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► To cite this version:

Géromine Collobert, Benoît Perez-Lamarque, Jean-Yves Dubuisson, Florent Martos. Gains and losses of the epiphytic lifestyle in epidendroid orchids: review and new analyses of succulence traits. *Annals of Botany*, 2023, 132 (4), pp.787-800. 10.1093/aob/mcad145 . hal-04943807

HAL Id: hal-04943807

<https://hal.science/hal-04943807v1>

Submitted on 12 Feb 2025

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1 RESEARCH IN CONTEXT

2

3 **Gains and losses of the epiphytic lifestyle in epidendroid orchids: review and new**

4 **analyses of succulence traits**

5

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17

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19

20 Word count: 9567

21 Tables: 1

22 Figures: 3

23 Supplementary data file(s): 1

1 **ABSTRACT**

2

3 **Background and Aims**

4 Epiphytism has evolved repeatedly in plants and has resulted in a considerable number of
5 species with original characteristics. Because the water supply is generally erratic compared
6 to soils, succulent forms in particular are widespread in epiphytic species. However, succulent
7 organs also exist in terrestrial plants, and the question of the concomitant evolution of
8 epiphytism and succulence has received little attention, not even in the epidendroid orchids,
9 which account for almost 67% of vascular epiphytes.

10 **Methods**

11 We built a new time-calibrated phylogenetic tree of Epidendroideae with 203 genera treated
12 in *Genera Orchidacearum*, from which we reconstructed the evolution of epiphytism as well
13 as traits related to water scarcity (stem and leaf succulence and the number of velamen
14 layers), while testing for the correlated evolution between both. Furthermore, we estimated
15 the ancestral geographic ranges to evaluate the paleoclimatic context in which epiphytism
16 evolved.

17 **Key Results**

18 Epiphytism evolved at least three times: 39.0 Myr ago in the common ancestor of the
19 Malaxideae and Cymbidieae that likely ranged from the Neotropics to Southeast Asia and
20 Australia, 11.5 Myr ago in the Arethuseae in Southeast Asia and Australia, 7.1 Myr ago in the
21 neotropical Sobralieae, and was notably lost in the Malaxidiinae, Collabieae, Calypsoeae,
22 Bletiinae, and Eulophiinae. Stem succulence is inferred to have evolved once, in a terrestrial
23 ancestor at least 4.1 Myr ago before the emergence of epiphytic lineages. If lost, stem
24 succulence was almost systematically replaced by leaf succulence in epiphytic lineages.

1 **Conclusions**

2 Epiphytism may have evolved in seasonally dry forests during the Eocene climatic cooling,
3 among stem-succulent terrestrial orchids. Our results suggest that the emergence of stem
4 succulence in early epidendroids was a key innovation in the evolution of epiphytism,
5 facilitating the colonisation of epiphytic environments that afterwards led to the greatest
6 diversification of epiphytic orchids.

7

8 **Keywords:**

9 Epidendroideae, Orchidaceae, vascular epiphytism, pseudobulbs, succulence traits, velamen,
10 ancestral reconstruction, key innovation, *Genera Orchidacearum*, hidden Markov model
11 (HMM).

1 INTRODUCTION

2

3 Epiphytism, or the ability of some plants to grow on the surface of other plants, is a major
4 component of tropical and subtropical forests on Earth (Benzing, 1990; Zotz, 2016),
5 representing about 9% of global vascular plant diversity (Zotz, 2013, 2016), and up to 50% of
6 the plant species in some tropical rainforests (Silvera and Lasso, 2016). Among epiphytes,
7 orchids are of particular interest, as they alone account for around 67% of all epiphytic
8 species (21,169 out of 31,311; (Zotz *et al.*, 2021)), and almost all of them belong to the
9 Epidendroideae (Zotz, 2013), the largest subfamily of Orchidaceae. Givnish *et al.* (2015)
10 demonstrated that several factors, including epiphytic lifestyle and tropical distribution, were
11 associated with higher net diversification rates in orchids. Although they did not specifically
12 address the evolution of epiphytism in the Orchidaceae, their taxon sampling (162 species
13 belonging to 18 tribes) allowed them to identify at least one transition to the epiphytic
14 lifestyle at the orchid family level, in the subfamily Epidendroideae, but this analysis was
15 carried out at the level of subtribes only. Two other ancestral estimations of lifestyle evolution
16 were conducted on more extensively sampled phylogenies, including 335 orchid species
17 (Chomicki *et al.*, 2015) and 312 Epidendroideae species (Freudenstein and Chase, 2015),
18 respectively. Freudenstein and Chase (2015) detected at least three independent origins of the
19 epiphytism in Epidendroideae, challenging the primary single origin of the epiphytic lifestyle
20 found by Givnish *et al.* (2015) and Chomicki *et al.* (2015). While the latter group includes
21 mainly epiphytic taxa in the tribes Cymbidieae and Vandaeae, the former group comprises both
22 terrestrial lineages that are rather basal in the tree and more recent epiphytic lineages, like the
23 Dendrobiinae which include the genus *Bulbophyllum*. In addition, confirming the early
24 assumption of Dressler (1981), these studies found multiple re-terrestrialization events

1 (Chomicki *et al.*, 2015; Freudenstein and Chase, 2015; Givnish *et al.*, 2015). Indeed, several
2 lineages nested in the Epidendroideae diversify on the ground and show features similar to
3 related epiphytic lineages, suggesting that reversions from the epiphytic lifestyle to the
4 terrestrial lifestyle may have occurred in more recent times. Finally, recent phylogenetic
5 works reappraising the phylogenetic relationships in Epidendroideae using genomic molecular
6 data (Givnish *et al.*, 2015; Li *et al.*, 2019; Serna-Sánchez *et al.*, 2021; Pérez-Escobar *et al.*,
7 2021) now provide a robust phylogenetic framework to assess the evolution of this group.

8
9 Although diverse and heterogeneous in terms of environmental conditions, epiphytic habitats
10 are characterised by an irregular water supply for plants (Benzing, 1987; Cribb, 1999; Zotz,
11 2016; Taylor *et al.*, 2022; Hietz *et al.*, 2022). Water shortage in epiphytic habitats could
12 explain why water catching and storing organs are commonly observed among epiphytic
13 plants, such as the tank-forming leaves holding water in some bromeliads (Givnish *et al.*,
14 2014; Zotz, 2016). Most epiphytic orchids and many Araceae have one or more layers of dead
15 epidermal cells surrounding the root, called a velamen, which helps to capture water running
16 off the surface of the tree (Cribb, 1999; Zotz and Winkler, 2013; Stern, 2014). Thickened
17 stems and leaves are also common to conserve and store water and nutrients to compensate
18 for their irregular supply (Cribb, 1999; Ng and Hew, 2000; Stern, 2014; Zotz, 2016; Yang *et*
19 *al.*, 2016; Niechayev *et al.*, 2019; Hietz *et al.*, 2022; Fu *et al.*, 2022). In addition,
20 Crassulacean Acid Metabolism (CAM) is a water-conserving trait widespread among
21 epiphytic plants (Luttge, 2004; Zotz, 2016; Niechayev *et al.*, 2019; Hietz *et al.*, 2022; Fu *et*
22 *al.*, 2022; Orlov *et al.*, 2022). With approximately 2100 species, the pantropical genus
23 *Bulbophyllum* in Epidendroideae illustrates the ecological and evolutionary success of these
24 characters (Gravendeel *et al.*, 2004; Gamisch and Comes, 2019). Even though traits related to

1 water scarcity (including succulence) were found to be more pronounced in epiphytes than in
2 terrestrial species (Rada and Jaimez, 1992; Zhang *et al.*, 2015; Hietz *et al.*, 2022), justifying
3 an ‘epiphytic syndrome’ according to Hietz *et al.* (2022), these features are not exclusive to
4 epiphytes, as they are also found in terrestrial plants (Zhang *et al.*, 2015; Zotz *et al.*, 2017;
5 Hietz *et al.*, 2022). For example, the velamen is a typical feature of epiphytic orchids, but is
6 also found in numerous terrestrial genera in many families, with *a priori* no epiphytic
7 ancestors from which they could have retained this feature (Zotz *et al.*, 2017). Therefore, as
8 stated by Zotz (2016): ‘in the absence of phylogenetic analyses, it usually remains unclear
9 whether a feature really represents an adaptation, or whether previously evolved traits simply
10 proved to be advantageous in an epiphytic environment’. The second scenario would
11 correspond to a key innovation as defined by Miller *et al.* (2023): ‘an organismal feature that
12 enables a species to occupy a previously inaccessible ecological state’.

13

14 Dressler (1981) hypothesized that water-deprived traits could have evolved as adaptations to
15 drought in seasonally dry tropical climates in terrestrial orchids, later facilitating the
16 transitions to the epiphytic niches. Indeed, an ancestral state estimation carried out by
17 Freudenstein and Chase (2015) suggested that the succulent stems, called pseudobulbs,
18 common in modern epiphytic orchids could indeed have arisen before the evolution of the
19 epiphytic lifestyle, but the method they used nevertheless returned an uncertain state. In the
20 present study, we aimed to test Dressler’s hypothesis, *i.e.* did traits related to water scarcity
21 appear in epiphytic lineages as adaptations (*i.e.* the adaptation hypothesis) or did they emerge
22 first in terrestrial ancestors as features that facilitated access to novel ecological states, here
23 the epiphytic environment (*i.e.* the key innovation hypothesis)? To address these questions,
24 we (i) analysed the evolutionary history of the epiphytic lifestyle and water-related traits in a

1 single state-of-the-art analysis, using the most recent and robust phylogeny and methods
2 available; (ii) ascertained the likelihood of an emergence of succulence traits prior to the
3 epiphytic lifestyle and estimated if transitions from terrestriality to the epiphytic lifestyle were
4 more frequent among drought-adapted lineages; and (iii) identified the paleoclimatic context
5 of the evolution of epiphytism, *i.e.* when and where water-related and the epiphytic lifestyle
6 arose and were lost.

