STUDIES

Correlated evolution of leaf and root anatomic traits in Dendrobium (Orchidaceae)

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Abstract

The whole-plant economic spectrum concept predicts that leaf and root traits evolve in coordination to cope with environmental stresses. However, this hypothesis is difficult to test in many species because their leaves and roots are exposed to different environments, above- and below-ground. In epiphytes, both leaves and roots are exposed to the atmosphere. Thus, we suspect there are consistent water conservation strategies in leaf and root traits of epiphytes due to similar selection pressures. Here, we measured the functional traits of 21 species in the genus Dendrobium, which is one of the largest epiphytic taxa in the family Orchidaceae, and used phylogenetically independent contrasts to test the relationships among traits, and between traits and the environment. Our results demonstrate that species with a thicker velamen tended to have thicker roots, a thicker root cortex and vascular cylinder, and a larger number of vessels in the root. Correspondingly, these species also had higher leaf mass per area, and thicker leaf lower cuticles. Leaf and root traits associated with water conservation showed significantly positive relationships. The number of velamen layers, leaf density and the ratio of vascular cylinder radius to root radius were significantly affected by the species' differing environments. Thus, traits related to water conservation and transport may play an important role in helping Dendrobium cope with the cool and dry conditions found at high elevations. These findings confirmed the hypothesis that leaf and root traits have evolved in coordination, and also provide insights into trait evolution and ecological adaptation in epiphytic orchids.

Keywords: Co-evolution; elevation; epiphyte; water conservation; water shortage.

Introduction

Trade-offs among functional traits reveal the strategies for plants to acquire and conserve resources (Wright and Westoby 2002; Kong et al. 2015), and provide insights into species distribution and ecosystem processes (Fortunel et al. 2012). These functional traits have been described as the ‘spectra’ to separate species with different adaptation strategies (Liese et al. 2017). On one end of the ecological axis are species with an acquisitive strategy. These species with low leaf mass per area (LMA) have higher photosynthetic rates but shorter lifespans (Reich et al. 1998; Westoby et al. 2002). On the other end of this axis are species with a conservative strategy. These species with denser tissue have greater resistance to mechanical damage and pathogen attack, leading to slower growth rates and longer lifespans (Poorter et al. 2008; Liu et al. 2010; Kong et al. 2015). Key traits related to resource acquisition and conservation should be considered as a part of the leaf and root functional coordination (Freschet et al. 2015).

The leaf economic spectrum (LES) concept has been widely applied. This concept hypothesizes that leaf functional traits may co-vary along a distinct spectrum among species (Wright et al. 2004; Somavilla et al. 2013; Poorter et al. 2014). Within the literatures on LES, similar trait spectra have been expanded to stems and roots, thus forming the whole-plant economic spectrum (Freschet et al. 2010; Kong et al. 2015; Díaz et al. 2016;
Valverde-Barrantes et al. 2017). Although the root is the main organ for resource acquisition, root traits receive the least attention in plant ecology research (Manschadi et al. 2006; Liese et al. 2017; Valverde-Barrantes et al. 2017; Kong et al. 2019). Research on root traits has been hampered due to constraints in observation and sampling, such that plant roots are labelled “the hidden hall” (Eshel and Beeckman 2013). Another reason for the complexity in evaluating the root trait syndrome is the linkage between leaf and root traits (Withington et al. 2006; Mommer and Weemstra 2012). According to the whole-plant economic spectrum hypothesis, leaf and root traits evolve in coordination (Freschet et al. 2010). However, studies on relationships between leaf and root traits across species have showed contrasting results. For example, Craine and Lee (2003) found nitrogen concentration and tissue density of leaves are correlated with those of fine roots. Tjoelker et al. (2005) found a concordance in leaf and root longevity. However, Withington et al. (2006) suggested tissue structure and longevity above-ground (leaves) can contrast markedly with those of below-ground (roots). Thus, more research into root traits is needed to resolve these contrasting findings. The decoupling of leaf and root traits may be caused by the following reasons. Firstly, differences in plant growth form may affect trait correlations (Reich et al. 1998; Withington et al. 2006; Liu et al. 2010). For example, among grass species, the acquisitive strategy is associated with low LMA, low leaf tissue density and low root tissue density (Ryser et al. 1997; Wahl and Ryser 2000), whereas among tree species, acquisitive strategy is associated with higher specific root length and smaller root diameters, but not root tissue density (Comas et al. 2002). This suggests that the trait correlations or plant strategies that have been widely observed in herbaceous plants cannot be directly extrapolated to woody plants (Liu et al. 2010). Secondly, the drivers of morphological variation in leaf and root traits may be different (Kembel and Cahill 2011; Valverde-Barrantes et al. 2017). Previous studies have suggested that phylogeny plays a major role in root trait variation (Kong et al. 2014; Reich 2014), whereas environmental factors may largely account for variations in leaf traits (Baraloto et al. 2012). Thus, when examining species-level responses to environmental changes, phylogeny should be considered (Ackerly and Donoghue 1998; Edwards 2006). Furthermore, leaf and root traits may be decoupled due to the differences in above- and below-ground environments (Freschet et al. 2015; Adair et al. 2019). For example, the availabilities of nutrients and water in soil are significantly higher, and more stable than that in atmosphere or canopy (Zott et al. 2010). However, it is not clear whether the association between leaf and root traits of epiphytes is stronger than that of terrestrial plants.

