatic substrates than in terrestrial substrates ( $13.97 \pm 25.06$  and  $7.28 \pm 9.22$  cm<sup>2</sup>, respectively). Particularly, the largest flowers were recorded during the rainy season in the aquatic substrate, where flowers were about three times larger than for the other interactions between

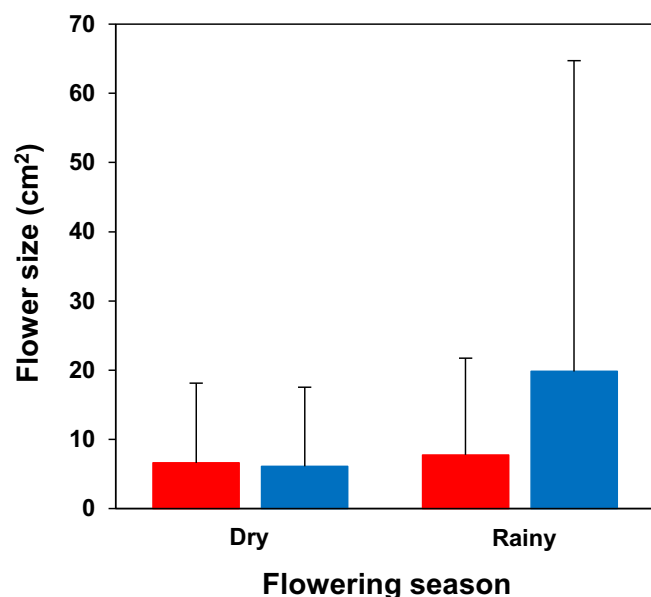
season and substrate (Figure 2). However, flower size showed the highest variation among plants within seasons and substrates (Table 1). Therefore, differences in flower size between seasons and substrates were not significant (Table 2).

Across the phylogeny, two of eight sister species pairs and groups that included species flowering in different seasons and/or substrates showed significant differences in flower size. *Commelina* and *Justicia* species flowering during the dry season had larger flowers than their sister species flowering in the rainy season (Table 3). We also found a significant phylogenetic signal for flower size ( $\lambda = 0.77$ ,  $p < 0.001$ ; Figure 3), indicating that this trait is phylogenetically constrained.

**TABLE 1** Percentage of variation (%) in flower size between seasons (rainy and dry) and substrates (aquatic and terrestrial).

Source of variation	Variation (%)
Season	9.1
Species	82.6
Individuals	8.3
Substrate	12.7
Species	78.0
Individuals	9.3

Note: For each factor (season and substrate), the variation among species and individuals within species is also shown.



**FIGURE 2** Effects of season and substrate (aquatic, blue; terrestrial, red) and their interaction on the variation in flower size (mean + SD) in 97 plant species sampled between January 2021 and December 2022 in the Pantanal wetland.

**TABLE 2** Summary of the phylogenetic generalized linear mixed model testing the effects of season, substrate, and the interaction season  $\times$  substrate on flower size of 97 plant species in the Pantanal wetland.

Variable	Value	df	<i>p</i>
Season	0.028	1	0.820
Substrate	0.053	1	0.836
Season $\times$ substrate	0.439	1	0.112

Note: Species was added as a random variable within the season  $\times$  substrate interaction, and individual was added as a random variable within species.

## DISCUSSION

Our study reveals a high variability in flower size across animal-pollinated species in the Pantanal, with the presence of a strong phylogenetic signal. This finding suggests that flower size exhibits phylogenetic conservatism or inertia, with closely related species showing convergent evolution for this trait. In other words, phylogeny determines flower size, indicating that the evolutionary history of plants constrains the adaptation of this trait in the Pantanal. In contrast, we did not detect any significant influence of water availability on community-level flower size variation, contrary to our expectations and water investment-based assumptions, which would potentially explain the appearance of smaller-flowered species under hot and dry conditions. Interestingly, we did find a trend toward larger flowers in aquatic substrates and during the rainy season, suggesting that water may still be relevant in determining variability and impose relevant selective pressures on flower size. In brief, the present study provides new insights into the evolutionary forces shaping flower size and adds nuances to the understanding about the adaptations of animal-pollinated plants in wetlands and seasonal tropical environments.

Our data indicate that many-flowered plants do not necessarily have smaller flowers, suggesting that the balance between size and number does not result in larger and less (or smaller and more) flowers. If the resource pool (e.g., water availability) for flower production is fixed, one would expect a trade-off between flower size and number, leading to a negative correlation between them (Ashman & Majetic, 2006; Goodwillie et al., 2010; Lanuza et al., 2023). However, a limitation of our study is that we did not account for the total number of flowers open by the sampled species and individuals during the flowering period. As a result, the number of flowers counted at the time of collection may not reflect the total number of flowers produced by an individual throughout the entire flowering season. Nevertheless, we confirmed that flower size, a relevant trait in animal-pollinated

**TABLE 3** Summary of the generalized linear mixed models testing the effect of season and/or substrate on flower size (mean  $\pm$  SD) of eight genera with two or more sister species across the phylogeny.

