Leaf physiological and anatomical responses of two sympatric *Paphiopedilum* species to temperature

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ABSTRACT

*Paphiopedilum dianthum* and *P. micranthum* are two endangered orchid species, with high ornamental and conservation values. They are sympatric species, but their leaf anatomical traits and flowering period have significant differences. However, it is unclear whether the differences in leaf structure of the two species will affect their adaptabilities to temperature. Here, we investigated the leaf photosynthetic, anatomical, and flowering traits of these two species at three sites with different temperatures (Kunming, 16.7 ± 0.2 °C; Puer, 17.7 ± 0.2 °C; Menglun, 23.3 ± 0.2 °C) in southwest China. Compared with those at Puer and Kunming, the values of light-saturated photosynthetic rate ($P_{\text{max}}$), stomatal conductance ($g_s$), leaf thickness (LT), and stomatal density (SD) in both species were lower at Menglun. The values of $P_{\text{max}}$, $g_s$, LT, adaxial cuticle thickness (CTad) and SD in *P. dianthum* were higher than those of *P. micranthum* at the three sites. Compared with *P. dianthum*, there were no flowering plants of *P. micranthum* at Menglun. These results indicated that both species were less resistance to high temperature, and *P. dianthum* had a stronger adaptability to high-temperature than *P. micranthum*. Our findings can provide valuable information for the conservation and cultivation of *Paphiopedilum* species. 

1. Introduction

Members of the genus *Paphiopedilum* (Orchidaceae) are world-famous ornamental orchids because of their unique flower shape and colors, large flowers, and long flower lifespan. They have been cultivated throughout the world for more than 100 years, but their commercial production is still limited (Averyanov et al., 2003; Liu et al., 2009). One of the important reasons for this is the insufficient understanding of the environmental adaptability of *Paphiopedilum* species, which limits the development of cultivation techniques. Meanwhile, due to illegal collection and habitat destruction, the number of wild populations and individuals of *Paphiopedilum* have drastically decreased (Liu et al., 2009). *Paphiopedilum* species are sensitive to environmental change. For example, the forest exploitation influences the light intensity and temperature in the natural distribution areas of *Paphiopedilum* (Averyanov et al., 2003). They are a particularly vulnerable group of plants which are amongst the first to disappear during degradation of primary native habitats. All known *Paphiopedilum* species are listed in Appendix I of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES, 2012), and are strictly protected by this international convention. Both the cultivation and conservation of *Paphiopedilum* species depend on knowledge of their environmental adaptabilities. However, only a few studies have focused on the adaptations of *Paphiopedilum* species to low temperature and light intensity (Zhang et al., 2011; Yang et al., 2017), and their environmental requirements and adaptive mechanisms remain unclear.

The genus *Paphiopedilum* comprises more than 70 species, which usually occur in limestone or mountainous forests of tropical and subtropical zones from Asia to the Pacific Islands (Cribb, 1998; Liu et al., 2009). Previous studies have suggested that *Paphiopedilum* plants are characterized by drought tolerance derived from their leaf anatomical traits, including fleshy leaves, thick leaves, thick cuticles, bigger adaxial epidermal cells, lower total stomata area and sunken stomata (Guan et al., 2011; Zhang et al., 2011; Yang et al., 2018). Stomatal and vein densities also tend to increase from terrestrial to...
facultative and epiphytic Paphiopedilum species (Zhang et al., 2012). The lack of guard cell chloroplasts affects the induction of photoynthesis in Paphiopedilum, which may reflect a physiological adaptation to water shortage (Assmann and Zeiger, 1985; Zhang et al., 2011). Therefore, previous studies infer that leaf morphological traits play an important function in reducing the loss of water (Guan et al., 2011; Zhang et al., 2011). However, these species vary largely in their growing environments, leaf morphology and anatomy, flowering period and floral lifespan (Guan et al., 2011; Zhang et al., 2012, 2017). Research on how leaf morphology and anatomy affect environmental adaptations of Paphiopedilum species through controlled experiments is still lacking.

Photosynthesis is a major mechanism for plants to assimilate carbon, and is extremely sensitive to environmental changes, such as light, temperature, and water (Long et al., 2006; Greer and Weedon, 2011; Toscano et al., 2018). Thus, the photosynthetic rate is a crucial trait reflecting a plant’s response to environmental changes. Usually, plants have a higher light-saturated photosynthetic rate under optimum temperature conditions (An et al., 2017). Both low and high temperatures can depress the photosynthetic rate and stomatal conductance of plants (Greer and Weston, 2010; Greer and Weedon, 2011; Wu et al., 2014). Low temperature can decrease photosynthetic parameters of Phalaenopsis plants (Pollet et al., 2009). Similarly, high temperature reduces relative growth rates by the direct effects of temperature on photosynthesis (Marchin et al., 2014). The photosynthetic rate is depressed, while respiration is promoted when plants are exposed to a temperature which is above the photosynthetic optimum. The vegetative growth is strongly inhibited due to the imbalance between carbon-fixation and consumption (Jersel, 2003). Recently, Yang et al. (2017) found that P. armeniacum and P. micranthum can use cyclic electron flow to protect photosystem I and photosystem II under chilling conditions. However, how the photosynthesis of Paphiopedilum plants respond to temperature changes remains unclear.

