



Research Article

The evolutionary rate of leaf osmotic strength drives diversification of *Primulina* species in karst regions

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Abstract Leaf water storage capacity and osmotic strength are important traits enabling species to adapt to environments that are often moisture limited. However, whether these drought tolerance traits are correlated with the species diversification rate (DR) of plant lineages is yet to be determined. In this study, we selected a species-rich genus (*Primulina*) of plants widely distributed in karst regions in which species frequently experience variable periods of drought. We measured water storage capacity-related traits (including leaf thickness and water content per mass) and saturated osmotic strength in the leaves of 58 *Primulina* species growing in a common garden. Subsequently, using phylogenetic methods, we examined the relationships between the rate of species diversification and the drought tolerance traits and between the species DR and evolutionary rates of these traits. We found that neither water storage capacity nor saturated osmotic strength showed significant relationships with the rate of species diversification. However, the evolutionary rate of saturated osmotic strength showed a significant correlation with the species DR, although no comparable significant relationship was detected regarding the evolutionary rate of water storage capacity. Our study indicates that the diversification among *Primulina* species has typically been accompanied by an extensive divergence of leaf osmotic strength but not a divergence in leaf water storage capacity. These findings will enhance our current understanding of how drought tolerance influences the diversification of plant species in karst regions.

Key words: diversification rate, karst, leaf drought tolerance, osmotic strength, phylogenetic analysis, water storage capacity.

1 Introduction

From both ecological and evolutionary perspectives, understanding the rates at which new species arise and phenotypic traits evolve is fundamental to establishing the species richness and phenotypic diversity (Venditti et al., 2011; Jetz et al., 2012; Cooney et al., 2017). In general, rates of species diversification would be positively correlated with evolutionary changes in phenotypic traits, and indeed, several of the proposed theories predict this relationship. For example, both the punctuated equilibrium (Gould & Eldredge, 1977) and adaptive radiation (Schluter, 2000) theories indicate that the rates of change in phenotypic traits and species diversification rate (DR) should be correlated at macroevolutionary scales. Furthermore, certain phenotypic traits have been proposed as the drivers of the rate of species

diversification in Hawaiian honeycreepers (Lovette et al., 2002), fish (Danely & Kocher, 2001; Rabosky et al., 2013), *Anolis* lizards (Losos et al., 2003), and major vertebrates (Cooney & Thomas, 2020).

Among plant species, leaf drought tolerance is an essential trait that enables species to adapt to environments that are often moisture limited (Karimi et al., 2012, 2018). Leaves characterized by a high water storage capacity are typically thick and have a high water content per mass, enabling species, such as cacti, to persist even when soils are devoid of available water (Meidner, 1952; Zhou et al., 2021). In addition, high osmotic strength can enhance water absorption and contribute to the maintenance of the hydration status of protoplasts in plants exposed to drought stress, as has been widely reported in previous studies

(Zhu, 2001; Karimi et al., 2012). However, whether leaf water storage capacity and osmotic strength are correlated with the rate of species diversification is yet to be sufficiently addressed. Two hypotheses could potentially explain the relationship between plant drought tolerance and species DR, the first of which proposes that higher drought tolerance confers a survival advantage under conditions of drought, thereby potentially reducing the risk of extinction, as, for example, a consequence of climate change (Holt, 1990), and also accelerates the rate of species diversification. The second hypothesis states that the rapid evolution of drought tolerance traits might also lead to a higher species DR. In this regard, the rapid evolution of drought tolerance traits would enable species to diversify more rapidly by facilitating the expansion of populations into drier environments inhabited, thereby reducing competition and providing additional opportunities (exploit ecological opportunities) for speciation (Kozak & Wiens, 2010; Fisher-Reid et al., 2012).

However, despite the proposal of the aforementioned hypotheses, no studies to date have examined the relationship between the rate of species diversification and leaf drought tolerance. Nonetheless, a few previous studies have addressed related topics concerning other traits. For example, Onstein et al. (2016) found that sclerophylly contributed to enhanced rates of species diversification of plants in the families Pomaderreae in Australia and Phylleaceae in the Cape, as sclerophyllous leaves prove advantageous to plants under conditions of water stress (i.e., during summer droughts) (Wright et al., 2004). Furthermore, Larter et al.

