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Research

Exceptional diversification of floral form in a specialized pollination mutualism

Jasen W. Liu[1,2], Diego Bogarín[3,4,5], Oscar A. Pérez-Escobar[6], Franco Pupulin[3], Adam P. Karremans[3,4], Zuleika Serracín[5], Eugenio Restrepo[7,8], Yongxuan Xie[9] and Santiago R. Ramírez[1,3]

[1]Center for Population Biology, University of California Davis, 1 Shields Ave, Davis, CA 95616, USA; [2]Smithsonian Environmental Research Center, 647 Contees Wharf Rd, Edgewater, MD 21037, USA; [3]Lankester Botanical Garden, University of Costa Rica, PO Box 302-7050, Cartago, 11501-2060, Costa Rica; [4]Naturalis Nationaal Naturrhistorisch Museum, Leiden, Zuid-Holland, 2333, the Netherlands; [5]Herbario UCH, Universidad Autónoma de Chiriquí, P.O. Box 0427, David, Chiriquí, 0427, Panama; [6]Royal Botanic Gardens, Kew, London, TW9 3AE, UK; [7]Grupo de Investigación Schultes, Fundación Ecotonos, Carrera 72 #13ª-56, Cali, 760033, Colombia; [8]Programa de Biología, Facultad de Ciencias Exactas y Naturales, Universidad de Caldas, Calle 65 #26-10, Manizales, 170004, Colombia; [9]College of Agricultural and Environmental Sciences, University of California Davis, 1 Shields Ave, Davis, CA 95616, USA

Author for correspondence:
Jasen W. Liu
Email: liuj2@si.edu

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Summary

- Floral morphology plays a key role in facilitating the mechanical fit between pollinators and plant reproductive organs, allowing for successful reproduction. In this study, we test how independent origins of pollination by scent-collecting male euglossine bees ('perfume flowers') have influenced the evolutionary dynamics of floral shape across the diverse neotropical Cymbidieae orchid radiation.
- We characterized morphological variation across 178 species (*c.* 5% of total diversity) spanning 86 genera (*c.* 54%) and 8 subtribes (100%) to provide the first quantitative description of morphospace within this clade.
- We fit a model of multivariate character evolution to a phylogenetically diverse subset of this dataset consisting of 67 orchid species across 65 genera to rigorously characterize the role of pollination mode on rates of flower shape evolution. Perfume flowers exhibit up to 7.8-fold higher rates of phenotypic evolution compared with plants utilizing other rewarding or deceptive mechanisms.
- Our results provide novel insights into the capacity for pollinators to influence the pace of floral evolution across a diverse radiation, providing an engine for trait diversification in some of the world's most floristically rich regions.

Introduction

A key objective of evolutionary biology was to understand the mechanisms underlying patterns of trait variation, particularly those within highly diverse groups of organisms. The study of flowers has provided important insights in developing our understanding of some of these mechanisms, particularly in light of mutualisms with pollinators (Darwin, 1862; Stebbins, 1951; Fenster *et al.*, 2004; Barrett, 2010; Armbruster, 2014; Kay & Anderson, 2025). For example, a considerable body of literature has documented covarying suites of floral traits in relation to specialized pollinator functional groups – 'pollination syndromes' (Fenster *et al.*, 2004; Dellinger, 2020). Strong patterns of convergence in floral form are often seen coincident with transitions to certain pollinator groups that facilitate efficient pollination, such as the repeated evolution of tubular corollas in hummingbird-pollinated taxa (Kay & Grossenbacher, 2022) or long nectar spurs in hawkmoth-pollinated 'moonflowers' (Johnson *et al.*, 2017). Furthermore, lineages exhibiting certain specialized pollination modes have been found to exhibit patterns of morphological stasis due to persistent pollinator-mediated selection on floral form (Davis *et al.*, 2014; Joly *et al.*, 2018; Kriebel *et al.*, 2020). Despite these suggestions that pollinators affect phenotypic evolution in flowers across macroevolutionary timescales, there are few empirical studies that rigorously characterize their role in shaping the pace of floral morphological evolution across diverse radiations.

Tropical American orchids of the Cymbidieae clade offer a compelling system in which to test evolutionary hypotheses about how pollinators influence the macroevolution of floral form. The roughly 3400 species of this clade originated from an Australasian dispersal event into tropical America 24–34 million years ago (Ma) (Givnish *et al.*, 2015, 2016; Pérez-Escobar *et al.*, 2017b). In their subsequent radiation, they have evolved a myriad of pollination mechanisms and reward strategies. Critically, there have been several independent origins of a highly specialized pollination system – androeuglossophily (Ramírez *et al.*, 2011; Liu *et al.*, 2025). These 'perfume flowers' (Vogel, 1966) are

exclusively pollinated by male euglossine bees that are attracted by scent and collect these chemical rewards for use in species-specific courtship displays (Dodson, 1966; Vogel, 1966, p. 19; Henske *et al.*, 2023; Liu *et al.*, 2025). Because female bees select mates based on the chemical blends acquired by males (Henske *et al.*, 2023), sexual selection has shaped the diversification of chemical preferences exhibited by male bees (Weber *et al.*, 2016). Scent operates as both a signal and reward in perfume flowers, differentiating its function from other attraction mechanisms where scent either signals the presence of a separate reward or is used to exploit sensory biases of pollinators expecting rewards in deceptive flowers.

The apparent ease of androeuglossophily in generating reproductive isolation via simple changes in scent production is thought to increase speciation rates in orchids, similar to processes identified in sexually deceptive species (Givnish *et al.*, 2015b). Additionally, previous work characterizing scent variation in the system identified rapid diversification of volatile phenotypes between closely related species (Liu *et al.*, 2024). These findings are consistent with quantitative genetic theory predicting rapid diversification of plants and their rewards in this pollination mode where runaway sexual selection acts synergistically with coevolutionary processes (Kiester *et al.*, 1984). How this framework extends to infer the pace of floral morphological evolution is unknown.

One striking characteristic of many perfume flowers in the Cymbidieae is the range of extreme strategies that they use to manipulate their pollinators. These strategies range from the forceful propulsion of pollen structures in *Catasetum* and manipulation of how pollinators slip and fall through the flowers of many *Gongora* species (Videos 1 and 2), to perhaps the most extreme mechanism – entrapment of pollinators in liquid-filled 'buckets' in *Coryanthes*, where the only escape is through a narrow tunnel containing the reproductive organs (Video 3). The specialized functional morphologies allowing for these complex pollination modes have captivated evolutionary biologists since the dawning of the field, yet their underlying dynamics have not been characterized in the context of recent phylogenetic constructions of orchids (Darwin, 1862; Romero & Nelson, 1986; Endress, 2016; Pérez-Escobar *et al.*, 2017b, 2024). We hypothesize that this diversity of pollination mechanisms within perfume flowers is associated with more rapid rates of phenotypic evolution across their independent origins compared with members of the Cymbidieae utilizing other pollinators.

