

## Variations in seed micromorphology of *Paphiopedilum* and *Cypripedium* (Cypripedioideae, Orchidaceae)

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### Abstract

The micromorphology of a seed is linked to its dispersal and germination, but evolutionary and ecological aspects in Orchidaceae remain unclear. We investigated the seed characters of *Paphiopedilum* and *Cypripedium* that might be associated with life form and involved in possible ecological adaptations. A phylogenetic comparative analysis of nine seed micromorphological characters was performed in 24 species from two genera with close phylogenetic relationships but significant differences in their ecological characteristics. Species within *Paphiopedilum* had larger embryos and a smaller percentage of air space (AS) than those of *Cypripedium* species. Compared with 16 terrestrial species, two epiphytic *Paphiopedilum* species had larger embryos and smaller AS. Those larger embryos might ensure more successful seedling establishment while the higher amount of air space in both terrestrial *Paphiopedilum* and *Cypripedium* may increase seed buoyancy and enable them to disperse over longer distances. Whereas AS and seed length (SL) are phylogenetically conservative, most other characters examined here had weak signals, indicating clear convergent evolution. Across species, SL was positively correlated with AS, indicating a high degree of seed size–dispersal coordination. These findings may imply a trade-off of seed characters in relation to the possible ecological adaptations required for seedling establishment versus dispersal.

**Keywords:** seed air spaces, seed dispersal, terrestrial versus epiphytic habits

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### Introduction

Subfamily Cypripedioideae (Orchidaceae; the slipper orchids) consists of five genera: *Selenipedium*, *Phragmipedium*, *Mexipedium*, *Paphiopedilum* and *Cypripedium* (Cox *et al.*, 1997). China has the greatest species diversity in *Paphiopedilum* and *Cypripedium* (Cribb, 1997, 1998). The plants are adapted to a wide variety of habitats, ranging from high elevations in the Himalayas to lowland tropical regions (Guo *et al.*, 2012). Species and hybrids in this subfamily are very popular in floriculture because of their large, peculiar and beautiful flowers, with a pouch-like lip, two fertile stamens, a shield-like staminode and a synsepal composed of fused lateral sepals (Bream, 1988; Cribb, 1997, 1998; Averyanov *et al.*, 2003; Koopowitz *et al.*, 2008; Liu *et al.*, 2009; Lan and Albert, 2011). The 79 species of *Paphiopedilum* are distributed mainly in tropical and subtropical forests from Asia to islands of the Pacific, with 27 of them occurring in south-western China, usually on karst limestone hills (Cribb, 1998; Chen *et al.*, 2005; Liu *et al.*, 2009). Plants of this genus display three contrasting life forms: terrestrial, facultative epiphytic and obligatory epiphytic (Liu *et al.*, 2009). By comparison, *Cypripedium* contains 51 species that are widely distributed in temperate and subtropical regions (Chen *et al.*, 2013). The 37 species in China mainly grow at elevations above 1800 m in the south-western region (Cribb, 1997; Chen *et al.*, 2013). All *Cypripedium* species are terrestrial (Chen *et al.*, 2013). Although both genera are closely related (Cox *et al.*, 1997), the two exhibit significant differences in distribution, developmental habitat, leaf morphology, physiology and life history strategies (Liu *et al.*, 2009; Guo *et al.*, 2012; Zhang *et al.*, 2012) thereby making them an ideal system for studying possible ecological adaptations by plants. Their previous taxonomic placement was generally based on floral characters (Liu *et al.*, 2009). However, the seed micromorphological traits of Cypripedioideae have received little scientific attention (Arditti *et al.*, 1979) and the

ecological significance of those characters remains unclear.

Orchid seeds are extremely small and numerous, and some species produce more than 1 million seeds per capsule (Arditti *et al.*, 1979). They comprise a frequently sculpted seed coat for protection as well as a simple embryo, which can vary significantly in volume but usually lacks an endosperm, the key source of nutrients to support germination (Dressler, 1993; Clements, 1999; Molvray and Chase, 1999; Swamy *et al.*, 2004). Seeds are usually dispersed by wind, but patterns can vary significantly across genera and species due to morphological characters, size, shape, colour and structure (Molvray and Kores, 1995; Aybeke, 2007).

