



Research article

Water-use strategies in flowers from a neotropical savanna under contrasting environmental conditions during flowering

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ABSTRACT

Flowers require high amounts of water, which ultimately may compromise pollinator attractiveness under water limitation. Water-use and -conservation strategies in leaves from hot and dry ecosystems are well documented, yet little is known about mechanisms of water allocation in flowers, particularly in tropical savanna ecosystems. We evaluated traits related to corolla water status in two *Kielmeyera* species that differ in flowering phenology and flower size: larger-flowered *K. regalis* blooms during the rainy summer and smaller-flowered *K. coriacea* blooms during the dry winter. To test the hypothesis that water demand in corollas increases with increasing vapor pressure deficit (VPD), we analyzed interspecific differences in corolla stomatal conductance and density, water content, and fresh and dry mass per unit area. We also performed hand-pollination and pollinator-exclusion experiments to determine variation in floral longevity. Corolla transpiration rates were higher in *K. coriacea* (157 vs 95 g-H₂O-m⁻²h⁻¹ for *K. coriacea* and *K. regalis*, respectively), and increased with VPD in both species. Stomatal density was 25-fold higher in *K. coriacea*, and corolla fresh and dry mass per unit of area were 47% and 21% higher, respectively, in *K. coriacea*, due to thick pectin-rich cell walls. The high pectin content increases water content in corollas of *K. coriacea*. Regardless of pollination, flowers lasted one day in *K. coriacea* and three in *K. regalis*. Our study suggests structure-function relationships of floral traits with flowering season, and that *K. coriacea* displays small and short-lived corollas with high water content to buffer the high evaporative demand during the dry period.

1. Introduction

In animal-pollinated plants, the production and maintenance of flowers require a considerable water allocation. Water is a limiting essential resource required for bud development and flower opening (van Doorn, 1997; Zhang et al., 2017), as well as for the maintenance of corolla turgor and temperature regulation, which is related to pollinator attraction (Patiño and Grace, 2002; Roddy et al., 2018, 2019). In fact, corolla water loss can significantly exceed that of leaves (Lambrecht, 2013; Teixeira and Valladares, 2014; Roddy et al., 2018), compromising floral physiological function (Galen et al., 1999; Lambrecht and Dawson, 2007). Water availability and temperature regulation are of paramount importance in flowers, since mating opportunities are spatially and temporally limited and thermal optima for processes involved in sexual reproduction are narrower than optima for development and growth functions (Erickson and Markhart, 2002; Fang et al., 2010).

Water allocation costs vary greatly with pollination-related flower traits such as floral longevity (Ashman and Schoen, 1994; Zhang et al., 2017), and even offset pollinator-mediated selection on floral attractiveness (Galen, 1999; Caruso, 2006; Gallagher and Campbell, 2017). Therefore, determining the factors that modulate water relations in flowers is critical to better understand their evolution and the ecological complexity of multiple plant-pollinator-environment interactions.

Because water availability is highly variable across habitats, water requirements and status during flowering may also reflect site-specific differences (Lambrecht and Dawson, 2007; Lambrecht, 2013; Teixeira et al., 2018). Transpirational cooling is a critical process to minimize floral overheating (Patiño and Grace, 2002; Teixeira and Valladares, 2014), and because water is particularly important under high evaporative demand of flowers, the detrimental effects of water shortage may be acute under dry conditions (Galen, 2005). Altogether, heat and drought can damage flowers and shorten floral longevity, ultimately

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reducing reproductive output (Konsens et al., 1991; Erickson and Markhart, 2002; Fang et al., 2010). Accordingly, high water demand in flowers from seasonal environments requires not only an adequate water supply from vascular tissues but also effective resource-conservation strategies to maintain floral physiological and reproductive functions (Chapotin et al., 2003; Feild et al., 2009; Roddy et al., 2018). Investigating the mechanisms related to floral water status is, thus, crucial to understand how flowers delay wilting to maintain turgor and pollinator attractiveness under hot and dry environments.

Leaf anatomical and physiological traits associated with temperature stress and water loss are globally well-documented in plants adapted to hot and dry conditions (e.g. Thompson, 2005; Lambers et al., 2008; Singh and Chaturvedi, 2018). For example, succulence is characteristic of thick water-storage leaves that increase their water content at the expense of a reduced specific area (Vendramini et al., 2002; Ogburn and Edwards, 2012). In fact, small-leaved species predominate in hotter and drier environments (Wright et al., 2017). Sclerophylly is also a central trait typically associated with cell wall thickness, thick cuticles and a subsequent high leaf dry mass per unit area, which ultimately influences stomatal regulation and water-use efficiency in long-lived evergreen leaves (Wright et al., 2004; John et al., 2017).

There is mounting evidence that floral water costs limit flower size, suggesting that small, water-conserving flowers are potentially advantageous in hot and dry environments, similarly to what has been shown for leaves (Lambrecht and Dawson, 2007; Gallagher and Campbell, 2017; Phillips et al., 2018). Heat and drought also reduce floral longevity to minimize flower maintenance costs in water-limited environments (Vesprini and Pacini, 2005; Teixeira and Valladares, 2015; Dudley et al., 2018). Unfortunately, only a handful of studies have concurrently examined anatomical and physiological traits underpinning water-use and -conservation strategies in flowers. Some large and heavy flowers from tropical dry forest species accumulate significant amounts of apoplastic polysaccharides and greater water retention in petals, which may temporarily buffer water deficits and maintain flower turgidity (Chapotin et al., 2003). Consistently, a high floral dry mass per unit area results in a more effective water-conservation strategy, an alleged adaptation to dry conditions (Zhang et al., 2017). Otherwise, the low density and functionality of stomata on petals may limit the regulation of corolla temperature and water loss (Lipayeva, 1989; Feild et al., 2009; Zhang et al., 2018). In this regard, examination of the presence and density of stomata on petals would provide further insights into the water balance and control in flowers (see Gleason, 2018).