(2017) have reported that species diversification in the genus *Callitris* coincided with the onset of arid conditions in Australia during the early Oligocene, which ascribes to the evolution of high stem drought tolerance. Similarly, Skelton et al. (2021) have documented North American oak species in more drought-prone regions have also evolved higher stem drought tolerance. Accordingly, the findings of these studies tend to indicate that the development of leaf tolerance to drought might drive the rate of plant diversification, particularly in those environments that are often moisture limited.

Karst landforms in tropical and subtropical regions, formed by the dissolution of limestone and other soft rocks, constitute an unrivaled biodiversity-rich landscape and have long been regarded as “natural laboratories” for ecological and evolutionary studies (Clements et al., 2006; Oliver et al., 2017). The soil in these areas is generally shallow, and the efficient subterranean drainage results in a rapid loss of water, consequently exposing the species inhabiting these areas to the frequent risk of drought (Zhang et al., 2011; Fu et al., 2015). Plants in the genus *Primulina* are widely distributed in the karst regions of southern China (from dry rock cliffs to the wet entrances of karst caves; Fig. 1). Additionally, all species in this genus are dwarf herbaceous plants that lack a main stem and typically have shallow roots. Moreover, most are characterized by thick leaves with a high water storage capacity (An Editorial Committee of Flora of China, 2013). Based on these traits, water storage capacity and osmotic adjustment are important features determining

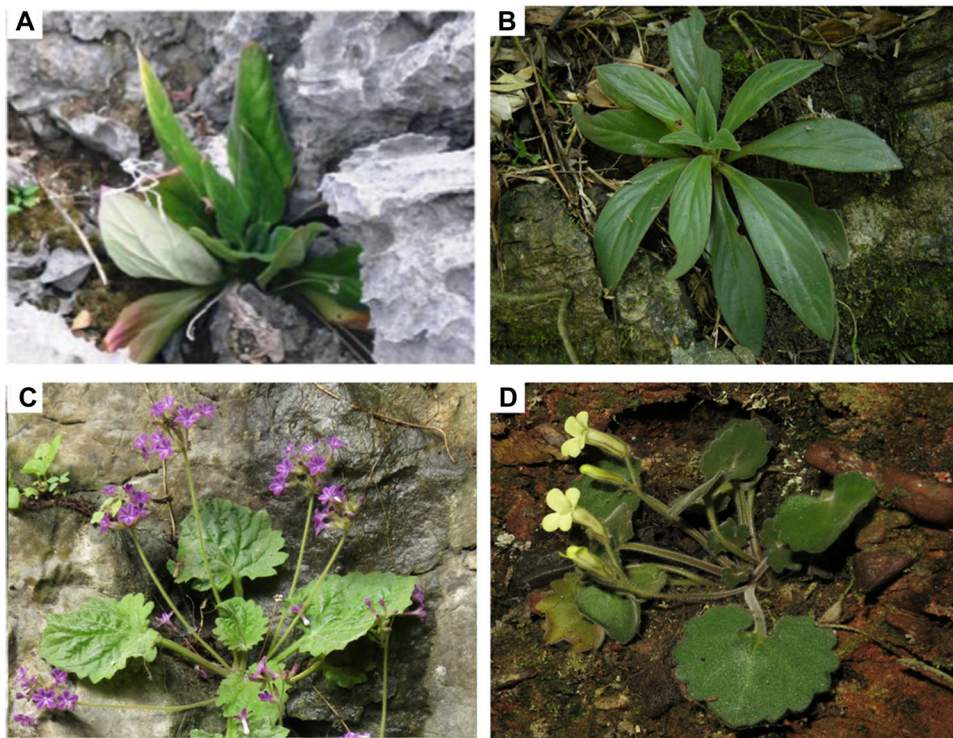


Fig. 1. Four *Primulina* species illustrating the natural distribution of these species from dry to wet habitats. **A**, *Primulina eburnea* (Hance) Y.Z. Wang on dry karst rock. **B**, *Primulina longgangensis* (W.T. Wang) Y.Z. Wang on karst rock. **C**, *Primulina tabacum* Hance on wet karst rock. **D**, *Primulina danxiaensis* (W.B. Liao, S.S. Lir. & R.J. Shen) W.B. Liao & K.F. Chung on wet danxia rock.

the drought tolerance capacities of species in karst habitats. Consequently, species of *Primulina* would appear to represent a good model for studying the relationships between leaf drought tolerance and the rate of species diversification.