Genus	Species	Flower size (cm <sup>2</sup> )				$\chi^2$	p
		Dry	Rainy	Aquatic	Terrestrial		
<i>Bacopa</i>	3	0.86 $\pm$ 0.21	0.85 $\pm$ 0.69	0.86 $\pm$ 0.21	0.85 $\pm$ 0.69	0.06	0.775
<i>Commelina</i>	2	0.44 $\pm$ 0.12	0.22 $\pm$ 0.07	...	...	50.81	<0.001
<i>Fridericia</i>	2	9.58 $\pm$ 2.34	8.50 $\pm$ 1.17	...	...	2.83	0.093
<i>Justicia</i>	2	2.11 $\pm$ 0.39	1.66 $\pm$ 0.41	...	...	10.96	0.001
<i>Ludwigia</i>	6	6.05 $\pm$ 6.33	2.75 $\pm$ 1.51	4.22 $\pm$ 4.14	1.62 $\pm$ 0.63 <sup>a</sup>	1.93	0.168
<i>Senna</i>	3	6.34 $\pm$ 1.25	6.80 $\pm$ 3.08	...	...	0.24	0.877
<i>Solanum</i>	4	4.90 $\pm$ 2.42	2.93 $\pm$ 0.40	...	...	1.69	0.195
<i>Utricularia</i>	2	...	...	0.61 $\pm$ 0.25	0.59 $\pm$ 0.20	0.15	0.695

Note: Species was added as a random variable within the season or substrate for the analyses comparing three or more species, while individual was added as a random variable within species in all the analyses (when two species were compared, the effect tested was species instead of season or substrate).

<sup>a</sup> $\chi^2 = 1.0$  and  $p = 0.312$ .

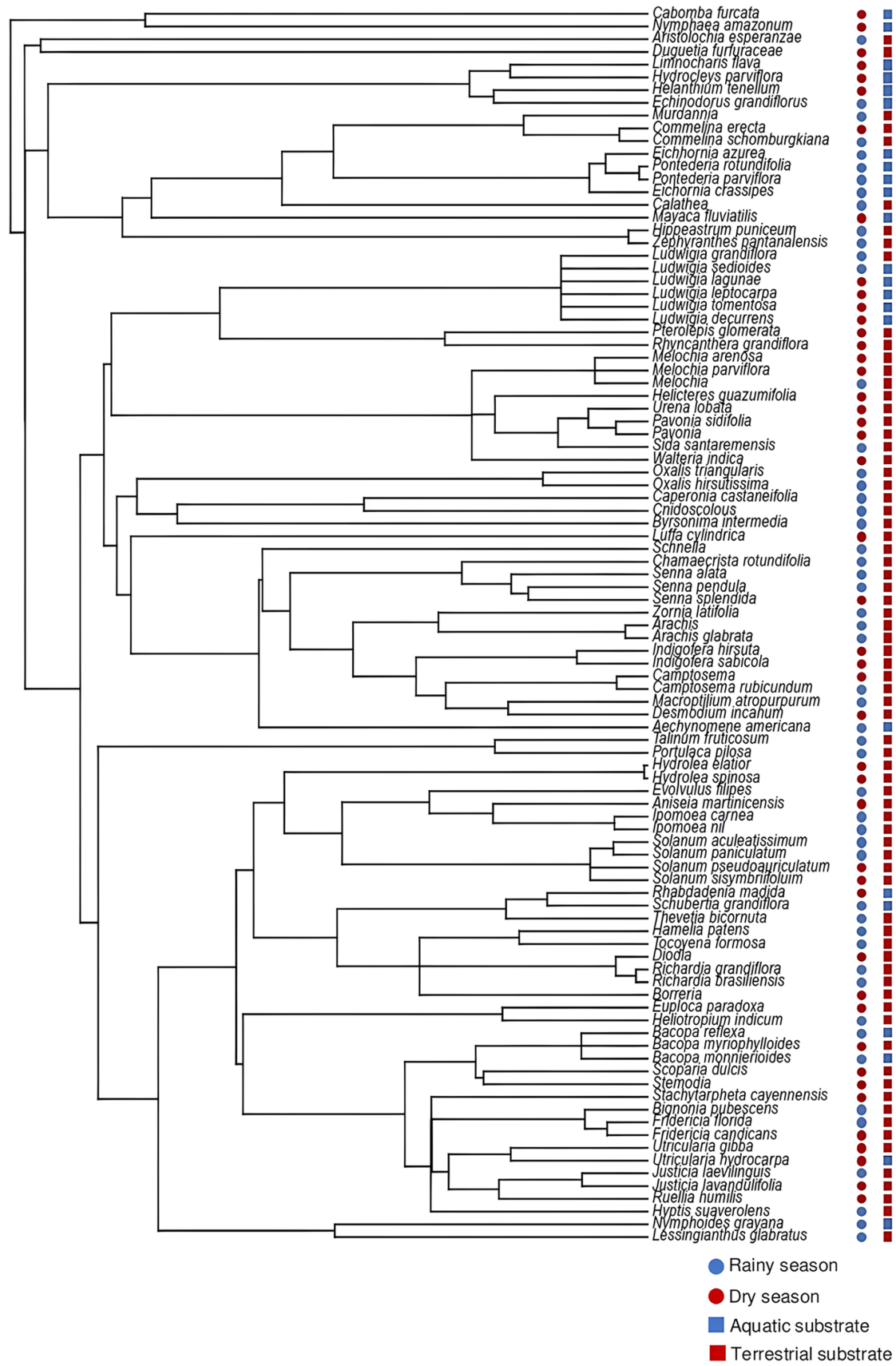
plants exposed to strong selective pressures imposed by a cost–benefit balance, shows a high interspecific variability regardless of flower number.