7

8 **MATERIALS AND METHODS**

9

10 *Phylogenetic analysis of Epidendroideae genera and divergence times*

11

12 In order to produce a well-sampled, robust, time-calibrated phylogenetic reconstruction of the
13 Epidendroideae at the genus level, we used a molecular dataset of three plastid genes (*matK*,
14 *psaB*, *rbcL*). Sequences were retrieved from GenBank for 203 placeholders representing each
15 genus of Epidendroideae listed in *Genera Orchidacearum* (Pridgeon *et al.*, 2005, 2009, 2014).
16 Whenever possible, all gene sequences came from the same voucher specimen (69% of the
17 genera), and if not from different specimens of the same species (14%), or from different
18 species (17%). GenBank accession numbers are provided in Supplementary Data Table S1.
19 Name acceptance by the WCSP (2020) of vouchers and genera was systematically checked.
20 Although synonyms of *Dendrobium*, *Cadetia* and *Epigeneium* were kept for reasons of fossil
21 calibration (see below). Sequences of each gene were aligned using MAFFT in Geneious
22 Prime 2021.2, manually checked, and then concatenated, resulting in a molecular dataset of
23 4649 characters. Best-fitting partition scheme was selected using ModelFinder in IQ-TREE
24 1.6.12 (Kalyaanamoorthy *et al.*, 2017).

1
2 A phylogenetic analysis based on these three genes is unlikely to provide strong support for
3 the deep relationships between the genera of Epidendroideae, but tribe relationships in
4 Epidendroideae have been studied several times using 74 to 78 plastid genes (Givnish *et al.*,
5 2015; Li *et al.*, 2019; Serna-Sánchez *et al.*, 2021; Pérez-Escobar *et al.*, 2021). Here we used
6 the multispecies coalescent tree inferred from 78 plastid genes by Pérez-Escobar *et al.* (2021)
7 as a backbone tree for tribe relationships. We used the classification of *Genera*
8 *Orchidacearum* (Pridgeon *et al.*, 2005, 2009, 2014) to circumscribe tribes and assign genera
9 to them, while correcting by the phylogenetic relationships of Pérez-Escobar *et al.* (2021). We
10 hereafter used the supra-generic classification of Chase *et al.* (2015) for convenience, with the
11 exception of the genus *Coelia* that we have kept in the subtribe Coeliinae (instead of
12 Calypsoinae).

13
14 Dated tree inference from the molecular dataset was performed in BEAST 2.6.6 (Bouckaert *et*
15 *al.*, 2019), with the backbone tree input as multiple monophyletic constraints. The stem age of
16 *Earina* and the crown age of *Dendrobium* (including *Cadetia* and *Epigeneium*) were calibrated
17 with the fossils of *Earina fouldenensis* and *Dendrobium winikaphyllum* (both dated at 23.2
18 Myr; (Conran *et al.*, 2009), respectively. The crown age of Epidendroideae was calibrated
19 using the fossil of *Succinantha baltica* estimated at least at 45 Myr (Poinar and Rasmussen,
20 2017). Despite having only been tentatively assigned to Epidendroideae but not to any extant
21 tribe, this fossil allows to put a minimal age to the Epidendroideae subfamily. Calibration
22 points were set with a log-normal distribution of mean = 1, and of standard deviation (SD) =
23 1.25 for the two points at 23.2 Myr, and SD = 2 for the crown age of Epidendroideae. The
24 higher SD on the calibration of the root of the Epidendroideae allows the node to take much

1 older values, encompassing the uncertainty of both the age estimation and the phylogenetic
2 position of *Succinanthera baltica*. We set a relaxed uncorrelated log-normal molecular clock,
3 and the birth-death process as tree prior. A diffuse gamma distribution ($\alpha = 0.001$ and $\beta =$
4 1000) was applied to both clock mean and birth rate priors. Other priors were left as default.
5 Site model averaging using bModelTest 1.2.1 was performed, as recommended by Bouckaert
6 and Drummond (2017), with mutation rate estimated and fixed mean substitution rate. We
7 performed two CoupledMCMC runs of 200 million steps each with eight chains, resampling
8 every 20,000 steps. Stationarity and convergence of runs were assessed using Tracer 1.7.1,
9 and trees were combined using LogCombiner 2.6.6 after discarding the first 12% as burn-in.
10 A maximum clade credibility (MCC) tree with median node heights was calculated using
11 TreeAnnotator 2.6.6.

12

13 *Lifestyle and water-related trait data set*

14

15 To estimate their evolutionary history, we scored the occurrence of epiphytic lifestyle and
16 water-related traits (see below) at the generic level, using *Genera Orchidacearum* (Pridgeon
17 *et al.*, 2005, 2009, 2014). While many traits are related to water limitations, such as specific
18 leaf area or water-use efficiency (*e.g.* Zhang *et al.*, 2015; Hietz *et al.*, 2022), only
19 morphological water-related traits were available. We thus retrieved the presence of stem
20 succulence (with two modalities if present: heteroblastic (one internode swollen) or
21 homoblastic (several internodes swollen) pseudobulbs/corms); the presence of leaf succulence
22 (including coriaceous leaves); and the minimum and maximum number of velamen layers
23 (also from Stern (2014) and Porembski and Barthlott (1988)) (Fig. 1). With regard to the
24 lifestyle, 35 genera included lithophytic species, but this lifestyle is sometimes equivocal

1 (Zotz, 2013, 2016), and as none of the genera sampled were entirely lithophytic, we did not
2 consider it. The genera were therefore assigned as epiphytic and/or terrestrial. With regard to
3 the leaf thickness, 14 genera (6.9%) were leafless, *i.e.* mycoheterotrophic (Fig. 2). For all
4 categorical traits, states were mutually non-exclusive, *i.e.* genera could include both epiphytic
5 species and terrestrial species (see frequencies of each state in Supplementary Data Table S2).
6 The number of velamen layers was missing for 66 genera out of 203 in the phylogeny
7 (32.5%).

8

9 *Test of correlated evolution between epiphytism and succulence traits*

10

11 Because the epiphytic habitat is characterised by irregular water-supply, epiphytic lifestyle
12 and succulent organs could have evolved in a correlated way. To test for their potential
13 correlated evolution throughout the phylogeny, we used the discrete dependent/independent
14 approach implemented in BayesTraits v4.0.0. For a given trait, we compared the fit of a
15 model where the epiphytic lifestyle and this trait evolved in a correlated way (“dependent
16 model”) to the fit of a model where both traits evolved independently (“independent model”)
17 using Bayes Factors (BF). We ran a reverse-jump (with an exponential prior of mean 10)
18 MCMC analysis for each model, with 5,500,000 iterations, a burn-in of 500,000, and used the
19 stepping stone sampler (Xie *et al.*, 2011) with 500 stones and 5000 iterations per stone.
20 Stationarity and convergence of two runs per model were assessed in Tracer v1.7.1. The log
21 BF were computed from the resulting marginal likelihoods, as $2 * (\log \text{marginal likelihood the}$
22 $\text{dependent model} - \log \text{marginal likelihood the independent model})$. We considered a $BF > 2$
23 to be significant support for the dependent model.

24

1 The test was replicated with another method, *i.e.* using the package corHMM v2.7.1 in R
2 v4.2.1 (R Core Team, 2022), which is a Hidden Markov model designed to allow for the
3 correlated evolution of several characters when estimating transition rates and inferring
4 ancestral states on a phylogeny (Boyko and Beaulieu, 2021). Two symmetric transition
5 matrices were used to correlate or decorrelate transition rates between states (Supplementary
6 Data Table S3). The maximum likelihood of each model was computed with the *corHMM*
7 function using 100 random restarts, and best-fitting model was determined by AICc
8 comparison. Compared with BayesTraits, corHMM allows some heterogeneity in the
9 transition rates across the whole phylogeny by assuming that the rates can depend on some
10 unobserved (“hidden”) traits.

11

12 *Estimation of transitions between ancestral lifestyles and succulence traits*

13

14 Freudenstein and Chase (2015) found that succulent stems could potentially have appeared
15 earlier than the epiphytic lifestyle. To ascertain the likelihood of an appearance of succulence
16 traits prior to the epiphytic lifestyle in a temporal framework, transition rates between states
17 were first estimated with *corHMM*. Polymorphic taxa were included in the analysis, as
18 *corHMM* can handle multiple states, although this is interpreted as uncertainty by the method
19 rather than polymorphism. We tested models with transition matrices assuming either equal,
20 symmetric, or all-different transition rates, and including or not one hidden rate category.
21 Then, 1000 stochastic character maps of the best-fitting model were generated using the
22 *makeSimmap* function and then summarised to estimate the posterior probability of each state
23 at ancestral nodes. Number of transitions between correlated states were estimated from the
24 posterior probability distribution of trait changes from the stochastic character mapping.

1

2 *Estimation of ancestral number of velamen layers and test of differences between lifestyles*

3

4 For the mean number of velamen layers in the root, we considered the trait to be continuous.

5 We used the R function `phytools::anc.ML` (Revell, 2012) with a Brownian Motion model of

6 continuous character evolution to estimate the ancestral states. The `caper::ppls` function was

7 used to test if the mean number of velamen layers in epiphytic taxa is significantly different

8 from that of terrestrial taxa, while accounting for the phylogenetic relatedness between

9 species. As several genera are both epiphytic and terrestrial, we repeated the PGLS ten times,

10 each time randomly assigning each polymorphic genus to be only epiphytic or terrestrial.

11

12 *Ancestral ranges of the Epidendroideae*

13

14 To address both the time periods and the biogeographic and palaeoclimatic context of the

15 emergence of the epiphytic lifestyle, we estimated the ancestral biogeographic ranges of

16 *Epidendroideae* on their time-calibrated MCC tree, using BioGeoBEARS (Matzke, 2013).

17 Bioregions were defined as in Givnish *et al.* (2016): North America, Neotropics, Africa,

18 Eurasia, Africa, Southeast Asia, Australia, and Pacific. The models DEC*, DEC*+J, DIVA*,

19 DIVA*+J, BayArea*, and BayArea*+J (excluding a null range) were tested, with a maximal

20 range size of 7, and thus 127 possible states. Time-stratified dispersal multipliers were set

21 following Givnish *et al.* (2016) (Supplementary Data Table S4). The best-fitting model was

22 chosen by AICc comparison (Supplementary Data Table S5) and likelihood-ratio tests

23 (Supplementary Data Table S6).