Leaf morphology and anatomy play an important role in response to changing environments, because they affect light capture, CO2 diffusion conductance, heat balance, water loss and storage of leaves. Leaf anatomical traits are significantly influenced by temperature. Plants will increase their leaf thicknesses under warming conditions (Soudzilowskia et al., 2013). Under high temperatures, plants may increase their leaf and adaxial cuticle thickness (Zheng et al., 2013; Zhou et al., 2019; Habermann et al., 2019), but decrease stomatal density (Ferris et al., 1996). Recently, Wu et al. (2018) found that stomatal density of Schima superba significantly decreases under warming conditions, while a non-significant change is observed in Syzygium rehderianum. Warm temperatures in the early spring, when shoots are emerging, appear favored, while high temperatures during a thesis appear detrimental, reducing both vegetative growth and flowering (Bleho et al., 2021). However, how the leaf anatomy of Paphiopedilum plants varies with the change of temperature remains unclear.

Phenotypic plasticity may play an important role in the establishment of plant population in novel environments, either after transplant to a new habitat or as a response to changing environments (Richards et al., 2006; Matesanz et al., 2010). Although the ability to respond to environmental change is often beneficial, phenotypic plasticity, just like any other aspect of the phenotype, cannot automatically be assumed to be adaptive (Richards et al., 2006). It is adaptive plasticity, however, that is of particular importance for ecological and evolutionary studies (Richards et al., 2006). For example, compared with stomatal length, stomatal density is relatively plastic (Richardson et al., 2001) and potentially adaptive to environmental change (Poulos et al., 2007; Sekiya and Yano, 2008). Thus, we speculate that large phenotypic plasticity indices of Paphiopedilum species may help them to tolerate the change of temperature.

In the present study, we investigated the responses of leaf photosynthetic and anatomical traits of P. dianthum and P. micranthum to different environmental temperatures. These two Paphiopedilum species can occur in the same natural habitats, but P. dianthum plants have a greater leaf, cuticle and epidermis thickness, and higher stomata density than P. micranthum (Zhang et al., 2012; Yang et al., 2018). In addition, P. dianthum flowers in autumn, while P. micranthum flowers in spring. This indicates that there may be differences in temperature adaptation between the two species. Thus, our aim was to address how do photosynthetic, anatomical traits and flowering performances of two sympatric species with different leaf anatomies respond to different temperatures. Such information will contribute to the conservation and cultivation of Paphiopedilum species. We speculate that the adaptation of the two Paphiopedilum species to temperature is related to their geographical distribution, and the plasticity of leaf anatomical traits may play an important role in the adaptations of two Paphiopedilum species to growth temperature.

2. Materials and methods

2.1. Study sites and plant materials

The study was carried out at Menglu, Puer, and Kunming in southwest China (Fig. 1). These three sites locate in tropical, southern subtropical, and mid-subtropical zone, respectively. The daily average temperature and air relative humidity was 23.3 ± 0.2 °C and 86% at Menglu, 17.7 ± 0.2 °C and 81% at Puer, and 16.7 ± 0.2 °C, and 73% at Kunming, respectively (Fig. 2).

In this study, two sympatric species, P. dianthum and P. micranthum, were chosen for the experiments. P. dianthum occurs on rocks in evergreen broad-leaved forests at an elevation of 1000–2250 m. P. micranthum occurs in rocky and bushy places or crevices of rocks in forests in limestone areas at an elevation 400–1700 m (Liu et al., 2009). In November 2018, two-year old artificially propagated plants (300 plants of each species at each site) were placed at three study sites. The plants were planted in porous plastic pots (10 cm × 15 cm) filled with bark and humus (7/3, v/v). We used 70% shade-net to keep the maximum light intensity at approximately 400–600 µmol m−2 s−1. These plants were watered as
needed, and fertilized with control release fertilizer (Osmocote, nitrogen: phosphate: potash = 14:14:14, Geldermalsen, Netherlands) about 0.5 g in each pot every month.