Variations in seed morphology serve as an important source of systematic characters for establishing relationships among species within a genus (Mathews and Levins, 1986; Ness, 1989; Larry, 1995). For example, Swamy *et al.* (2004) have used scanning electron microscopy to examine the morphologies of ten epiphytic orchid species and have found that the percentage of air space (AS) in seeds differs significantly among species. Chaudhary *et al.* (2014) have investigated the morphological characteristics of ten *Dendrobium* species using 13 quantitative trait descriptors, and have reported that, regardless of their phylogenetic associations, species from temperate regions have larger seed volumes and higher ratios of seed volume to embryo volume when compared with species from subtropical or tropical regions. Those researchers have suggested that the primary habitats for more phylogenetically related species are a function of climatic region as their prime habitat. However, the seed morphology in *Epipactis* is not correlated with biogeography (Arditti *et al.*, 1982) and Arditti *et al.* (1979) have proposed that the variations in micromorphology among several *Cypripedium* species could indicate taxonomic relationships. Hence, seed traits such as shape, colour, length and width are perhaps not only useful in taxonomy but might also be correlated with phylogenetic relationships (Clifford and Smith, 1969; Barthlott, 1976; Healey *et al.*, 1980). Nevertheless, those aspects have not previously been fully investigated.

Due to their horticultural significance, members within the Orchidaceae family have been over-collected, and their habitats have been lost or destroyed. All species are now listed in the Convention on International Trade in Endangered Species. In general, their dispersal mode and seed size are important factors that govern the establishment of new populations (Tsutsumi *et al.*, 2007). However, little is known about how certain seed traits might be correlated with possible ecological adaptations. Therefore, we investigated the micromorphological characters of 18 *Paphiopedilum* species and six *Cypripedium* species. We hypothesized that seed characters in these

two genera, such as internal air space and embryo volume, implicated as functional traits for the dispersal of dust seeds, differed between *Paphiopedilum* and *Cypripedium* and between life forms within these genera.

## Materials and methods

### Plant material

Wild plants of 18 *Paphiopedilum* and six *Cypripedium* species were collected from their natural environments (Table 1), then cultivated in a greenhouse at Kunming Institute of Botany, Chinese Academy of Sciences (102°41'E, 25°01'N) and pollinated manually during their normal period of flowering. Mature capsules were harvested from these greenhouse-grown plants for conducting our seed measurements. Because most *Paphiopedilum* species are endangered, it is difficult to collect seeds from their native habitats. Moreover, obtaining all seeds from a single location can reduce the effect of environmental heterogeneity on their development.

### Observations of seed morphology

Seeds were stained with safranin before being spread on a slide with a drop of water and protected with a cover slip. Samples were observed and photographed under a light microscope (Olympus-SZX 16, Olympus, Japan). Our morphological parameters included seed length (SL), width (SW), length/width (SL/SW) and volume (SV); embryo length (EL), width (EW), length/width (EL/EW) and volume (EV); and the percentage of air space (AS). All data were analysed with DP2-BSW software (Olympus). Values for SL, SW, EL and EW were recorded from approximately 20 seeds per species. Seed volume was calculated as  $SV = 2 \times (\text{half the width of the seed})^2 \times (\text{half the length of the seed}) \times \pi/3$ . Embryo volume was calculated as  $EV = 4/3\pi \times (\text{half the length of the embryo}) \times (\text{half the width of the embryo})^2$  (Arditti and Ghani, 2000). The amount of air space contained by the seed coat was calculated as  $AS = (\text{seed volume} - \text{embryo volume}) / (\text{seed volume}) \times 100\%$  (Arditti and Ghani, 2000).