Through our approach based on differences in water availability between seasons and substrates in a seasonally flooded wetland, we did not detect a significant influence of these factors on flower size of animal-pollinated plant communities in the Pantanal. As reported in other studies, flower size decreases in response to water limitation (Galen, 1999; Herrera, 2009; Phillips et al., 2018). This process occurs because larger flowers require greater water allocation for production and higher amounts of this resource for corolla transpiration than smaller flowers (Patiño & Grace, 2002; Teixido et al., 2019; Teixido & Valladares, 2014). Despite the non-significant effect of season and substrate on community-level flower size and the high variability of this trait across the studied species, we did find trends of larger flowers in aquatic species, particularly those flowering during the rainy season. We propose that this trend is not irrelevant as it suggests that abiotic factors related to water availability may still exert persistent evolutionary pressures on flower size in the Pantanal, beyond phylogenetic limitations. Our findings demonstrate the relevance of phylogeny and abiotic factors to understand the variation in flower size within plant communities flowering across contrasting environmental conditions.

Our particular findings on congeneric species suggest that seasonality can occasionally influence interspecific flower size variation. Interestingly, two sister species pairs exhibited larger flowers during the dry season than the rainy season, challenging the water investment-based assumptions typically associated with flower size. In a Brazilian savanna, two *Kielmeyera* species with

contrasting flowering phenology also exhibit differences in flower size, with the species blooming during the dry season producing smaller flowers due to the high evaporative demand during this period (Teixido et al., 2019). However, in scenarios where pollinator-mediated selective pressures (pollen limitation) outweigh water-mediated selection (water limitation), flowers can increase in size despite the associated costs (Caruso et al., 2019; Galen, 1999; Teixido et al., 2016). Particularly, the abundance and effectiveness of different functional groups of pollinators may exert relevant evolutionary pressures on this trait (Dellinger, 2020; Fenster et al., 2004; Rosas-Guerrero et al., 2014).

In the Pantanal, the availability of flowers and pollinators decreases during the dry season, increasing interspecific competition, reducing niche overlap among pollinators, and leading to greater functional specialization in pollination and specific plasticity in flower size (Souza et al., 2018). In this context, the assemblage and activity of pollinators during the flowering season may potentially determine the variation in flower size between congeneric species with different phenologies, favoring smaller-flowered species in the dry period. However, pollinator-mediated selection for larger flowers has been mostly reported among individuals within populations, yet little is known about the interspecific variation in flower size across multiple taxa in a community (Roddy et al., 2021). Within a given ecosystem, flower size divergence among plant species may mirror the preference for the most effective pollinator regardless of its abundance (e.g., bird-pollinated large flowers or insect-pollinated small flowers; Dellinger, 2020; Fenster et al., 2004). However, we did not address the assemblage of pollinators or visitation rates, factors that may indeed



**FIGURE 3** Phylogenetic relationship of the 97 species sampled between January 2021 and December 2022 in the Pantanal wetland and flower size (mean + SD) across the phylogeny. Circles show flowering season for each species, and squares show substrate where species occur. The phylogeny was reconstructed following Jin and Qian (2019).