The roots of tree- and rock-dwelling epiphytes are exposed to similar environments as their leaves (Zott and Winkler 2013). Epiphyte habitats supply irregular amounts of water, and the resultant water stress strongly inhibits epiphyte growth and survival (Zott 2005; Zott et al. 2010). In response to frequent drought stress, epiphytes have evolved ecophysiological adaptations (Zhang et al. 2018). Specifically, the aerial roots of epiphytes capture water via a special spongy structure called velamen, which absorbs water that flows down the tree trunk or rock surface (Roberts and Dixon 2008). Although velamen is not exclusive to epiphytes (Zott et al. 2017), its role in epiphytes’ physiology is especially important. Thick velamen significantly delays water loss (Zott and Winkler 2013), allowing epiphytes to survive in habitats where few other plants can survive, such as habitats with extremely small amounts of water availability (Roberts and Dixon 2008; Zott and Winkler 2013; Joca et al. 2017).

Plants can also respond to water availability by adjusting leaf traits (Wright et al. 2005; Qin et al. 2019). For example, plants can adapt to water shortage by regulating their stomatal area (SA), stomatal density (SD), leaf density (LD) and epidermis or cuticle thickness (Zhang et al. 2012). Although velamen has an important role in water conservation, few researches have tested the coordination between velamen thickness (VT) and leaf traits related to water conservation (Zott and Winkler 2013). Thus, it would be valuable to explore whether both leaf and root traits follow accordant trends in their water conservative strategies.

To address whether leaf and root traits in epiphytes show coordinated evolution in response to changing environments, we analysed the variations in leaf and root traits in species of the genus Dendrobium. All members within the genus are epiphytic or lithophytic (Zhu et al. 2009), and have roots that are easily observed and sampled. In addition, Dendrobium is one of the largest genera in Orchidaceae, and presents some of the most intricate taxonomic problems in the family (Xiang et al. 2013). Whether Dendrobium is monophyletic have been inconclusive to date (Schuiteman 2011; Takamiya et al. 2014). The phylogenetically independent contrast (PIC) method has been widely used in ecology to detect the evolutionary correlation among traits (Price 1997), because ignoring phylogenetic relationships among species included in a comparative analysis may lead to spurious conclusions due to high type I or type II errors (Morand and Poulin 2003). The correlated evolution between traits has been tested in large taxa by using a PIC method (e.g. angiosperm or specific clades (Grotkopp and Rejmánek 2007; Fortuneel et al. 2012; Zhang et al. 2012). However, previous studies into relationships among traits, and between plant traits and environmental factors in epiphytes mostly focused on above-ground organs, with particular emphasis on leaf traits, but rarely on the roots (Sun et al. 2014; Teixeira da Silva et al. 2016). The leaves and roots of epiphytes may experience similar selection pressures, but no study has been conducted to detect the evolutionary association between leaf and root traits of epiphytes, including Dendrobium.

Here, we determined the patterns of variation for 36 leaf and root traits in 21 species of Dendrobium, and used the PIC method to detect whether species traits co-varied with other traits and/or with the environment, and tried to answer following questions: (i) How do leaf and root traits vary with velamen thickness? (ii) Are there close associations between leaf and root traits in Dendrobium species? (iii) Are leaf and root traits shaped by phylogeny? We suspect leaf and root traits related to water conservation will coordinate along single axes of resource acquisition/conservation in Dendrobium species when their leaves and roots are exposed to similar environments.

Materials and Methods

Plant materials and study site

Twenty-one (21) species of Dendrobium, including epiphytes and lithophytes, were cultivated in a greenhouse at the Kunming Institute of Botany, Chinese Academy of Sciences (elevation 1990 m, 102°41’E, 25°01’N). Two species, D. kingianum and D. bracteoseum, were collected from Australia. Nine species (D. loddigesii, D. nobile, D. longicornu, D. crystallinum, D. crepidatum, D. chrysanthum, D. fimbriatum, D. chrysotoxum and D. thrysiflorum) were collected from the Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, and the remaining 11 species were grown at the Kunming Institute of Botany. Information on the natural habitat, growth form and altitude of the species was sourced from the Flora of China (Zhu et al. 2009; http://www.efloras.2020, Vol. 12, No. 4
org), Teixeira da Silva et al. (2016) and Simpson et al. (2018). To ensure that interspecific differences were not merely the result of plastic responses to variable growth conditions, plants were grown for >1 year in a greenhouse at the Kunming Institute of Botany. Plants were grown on a substrate that consisted of a mixture of 70 % bark (1 cm × 1 cm), 20 % moss and 10 % humus, at 18–27 °C, with a relative humidity of 50–70 %, and 20 % full sunlight. Water and fertilizer were supplied as needed. To avoid changes in root structure due to substrates, only aerial roots were selected as our test material.

**Phylogenetic tree**

A Phylogram was generated using concatenated data sets of nucleus gene: Internal Transcribed Spacers (ITS) and the chloroplast genes: rbcL, matK-trnK, trnH-psbA regions which were downloaded from GenBank (http://www.ncbi.nlm.nih.gov). Bulbophyllum odoratissimum was chosen as the outgroup because of its close relationship to Dendrobium (Freudenstein and Rasmussen 1999; Xiang et al. 2013). Numbers associated with nodes are maximum-likelihood bootstrap values. Multiple alignments were automatically performed using ClustalX v.2.0.11 and manual corrections through BioEdit v.7.0.9.0, generating a matrix in a NEXUS format for Bayesian analyses in MrBayes v3.2.2.x64. These analyses used the best-fit models selected with model selection criterion AIC by the software JModeltest v.2.1.4. In the Bayesian analyses, trees were generated by running Metropolis-coupled Monte Carlo Markov (MCMC) chains and sampling one tree every 100 generations for 1 000 000 generations, starting with a random tree. The phylogenetic relationships of the studied Dendrobium species and their ecological information are shown in Supporting Information—Fig. S1.