Here, we explore flower physiological and anatomical traits underpinning water-use strategies in flowers of two congeneric *Kielmeyera* (Calophyllaceae) species which flower in periods of contrasting environmental conditions in a seasonal Neotropical savanna. *Kielmeyera* comprises predominantly hermaphroditic species with large and solitary flowers widely distributed in the Brazilian Cerrado (Oliveira and Sazima, 1990; Ranieri et al., 2012). *K. coriacea* produces small flowers during the dry winter, whereas flowers of *K. regalis* are larger and produced during the wet summer. Therefore, the study system offers opportunities to examine the structure-function relationships of flower anatomical and physiological traits in congeneric species flowering at periods with contrasting environmental conditions. We performed field studies of corolla transpiration rates to determine microclimate-driven variation in patterns in flower water demand. We combined fieldwork with laboratory studies in both species to analyze potential interspecific differences in corolla stomata density, water content, and fresh and dry mass per unit of area. We also recorded floral longevity and performed an experiment to determine pollination-dependent potential variation for this trait. We expected corolla transpiration rates to be higher in flowers of *K. coriacea* due to higher air temperature and lower relative humidity that, combined, result in higher vapor pressure deficit (VPD; Feild et al., 2009; Lambrecht, 2013; Roddy et al., 2018). We also predicted structure-function relationships between anatomical and

physiological traits, with water-conservation strategies (shorter-lived flowers with higher water content and dry mass per unit of area) expressed predominantly in flowers of *K. coriacea* to buffer the water demands during the hot and dry period.

2. Material and methods

2.1. Species and study area

Kielmeyera coriacea Mart. is a small tree broadly distributed across the Amazonian and Cerrado biomes (Oliveira and Sazima, 1990; Brazilian Flora 2020 project: <http://floradobrasil.jbrj.gov.br/>). Flowering occurs in October, during the end of the dry season, and plants produce predominantly monoclinal (hermaphroditic), pinkish-white flowers 4–7 cm in diameter contained in several inflorescences (Oliveira and Sazima, 1990; Teixeira et al., 2018; Fig. S1). *Kielmeyera regalis* Saddi is an endemic shrub of *campo rupestre* from the Espinhaço Range, south-eastern Brazil (Saddi, 1987). The flowering period spans from January to March, during the wet season. Flowers are disc-shaped, hermaphroditic, individual and pink, ranging 7–10 cm in diameter (Saddi, 1987; Teixeira et al., 2019a; Fig. S1).

The study was conducted during the flowering peak in one population of *K. coriacea* (October 2016) and one population of *K. regalis* (February 2017), located at Serra do Cipó, southern region of the Espinhaço Range, Minas Gerais, Brazil (see Teixeira et al., 2018 for details). The area is characterized by high solar exposure, strong winds with a seasonal climate defined by wet summers (November to March) and dry winters (May to September), interspersed with a post-rain period in April and a post-dry period in October (Brito et al., 2017; see Fig. S2 for historical mean air temperature and relative humidity). The soils are acidic, extremely impoverished and shallow, with poor water-holding capacity, so that plants are water-stressed during the dry season.

2.2. Corolla size and floral longevity

Total corolla surface area (cm²) was determined to test for relationships between corolla size and water allocation for each species after flower growth ceased. A total of 20 plants per species were randomly selected and tagged during the flowering peak. We recorded corolla diameter (cm) in two flowers per plant using a metal tape measure (to the nearest mm). We also carefully excised one petal from each measured flower and photographed it on a black sheet to assess its surface area by means of image processing in ImageJ v1.51 (ImageJ, 2016; US National Institutes of Health, Bethesda, MD, USA). We subsequently multiplied each value by five to estimate corolla surface area (cm²), since flowers have five petals in both species. Then, we averaged corolla diameter and area per individual and species.

We experimentally manipulated potential variation in floral longevity by conducting three different treatments per species, each using ten individuals and three randomly selected individual flowers per plant. The treatments were: 1) flowers that were hand-pollinated with xenogamous pollen, 2) flowers that had pollen excluded by capping stigmas, and 3) unmanipulated naturally pollinated control flowers. Hand-pollination was carried out with five donor flowers collected from five different individuals within a 10-m radius of the recipient flower (Teixeira et al., 2019). We then deposited outcrossed pollen on the stigma 2 h after anthesis to ensure stigmatic receptivity by means of direct contact of anthers from donor flowers using circular movements until the stigmatic area was fully covered by pollen. Capping was carried out after flowers were totally open to avoid damage and early in the morning to ensure that no pollen was deposited on the stigma. Caps were made of yellow modelling clay covering the stigma (Teixeira and Valladares, 2015).

2.3. Corolla transpiration rates

During the flowering peak of each species, the 20 plants selected for corolla size were used. At each plant, two fully-expanded, sun-oriented flowers were measured ($N = 40$ flowers per species). Completely open flowers were measured only once between 08:00 a.m. and 13:00 p.m. during three consecutive days. On one petal of every flower we recorded stomatal conductance to water vapor, g_s ($\text{mmol m}^{-2} \text{s}^{-1}$), and corolla temperature with a portable steady-state porometer (Model AP4 Delta T Devices, Cambridge, UK). We assumed that both surfaces have the same conductances and only measured the abaxial surface (van Doorn, 1997; Patiño and Grace, 2002; Teixeira and Valladares, 2014). We also measured air temperature ($^{\circ}\text{C}$) and air relative humidity (%) for each stomatal conductance record using a data-logger (LI-1400, Decagon Devices, Pullman, WA, USA). We subsequently calculated VPD from air temperature and relative humidity. Then, corolla transpiration rate on a surface area basis, E_c ($\text{g H}_2\text{O m}^{-2} \text{h}^{-1}$), was calculated from air temperature, corolla temperature, relative humidity and g_s (Supplementary material). We also estimated the mean transpiration-mediated potential water loss along the VPD variation per species considering the mean corolla area and a mean transpiration rate per corolla ($\text{g H}_2\text{O corolla}^{-1} \text{h}^{-1}$) for each species.

2.4. Corolla anatomical traits

To study stomatal density and petal histochemistry, samples from median portion of petals were fixed in Karnovsky's solution (Karnovsky, 1965) for 24 h, dehydrated in an ethyl-alcohol series (Johansen, 1940) and subjected to pre-infiltration and infiltration in (2-hydroxyethyl)-methacrylate (Histo-resin embedding kit, Leica™, Heidelberg, Germany). Series of transversal sections ($5 \mu\text{m}$ thick) were obtained with a rotary microtome, adhered on slides and stained with 0.05% toluidine blue at pH 6.8 (O'Brien et al., 1964). Photomicrographs were acquired using a digital image capture system coupled to an Olympus BX-41 microscope (Olympus American Inc., Center Valley, PA, USA). For histochemical tests, we employed samples fixed in Karnovsky's solution and sectioned with a rotary microtome. We performed a Sudan red B test for lipids (Brundett et al., 1991) and an aqueous solution of ruthenium red to detect acidic polysaccharides (Johansen, 1940).