Despite the immense diversity of the tropical American Cymbidieae clade and the known importance of morphology in mediating their specialized pollinator interactions, there have been no attempts to rigorously characterize morphological variation of flowers across the group. As such, we lack a quantitative description of how floral morphological diversity is distributed across this major tropical radiation, whether particular clades occupy distinct regions of trait space, and how repeated origins of specialized pollination systems have contributed to overall morphological diversification. Without these data, there exists a major gap in our ability to infer the tempo of evolutionary change and consequently to evaluate whether shifts in pollination mode have modified the pace of phenotypic diversification and restructured the occupation of floral morphospace.

Here, we seek to address these gaps in knowledge by (1) constructing the first broad-scale floral morphospace for tropical American Cymbidieae orchids spanning over half of generic diversity and all subtribes, and (2) analyzing these morphological data within a phylogenetic comparative context to test the

Video 1 Pollinarium deposition on *Eulaema* sp. by *Catasetum maculatum* at Hacienda Baru, Costa Rica. Video taken by S. R. Ramírez.

Video 2 Pollination of *Coryanthes kaiseriana* by *Euglossa* sp. in Costa Rica. Video taken by A. P. Karremans.

Video 3 Pollination mechanism of *Gongora* where a scent collecting *Euglossa* sp. falls from the labellum and struggles against the anther cap. In this video, the anther cap was not removed and pollinarium deposition did not occur. Video taken by J. Dean near Viterbo, Colombia.

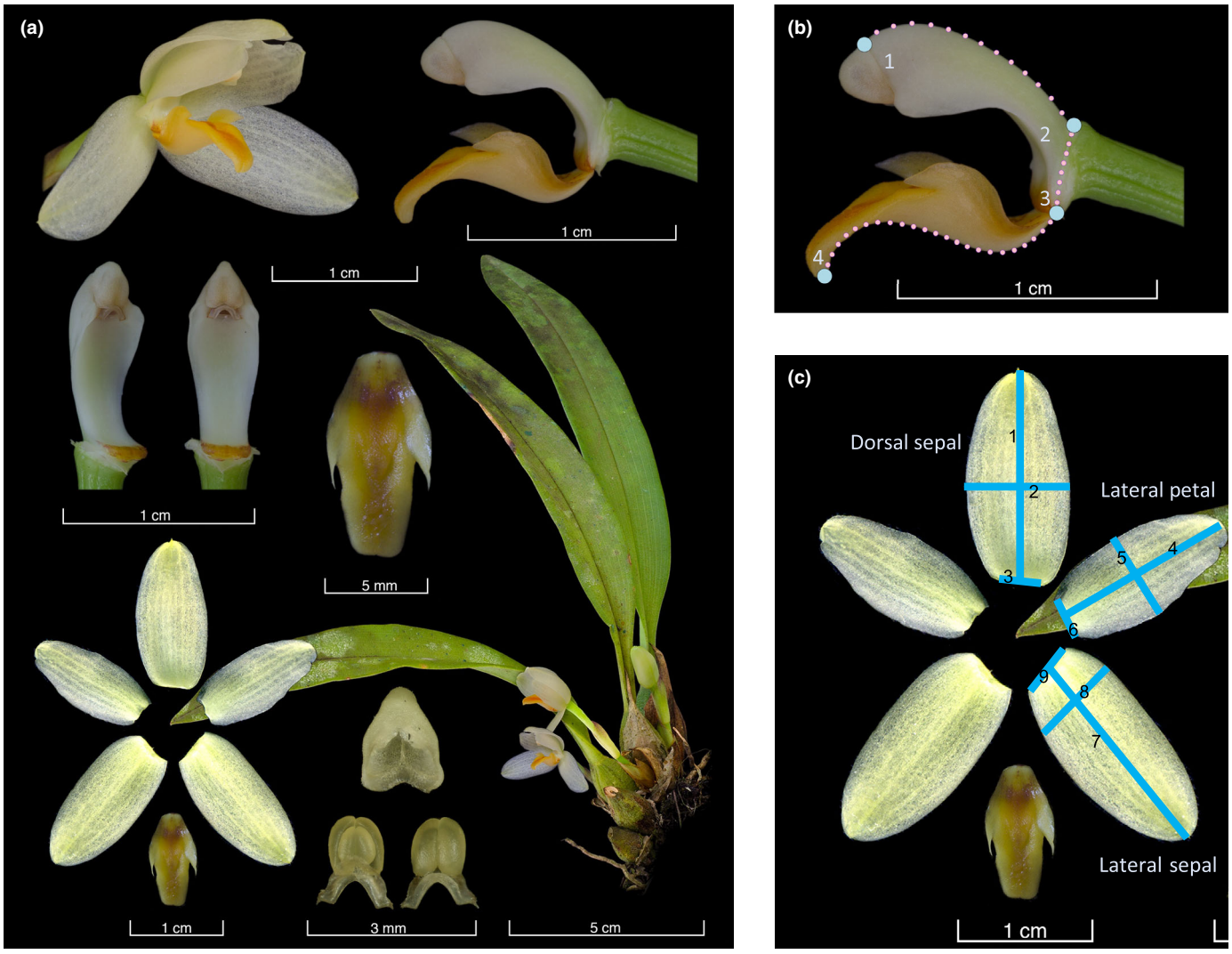

Fig. 1 Example of images used in this study. (a) A Lankester composite digital plate of *Mormolyca hedwigiae*. (b) Landmarking scheme of the reproductive structure. Landmarks (large pale circles) consist of (1) anther cap column junction, (2) column-ovary junction, (3) column foot-labellum junction, and (4) distal tip of the labellum. The smaller pink circles correspond to the evenly placed semilandmarks along traced curves. (C) Linear measurements taken of the perianth. Labelled measurements correspond to (1) length of the dorsal sepal, (2) maximum width of the dorsal sepal, (3) width at the base of the dorsal sepal, (4) length of the lateral petal, (5) maximum width of the lateral petal, (6) width at the base of the lateral petal, (7) length of the lateral sepal, (8) maximum width of the lateral sepal, and (9) width at the base of the lateral sepal.

hypothesis that androeuglossophily accelerates the pace of floral phenotypic evolution. By sampling broadly across genera and subtribes, we provide a comprehensive view on clade-wide patterns of morphological variation. Our comparative analyses reveal that perfume flowers exhibit exceptionally high rates of morphological evolution across their independent origins and provide new insights into how pollinators shape the landscape of floral evolution across diverse radiations.

Materials and Methods

Data collection

We characterized the morphology of flowers from 178 orchid species from the tropical American Cymbidieae radiation, representing *c.* 5.2% of accepted species, 54% of genera, and 100% of subtribes contained in this lineage. Orchid flowers share a conserved bauplan with other lilioid monocots consisting of three petals and three sepals (Fig. 1). However, orchids exhibit several lineage-specific novelties, most notably the modification of one petal into a 'labellum' structure and the fusion of reproductive organs into a 'column' structure. These modifications break the radial symmetry of the floral plan, creating a polarity amenable to the complex pollination mechanisms characteristic of this family. Labella have diversified greatly across the orchid radiation, usually acting to direct pollinators into a specific position to contact the column and facilitate pollination (Mondragón-Palomino & Theißen, 2009; Ackerman *et al.*, 2023). We focused on two sets of floral traits – the 'reproductive structure' consisting of the column and labellum, and the 'perianth' consisting of the remaining petals and sepals.