24

1 RESULTS

2

3 *Phylogenetic relationships and divergence times between Epidendroideae genera*

4

5 All epidendroid subtribes *sensu* Chase *et al.* (2015) were found monophyletic in the obtained
6 phylogenetic tree of 203 genera of Epidendroideae, except for the subtribes Cymbidinae and
7 Eulophiinae which were split into two and three clades, respectively. Posterior probabilities
8 (PP) and constrained nodes are presented in Supplementary Data Fig. S1. According to our
9 time-calibration based on three fossils (including a minimal age for the Epidendroideae based
10 on the fossil described in Poinar and Rasmussen (2017)), the crown age of Epidendroideae
11 was estimated at 47.4 Myr (95% Highest Posterior Density (95% HPD) interval = 63.2–45.0
12 Myr). Tribe crown ages and corresponding 95% HPD intervals are summed up in
13 Supplementary Data Table S7.

14

15 *Test of correlated evolution between epiphytism and succulence traits*

16

17 The evolution of leaf succulence was found to be correlated with the lifestyle using both
18 BayesTraits and corHMM (Table 1). The evolution of stem succulence was found to be
19 correlated with the lifestyle with BayesTraits only, while corHMM returned a non-significant
20 difference in AICc between the correlated and the uncorrelated models (Table 1). As both
21 traits were found significantly correlated using at least one method, we jointly estimated their
22 evolutionary histories with the lifestyle (using correlated matrices of transition between states)
23 in corHMM.

24

1 *Gains and losses of the epiphytic lifestyle*

2

3 The evolutionary history of the epiphytic lifestyle was consistent across corHMM models, *i.e.*
4 either with models correlated to leaf (Fig. 2) or stem (Supplementary Data Fig. S2)
5 succulence. The correlated model of evolution between the lifestyle and leaf succulence (Fig.
6 2) inferred that the most recent common ancestor (MRCA) of Epidendroideae was likely
7 terrestrial ($p = 0.99$) and ranged from the Neotropics to Southeast Asia and Australia ($p =$
8 0.58). In agreement with Freudenstein and Chase (2015), we found that the epiphytic lifestyle
9 likely appeared at least three times independently in ancestral nodes, namely in (i) the MRCA
10 of Malaxideae and Cymbidieae 39.0 Myr ago ($p = 0.98$, node PP = 1 (backbone node), 95%
11 HPD = 51.7–32.8 Myr) in Southeast Asia and Australia ($p = 0.94$, Fig. 3), (ii) in the MRCA of
12 *Dendrochilum* and *Panisea* in Arethuseae 11.5 Myr ago ($p = 0.98$, node PP = 0.98, 95% HPD
13 = 18.3–6.3 Myr) in Southeast Asia and Australia ($p = 0.94$, Fig. 3), and (iii) in the MRCA of
14 Sobralieae 7.1 Myr ago ($p = 0.71$, node PP = 1 (backbone node), 95% HPD = 17.1–1.7 Myr)
15 in the Neotropics ($p = 0.98$, Fig. 3).

16

17 Likewise, we inferred at least five transitions from the epiphytic lifestyle to terrestriality
18 (secondary terrestrialization) leading to diversification in (i) the MRCA of *Oberonioides* and
19 *Malaxis* in Malaxideae 17.0 Myr ago ($p = 0.89$, node PP = 1, 95% HPD = 25.9–10.1 Myr), (ii)
20 in the Collabieae excluding *Eriodes* 22.8 Myr ago ($p = 0.97$, node PP = 1, 95% HPD = 32.9–
21 14.5 Myr), (iii) in the MRCA of Calypsoeae 30.3 Myr ago ($p = 0.83$, 95% HPD = 40.7–22.8
22 Myr), (iv) in the MRCA of Bletiinae 12.4 Myr ago ($p = 0.95$, node PP = 1, 95% HPD = 20.7–
23 6.1 Myr), and (v) in the MRCA of *Eulophia* and *Oeceoclades* 12.6 Myr ago ($p = 0.97$, node
24 PP = 0.83, 95% HPD = 18.6–7.5 Myr).

1

2 Using the posterior probability distribution of trait changes from stochastic mapping,
3 transitions from the terrestrial to the epiphytic lifestyles were estimated to have occurred three
4 to seven times (95% HPD interval) in the phylogeny with a median of four changes. Reverse
5 transitions *i.e.*, from the epiphytic to the terrestrial lifestyle, were estimated to have occurred
6 ten to 16 times (95% HPD interval) in the phylogeny, with a median of 13.

7

8 *Gains and losses of drought-related traits (stem succulence, leaf succulence and velamen)*

9

10 Ancestral state estimation of stem succulence was consistent either when considering the
11 presence-absence of succulence (Supplementary Data Fig. S3) or when dividing the succulent
12 stems into heteroblastic (one internode swollen) or homoblastic (several internodes in
13 succession swollen) (Fig. 2, Supplementary Data Fig. S4). The absence of stem succulence
14 was inferred as ancestral in Epidendroideae ($p = 0.80$). Confirming the general assumption
15 (Dressler, 1981; Ng and Hew, 2000), stem succulence was inferred to be ancestrally
16 homoblastic, and to have appeared only once, in the MRCA of Nervilieae and Cymbidieae
17 43.1 Myr ago ($p = 0.82$, node PP = 1 (backbone node), 95% HPD = 57.3–36.6 Myr) (Fig 2,
18 Supplementary Data Fig. S4A) in the Neotropics, Southeast Asia and Australia ($p = 0.45$, Fig.
19 3) or in Southeast Asia and Australia only ($p = 0.45$, Fig. 3), thus predating the epiphytic
20 lifestyle by at least 4.1 Myr.

21 Heteroblastic succulent stems evolved repeatedly from homoblastic pseudobulbs at least eight
22 times throughout the phylogeny (distribution of changes from stochastic mapping: 95% HPD
23 [9, 16], median = 12). Stem succulence appeared to have been lost several times. Transition
24 rates (Fig. S4B) indicated that homoblastic pseudobulbs tended to be either lost (0.02

1 event/Myr) or to evolve into heteroblastic pseudobulbs (0.011 event/Myr). Heteroblastic stem
2 succulence derived predominantly from homoblasty (0.011 event/Myr) rather than from
3 absence of succulence ($3.5e-5$ event/Myr) and was lost as often as it was acquired (0.013
4 event/Myr), though mostly at tips (Supplementary Data Fig. S4).

5

6 Succulent leaves (Fig. 2, Supplementary Data Fig. S5) were inferred to have appeared several
7 times convergently (distribution of changes from stochastic mapping: 95% HPD [33, 52],
8 median = 43 in epiphytic taxa; 95% HPD [77, 112], median = 93 in terrestrial taxa), mostly at
9 tips, and particularly during the Oligocene in epidendroid lineages that were probably already
10 epiphytic at that time (*i.e.* in Podochileae, Epidendreae, Vandaeae, and at least twice in
11 Cymbidieae). However, even though succulent leaves evolved predominantly in epiphytic
12 groups, some terrestrial taxa also evolved it, for example in genera *Oberonioides*, *Acrolophia*,
13 or *Cyrtopodium*. In Vandaeae, the appearance of leaf succulence 26.6 Myr ago ($p = 0.76$, node
14 PP = 1, 95% HPD = 36.5–19.3 Myr) was inferred to coincide with the loss of stem succulence
15 ($p = 0.86$, node PP = 1, 95% HPD = 36.5–19.3 Myr).

16

17 The number of velamen layers (Supplementary Data Fig. S6) tended to be ancestrally low in
18 Epidendroideae, and seems to have increased in the MRCA of *Panisea* and *Bulleyia* in
19 Arethuseae, in Dendrobiinae, in Collabieae, in Laeliinae, and in most of Cymbidieae, as well
20 as in *Govenia* (tribe Calypsoeae). In Vandaeae, most genera miss data on velamen, thus
21 inheriting ancestral state without change, hence some genera may actually have a high
22 number of velamen layers in this tribe. As expected, primarily terrestrial tribes tended to have
23 only a few velamen layers, but some secondary terrestrial genera, *i.e.* *Govenia*, *Cyrtopodium*,
24 and the subtribe Eulophiinae have among the highest numbers of layers. All PGLS models

1 indicated that terrestrial taxa tended to have fewer velamen layers than epiphytic ones, but a
2 high number of velamen layers was not significantly associated with epiphytic lifestyle (range
3 of F-statistic [2.2–0.099] on 1 and 135 DF, p-value [0.14–0.75]).

4

5 *Transition rates between correlated lifestyle and succulence traits*

6

7 Using the posterior probability distribution of trait changes from the stochastic character
8 mapping, we estimated that transitions from terrestrial to epiphytic lifestyles only occurred in
9 lineages with succulent stems (0.0087 event/Myr, Fig. 2C(i)), while such transitions were
10 almost impossible in non-succulent lineages (1e-9 event/Myr, Fig. 2C(ii)). Conversely, we
11 reported that transitions from terrestrial to epiphytic lifestyles occurred in lineages without
12 thickened leaves (0.0041 and 0.011 event/Myr, Fig. 2D(iii, iv)), with almost no transitions
13 between terrestrial and epiphytic species with succulent leaves (1e-9 event/Myr, Fig. 2D(v)).

14

15 **DISCUSSION**

16

17 *Where and when did the epiphytic lifestyle and succulence traits evolved?*

18

19 The first occurrence of the epiphytic lifestyle in Epidendroideae was inferred at the end of the
20 Eocene in a range encompassing the Neotropics, Southeast Asia, and Australia (Figs. 2 and
21 3). Over the course of the Eocene, the global climate gradually became cooler and drier
22 (Bohaty *et al.*, 2009; Bush *et al.*, 2011), leading to a major retraction of megathermal forests
23 (Bush *et al.*, 2011) and to the development of new open and drier habitats (Bobe, 2006;
24 Woodcock and Meyer, 2020). Even though geographical ranges were different between

1 Aizoaceae and Epidendroideae, this origin coincides with the origin of Aizoaceae, an entirely
2 African succulent group (41.5 Myr, 95% HPD = 38.7–56.4 Myr; (Arakaki *et al.*, 2011; Klak
3 *et al.*, 2017). In several groups, succulence evolved in arid desert systems and in semi-arid
4 systems such as savannas (Ringelberg *et al.*, 2020; Anest *et al.*, 2021). Likewise, stem
5 succulence in Epidendroideae could have been an adaptation to drier, seasonal forests or
6 savannas. With at least ancestral stem succulence (Fig. 2), epiphytism could have arisen in
7 seasonally dry forests or dry microhabitats, maybe primarily on cliffs and rocky areas as
8 hypothesised by Dressler (1981), among taxa already adapted to drought. This conclusion is
9 also consistent with Frenzke *et al.* (2016), who hypothesised as well that the terrestrial
10 ancestor of epiphytic *Peperomia* (Piperaceae) already showed water-related traits potentially
11 facilitating epiphytic life such as succulence or CAM metabolism.