We determined stomatal density on five fully-expanded petals (from different flowers) taken from five individuals per species ($N = 25$ petal samples per species). Petal samples were fixed in FAA (37% formaldehyde:glacial acetic acid:50% ethanol; 5:5:90v/v; see Johansen, 1940) for 24 h, then stored in 50% ethanol until analysis and prepared for routine light microscopy. To determine stomatal density on petals, hand-cut abaxial paradermal sections of the median portions were obtained with a razor blade, mounted in 50% aqueous glycerine and analyzed using an Olympus BX-41 light microscope (Olympus American Inc., Center Valley, PA, USA). The abaxial face of the central portion of petals was studied, being analyzed five fields (1 mm^{-2} each) from each sample.

Petal surface was studied with scanning electron microscopy with special attention to cuticle, stomata and epicuticular waxes. Fully-expanded petals were collected, fixed in 2.5% glutaraldehyde (0.1 M phosphate buffer, pH 7.2), dehydrated in an ethyl-alcohol series, submitted to critical point drying and coated with 10 nm of gold (Robards, 1978). Samples were examined using a Quanta SEM 200 (Thermo Fisher Scientific, Grand Island, NY, USA) scanning electron microscope at 12–20 kV, and all images were processed digitally.

2.5. Corolla water content

To estimate corolla water content, two recently opened flowers per each of 20 plants used for corolla transpiration rates were harvested and petals were immediately removed and weighed to the nearest 0.1 mg with a microbalance (ELB2000S, Shimadzu, Kyoto, Japan). Two

randomly selected fully developed pre-anthesis flower buds per plant were harvested and kept in alcohol 70% to estimate corolla dry mass. Flower buds contain similar amounts of dry mass as open flowers and differences in fresh mass are only due to water allocation (Galen, 2005). In the lab, we removed petals from each bud to exclusively determine masses of corollas, which were subsequently oven-dried for 2 days at 60°C and weighed to the nearest 0.1 mg with a microbalance (AUW220D, Shimadzu, Kyoto, Japan). Then, we recorded corolla water content (% and g corolla^{-1}) from corolla fresh and dry mass (g) for each species. We did not use the petals removed from opened flowers to calculate dry mass because we also measured flower fresh and dry mass (results not shown). In this regard, despite using recently opened flowers, we were not able to control any pollen removal or pollen deposition on stigmas. We also determined corolla fresh and dry mass per unit of area (g cm^{-2}) per individual and species. Subsequently, we assessed corolla water content per unit of area (g cm^{-2}) as the difference between corolla fresh mass and corolla dry mass per unit of corolla area. Lastly, we estimated the mean time to corolla desiccation (h) per species along the VPD variation, considering the mean transpiration rates per corolla ($\text{g H}_2\text{O corolla}^{-1} \text{h}^{-1}$) and corolla water content (g corolla^{-1}) for each species, and assuming no further water is supplied.

2.6. Statistical analysis

To test for significant differences in transpiration rates between species, VPD and the interaction of species \times VPD (fixed factors), we fitted a model by means of a Generalized Linear Mixed Model (GLMM), including plant nested within species (random factor). A significant interaction indicates a differential effect of VPD depending on the species (i.e. different transpiration rates per species with increasing VPD). We assumed a quasi-Poisson error distribution because of overdispersion of data (Ver Hoef and Boveng, 2007). The model was analyzed using the restricted maximum likelihood (REML). For each specific relationship between corolla transpiration rates and VPD, we calculated pseudo-coefficients of determination (R^2) as $1 - (D_{\text{model}}/D_{\text{null}})$, where D_{model} and D_{null} are the residual deviances of the regression model and of the corresponding null model, respectively. In each case, we also explored both linear and quadratic regression model, and then chose the best-fitted adjustment (i.e. the one that increased the coefficient of determination).

To determine the influence of the species on stomatal density, we fitted an additional GLMM with a quasi-Poisson error distribution using a log link function. Species (fixed factor) and plant nested within species (random factor) were included in the model, which was analyzed using REML. To evaluate whether corolla water content, corolla fresh and dry mass per unit of area varied between species and plant nested within species (random factor), we also fitted GLMMs for every response variable. In all cases, we assumed a quasi-Poisson distribution to reduce variance overdispersion with a log link function. All GLMMs were conducted using the MASS package (Venables and Ripley, 2013) in R software (R Development Core Team, 2018).

To test the effects of species and VPD on mean transpiration rates per corolla and mean time to corolla desiccation, we conducted Generalized Linear Models (GLM) with a quasi-Poisson error distribution using a log link function for each response variable. Species, VPD and interaction of species \times VPD were included in the model. For each specific relationship between the response variables and VPD we calculated pseudo-coefficients of determination (R^2) as explained above. GLMs were run in R software (R Development Core Team, 2018).

3. Results

3.1. Corolla size and floral longevity response

Corolla area and diameter in *K. regalis* were about 1.5- and 2.5-times larger, respectively, than those in *K. coriacea* (Fig. 1 and S1). Regardless

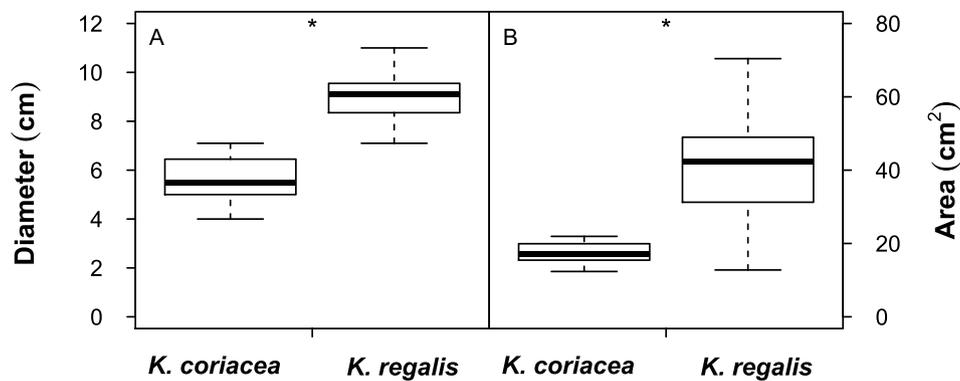


Fig. 1. Mean (\pm SE) corolla diameter (A) and area (B) in *K. coriacea* and *K. regalis*. * $P < 0.05$.

of pollination treatment, flowers lasted fully open and functional for one day in *K. coriacea* and for three days in *K. regalis*. After that time, corollas started to close and wilt and remained on the flower for 2–3 days in *K. coriacea* and 6–7 days in *K. regalis*.