**Sampling and measurement**

To minimize the confounding effect of plant age, for each species, at least six mature individuals were randomly selected, and three healthy, mature leaves and roots from each individual were collected. Leaves were selected in the middle part of the leaf (avoiding the main vein) and roots were sampled ~2 cm above the apex of new viable roots. After collection, samples were sealed in plastic bags, and anatomical traits were immediately measured. Collection and measurement were conducted during the wet season (from July to September 2018).

After measuring the fresh mass (M_{dry}) of leaves, the leaf area (LA) was measured with a Li-Cor 3000A area meter (Li-Cor Inc., Lincoln, NE, USA), and leaves were then oven-dried for 48 h at 70 °C until reaching a constant mass to obtain leaf dry mass (M_{dry}). Water content (WC, %) was calculated as \(\frac{M_{\text{dry}} - M_{\text{wet}}}{M_{\text{wet}}} \times 100\%\). Leaf mass per area (LMA) was calculated as M_{\text{dry}}/LA.

To characterize leaf anatomical traits, we cut 5-mm × 2-mm sections from the middle part of the leaf (avoiding the main vein) with a freezing microtome (CM3050S, Leica, Germany). Leaf thickness (LT), upper epidermal thickness (UET), lower epidermal thickness (LET), upper cuticle thickness (UCT) and lower cuticle thickness (LCT) were measured with the software ImageJ v.1.43u (National Institutes of Health, Bethesda, MD, USA). Leaf density (LD, kg m\(^{-2}\)) was calculated as leaf dry mass per unit volume, which was calculated as LA × LT (Sun et al. 2014).

For stomatal traits, abaxial nail varnish peels were taken centrally, midway between the midrib and margin (Sack et al. 2003), transferred to glass slides after drying and then photographed under an optical microscope. The images were measured using ImageJ. Stomatal density (SD) was measured as the number of stomata per unit area, and was calculated as the mean value of >36 images from each species (6 images per leaf). Stomatal length (SL) and width (SW) were averaged from 60 randomly selected stomata for each species. Stomatal area (SA) was estimated by the formula 1/4 × π × SL × SW (Sun et al. 2014).

To measure vein density (VD), the leaves were boiled for 30 min in 5 % NaOH and washed with distilled water three times, then bleached in 5 % sodium hypochlorite until the mesophyll was transparent. The leaves were then stained for 2 min with 1 % toluidine blue, mounted on glass slides and photographed. Total vein length was measured with ImageJ, and VD was calculated as total vein length per leaf area (LA).

To examine root anatomical traits, we used a freezing microtome to cut 4-mm-thick sections ~2 cm from the root apex and photographed the cross sections with an optical microscope. Velamen thickness (VT) and root radius (r) were measured with ImageJ. The area of velamen (A_{vel}) was calculated as the whole cross-section area minus the area within the epidermis. We measured the length (vcl) and width (vcw) of ~100 randomly selected velamen cells. The area of each velamen cell (A_{cell}) was calculated as vcl × vcw. Exodermic, endodermic and passage cells were counted using ImageJ. The number of vessel (N_{ves}) referred to the number of primary xylem vessels. To determine vessel diameter (D_{ves}) and vessel area (A_{ves}), we measured all the primary xylem vessels.

**Data analysis**

Before analysis, all data were log10 transformed to improve normality and homoscedasticity. Comparison of traits among different groups was conducted by a one-way ANOVA. A PIC method was used to detect whether species traits co-varied with other traits or with the environment (Price 1997; Purvis and Webster 1999) by employing the ‘ape’ package in R v.3.4.4. Any PIC correlations were evaluated with a ‘Pearson’ correlation in R package.

To evaluate the evolutionary history of leaf and root traits, we first tested for a phylogenetic signal in each trait using the K-statistic, which is based on a ‘Brownian motion model’ of trait evolution (Blomberg et al. 2003). The K metric can be used to assess phylogenetic conservatism. K > 1 indicates that a trait value is more conserved than expected from Brownian motion. K < 1 indicates that a trait value is significantly less conserved than expected from Brownian motion, and instead demonstrates significant lability, while K = 1 shows that a trait value is as expected from a Brownian motion model (Blomberg et al. 2003). The K-statistic was estimated using the ‘picante’ package in R program. We used the ‘Rtsne’ package in R to compute t-SNE dimensional reduction (Van der Maaten and Hinton 2008) and grouped traits and species to distinct clusters. The ‘Rtsne’ was run with ‘perplexity = 5’.

A principal component analysis (PCA) was performed with the ‘prcomp’ function of the ‘vegan’ package in R program to analyse the associations among the traits. Multidimensional scaling (MDS) was conducted in SPSS 16.0 (SPSS Inc., Chicago, IL, USA) and was also used to verify the relationships of the traits.

**Results**

**Variations in anatomical traits among species**

In total, 22 root traits and 14 leaf traits of 21 Dendrobium species were studied. Coefficient of variation (CV) defined as the ratio
of the standard deviation to the mean was used to measure trait variability. Leaf traits varied more than root traits for all *Dendrobium* species (Table 1). Leaf dry mass (*M*<sub>L(D)</sub>, CV = 145 %) and fresh mass (*M*<sub>L(F)</sub>, CV = 117 %) had the largest variation, while leaf area (LA) also varied greatly (CV = 97 %). Leaf water content (WC) had the smallest variation (CV = 15 %). For root traits, the area of vessels in cross section showed the greatest variation (A<sub>n</sub>, CV = 88 %), while the area of velamen in cross section (A<sub>vel</sub>) also varied greatly (CV = 75 %). The ratio of radius of vascular cylinder to root radius (R<sub>r</sub>/R<sub>s</sub>) showed the smallest variation (CV = 17 %).

