Seed Science Research

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Seed morphology and its relationships with phylogeny, lifeform and distribution of African orchid species

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Research Paper

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Abstract

Variation in orchid seed size and shape can be linked to phylogenetic relationships, habitat preferences, germination behaviour or dispersal strategies. To investigate this, we compared 45 orchid species from 29 genera collected across different localities in Cameroon using optical microscopy. We categorized each species according to lifeform (38 epiphytic vs. 7 terrestrial), altitudinal range (11 mountain vs. 34 lowland) and geographic distribution (28 widespread vs. 17 range-restricted). We analysed seed morphology using phylogenetic signal tests, analysis of variance and principal component analysis. Our results confirm a clear distinction between epiphytic and terrestrial species, with intermediate morphologies observed in genera encompassing species with both lifeforms (*Cynorkis, Graphorkis, Habenaria* and *Liparis*). Certain traits, such as seed length and seed air space, show a strong phylogenetic signal, suggesting that these traits are more linked to ancient evolutionary history than to recent ecological adaptation. Among the 38 epiphytic species, no consistent relations were found between seed traits and either geographic range or altitudinal distribution. Our findings suggest that the variation observed in seed morphology among African orchids is influenced more by phylogenetic relationships than by present-day distribution.

Introduction

Orchids are a highly diverse and ecologically significant group of plants with over 25 000 species distributed worldwide (Givnish et al., 2016). In tropical Africa, orchids are widespread, with many species found in the Central African region (Govaerts et al., 2025). This region is home to approximately 600 orchid species, with Cameroon (~450 species) being the country with higher species richness (Droissart et al., 2025). It is estimated that more than 30 new species have been discovered and described from this area in the last 20 years (e.g. WWF, 2024). At the same time, the large Angraecineae subtribe, which includes approximately 350 epiphytic species distributed in Central Africa, has been the focus of extensive sampling and phylogenetic studies in recent years (Simo-Droissart et al., 2018; Farminhão et al., 2021). Notwithstanding their numerical significance and the substantial threats confronting numerous species, the study of orchids in this region has been limited, and much remains to be discovered about their morphology, ecology, and evolution.

One important approach to study orchids is through the use of morphometric analysis, which involves the measurement and analysis of various morphological features of the plant. Morphometric analysis has been widely used in botanical research, particularly for understanding the systematics and evolution of plant species (e.g. Henderson, 2006; Cope et al., 2012; Simo-Droissart et al., 2016; Portillo et al., 2020). By analysing morphological traits such as leaf shape, flower size or seed morphology, researchers can better understand the relationships between different orchid species and the factors that have influenced their evolution (Shipunov and Bateman, 2005; Bateman and Rudall, 2006, 2023). Orchidaceae represent an excellent model family as they combine well-characterized lifeforms and floral variation with repeated evolutionary shifts that can be mapped onto modern phylogenies (Collobert et al., 2023).

Orchid seeds are generally described as 'dust-like', because they are small compared to most other Angiosperms (Arditti and Ghani, 2000). In reality, orchid seeds show extreme variations in size and shape, which could explain both phylogenetic relationships and ecological adaptations (Nakanishi, 2022; Alfaro Pinto et al., 2023; Collier et al., 2023; Lee and Yeung, 2023). For instance, the width and length of orchid seeds can respectively vary by a ratio of 90 to 120×

depending on the species (Arditti and Ghani, 2000). Some studies have highlighted that certain seed characters, such as testa ornamentation or seed size, are phylogenetically conserved and thus could provide valuable taxonomic characters to support species delimitation (Aytaş Akçin et al., 2010; Alomia et al., 2016; Rewicz et al., 2022). Other studies tend to show that the morphometric diversity of orchid seeds may be more related to different ecological adaptations and dispersal modes than to taxonomy, biogeographical origin or lifestyle. In fact, orchid seed characters may be related to seed dispersal (Diantina et al., 2020; Aprilianti et al., 2021), dormancy (Prasongsom et al., 2017) or germination (Oikonomidis and Thanos, 2024) and thus represent important traits for survival and reproduction in various environmental conditions. To date, despite the high diversity and ecological significance of orchids in Africa, relatively little is known about morphometric variations of orchid seeds in this region (but see Kurzweil, 1993; Barthlott et al., 2014; Gamarra et al., 2018).

Therefore, the present study aimed to fill this knowledge gap by conducting an extensive analysis of seed morphometry in selected African orchid genera and species occurring in Cameroon. By analysing a range of morphological features of seeds from multiple species and genera, we hope to highlight the importance of seed morphometry as a tool for understanding the evolution and ecology of orchids, particularly in the context of their distribution and adaptation to the diverse habitats found in Central Africa. The main question we want to address here is whether seed morphometry is linked to the current distribution of species, or whether it is more related to a phylogenetic determinism.

Material and methods

Cameroonian orchid seed bank

The orchid seeds used in this study were obtained from a seed bank developed since 2014 at the Plants Systematics and Ecology Laboratory of the Higher Teachers' Training College in Yaoundé, Cameroon. The seeds were obtained from mature fruits that were either collected in the field or from living plants cultivated in the Yaoundé orchid shade house (Stévart et al., 2020). The seeds obtained from mature fruits were dried using the same protocol: placed in a desiccator containing a lithium chloride solution for seven days, reducing their relative humidity to approximately 12% (Hosomi et al., 2012; Seaton et al., 2018). The seeds were then stored in labelled microtubes in a freezer (−20 °C). The orchid seed bank in Yaoundé may contain several collections of the same species, but for the present study, we only used one collection/sample per species, ensuring that the seeds in that collection had fully developed/mature embryos.