3.2. Corolla transpiration rates

Air temperature was significantly higher and air relative humidity significantly lower during blooming of *K. coriacea* compared to blooming of *K. regalis* (Table 1; see also Fig. S2 for further details of air temperature and relative humidity during the study seasons). Air temperature and relative humidity were negatively correlated during flowering of each species ($r_p = -0.87$, $P < 0.001$, $N = 40$ for *K. coriacea* and $r_p = -0.81$, $P < 0.001$, $N = 40$ for *K. regalis*). Therefore, VPD was significantly higher during the dry season (Table 1). However, corolla temperature was similar in both species (mean \pm SD = 28.3 ± 1.5 °C for *K. coriacea* and 27.4 ± 2.1 °C for *K. regalis*; $\chi^2 = 0.04$, $P = 0.841$, $N = 40$). Corolla stomatal conductance and transpiration rates per unit area were about 1.5-times higher in *K. coriacea* (Fig. 2A and B). VPD positively affected corolla transpiration rates and this effect was greater in *K. coriacea* (species \times VPD significant: Table 2, Fig. 2B). Mean (\pm SE) transpiration rate per corolla was significantly about 1.5-times higher in *K. regalis* than in *K. coriacea* (0.38 ± 0.10 and 0.27 ± 0.07 g H₂O corolla⁻¹ h⁻¹, respectively) and increased similarly with VPD in both species (Fig. 2C).

3.3. Corolla anatomical traits

The number of stomata per unit of area on petals was about 25-times higher in *K. coriacea* than in *K. regalis* (22.3 ± 9.5 vs 0.9 ± 1.2 mm⁻²; $t_{1,8} = -7.02$, $P < 0.001$). Corollas of both species had stomata on both sides of petals (Fig. 3A–C), although more frequently on the abaxial face, on which stomata density was evaluated. However, teratological stomata, on which the pores were coated by cuticle, were frequent on the adaxial side (Fig. 3A). In both species, petals showed a strong dimorphism between the adaxial and abaxial faces, especially on the midrib. Petals of both species have glabrous epidermis on the entire adaxial surface (Fig. 3A and B) and on the edges

Table 1

Mean (\pm SD) air temperature (Temp, °C), relative humidity (RH, %) and vapor pressure deficit (VPD, kPa) recorded during the measurements of corolla transpiration rates in *K. coriacea* and *K. regalis* at Serra do Cipó, southeastern Brazil. Different letters for each column indicate significant differences ($P < 0.05$).

Species	Temperature	RH	VPD
<i>K. coriacea</i>	29.4 ± 1.1^a	39.8 ± 2.2^a	2.5 ± 0.2^a
<i>K. regalis</i>	26.9 ± 2.3^b	48.8 ± 4.8^b	1.8 ± 0.3^b

of the abaxial face. Branched non-glandular trichomes occur on the abaxial face in *K. regalis* (Fig. 3C) while simple non-glandular trichomes were observed in *K. coriacea* (Fig. 3D). Trichomes formed dense patches on the midrib, which corresponds to the exposed portion of petals to the environment in floral buds. The epidermis of petals presents a thin cuticle in both species, although it showed some differences in cell-wall composition. In *K. regalis*, the epidermis shows a typical pectocellulosic cell wall (Fig. 4A and B). In contrast, pectin presence in cell walls is noticeably higher in *K. coriacea*, as shown by ruthenium red test (Fig. 4C–E). The mesophyll of petals is composed by thin-walled parenchyma cells interspersed with well-developed intercellular spaces in both species (Fig. 4B and C). However, pectin was observed within and between all intercellular spaces in *K. coriacea* petal mesophyll, even through some stomatal pores (Fig. 4C–E).

3.4. Corolla water content

Corolla fresh mass per unit of area in *K. coriacea* was significantly about 2-fold higher and corolla dry mass per unit of area about 25% higher than in *K. regalis* (Fig. 5A and B). Consistently, corollas of *K. coriacea* had greater water content than corollas of *K. regalis* in terms of both percentage and per unit of area (Fig. 5C and D). The influence of specific corolla size on petal water and carbon allocation determines a negative allometric relationship between species since water content, fresh and dry mass per unit of area decreased in larger corollas of *K. regalis*. When considering mean corolla area, mean water content (g \pm SE) was similar between species (1.09 ± 0.17 and 1.14 ± 0.15 for *K. coriacea* and *K. regalis*, respectively). Mean time to corolla desiccation (h \pm SE) was significantly higher in *K. coriacea* (4.3 ± 0.7) than in *K. regalis* (3.3 ± 0.7), but decreased faster with increasing VPD in *K. coriacea* (Fig. 6).

4. Discussion

Assessments of flower water-use and -conservation strategies have critical implications for understanding how drought affects flower traits and subsequent plant reproduction (Phillips et al., 2018). Our multi-trait approach in two congeneric Neotropical *Kielmeyera* species demonstrates structure-function relationships in anatomical, physiological and other functional flower traits related to water-conservation linked with microclimate and flowering water requirements. We found an interspecific variation in corolla transpiration rates, which were higher in *K. coriacea* (dry season blooming), but increased linearly with VPD in both species. The more physiologically water-demanding flowers of *K. coriacea* had abundant pectin presence in intercellular spaces of petals, with a subsequent disproportionate increase in corolla dry mass and water content. Altogether, this set of traits translated into shorter-lived flowers, limited to only one day, regardless of pollination treatment. Overall, the results reported here reveal flower hydraulic