2.2. Measurement of photosynthetic gas exchange

All measurements of leaf gas exchange were performed in an open infrared gas exchange system with an integrated fluorescence chamber (LI-6400-40; Li-Cor, Lincoln, NE, USA) in June and December 2019. Before photosynthetic measurements, the leaf steady-state conditions of December 2019. Before photosynthetic measurements, the leaf chamber (LI-6400-40; Li-Cor, Lincoln, NE, USA) in June and transpiration rate (Tr), and instantaneous water use efficiency (WUEi) were obtained during the measurements of light response curves which were fitted with the software of Photosynthesis (LI-COR Application). The phenotypic plasticity index was calculated for each measured variable as the difference of photosynthesis (LI-COR Application). The phenotypic plasticity index was calculated for each measured variable as the difference between maximum and minimum values divided by the maximum value (Valladares et al. 2000). The map of natural distribution of Paphiopedilum species was performed on the software of ArcGIS 10.2 (Esri, Inc., CA, USA). Graphic images were produced using the Sigma Plot 10.0 package (Systat Software Inc., CA, USA).

3. Results

3.1. Leaf photosynthesis at different sites

The leaf photosynthetic rates \(\left(P_n\right)\) of \(P.\ dianthum\) and \(P.\ micranthum\) increased greatly with the increase in PPFDs when the light intensity below 300 \(\mu\text{mol} \text{m}^{-2} \text{s}^{-1}\) at the three sites (Fig. 3). There were slightly changes of leaf photosynthetic rates of the two Paphiopedilum species with the increase in PPFDs when the light intensity over 300 \(\mu\text{mol} \text{m}^{-2} \text{s}^{-1}\) at the three sites. Compared with Puer and Mengluan, the two tested species had a higher \(P_{\text{max}}\) and \(g_s\) at Kunming (Table 1). For example, \(P_{\text{max}}\) for \(P.\ dianthum\) at Kunming (5.24 ± 0.29 \(\mu\text{mol} \text{CO}_2 \text{m}^{-2} \text{s}^{-1}\)) was significantly higher than at Mengluan (3.24 ± 0.32 \(\mu\text{mol} \text{CO}_2 \text{m}^{-2} \text{s}^{-1}\)). Similarly, the \(g_s\) at Kunming (97.0 ± 11.5 mmol \(\text{H}_2\text{O} \text{m}^{-2} \text{s}^{-1}\)) was also significantly higher than at Mengluan (39.4 ± 14.4 mmol \(\text{H}_2\text{O} \text{m}^{-2} \text{s}^{-1}\)). For \(P.\ micranthum\), the \(P_{\text{max}}\) (3.00 ± 0.10 \(\mu\text{mol} \text{CO}_2 \text{m}^{-2} \text{s}^{-1}\)) and \(g_s\)
Fig. 3. Responses of photosynthetic rate (Pn) to photosynthetic photon flux density (PPFD) in Paphiopedilum dianthum (A) and P. micranthum (B) at the three study sites. The light response curve is fitted with Exponential Rise to Maximum (single, two parameters) in Sigma Plot 10 package. Each data point represents mean ± SE for five measurements from individual plants.

### Table 1
Leaf photosynthetic, anatomical and physiological traits of *Paphiopedilum dianthum* and *P. micranthum* at the three study sites.