To explore the seed diversity of Central African orchids, we have selected and studied samples representing 45 taxa (including species and infra-specific ranks) belonging to 29 genera, 10 subtribes, 8 tribes and 3 subfamilies (see Table 1). The selection is primarily based on the availability and quality of the seed samples in the seed bank. Our aim is to cover as many genera as possible, and, wherever possible, to include two species per genus. Species were also selected according to their geographical distribution (widespread vs. range-restricted), their habitat (lowland vs. mountain) and their lifeforms (terrestrial vs. epiphytic) (Table 1). We have followed the classification of POWO (2025), for the scientific names, with the exception of the genera *Dolabrifolia* (Pfitzer) Szlach. and Romowicz and *Eichlerangraecum* Szlach., Mytnik and Grochocka. Recent molecular data (Farminhão et al., 2021) have confirmed that these genera do indeed represent distinct genera within the polyphyletic genus *Angraecum* Bory.

Photography and measuring using a light microscope

The seeds were photographed under a light microscope, and all morphometric measurements were performed on those seed photographs. The photographic equipment consisted of a digital SLR camera (Canon EOS 760D) connected to a computer mounted on a light microscope (Zeiss Axio Lab A1). The photographs were mainly taken at two magnifications: ×40 and ×5. A series of 6–10 images were taken at each magnification and stacked together into a clear image using the freeware 'focus-stack' (https://github.com/PetteriAimonen/focus-stack; Forster-Heinlein et al., 2004). At ×5 magnification, the series of photographs had to show at least five seeds, while at ×40 magnification, the series of photographs had to show an entire seed to produce an illustrated catalogue of the seeds examined (Appendix 1). For seeds that were not visible in their entirety at ×40 magnification, ×10 magnification was used.

All measurements were made on photographs taken at ×5 magnification, with five seeds per individual measured, to capture within-individual variation. As stated above, the five seeds per species were obtained from the same collection/sample (i.e. corresponding to one or several seedpods that were collected at the same time on the same individual). Measurements were made using the OPTIKA PROVIEW software, which allows the image to be calibrated using a micrometre slide. Each time a measurement is taken, the software directly generates a cross-rule. We measured four main parameters (see Appendix 2): the seed length (SL, in µm), the seed width (SW, in µm), the seed surface (SS, in μm^2) and the embryo surface (ES, in μm^2). We have also derived two calculated parameters. The seed air space (SAS) to obtain an estimate of the space/volume of air in the seed that is less dependent on seed size. It was calculated as the difference between the seed surface and the embryo surface divided by the seed surface and multiplied by 100 ((SS − ES)/SS × 100). We developed this new approach because it takes into account the different shapes of the individual seeds compared to the method of Arditti and Ghani (2000), commonly used in the literature and which assimilates the orchid seed to two cones joined at the base. Finally, we also calculated the ratio between seed length (SL) and seed width (SW).

Statistical approach

To assess the link between seed morphology and phylogenetic relationship among the 29 studied orchid genera, a phylogenetic tree was reconstructed using data from the NCBI global database (Schoch et al., 2020) and a recent revision of the 18 genera belonging to the Angraecinae subtribe (Farminhão et al., 2021). The Interactive Tree Of Life (iTOL) online tool was used to produce the final topology of the phylogenetic tree (Letunic and Bork, 2024).

Using the phylogenetic tree, we assessed the phylogenetic signal of each morphometric parameter using the phylosig function (Pagel, 1999) from the *Phytools* R package (Revell, 2024) using the lambda method. This method allows us to assess how much of the variation in a trait between different species/genera is explained by their evolutionary relationships. The method is based on a statistic called Pagel's lambda (λ), which ranges from 0 to 1. If λ is close to 0, it means that the trait variation is not influenced by evolutionary history of the studied taxa (i.e. the trait is evolving independently

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Table 1 Systematics, distribution and lifeforms of the 45 African orchid taxa investigated. Taxa are grouped by subfamily and by tribe–subtribe. Among the range-restricted taxa, we distinguished between the 'rare/endemic' taxa, which are those that have been recently discovered and/or are endemic to Atlantic Central Africa (see Droissart, 2009), and the 'rare/disjunct' taxa, which are those that are only known from two or three localities, usually more than 1000 km apart. All 'rare' taxa usually have an area of occupancy (sensu IUCN, 2022) not exceeding 2000 km^2. Seeds morphology are illustrated in Appendix 1