### Statistical analysis

Phylogenetic relationships among species of *Paphiopedilum* and *Cypripedium* were determined according to the methods described by Cox *et al.* (1997) and Li *et al.* (2011), respectively. The phylogenetic signal (*K* statistic) for each trait was calculated using 'picante' based on the R package. Such *K* statistics evaluate the strength of

**Table 1.** Micromorphometric data for seeds and embryo-related characters (mean ± SE) from 18 *Paphiopedilum* species and six *Cypripedium* species

Species	n	Life form	Elevation (m)	Seed				Embryo				
				Length (µm)	Width (µm)	Length/width	Volume (× 10 <sup>-3</sup> mm <sup>3</sup> )	Length (µm)	Width (µm)	Length/width	Volume (× 10 <sup>-3</sup> mm <sup>3</sup> )	Air space (%)
<i>P. appletonanum</i>	20	Facultative	300–1200	1484.6 ± 35.8	183.8 ± 3.9	8.1 ± 0.2	13.4 ± 0.7	264.9 ± 8.3	123.3 ± 3.0	2.2 ± 0.1	2.2 ± 0.1	83.4 ± 1.1
<i>P. areeanum</i>	19	Terrestrial	500–2000	567.3 ± 15.0	189.4 ± 4.0	3.0 ± 0.1	5.3 ± 0.2	295.3 ± 7.2	151.2 ± 3.9	2.0 ± 0.1	3.6 ± 0.2	32.6 ± 2.9
<i>P. armeniacum</i>	20	Facultative	1400–2250	531.3 ± 16.5	185.2 ± 4.3	2.9 ± 0.1	4.8 ± 0.2	247.8 ± 9.4	123.2 ± 3.9	2.0 ± 0.1	2.0 ± 0.2	56.8 ± 3.1
<i>P. barbigerum</i>	20	Facultative	800–1000	492.2 ± 12.9	181.6 ± 5.2	2.8 ± 0.1	4.3 ± 0.2	247.6 ± 7.9	145.3 ± 4.8	1.7 ± 0.1	2.9 ± 0.2	33.8 ± 3.8
<i>P. bellatulum</i>	20	Terrestrial	1000–1800	758.3 ± 11.2	185.7 ± 4.5	4.1 ± 0.1	6.9 ± 0.4	279.2 ± 3.7	133.6 ± 2.9	2.1 ± 0.1	2.6 ± 0.1	61.1 ± 1.8
<i>P. concolor</i>	20	Facultative	300–1400	637.9 ± 17.9	195.0 ± 5.4	3.3 ± 0.2	6.4 ± 0.4	231.0 ± 6.2	144.9 ± 4.4	1.6 ± 0.1	2.6 ± 0.2	57.9 ± 3.1
<i>P. dianthum</i>	20	Epiphytic	550–2250	586.1 ± 14.0	199.2 ± 4.6	3.0 ± 0.1	6.1 ± 0.2	271.3 ± 6.2	153.0 ± 3.2	1.8 ± 0.1	3.4 ± 0.8	43.9 ± 2.9
<i>P. emersonii</i>	20	Facultative	600–800	651.7 ± 22.1	198.9 ± 3.3	3.3 ± 0.1	6.8 ± 0.3	218.8 ± 4.2	116.3 ± 3.3	1.9 ± 0.1	1.6 ± 0.1	76.3 ± 1.5
<i>P. gratrixianum</i>	20	Facultative	1800–1900	754.0 ± 25.0	156.3 ± 4.2	4.9 ± 0.2	5.0 ± 0.4	312.7 ± 9.0	122.7 ± 3.4	2.6 ± 0.1	2.5 ± 0.1	48.1 ± 2.0