| Species          | Traits            | Menglung (alt. 570 m) | Puer (alt. 1302 m) | Kunming (alt. 1990 m) | Plasticity index (%) |
|------------------|-------------------|------------------------|--------------------|------------------------|---------------------|
| *P. dianthum*    | Rd (µmol m⁻² s⁻¹) | 0.64 ± 0.24ᵃ           | 0.68 ± 0.03ᵃ       | 0.48 ± 0.04ᵇ           | 29.4                |
|                  | Pmax (µmol CO₂ m⁻² s⁻¹) | 3.24 ± 0.32ᵇ         | 4.80 ± 0.32ᵃ       | 5.24 ± 0.29ᵃ           | 38.2                |
|                  | gₑ (µmol H₂O m⁻² s⁻¹) | 39.4 ± 14.4ᵇ         | 61.7 ± 8.0ᵇ        | 97.0 ± 11.5ᵃ           | 59.4                |
|                  | Tₑ (µmol H₂O m⁻² s⁻¹) | 0.74 ± 1.7ᵃ          | 0.54 ± 0.10ᵃ       | 0.71 ± 0.07ᵇ           | 27.0                |
|                  | WUE (µmol CO₂ mmol⁻¹ H₂O⁻¹) | 6.87 ± 4.17ᵃ       | 7.53 ± 1.05ᵃ       | 7.04 ± 0.28ᵇ           | 8.8                 |
|                  | LT (µm)           | 1043.4 ± 19.7ᵇ       | 1371.8 ± 33.0ᵃ     | 1223.1 ± 18.1ᵇ         | 23.9                |
|                  | CTₑ (µm)          | 34.57 ± 1.38ᵃ        | 35.04 ± 1.18ᵃ      | 31.54 ± 1.71ᵃ           | 10.0                |
|                  | CTₚ (µm)          | 24.35 ± 0.89ᵇ        | 23.27 ± 0.70ᵇ      | 26.19 ± 1.20ᵇ           | 11.1                |
|                  | Etₑ (µm)          | 304.6 ± 16.0ᵇ        | 510.9 ± 13.5ᵇ      | 451.4 ± 16.4ᵇ           | 40.4                |
|                  | Etₚ (µm)          | 59.42 ± 1.75ᵇ        | 54.45 ± 1.42ᵇ      | 60.70 ± 1.79ᵇ           | 10.3                |
|                  | MT (µm)           | 613.9 ± 10.1ᵇ        | 715.4 ± 23.8ᵃ      | 659.4 ± 16.4ᵇ           | 14.2                |
|                  | SD (mm⁻²)         | 36.0 ± 0.4ᵃ          | 45.3 ± 2.2ᵃ        | 39.6 ± 2.1ᵇ            | 20.5                |
|                  | Aₑ (µm⁻²)         | 1296.8 ± 27.8ᵃ       | 1463.6 ± 23.2ᵃ     | 1477.4 ± 29.2ᵇ         | 12.1                |
|                  | LMA (g m⁻²)       | 146.4 ± 8.0ᵃ         | 165.2 ± 5.5ᵇ       | 161.1 ± 7.8ᵇ           | 11.4                |
|                  | Cₑmax (%)         | 49.38 ± 0.24ᵃ        | 48.30 ± 0.39ᵇ      | 47.53 ± 0.40ᵇ           | 3.7                 |
|                  | Nₑmax (%)         | 1.05 ± 0.09ᵇ         | 0.86 ± 0.10ᵇ       | 1.11 ± 0.10ᵇ            | 22.5                |
|                  | Pₑmax (%)         | 1.29 ± 0.19ᵃ         | 1.06 ± 0.09ᵇ       | 1.20 ± 0.05ᵇ            | 17.8                |
| *P. micranthum*  | Rd (µmol m⁻² s⁻¹) | 0.26 ± 0.04ᵇ         | 0.33 ± 0.08ᵇ       | 0.13 ± 0.04ᵇ           | 60.6                |
|                  | Pmax (µmol CO₂ m⁻² s⁻¹) | 1.62 ± 0.04ᵇ       | 2.10 ± 0.05ᵇ       | 3.00 ± 0.10ᵇ           | 46.0                |
|                  | gₑ (µmol H₂O m⁻² s⁻¹) | 31.1 ± 5.5ᵇ         | 46.5 ± 3.3ᵇ        | 60.8 ± 11.1ᵇ           | 48.8                |
|                  | Tₑ (µmol H₂O m⁻² s⁻¹) | 0.26 ± 0.05ᵇ       | 0.46 ± 0.05ᵇ       | 0.35 ± 0.04ᵇ           | 43.5                |
|                  | WUEₑ (µmol CO₂ mmol⁻¹ H₂O⁻¹) | 6.87 ± 1.64ᵃ     | 5.91 ± 1.23ᵃ       | 7.67 ± 0.74ᵇ           | 22.9                |
|                  | LT (µm)           | 949.8 ± 20.0ᵃ        | 905.2 ± 16.7ᵇ      | 1041.0 ± 35.9ᵇ         | 13.0                |
|                  | CTₑ (µm)          | 22.05 ± 0.83ᵇ       | 23.45 ± 1.04ᵇ      | 27.24 ± 2.35ᵇ           | 19.0                |
|                  | CTₚ (µm)          | 19.77 ± 0.70ᵇ       | 18.54 ± 1.02ᵇ      | 19.24 ± 1.15ᵇ           | 6.2                 |
|                  | Etₑ (µm)          | 165.0 ± 6.6ᵃ        | 156.2 ± 5.4ᵇ       | 164.6 ± 10.1ᵇ           | 5.4                 |
|                  | Etₚ (µm)          | 68.02 ± 2.69ᵇ       | 68.87 ± 2.64ᵇ      | 66.57 ± 2.09ᵇ           | 3.3                 |
|                  | MT (µm)           | 674.7 ± 16.1ᵇ       | 644.0 ± 15.3ᵇ      | 759.42 ± 29.1ᵇ         | 15.2                |
|                  | SD (mm⁻²)         | 16.0 ± 6.6ᵃ         | 19.2 ± 1.0ᵇ        | 17.6 ± 0.6ᵇ            | 16.7                |
|                  | LMA (g m⁻²)       | 177.4 ± 10.6ᵇ       | 170.1 ± 7.6ᵇ       | 160.4 ± 8.4ᵇ           | 9.6                 |
|                  | Cₑmax (%)         | 43.86 ± 0.20ᵃ       | 44.02 ± 0.43ᵇ      | 43.44 ± 0.19ᵇ           | 1.3                 |
|                  | Nₑmax (%)         | 0.90 ± 0.07ᵃ        | 0.77 ± 0.10ᵃ       | 0.92 ± 0.07ᵇ           | 16.3                |
|                  | Pₑmax (%)         | 0.53 ± 0.04ᵃ        | 0.49 ± 0.05ᵇ       | 0.54 ± 0.04ᵇ           | 9.2                 |