In terms of the function that the leaf and root traits reflected, the traits related to water conservation showed relatively large variation. Among leaf traits, the CV values for leaf mass per area (LMA), leaf thickness (LT), upper cuticle thickness (UCT), lower cuticle thickness (LCT) were 47 %, 49 %, 46 % and 55 %, respectively. Among root traits, the CV values for velamen thickness (VT) and A<sub>vel</sub> were 46 % and 75 %, respectively. Correlations between leaf and root traits in *Dendrobium*

For leaf traits, significant positive correlations were observed between LMA and LT (r = 0.85), UCT and LCT (r = 0.72 and 0.79, respectively) and lower epidermal thickness (LET, r = 0.68) [see Supporting Information—Table S1]. Leaf thickness (LT) was positively correlated with M<sub>L(F)</sub> (r = 0.56), LMA (r = 0.85), UCT and LCT (r = 0.62 and 0.73, respectively), UET and LET (r = 0.53 and 0.74, respectively). Stomatal density (SD) was positively correlated with leaf density and vein density (LD and VD, r = 0.56 and 0.45, respectively), but negatively correlated with LET (r = −0.52). The LET was also positively correlated with UET, UCT and stomatal area (SA, r = 0.86, 0.58 and 0.46, respectively).

Traits related to root velamen were strongly correlated with root radius (r), whether or not phylogenetic effects were considered [see Supporting Information—Table S2]. For instance, VT and A<sub>vel</sub> were positively correlated with root radius (r = 0.96 and 0.99, respectively). Velamen thickness (VT) was also negatively correlated with LCT (r<sub>VT/LCT</sub> = −0.52). The correlation coefficient for the root cortex thickness/radius (RCT/r) was lower in *Dendrobium* species (r<sub>RCT/r</sub> = −0.54) than in D. grandiflorum (r<sub>RCT/r</sub> = −0.72). Correlations between leaf and root traits in *Dendrobium* species. SD: standard deviation; CV: coefficient of variation (%).

### Table 1. Variations in leaf and root traits of tested *Dendrobium* species. SD: standard deviation; CV: coefficient of variation (%).

| Traits | Abbr. | Function | Unit | Range | Mean | SD | CV (%) |
|--------|-------|----------|------|-------|------|----|--------|
| Leaf fresh mass | M<sub>L(F)</sub> | Growth performance | g | 0.05–2.42 | 0.54 | 0.63 | 117.49 |
| Leaf dry mass | M<sub>L(D)</sub> | Growth performance | g | 0.0072–0.49 | 0.08 | 0.12 | 145.04 |
| Water content | WC | Water status | % | 52.28–98.13 | 82.31 | 12.17 | 14.79 |
| Leaf area | LA | Water loss | cm<sup>2</sup> | 2.02–40.96 | 12.32 | 11.90 | 96.58 |
| Leaf mass per area | LMA | Water conservation | g m<sup>−1</sup> | 18.50–139.18 | 57.34 | 27.16 | 47.36 |
| Leaf density | LD | Water conservation | kg m<sup>−3</sup> | 57.62–210.33 | 138.70 | 46.63 | 33.62 |
| Vein density | VD | Water transport | mm mm<sup>−2</sup> | 1.50–5.64 | 2.80 | 1.08 | 38.39 |
| Leaf thickness | LT | Water conservation | µm | 157.31–899.75 | 446.11 | 217.42 | 48.74 |
| Upper epidermal thickness | UET | Water conservation | µm | 19.26–70.93 | 38.32 | 11.24 | 29.32 |
| Upper cuticle thickness | UCT | Water conservation | µm | 2.36–18.10 | 7.26 | 3.31 | 45.64 |
| Lower epidermal thickness | LET | Water conservation | µm | 9.55–56.69 | 24.44 | 9.93 | 40.65 |
| Lower cuticle thickness | LCT | Water conservation | µm | 1.01–12.75 | 5.92 | 3.25 | 54.89 |
| Stomatal density | SD | Water loss | No. per mm<sup>2</sup> | 36.72–108.16 | 67.44 | 22.22 | 32.95 |
| Stomatal area | SA | Water loss | µm<sup>2</sup> | 261.9–1160.0 | 623.39 | 194.38 | 31.18 |
| Layer of velamen | LV | Water conservation | No. | 3–10 | 5.76 | 2.05 | 35.53 |
| Velamen thickness in cross section | VT | Water conservation | µm<sup>2</sup> | 87.22–589.31 | 305.32 | 140.96 | 46.17 |
| Root radius in cross section | r | Water absorbability | µm | 413.02–1550.99 | 843.18 | 280.79 | 33.30 |
| Velamen thickness/radius | VT/r | Water conservation | % | 18.59–46.96 | 34.83 | 7.06 | 20.26 |
| Velamen area in cross section | A<sub>vel</sub> | Water conservation and storage | µm<sup>2</sup> | 0.23–4.65 | 1.49 | 1.12 | 75.09 |
| Unit velamen cell length | vcl | Water storage | µm | 20.45–72.71 | 43.14 | 13.92 | 32.28 |
| Unit velamen cell width | vcw | Water storage | µm | 13.46–45.59 | 27.67 | 7.67 | 27.73 |
| Velamen cell length/width | vcl/vcw | Water storage | µm | 0.85–2.49 | 1.58 | 0.41 | 25.86 |
| Area of velamen cell | A<sub>vel</sub> | Water storage | µm<sup>2</sup> | 769.3–4472.1 | 1849.88 | 984.59 | 53.22 |
| Number of exodermis cell | N<sub>exd</sub> | Water transport | No. | 70–196 | 115.76 | 31.28 | 27.02 |
| Number of exodermis passage cell | N<sub>exp</sub> | Water transport | No. | 1–13 | 6.85 | 3.05 | 44.49 |
| Ratio of passage cell to exodermis cell | expc% | Water transport | % | 1.28–11.25 | 6.31 | 2.92 | 46.27 |
| Number of endodermis cell | N<sub>end</sub> | Water transport | No. | 32–100 | 54.95 | 17.94 | 32.65 |
| Number of endodermis passage cell | N<sub>expd</sub> | Water transport | No. | 3.33–14 | 8.8 | 2.77 | 31.52 |
| Ratio of passage cell to endodermis cell | enpd% | Water transport | % | 4.76–20.00 | 16.6 | 3.96 | 23.88 |
| Number of vessel | N<sub>ves</sub> | Water transport | No. | 7–20 | 11.71 | 3.95 | 33.73 |
| Diameter of vessel | D<sub>ves</sub> | Water transport | µm | 13.74–65.43 | 27.62 | 10.61 | 38.40 |
| Area of vessel in cross section | A<sub>ves</sub> | Water transport | µm<sup>2</sup> | 152.12–2782.72 | 614.09 | 538.15 | 87.63 |
| Root cortex thickness | RCT | Water storage | µm | 157.25–624.20 | 291.48 | 104.54 | 35.87 |
| Root cortex thickness/radius | RCT/r | Water storage | % | 21.53–44.26 | 35.10 | 6.09 | 17.35 |
| Radius of vascular cylinder | R<sub>r</sub> | Water transport | µm | 121.37–462.87 | 246.96 | 85.52 | 34.63 |
| Radius of vascular cylinder/radius | R<sub>r</sub>/r | Water transport | % | 21.22–41.04 | 29.61 | 4.98 | 16.83 |
| Elevation | EL | m | 700–2500 | 900 | 297 | 33.74 |
positively correlated with the number of exodermis cells (N_exo, \( r = 0.69 \)) and endodermis cells (N_en, \( r = 0.67 \)), and the number of vessels (N_vel, \( r = 0.62 \)). Meanwhile, VT was positively correlated with root cortex thickness (RCT) and radius of vascular cylinder (R_M, \( r = 0.78 \) and 0.83, respectively), but negatively correlated with R_CT/@r (\( r = -0.52 \)). The N_vel was not only positively correlated with the variables associated with velamen including LV, VT and A_vel (\( r = 0.59 \), 0.62 and 0.67, respectively), but also positively correlated with N_exo, N_en and N_vel, (\( r = 0.87 \), 0.98 and 0.48, respectively).