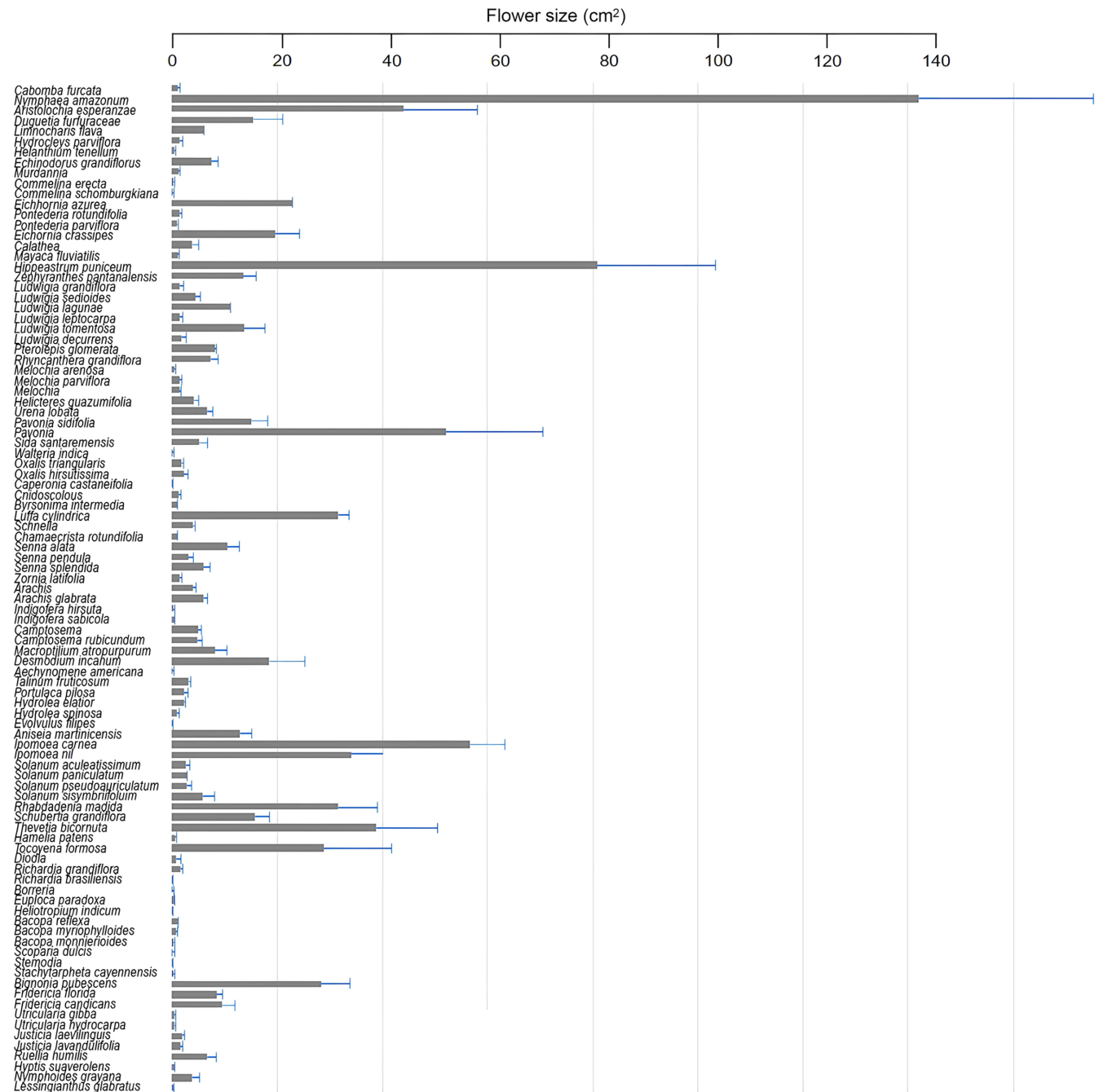


FIGURE 3 (Continued)

vary with seasonality and have a significant effect on flower size.

Simultaneously, the presence of florivores may also have a considerable effect on flower size. A recent study reported that this trait was decisive in the incidence and intensity of florivory in animal-pollinated plants in the Pantanal, where larger-flowered species suffered greater attack regardless of season and substrate (Ortiz et al., 2023). Although the variation in florivory patterns did not show a relationship with abiotic factors

associated with water availability, the relevance of the interaction between florivores and flower size suggests that this process may involve significant selective pressures on the trait. Altogether, the relationship between plants and their floral visitors reveals the importance of further considering biotic factors for an in-depth understanding of flower size variation.

