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RESEARCH ARTICLE

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The evolution of floral ontogenetic allometry in the Andean genus *Caiophora* (Loasaceae, subfam. Loasoideae)

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The astounding variety of angiosperm flower morphologies has evolved in response to many selective forces. Flower development is highly coordinated and involves developmental associations between size and shape, ontogenetic allometry, which in turn affect the morphology of mature flowers. Although ontogenetic allometries can act as a developmental constraint and may influence adaptive evolution, allometries can evolve themselves and may change rapidly in response to selection. We explored the evolution of ontogenetic allometry in the flowers of 11 species of Loasoideae. Seven species belong to *Caiophora*, which radiated recently in the central Andes, and contains species that are pollinated by bees, hummingbirds, and small rodents. According to a previous study, the diversification of *Caiophora* involved departures from simple allometric scaling, but the changes to allometry that enabled flower diversification have not been explored yet. We characterized the ontogenetic allometry of each species with the methods of geometric morphometrics. We studied the evolution of allometries by constructing allometric spaces, in which the allometry of each species is represented by a point and the arrangement of points indicates the relations among allometric trajectories. To examine the history of changes of ontogenetic allometries, we projected the phylogeny into the allometric spaces. Inspection of allometric spaces suggests that ontogenetic variation is limited to a few dominant features. The allometries of the two main functional flower parts under study differ in their evolutionary labilities, and patterns of variation reflect pollination systems, differences in structural organization, and abiotic environmental factors.

1 | INTRODUCTION

Flower morphology underwent enormous evolutionary changes, adapting to a wide arrange of environmental conditions, including different mating and pollination scenarios (Barrett, 2013; Fenster, Armbruster, Wilson, Dudash, & Thomson, 2004; Strauss & Whittall, 2006). Duplication, loss or merging of floral structures, homeotic changes of flower organs, and changes in flower symmetry are among the mechanisms that enabled floral structure to evolve (Becker, Alix, & Damerval, 2011; Endress, 2011; Glover, Airoldi, Brockington, Fernández-Mazuecos, & Martínez-Pérez, 2015). Even among taxa that share the same floral bauplan, evolutionary changes in the sizes, shapes, and arrangement of floral structures produced extensive variation in floral morphology (Gardner et al., 2016; Gómez, Torices, Lorite, Klingenberg, & Perfectti, 2016; McCarthy et al., 2016). These changes were accompanied by alterations of floral development, which were reflected in the respective patterns of ontogenetic

allometry, the association between size and shape during development.

During development, from the initiation of primordia through anthesis to wilting, flowers undergo coordinated changes in size and shape, resulting in specific patterns of ontogenetic allometry. Evolutionary changes of morphological traits in functional (mature) flowers can occur by extending or truncating ancestral allometric trajectories, a phenomenon that has been discussed as ontogenetic scaling, often in connection to heterochrony (Gould, 1975; Klingenberg, 1998; Li & Johnston, 2000; Strelin, Benitez-Vieyra, Fornoni, Klingenberg, & Cocucci, 2016). If changes of this kind evolve readily and the directions of ontogenetic trajectories remain relatively constant, morphological variation among taxa will be concentrated along the trajectories, so that ontogenetic allometry can be viewed as a developmental bias or constraint favoring evolutionary changes along the allometric trajectory (Arthur, 2002; Gould, 2002; Voje, Hansen, Egset, Bolstad, & Pélabon, 2013). Yet, ontogenetic allometries can also evolve themselves, and such alterations in allometry may be a mechanism for diversification of floral shapes. Variation in ontogenetic allometries can involve differences in the pattern of growth-related shape change, as well as differences in the strength of ontogenetic allometries (Figure 1). Interestingly, population differences in ontogenetic allometry of flower traits have been found to be associated with differences in pollination systems (Hazle & Canne-Hilliker, 2005; Summers, Hartwick, & Raguso, 2015). Accordingly, evolutionary changes in ontogenetic allometry of flowers may play adaptive roles and are themselves an important subject of study.