We collected most measurements from 'Lankester composite digital plates' (LCDPs) – composite arrangements of photographs of dissected flowers that have become increasingly common in botanical research over the past decade (Fig. 1). These plates consist of photographic compositions illustrating the diagnostic characters of each species, with multiple views of dissected flowers. These plates are further supported by voucher specimens deposited in the Lankester Botanical Garden Herbarium. Additionally, we photographed dissected flowers taken from private and public collections and used comparable images from published studies to obtain measurements (Supporting Information Table S1).

We conducted two sets of morphological analyses – a shape analysis using geometric morphometrics from lateral views of the column and labellum (the 'reproductive structure'), and linear and curved measurements of the sepals and remaining unmodified petals (the 'perianth'). Data for the reproductive structure were collected using StereoMorph v.1.6.5 (Olsen & Westneat, 2015). We placed four homologous landmarks corresponding to the intersection of the column with the anther cap, the intersection of the column with the base of the flower, the intersection of the base of the flower with the labellum, and the distal tip of the labellum. Three sets of curves were drawn between these points that corresponded to the column, column foot, and labellum, with 15, 25, and 10 equidistant points sampled from these regions, respectively. For monoecious species represented in the dataset (five species of perfume flowers in *Catasetum* and *Cycnoches*), we only used staminate (i.e. functionally 'male') flowers to conserve the anther cap landmark, as there was no clear analogous landmark in the pistillate flowers. Thus, we note that our estimates of shape variation systematically underestimate variation in perfume flowers.

For the perianth, we took 15 linear measurements per flower corresponding to five measurements of the organ lengths, five measurements of organs at their points of attachment, and five measurements of maximum widths across organs. Due to the presence of missing data from occasional broken and missing organs, we then averaged paired measurements (e.g. left and right petal lengths) for a total of nine final measurements that were present in every species. These final measurements consisted of the dorsal sepal length, dorsal sepal base, dorsal sepal maximum width, lateral petal length, lateral petal base, lateral petal maximum width, lateral sepal length, lateral sepal base, and lateral sepal maximum width (Fig. 1).

Although within-species replication was limited for many taxa (median = 2 flowers per species, range 1–8), Procrustes ANOVA (quantifying the proportion of shape variation in a matrix of aligned landmark coordinates attributable to different factors; Goodall, 1991) in a subset of 14 species with more than or equals to four flowers per species indicated that among-species variance greatly exceeded within-species variance (94.77% vs 1.66% of variance). We therefore interpret the dataset as appropriate for the broad macroevolutionary comparisons that we conducted. Future studies at finer levels of taxonomic organization would benefit from a more intensive sampling scheme to more rigorously characterize intraspecific variation in floral morphology.

Phylogeny We used the time-calibrated phylogenetic hypothesis of the Neotropical Cymbidieae proposed in Pérez-Escobar *et al.* (2017a,b) inferred from four plastid and one nuclear loci across 816 species pruned to our focal species. This tree was selected based on its dense taxon sampling and generally strong support for genus-level relationships. Where sample species in our dataset were not present in the phylogeny, we substituted them with a congener or, if possible, a member of the same subgenus (Table S4). Because our goal was to test broad-scale differences in rates across major pollination categories rather than species-specific evolutionary histories, these substitutions were used as a pragmatic approximation at the scale of inference (mostly genus level) targeted here. We nevertheless recognize that this approach introduces phylogenetic uncertainty, particularly near the tips, and therefore interpret fine-scale lineage-specific patterns cautiously.

Controlling for size and log-shape ratios

As the primary goal of this study was to understand shape evolution across Cymbidieae orchids, we removed the effect of size before downstream analyses. For the shape analysis of the labellum and column, we performed a generalized Procrustes analysis to center and align coordinates of each specimen in a standardized shape space independent of size. As size and shape can still vary evolutionarily due to allometry, we used the *procD.pgls* function in geomorph v.4.0.6 (Adams & Otárola-Castillo, 2013) to perform a Procrustes ANOVA of shape vs log-transformed centroid-size (square root of summed squared distances of landmarks from their center within each specimen).

We used another method of size-correction – log-shape ratios – to process our dataset of linear measurements. These ratios were computed by dividing each trait measurement by the geometric mean of the petal and sepal lengths that captured the major dimensions of floral size, and then taking the logarithm of this value. We then applied the *phylolm* function from phylolm v.2.6.2 to quantify the relationship between each transformed trait and log-transformed size (Tung Ho & Ané, 2014).

Classification of pollination modes

Tropical American Cymbidieae orchids exhibit a diversity of pollination rewards, including the presentation of oils, nectar, resin, edible trichomes, and perfume; in addition to mimicry of those rewards and of mates (Ramírez *et al.*, 2011; Castro & Singer, 2019; Ackerman *et al.*, 2023). In most orchids, traits such as scent and color either signal rewards or act as cues for unrewarding flowers exploiting pre-existing mutualisms, thus mediating pollinator behavior. The distinction between these strategies is unclear – even some species that present rewards are thought to provide them in such low quantities that their validity as true mutualisms is ambiguous (Chase *et al.*, 2009; Davies & Stpiczyńska, 2012; Davies *et al.*, 2013). In perfume flowers, however, scent acts as both the signal and reward and is thus functionally distinct from all other pollination systems.

We utilized a large published dataset of orchid pollination and rewards (Ackerman *et al.*, 2023) to code pollination modes. In addition to species with direct observations, species that contained nectar, trichome, oil, or resin rewards, as determined via chemical and histological studies, were characterized as nonperfume flowers, as perfume flowers do not produce these additional rewards (Vogel, 1966; Dressler, 1968). This stricter scheme resulted in 67 species, of which 23 were perfume flowers and 44 were nonperfume flowers, reflecting 13 and 28 genera, respectively, corresponding to *c.* 2% of total species, 27% of genera, and 88% of subtribes.

As this strict method resulted in oversampling of two of the major subtribes (the Catasetinae and Stanhopeinae) containing perfume flowers (Fig. S1), we used another method to impute pollination mode resulting in more even sampling. If at least two members of a genus produced identical rewards or had multiple observations of the same pollination type, we considered congeners without those standards of evidence to exhibit the same pollination type. We justified this approach as intrageneric variation in androeuglossophily has not been extensively documented in orchids, with only two cases known: *Dichaea*, where transitions toward variants of selfing have been documented (Nunes *et al.*, 2018; Ackerman *et al.*, 2023) and *Trichocentrum*, a genus exhibiting several different reward mechanisms, including nectar, oil, and deceit. Furthermore, the ease of attracting large numbers of male orchid bees to chemical baits has led to extensive genus-level sampling of perfume–flower pollination systems (Dressler, 1968; Roubik & Ackerman, 1987; Ramirez, 2019; Liu *et al.*, 2025), supporting the inference that perfume–flower pollination is often conserved within sampled genera.

This more relaxed approach resulted in a dataset of 140 species that was used to test whether including a broader range of taxa changed the main comparative results. Of these species, 36 were perfume flowers and 104 were nonperfume flowers, reflecting 25 and 40 genera (4.1% of species, 41% of genera, and 100% of subtribes), respectively, more closely reflecting actual composition of the clade (Fig. S1). Perfume flowers identified in this latter scheme and not in the former were all members of clades in which all species with pollination studies were confirmed to be androeuglossophilous, suggesting that major overestimation of perfume flower diversity is unlikely. While this approach introduces a higher risk of misclassification, including a more densely sampled scheme provided a more comprehensive view of broad evolutionary patterns across the Cymbidieae radiation.