Several leaf and root traits were positively correlated (Table 2). N_lam was positively correlated with LV (\( r = 0.47 \)), VT (\( r = 0.50 \)), the ratio of velamen thickness to radius (VT/R_M, \( r = 0.58 \)), A_vel (\( r = 0.45 \)), N_vel (\( r = 0.48 \)) and N_en (\( r = 0.45 \)), but negatively correlated with the ratio of root cortex thickness to root radius (RCT/R_CT/@r = -0.53; Fig. 1). Leaf water content (WC) was negatively correlated with A_vel, D_vel and VT (\( r = -0.62 \), -0.54 and -0.46, respectively; Fig. 1). Leaf area (LA) was positively correlated with VT/R_CT/@r (\( r = 0.47 \)) and negatively correlated with R_CT/@r (\( r = -0.55 \)). Leaf density (LD) was positively correlated with N_vel and N_en (\( r = 0.45 \) and 0.49, respectively). There were also positive correlations between LCT and VT (\( r = 0.53 \)), VT/R_CT/@r (\( r = 0.46 \), A_vel (\( r = 0.53 \)) and root radius (\( r = 0.51 \)).

Interestingly, LMA was positively correlated with root traits related to water absorbability (root radius, \( r = 0.57 \)), water storage (A_vel, A_en, and R_CT/@r = 0.58, 0.50 and 0.47, respectively), water transport (N_vel, N_exo, N_en, R_CT/@r = 0.54, 0.54 and 0.51, respectively) and water conservation (LV, VT, VT/R_CT/@r = 0.51, 0.59 and 0.48, respectively; Fig. 2).

### Influence of phylogeny and elevation on leaf and root traits in *Dendrobium*

To test whether variations observed in leaf and root traits were shaped by phylogeny or environmental factors, we tested traits in 21 *Dendrobium* species for phylogenetic signals using the K-statistic (Table 3). Almost all the traits showed a weak phylogenetic signal, except UET. This finding indicated that the effect of ecological variation on these traits overshadowed evolutionary constraints, especially LD (K = 0.721, \( P = 0.004 \)), LV (K = 0.805, \( P = 0.035 \)) and R_CT/@r (K = 0.606, \( P = 0.041 \)).

We found that leaf traits such as LD and SD, root traits such as N_vel and N_en were positively correlated with elevation (Fig 3A). In Yunnan Province, increase in elevation is accompanied by decreasing temperature, relative humidity and precipitation (Fig 3B). These findings indicated that leaf and root traits in *Dendrobium* were affected by temperature and moisture level.