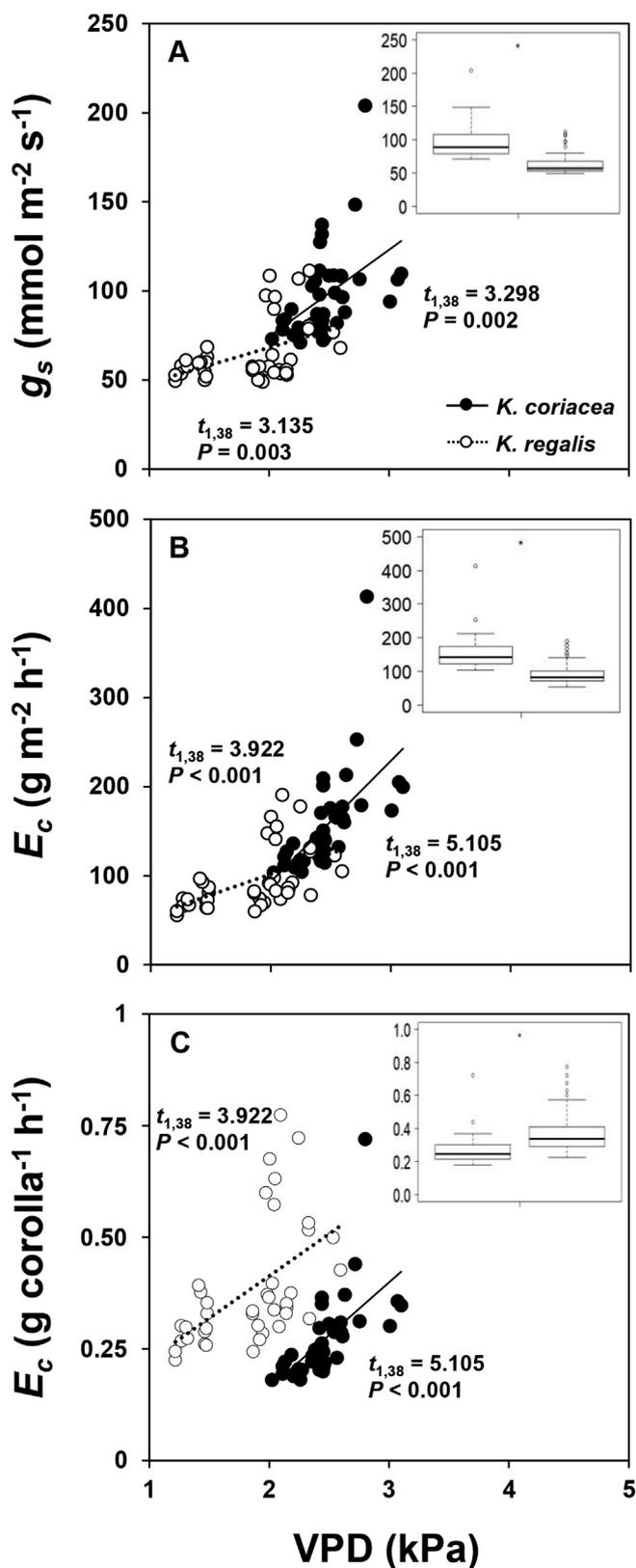


Fig. 2. Corolla stomatal conductance (A), transpiration rates (B) and transpiration rates per corolla (C) versus vapor pressure deficit (VPD) in *K. coriacea* and *K. regalis*. Smaller plots within the boxes show means (\pm SE) for each species. t -values show the coefficients (estimate/SE) for each quasi-Poisson regression. * $P < 0.05$.

Table 2

Summary table of the results of the general linear mixed model (GLMM) for differences in transpiration rates ($\text{g H}_2\text{O m}^{-2}\text{h}^{-1}$) between species, vapor pressure deficit (VPD) and their interaction (fixed factors), and plants (random factor nested within species). Transpiration rates were modeled as a quasi-Poisson error distribution because of overdispersion. Sample size was two measures taken from two flowers on each of 20 individuals per species ($N = 80$). $\sigma^2 \pm \text{SE}$ shows the mean residual variance \pm standard error of the random effect.

Variable	$\sigma^2 \pm \text{SE}$	df	χ^2	P
Species		1	10.57	0.001
VPD		1	28.93	< 0.001
Species \times VPD		1	5.05	0.024
Plant (Species)	0.17 \pm 2.00			

strategies to cope with drought.

4.1. Comparative water-related physiological traits

We recorded high water demand associated with physiological costs of corolla maintenance. Interestingly, corolla transpiration rates in our system, especially for *K. coriacea*, largely exceed those for species in a Mediterranean ecosystem (Blanke and Lovatt, 1993; Teixeira and Valladares, 2014). Corolla water loss in our study species is also comparable to studies considering different transpiration units from mesic temperate forests. Water use in corollas of *K. coriacea* ($2.4 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$) and, to a lesser extent, of *K. regalis* ($1.4 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$) are noticeably higher than that measured in the giant-flowered *Magnolia grandiflora* ($0.7 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$; Feild et al., 2009) and large-flowered *Calycanthus* species ($0.1\text{--}1.1 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$; Roddy et al., 2018). Therefore, water requirements related to corolla physiological maintenance are considerably high in *Kielmeyera* species, especially during the dry season.

Following our expectations, we detected that interspecific differences in corolla transpiration rates were dependent on VPD. As predicted, corollas of *K. coriacea* lost water more rapidly than those of *K. regalis*, particularly under high VPD conditions, agreeing with studies across a diverse range of ecosystems (Feild et al., 2009; Lambrecht, 2013; Teixeira and Valladares, 2014; Roddy et al., 2018). Although we measured interspecific variation in corolla transpiration rates across different ranges of VPD in two contrasting seasons, the elevated rates of water loss by corollas of *K. coriacea* under higher VPD suggest an effective transpirational cooling, as reported for other species (Patiño and Grace, 2002; Teixeira and Valladares, 2014). The lack of interspecific differences in corolla temperature, despite contrasting microclimatic conditions, provides support to the idea that thermal optima for processes contributing to sexual reproduction are constrained (Patiño and Grace, 2002; Fang et al., 2010; Teixeira and Valladares, 2014). In this regard, similar corolla temperature between *K. coriacea* and *K. regalis* suggests that higher transpiration rates are linked with a required overheating avoidance in corollas during the dry season.