We treat the strict coding scheme as the primary dataset for inference, and the expanded relaxed scheme as a sensitivity analysis used to assess whether the main comparative results were robust to denser taxon sampling. Both schemes of filtering the data resulted in overall quantitatively similar results in our comparative methods (Table S3; Figs S2, S3).

Phylogenetic comparative methods

To understand the evolutionary history of pollination mode evolution in the Cymbidieae, we used the *make.simmaps* function in PHYTOOLS v.2.0 to generate 1000 stochastic character maps, using the equal rates model which exhibited a lower AICc than the all rates different model (Bollback, 2006; Revell, 2012). As the tropical American Cymbidieae orchids originated from a dispersal event from a continent where euglossine bees are not present (Pérez-Escobar *et al.*, 2017b), we fixed the root of the tree to have the nonperfume flower character state.

We used phylogenetic principal components analysis (pPCA) to visualize and interpret major patterns of variation in our trait spaces (Revell, 2009). For the reproductive structure dataset, we performed pPCA on the Procrustes transformed coordinates using the *gm.prcomp* function in GEOMORPH v.4.0.6. For the perianth dataset, pPCA was performed using the correlation rather than covariance matrix among the variables, ensuring more equal contribution of each of the variables that originally exhibited contrasting levels of variance. Though there were nine total principal component scores, only the first eight scores portrayed floral variation, since one degree of freedom was lost when applying log-shape ratios. To quantify occupation of morphological space by orchids of each pollination mode, we used the *morphol.disparity* function in GEOMORPH v.4.0.6 to compare their levels of shape variance.

We utilized a multiple state-specific rates of continuous-character evolution model (MuSSCRat; May & Moore, 2020) to understand the effect of pollination on floral morphological diversification. In contrast to other methods developed for continuous trait evolution, this Bayesian approach explicitly models background rate variation, a feature we expect to be present across this hyperdiverse radiation, to robustly estimate rate parameters associated with discrete states (Fig. S4). MuSSCRat uses an uncorrelated log-normal (UCLN) clock to place independent parameters on each branch to model background rate variation that cannot be attributed to discrete traits of interest (analogous to the UCLN relaxed clock model for molecular evolution). Critically, this approach allows for avoiding the 'straw-man effect', where all variation in continuous trait evolution is inaccurately attributed to a discrete trait of interest because the null hypothesis is no rate variation (May & Moore, 2020). Furthermore, in contrast to individually modelling separate morphological traits, this framework more accurately captures the multivariate nature of our complex morphological datasets.

The reversible-jump Monte Carlo Markov Chain (MCMC) ran for 500 000 generations for the first 5 PC's of labellum-column shape explaining 90% of variation and for 1000 000 generations for the log-shape ratio dataset, with the initial 10% of iterations discarded as burn-in. Due to strong covariance between lateral petal and lateral sepal lengths in the shape ratio dataset leading to issues with estimations of correlation structure and model convergence in some test runs, we removed the latter trait. We observed poor model mixing with low acceptance rates for reversible-jump moves under default settings. Thus, we increased the weights of mvRJSwitch from 1 to 10 to more frequently propose transitions between models with and without rate shifts. Additionally, we replaced the single default mvScale proposal ($\lambda = 1$, weight = 1) with three scale proposals ($\lambda = 0.1$, 1, and 10), each with increased weights of 3 to facilitate