12

13 In Arethuseae, epiphytism likely appeared during the Miocene in the Southeast Asian and
14 Australian range, when the climate was cooling again after the Mid-Miocene climatic
15 optimum and moist megathermal forests were restricted to the tropical zone, with the
16 exception of the Australasian region (Bush *et al.*, 2011). In Southeast Asia, everwet climates
17 predominated, but some regions could have been wet but seasonal, with open vegetation
18 (Bush *et al.*, 2011). Thus, it is unclear if epiphytic Arethuseae most likely appeared in
19 seasonal or everwet forests, and in addition the ancestral stem succulence could have
20 facilitated the transition to the epiphytic habitat in either environment. Moreover,
21 Coelogyninae are at present predominantly inhabiting everwet forests, and occur less
22 frequently in areas with seasonal climates (Pridgeon *et al.*, 2005).

23

24 Our interpretation that the epiphytic lifestyle could have evolved in seasonally dry forests

1 generally contrast with the presumed evolution of other epiphytic lineages, such as
2 Bromeliaceae (Givnish *et al.*, 2014), ferns (Schuettpelez and Pryer, 2009; Chen *et al.*, 2022),
3 Lycopodiaceae (Wikström *et al.*, 1999) or thalloid liverworts (Bechteler *et al.*, 2021), which
4 were rather found to have originated mostly in rainforests or moist montane forests. In some
5 of these other taxa, epiphytism is indeed unrelated to drought-tolerant traits, or drought-
6 tolerant traits evolved as adaptations to the epiphytic lifestyle. Even among the
7 Epidendroideae, the last appearance of the epiphytic lifestyle likely occurred in Sobralieae in
8 absence of any type of succulence 7.1 Myr ago (95% HPD = 17.1–1.7 Myr), at the end of the
9 Miocene, in the Neotropics. In tank bromeliads (Bromeliaceae), epiphytism evolved around
10 5.9 Myr in the late Miocene, in the Atlantic forest region of Neotropics, during a global
11 cooling of the climate, synchronously with the uplift of the Serra do Mar which would have
12 favoured cooler, rainier, more humid conditions in the Atlantic forest region (Givnish *et al.*,
13 2014). Moreover, humid montane habitats were found to have favoured epiphytism in
14 Bromeliaceae (Givnish *et al.*, 2014). By the Miocene, mountain uplift had created abundant
15 topographic relief across the Neotropics (Potter and Szatmari, 2009; Givnish *et al.*, 2014;
16 Martins *et al.*, 2018). The lack of succulent organs in Sobralieae is suggestive that this tribe
17 could have evolved in humid, montane habitats.

18

19 Nevertheless, the hypothesis that epiphytism in all Epidendroideae evolved in everwet forests
20 should not be rejected: in Epidendroideae the pre-adaptation to drought would also have been
21 beneficial in everwet habitats. Indeed, CAM photosynthesis, a water-conserving metabolism,
22 could have been selected in high rainfall habitats as has been observed for *Bulbophyllum*
23 (Gamisch *et al.*, 2021), and many species of Epidendroideae with succulent organs now grow
24 in the shade of everwet forests (Pridgeon *et al.*, 2005, 2009, 2014). On the other hand, in the

1 genus *Crassula* (Crassulaceae) succulence is also found in species of mesic or wet
2 environments, and has been suggested to be evolutionarily conserved after having been
3 selected in ancestral dry microhabitats (Fradera-Soler *et al.*, 2021).

4
5 *Succulence traits as key innovations for the evolution of epiphytic epidendroid orchids*

6
7 Stem succulence likely evolved prior to the epiphytic lifestyle, and epiphytes evolved far
8 more frequently among lineages with thickened stems than among lineages without this
9 feature (Fig. 2). It is likely that stem succulence emerged as an adaptation to water shortage in
10 seasonally dry climates, and that it proved to be advantageous in an epiphytic environment.
11 Moreover, even though stem succulence has sometimes been lost in epiphytic lineages, it was
12 almost systematically offset by the appearance of leaf succulence, as in Vandaeae or
13 Pleurothallidinae for example (Fig. 2). We thus propose that stem succulence has been a key
14 innovation (as defined by Miller *et al.* (2023): ‘an organismal feature that enables a species to
15 occupy a previously inaccessible ecological state’) for the evolution of epiphytism in
16 Epidendroideae.

17
18 The definition of key innovation as stated by Miller *et al.* (2023) does not include an
19 enhanced diversification, because, as they explain, ‘the expectation that key innovations
20 should result in increased species richness or adaptive radiation is conceptually problematic
21 [...] because it conflates two distinct evolutionary phenomena: diversification in species
22 richness, and shifts in ecology’. Nevertheless, it would be interesting to test the impact of
23 succulence traits on the diversification of epiphytes (Miller and Stroud, 2021), for example
24 using trait-dependent diversification analyses such as Hidden State Speciation and Extinction

1 models (Beaulieu and O'Meara, 2016; Herrera-Alsina *et al.*, 2019; Nakov *et al.*, 2019).
2 However, in order to be accurate these analyses require robust species-level phylogenies, with
3 a sampling fraction ideally > 50%, which is not the case of the phylogenetic tree we were able
4 to produce until now. Nevertheless, Givnish *et al.* (2015) detected a shift towards higher
5 diversification rates in the MRCA of Arethuseae and Cymbidieae, corresponding
6 approximately to the first appearance of epiphytism, and subsequently including the majority
7 of epiphytic Epidendroideae. Stem succulence, by facilitating the transition to the epiphytic
8 lifestyle, would have indirectly enabled diversification bursts within the Epidendroideae.

9

10 In this study we focused on a few traits, however there are other traits that could also have
11 significantly contributed to the evolution of epiphytism and the diversification of orchids,
12 notably the water-conserving CAM photosynthesis (Silvera *et al.*, 2009; Givnish *et al.*, 2015;
13 Silvera and Lasso, 2016) which is often associated to succulent organs (Niechayev *et al.*,
14 2019). Even though recent studies did not find a link between CAM photosynthesis and an
15 increase in diversification rates, at least in *Bulbophyllum* (Gamisch *et al.*, 2021; Hu *et al.*,
16 2022), an estimation of the evolution of CAM photosynthesis has been conducted by Silvera
17 *et al.* (2009) at subtribe and genera levels, then by Givnish *et al.* (2015) at subtribe level, and
18 in spite of high uncertainties in the ancestral state estimations and/or in tree topology, their
19 results nevertheless indicate that CAM photosynthesis could also have appeared prior to
20 epiphytism. In the future, further investigation of the evolutionary history of CAM
21 metabolism in Epidendroideae could thus be interesting.

22

23 *Secondary terrestrial genera mostly retained the epiphytic ancestral drought-related traits*

24

1 Epiphytism has probably been lost multiple times during the Oligocene and the Miocene, in
2 different geographical ranges (Figs. 2 and 3). As reported by Chen *et al.* (2022) in the fern
3 family Polypodiaceae, re-terrestrialization in Calypsoeae likely occurred during the Oligocene
4 in Southeast Asia and the Neotropics, when a glaciation led to the decrease of the area of
5 broad-leaved forests and available habitats, likely increasing the competition in upper
6 canopies (Chen *et al.*, 2022). Opening seasonal forests and savannas may have promoted the
7 re-terrestrialization of epiphytes, by allowing more light to reach the floor than broad-leaved
8 forests thus releasing the competition for light in the understory (Dressler, 1981; Wikström
9 *et al.*, 1999), and by allowing plants to occupy a wider range of habitats (Chen *et al.*, 2022).
10 In Collabieae, Malaxideae, Bletinae, and Eulophiinae, epiphytism was likely lost during the
11 Miocene. This geological epoch was characterised by fluctuations in the global climate, with
12 rapidly changing environments and temperatures, and by widespread topographic changes
13 which created new habitats (Potter and Szatmari, 2009; Bush *et al.*, 2011), enhancing the
14 diversification of many lineages (Potter and Szatmari, 2009). Hence, as hypothesised by Chen
15 *et al.* (2022) for Polypodiaceae, it is likely that new habitats created by climate and
16 topographical changes could have favoured the re-terrestrialization and subsequent
17 diversification of the secondary terrestrial clades in Epidendroideae, but also of the epiphytic
18 taxa which seem to have diversified mostly during the Miocene (Supplementary Data Fig. S7,
19 S8). In Eulophiinae, whose diversity is centered on Madagascar (Bone *et al.*, 2015), re-
20 terrestrialization occurred between 12 and 13 Myr ago, *i.e.* simultaneously with the
21 appearance of the cactiform stem succulence in the genus *Euphorbia* sections *Goniostema*,
22 *Denisophorbia* and *Deuterocalli* which represent about 70% of the diversity of *Euphorbia* in
23 Madagascar (Aubriot, 2012). The cactiform stem succulence has been found to prevail in
24 areas with seasonal drought but a reliable season of precipitation (Evans *et al.*, 2014), which

1 means that seasonal dry forests may have been present in Madagascar at this time and thus
2 could have been favourable to the re-terrestrialization of Eulophiinae.