In this study, we examined leaf drought tolerance traits, namely water storage capacity and saturated osmotic strength, in the leaves of 58 *Primulina* species, representing 48% (58 of 121) of the species in this genus. We investigated the relationships between these traits and the rate of species diversification using phylogenetic methods and examined the relationship between the evolutionary rates of leaf drought tolerance and species DR. Specifically, we aimed to test the following hypotheses: (i) a higher leaf drought tolerance would accelerate the rate of species diversification via a reduction in the risk of extinction (i.e., a significant positive relationship between the rate of species diversification and leaf drought tolerance) (H1); and (ii) a higher leaf drought tolerance would accelerate the rate of species diversification via an increase in the ability to exploit ecological opportunities (i.e., a significant positive relationship between the DR and rate of leaf drought tolerance evolution) (H2). Furthermore, given that most *Primulina* species have thick leaves and shallow roots, we hypothesized that water storage capacity would be more important than osmotic strength in influencing the rate of *Primulina* diversification (H3).

2 Material and Methods

2.1 Species and growth conditions

Experiments in this present study were conducted at the Gesneriaceae Conservation Center in the Fairy Lake Garden, Shengzhen, China (22°34'N, 114°09'E). All species were collected from the field in southwest China or southeastern Asia and transplanted to flowerpots in the aforementioned conservation center, which comprises a greenhouse, in which 79 species with accepted names are maintained. During the experimental period, the mean daytime temperature was $27 \pm 3^\circ\text{C}$, relative humidity was $70\% \pm 10\%$, and sunny day irradiance was $\sim 1000 \mu\text{mol}/\text{m}^2$ per s. Individual plants were planted in 5 L pots containing the same compost and sand substrate, following a randomized design and were watered daily with a sufficient amount of water. We ultimately selected 58 species for inclusion in the study, initially having established phylogenetic relationships based on a tree constructed by Kong et al. (2017) and excluding species with less than three individuals. To determine leaf drought tolerance traits, we randomly selected fully expanded mature leaves from three to five individuals of each species. All leaves sampled have a similar size for a particular species. We sampled one to three leaves from each individual given that some species have large and thick leaves (e.g., *Primulina eburnea*), whereas others have small and thin leaves (e.g., *Primulina yangshuoensis*). In principal, more leaves were collected per individual from species with small leaves.

2.2 Leaf drought tolerance traits

The leaf drought tolerance traits, including water storage capacity and saturated osmotic strength, were measured

using fully saturated leaves, for the former of which we assessed leaf thickness (mm) and water content per mass (g/g).

Saturated osmotic strength can serve as an index of the osmotic capacity of plants, which is an important trait with respect to predicting drought tolerance (Patakas et al., 2002; Hessini et al., 2009; Bartlett et al., 2012a, 2012b; Katuwal et al., 2020). In the present study, we estimated saturated osmotic strength in *Primulina* as the capacity of osmotic adjustment. Mature fully expanded leaves were sampled from three to five mature individuals of each species during early dusk, sealed in black plastic bags containing a moist towel, and immediately transported to the laboratory. All petioles of the sampled leaves were subsequently re-cut under water using a razor blade. The base of the petioles was placed in a beaker containing water, ensuring that the leaves remained above the water surface, after which the beakers were placed in black plastic bags for 12 h to ensure that the leaves became water saturated.

A dry towel was used to remove water from the leaf surface, after which the samples were immediately frozen in liquid nitrogen and then ground to produce a fine powder to determine saturated leaf osmotic strength. Resuspensions of the powdered material were then centrifuged for 5 min at 5000g, and the resulting supernatants were re-centrifuged for 10 min at 10 000g. The supernatants thus obtained were collected for determinations of saturated osmotic strength (mOsm/kg H₂O) using an OM806 osmometer (Loser, Giessen, Germany).

For each *Primulina* species, hand-cut cross-sections from three to five mature fully expanded leaves were used to determine leaf thickness. Cross-sections were cut at the base, mid-section, and tip of each leaf, and the thickness of these sections was determined using an Optec upright microscope (Chongqing Optec Instrument Co. Ltd, Chongqing, China) equipped with a digital camera and a computerized image analysis system (OPTPro 2012 version 4.0; Optec Software). A further three to five leaves obtained from each species were used to determine fresh weights. The leaves were then oven-dried for 72 h at 70°C to determine dry weights, with the water content per mass being determined using the following equation: (fresh weight–dry weight)/fresh weight.