<i>P. henryanum</i>	20	Facultative	900–1300	563.3 ± 13.1	194.0 ± 5.5	3.0 ± 0.1	5.6 ± 0.3	265.8 ± 7.4	153.8 ± 4.9	1.8 ± 0.1	3.4 ± 0.2	39.8 ± 2.9
<i>P. hirsutissimum</i>	21	Facultative	300–1500	717.8 ± 15.9	169.4 ± 4.3	4.3 ± 0.1	5.5 ± 0.3	274.4 ± 6.8	131.8 ± 3.0	2.1 ± 0.1	2.5 ± 0.2	52.6 ± 2.3
<i>P. insigne</i>	18	Terrestrial	1200–1600	565.4 ± 22.6	189.5 ± 6.1	3.1 ± 0.2	5.3 ± 0.4	293.4 ± 11.4	153.2 ± 5.2	2.0 ± 0.1	3.6 ± 0.2	31.1 ± 3.1
<i>P. malipoense</i>	20	Terrestrial	800–1000	990.7 ± 43.6	160.6 ± 2.9	6.2 ± 0.3	6.8 ± 0.4	249.4 ± 10.5	117.5 ± 3.7	2.1 ± 0.1	1.9 ± 0.2	71.7 ± 2.1
<i>P. micranthum</i>	20	Terrestrial	400–1700	901.7 ± 21.7	164.4 ± 4.9	5.6 ± 0.2	6.6 ± 0.5	226.0 ± 7.3	114.9 ± 3.3	2.0 ± 0.1	1.6 ± 0.1	74.9 ± 1.4
<i>P. purpuratum</i>	20	Terrestrial	1200–1500	1530.9 ± 59.2	167.6 ± 4.6	9.2 ± 0.3	11.7 ± 1.0	218.6 ± 8.7	94.6 ± 3.3	2.4 ± 0.1	1.1 ± 0.1	90.5 ± 0.8
<i>P. venustum</i>	21	Terrestrial	1100–1600	1066.4 ± 26.8	168.6 ± 2.9	6.3 ± 0.1	8.0 ± 0.4	232.1 ± 5.8	115.6 ± 3.1	2.0 ± 0.1	1.7 ± 0.1	78.3 ± 2.3
<i>P. villosum</i>	20	Epiphytic	1800–2200	753.0 ± 20.1	187.1 ± 5.1	4.1 ± 0.2	7.0 ± 0.4	289.5 ± 11.0	157.6 ± 3.8	1.9 ± 0.1	3.8 ± 0.2	44.4 ± 2.6
<i>P. wardii</i>	20	Terrestrial	1200–2500	1397.7 ± 42.4	182.9 ± 5.1	7.8 ± 0.3	12.3 ± 0.7	233.1 ± 6.2	100.3 ± 2.0	2.3 ± 0.1	1.2 ± 0.3	89.5 ± 0.7
<i>C. farrei</i>	20	Terrestrial	2000–3400	2031.0 ± 36.7	205.1 ± 6.1	10.1 ± 0.4	22.7 ± 1.4	230.6 ± 4.2	107.7 ± 3.2	2.2 ± 0.1	1.4 ± 0.1	93.5 ± 0.4
<i>C. flavum</i>	23	Terrestrial	1800–3500	1304.8 ± 26.2	363.1 ± 10.5	3.7 ± 0.2	45.7 ± 2.7	232.6 ± 5.3	126.0 ± 4.2	1.9 ± 0.1	2.0 ± 0.2	95.5 ± 0.3
<i>C. guttatum</i>	20	Terrestrial	500–4000	961.4 ± 17.4	231.8 ± 7.0	4.2 ± 0.1	13.8 ± 0.9	203.1 ± 3.3	109.2 ± 2.1	1.9 ± 0.1	1.3 ± 0.1	90.2 ± 0.5
<i>C. plectrochilum</i>	20	Terrestrial	1000–3600	843.6 ± 11.7	209.4 ± 4.9	4.1 ± 0.1	9.8 ± 0.5	265.9 ± 4.9	158.3 ± 4.3	1.7 ± 0.0	3.6 ± 0.3	63.4 ± 1.7
<i>C. tibeticum</i>	20	Terrestrial	2300–4200	1301.4 ± 27.4	322.0 ± 7.7	4.1 ± 0.2	35.5 ± 1.6	207.8 ± 7.1	120.7 ± 4.4	1.7 ± 0.1	1.7 ± 0.1	95.2 ± 0.4
<i>C. yunnanense</i>	20	Terrestrial	2700–3800	857.9 ± 24.7	276.5 ± 8.0	3.2 ± 0.1	17.4 ± 1.1	196.5 ± 5.2	112.5 ± 3.7	1.8 ± 0.1	1.4 ± 0.1	92.1 ± 0.5