The evolution of allometric patterns can be studied using multivariate ordinations of allometric vectors by principal component analysis (PCA) (Klingenberg & Froese, 1991; Klingenberg & Spence, 1993; Solignac, Cariou, & Wimitzky, 1990), an approach that has more recently been called allometric spaces (Gerber, Eble, & Neige, 2008; Wilson & Sánchez-Villagra, 2010). In an allometric space, each point represents the allometry of a taxon, and the relative arrangement of these points, in combination with phylogenetic information, can therefore provide information on the evolution of allometry. Most studies using allometric spaces are based on traditional morphometrics and have characterized ontogenetic allometry as the first principal component of a set of distance measurements (Jolicoeur, 1963; Klingenberg, 1996). In this study, we extend the approach of allometric spaces to geometric morphometrics (Dryden & Mardia, 2016; Klingenberg, 2010; Zelditch, Swiderski, & Sheets, 2012). Accordingly, we analyze ontogenetic allometry using multivariate regressions of the shape of floral structures on their sizes (Klingenberg, 2016; Monteiro, 1999).

We apply the approach of allometric spaces to examine how ontogenetic allometry evolved in the Andean genus *Caiophora* (Loasaceae, subfam. Loasoideae). The adaptive radiation in *Caiophora* involved at least one transition from the ancestral condition of bee- to hummingbird-pollination, one transition from hummingbird- to small rodent-pollination, and at least one reversion from hummingbird- to bee-pollination (Strelin, Arroyo, Fliesswasser, & Ackermann, 2017). These transitions were accompanied by changes in flower morphology (Strelin, Benitez-Vieyra, Ackermann, & Cocucci, 2016), and took place during the last 10 Myr (most of them took place during the last 5 Myr), following the uplift of different Andean mountain ranges (Strelin et al., 2017).

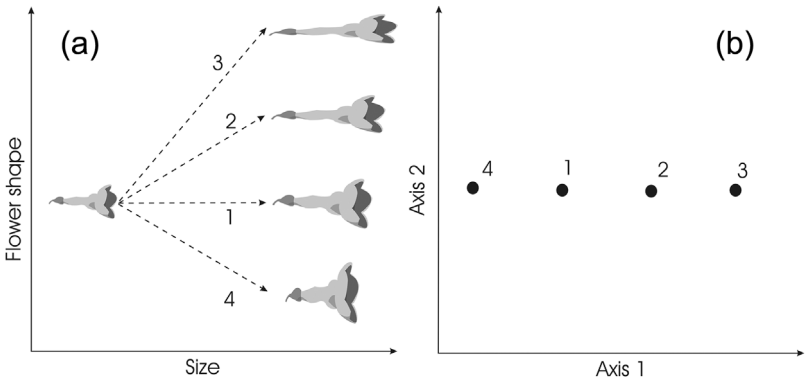

FIGURE 1 Variation of allometries as plots of shape versus size and in the corresponding allometric space. (a) Four hypothetical examples of different ontogenies. Taxon 1 shows isometric growth, with no shape change. Taxa 2 and 3 have the same type of allometric shape change, but taxon 3 has a stronger allometry than taxon 2, because it has a greater shape change per unit of increase in size. Taxon 4 has an allometric shape change that is the opposite of the change in taxa 2 and 3, but the strength of allometries is the same in taxa 2 and 4. (b) The allometric space, in which each of the allometries is represented by a single point. Because the allometries in this simplified example only involve a single aspect of shape (elongation versus shortening/widening, the vertical axis in panel [a]), they occupy only one dimension in the allometric space (no variation along axis 2)