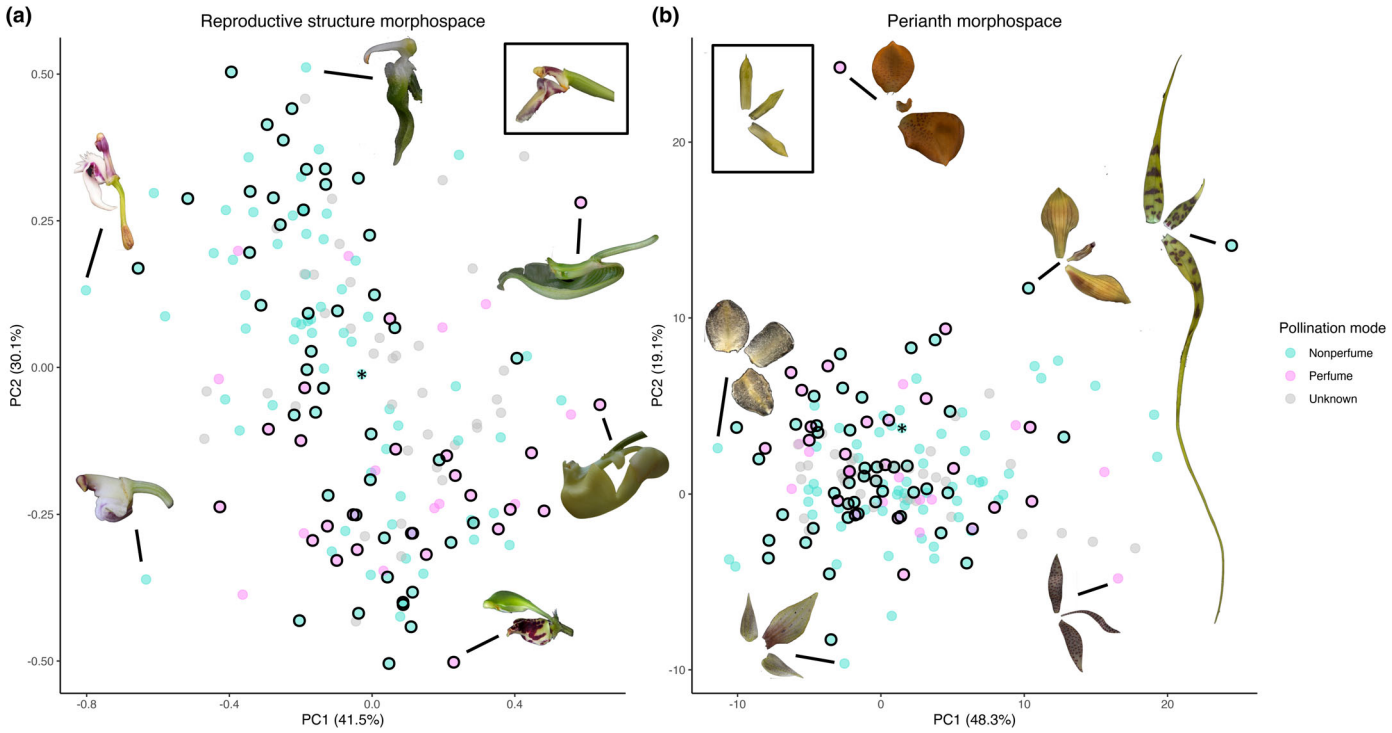

Fig. 2 Morphospaces of the neotropical Cymbidieae flower comparing axes of variation in (a) the reproductive structure and (b) the perianth. Points represent species means and are colored by pollination mode in the relaxed scheme while values on the *x* and *y* axis correspond to proportions of total morphological variance explained by the first and second principal components, respectively. Closed points represent species with pollination modes categorized in the strict scheme. Images on the top right of (a) and top left of (b) in boxes correspond to *Camaridium bracteatum*, a species close to the mean phenotype in both sets of traits (asterisked point in either panel). Other represented species in (a) are (from center above, going counterclockwise): *Ornithocephalus inflexus, Cyrtochilum ramosissimum*, *Cyrtochilum myanthum*, *Kegeliella kupperi*, *Coryanthes hunteriana*, and *Clowesia dodsoniana E.Aguirre*. Other represented species in (b) are (from center above, going counterclockwise): *Gongora armeniaca*, *Trichocentrum morenoi*, *Telipogon biolleyi*, *Polycycnis barbata*, *Brassia caudata*, and *Trigonidium egertonianum*. Photographs copyright the authors except for the following, modified with permission: *Clowesia dodsoniana* from Tamayo-Cen *et al*. (2022), *Cyrtochilum ramosissimum* from Dalström (2020) copyright Carlos Jerez, *Cyrtochilum myanthum* from Gutiérrez Morales *et al*. (2018), and *Trichocentrum morenoi* copyright Gustavo Montealegre. Images are not to scale.

exploration of the MCMC across different magnitudes of rate multipliers.

We specified a prior of five rate shifts, corresponding roughly to the average number of transitions to androeuglossophily identified across 1000 stochastic character-mapping simulations. To assess the effects of specifying different number of rate shifts, we performed both sets of analyses using priors of 1, 5, and 10 rate shifts and identified qualitatively similar results (Figs S5, S6). We ran the model in RevBayes v.1.2.1 (Höhna *et al*., 2016) and used Tracer v.1.7.2 (Rambaut *et al*., 2018) to verify convergence of the MCMCs (ESS >> 200 for primary model parameters). Data were visualized and plotted using RevGadgets v.1.2.1 and ggtree v.3.4.4 (Yu *et al*., 2017; Tribble *et al*., 2022). Scripts and data used for this study are available on Dryad.

Results

Floral morphospace

We identified a broad diversity of morphologies in both sets of floral traits. The primary axis of morphological variation in the reproductive structure, explaining over 40% of variation in the data, contrasts pouched labella, as found in the highly modified flowers of the 'bucket orchid' *Coryanthes hunteriana* Schltr., to highly reflexed labella, as exhibited by several species of *Cyrtochilum* (Fig. 2a). The second major axis of variation explaining *c.* 30% of morphological variance describes a continuum from constricted tubes, formed by parallel positioning between the column and labellum, to structures where the labellum is positioned roughly 180 degrees from the column. The former phenotype is common among many species of the Maxillariinae (i.e. 'gullet flowers' described in the pollination biology literature of orchids; van der Pijl & Dodson, 1966), while the latter phenotype is common among many species of the Oncidiinae, where the expanded flat labella operate as landing pads for bees seeking oil rewards (Fig. S7A).

The primary axis of variation in the perianth dataset explaining almost 50% of the total variance captures elongation (Fig. 2b). Species with high values of PC1 exhibit exaggeratedly long petals and sepals, as exemplified by the 'spider orchid' *Brassia caudata*m (L.) Lindl., while those with low values along this axis of variation exhibit squat, rounded floral organs (Table S5). The second PC, explaining *c.* 20% of morphological variation, contrasts species with long petals relative to the sepals or vice versa. Mirroring the

Table 1 Comparison of results from stochastic character mapping, morphological disparity analyses, and evolutionary rate modelling among pollination modes.

Pollination mode (*n*)	Time in tree (%)	Morphological disparity	Multivariate rate
Perfume (23)	27.1	0.219 (reproductive structure) 9.71 (perianth)	1.547 (reproductive structure) 1.773 (perianth)
Nonperfume (44)	72.9	0.177 (reproductive structure) 8.42 (perianth)	0.453 (reproductive structure) 0.227 (perianth)

'Time in tree (%)' corresponds to the percentage of time spent in each state across 1000 simulations of stochastic character mapping, 'Morphological disparity' corresponds to Procrustes variance of each group from analyses of morphological variation, while 'Multivariate rate' corresponds to state-dependent rates obtained from MuSSCRat.

reproductive structure dataset, this axis roughly divides the morphospace between the diverse Maxillariinae and Oncidiinae clades (Fig. S7B).

There was only a modest effect of size on shape in both datasets. While the effect of size was significant on the reproductive structure shape ($P = 0.023$), the overall relationship was weak ($r^2 = 0.017$). Across the comparisons of log-shape ratios with floral size, the largest r^2 value was 0.062 with no significant positive or negative relationships, confirming an overall weak relationship between size and shape in the transformed variables (Table S2).

In both the relaxed and strict schemes, perfume flowers occupy similar amounts of morphospace across both sets of traits compared with flowers of all other pollination mechanisms (Tables 1, S4). While perfume flowers were found to exhibit slightly higher Procrustes variances across both sets of traits in both schemes, differences were nonsignificant (highest ratio of perfume to nonperfume variance = 1.24 for reproductive structure in relaxed scheme, $P > 0.05$ for all comparisons). Notably, neither scheme of pollination coding resulted in major systematic biases toward or against regions of morphospace encompassed within the entire dataset (Fig. 2).

Stochastic character mapping

We used stochastic character mapping to characterize the history of pollination mode evolution across the Cymbidieae phylogeny, recovering a mean of 4.14 transitions from nonperfume flowers to perfume flowers and 0.82 reversions, with lineages spending approximately three times as much evolutionary time in the nonperfume flower state (Fig. 3; Table 1). The proportion of evolutionary time spent in each state was similar in the relaxed scheme (Table S3), while more transitions and reversions were identified (5.16 and 2.29, respectively), reflecting a gain of androeuglossophily in a clade of twig epiphytes within the Oncidiinae (*Notylia* and *Macroclinium*) and an additional loss of androeuglossophily in the Zygopetalinae (*Warczewiczella discolor* (Lindl.) Rchb.f.) that were unsampled in the strict scheme.

Evolutionary rate modelling

Pollination mode had a strong effect on the rate of floral evolution, with posterior probabilities of state dependence at 0.99 and 1.0 for the reproductive structure and perianth, respectively in the strict scheme (Fig. 4). Critically, perfume flowers evolved at 3.4-fold and 7.8-fold the pace of nonperfume flowers for these two sets of traits (Fig. 4), accounting for their equivalent amounts of floral morphospace filled despite having a third of evolutionary time to accumulate this variation. These patterns emerge against a complex background of rate heterogeneity, characteristic of diverse radiations (Fig. S4). Slightly weaker but still strong evidence for state dependence was identified in the relaxed scheme, with posterior probabilities of 0.94 and 1.0 in the reproductive structure and perianth, respectively, with perfume flowers exhibiting 1.9-fold and 5.3-fold the pace of nonperfume flowers.