**Table 2.** Micromorphometric data for seeds and embryo-related characters of *Paphiopedilum* and *Cypripedium*

Character	<i>Paphiopedilum</i>	<i>Cypripedium</i>	Z	P
	(n = 359) Mean ± SE	(n = 123) Mean ± SE		
Seed length (µm)	833.12 ± 18.28	1218.84 ± 38.11	-9.87	0.000
Seed width (µm)	180.92 ± 1.25	270.31 ± 6.24	-13.57	0.000
Seed length/seed width	4.73 ± 0.11	4.85 ± 0.22	-0.45	0.650
Seed volume (× 10 <sup>-3</sup> mm <sup>3</sup> )	7.10 ± 0.17	24.67 ± 1.33	-14.80	0.000
Embryo length (µm)	258.05 ± 2.33	223.00 ± 2.95	-7.83	0.000
Embryo width (µm)	130.49 ± 1.30	122.47 ± 2.16	-3.53	0.000
Embryo length/embryo width	2.02 ± 0.02	1.86 ± 0.03	-4.41	0.000
Embryo volume (× 10 <sup>-3</sup> mm <sup>3</sup> )	2.43 ± 0.06	1.89 ± 0.09	-5.58	0.000
Air space (%)	59.52 ± 1.15	88.48 ± 1.06	-13.36	0.000

the phylogenetic signal while the *P* value represents the quantile of the observed phylogenetically independent contrast (PIC) variance versus the null distribution. Traits with *P* values < 0.05 have non-random phylogenetic signals. Cases where the *K* value is < 1 indicate convergent traits, whereas *K* > 1 means that the characters are more conserved than would be presumed from a Brownian expectation (Blomberg *et al.*, 2003). Relationships among variables were examined using both pairwise Pearson and PIC correlations. All statistical analyses were performed with R software v. 2.15.0 (R Development Core Team, 2012). Differences in seed traits among life forms were determined by the *post-hoc* (LSD) test. All of those statistical analyses were performed using SPSS 16 (SPSS, Chicago, Illinois, USA), with measured variables presented as mean ± SE.

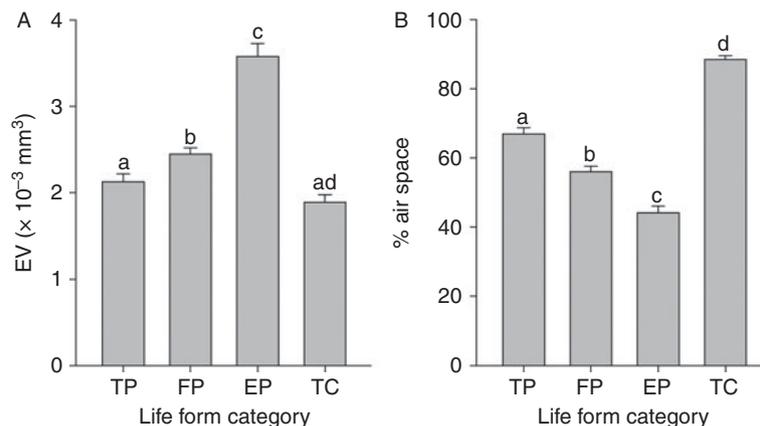
## Results

Nine morphological characters of orchid seeds and embryos were examined across all studied species

(Table 1). Values for SL, SW, SV and AS were significantly smaller for *Paphiopedilum* species than for *Cypripedium* species, while those for EL, EW and EV were significantly larger in *Paphiopedilum* species (Table 2). The ratio of SL to SW did not differ between the two genera (*Z* = -0.45, *P* = 0.650).

A statistical analysis showed that EV and AS differed significantly among life forms for *Paphiopedilum* species (Fig. 1). Seeds from epiphytic plants had larger embryos and smaller percentages of air space than did terrestrial plants, while values for EV in facultative species were larger than in terrestrial species but smaller than in epiphytic species. The smaller embryos in terrestrial plants resulted in a larger amount of air space for *Paphiopedilum* seeds. Although AS was significantly smaller for terrestrial *Paphiopedilum* species than for terrestrial *Cypripedium* species, embryo volumes did not differ between the two.