Different letters indicated significant differences between study sites and the maximum value was bold (P < 0.05, based on ANOVA, followed by Tukey’s tests for comparison). Values were means ± SE (n = 5). Rd, respiration rate in dark; Pmax, light-saturated photosynthetic rate; gₑ, stomatal conductance; Tₑ, transpiration rate; WUEₑ, instantaneous water use efficiency; LT, leaf thickness; CTₑ, adaxial cuticle thickness; CTₚ, abaxial cuticle thickness; ETₑ, adaxial epidermis thickness; ETₚ, abaxial epidermis thickness; MT, mesophyll thickness; SD, stomatal density; Aₑ, stomatal apparatus area; LMA, leaf dry mass per unit area; Cₑmax, leaf carbon concentration; Nₑmax, leaf nitrogen concentration; Pₑmax, leaf phosphorus concentration.

(61.8 ± 11.1 mmol H₂O m⁻² s⁻¹) were significantly higher at Kunming than those at Menglung (1.62 ± 0.04 µmol CO₂ m⁻² s⁻¹, 31.1 ± 5.5 mmol H₂O m⁻² s⁻¹, respectively). However, except for the Pmax being significantly higher at Kunming than at Menglung for *P. micranthum*, there was no significant difference in gₑ for the two species between Puer and Menglung. The Rd and Tₑ of the two species had no significant differences among the three sites, except the Tₑ value of *P. micranthum* was significantly higher at Puer than at Menglung. Moreover, the values of Pmax, gₑ, and Tₑ of the two species were significantly higher in summer than in winter (Table 2).
Kunming, but the petal length, lip length and dorsal sepal length was significantly larger at Menglun than at Puer and Kunming. The Cmass was significantly larger at Menglun than at Kunming and Puer. However, no significant difference was found in the CTad, CTab, ETab, As, LMA, Nmass, and Pmass in P. micranthum among the three sites. For P. micranthum, the LT, CTad, and MT were significantly thicker at Kunming than at Puer and Menglun. The change trend in the SD among the three sites was similar to that of P. dianthum. However, the values of CTab, ETab, ETab, As, LMA, Cmass, Nmass, and Pmass in P. micranthum was not significantly different among the three sites. The plasticity indices of LT, CTab, ETab, ETab, SD, LMA, Cmass, Nmass, and Pmass in P. micranthum among the three sites were higher than those of P. micranthum. Nevertheless, except for the ETab and As, the CTab, CTab, SD, Cmass, Pmass, Rg, Pmax, and Tr of P. dianthum were significantly higher than those of P. micranthum (Table 3).

### 3.2. Leaf functional traits at different sites

There were significant differences in many leaf anatomical and chemical traits for P. dianthum and P. micranthum among the three sites (Table 1). For P. dianthum, the values of LT, ETab, and MT were significantly higher at Puer than at Kunming and Menglun. The SD was larger at Puer than at Menglun. The Cmass was significantly higher at Menglun than at Kunming and Puer. However, no significant difference was found in the CTab, CTab, ETab, As, LMA, Nmass, and Pmass in P. micranthum among the three sites. For P. micranthum, the SD, CTab, and MT were significantly larger at Kunming than at Puer and Menglun. The control experiment was set up with the highest (42°C) of natural distribution area of the two species was the lowest at Menglun, where the annual average temperature and air relative humidity were the highest (Fig. 2, Table 1). Compared with that at Kunming, the Pmax of P. dianthum was decreased by 38% at Menglun, while the Pmax of P. micranthum was decreased by 46%. Contrary to our result, an increased temperature increases the Pmax of Dryas Octopetala var. asiatica and Rhododendron confertissimum (Zhou et al., 2019). Such inconsistency may be caused by differences in the optimum temperature requirement of different species. Both P. dianthum and P. micranthum are distributed in limestone areas above 1000 m a.s.l. in subtropical Asia (Cribb, 1998), where the temperature is not too high. However, Menglun (alt. 570 m) locates at the northern edge of the tropics has high air relative humidity and temperature. The summer temperature at Menglun may exceed the annual average temperature (about 16 °C) of natural distribution area of the two species. Thus, the Pmax at Menglun may be depressed by high temperatures. Previous studies have suggested that the gs and Rubisco activity of plants decrease under high temperature conditions (Greer and Weston, 2010; Greer and Weedon, 2011; Wise et al., 2004). Compared with P. dianthum, the Pmax of P. micranthum showed significant opposite trends. However, the floral buds of P. micranthum were aborted before blossoming at Menglung, while the plants at Puer and Kunming flowered.