Discussion

We characterized the primary axes of shape variation across a large sample of the hyperdiverse tropical American Cymbidieae radiation, obtaining a sample capturing over half of the genera in the group. We identified a strong effect of phylogeny across both morphological datasets, with the second major axis of variation being strongly structured by subtribes within the radiation. Furthermore, we identified a strong effect of androeuglossophily increasing the tempo of floral shape evolution in tropical American Cymbidieae orchids, resulting in an exceptional expansion of morphospace across origins of this pollination mode. These patterns stand out against a complex background of rapid morphological evolution characteristic of one of the most spectacular radiations of flowering plants in the world's richest botanical hotspots (Gentry, 1982; Pérez-Escobar *et al.*, 2017b). Rather than mediating the evolution of predictable morphologies across or stasis within each origin of androeuglossophily, we demonstrate that the repeated origins of perfume flowers in the Cymbidieae are associated with substantial increases in phenotypic evolutionary rates. This pattern is consistent with a scenario of male euglossine bee pollination fueling the generation of floral disparity across a large radiation of orchids.

Due to their range of bizarre appearances and complex pollination mechanisms, perfume flowers have long captured the attention of evolutionary biologists, botanists, and horticulturists alike (Darwin, 1862). Many species manipulate the movement of their male euglossine bee pollinators – from causing them to fall through open dimensions of the flowers (e.g. *Gongora* and *Stanhopea*) to forcing them to crawl through narrow passages to

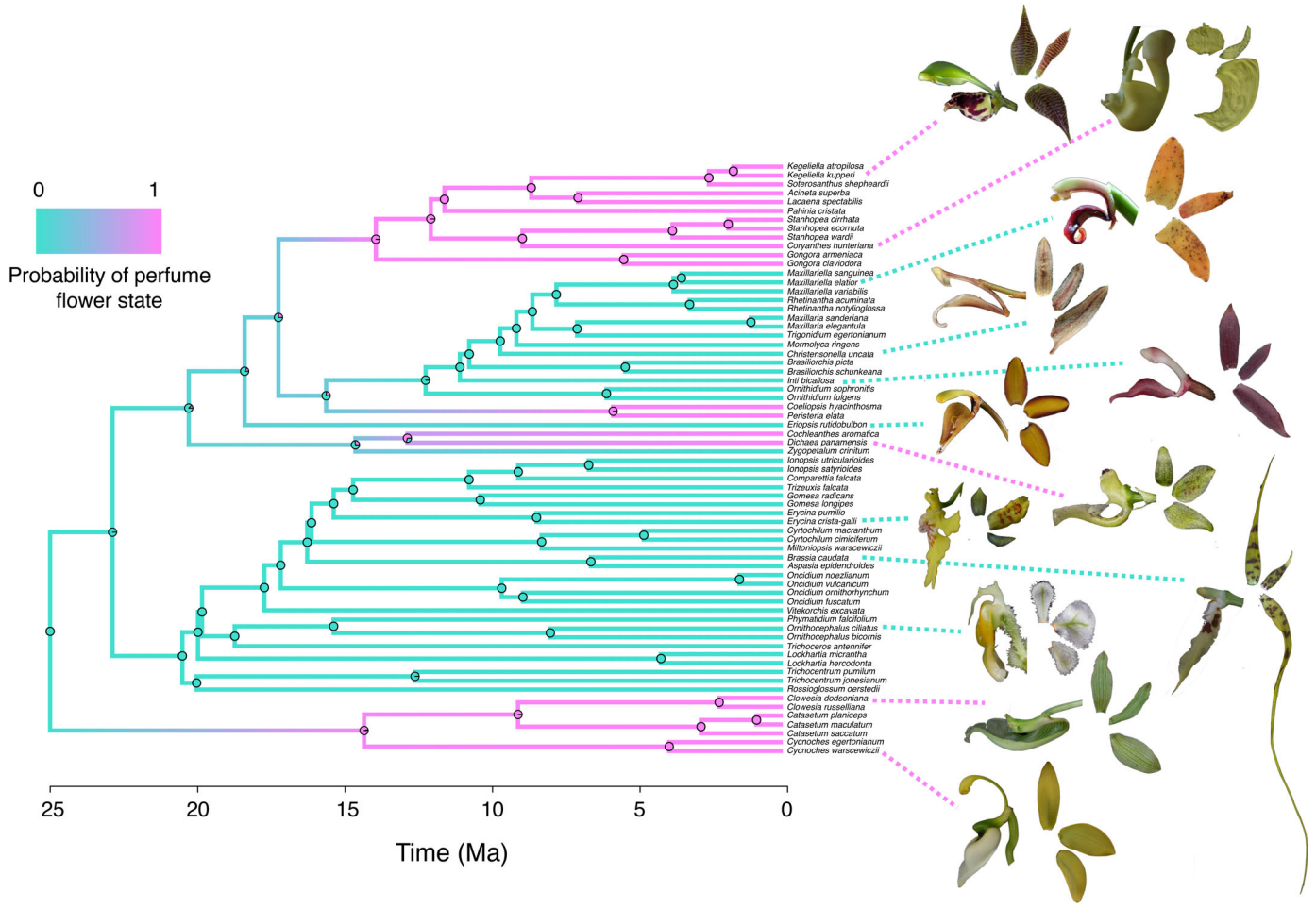

Fig. 3 Results of stochastic character mapping of pollination mode across the tropical American Cymbidieae clade. Shown is the density of pollination mode across this phylogeny estimated across 1000 stochastic character maps using the densityMap function in phytools. Pie charts at each node indicate the frequency of each state across these character maps. Photographs of the reproductive structure and perianth of selected taxa are shown to the right. Images are not to scale.

escape drowning (e.g. *Coryanthes*). Additionally, many species employ complex strategies of pollinarium placement, ranging from enantiostyly in *Mormodes* (Domingos-Melo *et al.*, 2025) to the hinged labellum of *Peristeria* and *Gongora* subgenus *Acropera* causing visiting bees to be propelled into the column (Romero & Nelson, 1986; Hetherington-Rauth & Ramírez, 2015). These mechanisms contrast with more passive pollination strategies found in other modes among orchids. For example, 'gullet flowers' are a common feature of many members of the hyperdiverse Maxillariinae clade, which simply require crawling in and out of a passage created by the petals and sepals to ensure proper placement of pollinaria (van der Pijl & Dodson, 1966).

Our comparative analyses revealed over three- and sevenfold increases in overall evolutionary rates of the reproductive and perianth structures, respectively, in perfume flowers compared with flowers utilizing other pollination mechanisms. Notably, we found that some of the fastest overall rates of evolution in these structures occur in the Catasetinae and the Stanhopeinae, respectively, two groups of orchids that have been extensively studied in the context of androeuglossophily (Roubik & Ackerman, 1987; Milet-Pinheiro & Gerlach, 2017; Liu *et al.*, 2024). This increased tempo of evolutionary change has allowed perfume flowers to accumulate equivalent amounts of morphological variation found in related nonperfume lineages in only around a quarter of the total evolutionary time in the clade, resulting in some of the most extreme floral morphologies and pollination mechanisms in the orchids and across flowering plants.