Across species, SL and AS showed strong phylogenetic conservatism whereas SW, SV, EL, EW and EV were significantly convergent for all examined species (Table 3). For example, SL was positively correlated



**Figure 1.** Differences in embryo volume (A) and percentage of air space (B) for 18 *Paphiopedilum* species and six *Cypripedium* species as function of life form. TP, Terrestrial species of *Paphiopedilum*, *n* = 158 plants; FP, facultative species of *Paphiopedilum*, *n* = 161; EP, epiphytic species of *Paphiopedilum*, *n* = 40; TC, terrestrial species of *Cypripedium*, *n* = 123.

**Table 3.** Phylogenetic signal (*K*) of seed characters in 18 *Paphiopedilum* species and six *Cypripedium* species

	<i>K</i>	<i>P</i>
Seed length	1.058	0.002
Seed width	0.507	0.007
Seed length/seed width	0.603	0.024
Seed volume	0.603	0.003
Embryo length	0.335	0.498
Embryo width	0.279	0.766
Embryo length/embryo width	0.283	0.740
Embryo volume	0.775	0.004
Air space	3.130	0.001

with AS. When phylogeny was not considered, EV was negatively correlated with AS (Table 4).

## Discussion

Seed micromorphological characters differed significantly among species sampled from *Paphiopedilum* and *Cypripedium* orchids. *Paphiopedilum* plants produce significantly smaller seeds with relatively larger embryos and smaller percentages of air space. The AS variable is an important parameter because the seeds of most orchids are wind dispersed, implying that seeds with higher AS float in the air for a longer time and, thus, can spread to more distant places (Swamy *et al.*, 2004, 2007). Traits for SL and AS varied significantly across species, with both characters exhibiting clear phylogenetic signals that were evidence of a high level of conservatism and a distinct evolutionary shift among species. However, most of the other characters examined here showed weak signals and were clearly convergent, possibly because of a departure from Brownian motion that might have occurred due to adaptive evolution that would not have been correlated with phylogeny. Few studies of

orchid seed traits have incorporated explicit phylogenetic methods (Chemisquy *et al.*, 2009). Therefore, further research using such comparative approaches should be performed with orchids.

The patterns of seed growth during maturation implied that elongation resulted from the lengthening of testa cells rather than an increase in overall cell numbers, thus improving buoyancy for wind dispersal (Arditti *et al.*, 1980). Whether or not phylogeny was considered, SL in *Paphiopedilum* and *Cypripedium* was positively correlated with AS, indicating that those two parameters have an evolutionary association. These findings support the belief that the development and function of SL and AS are coordinated and that both are important for optimizing dispersal, i.e. lighter weights and larger free air space increase the distance that can be travelled (Tsutsumi *et al.*, 2007; Chaudhary *et al.*, 2014). Our data showed that *Cypripedium* species from high elevations – at relatively low atmospheric pressure – produced longer seeds with larger AS values when compared with the *Paphiopedilum* species. Similar results were obtained when only the terrestrial life forms of each genus were analysed. Thus, we can conclude that the percentage of air space for seeds in subfamily Cypripedioideae (Orchidaceae) may be a function of elevation at which they are produced.

The larger embryos and smaller AS values for our epiphytic *Paphiopedilum* species versus terrestrial species are in agreement with a report by Tsutsumi *et al.* (2007), who used phylogeny and comparative methods to investigate the genus *Liparis* in Japan. That research group showed that epiphyte seeds with smaller air spaces had lower capacity for dispersal than seeds of terrestrial plants, where the percentage of air space was greater. Other data suggested that life form is also correlated with AS in *Paphiopedilum*. However, our study indicated that the terrestrial species *P. areeanum* and *P. insigne* did not fit closely within this overall habitat-connected classification; both had approximately 32% AS compared with

**Table 4.** Correlation of Pearson's regressions (upper right of the diagonal) and phylogenetic independent contrast correlations (lower left of the diagonal) among seed micromorphometric characters