2.3 Phylogenetic tree and species DR

The phylogenetic tree used in the present study was adapted from that constructed by Kong et al. (2017) based on analyses of nine noncoding regions of chloroplast DNA (*atpB-rbcL*, *rpl32-trnL*, *trnL-trnF*, *rpoBtrnC*, *trnC-petN*, *ndhA_intron*, *ndhH-rps15-ycf1*, *ycf1_1*, and *ycf1_2*) and the nuclear ribosomal internal transcribed spacer (ITS). Using the Bayesian information criterion implemented in PARTITIONFINDER 1.1.1 (Lanfear et al., 2012), we selected nucleotide models and MRBAYES 3.2.6 (Ronquist et al., 2012) to construct the tree. Finally, we obtained a consensus phylogenetic tree containing 201 tips. After matching accepted species in The Plant List 2013, version 1.1, and accepted species maintained in the Conservation Center, 79 species remained in the phylogenetic tree, 21 of which were subsequently discarded, as there were fewer than three individual plants for these species in the Conservation Center. After excluding these

species, the remaining 58 species were used for determinations of leaf drought tolerance, containing 48% (58 of 121) species of this genus, which could fulfill the phylogenetic models in calculating DR (Liu et al., 2020).

The DR of each species in the tree was estimated as the inverse of the mean equal-split measure (Jetz et al., 2012) using the R package PICANTE version 1.8 (Redding & Mooers, 2006). Species-level lineage DR for species i is the inverse of its mean equal splits measure:

$$DR_i = \left(\sum_{j=1}^{N_i} l_j \frac{1}{2^{j-1}} \right)^{-1} \quad (1)$$

where N_i is number of edges on path from species i to the root and l_j is length of the edge j . Using this method, we obtained independent DR estimates for each species, thereby enabling us to determine thousands of different rates across species.

We also used BAMM 2.5.0 (Rabosky, 2014) to determine the DR of each species, which used a reversible-jump MCMC to sample a large number of possible species DRs from a given time-calibrated phylogeny. The model type set as “speciationextinction.” The MCMC was run for 30 million generations and sampled every 1000 generations. Prior values were selected using function setBAMMpriors. Postrun analyses were performed using R package BAMMTOOLS version 2.1.7 (Rabosky et al., 2014). Using the getTipRates function in BAMMTOOLS, we finally determined the net DR of each species.

2.4 Estimating evolutionary rate of drought tolerance

For determinations of the evolutionary rate of leaf drought tolerance, we initially evaluated the fit of three variables (leaf thickness, water content per mass, and saturated osmotic strength) to the following four standard evolutionary models: Brownian motion (BM), estimated lambda (LA), Ornstein–Uhlenbeck (OU; single peak), and white noise (WN). For each of these models, we used the function fitContinuous in the R package GEIGER version 2.0 (Pennell et al., 2014) to estimate the Akaike information criterion (AIC) of each model in each case, selecting the best-fit model with the lowest AIC value (Table S1). Given that the WN model is inappropriate for ancestral reconstructions and may be erroneously selected if we found this model to have the best fit, we used the second-best model instead, as described by Jezkova & Wiens (2016) and Liu et al. (2020). The trees were then transformed based on the best-fitting model, and the ancestral values for each node were reconstructed using phylogenetic generalized least squares (PGLS) in the R package CAPER version 0.5.2 (Orme, 2013). To estimate the evolutionary rate for each species for each variable, we used the absolute difference between the estimated values at its most recent ancestral node and divided the value obtained for this species by the age at that node (Liu et al., 2020). Although alternative approaches yielded similar results, the main results used the mean values and best-fitting models (Table S2).

We also used BAMM 2.5.0 (Rabosky, 2014) to determine the evolutionary rate of drought tolerance for each species. The model type set as “trait,” the other parameters were set

as the same as the DR estimation described above. Using the getTipRates function in BAMMTOOLS (Rabosky et al., 2014), we determined the evolutionary rate of drought tolerance for each species.