Group	Taxon name	Geographical distribution	Altitudinal distribution	Lifeform
Epidendroideae				
Vandeae – Angraecinae				
	Aerangis collum-cygni Summerh.	Wide	Lowland (<800 m)	Epiphyte
	Aerangis gravenreuthii (Kraenzl.) Schltr.	Rare/disjunct	Mountain (>800 m)	Epiphyte
	Ancistrorhynchus schumannii (Kraenzl.) Summerh.	Rare/endemic	Mountain (>800 m)	Epiphyte
	Ancistrorhynchus tenuicaulis Summerh.	Wide	Lowland (<800 m)	Epiphyte
	Bolusiella zenkeri (Kraenzl.) Schltr	Wide	Lowland (<800 m)	Epiphyte
	Calyptrochilum aurantiacum P.J.Cribb and Laan	Rare/disjunct	Lowland (<800 m)	Epiphyte
	Calyptrochilum christyanum (Rchb.f.) Summerh.	Wide	Lowland (<800 m)	Epiphyte
	Calyptrochilum emarginatum (Afzel. ex Sw.) Schltr.	Wide	Lowland (<800 m)	Epiphyte
	Cyrtorchis chailluana (Hook.f.) Schltr.	Wide	Lowland (<800 m)	Epiphyte
	Cyrtorchis okuensis Droissart, Azandi and M.Simo	Rare/endemic	Mountain (>800 m)	Epiphyte
	Diaphananthe bidens (Afzel. ex Sw.) Schltr.	Wide	Lowland (<800 m)	Epiphyte
	Diaphananthe vesicata (Lindl.) Schltr.	Wide	Lowland (<800 m)	Epiphyte
	Dolabrifolia aporoides (Summerh.) Szlach. and Romowicz	Wide	Lowland (<800 m)	Epiphyte
	Dolabrifolia disticha (Lindl.) Szlach. and Romowicz	Wide	Lowland (<800 m)	Epiphyte
	Eichlerangraecum birrimense (Rolfe) Szlach., Mytnik and Grochocka	Wide	Lowland (<800 m)	Epiphyte
	Eichlerangraecum eichlerianum var. *curvi-calcaratum* (Szlach. and Olszewski) Szlach., Mytnik and Grochocka	Rare/endemic	Lowland (<800 m)	Epiphyte
	Kylicanthe arcuata Descourv., Stévart and Droissart	Rare/endemic	Mountain (>800 m)	Epiphyte
	Listrostachys pertusa (Lindl.) Rchb.f.	Wide	Lowland (<800 m)	Epiphyte
	Microcoelia microglossa Summerh.	Wide	Lowland (<800 m)	Epiphyte
	Plectrelminthus caudatus (Lindl.) Summerh. var. *caudatus*	Wide	Lowland (<800 m)	Epiphyte
	Podangis rhipsalisocia (Rchb.f.) Summerh.	Wide	Lowland (<800 m)	Epiphyte
	Rangaeris muscicola (Rchb.f.) Summerh.	Wide	Lowland (<800 m)	Epiphyte
	Rhipidoglossum pulchellum var. *genic-ulatum* (Summerh.) Szlach. and Olszewski	Rare/disjunct	Mountain (>800 m)	Epiphyte
	Rhipidoglossum rutilum (Rchb.f.) Schltr.	Wide	Lowland (<800 m)	Epiphyte
	Summerhayesia laurentii (De Wild.) P.J.Cribb	Wide	Lowland (<800 m)	Epiphyte
	Triceratorhynchus comptus (Summerh.) Szlach., Oledrz. and Mytnik	Rare/disjunct	Lowland (<800 m)	Epiphyte
	Triceratorhynchus sp. nov.	Rare/endemic	Mountain (>800 m)	Epiphyte

(*Continued*)

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Table 1 (*Continued.*)

Group	Taxon name	Geographical distribution	Altitudinal distribution	Lifeform
Epidendroideae				
	Tridactyle anthomaniaca (Rchb.f.) Summerh.	Wide	Lowland (<800 m)	Epiphyte
	Tridactyle filifolia (Schltr.) Schltr.	Rare/disjunct	Mountain (>800 m)	Epiphyte
Vandeae – Polystachyinae				
	Polystachya alpina Lindl.	Rare/endemic	Mountain (>800 m)	Epiphyte
	Polystachya cultriformis (A. Thouars) Spreng.	Wide	Lowland (<800 m)	Epiphyte
	Polystachya paniculata (Sw.) Rolfe	Wide	Lowland (<800 m)	Epiphyte
Collabieae – Collabiinae				
	Ancistrochilus rothschildianus O'Brien	Wide	Lowland (<800 m)	Epiphyte
Cymbidieae – Eulophiinae				
	Eulophia euglossa (Rchb.f.) Rchb.f.	Wide	Lowland (<800 m)	Terrestrial
	Graphorkis lurida (Sw.) Kuntze	Wide	Lowland (<800 m)	Epiphyte
Malaxideae – Dendrobiinae				
	Bulbophyllum aff. *saltatorium* Lindl.	Rare/endemic	Lowland (<800 m)	Epiphyte
	Bulbophyllum encephalodes Summerh.	Rare/disjunct	Mountain (>800 m)	Epiphyte
	Bulbophyllum sandersonii subsp. *stenopetalum* (Kraenzl.) J.J.Verm.	Wide	Lowland (<800 m)	Epiphyte
Malaxideae – Malaxidinae				
	Liparis aff. *winklerii* Schltr.	Rare/endemic	Lowland (<800 m)	Terrestrial
	Liparis nervosa (Thunb.) Lindl	Wide	Lowland (<800 m)	Terrestrial
Podochileae – Eriinae				
	Porpax macrantha subsp. *lejolyana* (Stévart, Droissart and M.Simo) Schuit., Y.P. Ng and H.A. Pedersen	Rare/endemic	Mountain (>800 m)	Epiphyte
Tropidieae – Tropidiinae				
	Corymborkis corymbis Thouars	Wide	Lowland (<800 m)	Terrestrial
Orchidoideae				
Orchideae – Orchidiineae				
	Cynorkis aff. *debilis* (Hook.f.) Summerh	Rare/endemic	Mountain (>800 m)	Terrestrial
	Habenaria procera (Afzel. ex Sw.) Lindl	Wide	Lowland (<800 m)	Terrestrial
Vanilloideae				
Vanilleae – Vanillineae				
	Vanilla ochyrae Szlach. and Olszewski	Rare/endemic	Lowland (<800 m)	Terrestrial

in each species/genera). Conversely, if λ tends to 1, this means that trait tends to evolve in a way that is consistent with its phylogenetic tree. We also calculated the local phylogenetic association index (LIPA) using the lipaMoran function (Anselin, 1995) from the *phylosignal* R package (Keck et al., 2016). This index allows the identification of phylogenetic autocorrelation hotspots (i.e. taxa with similar traits values due to their phylogenetic proximity). The LIPA can be positive, negative or equal to zero. If LIPA < 0, we speak of negative phylogenetic autocorrelation (i.e. trait values are less similar or not similar between phylogenetically close species), for LIPA $= 0$, there is no phylogenetic autocorrelation (i.e. trait values are random and independent of phylogeny), for LIPA > 0, it is positive phylogenetic autocorrelation (i.e. the trait values are similar between phylogenetically close species). The lipaMoran function performs a non-parametric randomization test on each LIPA value and returns a p-value. A p-value < 0.05 indicates a positive and significant LIPA.