In addition to the microclimate-based environmental conditions, species-specific factors relative to stomatal density may help explaining differences in corolla transpiration rates. Consistent with the values reported for *K. regalis*, many species lack stomata on petals or they are sparse and frequently non-functional (Hew et al., 1980; Lipayeva, 1989; van Doorn, 1997; Patiño and Grace, 2002; Roddy et al., 2016). This suggests that water loss across cuticular conductance in *K. regalis* may play a more important role than stomatal conductance in determining transpiration from corollas (Patiño and Grace, 2002; Roddy et al., 2016, 2018). Otherwise, mean stomatal density on *K. coriacea* petals stands as the highest values measured on corollas to date ($14.2\text{--}24.3 \text{ mm}^{-2}$; Patiño and Grace, 2002; Feild et al., 2009; Roddy et al., 2016) and largely exceeds the mean density recently reported for 18 angiosperm species (12.09 mm^{-2} ; Zhang et al., 2018). Therefore, high stomatal

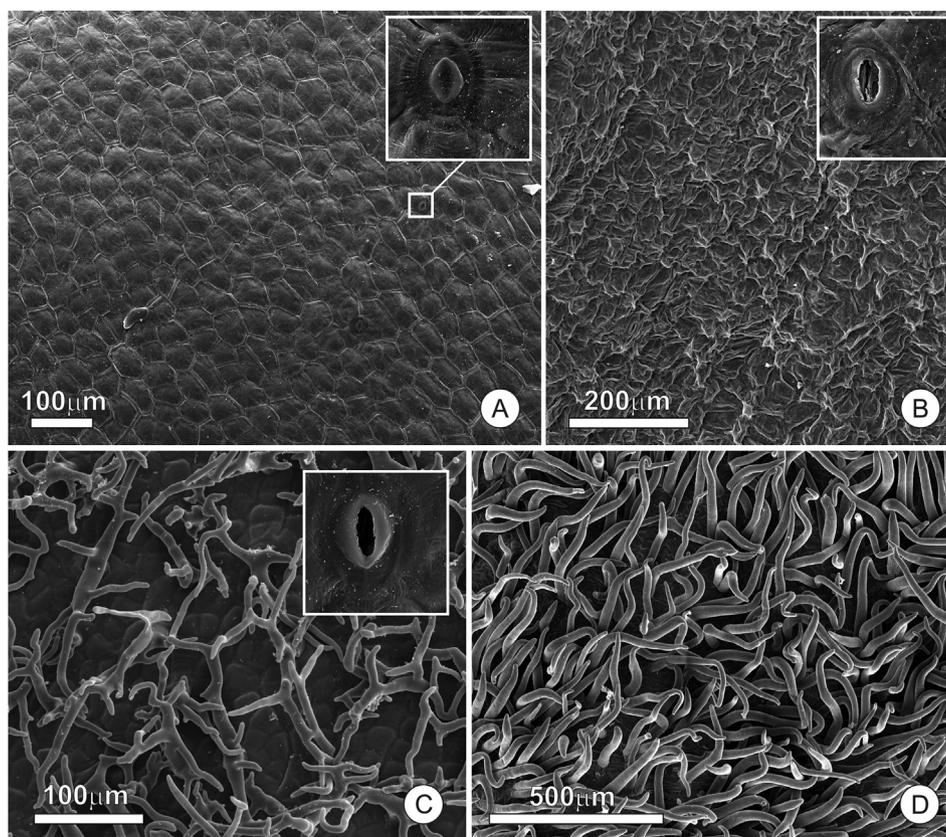


Fig. 3. Micromorphology of petal surface of *Kielmeyera regalis* (A, C) and *K. coriacea* (B, D). A–B. Adaxial surface; note the glabrous epidermis and a teratological (A) and normal stoma (B). C–D. Abaxial surface at midrib. C. Branched non-glandular trichomes and a normal stoma. D. Simple trichomes.

density in petals of *K. coriacea* may potentially reflect the corolla water demand and sustain the high rates of transpiration.

Studies focused on leaves have broadly reported that high stomatal densities are associated with increases in transpiration as a response to high evaporative demand (e.g. Woodward and Kelly, 1995; Xu and Zhuo, 2008; Caine et al., 2019). Similarly, species with lower stomatal density are more conservative and efficient in water-use (see also Bertolino et al., 2019). Higher stomatal density in *K. coriacea* is consistent with high corolla transpiration rates of this species, but contrasts with water-use efficiency under dry conditions. However, floral physiology and water relations may not be comparable with those of leaves, as flowers are directly involved in reproduction during a limited period of time and comprise a wider set of drought strategies than leaves (Roddy et al., 2019). In a previous study, corolla transpiration rates in *K. coriacea* showed a negative quadratic response with increasing VPD during an unusually hot and dry flowering period, reaching the lowest values at about 4 kPa (Teixido et al., 2018). This pattern suggests that a higher stomatal density on petals may, besides sustaining the high evaporative demand, be critical to regulate water loss under the hottest and driest conditions (i.e. high VPD; Roddy et al., 2018).

Flower colour is one unexplored trait that might explain differences in corolla transpiration. This trait is strongly related to climatic conditions and UV absorption, which subsequently determine corolla temperature (Koski and Ashman, 2016). For example, dark-coloured and UV-absorbing flowers are more frequent in environments experiencing higher UV-B irradiance than white flowers (Dyer, 1996; Patiño and Grace, 2002; Koski and Ashman, 2016). In our study system, it is unclear whether flowers receive differences in the amount and intensity of sunlight between seasons and how this could influence on corolla temperature between pink (*K. regalis*) and white (*K. coriacea*) flowers. However, we did not detect any interspecific difference in corolla temperature, despite higher transpiration rates per unit of corolla area

and increased VPD during the flowering of *K. coriacea*, which suggests a high evaporative cooling in this species during the dry season.

4.2. Comparative water-related structural traits

Our results solidly support the hypothesis that corolla anatomical traits and water content are coupled with the transpirational response. Indeed, physiologically water-demanding corollas of *K. coriacea* disproportionately had greater dry mass and water content per unit of area. As broadly reported for leaves, high dry mass per unit of area facilitates water transport and provides drought tolerance (e.g. Wright et al., 2004; John et al., 2017). Similarly, corolla dry mass per area has been recently proposed as an adaptation to maintain water in petals (Zhang et al., 2017). Interestingly, corolla dry mass per unit of area in *K. coriacea* (Fig. 5) is close to the maximum values reported by Zhang et al. (2017) for large-flowered orchids, whereas *K. regalis* fits into intermediate dry mass values (range: 2.6–6.2 mg cm⁻²). Our data reflect that a high corolla carbon investment per unit of area in *K. coriacea* is linked to a suite of water-related functional traits in flowers.