2.5 Relationship between species DR, leaf drought tolerance, and rate of leaf drought tolerance

To establish whether leaf drought tolerance or rate of leaf drought tolerance evolution is correlated with the rate of *Primulina* diversification, we performed PGLS regression analysis (Martins & Hansen, 1997), using the R package CAPER version 0.5.2 (Orme, 2013) to estimate the relationships between the species DR and the two tolerance variables. Branch lengths were transformed based on the maximum-likelihood estimated values of the phylogenetic signal lambda (Pagel, 1999; Freckleton et al., 2002), with kappa and delta each fixed at 1.

3 Results

Our assessment of the thickness of *Primulina* leaves revealed a relatively large variation among species, ranging from 0.025 to 1.589 mm, with a mean value of 0.721 ± 0.362 mm. The average water content per mass was $0.954 \pm 0.017 \times 100\%$ (ranging from 0.851 to 0.972), whereas osmotic strength was 181.647 ± 41.357 mOsm/kg H₂O (ranging from 80 to 300) (Fig. 2).

We found that neither leaf water storage capacity (leaf thickness and water content per mass) nor leaf saturated osmotic strength showed a significant correlation with the species DR of *Primulina* ($P > 0.050$, $r^2 < 0.020$; Table 1). Similarly, we detected no significant correlation with respect to the relationship between the evolutionary rate of leaf water storage capacity and species DR (DR–leaf thickness rate: $P = 0.106$, $r^2 = 0.046$; Fig. 3A; DR–water content per mass rate: $P = 0.295$, $r^2 = 0.020$; Fig. 3B). In contrast, the evolutionary rate of leaf saturated osmotic strength was found to show a significant correlation with the species DR ($P < 0.001$, $r^2 = 0.233$; Fig. 3C). Although the evolutionary rates of leaf drought tolerance were based on the best-fitting models (Table S1), similar results were obtained using alternative models to estimate the evolutionary rate of leaf drought tolerance (Table S2).

Similar results were obtained by using BAMM to estimate species DR and evolutionary rate of drought tolerance. The relationship between evolutionary rate of leaf water storage capacity and species DR were not significant (DR–leaf thickness rate: $P = 0.500$, $r^2 = 0.008$; DR–water content per mass rate: $P = 0.391$, $r^2 = 0.013$) while evolutionary rate of leaf saturated osmotic strength and species DR was significantly correlated (DR–leaf saturated osmotic strength $P < 0.001$, $r^2 = 0.318$; Table S3).

4 Discussion

Water storage capacity and osmotic strength play essential roles in determining plant survival, particularly in moisture-limited environments (Tardieu, 2013; Forner et al., 2018). However, if and how these leaf drought tolerance traits influence the rate of species diversification is yet to be