The analysis based on the t-distributed stochastic neighbourhood embedding (t-SNE) showed the clustering results of the species and traits among *Dendrobium* (Fig. 4). The species were separated by the zero axis vertical to t-SNE 1. One group of species was those with thick roots and the other was those with thin roots (Fig. 4A). The leaf and root traits were gathered into four parts with different functions, and both leaf and root traits were included in each part (Fig. 4B). We also used the PCA and MDS to compare the leaf and root traits among *Dendrobium* species [see Supporting Information—Fig. S2], and obtained results consistent with the t-SNE. This indicated that the functional traits tended to coupling between leaves and roots.

### Discussion

**Coordinated evolution of leaf and root traits within *Dendrobium***

Our study suggests that leaf and root traits in *Dendrobium* have evolved in coordination to cope with water stress, which is

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**Table 2. Pearson’s correlation coefficients among leaf and root traits across 21 *Dendrobium* species. Data were corrected by PCs. Significant correlations are showed in boldface. See Table 1 for definitions of abbreviations. Asterisks denote significant levels: ** \( P \leq 0.01 \), * \( P \leq 0.05 \).**

| Variables | LV | VT | D_vel | N_vel | N_en | N_exo | D_exo | A_vel | M_exo | M_en | WC | LA | A_lam | A_en | A_Lam | R_CT/@r | R_CT | R_M |
|-----------|----|----|-------|-------|------|-------|-------|-------|-------|------|-----|----|-------|------|-------|--------|------|-----|
| M_exo     | 0.21 | 0.11 | 0.02  | 0.24  | 0.07 | 0.52  | 0.35  | 0.10  | 0.31  | 0.56 | 0.47| 0.32| 0.16  | 0.16 | 0.25  | 0.14  | 0.20 | 0.47|
| M_en      | 0.47 | 0.50 | 0.38  | 0.58  | 0.45 | 0.34  | 0.28  | 0.26  | 0.30  | 0.43 | 0.31| 0.44| 0.32  | 0.38 | 0.41  | 0.32  | 0.38 | 0.48|
| WC        | 0.45 | 0.31 | 0.38  | 0.29  | 0.40 | 0.49  | 0.48  | 0.41  | 0.38  | 0.34 | 0.33| 0.40| 0.46  | 0.43 | 0.47  | 0.41  | 0.46 | 0.47|
| LA        | 0.18 | 0.31 | 0.38  | 0.30  | 0.39 | 0.41  | 0.38  | 0.38  | 0.38  | 0.39 | 0.39| 0.42| 0.40  | 0.42 | 0.43  | 0.42  | 0.43 | 0.47|
consistent with our hypothesis. Roots are the major organ for absorbing water and nutrients (Pregitzer et al. 2002; Guo et al. 2008; Liese et al. 2017). Most researches have focused on ‘fine roots’, which are defined as those <2 mm in diameter (Mommer and Weemstra 2012; Kong et al. 2014). For absorptive roots, the radius is a key trait because thicker roots have greater dependence on mycorrhizal fungi and may lead to a different absorptive strategy compared to thinner roots (Guo et al. 2008; Kong et al. 2015; Ma et al. 2018). In our study, the radius of the thickest root (1551 µm) was nearly 4-fold greater than the thinnest root (413 µm). Even the thinnest root exceeds the standard for thick roots (diameter > 470 µm) in a previous study (Kong et al. 2014). This indicates that the root traits of Dendrobium in this study may be different to the thin root traits of other plants. Meanwhile, root radius had significant positive relationships with velamen thickness, root cortex thickness and radius of vascular cylinder (Fig. 5A). This finding indicates that the variation in root radius may arise from the combined thickening of the velamen, cortex and vascular cylinder. We also found that the ratio of velamen thickness to root radius (VT/r) was positively correlated with root radius, but there were no relationships between root radius and the ratio of root cortex thickness to root radius (RCT/r), and the ratio of radius of vascular cylinder to root radius (R vc/r) (Fig. 5B). This suggests that the thicker roots of Dendrobium may be caused by

Figure 1. (A) Leaf water content was negatively correlated with root traits: cross-section area of vessel, diameter of vessel and velamen thickness (white circle); (B) leaf dry mass was positively correlated with root traits: velamen thickness and number of vessel, but negatively with the ratio of root cortex thickness to radius (black circle). Significance levels are expressed as follows: *P ≤ 0.05; **P ≤ 0.01. Data were corrected by PICs.
a higher proportion of velamen. Thus, velamen thickness was a proxy for the root radius. This result conflicts with some previous researches on tree species that find a negative relationship between root density and root diameter, because the lower density of thick roots is caused by a larger proportion of root cortex (Chen et al. 2013; Kong et al. 2015). This may be because the roots of Dendrobium plants are exposed to the atmosphere. Velamen plays a crucial role in arboreal habitats (Joca et al. 2017). Although thicker velamen (due to greater numbers of cell layers) incurs greater construction costs (Enquist et al. 1999), it confers greater resistance to water loss and mechanical damage (Zotz and Hietz 2001). Thus, velamen is an important

Figure 2. (A) Correlations between leaf mass per area (LMA) and root traits related to water transport (black circle), (B) water absorption and conservation (white circle), (C) water storage (grey circle). Significance levels are expressed as follows: *$P \leq 0.05$; **$P \leq 0.01$. Data were corrected by PICs.
regulator to enhance the adaptability of Dendrobium plants to the environment.