Beyond floral visitors, non-mutually exclusive alternatives not considered in this study may constitute concurrently interacting actions with the relationship

between water and flower size, resulting in confounding effects that hinder more robust conclusions. In this regard, sample size may be limited, and the addition of more species may be relevant to understand the effects of season and substrate on flower size variation. Conversely, the effect of water availability may be obscured when comparing species from different families and taxonomic levels as this may amplify the phylogenetic signal for flower size. Other explanations, however, may be more plausible for interpreting our results. Certainly, flower size seems to be strongly conserved across phylogenies, regardless of the taxonomic level considered (Armbruster et al., 1999; Oguro & Sakai, 2015; Teixeira et al., 2017). Moreover, water shortage has been shown to drive variation in flower size across different families within plant communities (Phillips et al., 2018).

Otherwise, the presence of species that flower year-round may help to explain the high variability in flower size as seasonal modifications in this trait modulated by plastic responses have been reported in the Pantanal (Catian et al., 2019; Gomes & Aoki, 2015). For example, macrophyte communities exhibit a high phenological variation, where different life forms (amphibious, emergent, floating, submerged) adjust their flowering allocation throughout the year depending on the monthly variations in flood regime (Catian et al., 2019). Lastly, water evaporation through the leaves may influence flower size. However, while flowers are mostly heterotrophic and short-lived compared to leaves, they are crucial in early reproductive success, affecting fitness. Several studies have shown that flowers are constrained by different physiological factors, with water loss from flowers greatly exceeding that of leaves (e.g., Teixeira & Valladares, 2014). This results in no correlation between leaf and flower size, indicating that flower and leaf economic traits evolved independently (Roddy et al., 2019; Zhang et al., 2017).

We found that phylogeny determined the variation in flower size, reinforcing the phylogenetic inertia of this trait (Armbruster et al., 1999; Teixeira et al., 2017). Thus, closely related species across the phylogeny tend to share similar flower size with a strong phylogenetic signal. In the Pantanal, where diverse floristic elements from the Amazon and the Cerrado co-occur, the evolutionary history of plants sharing common biogeographic origins may be more relevant than in other wetlands and seasonal tropical environments (Pott & Pott, 2021), potentially playing a significant role in determining flower size. Likewise, the observed phylogenetic signal may reflect ecological preferences, with closely related species displaying consistent flower size due to shared adaptations to similar environments and/or seasonal conditions. For instance, this would imply that species within clades

comprising typically aquatic taxa (e.g., *Eichhornia*, *Ludwigia*, *Nymphaea*, *Pontederia*) tend to have larger flowers than terrestrial species. Despite phylogenetic limitations, flower size is still considered a labile trait across ecosystems and lineages that easily adapts to selective pressures exerted by abiotic factors such as water availability (Kuppler & Kotowska, 2021). Therefore, flower size evolution may be complex and reflect the result of the combined action of multiple interacting factors. This complexity reinforces the importance of considering the phylogenetic relationships among plants as a fundamental determinant factor on flower size, apparently surpassing immediate ecological pressures.

To conclude, this study provides valuable contributions to unravel the effects of water-related environmental factors and the influence of phylogeny on flower size variation. The presence of a phylogenetic signal for this trait suggests that the evolutionary history of plants imposes a selective constraint on flower size. However, the trend toward larger flowers under wetter conditions, particularly in species of aquatic substrates during the rainy season, highlights the potential role that the pressure imposed by water availability and adaptation to the environment may play in determining flower size in the world's largest tropical wetland. This study contributes to a more comprehensive understanding of the evolution of this essential trait for the reproductive success of animal-pollinated plants in ecosystems with marked seasonality in water availability. We recommend that future research considers the potential multiple effects of phylogeny, environmental conditions, and flower–animal interactions on flower size across different ecosystems and plant communities.

#### AUTHOR CONTRIBUTIONS

Layla M. A. Rodrigues and Alberto L. Teixeira conceived and designed research. Layla M. A. Rodrigues and Camila Aoki collected the data. Layla M. A. Rodrigues, Thais B. Zanata, and Alberto L. Teixeira analyzed the data. Layla M. A. Rodrigues, Thais B. Zanata, Camila Aoki, and Alberto L. Teixeira wrote and edited the manuscript.

#### ACKNOWLEDGMENTS

We thank Gaby Ortiz, Larissa Lavoratti, Leticia Aquino, and Anderson Faria for fieldwork support. Ana Paula Caetano, Mariana O. Duarte, Temilze G. Duarte, and three anonymous reviewers provided suggestions during the earlier versions of the manuscript. Arnildo Pott, Vali J. Pott, Lucia Lohmann, and Miriam Kaehler assisted with plant identification. The drivers of the UFMT provided transport logistics. Layla M. A. Rodrigues was funded with a MsC scholarship from the Coordenação de

Aperfeiçoamento de Pessoal de Nível Superior (CAPES, Brazil, Finance Code 001). The study was also partially funded by the Universidade Federal de Mato Grosso do Sul (UFMS/MEC).

## CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

## DATA AVAILABILITY STATEMENT

Data (Rodrigues et al., 2025) are available from Figshare: <https://doi.org/10.6084/m9.figshare.28506677.v1>.

## REFERENCES

- Ackerly, D. D. 2009. "Conservatism and Diversification of Plant Functional Traits: Evolutionary Rates Versus Phylogenetic Signal." *Proceedings of the National Academy of Sciences of the United States of America* 106: 19699–706.
- Armbruster, W. S., V. S. Di Stilio, J. D. Tuxill, T. C. Flores, and J. L. Velasquez Runk. 1999. "Covariance and Decoupling of Floral and Vegetative Traits in Nine Neotropical Plants: A Re-Evaluation of Berg's Correlation-Pleiades Concept." *American Journal of Botany* 86: 39–55.
- Ashman, T.-L., and C. J. Majetic. 2006. "Genetic Constraints on Floral Evolution: A Review and Evaluation of Patterns." *Heredity* 96: 343–352.
- Bell, G. 1985. "On the Function of Flowers." *Proceedings of the Royal Society of London. Series B. Biological Sciences* 224: 223–265.
- Blomberg, S. P., and T. Garland, Jr. 2002. "Tempo and Mode in Evolution: Phylogenetic Inertia, Adaptation and Comparative Methods." *Journal of Evolutionary Biology* 15: 899–910.
- Caruso, C. M., K. E. Eisen, R. A. Martin, and N. Sletvold. 2019. "A Meta-Analysis of the Agents of Selection on Floral Traits." *Evolution* 73: 4–14.
- Catian, G., E. Scremin-Dias, and A. Pott. 2019. "Reproductive Phenology of Macrophyte Community in Response to Wetland Flooding Cycle." *Oecologia Australis* 23: 856–873.
- da Nunes Cunha, C., I. Bergier, W. Moraes Tomas, G. A. Damasceno-Júnior, S. A. Santos, V. A. Assunção, A. L. B. Sartori, et al. 2023. "Classificação dos Macrohabitats do Pantanal Matogrossense para Fins de Gestão: Atualização para Políticas Públicas e Manejo de Áreas Protegidas." *Biodiversidade Brasileira* 13: 1–26.
- da Nunes Cunha, C., L. Rebellato, and C. P. Costa. 2010. "Vegetação e Flora: Experiência Pantaneira no Sistema de Grade." In *Biodiversidade no Pantanal de Poconé*, edited by I. M. Fernandes, C. A. Signor, and J. Penha, 37–57. Manaus: Attema.
- Dellinger, A. S. 2020. "Pollination Syndromes in the 21st Century: Where Do We Stand and Where May We Go?" *New Phytologist* 228: 1193–1213.
- Fenster, C. B., W. S. Armbruster, P. Wilson, M. R. Dudash, and J. D. Thomson. 2004. "Pollination Syndromes and Floral Specialization." *Annual Review of Ecology, Evolution, and Systematics* 35: 375–403.
- Galen, C. 1999. "Why Do Flowers Vary? The Functional Ecology of Variation in Flower Size and Form within Natural Plant Populations." *BioScience* 49: 631–640.
- Gervasi, D., and F. Schiestl. 2017. "Real-Time Divergent Evolution in Plants Driven by Pollinators." *Nature Communications* 8: e14691.
- Gomes, A. C., and C. Aoki. 2015. "Efeito da Sazonalidade Hídrica Sobre a Fitossociologia de Macrófitas Aquáticas em uma Lagoa no Pantanal, Brasil." *Revista de Biologia Neotropical* 12: 1–7.