### 3.3. Flowering performance at different sites

The flowering performance of P. dianthum was significantly different at the three sites (Table 4). The flower number was significantly fewer at Menglun than at Puer and Kunming. The scape length was significantly larger at Menglun than at Puer and Kunming, but the petal length, lip length and dorsal sepal length were aborted before blossoming at Menglung, while the plants at Puer and Kunming flowered.

### Table 2

Differences in photosynthetic traits in Paphiopedilum dianthum and P. micranthum between summer and winter at the three study sites.

| Species       | Traits                        | Summer     | Winter    | P value |
|---------------|-------------------------------|------------|-----------|---------|
| P. dianthum   | Rg (μmol m⁻² s⁻¹)             | 0.60 ± 0.06| 0.23 ± 0.11| 0.047   |
|               | Pmax (μmol CO₂ m⁻² s⁻¹)       | 4.43 ± 0.01| 0.71 ± 0.22| 0.005   |
|               | gs (mmol H₂O m⁻² s⁻¹)         | 66.0 ± 16.9| 8.3 ± 2.9  | 0.028   |
|               | Tr (mmol H₂O m⁻² s⁻¹)         | 0.06 ± 0.06| 0.13 ± 0.07| 0.004   |
|               | WUEi (μmol CO₂ mmol H₂O⁻¹)    | 7.15 ± 0.20| 9.50 ± 3.93| 0.611   |
| P. micranthum | Rg (μmol m⁻² s⁻¹)             | 0.24 ± 0.06| 0.23 ± 0.14| 0.943   |
|               | Pmax (μmol CO₂ m⁻² s⁻¹)       | 2.24 ± 0.40| 0.81 ± 0.40| 0.066   |
|               | gs (mmol H₂O m⁻² s⁻¹)         | 460 ± 8.7  | 140 ± 5.0  | 0.033   |
|               | Tr (mmol H₂O m⁻² s⁻¹)         | 0.36 ± 0.06| 0.13 ± 0.02| 0.021   |
|               | WUEi (μmol CO₂ mmol H₂O⁻¹)    | 6.82 ± 0.51| 8.46 ± 5.37| 0.789   |

Values were means ± SE (n = 3). Analysis of difference based on t tests of independent samples and the maximum value was bold. The abbreviations are the same as Table 1.

### Table 3

Differences in leaf anatomical and photosynthetic traits between Paphiopedilum dianthum and P. micranthum at the three study sites.

| Traits               | P. dianthum | P. micranthum | P value |
|----------------------|-------------|---------------|---------|
| LT (μm)              | 1212.8 ± 94.9| 965.3 ± 40.0  | 0.074   |
| CTab (μm)            | 33.72 ± 1.10| 24.25 ± 1.55  | 0.008   |
| CTab (μm)            | 24.60 ± 0.85| 19.18 ± 0.36  | 0.004   |
| ETab (μm)            | 422.3 ± 61.3| 161.9 ± 2.9   | 0.051   |
| ETab (μm)            | 58.19 ± 1.91| 67.82 ± 0.67  | 0.009   |
| MT (μm)              | 662.9 ± 29.4| 692.6 ± 34.6  | 0.549   |
| SD (mm⁻²)            | 40.30 ± 2.71| 17.60 ± 0.92  | 0.001   |
| As (μm⁻²)            | 1413.2 ± 57.4| 2473.6 ± 150.7| 0.003   |
| LMA (g m⁻²)          | 157.6 ± 5.7 | 169.3 ± 4.9   | 0.195   |
| Cmass (%)            | 48.40 ± 0.54| 43.77 ± 0.17  | 0.001   |
| Nmass (%)            | 1.015 ± 0.08| 0.86 ± 0.05   | 0.182   |
| Pmass (%)            | 1.18 ± 0.07 | 0.52 ± 0.02   | 0.001   |
| Rg (μmol CO₂ m⁻² s⁻¹)| 0.60 ± 0.06 | 0.24 ± 0.06   | 0.013   |
| Pmax (μmol CO₂ m⁻² s⁻¹)| 4.43 ± 0.61| 2.24 ± 0.40   | 0.04    |
| gs (mmol H₂O m⁻² s⁻¹)| 66.0 ± 16.9 | 46.0 ± 8.7    | 0.351   |
| Tr (mmol H₂O m⁻² s⁻¹)| 0.66 ± 0.06 | 0.36 ± 0.06   | 0.023   |
| WUEi (μmol CO₂ mmol H₂O⁻¹)| 7.15 ± 0.20| 6.82 ± 0.51   | 0.578   |