3

4 If we examine the evolutionary history of succulence traits in Epidendroideae, it appears that,
5 on the whole, secondary terrestrial taxa retained their ancestral succulent stems (Fig. 2). In
6 Calypsoeae and Blettiinae, this stem succulence takes the form of underground corms, while in
7 Malaxideae, Collabieae and Eulophiinae it is pseudobulbs (Pridgeon *et al.*, 2005, 2009).
8 However, pseudobulbs are sometimes more or less buried, for example in Collabieae genus
9 *Ipea* (Pridgeon *et al.*, 2005; Descourvières, 2011), or in *Eulophia graminea* (Pemberton *et*
10 *al.*, 2008). Actually, burying succulent structures could better protect them from above-
11 ground temperatures extremes and drought, especially in seasonal climates (Pemberton *et al.*,
12 2008; Kumar *et al.*, 2022). In addition, Kumar *et al.* (2022) showed that some species in
13 Malaxideae are secondary epiphytes, and that they also retained the perennating organ
14 structure and leaf texture of their parent lineage. This tends to support the hypothesis that
15 seasonal forests could have been the cradle to at least part of both epiphytes and terrestrial
16 Epidendroideae diversity.

17

18 The current ecology of secondary terrestrials is also not exactly the same as the primary
19 terrestrial taxa. Indeed, unlike the primary terrestrial taxa, many secondary terrestrials are also
20 found as lithophytes, or even as occasional epiphytes (Pridgeon *et al.*, 2005, 2009, 2014).
21 Even though there are differences between the lithophytic and epiphytic habitats, they still
22 share similar rooting conditions (Zotz, 2016), and similar mycorrhizal fungal communities are
23 shared by orchids between the two habitats (Xing *et al.*, 2019; Qin *et al.*, 2020). Moreover,
24 lithophytic Malaxideae were not found on the rock itself but in the humus-rich and mossy

1 substrate (Hermans *et al.*, 2020), resembling some epiphytic conditions. Even among genera
2 that were described as fully terrestrial in *Genera Orchidacearum* (Pridgeon *et al.*, 2005, 2009,
3 2014), some may not always really root in the soil.

4
5 While epiphytes tend to have slightly more velamen layers than terrestrials, the re-
6 terrestrialization did not particularly lead to a reduction of the number of velamen layers, and
7 this number may have even increased in some secondary terrestrial clades (Supplementary
8 Data Fig. S9). The velamen allows a quick uptake and long retention (increasing with
9 velamen size) of water and nutrients, which is beneficial in environments with low and
10 intermittent water and nutrient supply (Zotz and Winkler, 2013). Other important functions
11 are mechanical protection and, in exposed roots, reduction of heat load (Zotz and Winkler,
12 2013; Zotz *et al.*, 2017) and UV protection (Chomicki *et al.*, 2015). Hence, velamentous roots
13 may have been beneficial in secondary terrestrial taxa with lithophytic-like ecology, whose
14 roots can be exposed to a scarce supply of water and UV radiation as for epiphytic taxa.
15 Furthermore, even rooted in soil, numerous taxa in Orchidaceae and other families are
16 velamentous, for the velamen minimises the water loss in dry soils without damaging the root
17 unlike other, nonvelamentous, taxa (Zotz and Winkler, 2013; Zotz *et al.*, 2017). Indeed, Zotz
18 *et al.* (2017) found that terrestrial species were most prominent in seasonally dry habitats,
19 which is consistent with our hypothesis of re-terrestrialization events in seasonally dry
20 environments. In addition, Zotz *et al.* (2017) suggested that velamen predated epiphytism, and
21 thus could have been another key innovation (in addition to stem succulence as shown in this
22 study) for the evolution of epiphytism, a view also supported by our results. As well, this is
23 consistent with the hypothesis that epiphytism appeared in mostly seasonally dry climates.

24

1 Finally, considering that secondary terrestrials mostly retained their ancestral velamen and
2 succulence traits, we could wonder why some of these taxa are no longer found as epiphytes.
3 The minute orchid seeds should not have constrained dispersal between ground and aerial
4 environments, although different communities of suitable mycorrhizal fungi should (Martos *et*
5 *al.*, 2012). Indeed, given that orchids germinate with mycorrhizal fungi, we hypothesise that
6 these taxa could no longer colonise the epiphytic habitat because of an evolutionary change in
7 their association with mycorrhizal fungal symbionts, which are different between the ground
8 and the trees (Martos *et al.*, 2012). Mycorrhizal shifts are also suggested by the fact that all
9 terrestrial lineages derived from epiphytic ancestors have evolved strategies of
10 mycoheterotrophy by parasitizing soil-dwelling, ectomycorrhizal or saprotrophic fungi.
11 Mycoheterotrophy is also often seen as an adaptation to shaded forest understorey (Martos *et*
12 *al.*, 2009). Likewise, even though light conditions of epiphytes span the entire gradient from
13 deep shade to full radiation, many epiphytes may not tolerate shade, and low light in the
14 understorey could thus promote epiphytism (Gravendeel *et al.*, 2004; Zotz, 2013). Therefore,
15 because most of Epidendroideae taxa evolved epiphytism, and primary and secondary
16 terrestrial taxa have often evolved mycoheterotrophy, access to light may have been a major
17 constraint in the evolution of Epidendroideae, leading to the evolution of either epiphytism or
18 mycoheterotrophy.

19

20 *Assumptions and intrinsic limitations of methods*

21

22 We identified two main methodological limitations intrinsic to the methods we used. First,
23 even though our phylogeny is robust and congruent with previous studies based on a higher
24 number of plastid markers, the divergence times were based on only three fossils (including a

1 fossil with an uncertain phylogenetic position), while the Epidendroideae are very much
2 diversified with no less than 23,246 species listed (WCSP, 2021). In addition, divergence time
3 confidence intervals are large. Because we allowed the model to infer much older values than
4 our fossil calibrations, such large intervals are partly artefacts of the probability distribution
5 we used for calibration points combined with the relaxed molecular clock. Nevertheless, new
6 orchid fossils would be beneficial to ascertain the divergence times of the subfamily.
7 Second, models of ancestral state estimation rely on several assumptions. For instance
8 discrete state models assumes that transition rates between states are constant throughout the
9 phylogeny, even though hidden states in corHMM alleviate to some extent this assumption.
10 Moreover, all transitions are likely to be slightly underestimated due to the processing of
11 polymorphic traits as uncertainties and not as polymorphisms by corHMM; in other words,
12 we only estimated transitions between sampled genera, but were not able to measure within-
13 genus transitions.

14

15 **CONCLUSION**

16

17 Our analyses show that drought-related traits probably did not emerge as adaptations to the
18 epiphytic lifestyle. Rather, epiphytes would have appeared among already drought-adapted
19 terrestrial lineages in seasonally-dry forests, possibly driven by light availability. Particularly,
20 the ancestral stem succulence would have favoured the multiple colonization of aerial
21 environments. This character would have evolved once in the form of a homoblastic
22 pseudobulb, later evolving convergently towards heteroblastic pseudobulbs. When lost, stem
23 succulence would have almost always been offset by leaf succulence, indicating that succulent
24 organs could have actually been a key innovation for the emergence of the epiphytic lifestyle.

1 Other physiological and morpho-anatomical traits, such as the CAM photosynthesis, should
2 be further investigated in combination with diversification analyses, to further comprehend
3 the macroevolutionary history of epiphytism in Epidendroideae.

4

5 **ACKNOWLEDGEMENTS**

6

7 We thank B. Bytebier et T. Stévant for constructive feedback on these results. We also thank
8 the Editor and the two anonymous reviewers for their constructive feedbacks.

1 LITERATURE CITED

- Anest A, Charles-Dominique T, Maurin O, Millan M, Edelin C, Tomlinson KW. 2021.** Evolving the structure: climatic and developmental constraints on the evolution of plant architecture. A case study in *Euphorbia*. *New Phytologist* **231**: 1278–1295.
- Arakaki M, Christin P-A, Nyffeler R, et al. 2011.** Contemporaneous and recent radiations of the world's major succulent plant lineages. *Proceedings of the National Academy of Sciences* **108**: 8379–8384.
- Aubriot X. 2012.** *Radiations évolutives," innovations clés" et notions d'espèces dans le genre Euphorbia L. à Madagascar*. PhD Thesis, Muséum national d'histoire naturelle, Paris, France.
- Beaulieu JM, O'Meara BC. 2016.** Detecting Hidden Diversification Shifts in Models of Trait-Dependent Speciation and Extinction. *Systematic Biology* **65**: 583–601.
- Bechteler J, Schäfer-Verwimp A, Glenny D, et al. 2021.** The evolution and biogeographic history of epiphytic thalloid liverworts. *Molecular Phylogenetics and Evolution* **165**: 107298.
- Benzing DH. 1987.** Vascular Epiphytism: Taxonomic Participation and Adaptive Diversity. *Annals of the Missouri Botanical Garden* **74**: 183.
- Benzing DH. 1990.** *Vascular Epiphytes: General Biology and Related Biota*. Cambridge: Cambridge University Press.
- Bohe R. 2006.** The evolution of arid ecosystems in eastern Africa. *Journal of Arid*

Environments **66**: 564–584.

Bohaty SM, Zachos JC, Florindo F, Delaney ML. 2009. Coupled greenhouse warming and deep-sea acidification in the middle Eocene. *Paleoceanography* **24**.

Bone RE, Cribb PJ, Buerki S. 2015. Phylogenetics of Eulophiinae (Orchidaceae: Epidendroideae): evolutionary patterns and implications for generic delimitation. *Botanical Journal of the Linnean Society* **179**: 43–56.

Bouckaert RR, Drummond AJ. 2017. bModelTest: Bayesian phylogenetic site model averaging and model comparison. *BMC Evolutionary Biology* **17**: 42.

Bouckaert R, Vaughan TG, Barido-Sottani J, et al. 2019. BEAST 2.5: An advanced software platform for Bayesian evolutionary analysis (M Perteua, Ed.). *PLOS Computational Biology* **15**: e1006650.

Boyko JD, Beaulieu JM. 2021. Generalized hidden Markov models for phylogenetic comparative datasets (N Cooper, Ed.). *Methods in Ecology and Evolution* **12**: 468–478.

Bush M, Flenley J, Gosling W (Eds.). 2011. *Tropical Rainforest Responses to Climatic Change*. Berlin, Heidelberg: Springer Berlin Heidelberg.

Chase MW, Cameron KM, Freudenstein JV, et al. 2015. An updated classification of Orchidaceae. *Botanical Journal of the Linnean Society* **177**: 151–174.

Chen C, Hyvönen J, Schneider H. 2022. Re-terrestrialization in the phylogeny of epiphytic plant lineages: Microsoroid ferns as a case study. *Journal of Systematics and*

Evolution: jse.12899.