This suggests rapid diversification of the genus responding to Andean orogeny and the concomitant changes in the pollination environment (Strelin et al., 2017). Ontogenetic scaling cannot account for the evolution of floral shapes in *Caiophora*, and there is evidence that significant departures from the ancestral pattern of flower ontogenetic allometry accompanied this diversification (Strelin, Benitez-Vieyra, Fornoni, Klingenberg, & Cocucci, 2016). Therefore, it seems promising to use allometric spaces for exploring the evolution of ontogenetic allometry and the specific changes that accompanied the evolution of different pollination strategies. This study investigates ontogenetic allometries of flowers in seven *Caiophora* species (including bee-, hummingbird-, and rodent-pollinated species) and four species in their allied bee-pollinated genera, *Loasa* and *Blumenbachia*. Ontogenetic allometries for two functional floral parts involved in pollination (corolla and staminode complex) were first characterized using multivariate regression of shape on size in each taxon (Klingenberg, 2016; Monteiro, 1999). Allometric spaces were then obtained from multidimensional ordinations of ontogenetic allometries using PCA. Finally, the phylogeny of these species was projected into the allometric space (Klingenberg & Ekau, 1996; Sidlauskas, 2008) to visualize the phylogenetic history of the evolution of ontogenetic allometries. This combination of methods is a powerful approach to investigate how allometries evolve and their role in morphological diversification.

2 | MATERIALS AND METHODS

2.1 | Study system

The Andean genus *Caiophora* (Loasaceae, subfam. Loasoideae) is monophyletic and radiated recently into more than 50 species (Ackermann & Weigend, 2006; Strelin et al., 2017). Adaptation to vertebrate pollination (Ackermann & Weigend, 2006; Strelin, Benitez-Vieyra, Ackermann, et al., 2016), in concert with the uplift of different Andean mountain ranges, may have triggered this radiation (Strelin et al., 2017). While early diverging *Caiophora* species, as well as species in its allied genera, *Loasa*, *Blumenbachia* and *Scyphanthus*, are bee-pollinated (Ackermann & Weigend, 2006; Strelin et al., 2017), some species in *Caiophora* evolved adaptations to hummingbird pollination (Strelin, Benitez-Vieyra, Ackermann, et al., 2016). The genus also includes a single species pollinated by small rodents, *Caiophora coronata* (Cocucci & Sérsic, 1998), which presumably evolved from a hummingbird-pollinated ancestor (Strelin et al., 2017). In addition, at least one reversal from hummingbird to bee pollination took place in *Caiophora* (Strelin et al., 2017).

Loasoideae presents highly complex flower morphology (Figure 2a) consisting of a divided corolla with pouch-shaped petals protecting the stamens, and a whorl of five androecium derived staminode complexes. The three outer staminodes of each complex are united into a nectar scale and bear the two inner, free staminodes (Brown & Kaul, 1981). This structural complex mediates flower-pollinator fit, conditioning nectar harvesting (Ackermann & Weigend, 2006; Hufford, 2003). The shapes of the corolla and the staminode complex were under pollinator selection in Loasoideae (Strelin, Benitez-Vieyra, Ackermann, et al., 2016). Bee-pollinated species have small pendulous flowers, which require the pollinator to land and hold onto the flower by grappling the nectar scales. These flowers present open corollas, which make the nectar scales visible and easy to grasp (Weigend, 2004). The staminode complex participates in a stamen release mechanism, which is activated when bees insert their proboscis between the scale and the two protruding staminodes (which block the nectar scale) and move the scale outwards to access the nectar (Figure 2b; Weigend, Ackermann, & Henning, 2010). Unlike bee-pollinated species, flowers of hummingbird-pollinated species present narrower corollas and staminode complexes with conspicuous staminodes that noticeably protrude beyond the nectar scale opening. These staminodes ensure the contact of the hummingbird head with fertile flower structures by guiding the pollinator's beak toward the nectar container (Figure 2c; M. Weigend, pers. comm.). The staminodes are markedly reduced in the rodent pollinated species since they do not participate in pollination (Figure 2d; Strelin, Benitez-Vieyra, Ackermann, et al., 2016).

2.2 | Study design and data collection

This study includes seven species of *Caiophora* and four bee-pollinated species of two allied genera, *Loasa* and *Blumenbachia*. The sampling of *Caiophora* includes four bee-pollinated species and two hummingbird-pollinated species. Hummingbirds were also reported to visit *Caiophora lateritia*, which is one out of the four selected bee-pollinated *Caiophora* species (Ackermann & Weigend, 2006; Strelin, Benitez-Vieyra, Ackermann, et al., 2016). We also included the single rodent-pollinated species, *C. coronata*. The sampling for each species included between 8 and 25 individual plants of the same population; the number of individuals sampled for each species depended on the availability of plant material in the field (Table S1). One flower in anthesis and four flower buds, covering a range of flower bud diameter from approximately 3–20 mm in large-flowered species and from approximately 3–10 mm in small-flowered species, were sampled from each individual. Samples were kept in 70% ethanol and later dissected. The petal and the staminode complex were photographed in lateral view using a Leica M420 stereomicroscope (Heerbrugg, Switzerland).