Values were means ± SE (n = 3). Analysis of difference based on t tests of independent samples and the maximum value was bold. The abbreviations are the same as Table 1.

### Table 4

Differences in flowering performance of Paphiopedilum dianthum at the three study sites.

| Traits               | Study sites      |
|----------------------|------------------|
| Number of flowers per scape | Menglung | Puer | Kunming |
| Scape length (cm)    | 35.02 ± 0.96     | 27.89 ± 0.50  | 28.09 ± 0.80  |
| Petal length (cm)    | 9.21 ± 0.39b     | 11.69 ± 0.17a | 11.80 ± 0.16a |
| Lip length (cm)      | 4.54 ± 0.10b     | 4.84 ± 0.04a  | 4.83 ± 0.04a  |
| Dorsal sepal length (cm) | 4.53 ± 0.13b | 5.11 ± 0.07b | 5.29 ± 0.05a |

Values were means ± SE (n = 5). Different letters indicated significant differences between study sites and the maximum value was bold (P < 0.05, based on ANOVA, followed by Tukey’s tests for comparison).
at Mengln was reduced by a greater proportion. These results showed that the two Paphiopedilum species had weak tolerance to high temperature at Mengln, and P. micranthum was less tolerant to high temperature than P. dianthus. The reason why P. micranthum was less tolerant to high temperature than P. dianthus was that the former was more geographically distributed in the North (Fig. 1). In addition, the photosynthetic rates of the two Paphiopedilum species in winter were significantly lower than that in summer. This might be related to the seasonal variation in the vigor of Paphiopedilum plants which was affected by seasonal climate. Thus, growth temperature was a key factor affecting the photosynthetic performances of two Paphiopedilum species.

The changes in leaf anatomical traits at the three sites were species-specific. For P. dianthus, the values of LT, ETad, MT, and SD were significantly higher at Puer than those at Mengln, but were not significantly different between Puer and Kunming. This indicated that leaf anatomical traits of P. dianthus were significantly affected by temperature. Previous studies have suggested that LT is significantly reduced under warming environments (Jin et al., 2011; Yang et al., 2011; Zheng et al., 2013; Habermann et al., 2019; Zhou et al., 2019). In this study, the change of LT of P. dianthus was 23.5% which was mainly due to the change of ETad and MT. Thus, high temperature might decrease LT mainly via reducing the ETad and MT of P. dianthus. A high temperature is usually accompanied by high water loss. To reduce water loss via stomata, plants usually regulate the number or area of stomata (Xu and Zhou, 2008; Cruz et al., 2019). Here, the SD in two Paphiopedilum species reduced significantly under warmer conditions. This is consistent with the result from a study on S. superba (Wu et al., 2018). Thus, the low photosynthetic rate in P. dianthus at Mengln was related to the reduced SD. For P. micranthum, the values of LT, CTad, and MT were significantly higher at Kunming than those at Mengln. This is consistent with previous results (Bacelar et al., 2004; Cassola et al., 2019). The increase in cuticle thickness is a response of plant development to water deficits (Chen et al., 2020). Plants may increase the CTad to prevent water loss under conditions of high transpiration demand (Bacelar et al., 2004). There were no significant differences in the values of CTad, ETad, ETab, and A; among the three sites. The LT and SD of the two Paphiopedilum species are important traits in response to the changing growth temperatures. Thus, the adjustments of LT and SD of Paphiopedilum species may play essential roles in plant responses to different environmental temperatures. In addition, some leaf anatomical traits of P. dianthus were significantly higher than those of P. micranthum, such as the thickness of cuticles and stomatal densities. Thus, these variations in leaf anatomical traits may help P. dianthus to adapt to environmental change better than P. micranthum.