As sample size exceeded 30 observations per group and residual diagnostics (Q–Q plots and residuals-versus-fitted plots) did not indicate substantial violations of ANOVA assumptions, we

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analysed each morphometric parameter using a one-way ANOVA. Pairwise differences among species were then assessed using Tukey's HSD post-hoc test, with a significance level of $\alpha = 0.05$. As an additional robustness check, we also verified that the main conclusions were consistent with a non-parametric alternative (Kruskal–Wallis test followed by Dunn's post-hoc test with *p*-value adjustment for multiple comparisons), which further supports the reliability of our results.

To observe the distance between species regarding their lifeforms, and their altitudinal and geographic distributions, we performed a principal component analysis (PCA) using the PCA function of the *FactoMineR* R package (Lê et al., 2008), based on the six morphometric parameters detailed above. We subsequently performed a hierarchical clustering on the principal component scores using the HCPC function, with Euclidean distance as the dissimilarity measure, to evaluate whether the clustering pattern was driven by predefined species groups. When clustering did not clearly reflect the predefined groups, we further tested for group differences by applying a non-parametric Kruskal–Wallis test to individual scores on the first two PCA axes.

All the above analyses were performed using R software version 4.5.1 (R Core Team, 2025).

Results

Seed morphometry and phylogenetic relationship

In this study, the seeds of 45 taxa in 29 genera were photographed (Appendix 1) and examined, whose phylogenetic relationships are shown in Fig. 1. Visual examination of the tree shows a clear difference in size between epiphytic and terrestrial species. The most remarkable variation is the average seed surface of terrestrial orchid seeds (115 304.38 ± 72 044.36 μm^2), which is nine times larger than that of epiphytes (12 526.34 ± 6931.22 μm^2) (see Appendix 3 for exact measurements for each species). The Fig. 1 also highlights a large diversity in shape and size among the 18 genera of the monophyletic Angraecinae subtribe.

Variability in size is considerable within the studied species (Appendix 3). The length of the seeds varies by a factor of 12 between the longest (*Corymborkis corymbis*, 2062.0 ± 162.6 µm) and the shortest (*Bulbophyllum* aff. *saltatorium*, 131.4 ± 15.6 µm), and these two species also present the maximum and minimum values for the seed surface. *Vanilla ochyrae* seed, the widest one (387.0 ± 49.7 µm), is 11 times wider than the 'thinnest' one (*Microcoelia microglossa*, 34.6 ± 6.1 µm). The embryo surface varied between 154324.0 μm^2 (*Vanilla ochyrae*) and 2556.8 μm^2 (*Summerhayesia laurentii*). The SAS ranged from ∼96% in *Corymborkis corymbis* to ∼16% in *Vanilla ochyrae*.

The test of phylogenetic signal in the morphometric traits (Fig. 2) showed a high signal intensity for length ($\lambda = 0.5$) and SAS ($\lambda = 0.81$). This reflects a link between phylogenetic relationship among studied genera and these traits. Width, length to width ratio, seed surface and embryo surface show almost no phylogenetic signal ($\lambda \approx 0$). The local phylogenetic association index shows values close to zero for most of the traits measured for each genus (Fig. 3), indicating that there is no phylogenetic autocorrelation. Only *Liparis* and *Bulbophyllum* showed significant positive values of autocorrelation for length, SAS, length to width ratio (SL/SW) and seed surface (Fig. 3). *Summerhayesia* and *Tridactyle* also showed significant positive autocorrelation for SASs (Fig. 3). We also noted that only the length and SAS did not show negative autocorrelation in all 29 genera (Fig. 3).

Seed morphometry and species' ecology

The analysis of variance carried out on life forms, altitudinal range and geographical distribution using the six measured or calculated morphometric traits (Fig. 4), shows significant differences only for the lifeform and for all traits except the ratio between seed length and seed width. There are no significant differences between the species with different geographical distribution (widespread vs. range-restricted) or altitudinal range (lowland vs. mountain).

Multivariate analysis performed on the seed morphology of 45 orchid species also separates epiphytes from terrestrial orchids (Fig. 5A). Most of the epiphytic species are grouped along the first two axes of PCA, which account for 86.3% of the explained variance. Terrestrial orchids are stretched along axis 1 of the PCA, which accounts for 53.7% of the total variance (Fig. 5A). This axis is correlated with seed length, seed width, and SAS (Appendix 4).

Following the PCA, we performed a hierarchical classification based on the principal components (HCPC) and obtained four different groups (Fig. 5B). The first, red-coloured group contains only epiphytic orchids. The second green-coloured group contains epiphytic orchids (*Graphorkis lurida*) and terrestrial orchids belonging to genera that also have epiphytic species. The third group is represented by a liana-like species (*Vanilla ochyrae*) whose lifeform is initially terrestrial and eventually becomes epiphytic as the plant grows. The last group contains two terrestrial orchids. The dispersal gradient of terrestrial orchid species along the axis 1 of the PCA and the grouping of *Graphorkis lurida* with terrestrial orchids tend to reflect an evolutionary process from one lifeform to another.

Seed morphometry and epiphytic species distribution

Based on the seed morphometric variables measured on epiphytic species, no correlation could be established between the seed morphology and the geographical and altitudinal distributions of the species to which they belong. The PCA based on morphological characteristics shows a grouping of epiphytic species regardless of their distribution (Fig. 6), although some species such as *Ancistrochilus rothschildianus, Graphorkis lurida, Microcoelia microglossa, Summerhayesia laurentii* stand out from the group. In addition, the Kruskal–Wallis test carried out on axes 1 and 2 (which account for 83.5% of the total variance) of the PCA shows that there is no significant difference between the morphology of the seeds of mountain orchids (>800 m) and lowland orchids (<800 m) (*P*-value of 0.64 on axis 1 and 0.59 on axis 2). Similarly, there was no significant difference between the seeds of orchids with a wide distribution and those with a restricted distribution (*P*-value = 0.71 on axis 1 and 0.20 on axis 2).