Because both the corolla and staminode complex structures have a functional role during pollination in Loasoideae, we characterized the size and shape of both

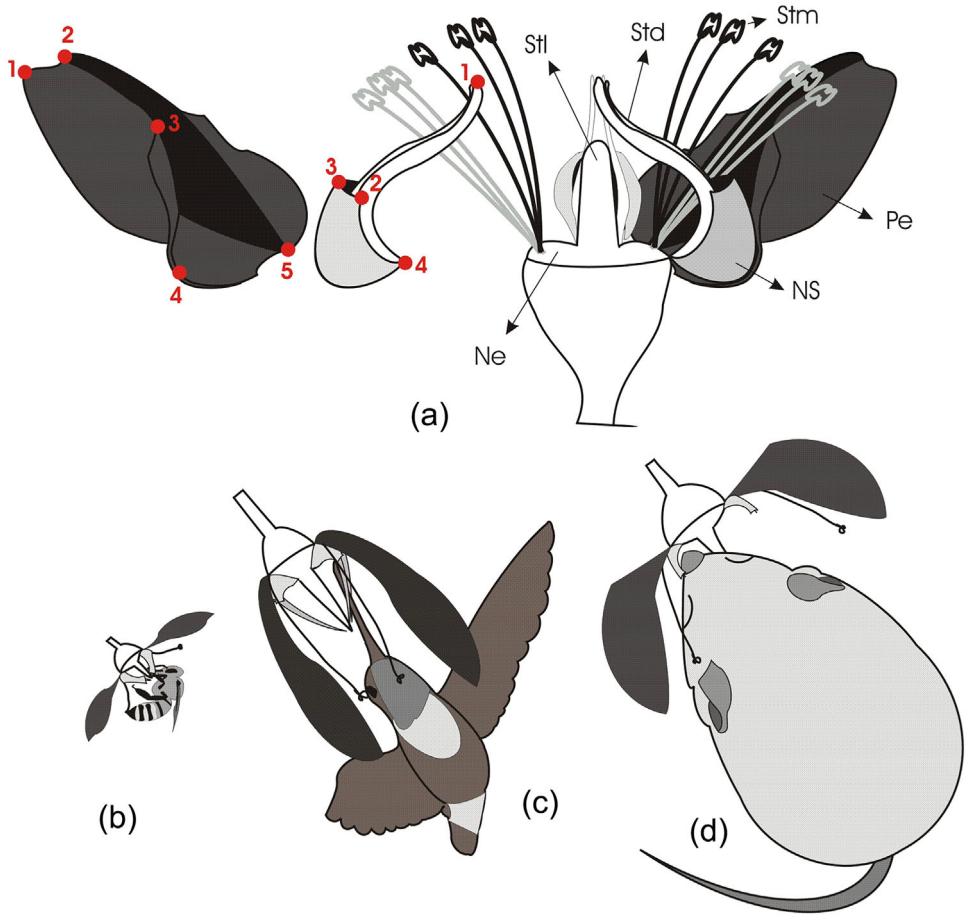

FIGURE 2 Loasoideae flowers and pollination modes. (a) Schematic representation of a typical Loasoideae flower, indicating the name of each floral structure: Pe, petal; NS, nectar scale; Std, staminode; Stm, stamen; Stl, style; Ne, nectary. The landmarks representing the shape of the petal and the staminode complex are in red (numbers correspond to those in Figure S2). (b) Bee-pollinated flower. (c) Hummingbird-pollinated flower. (d) Flower of *Caiophora coronata*, the species pollinated by small rodents. Notice the reduced staminodes

with geometric morphometric methods. The petal shape was used to represent corolla shape because developmental changes in the shape of the separate petals are easier to follow than developmental changes in the corolla as a whole.