Chomicki G, Bidet LPR, Ming F, et al. 2015. The velamen protects photosynthetic orchid roots against UV-B damage, and a large dated phylogeny implies multiple gains and losses of this function during the Cenozoic. *New Phytologist* **205**: 1330–1341.

Conran JG, Bannister JM, Lee DE. 2009. Earliest orchid macrofossils: Early Miocene *Dendrobium* and *Earina* (Orchidaceae: Epidendroideae) from New Zealand. *American Journal of Botany* **96**: 466–474.

Cribb PJ. 1999. II Morphology In: Genera Orchidacearum. *Genera Orchidacearum: Volume 1: Apostasioideae and Cyripedioideae*. Oxford, New York: Oxford University Press, 13–23.

Descourvières P. 2011. *Encyclopédie des orchidées tropicales*. Paris: Ulmer.

Dressler RL. 1981. *The orchids : natural history and classification*. Cambridge, Mass. : Harvard University Press.

Evans M, Aubriot X, Hearn D, et al. 2014. Insights on the Evolution of Plant Succulence from a Remarkable Radiation in Madagascar (*Euphorbia*). *Systematic Biology* **63**: 697–711.

Fradera-Soler M, Rudall PJ, Prychid CJ, Grace OM. 2021. Evolutionary success in arid habitats: Morpho-anatomy of succulent leaves of *Crassula* species from southern Africa. *Journal of Arid Environments* **185**: 104319.

Frenzke L, Goetghebeur P, Neinhuis C, Samain M-S, Wanke S. 2016. Evolution of

Epiphytism and Fruit Traits Act Unevenly on the Diversification of the Species-Rich Genus *Peperomia* (Piperaceae). *Frontiers in Plant Science* **07**.

Freudenstein JV, Chase MW. 2015. Phylogenetic relationships in Epidendroideae (Orchidaceae), one of the great flowering plant radiations: progressive specialization and diversification. *Annals of Botany* **115**: 665–681.

Fu Z, Martin CE, Do J, Ho C-L, Wagner B. 2022. Functional relationship between leaf/stem pseudobulb size and photosynthetic pathway in the Orchidaceae (B Beres, Ed.). *Canadian Journal of Plant Science* **102**: 419–426.

Gamisch A, Comes HP. 2019. Clade-age-dependent diversification under high species turnover shapes species richness disparities among tropical rainforest lineages of *Bulbophyllum* (Orchidaceae). *BMC Evolutionary Biology* **19**: 93.

Gamisch A, Winter K, Fischer GA, Comes HP. 2021. Evolution of crassulacean acid metabolism (CAM) as an escape from ecological niche conservatism in Malagasy *Bulbophyllum* (Orchidaceae). *New Phytologist* **231**: 1236–1248.

Givnish TJ, Barfuss MHJ, Ee BV, et al. 2014. Adaptive radiation, correlated and contingent evolution, and net species diversification in Bromeliaceae. *Molecular Phylogenetics and Evolution* **71**: 55–78.

Givnish TJ, Spalink D, Ames M, et al. 2015. Orchid phylogenomics and multiple drivers of their extraordinary diversification. *Proceedings of the Royal Society B: Biological Sciences* **282**: 20151553.

Givnish TJ, Spalink D, Ames M, et al. 2016. Orchid historical biogeography,

- diversification, Antarctica and the paradox of orchid dispersal. *Journal of Biogeography* **43**: 1905–1916.
- Gravendeel B, Smithson A, Slik FJW, Schuiteman A. 2004.** Epiphytism and pollinator specialization: drivers for orchid diversity? (PT Pennington, QCB Cronk, and JA Richardson, Eds.). *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences* **359**: 1523–1535.
- Hermans J, Verlynde S, Cribb P, Ramandimbisoa B, Hervouet J-M, Bernet P. 2020.** Malaxideae (Orchidaceae) in Madagascar, the Mascarenes, Seychelles and Comoro Islands. *Kew Bulletin* **75**: 1.
- Herrera-Alsina L, Van Els P, Etienne RS. 2019.** Detecting the Dependence of Diversification on Multiple Traits from Phylogenetic Trees and Trait Data (M Alfaro, Ed.). *Systematic Biology* **68**: 317–328.
- Hietz P, Wagner K, Nunes Ramos F, et al. 2022.** Putting vascular epiphytes on the traits map. *Journal of Ecology* **110**: 340–358.
- Hu A-Q, Gale SW, Liu Z-J, Fischer GA, Saunders RMK. 2022.** Diversification Slowdown in the *Cirrhopetalum* Alliance (*Bulbophyllum*, Orchidaceae): Insights From the Evolutionary Dynamics of Crassulacean Acid Metabolism. *Frontiers in Plant Science* **13**: 794171.
- Kalyaanamoorthy S, Minh BQ, Wong TK, Von Haeseler A, Jermin LS. 2017.** ModelFinder: fast model selection for accurate phylogenetic estimates. *Nature methods* **14**: 587–589.

- Klak C, Hanáček P, Bruyns PV. 2017.** Out of southern Africa: Origin, biogeography and age of the Aizooideae (Aizoaceae). *Molecular Phylogenetics and Evolution* **109**: 203–216.
- Kumar P, Li J, Gale SW. 2022.** Integrative analyses of *Crepidium* (Orchidaceae, Epidendroideae, Malaxideae) shed more light on its relationships with *Dienia*, *Liparis* and *Malaxis* and justify reinstatement of narrow endemic *C. allanii*. *Botanical Journal of the Linnean Society* **198**: 285–305.
- Li Y-X, Li Z-H, Schuiteman A, et al. 2019.** Phylogenomics of Orchidaceae based on plastid and mitochondrial genomes. *Molecular Phylogenetics and Evolution* **139**: 106540.
- Luttge U. 2004.** Ecophysiology of Crassulacean Acid Metabolism (CAM). *Annals of Botany* **93**: 629–652.
- Martins AC, Bochner T, Pérez-Escobar OA, Chomicki G, Monteiro SHN, De Camargo Smidt E. 2018.** From tree tops to the ground: Reversals to terrestrial habit in *Galeandra* orchids (Epidendroideae: Catasetinae). *Molecular Phylogenetics and Evolution* **127**: 952–960.
- Martos F, Dulormne M, Paillet T, et al. 2009.** Independent recruitment of saprotrophic fungi as mycorrhizal partners by tropical achlorophyllous orchids. *New Phytologist* **184**: 668–681.
- Martos F, Munoz F, Paillet T, Kottke I, Gonneau C, Selosse M-A. 2012.** The role of epiphytism in architecture and evolutionary constraint within mycorrhizal networks of tropical orchids. *Molecular Ecology* **21**: 5098–5109.

- Matzke NJ. 2013.** *Probabilistic historical biogeography: new models for founder-event speciation, imperfect detection, and fossils allow improved accuracy and model-testing*. PhD Thesis, University of California, Berkeley, USA.
- Miller AH, Stroud JT. 2021.** Novel Tests of the Key Innovation Hypothesis: Adhesive Toepads in Arboreal Lizards (J Uyeda, Ed.). *Systematic Biology* **71**: 139–152.
- Miller AH, Stroud JT, Losos JB. 2023.** The ecology and evolution of key innovations. *Trends in Ecology & Evolution* **38**: 122–131.
- Nakov T, Beaulieu JM, Alverson AJ. 2019.** Diatoms diversify and turn over faster in freshwater than marine environments*. *Evolution* **73**: 2497–2511.
- Ng CKY, Hew CS. 2000.** Orchid pseudobulbs – “false” bulbs with a genuine importance in orchid growth and survival! *Scientia Horticulturae* **83**: 165–172.
- Niechayev NA, Pereira PN, Cushman JC. 2019.** Understanding trait diversity associated with crassulacean acid metabolism (CAM). *Current Opinion in Plant Biology* **49**: 74–85.
- Orlov NM, Viktorova VA, Eskov AK. 2022.** CAM (Crassulacean Acid Metabolism) Photosynthesis in Vascular Epiphytes. *Biology Bulletin Reviews* **12**: 527–543.
- Pemberton RW, Collins TM, Koptur S. 2008.** An Asian orchid, *Eulophia graminea* (Orchidaceae: Cymbidieae), naturalizes in Florida. *Lankesteriana* **8**: 5–14.
- Pérez-Escobar OA, Dodsworth S, Bogarín D, et al. 2021.** Hundreds of nuclear and plastid loci yield novel insights into orchid relationships. *American Journal of Botany* **108**:

1166–1180.

Poinar G, Rasmussen FN. 2017. Orchids from the past, with a new species in Baltic amber. *Botanical Journal of the Linnean Society* **183**: 327–333.

Porembski S, Barthlott W. 1988. Velamen radicum micromorphology and classification of Orchidaceae. *Nordic Journal of Botany* **8**: 117–137.

Potter PE, Szatmari P. 2009. Global Miocene tectonics and the modern world. *Earth-Science Reviews* **96**: 279–295.

Pridgeon AM, Cribb P, Chase MW, et al. (Eds.). 2005. *Genera Orchidacearum Volume 4: Epidendroideae (Part 1)*. Oxford, New York: Oxford University Press.

Pridgeon AM, Cribb P, Chase MW, et al. (Eds.). 2009. *Genera Orchidacearum Volume 5: Epidendroideae (Part II)*. Oxford, New York: Oxford University Press.

Pridgeon AM, Cribb PJ, Chase MW, et al. (Eds.). 2014. *Genera Orchidacearum Volume 6: Epidendroideae (Part 3)*. Oxford, New York: Oxford University Press.

Qin J, Zhang W, Zhang S-B, Wang J-H. 2020. Similar mycorrhizal fungal communities associated with epiphytic and lithophytic orchids of *Coelogyne corymbosa*. *Plant Diversity* **42**: 362–369.

R Core Team. 2022. R: A Language and Environment for Statistical Computing.

Rada F, Jaimez R. 1992. Comparative Ecophysiology and Anatomy of Terrestrial and Epiphytic *Anthurium bredemeyeri* Schott in a Tropical Andean Cloud Forest. *Journal of Experimental Botany* **43**: 723–727.