We show anatomical traits of corollas that reveal as increased dry mass per unit of area in *K. coriacea* is closely related to the structure of petal surfaces and cell walls that seem to enhance water retention, like conspicuous presence of pectins. Cell wall properties such as pectin and cellulose content can influence the flux of foliar water uptake and determine the water holding capacity in leaves (Voragem et al., 2009; Berry et al., 2019). Species from seasonally dry tropical forests that bloom during the dry season produce considerable amounts of other polysaccharides (as mucilage) in corolla tissues, which seem to guarantee a substantial water supply to remain functionally active despite high evaporative demands (Chapotin et al., 2003). Pectinous mucilage is also a common adaptation of seeds to dry habitats in many families of angiosperms (Kreitschitz and Gorb, 2017). In *K. coriacea*, the pectin-

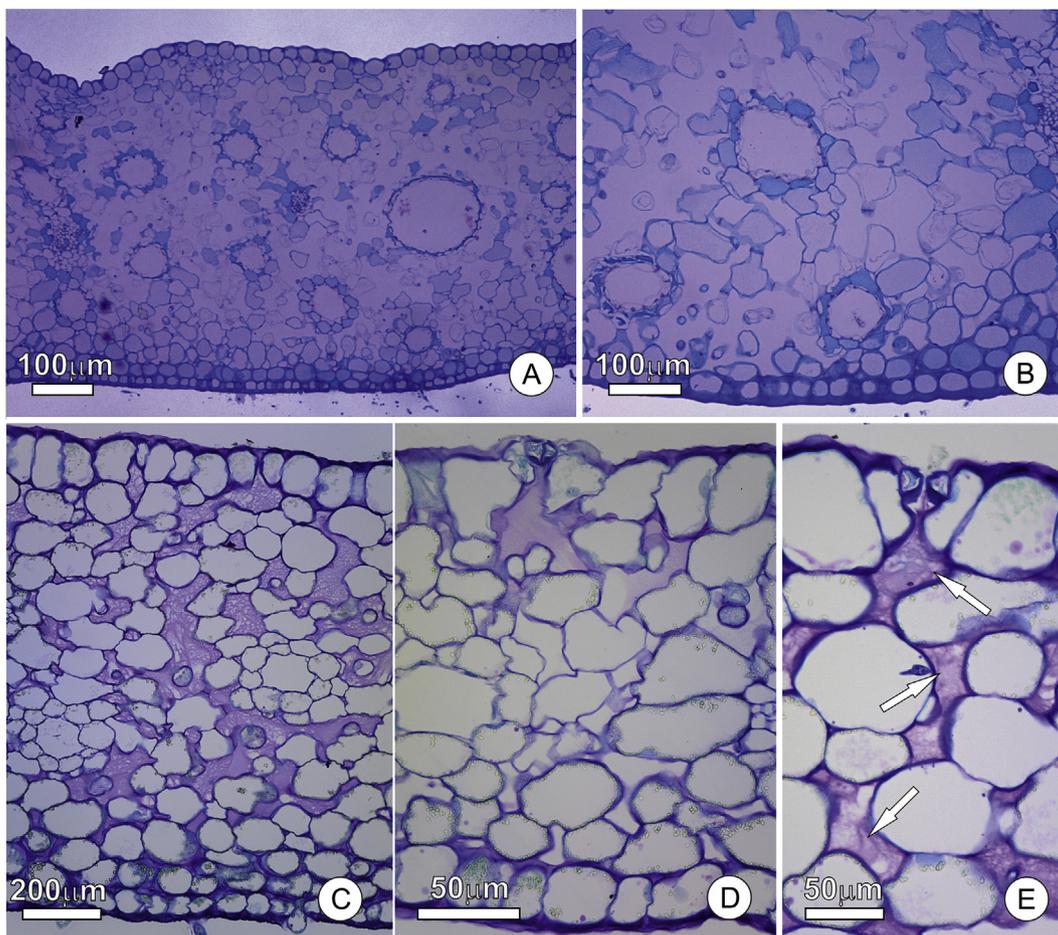


Fig. 4. Petal anatomy of *Kielmeyera regalis* (A–B) and *K. coriacea* (C–E) at median third of petal blade. Transverse sections showing mesophyll cells with vacuolated cells and reserve absence. A–B. Petal showing mesophyll with thin-walled parenchyma cells interspersed with well-developed intercellular spaces. C–E. Petal showing thin-walled mesophyll cells, but with a conspicuous presence of pectin within intercellular spaces (arrows in E).

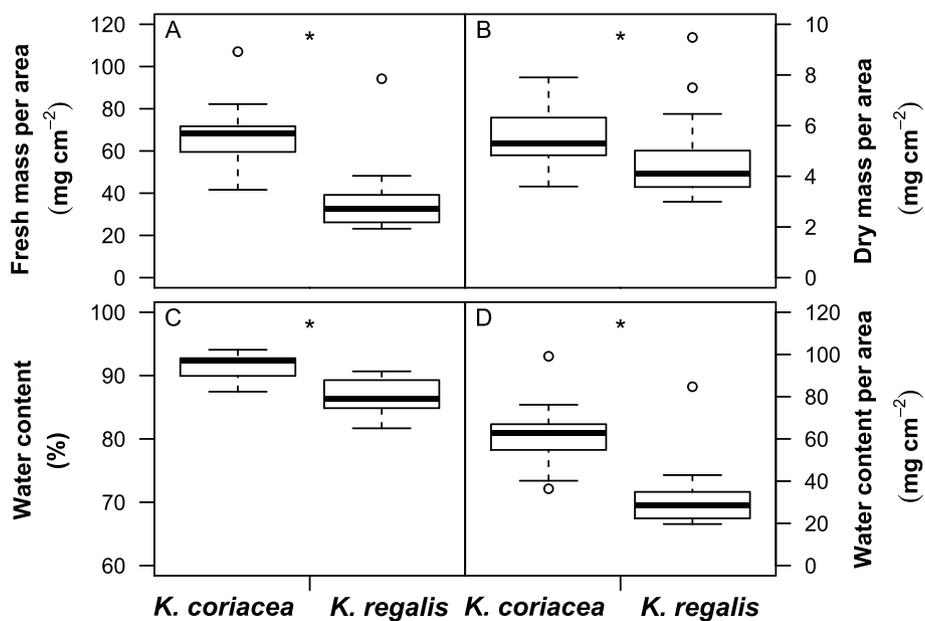


Fig. 5. Mean (± SE) corolla fresh mass (A) and dry mass (B) per unit area, and corolla water content (%; C; per unit of area, D) in *K. coriacea* and *K. regalis*. **P* < 0.05.