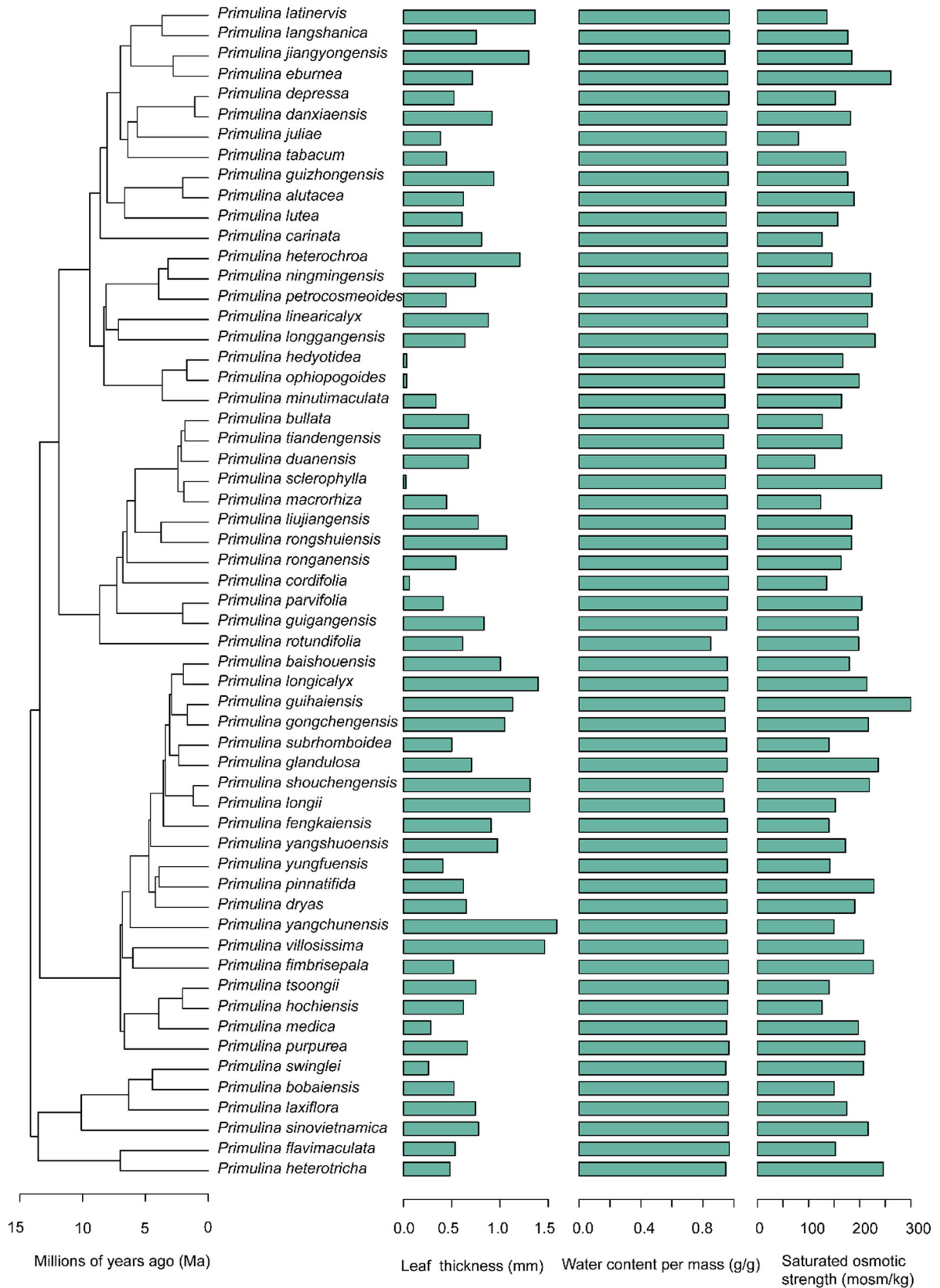


Fig. 2. A time-calibrated phylogeny of 58 *Primulina* species (based on Kong et al., 2017). The bars on right were leaf thickness, water content per mass and saturated osmotic strength, respectively.

sufficiently determined. In the present study, we examined the relationship between the rate of species diversification and leaf drought tolerance in *Primulina* based on phylogenetic methods for the first time. We found that speciation in *Primulina* is typically associated with an extensive divergence in osmotic strength, although not by a divergence in water storage capacity. Our study supported punctuated equilibrium (Gould & Eldredge, 1977) and adaptive radiation (Schluter, 2000) theories in *Primulina*, suggesting species diversification in karst regions via enhancing the capacity to exploit different niches characterized by drought. Thus, we believe that the findings of this study will contribute to advancing our current understanding of how leaf drought tolerance influences the diversification of plant species in karst regions.

4.1 Relationship between DR and drought tolerance traits

Although a higher drought tolerance has been proposed to enable species to reduce the risk of extinction in drought-prone environments and thereby enhance the rate of species diversification, this hypothesis is yet to be sufficiently tested. For example, Larter et al. (2017) found that the diversification of a *Callitris* clade coincided with the onset of arid conditions in Australia during the early Oligocene, indicating a potentially positive relationship between the rate of

diversification and drought tolerance. Unexpectedly, we were unable to detect any significant positive association between leaf drought tolerance (i.e., water storage capacity and saturated osmotic strength) and the rate of species diversification, as thus reject our first hypothesis (H1) stating that a higher leaf drought tolerance would accelerate the rate of species diversification via a reduction in the risk of extinction. Conceivably, this lack of association could be attributable to the fact that most species of *Primulina* have allopatric distributions and are calciphiles growing in limestone habitats in which population densities are typically low (Hao et al., 2014). Thus, allopatric diversification might play a more prominent role in the species diversification of these plants. A higher leaf drought tolerance in some species would enable them to extend their range across a wider diversity of environments in karst regions. However, a wider range of distribution would potentially promote the gene flow among species (Gaston, 2003), thereby reducing their rate of species diversification. Accordingly, this might contribute to counteracting the positive effect of high leaf drought tolerance in reducing the rate of extinction, which warrants a more in-depth examination in further studies.