The value of Cmass in the two species was higher at Mengln than at Kunming, but there were no significant differences in Nmass, Pmass, and LMA among the three sites. Compared with P. dianthus, the values of Nmass and Pmass were higher than those in P. micranthum, while the value of LMA was opposite in the two species. Previous studies have also suggested that the plants with higher LMA have lower values of Nmass and Pmass (Guan et al., 2011), and LMA shows a significant increase in drought environments (Wang et al., 2011; Toscano et al., 2018). This indicated that the two Paphiopedilum species were not stressed by water availability in summer. However, the two species become dormant during winter, and no new leaves are produced. Thus, further research is still required to illustrate the response of Paphiopedilum species to water status.

Pmax is influenced by leaf anatomical and physiological traits. Leaf photosynthesis depends on the concentration of CO2 which arrives at the carboxylation site (Muir et al., 2014). Stomata, intercellular airspaces, cells, plasma membrane, cytosol, and chloroplast envelopes and stroma are the major barriers that limit atmospheric CO2 arrival at the carboxylation site (Flexas et al., 2012; Muir et al., 2014). A previous study has found that there is a significantly positive correlation between photosynthetic rate and gS (Gago et al., 2016). The gS is determined by SD, stomatal size and whether the stomata are open (Frankas and Beerling, 2009; De Boer et al., 2016). In our study, the change trend of Pmax was same with gS, SD, LT, CTad, ETad, but opposite with ETab and A; among the three sites (Table 1). This is similar to previous studies which found that the Pmax increases with SD (Xu and Zhou, 2008; Jin et al., 2011; Yang et al., 2018). There was no significant difference in the A; among the three sites, which further proved that the two species may not be stressed by drought (Xu and Zhou, 2008). In addition, the increase in ETab may decrease the mesophyll conductance, which is positively correlated with the photosynthetic rate (Grassi and Magnani, 2005). Thus, leaf anatomy plays an important role in regulating photosynthesis of two Paphiopedilum species in different growth environments.

Temperature has a vital influence on flowering traits, the flowering of Doritaenopsis 'Newberry Parfait' was completely suppressed under high-temperature (Newton and Runkle, 2009). However, high temperature can increase the inflorescence size of Phalaenopsis hybrid (Lee and Lin, 1984), and leads to the reduction of flower number and size of tepals in Phalaenopsis hybrid and Tolumnia dianthum (Newton and Runkle, 2008; Marchin et al., 2014). In our study, the flowering performance of the two Paphiopedilum species were significantly different at the three sites. The number of flowers of P. dianthus was significantly fewer, and no normal flowers developed on the P. micranthum plants at Mengln. High temperature significantly decreased the length of petal, dorsal sepal, and lip of P. dianthus (Table 4). We inferred that there was a trade-off between inflorescence size and tepals size of Paphiopedilum species under high temperature conditions. These results not only indicated that high temperature inhibited the flowering performances of the two Paphiopedilum species, but also revealed that P. dianthus might be tolerant to high temperature better than P. micranthum.

Plant plasticity has been recognized as a vital aspect of how plants develop, function and evolve in their environments (Sultan, 2000). High phenotypic plasticity is not only associated with wide geographical distribution of a species, but it also demonstrates the ability of plants to adapt to new environments (Sultan, 2000; Velikova et al., 2020). Moreover, greater phenotypic plasticity would enable a plant to respond more rapidly to an adverse environment in a short time, which shows that these species may accelerate the process of adaptation in new environments (Ghalambor et al., 2007; McLean et al., 2014). In our study, compared with P. micranthum, higher plasticity indexes of P. dianthus were found in LT, CTad, CTab, ETad, ETab, SD, LMA, Cmass, Nmass, and Pmass. In particular, the plasticity indexes of LT, ETad, and SD of P. dianthus were larger than those of P. micranthum. Similar to our hypothesis, these results indicated that the stronger adaptability of P. dianthus than P. micranthum was related to the plasticity of leaf traits under the tested environments.

In conclusion, we focused on the leaf photosynthetic, anatomical, and flowering responses of P. dianthus and P. micranthum to different environmental temperatures in southwest China. The photosynthetic rate and flowering performance of the two species were strongly affected by temperature. However, compared with P. micranthum, the photosynthetic rate of P. dianthus was less sensitive to high temperature. The leaf thickness, mesophyll thickness, and stomatal density were lower at Mengln than those at Puer and Kunming. Furthermore, a larger plasticity of leaf anatomical traits makes P. dianthus more adaptable to the tested environments. This study indicates that P. dianthus plants can more effectively regulate their leaf structure to respond to various environmental temperatures than P. micranthum. The findings will contribute to the conservation and utilization of Paphiopedilum species.
Author contributions

S.B.Z. and J.Q.F. designed the study; J.Q.F. carried out the experiments; J.Q.F. and S.B.Z. analyzed the data; J.Q.F., J.H.W. and S.B.Z. wrote and revised this manuscript.

Declaration of competing interest

The authors declare no competing financial interest.

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