- Goodwillie, C., R. D. Sargent, C. G. Eckert, E. Elle, M. A. Geber, M. O. Johnston, and A. A. Winn. 2010. "Correlated Evolution of Mating System and Floral Display Traits in Flowering Plants and Its Implications for the Distribution of Mating System Variation." *New Phytologist* 185: 311–321.
- Herrera, J. 2009. "Visibility vs. Biomass in Flowers: Exploring Corolla Allocation in Mediterranean Entomophilous Plants." *Annals of Botany* 103: 1119–27.
- Jin, Y., and H. Qian. 2019. "V. PhyloMaker: An R Package that Can Generate Very Large Phylogenies for Vascular Plants." *Ecography* 42: 1353–59.
- Junk, W. J., and C. da Nunes Cunha. 2012. "Pasture Clearing from Invasive Woody Plants in the Pantanal: A Tool for Sustainable Management or Environmental Destruction?" *Wetlands Ecology & Management* 20: 111–122.
- Junk, W. J., C. da Nunes Cunha, K. M. Wantzen, P. Petermann, C. Strüssman, M. I. Marques, and J. Adis. 2006. "Biodiversity and Its Conservation in the Pantanal of Mato Grosso, Brazil." *Aquatic Sciences* 68: 278–309.
- Kuppler, J., and M. M. Kotowska. 2021. "A Meta-Analysis of Responses in Floral Traits and Flower-Visitor Interactions to Water Deficit." *Global Change Biology* 27: 3095–3108.
- Lanuza, B. J., R. Rader, J. Stavert, L. K. Kendall, M. E. Saunders, and I. Bartomeus. 2023. "Covariation among Reproductive Traits in Flowering Plants Shapes Their Interactions with Pollinators." *Functional Ecology* 37: 2072–84.
- Machado, I. C., and A. V. Lopes. 2004. "Floral Traits and Pollination Systems in the Caatinga, a Brazilian Tropical Dry Forest." *Annals of Botany* 94: 365–376.
- Morellato, L. P. C., M. G. G. Camargo, and E. Gressler. 2013. "A Review of Plant Phenology in South and Central America." In *Phenology: An Integrative Environmental Science*, edited by M. D. Schwartz, 91–113. Dordrecht: Springer.
- Münkemüller, T., S. Lavergne, B. Bzeznik, S. Dray, T. Jombart, K. Schifffers, and W. Thuiller. 2012. "How to Measure and Test Phylogenetic Signal." *Methods in Ecology & Evolution* 3: 743–756.
- Oguro, M., and S. Sakai. 2015. "Relation between Flower Head Traits and Florivory in Asteraceae: A Phylogenetically Controlled Approach." *American Journal of Botany* 102: 407–416.
- Oliveira, C. S., J. V. S. Messeder, A. L. Teixeira, M. R. R. Arantes, and F. A. O. Silveira. 2021. "Vegetative and Reproductive Phenology in a Tropical Grassland-Savanna-Forest Gradient." *Journal of Vegetation Science* 32: e12997.
- Ortiz, G. L., Y. Columbano, M. V. de Melo, M. G. Boaventura, C. Aoki, T. Cornelissen, C. S. Souza, and A. L. Teixeira. 2023. "Among-Species Variation in Flower Size Determines Florivory in the Largest Seasonally Flooded Tropical Wetland." *American Journal of Botany* 110: e16186.
- Pagel, M. 1999. "Inferring the Historical Patterns of Biological Evolution." *Nature* 401: 877–884.
- Patiño, S., and J. Grace. 2002. "The Cooling of Convolvulaceous Flowers in a Tropical Environment." *Plant Cell and Environment* 25: 41–51.