The tpsDig software (Rohlf, 2006) was used to digitize five landmarks on the petal (Figure 2a) and four landmarks on the staminode complex (Figure 2a) of flowers and developing buds of the 11 focal species. Based on morphological and anatomical evidence, the landmarks placed on each flower structure are assumed to be homologous across species (for exact definitions of the landmarks, see Figure S2).

2.3 | Ontogenetic allometry

To extract shape information from the landmark coordinates, we applied a Procrustes fit using the MorphoJ software (Klingenberg, 2011). As a measure of size of each floral structure, centroid size was computed.

Ontogenetic allometry can be described by measuring how several traits covary during growth, for example, several distance measurements on a structure taken at different developmental stages (Huxley–Jolicoeur school), or as the developmental relationship between the size and the shape of a structure, for example, how flower proportions change during flower growth (Gould–Mosimann school; for more details on this distinction, see Klingenberg, 2016). Fundamentally, these approaches to characterize allometry are logically equivalent, as they both describe how shape changes during growth, and mutually compatible results are expected from both approaches (Klingenberg, 2016). In geometric morphometrics, size and shape are usually quantified separately, following the logic of the Gould–Mosimann school (Klingenberg, 2016). Accordingly, to characterize ontogenetic allometry, we used a multivariate regression of the shape (Procrustes coordinates) on the log-transformed centroid size of each flower structure and in each species separately (Monteiro, 1999). The log-transformed centroid size was used instead of the raw centroid size, because it yields a more linear relationship between size and shape for ontogenetic allometry (Klingenberg, Duttke, Whelan, & Kim, 2012). Because shape changes are often concentrated in the range of smaller sizes in ontogenies and there is relatively

little shape variation among larger samples, log-transformation of the size axis often achieves a better linear relationship (Klingenberg, 2016; Klingenberg et al., 2012) and thus simplifies the comparison of allometric trajectories among taxa. Unlike the Huxley–Jolicoeur approach, where log transformation of measurements is a key part of the theoretical justification why allometric trajectories are expected to be linear (Huxley, 1932), the precise form of the relation between size and shape in the Gould–Mosimann approach is open and therefore investigators are free to choose freely whether to use raw or log-transformed values of the size measure. A permutation test (Good, 2000) was performed for each multivariate regression in order to assess the statistical significance of the association between size and shape.

The resulting regression vectors represent the expected change in the relative landmark positions per unit of increase of the log-transformed centroid size. Since the landmarks are homologous among taxa and allometric vectors were computed in the same way in each species, the ontogenetic vectors are also homologous. Therefore, it is possible to compare the ontogenetic vectors of different taxa directly.

The strength of allometry (Figure 1) can be quantified as the length of the allometric regression vectors, which indicates the amount of shape change expected per unit of increase in size. In this study, this quantity is in units of Procrustes distance per unit of increase of log-transformed centroid size, where one unit corresponds to an increase of centroid size by a factor of 2.718 (Euler's number). The length of the regression vector can be computed as the norm of the vector, which is the square root of its inner product, or equivalently, the square root of the sum of squared regression coefficients (the latter version can be used to compute this quantity with any spreadsheet from standard output of morphometrics or statistics programs). For testing the presence of a phylogenetic signal in the strength of allometry, we used a permutation test that simulates the null hypothesis of no phylogenetic signal by randomly swapping the values among taxa (Laurin, 2004), with 10,000 repeats of the swapping procedure for each test.

2.4 | Allometric spaces

To explore the evolution of ontogenetic allometry for each flower structure, allometric spaces were obtained (Gerber et al., 2008; Klingenberg & Froese, 1991). Each dimension of an allometric space expresses variation among taxa in the growth-related changes for the respective shape variable. The allometry of each taxon is represented by a point in this allometry space, and distances between points represent differences between ontogenetic allometries (Figure 1). Allometric spaces are obtained from a multivariate ordination of allometric vectors, usually using principal components of the vectors themselves (Gerber et al., 2008; Klingenberg & Froese, 1991; Klingenberg & Spence, 1993; Wilson, 2013; Wilson & Sánchez-Villagra, 2010). An alternative method, which is likely to give broadly similar results in practice, is to use a multidimensional scaling analysis based on the angles between allometric vectors (Frédérich & Vandewalle, 2011; Urošević, Ljubisavljević, & Ivanović, 2013).