Discussion

The morphometry of orchid seeds and its taxonomic significance

Seed morphology is a well-established taxonomic tool for studying orchid diversity (Gamarra et al., 2008, 2012; Barthlott et al., 2014). Our results confirm that some seed traits, such as length and SAS, exhibit strong phylogenetic signals for African orchid species. In this context, seed morphometry appears to provide valuable complementary information about the phylogenetic placement of species (Collier et al., 2023). However, the strength of phylogenetic signals may varies depending on the taxonomic scale (Keck et al., 2016). Our local phylogenetic analysis (LIPA) of selected African

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Figure 1. Link between phylogenetic relationships and 'visual' seed morphology of the 29 African orchid genera studied in this paper. The topology of the cladogram was constructed using data from the NCBI global database (Schoch et al., 2020) and a recent revision of 18 genera of the Angraecinae subtribe (Farminhão et al., 2021). All the seeds illustrated are reproduced to the same scale to facilitate visual size comparisons. Green and Orange bullets indicate the main lifeform of the species belonging to each genus. The species used to illustrate each genus are indicated in Appendix 3. *Cynorkis, Graphorkis, Habenaria* and *Liparis* contain species with different lifeforms.

orchid genera revealed a heterogeneous signal. For example, genera such as *Liparis* and *Bulbophyllum* displayed significant phylogenetic structuring for seed length, surface area, length-to-width ratio, and SAS, whereas most of the other genera showed no clear patterns (Fig. 3). This suggests that the evolutionary history of individual genera strongly shapes the expression of seed traits, while in others these characters may have undergone ecological filtering or convergence. Traits lacking a phylogenetic signal are particularly interesting, as they may reflect adaptive evolution decoupled from ancestry (Desdevises, 2018).

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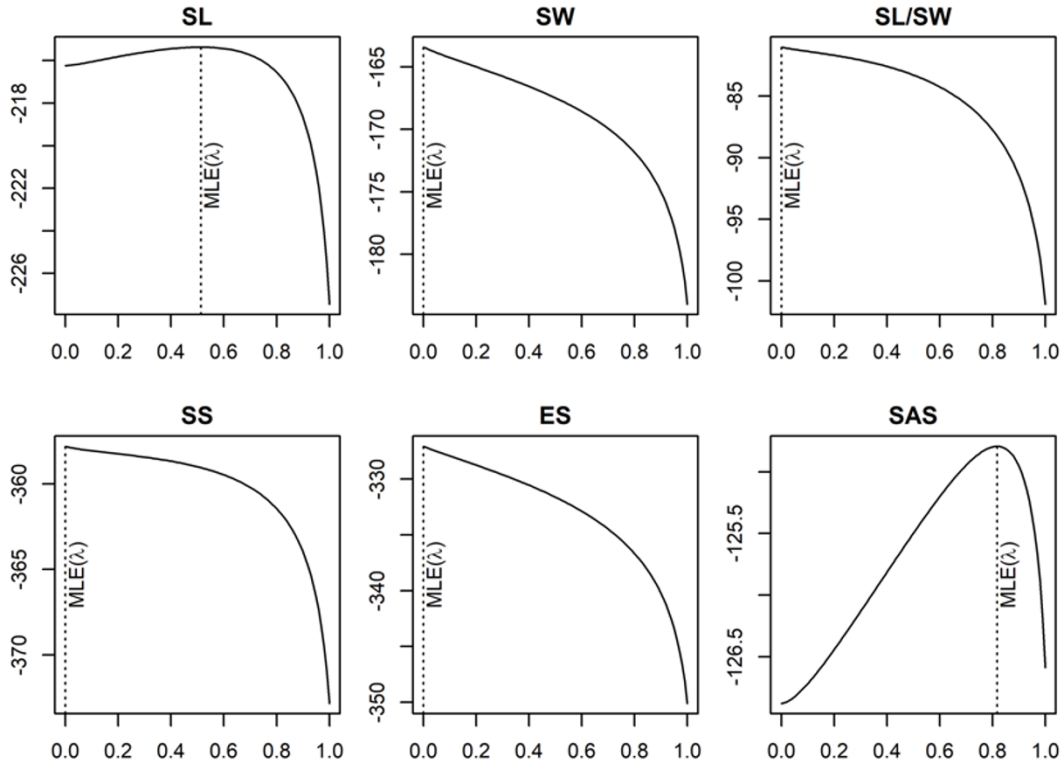

Figure 2. Value of the phylogenetic signal of the six morphometric traits measured for the 29 studied orchid genera. The *x*-axis represents the value of the phylogenetic signal (Pagel's lambda (λ) tends to 1 when seed traits evolve in a way that is consistent with its phylogenetic tree) and the *y*-axis the log-likelihood associated with λ. SL, seed length; SW, seed width; SS, seed surface; ES, embryo surface; SAS, seed air space.