- Pearse, W. D., J. Cavender-Bares, A. Puvis, and M. R. Helmus. 2014. "Metrics and Models of Community Phylogenetics." In *Modern Phylogenetic Comparative Methods and Their Application in Evolutionary Biology—Concepts and Practice*, edited by L. Z. Garamszegi, 451–464. Berlin: Springer-Verlag.
- Phillips, B. B., R. F. Shaw, M. J. Holland, E. L. Fry, R. D. Bardgett, J. M. Bullock, and J. L. Osborne. 2018. "Drought Reduces Floral Resources for Pollinators." *Global Change Biology* 24: 3226–35.
- Pott, A., and V. J. Pott. 1994a. *Plantas do Pantanal*. Corumbá: Empresa Brasileira de Pesquisa Agropecuária, Centro de Pesquisa Agropecuária do Pantanal.
- Pott, A., and V. J. Pott. 1994b. *Plantas Aquáticas do Pantanal*. Corumbá: Empresa Brasileira de Pesquisa Agropecuária, Centro de Pesquisa Agropecuária do Pantanal.
- Pott, A., and V. J. Pott. 2021. "Flora of the Pantanal." In *Flora and Vegetation of the Pantanal Wetland*, edited by G. A. Damasceno-Junior and A. Pott, 39–228. Cham: Springer.
- R Core Team. 2022. *R: A Language and Environment for Statistical Computing*. Vienna: R Foundation for Statistical Computing. <https://www.R-project.org/>.
- Revell, L. J. 2012. "Phytools: An R Package for Phylogenetic Comparative Biology (and Other Things)." *Methods in Ecology & Evolution* 3: 217–223.
- Roddy, A. B., G. F. Jiang, K. Cao, K. A. Simonin, and C. R. Brodersen. 2019. "Hydraulic Traits Are More Diverse in Flowers than in Leaves." *New Phytologist* 223: 193–203.
- Roddy, A. B., C. Martínez-Perez, A. L. Teixeira, T. G. Cornelissen, M. E. Olson, R. S. Oliveira, and F. A. O. Silveira. 2021. "Towards the Flower Economics Spectrum." *New Phytologist* 229: 665–672.
- Rodrigues, L. M. A., T. B. Zanata, C. Aoki, and A. L. Teixeira. 2025. "Data From: Untangling the Effects of Water Availability on Flower Size: Insights from the Largest Tropical Wetland." Figshare. Dataset. <https://doi.org/10.6084/m9.figshare.28506677.v1>.
- Rosas-Guerrero, V., R. Aguilar, S. Martín-Rodríguez, L. Ashworth, M. Lopezaraiza-Mikel, J. M. Bastida, and M. Quesada. 2014. "A Quantitative Review of Pollination Syndromes: Do Floral Traits Predict Effective Pollinators?" *Ecology Letters* 17: 388–400.
- Schemske, D. W., and H. D. Bradshaw. 1999. "Pollinator Preference and the Evolution of Floral Traits in Monkeyflowers (*Mimulus*)." *Proceedings of the National Academy of Sciences of the United States of America* 96: 11910–15.
- Schneider, C. A., W. S. Rasband, and K. W. Eliceiri. 2012. "NIH Image to ImageJ: 25 Years of Image Analysis." *Nature Methods* 9: 671–75.
- Smith, S. A., and J. W. Brown. 2018. "Constructing a Broadly Inclusive Seed Plant Phylogeny." *American Journal of Botany* 105: 302–314.
- Sokal, R. R., and F. J. Rohlf. 1995. *Biometry: The Principles and Practice of Statistics in Biological Research*, 3rd ed. San Francisco: W. H. Freeman and Co.
- Souza, C. S., P. K. Maruyama, C. Aoki, M. R. Sigrist, J. Raizer, C. L. Gross, and A. C. de Araujo. 2018. "Temporal Variation in Plant–Pollinator Networks from Seasonal Tropical Environments: Higher Specialization When Resources Are Scarce." *Journal of Ecology* 106: 2409–20.
- Stanton, M. L., A. A. Snow, and S. N. Handel. 1986. "Floral Evolution: Attractiveness to Pollinators Increases Male Fitness." *Science* 232: 1625–27.
- Teixido, A. L., M. Barrio, and F. Valladares. 2016. "Size Matters: Understanding the Conflict Faced by Large Flowers in Mediterranean Environments." *Botanical Review* 82: 204–228.
- Teixido, A. L., R. L. C. Dayrell, A. J. Arruda, L. O. Azevedo, P. A. Junqueira, J. V. S. Messeder, and F. A. O. Silveira. 2018. "Differential Gender Selection on Flower Size in Two Neotropical Savanna Congeneric Species." *Plant Ecology* 219: 89–100.
- Teixido, A. L., B. Guzmán, V. G. Staggemeier, and F. Valladares. 2017. "Phylogeny Determines Flower Size-Dependent Sex Allocation at Flowering in a Hermaphroditic Family." *Plant Biology* 19: 963–972.
- Teixido, A. L., V. B. Leite-Santos, E. A. Paiva, and F. A. O. Silveira. 2019. "Water-Use Strategies in Flowers from a Neotropical Savanna under Contrasting Environmental Conditions during Flowering." *Plant Physiology & Biochemistry* 144: 283–291.
- Teixido, A. L., and F. Valladares. 2014. "Disproportionate Carbon and Water Maintenance Costs of Large Corollas in Hot Mediterranean Ecosystems." *Perspectives in Plant Ecology, Evolution & Systematics* 16: 83–92.
- Venables, W. N., and B. D. Ripley. 2013. *Modern Applied Statistics with S*. New York: Springer.
- Ximenes, L. D. S. V., V. J. Pott, and C. Aoki. 2017. "Plantas Aquáticas do Parque Natural Municipal da Lagoa Comprida, Aquidauana, Mato Grosso do Sul, Brasil." *Boletim do Museu Paraense Emílio Goeldi-Ciências Naturais* 12: 187–195.
- Zhang, F. P., Y. J. Yang, Q. Y. Yang, W. Zhang, T. J. Brodribb, G. Y. Hao, and S. B. Zhang. 2017. "Floral Mass per Area and Water Maintenance Traits Are Correlated with Floral Longevity in *Paphiopedilum* (Orchidaceae)." *Frontiers in Plant Science* 8: 501. <https://doi.org/10.3389/fpls.2017.00501>.

## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

**How to cite this article:** Rodrigues, Layla M. A., Thais B. Zanata, Camila Aoki, and Alberto L. Teixeira. 2025. "Untangling the Effects of Water Availability on Flower Size: Insights from the Largest Tropical Wetland." *Ecosphere* 16(5): e70244. <https://doi.org/10.1002/ecs2.70244>