We note an apparent inverse relationship between contemporary morphological and species diversity across the tropical American Cymbidieae. This pattern is consistent with the history of systematic revisions in the two most diverse clades – the Maxillariinae and the Oncidiinae – that are predominantly composed of nonperfume flowers (Whitten *et al.*, 2007; Chase *et al.*, 2009; Neubig *et al.*, 2012). In these clades, multiple major upheavals of taxonomic hypotheses were carried out with the advent of genetic data, revealing pervasive morphological convergence in floral form, likely in response to the utilization of the same guilds of pollinators within both clades (bees in the Meliponini and

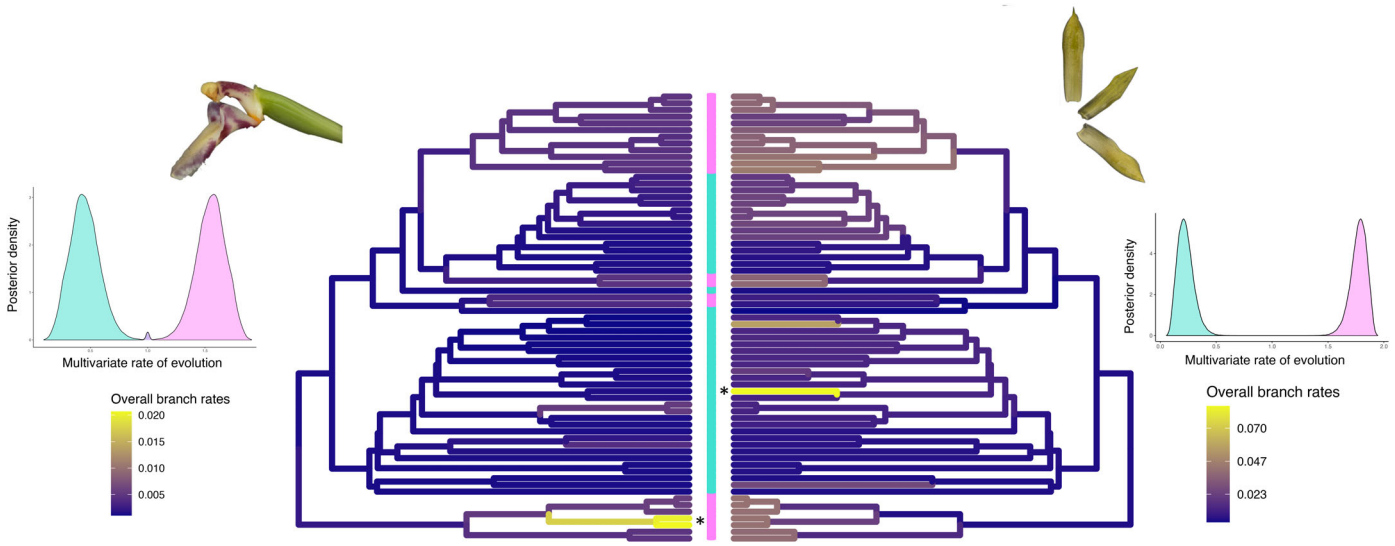

Fig. 4 Floral morphological evolution of the reproductive structure (left) and perianth (right) across the Cymbidieae radiation using the strict scheme of pollinator coding described in the methods. Branch colors on the phylogenies indicate overall rates of evolution, with dark purple indicating lower rates and yellow indicating higher rates. Bars between the phylogenies represent character states at the tips, with pink corresponding to perfume flowers and turquoise corresponding to nonperfume flowers. Plots below representative images of the floral structures represent posterior estimates of state-dependent evolutionary rates, with pink representing perfume flowers and turquoise representing nonperfume flowers. Regions of this distribution *c*. 0 on the *x*-axes correspond to iterations of the MCMC where no state dependence was identified (i.e. both groups exhibited identical rates of morphological evolution). Asterisks correspond to the taxa exhibiting the fastest overall rates of morphological evolution for either set of traits (*Clowesia* for the reproductive structure and *Brassia caudata* for the perianth).

Centridini tribes, respectively; Papadopulos *et al*., 2013). The presence of rapid phenotypic evolution in lineages that utilize alternative strategies within each clade mirrors the patterns of release from constraint found in previous studies of pollinator-mediated morphological stasis (Davis *et al*., 2014). For example, some of the fastest overall rates of reproductive structure evolution outside of perfume flowers occur in *Cryptocentrum latifolium* Schltr., a putatively moth-pollinated member of the Maxillariinae while the fastest overall rates of perianth evolution occurred in *Brassia caudata* (L.) Lindl. in the Oncidiinae (asterisk on the right side in Fig. 4), a species whose elongated flowers mimic spiders to attract pompilid wasps in search of larval hosts (van der Pijl & Dodson, 1966).

Elucidating the exact mechanisms underlying how androeuglossophily has fueled faster floral evolution is challenging. However, multiple nonmutually exclusive hypotheses can be invoked to explain this pattern. The first is that as scent acts simultaneously as both a signal and a reward in perfume flowers, selection on morphological elements of floral displays mediating constancy may be relaxed. Male euglossine bees readily collect scents from diverse sources outside of orchid flowers, such as aromatic leaves, feces, and even filter paper soaked with pure compounds, indicating a lack of context-dependent visual cues to elicit such behavior (Whitten *et al*., 1993; Pemberton & Wheeler, 2006; Henske *et al*., 2025). Consequently, there may be reduced constraint on the evolution of morphologies across the floral reproductive apparatus. Evidence for this hypothesis could manifest as reduced evolutionary covariance (*i.e.* integration) between the structures due to functional decoupling allowing for independent evolutionary trajectories across the floral phenotype.

Consistent with this scenario, we observe that perfume flowers lack significant integration between the perianth and reproductive structures while nonperfume flowers exhibit strong integration (Methods S1; Fig. S8). These patterns contrast with generally high integration across our dataset within the perianth and reproductive structures across pollination systems, suggesting that elevated evolutionary rates in perfume flowers are not due to a general release of developmental constraint, but rather to reduced coupling between floral modules (Fig. S9). Integration due to coupled function among these sets of traits is expected in the two most diverse clades containing nonperfume flowers, the Maxillariinae, and the Oncidiinae. Gullet flowers found in many members of the former group are formed via physical coupling of the perianth with the reproductive structure, forcing pollinators to contact the column as they enter this constricted structure to collect rewards. In the latter group, flowers of many species mimic flowers of various species across the Malpighiaceae that offer oil to female *Centris* bees, with functional coupling across the flower expected to create this visual similarity (Papadopulos *et al*., 2013; Castro & Singer, 2019).

Several studies have suggested a role of reduced integration across the flower (*i.e.* modularity) facilitating more rapid phenotypic evolution. For example, increased modularity was found to increase evolutionary rates in the Merianieae tribe of Melastomataceae, allowing for rapid transitions between divergent pollination modes (Dellinger *et al*., 2019). Additionally, modularity in concordance with developmental hypotheses was identified in

Malagasy *Bulbophyllum* orchids, allowing for independent evolution of different floral components (Artuso *et al.*, 2022). Our data are consistent with this framework, with weak integration in the perfume flowers perhaps contributing to their exceptional diversification in floral form into extreme areas of shape space facilitating evolution of their complex pollination mechanisms. Future work investigating integration across a larger set of floral traits, especially in 3D, would provide further clarity into this interpretation. Additionally, characterizing finer-scale patterns of integration at the intraspecific level using quantitative genetic methods would allow for the prediction of how microevolutionary processes promote or constrain divergence within and between populations (Bolstad *et al.*, 2014).