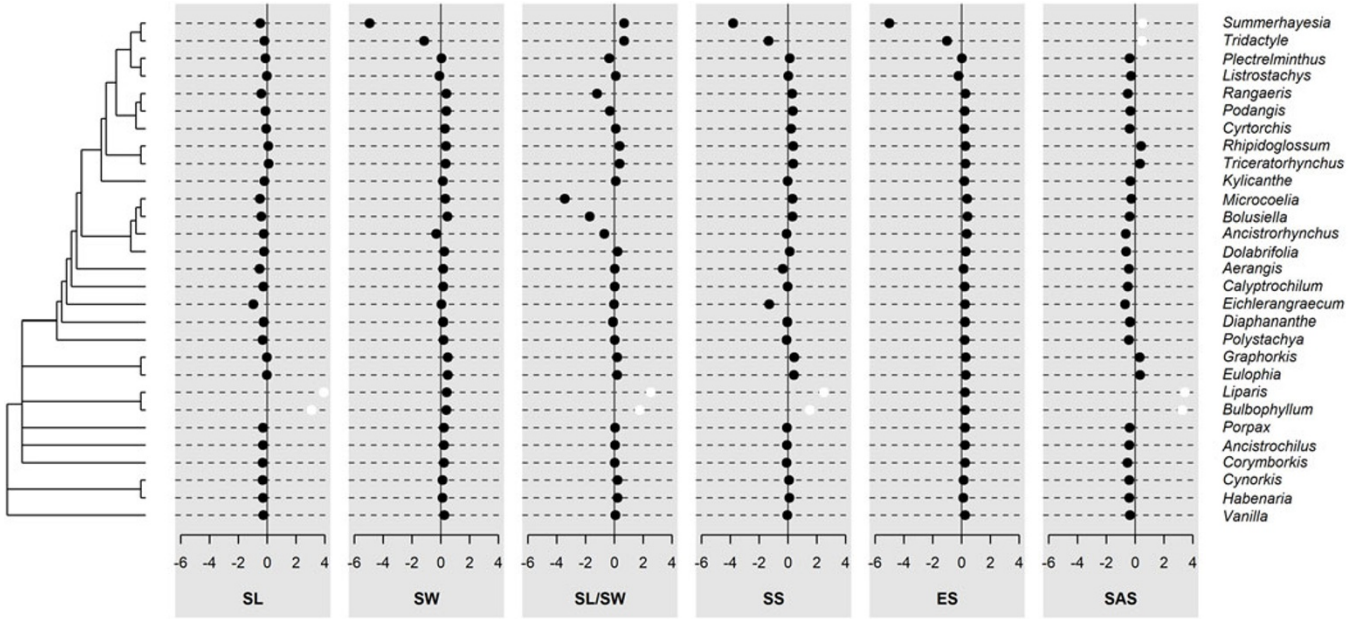

Figure 3. Values of the local phylogenetic association index (LIPA) for six morphometric characteristics of the 29 studied orchid genera. White points indicate significant LIPA values and phylogenetic autocorrelation hotspots (genera whose values for a trait are similar due to their phylogenetic proximity). SL, seed length; SW, seed width; SS, seed surface; ES, embryo surface; SAS, seed air space.

Using scanning electron microscopy (SEM), Barthlott et al. (2014) also identified fine features of the testa cells as key taxonomic criteria in orchids. Although our optical microscopy study does not permit ultrastructural observation, we believe that our methodological approach is still suitable for large-scale quantitative morphometry. Light microscopy has several advantages: it is widely accessible, cost-effective and non-destructive, and it preserves specimen colouration. Although SEM provides superior

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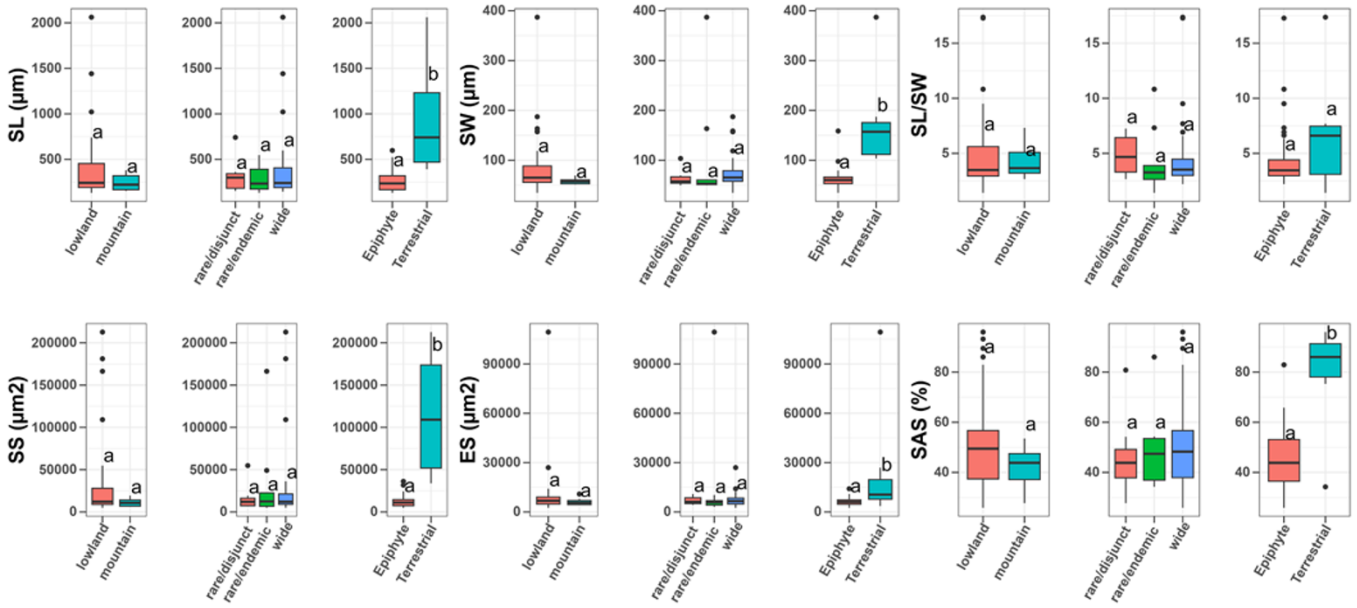

Figure 4. Boxplots for each morphometric character among different geographical (rare/disjunct = 8 species; rare/endemic = 9 species; wide = 28 species), altitudinal (lowland = 35 species; mountain = 10 species) and lifeform (epiphyte = 38 species; terrestrial = 7 species) groups. The boxplots with different letters show a significant difference at the 0.05 significance level using the Tukey test. SL, seed length; SW, seed width; SS, seed surface; ES, embryo surface; SAS, seed air space.