	SL	SW	SL/SW	SV	EL	EW	EL/EW	EV	AS
SL		0.241	<b>0.875**</b>	0.605*	-0.418*	<b>-0.658**</b>	0.406	-0.620*	<b>0.789**</b>
SW	0.289		-0.250	<b>0.898**</b>	-0.444*	-0.060	0.406*	-0.620	0.789*
SL/SW	<b>0.913**</b>	0.643*		0.163	-0.214	-0.653*	0.638*	-0.555*	0.573*
SV	0.261	<b>0.821**</b>	0.129		-0.463*	-0.311	-0.122	-0.381	<b>0.671**</b>
EL	0.118	0.107	0.114	0.042		0.652*	0.261	<b>0.795**</b>	<b>-0.783**</b>
EW	0.392	0.307	0.480*	0.072	0.427*		-0.554*	<b>0.972**</b>	<b>-0.813**</b>
EL/EW	0.542*	0.429*	0.636*	0.049	0.293	<b>0.727**</b>		-0.358	0.172
EV	0.291	0.213	0.357	0.043	0.601*	<b>0.968**</b>	0.557*		<b>-0.861**</b>
AS	0.583*	0.088	0.453*	0.366	0.408	<b>0.753**</b>	0.4598	<b>0.758**</b>	

\* Correlation is significant at the 0.05 level (two-tailed).

\*\* Correlation is significant at the 0.01 level (two-tailed).

60–90% for other terrestrial species in that genus. The life form for these two species was quoted from previous taxonomic references, in which *P. areeanum* (*P. rhizomatosum*) was described as a new species based on a cultivated plant sampled from northern Myanmar (Chen and Liu, 2002) while *P. insigne* was recorded in *Flora of China* although it still lacks a collected specimen (Lang *et al.*, 1999). No observations were described for the habitats of those two species. Therefore, in the absence of such details, it is possible that they are not obligate terrestrial plants as stated earlier, but instead are facultative epiphytic or epiphytic species.

The large percentage of air space in Orchidaceae is thought to be beneficial to long-distance dispersal because of enhanced seed buoyancy (Arditti and Ghani, 2000; Augustine *et al.*, 2001; Tsutsumi *et al.*, 2007; Chaudhary *et al.*, 2014). The height at which seeds are released from a plant may also be a critical factor that results in greater dispersal distances despite those seeds having smaller/fewer air spaces (Murren and Ellison, 1998). Compared with epiphytic species, higher AS values in seeds of terrestrial species can help spread them further along the forest floor where wind speeds are lower.

Our morphometric comparisons in *Paphiopedilum* showed that embryo volumes differed significantly among life forms. That is, seeds of epiphytic species had larger embryos than those of terrestrial species, allowing the former type to germinate earlier (Chaudhary *et al.*, 2014). Chase and Pippen (1988) have found hooked testa extensions in some epiphytic orchids. However, we did not observe any special extensions on the seed coats of *Paphiopedilum* epiphytes. At the seedling development stage, adhesive organs, such as rhizoids or emerging roots, facilitate the growth of those seedlings on trees or stones. Consequently, larger embryos may play an important role in seedling establishment by epiphytic species (Chaudhary *et al.*, 2014) because they can store more nutrients that provide an advantage in poor environments. Moreover, those larger embryos mean that epiphytic *Paphiopedilum* species have higher establishment success even though greater seed dispersibility is sacrificed. Furthermore, having smaller AS values may not present a challenge for these epiphytes, because seed dispersal is better from greater heights. All of these characteristics probably reflect a trade-off between seedling establishment and dispersibility that comes from this divergence in embryo size and percentage of air space between epiphytic and terrestrial species.

In summary, both life form and phylogeny have significant effects on embryo volumes and the percentage of air space in *Paphiopedilum* and *Cypripedium*. To a certain extent, the importance of seed morphology in Cypripedioideae is associated with the

possible ecological adaptations required for seed dispersal or establishment of new populations. More air space means that seeds of terrestrial species can travel further and land where habitats are most suitable for germination. By contrast, larger embryos in epiphytic species can benefit seedling recruitment. We noted a correlated evolution between seed length and percentage of air space in the two genera that implies an evolutionary trade-off between seedling establishment and dispersal within the Cypripedioideae subfamily. Our findings provide new insights into the development of seed micromorphology characteristics related to those two factors for orchids growing under different ecological conditions, and are useful to the understanding of possible ecological significances for seed micromorphology in Cypripedioideae.

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### Conflicts of interest

None.

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