4.2 Relationship between species DR and evolutionary rate of leaf drought tolerance

We found the phylogenetic signals were weak (Table S4) for all three drought tolerance traits, indicating that drought tolerance traits were divergent in *Primulina*. This enables *Primulina* plants to adapt to different water niche, thus potentially leading to their diversification in karst regions. Somewhat unexpectedly, we found that the evolutionary rate of leaf saturated osmotic strength, although not that of water storage capacity, had the effect of accelerating the rate at which *Primulina* diversifies. This provides partial support for our second hypothesis (H2), stating that a higher rate of leaf drought tolerance evolution would accelerate the rate of *Primulina* diversification via an increase in the ability of different species to exploit ecological opportunities. In contrast, we could reject our third hypothesis (H3) that

Table 1 Relationship between species diversification rate (DR) and water storage capacity (including leaf thickness [thick] and water content per mass [wc.mass]) and between DR and osmotic strength (osmotic)

	<i>P</i>	Adjusted <i>r</i> ²	
DR–thick	0.323	0.017	–
DR–wc.mass	0.836	0.001	–
DR–osmotic	0.431	0.011	+

*r*² and *P* values are based on phylogenetic generalized least squares (PGLS). + and – indicate positive and negative relationship, respectively.

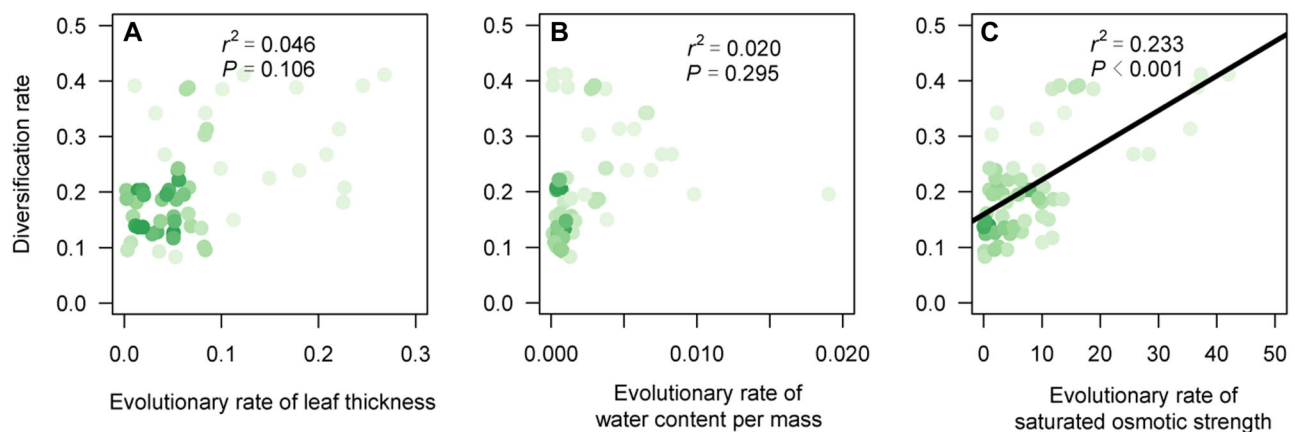


Fig. 3. Relationships between the rate of species diversification and (A and B) the rate of water storage capacity and (C) the rate of saturated osmotic strength. **A**, Diversification rate versus the rate of leaf thickness. **B**, Diversification rate versus the rate of water content per mass. **C**, Diversification rate versus the rate of saturate osmotic strength. *r*² and *P* values are based on phylogenetic generalized least squares (PGLS) analysis. Darker colors indicate a greater overlap of data points.

water storage capacity would be more important than osmotic strength in influencing the rate of species diversification.