Species with thicker root velamen had a higher leaf mass per area (LMA) and thicker leaf lower cuticle thickness (LCT). Meanwhile, in the species with higher leaf water content, the velamen thickness and area tended to be thinner in roots. Leaf dry mass is commonly used to measure the leaf strength and durability (Portillo-Estrada et al. 2015). The leaves with higher dry mass always have thicker laminas and higher tissue density because of their greater concentration of fibres and cell walls (Shipley and Vu 2002). Leaf mass per area (LMA) is used as an indicator of water and nutrient retention in plants (Witkowski and Lamont 1991), and a higher LMA represents a more conservative strategy. Greater LMA also brings a greater cost to the plant (Westoby et al. 2002). The average LMA is well known to be higher in low rainfall environments, owing to thicker leaves, denser tissue or both (Cunningham et al. 1999; Niinemets 2001). Likewise, leaf lower cuticle thickness is also related to water conservation (Zhang et al. 2012; Sun et al. 2014). All of the leaf traits mentioned above were correlated with root traits. This greatly supported our hypothesis that leaf and root traits were coordinated in terms of water conservation.

Leaf area was positively correlated with the ratio of velamen thickness to root radius (VT/r), but negatively correlated with the ratio of root cortex thickness to root radius (RCT/r). Leaf surfaces are the primary border of energy and mass exchange. Some important processes such as evapotranspiration and photosynthesis are directly proportional to leaf area (Agrawal et al. 2009). Previous studies have shown that lower leaf area helps plants prevent water loss in xeric conditions (Qin et al. 2019). The roots with larger proportions of velamen have a higher capacity for water conservation, but a larger leaf area means greater water loss. This may be because species with a conservative water use strategy tends to generate a larger total leaf area to offset the costs of construction in water conservation tissue (Reich et al. 1992; Westoby et al. 2002).

The results of the t-SNE showed that leaf and root traits were gathered, and not separated by functional category (Fig. 4B). This result is consistent with PCA and MDS. This provided further evidence that leaf and root traits coordinate to improve water utilization. Improvement of water utilization depends on the coupling of functional trait categories, supporting the idea of a whole-plant-based strategy. We also found the species were separated into two axes (Fig. 4A). This suggests that root radius may have an effect in driving leaf and root trait spectra, which is consistent with the findings in a previous study (Kong et al. 2015).

### The environment drives variation in water conservation traits within Dendrobium

Most leaf and root traits, especially the number of velamen layers, leaf density and the ratio of vascular cylinder radius to root radius, varied in response to the environments. This variation helps Dendrobium plants adapt to water stress. Two pieces of evidence support this finding: the patterns of leaf and root trait variations were consistent with the responses to environmental conditions in the arboreal habitats of Dendrobium (as discussed above), and leaf and root traits were correlated with elevational distribution.

No strong phylogenetic signal was detected in all leaf and root traits. This indicated that the effect of ecological factors on these traits overshadowed evolutionary constraints. Leaf density, layer of velamen and the ratio of vascular cylinder radius to root radius showed high adaptability to the environments (Table 3). This suggested that the environment, not phylogeny, was the main driver of leaf and root traits variation in Dendrobium. Leaf density responds generally to the changes in moisture (Xu and Zhou 2008). High leaf density can help plants cope with water stress (Witkowski and Lamont 1991). The increase in velamen layer numbers confers greater resistance to water loss and mechanical damage (Zotz and Hietz 2001). The vascular cylinder is responsible for the transport of water and nutrients to the shoot (Mellor et al. 2016). Ribeiro et al. (2019) reported that the increase in vascular cylinder diameter of Glycine max seedlings alleviates the effect aroused by water deficits. All these traits showed a strong relationship with environmental factors, and indicated that Dendrobium have a great capacity to withstand drought stress. But somewhat contradictory to our result, a study on leaf functional traits in Dendrobium found that phylogeny has a significant effect on leaf density and leaf upper cuticle thickness, although most traits measured also have weak signals (Sun et al. 2014). The discrepancy was probably caused by

### Table 3. Phylogenetic signals of leaf and root traits in 21 Dendrobium species. Significant correlations are shown in boldface. Asterisks denote significant levels: **P ≤ 0.01; *P ≤ 0.05, respectively.

| Trait | Phylogenetic signal | K    | P    |
|-------|---------------------|------|------|
| Leaf fresh mass (M_Lm) | 0.425 | 0.664 |
| Leaf dry mass (M_Lm)  | 0.439 | 0.416 |
| Water content (WC)   | 0.584 | 0.082 |
| Leaf area (LA)       | 0.487 | 0.230 |
| Leaf mass per area (LMA) | 0.402 | 0.723 |
| Leaf density (LD)    | 0.721 | 0.004** |
| Vein density (VD)    | 0.385 | 0.742 |
| Leaf thickness (LT)  | 0.366 | 0.854 |
| Upper epidermal thickness (UET) | 1.223 | 0.253 |
| Upper cuticle thickness (UCT) | 0.64  | 0.749 |
| Lower epidermal thickness (LET) | 0.861 | 0.531 |
| Lower cuticle thickness (LCT) | 0.554 | 0.803 |
| Stomatal density (SD) | 0.723 | 0.475 |
| Stomatal area (SA)   | 0.92  | 0.363 |
| Layer of velamen (LV) | 0.805 | 0.035* |
| Velamen thickness (VT) | 0.48  | 0.558 |
| Root radius (r)      | 0.392 | 0.889 |
| Velamen thickness/radius (VT/r) | 0.639 | 0.116 |
| Velamen area in cross section (A_v) | 0.433 | 0.755 |
| Unit velamen cell length (vcl) | 0.6  | 0.361 |
| Unit velamen cell width (vcw) | 0.594 | 0.046 |
| Velamen cell length/width (vcl/vcw) | 0.579 | 0.343 |
| Area of velamen cell (A_v) | 0.382 | 0.828 |
| Number of exodermis cell (N_ex) | 0.446 | 0.605 |
| Number of exodermis passage cell (N_exop) | 0.361 | 0.831 |
| Ratio of passage cell to exodermis cell (exopc%) | 0.367 | 0.809 |
| Number of endodermis cell (N_en) | 0.547 | 0.494 |
| Number of endodermis passage cell (N_enop) | 0.37  | 0.802 |
| Passage cell/endodermis cell (enpc%) | 0.404 | 0.769 |
| Number of vessel (N_ves) | 0.454 | 0.843 |
| Diameter of vessel (D_ves) | 0.527 | 0.244 |
| Area of vessel in cross section (A_ves) | 0.56  | 0.145 |
| Root cortex thickness (RCT) | 0.338 | 0.913 |
| Root cortex thickness/radius (RCT/r) | 0.641 | 0.152 |
| Radius of vascular cylinder (R_ves) | 0.533 | 0.414 |
| Vascular cylinder radius/radius (R_/r) | 0.606 | 0.041* |
different materials, a wider diversity of species and cultivation conditions than in our study.