A second hypothesis is that the rapid accumulation of euglossine bee species in the last 10 million years (Myr) (Ramírez *et al.*, 2010) facilitated expansion of morphospace in perfume flowers through selection for pollinator partitioning. The high levels of diversity in sympatric bee populations, with up to 66 species in northwestern Amazonian regions (Padrón *et al.*, 2018), may have facilitated rapid morphological evolution causing differential pollinarium placement on bees to minimize potential costly hybridization between interfertile orchid taxa (Hills *et al.*, 1972). In the Cymbidieae, many species and even genera are interfertile as shown by an extensive history of horticultural crosses (https://www.rhs.org.uk/plants/horticulture-hub/plant-registration/orchid-hybrids/orchid-hybrid-lists). While the arrival of Cymbidieae orchids to the Americas was coincident with the origin of perfume collection in euglossine bees (both *c.* 34 Ma), predominantly androeuglossophilous lineages exhibit much younger ages (*c.*15 Myr for the Stanhopeinae and the Catasetinae, with many species originating in the past 5 Myr; Pérez-Escobar *et al.*, 2017a,b), consistent with the timing of euglossine diversification. These findings across perfume flowers parallel morphological expansion facilitated by floral character displacement within systems lacking major pollinator shifts, such as bat-pollinated *Burmeistera* (Muchhala & Potts, 2007) and bumble bee-pollinated *Pedicularis* (Eaton *et al.*, 2012), albeit spanning a greater phylogenetic breadth. Future studies investigating distribution of floral traits within communities to test for character displacement in perfume flowers vs nonperfume flowers would provide support for this hypothesis.

The floral measurements utilized in the design of this study provide a set of standardized dimensions to be taken in future studies permitting the generation of large orchid floral morphological datasets, such as those that have been created in fishes and birds (Price *et al.*, 2019; Tobias *et al.*, 2022). The increasing popularity of Lankester composite digital plates across botanical institutions will further provide a means for measuring these characteristics. While we note the inherent limitations of our sampling in terms of species diversity (5.2% of species diversity of the Cymbidieae for morphospace analysis and *c.* 2% for the strict pollinator-coding scheme used for comparative analyses), this sampling is comparable to several other recent studies connecting ecology to phenotypic evolution at broad scales using similar methodologies (e.g. 44/17 000 percomorph fishes in Corn *et al.*, 2021; 340–815/47 000 spiders in Wolff *et al.*, 2021; 207/6100 extant mammals in Goswami *et al.*, 2022; 228/14 000 acanthomorph fishes in Roberts-Hugghis *et al.*, 2023). Future work integrating across new results from pollination biology research along with combined efforts across botanical institutions to generate standardized images for morphological analysis will provide exciting avenues to understand the evolution of the famously complex orchid floral phenotype.

We note that future studies would greatly benefit from collection of 3D geometric morphometric data and color. The former would allow for characterization of the relative positioning of all organs in intact floral specimens, allowing for understanding evolution of this critical component of functional morphology. Such work has already been performed in several groups of flowering plants (Dellinger *et al.*, 2019; Reich *et al.*, 2020; Artuso *et al.*, 2022), and would increase our capacity to uncover further axes of morphological evolution in orchids. Quantification of color would provide another dimension of phenotypic variation with a critical role in pollinator attraction, with future studies perhaps investigating its evolutionary dynamics with morphology. Photogrammetry (i.e. inferring 3D shape from multiple 2D photos taken from different angles) along with the inclusion of color standards would be readily amenable for the construction of future LCDP's (Leménager *et al.*, 2023).

Our results provide new perspectives on the influences of pollination on the dynamics of floral evolution. Using a series of rigorous phylogenetic comparative methods, we reconstructed the evolutionary history of pollination in the tropical American Cymbidieae radiation and characterized the substantial impact of male euglossine pollination in triggering the exceptional diversification of functional morphology in perfume flowers. This shift in the adaptive landscape has allowed this group of plants to evolve some of the most complex pollination mechanisms that have captivated evolutionary scientists for over a century.

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used generative AI to troubleshoot code for data analysis and visualization. After using these tools, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

Competing interests

None declared.

Author contributions

JWL designed the study with input from SRR. JWL collected data, performed the major analyses, and wrote the first draft of the manuscript. DB, FP, APK, ZS, and ER created many of the digital plates from which most of the data were collected. OAP provided the phylogenetic hypothesis required for the comparative analyses. YX collected data and performed exploratory analyses. SRR facilitated the collaboration between Lankester Botanical Garden and UC Davis. All authors provided comments on the final draft.

ORCID

Diego Bogarín https://orcid.org/0000-0002-8408-8841
Adam P. Karremans https://orcid.org/0000-0001-5987-7710
Jasen W. Liu https://orcid.org/0009-0002-3353-1409
Oscar A. Pérez-Escobar https://orcid.org/0000-0001-9166-2410
Franco Pupulin https://orcid.org/0000-0001-6780-8395
Santiago R. Ramírez https://orcid.org/0000-0003-1306-1315
Eugenio Restrepo https://orcid.org/0000-0002-7037-1670
Zuleika Serracín https://orcid.org/0000-0002-8412-3555

Data availability

Processed data along with R and RevBayes scripts to replicate the results have been archived in Dryad at https://doi.org/10.5061/dryad.pk0p2nh1z. LCDPs generated for this study are available in Notes S1.

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Fig. S1 Sampling metrics across the study by subtribe at two taxonomic scales representing species diversity and generic diversity.

Fig. S2 Results of evolutionary modelling of orchid evolution using the relaxed scheme.

Fig. S3 State-specific and background rates of morphological evolution across the Cymbidieae using the relaxed scheme.

Fig. S4 State-specific and background rates of morphological evolution across the Cymbidieae using the strict scheme.

Fig. S5 Influence of different rate shift priors (1, 5, and 10 for low, medium, and high, respectively) on total number of shifts and the ratio of rates of evolution of perfume flower to nonperfume flowers in the strict scheme.

Fig. S6 Influence of different rate shift priors (1, 5, and 10 for low, medium, and high, respectively) on total number of shifts and the ratio of rates of evolution of perfume flower to nonperfume flowers in the relaxed scheme.

Fig. S7 Morphospaces of the neotropical Cymbidieae flower comparing axes of variation in the reproductive structure and the perianth colored by subtribe.

Fig. S8 Results of a two-block phylogenetic partial least-squares analysis comparing evolutionary integration in Cymbidieae flowers between the perianth and reproductive structure across perfume and nonperfume flowers.

Fig. S9 Results of a two-block phylogenetic partial least-squares analysis comparing evolutionary integration in Cymbidieae flowers within the reproductive structure and reproductive structure.

Methods S1 Methods used to describe the partial least-squares analysis used to compute integration.

Notes S1 LCDPs generated by the authors for this study.

Table S1 Sources of additional species not sampled during this study.

Table S2 Results of phylogenetic regression of log(size) on log-shape ratios for each perianth trait.

Table S3 Comparison of results from stochastic character mapping, morphological disparity analyses, and evolutionary rate modelling among pollination modes using the relaxed scheme.

Table S4 Substitutions of species made in this study.

Table S5 Loadings of each principal component of perianth variation.

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