Several theories propose that speciation is typically associated with an extensive divergence of critical traits (evolutionary rate of critical traits). Among these, the adaptive radiation theory maintains a coincidence between the evolution of ecological diversity and the rapid divergence of lineages, as lineages typically undergo rapid ecological differentiation to fill unoccupied niche space (Schluter, 2000). The hypothesis of punctuated equilibrium (Gould & Eldredge, 1977) similarly predicts that most evolutionary changes occur during speciation events, indicating a positive relationship between the rates of species diversification and the evolutionary rates of critical traits (Ricklefs, 2006; Bokma, 2008). Consistent with these assumptions, the findings of some previous studies have indicated significant positive correlations between the rate of diversification and the evolutionary rate of specific phenotypic traits (Rabosky et al., 2013; Cooney & Thomas, 2020). In the present study, we similarly identified a significant positive relationship between the evolutionary rate of osmotic strength and the rate at which species of *Primulina* diversify, thereby indicating that a divergence in osmotic strength has played a prominent role in the species diversification of *Primulina*.

In contrast, we found little evidence to indicate a significant relationship between the evolutionary rate of water storage capacity and the rate of species diversification, which we speculate could be attributed to one or more of the following factors. First, water storage capacity might have little effect on the rate at which *Primulina* species diversify. As indicated by the nonadaptive radiation hypothesis, the diversification of traits shows no association with natural selection (Czekanski-Moir & Rundell, 2019). For example, Gulick (1872) found that the most species-rich groups of land snails in Hawaii have distinguishable shell characters, although their habitats are essentially similar.

Second, water storage capacity might represent a key trait in the exaptive radiation of *Primulina*. The exaptive radiation hypothesis proposes that certain “key innovation” traits might have evolved prior to evolutionary radiations (Simões et al., 2016). When the effects of climate change manifested, these traits may have conferred certain survival-related advantages among the species possessing them, whereas those lacking such traits would be vulnerable to extinction (Lieberman, 2012). The vacant niche space thus created would thereby provide opportunities for diversification of the surviving clades. Consistent with this scenario, Bouchenak-Khelladi et al. (2015) found that numerous species' radiation events have been associated with physiological characters that evolved prior to radiation, although not necessarily associated with speciation events. Accordingly, this would account for our inability to detect any evolutionary association between leaf drought tolerance divergence and species diversification in the present study.

Third, we established that all species' mean water content per mass was 0.95 (ranging from 0.85 to 0.97; Fig. 2). Based on our observations, water content per mass may have already evolved to a very high degree in this genus. This reduces the likelihood of any further enhancement in water content capacity to accelerate the rate of species

diversification (Crouch & Ricklefs, 2019), implicating our finding of a nonsignificant relationship between the evolutionary rate of water content capacity and the rate of species diversification. However, this should not imply that water storage capacity is unimportant from the perspective of *Primulina* diversification; rather, more evidence is needed to establish how historical water storage capacity has influenced the diversification process in *Primulina*.

4.3 Potential sources of error

In the present study, we have included as many species as possible. Finally, about 50% species were sampled in the genus of *Primulina*. The incomplete species sampled in our study might affect the estimations of species DR and evolutionary rate of drought tolerance for the genus. This is a limitation of our current study, and more species should be included in future studies.

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Supplementary Material

The following supplementary material is available online for this article at <http://onlinelibrary.wiley.com/doi/10.1111/jse.12914>/supinfo:

Table S1. Best fitting model for species diversification rate (DR), water storage capacity, saturate osmotic strength, rate of water storage capacity, and rate of osmotic strength. Significant *P*-values (<0.05) and best model chosen by the Akaike information criterion (AIC) are shown in bold. BM, Brownian motion; LA, estimated lambda; OU, Ornstein–Uhlenbeck; WN, white noise.

Table S2. Relationship between species diversification rate (DR) and rate of drought tolerance when the rates of drought tolerance were estimated from different models. Significant *P*-values (<0.05) and best model chosen by Akaike information criterion (AIC) are shown in bold. + indicates a positive relationship. BM, Brownian motion; LA, estimated lambda; OU, Ornstein–Uhlenbeck; WN, white noise.

Table S3. Correlations between species diversification rate (DR) and rate of drought tolerance when DR and the rates of drought tolerance were estimated by using BMM. + and – indicate positive and negative relationship, respectively.

Table S4. Phylogenetic signal of leaf thickness, water content per mass and saturated osmotic strength. Phylogenetic signal was estimated by the function “phylosig” in the R package phytools version 0.6-99 (Revell, 2012). It was also tested if the observed signal differed significantly from zero.