- Revell LJ. 2012.** phytools: an R package for phylogenetic comparative biology (and other things). *Methods in Ecology and Evolution* **3**: 217–223.
- Ringelberg JJ, Zimmermann NE, Weeks A, Lavin M, Hughes CE. 2020.** Biomes as evolutionary arenas: Convergence and conservatism in the trans-continental succulent biome (A Moles, Ed.). *Global Ecology and Biogeography* **29**: 1100–1113.
- Schuettpelz E, Pryer KM. 2009.** Evidence for a Cenozoic radiation of ferns in an angiosperm-dominated canopy. *Proceedings of the National Academy of Sciences* **106**: 11200–11205.
- Serna-Sánchez MA, Pérez-Escobar OA, Bogarín D, et al. 2021.** Plastid phylogenomics resolves ambiguous relationships within the orchid family and provides a solid timeframe for biogeography and macroevolution. *Scientific Reports* **11**: 6858.
- Silvera K, Lasso E. 2016.** Ecophysiology and Crassulacean Acid Metabolism of Tropical Epiphytes In: Goldstein G, Santiago LS, eds. Tree Physiology. *Tropical Tree Physiology*. Cham: Springer International Publishing, 25–43.
- Silvera K, Santiago LS, Cushman JC, Winter K. 2009.** Crassulacean Acid Metabolism and Epiphytism Linked to Adaptive Radiations in the Orchidaceae. *Plant Physiology* **149**: 1838–1847.
- Stern WL. 2014.** *Anatomy of the Monocotyledons Volume X: Orchidaceae* (M Gregory and DF Cutler, Eds.). Oxford University Press.
- Taylor A, Zotz G, Weigelt P, et al. 2022.** Vascular epiphytes contribute disproportionately to global centres of plant diversity (RE Onstein, Ed.). *Global Ecology and Biogeography*

31: 62–74.

WCSP. 2020. *World Checklist of Selected Plant Families*. <http://wcsp.science.kew.org/>. 27 Nov. 2020.

WCSP. 2021. *World Checklist of Selected Plant Families*. <http://wcsp.science.kew.org/>. 29 Jan. 2021.

Wikström N, Kenrick P, Chase M. 1999. Epiphytism and terrestrialization in tropical *Huperzia* (Lycopodiaceae). *Plant Systematics and Evolution* **218**: 221–243.

Woodcock DW, Meyer HW. 2020. The Piedra Chamana fossil woods and leaves: a record of the vegetation and palaeoenvironment of the Neotropics during the late middle Eocene. *Annals of Botany* **125**: 1077–1089.

Xie W, Lewis PO, Fan Y, Kuo L, Chen M-H. 2011. Improving Marginal Likelihood Estimation for Bayesian Phylogenetic Model Selection. *Systematic Biology* **60**: 150–160.

Xing X, Jacquemyn H, Gai X, et al. 2019. The impact of life form on the architecture of orchid mycorrhizal networks in tropical forest. *Oikos* **128**: 1254–1264.

Yang S-J, Sun M, Yang Q-Y, Ma R-Y, Zhang J-L, Zhang S-B. 2016. Two strategies by epiphytic orchids for maintaining water balance: thick cuticles in leaves and water storage in pseudobulbs. *AoB PLANTS* **8**: plw046.

Zhang S-B, Dai Y, Hao G-Y, Li J-W, Fu X-W, Zhang J-L. 2015. Differentiation of water-related traits in terrestrial and epiphytic *Cymbidium* species. *Frontiers in Plant Science*

06.

Zotz G. 2013. The systematic distribution of vascular epiphytes - a critical update: Vascular Epiphytes. *Botanical Journal of the Linnean Society* **171**: 453–481.

Zotz G. 2016. *Plants on Plants – The Biology of Vascular Epiphytes*. Cham: Springer International Publishing.

Zotz G, Schickenberg N, Albach D. 2017. The velamen radicum is common among terrestrial monocotyledons. *Annals of Botany* **120**: 625–632.

Zotz G, Weigelt P, Kessler M, Kreft H, Taylor A. 2021. EpiList 1.0: a global checklist of vascular epiphytes. *Ecology* **102**.

Zotz G, Winkler U. 2013. Aerial roots of epiphytic orchids: the velamen radicum and its role in water and nutrient uptake. *Oecologia* **171**: 733–741.

1 TABLES AND FIGURES

2

3 Table 1

4 Tests of correlated evolution between epidendroid orchid lifestyle (epiphytic/terrestrial) and
5 stem (absence/presence), or leaf succulence (absence/presence) using two methods
6 (BayesTraits and corHMM). The log marginal likelihood (LML) of the dependent or
7 independent models, and the log Bayes factor (BF), were calculated using BayesTraits. We
8 considered a $BF > 2$ to be significant support for the dependent (*i.e.* correlated evolution)
9 model. Symmetric matrices were used in corHMM to correlate or decorrelate the two traits
10 (see Supplementary Data Table S3 for more details on these matrices). A delta AICc between
11 0 and 2 indicates that the AICc values are not significantly different. A delta AICc > 2
12 indicates that the correlated model fits the data significantly better.

13

14 Figure 1

15 Illustration of morpho-anatomical water-related traits observed among epiphytic and
16 terrestrial epidendroid orchids. (A) Homoblastic pseudobulbs (composed of several
17 internodes) of the terrestrial *Oeceoclades pulchra* (Thouars) P.J.Cribb & M.A.Clem.
18 (Cymbidieae, Eulophiinae). (B) Heteroblastic pseudobulbs of the epiphytic *Bulbophyllum*
19 *nutans* (Lindl.) Rchb.f. species complex (Malaxideae, Dendrobiinae). (C) Succulent leaves of
20 the epiphytic *Holcoglossum pumilum* (Hayata) L.J.Chen, X.J.Xiao & G.Q.Zhang (Vandaeae,
21 Aeridinae). Photograph by sunoochi from Sapporo, Hokkaido, Japan, CC BY 2.0,
22 <https://commons.wikimedia.org/w/index.php?curid=83337314>. (D) Multi-layered velamen,
23 cross-section of a root of *O. pulchra*.

24

1 **Figure 2**

2 Evolution of lifestyle and succulent stems and leaves in epidendroid orchids. (A) and (B)
3 Ancestral state estimation of lifestyles in the Epidendroideae genus tree with corHMM. Pie
4 charts at nodes represent ancestral lifestyles and their probabilities under correlated evolution
5 with leaf succulence. For succulent stems and leaves, only significant state changes, *i.e.* state
6 probability > 0.5 while < 0.5 in the ancestral node, were indicated by coloured triangles (see
7 Supplementary Data Figs. S4A and S5 for full ancestral state estimations of stem and leaf
8 succulence respectively). The character states of the present genera are represented on the
9 right side of the tree by coloured boxes. In addition, genera with mycoheterotrophic species
10 are indicated by a mushroom symbol. Non-monophyletic subtribes are indicated by stars after
11 the subtribe name. (C) Diagram of transition rates (in events/Myr) between lifestyles and
12 presence/absence of succulent stems or (D) leaves estimated under correlated evolution in
13 corHMM with a symmetric and an all-rate-different transition matrices, respectively.

14

15

16 **Figure 3**

17 Estimation of ancestral geographic ranges in relation to the evolution of epiphytism.
18 Ancestral ranges were estimated in the dated phylogenetic tree of the 203 genera of
19 epidendroid orchids using the BayAreaLike*+J model in BioGeoBEARS. The different
20 geographical areas occupied by the present-day Epidendroideae genera are represented by
21 unique colours. The pie charts at nodes represent the ancestral geographic ranges in
22 proportion to their likelihood. These are solid-coloured when the range comprises a single
23 area (*e.g.* Neotropics), or striped when the range comprises several geographical areas (*e.g.*, a
24 range represented by orange and light blue bands includes both Southeast Asia and Australia).

- 1 The geographical ranges of extant genera are represented on the right-hand side of the tree by
- 2 coloured boxes. Significant lifestyle changes, *i.e.* state probability > 0.5 while < 0.5 in the
- 3 ancestral node, were indicated by coloured triangles (see Fig. 2 for full ancestral state
- 4 estimation of lifestyles).