We found that elevational distribution was positively correlated with root traits such as the number of endodermis cell and vessel, and with leaf traits such as leaf density and stomatal density. All these traits are related to water use efficiency. The endodermis not only separates the vascular cylinder and provides a diffusion barrier (Roppolo et al. 2011), but also functions as a protective layer during drought (Ranathunge et al. 2003). When plants are deprived of water, the endodermis resists water movement from the stele to the outside, allowing internal layers to survive (Stasovski and Peterson 2011). A previous study has shown that water transport efficiency is promoted by increased number of vessels with a larger diameter (Dickison 2000). In contrast, drought can lead to a higher proportion of narrower (less efficient) vessels and decreased vessel numbers (Durante et al. 2011; Jupa et al. 2015). Moreover, some studies have shown that a water deficit leads to an increase in stomatal

Figure 3. Elevation is positively correlated with (A) root traits (black circle): number of vessel and endodermis cell, and leaf traits (white circle): leaf density and stomatal density. Significance levels are expressed as follows: * $P \leq 0.05$; ** $P \leq 0.01$. Data were corrected by PICs. (B) Variations of relative humidity, precipitation and temperature with elevation in Yunnan Province. Each scatterplot represents a meteorological station ($n = 119$).
density, which is positively correlated with water use efficiency (Martínez et al. 2007; Xu and Zhou 2008).

Taken together, the significant correlations between elevation with endodermis and vessels number, leaf density and stomatal density indicated that a higher elevation tended to select traits that increased water use efficiency in Dendrobium. In Yunnan Province, high elevation is often accompanied by lower temperature and humidity (Fig. 3B). The number of epiphytic orchid species decreases with increasing elevation (Zhang et al. 2015). This indicates that a low moisture level is an important factor limiting the distribution of epiphytic orchids in high-altitude areas. We speculated that the species with thicker velamen may be more adapted to higher elevations as the velamen has the function of retaining moisture and warmth in roots. Although endodermis and vessels number were positively correlated with velamen thickness, elevation was not correlated with velamen thickness. It would be helpful to investigate the role

Figure 4. t-SNE (t-distributed stochastic neighbourhood embedding) visualization to compare the species and traits of Dendrobium. (A) Species were separated along zero axis of t-SNE 1. Dot colour was used to denote the relative size of the species root radius, and darker colours were used to denote thicker roots. (B) Traits were gathered in several parts with different function and belonging to different organs. Each dot denotes a trait. Colours denote corresponding function. The circle and triangle represent leaf and root traits, respectively.
of temperature and moisture levels in measuring the capacity for plants to adapt to the potential changing environmental conditions in the future.

**Conclusions**

We proposed a model of interaction between leaf and root traits of *Dendrobium* which is an important epiphytic taxon. The majority of leaf and root traits were shaped by the environment rather than evolutionary constraints. To maintain water balance and improve water use efficiency, leaf and root traits showed close coordination in *Dendrobium*. The traits related to water uptake and conservation might play an important role in helping *Dendrobium* species to adapt to cold and dry conditions at high elevations. The results of this study confirmed the plant economic hypothesis, which states that plant populations adapt to the environment through coordinated leaf and root trait evolution. These findings improve our understanding of the interactive pattern of leaf and root traits in epiphytes.

**Supporting Information**

The following additional information is available in the online version of this article—

- **Figure S1.** Phylogenetic relationships and ecological information across 21 *Dendrobium* species.
- **Figure S2.** (A) Principal component analysis (PCA) and (B) multidimensional scaling (MDS) are used to compare leaf and root traits among 21 *Dendrobium* species.
- **Table S1.** Coefficients of Pearson’s correlations and phylogenetically independent contrast correlations among leaf traits, and between leaf traits and elevation across 21 *Dendrobium* species.
- **Table S2.** Coefficients of Pearson’s correlations and phylogenetically independent contrast correlations among root traits across 21 *Dendrobium* species.

![Figure 5. Correlations of root radius with velamen thickness, root cortex thickness and radius of vascular cylinder (A), and with the ratio of velamen thickness to radius, ratio of root cortex thickness to radius and ratio of vascular cylinder radius to root radius (B). Significance levels are expressed as follows: ***P ≤ 0.001; **P ≤ 0.01. Data were corrected by PICs.](image)
Data Availability
All data used in this study are available at https://osf.io/8dkur.

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Contributions by the Authors
All authors conceived and designed the experiments. Y.Q. performed the experiments, collected and analysed the data before wrote the first draft, J.H. collected and identified the species. S.Z. revised the manuscript and gave final approval for its publication.

Conflict of Interest
None declared.

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Compliance with Ethical Standards
This article does not contain any studies with human participants or animals performed by any of the authors.

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