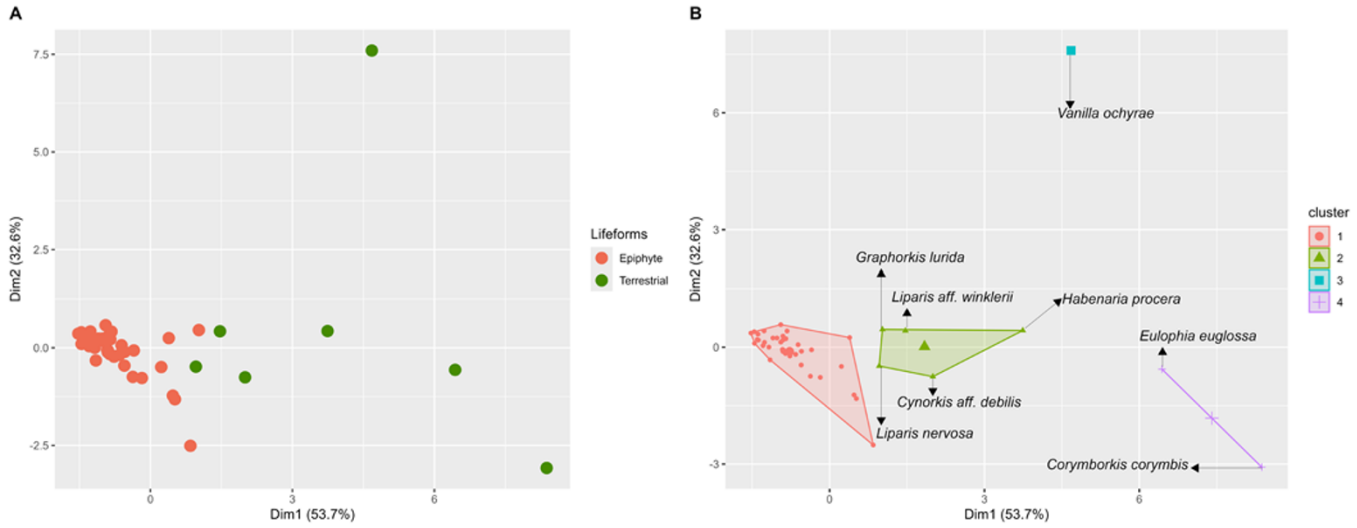

Figure 5. Morphometric variation between seeds of 45 African orchid species. (A) Principal Component Analysis (PCA) biplot for six seed characteristics comparing 45 orchids' taxa occurring in Cameroon with two lifeforms. (B) Hierarchical clustering (Euclidian distance) on principal component scores at the 0.05 significance level.

resolution and ultrastructural detail, the cost and required sample preparation may limit its applicability in comparative studies such as ours. Future studies could combine both approaches, using optical microscopy for large datasets and SEM for detailed analyses of critical taxa.

Morphology of orchid seeds and species ecology

Seed morphology offers insights into evolutionary mechanisms within Orchidaceae (Fan et al., 2020). Phylogenomic studies indicate that the ancestral condition of orchids was terrestrial, followed by multiple independent transitions to epiphytism (Zhang et al., 2023). Our results are consistent with this evolutionary trajectory, since African seed morphology clearly differentiates terrestrial from epiphytic orchids. Terrestrial seeds tend to be longer and show highest SASs than epiphytic ones (Fig. 3). Such differences in seed structure are thought to optimize dispersal under contrasting ecological conditions (Diantina et al., 2020; Fan et al., 2020).

Interestingly, genera that include both terrestrial and epiphytic species, such as *Cynorkis, Graphorkis, Habenaria* and *Liparis*, tend to exhibit intermediate seed morphologies (Fig. 5). This intermediate phenotype may reflect ecological plasticity and/or ongoing transitions between lifeforms, corroborating the dynamic evolutionary history of Orchidaceae. However, we were unable to link these intermediate/transitional genera to specific environmental conditions or distribution patterns.

Our findings also reinforce the idea that seed size and the proportion of air cavities are directly linked to ecological adaptations

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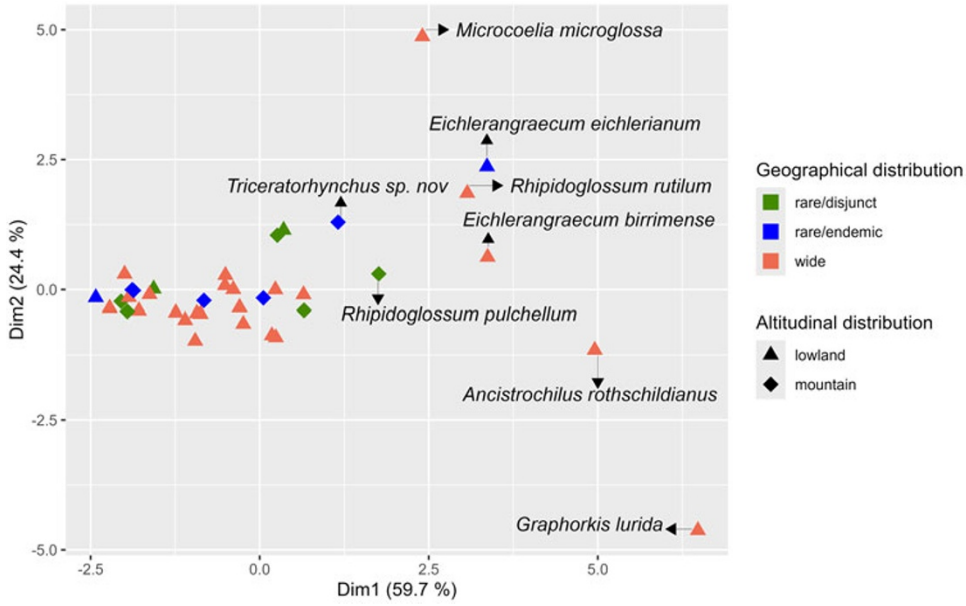

Figure 6. Morphometric variation between seeds of epiphytic African orchid species in relation to their geographical and altitudinal distributions. The graph shows the results of a Principal Component Analysis (PCA) for six seeds characteristics and comparing 38 species with contrasted geographical range (rare/disjunct = 7 species; rare/endemic = 7 species; wide = 24 species) and altitudinal distribution (lowland = 28 species; mountain = 10 species).