1 **Table 1**

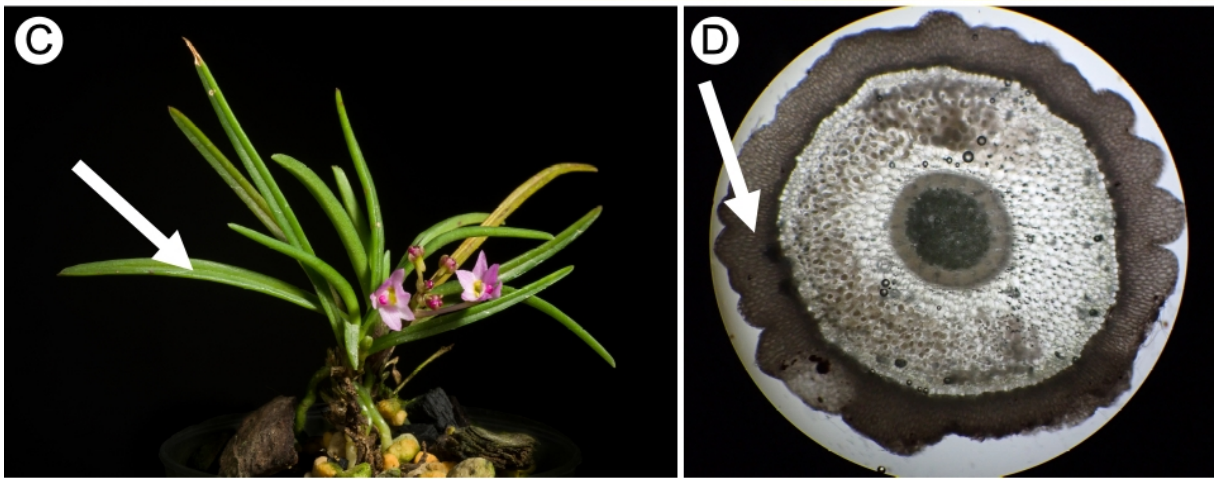
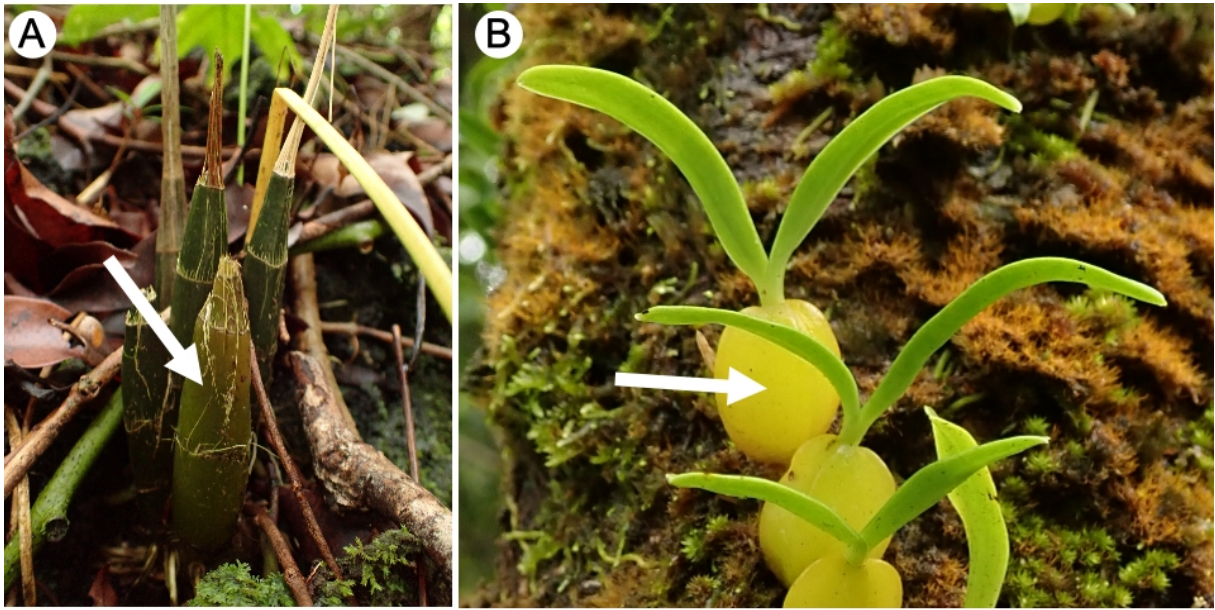
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	BayesTraits			corHMM		
Tested correlation	LML of independent model	LML of dependent model	log BF	AICc correlated matrix	AICc uncorrelated matrix	delta AICc
Lifestyle~Stem succulence	-147.4	-144.0	6.7	276.9	278.8	1.9
Lifestyle~Leaf succulence	-183.1	-172.8	20.7	324.4	344.5	20.2

3

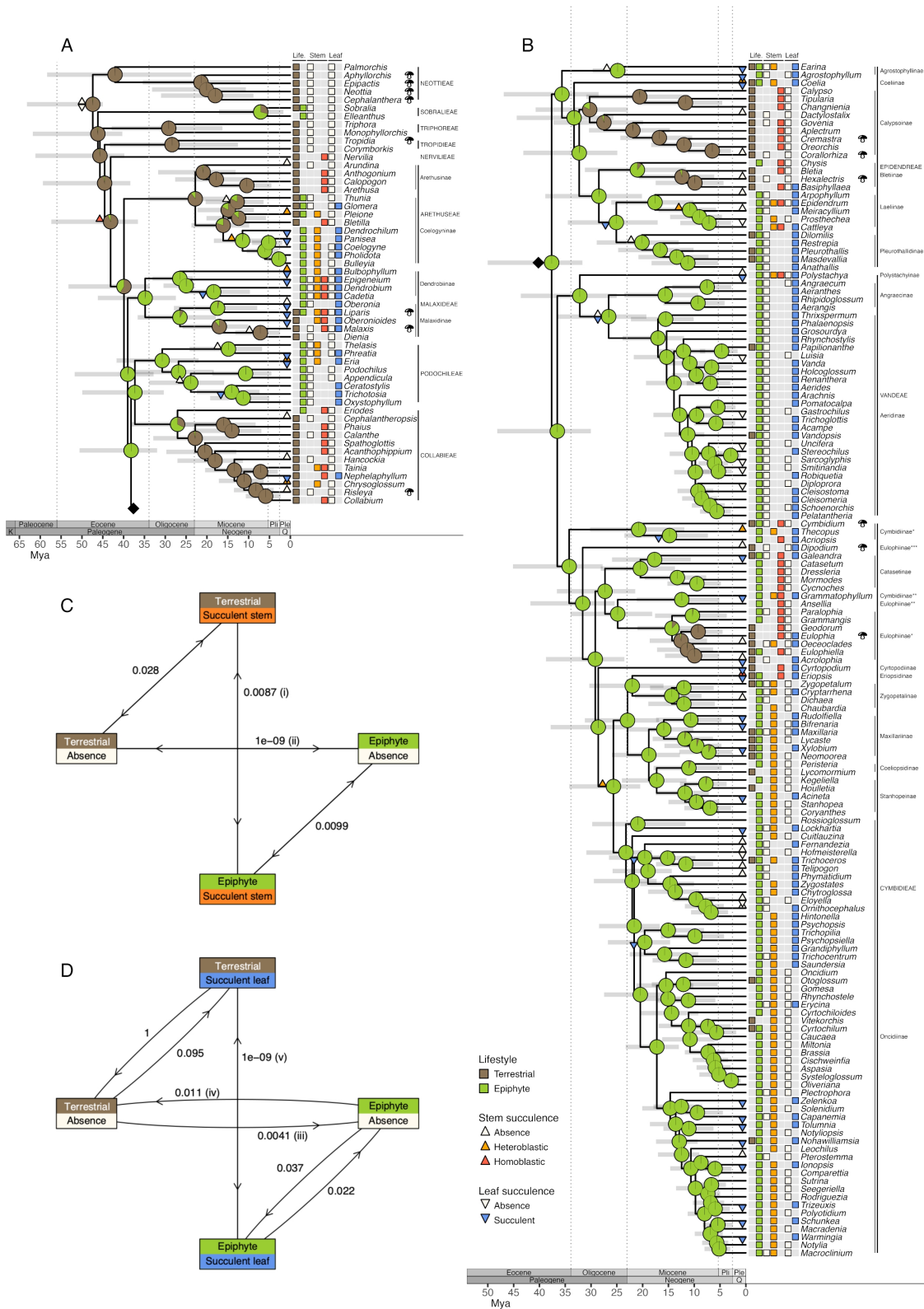
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1 Figure 1



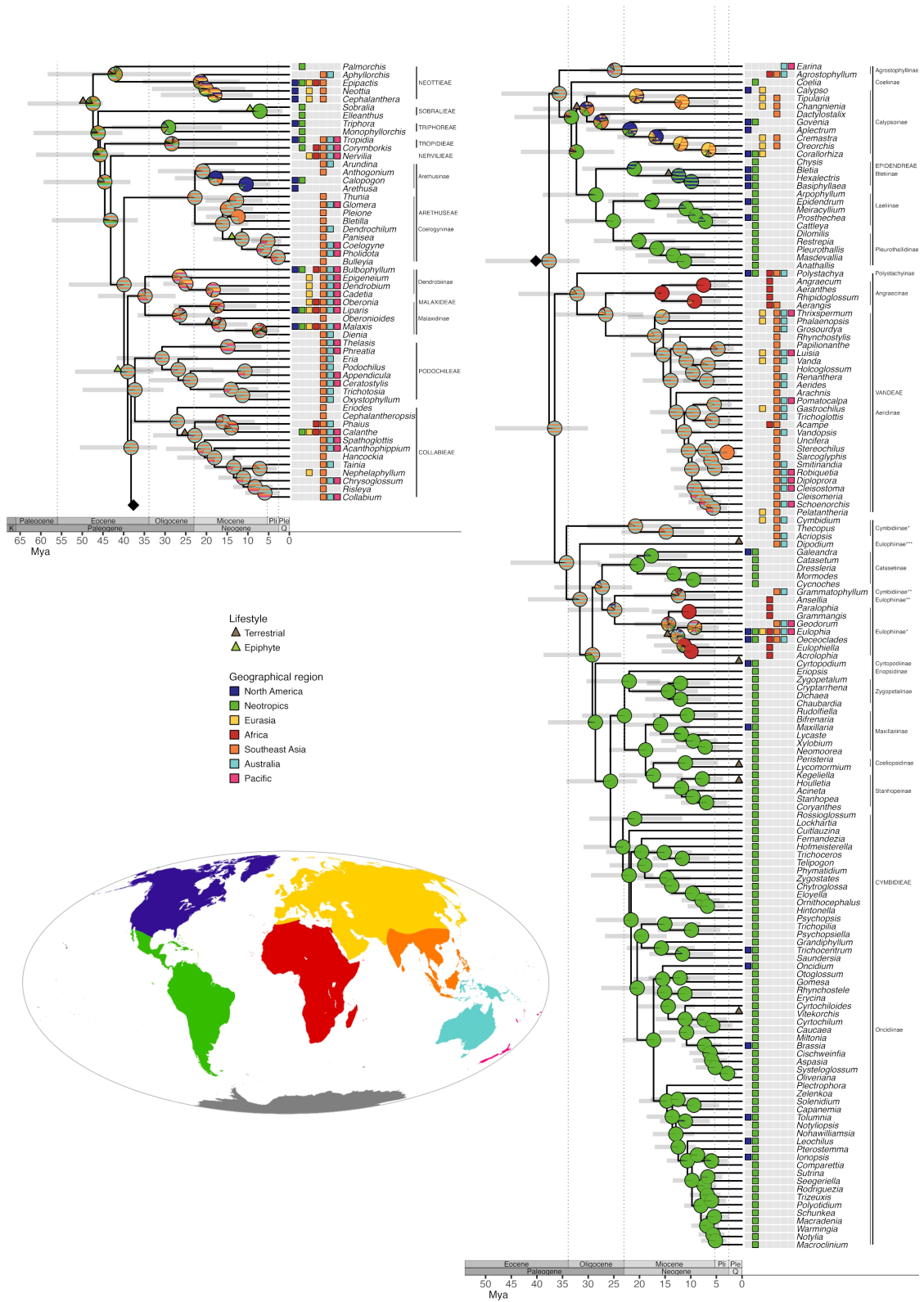
2

1 Figure 2



2

1 Figure 3



2