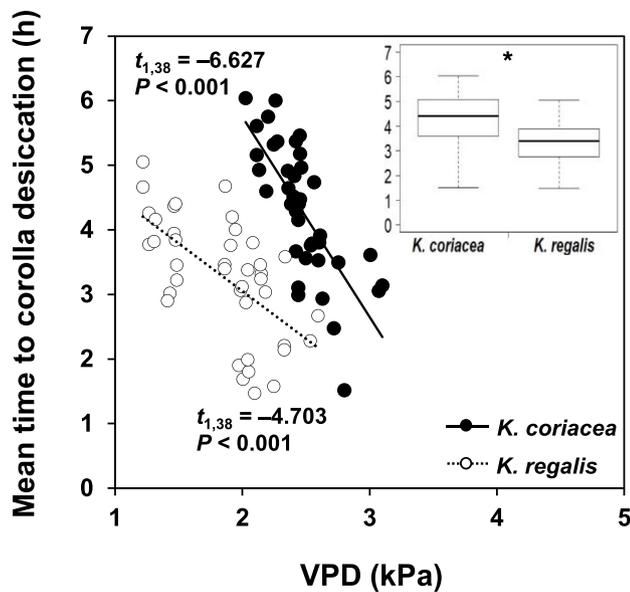


Fig. 6. Mean time to corolla desiccation along VPD variation in *K. coriacea* and *K. regalis*. Smaller plot within the box show means (\pm SE) for each species. * $P < 0.05$.

rich epidermal surface and the hydrophilic pathways interspersed in cell walls of petals may consequently explain the water content of corollas. Interestingly, our findings also improve our understanding of how and where the water that contributes to corolla physiological and structural maintenance is stored.

Water content in *K. coriacea* corollas exceeds the values of other large-flowered plants, including those from dry tropical forests (80–86%; Chapotin et al., 2003; Feild et al., 2009). Altogether, our results suggest that a disproportionate high pectin production and water content in corollas of the smaller-flowered *K. coriacea* may represent a significant water supply to face the high transpirational demands. Indeed, high carbon and water investment in corollas may confer a higher shielding flowers from drought stress (Roddy et al., 2019). Without a high-water content and assuming no additional water import, corollas could rapidly desiccate under the transpiration rates that we measured. In fact, corollas of *K. coriacea* wilt after one day so most, or part, of water demand seems to rely on stored water in pectin-rich petals rather than a continuous delivery from xylem or phloem. This is particularly relevant and may also help disentangle the interplay between stomatal density, high evaporative demands and water conservation strategies in corollas of *K. coriacea* during the dry season. In this regard, stomata may indeed contribute to the transpirational process to avoid floral overheating and, at a lesser extent, regulate water loss, whereas pectin-related water retention in petals mostly tempers this control and supplies the water demands. Over time, corolla water loss can easily exceed water storage and lead to corolla wilting (Roddy et al., 2018). As a consequence, the elevated water demand from corollas and the high VPD during the dry season seem to impose a strong filter to floral longevity, potentially limiting the chances of receiving pollinators, but ultimately increasing water use efficiency by ephemeral flowers. Instead, large corollas of *K. regalis* may be mainly hydrated by vascular tissues to avoid desiccation during floral life span and sustain longer-lived flowers when more water is available, but reliable estimates of hydraulic capacitance and conductance of corollas are required to draw solid conclusions (Chapotin et al., 2003; Feild et al., 2009; Roddy et al., 2016, 2018).

As expected, we found empirical evidence that more water-demanding corollas of *K. coriacea* were short-lived. More interestingly, we detected that floral longevity in this species is limited to only one day, regardless of pollination treatment. Thus, elevated physiological costs

seem to exceed female fitness gain and to interrupt flower function independent of fruit and seed production. We are aware that a multiple array of abiotic and biotic factors, including phylogenetic constraints, may determine floral longevity beyond corolla maintenance costs (e.g. Primack, 1985; Vesprini and Pacini, 2005; Teixeira et al., 2019). However, our predictions and subsequent results are consistent with the assumptions based on a reduced floral life span to minimize corolla water loss under dry and hot conditions (Ashman and Schoen, 1994; Vesprini and Pacini, 2005; Teixeira and Valladares, 2015; Dudley et al., 2018). Although we cannot exclude the effects of other drivers of floral longevity, it is reasonable to hypothesize that the production of ephemeral flowers with high water content may be a strategy to deal with elevated transpirational requirements during periods of water stress. Otherwise, flowers of *K. regalis* lasted fully open for three days, although it was also independent of pollination. Recently, Teixeira et al. (2019) demonstrated that the retention of pollinated and reward-free old flowers in *K. regalis* maximizes pollinator visitation by increasing individual floral display, which shows that this large-flowered species with longer-lived flowers may tolerate a moderate level of corolla physiological costs in order to enhance mating opportunities in a pollen-limited and water-availability scenario.

Overall, our results suggest that to buffer the water costs of flowering during the dry season, *K. coriacea* produces small and short-lived corollas with high water content. However, multispecies comparisons between seasons are needed to determine structure-function relationships and phylogenetically-controlled coordinated evolution of the flower trait set linked to flowering requirements and water-conservation strategies. Some attractiveness-related floral traits, such as flower size, and dry mass and water investment to corollas are strongly adaptive and conserved among phylogenetically diverse lineages (Roddy et al., 2016; Teixeira et al., 2017), which reduces the evolutionary potential of floral traits in response to novel selective pressures (see Opedal, 2019 for flower size). In contrast, other studies have reported that hydraulic and resource allocation traits of flowers lack phylogenetic signal (Oguro and Sakai, 2014; Roddy et al., 2019), suggesting convergence among distantly related species that have adapted into similar environmental conditions.

Credit author statement

Alberto L. Teixeira: Conceptualization, Methodology, Validation, Formal Analysis, Investigation, Data Curation, Writing – Original Draft, Writing – Review & Editing, Visualization, Project Administration. Victor B. Leite-Santos: Methodology, Data Curation, Writing – Review & Editing. Élder A. S. Paiva: Conceptualization, Methodology, Validation, Investigation, Resources, Writing – Original Draft, Writing – Review & Editing, Supervision, Funding Acquisition. Fernando A. O. Silveira: Conceptualization, Investigation, Resources, Writing – Review & Editing, Supervision, Project Administration, Funding Acquisition.

Declaration of competing interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.plaphy.2019.10.004>.

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