(Arditti and Ghani, 2000). Chaudhary et al. (2014) demonstrated that temperate *Dendrobium* species have larger air spaces than their tropical counterparts, facilitating flotation and thereby enhancing their dispersal. In our dataset, the consistently higher air content in terrestrial seeds might be linked to their in low-wind understory habitats, as their potential for long-distance anemochory is reduced compared to epiphytes exposed to canopy-level winds. Animal-mediated dispersal provides complementary pathways, with both endozoochory and ectozoochory now demonstrated in *Vanilla*: mammals consume indehiscent fleshy fruits and defecate viable seeds, while bees remove seeds from dehiscent fruits attracted by vanillin volatiles (Karremans et al., 2023). This highlights how habitat structure and dispersal ecology interact to shape orchid seed morphology.

Morphology of orchid seeds and species distribution patterns

A fundamental question guiding this research was to ascertain whether African orchids with restricted distribution possess seeds with a distinct morphology. The link between orchid seed morphology and dispersal capacity has long been hypothesized (Arditti and Ghani, 2000); seeds with higher air content enjoy prolonged suspension and may disperse farther. In contrast to this expectation, our study did not detect a significant correlation between seed air content and either geographical or altitudinal distribution of species. This aligns with Aprilianti et al. (2021), who showed that in *Bulbophyllum* and *Dendrobium*, SAS does not predict distribution range but may instead influence micro-scale dispersal mechanisms.

The majority of the range-restricted species selected for this study (11 out of 17, see Table 1) are found in montane forests above 800 m, which are a scarce and more and more threatened habitat in Central Africa (Abera et al., 2024). Cuni-Sanchez et al. (2021) estimated that these forests occupy less than 5% of Cameroon's primary humid forest area. Droissart et al. (2009) also demonstrated that the majority of orchid species endemic to Atlantic Central Africa are disproportionately concentrated in mountainous regions. Such mountain systems have been shown to function as 'cradles' of diversification, where topographic complexity, habitat heterogeneity and isolation promote lineage splitting and the emergence of narrowly distributed taxa, like the studied 'rare/endemic' putative new species (*Bulbophyllum* aff. *saltatorium, Cynorkis* aff. *debilis, Liparis* aff. *winklerii, and Triceratorhynchus* sp. nov.). Alternatively, such systems can be regarded as 'museums' that preserve diversity through time by providing relatively stable refugial condition (Couvreur et al., 2021). The latter role is consistent with the persistence of the studied 'rare/disjunct' species (e.g., *Aerangis gravenreuthii, Bulbophyllum encephalodes, Calyptrochilum aurantiacum, Rhipidoglossum pulchellum* var. *geniculatum, Triceratorhynchus comptus, Tridactyle filifolia*).Other ecological or biotic factors appear relevant to explaining distribution patterns of tropical orchids. Duffy and Johnson (2017) suggest that pollinator distributions may limit the geographical ranges of flowering plants with specialized pollination systems, which are frequently observed in orchids. Symbiotic associations with mycorrhizal fungi are critical to orchid establishment, and their absence or patchy distribution can strongly limit species ranges (Waud et al., 2016; Li et al., 2021). Likewise, for epiphytic orchids, substrate specificity plays a major role: bark type influences seedling establishment (Birindwa et al., 2023), while microclimatic variables such as humidity, temperature and light availability drive preferences for particular phorophytes (Kimpouni et al., 2017). These multi-factorial constraints likely explain local clustering and range restriction, and may override potential dispersal ability conferred by seed morphology.

Conclusion

The morphometric analysis of central African orchid seeds reveals a complex interplay between phylogenetic constraints and ecological adaptations. We confirmed a consistent pattern whereby epiphytic species possess smaller seeds with reduced air content

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compared to their terrestrial counterparts, suggesting that the evolutionary transition to epiphytism involved specific modifications of seed morphology. Some orchid seed traits appear to be phylogenetically conserved, making them potentially valuable for systematic studies within the family. Integrating such morphometric data with emerging phylogenomic datasets could help resolving taxonomic uncertainties and reconstructing the evolutionary history of seed adaptations in orchids.

Contrary to expectations, we could not establish any clear link between seed morphology and distribution patterns among epiphytic species, regardless of their geographical range or elevation preferences. This finding indicates that factors other than seed dispersal capacity, including mycorrhizal associations, host tree specificity and local environmental conditions, are probably more important in determining where orchid species can successfully establish and persist. As no germination data like that used by Oikonomidis and Thanos (2024) are currently available for the Central African orchid species investigated here, there is a significant knowledge gap in regional seed biology. Targeted comparative studies of germination behaviour would be especially valuable in testing evolutionary hypotheses and informing conservation and restoration strategies for Orchidaceae in Central Africa.

Moving forward, we suggest that research efforts should focus on expanding morphometric surveys to encompass a wider taxonomic representation across African Orchidaceae, while also incorporating functional studies that directly measure dispersal effectiveness and germination success. Additionally, distribution models would benefit from explicitly accounting for the multiple and complex ecological interactions that orchids depend upon. This multivariate approach should provide deeper insights into the mechanisms driving diversification in this remarkably species-rich plant family.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S0960258526100117.

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Author contributions. P.D.A. conceived the study under direction of V.D., B.S. P.D.A. designed and performed the analysis with assistance from V.D. P.D.A., and V.D. collected the data. M.S.D. contributed to the interpretation of the results. V.D. and P.D.A. wrote the manuscript with the assistance of M.S.D and B.S.

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Competing interests. The authors declare none.

Data availability. All data generated or analysed during this study are included in the article. Please contact the corresponding author for more data requests.

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