For each flower structure, the ontogenetic vectors obtained from multivariate regression analyses for the 11 taxa were used as observations in a PCA to analyze the allometric spaces (Klingenberg & Froese, 1991; Klingenberg & Spence, 1993). The PCA needs to use the covariance matrix of the allometric vectors (and not the correlation matrix, which is the default in many statistics programs) because the scaling of variables of the ontogenetic vectors reflects the allometry that is of interest, and any standardization would destroy this scaling (and, in the context of geometric morphometrics, also the scaling that reflects the Procrustes geometry). Because the allometric vectors have the same variables as the morphospace in which allometry is characterized (log-transformed measurements in traditional morphometrics, landmark coordinates in geometric morphometrics), the allometric space and corresponding morphospace share the same coordinate system and are thus closely related. In the context of geometric morphometrics, it is therefore possible to use the usual tools for visualizing shape changes (Klingenberg, 2013) to display the morphological meaning of the PC axes. Because the observations in the PCA are allometric vectors for the taxa in the study, the PCs obtained in the analysis are those axes that account for the maximum amount of variation among allometries of different taxa. For this study, ontogenetic vectors were imported into R (R Core Team, 2016), where two separate PCAs (one for each flower structure) were run using the function *prcomp*. The resulting PC coefficients (eigenvectors) were imported back into MorphoJ (Klingenberg, 2011) in order to visualize the changes in allometric vectors associated to each PC.

To investigate the evolutionary history of changes in allometry, we projected the phylogeny of Loasoideae (Strelin et al., 2017), pruned to include only the 11 species for which ontogenetic allometry data were available, into the scatter plots of the first two PCs of allometric spaces (Gómez et al., 2016; Klingenberg & Ekau, 1996; Sidlauskas, 2008). The position of internal nodes in the allometric spaces was determined following Sidlauskas (2008), using a maximum-likelihood algorithm which is mathematically equivalent to weighted squared-change parsimony (Sidlauskas, 2008). This was done with the *phylomorphospace* function of the *phytools* package in R (Revell, 2012). In analogy with the expression "phylomorphospace" (Sidlauskas, 2008) for scatter plots showing a phylogeny projected into a space derived from a morphometric analysis, it might seem tempting to coin an

expression such as "phyloallometric space" (to call it phylomorphospace would be incorrect because we are dealing with an allometric space, not a morphospace). We recommend against this type of name, however, because it might suggest to unwary readers that there is something special about the space in relation to phylogeny. Such an impression would be mistaken, because the underlying space (allometric space or morphospace) is exactly the same whether or not a phylogeny is projected into it —the space is altered no more than drawing a phylogenetic tree onto a sheet of paper transforms it into a "phylopaper."

As a statistical assessment of the phylogenetic signal in the variation of allometries among species, we used a permutation test that simulated the null hypothesis of no phylogenetic signal by randomly exchanging allometric vectors among taxa, a direct equivalent of a test widely used to assess the phylogenetic signal in the average shapes of species (Klingenberg & Gidaszewski, 2010). For each floral structure, the permutation test used 10,000 randomization rounds.

3 | RESULTS

3.1 | Ontogenetic allometries

The permutation tests associated with the regression analyses indicated that all taxa displayed statistically significant ontogenetic allometry ($p < 0.0001$ in all cases). Size predicted between 16.57% and 62.84% of shape variation within taxa for the petal and between 9.38% and 50.84% for the staminode complex (Table 1).

The shape changes associated with ontogenetic allometry differed among taxa, but there were also general patterns shared among them (Figure 3). Petal growth in all species involves a progressive straightening of the petal base associated with opening of the flower (Figure 3). The extent of this straightening differs to some degree among taxa. Furthermore, in some species such as *C. lateritia* and *L. acerifolia*, a relative reduction in the size of the tip of the petal takes place during growth. For the staminode complex, a common feature is that the nectar scale overgrows the staminode, more so in some species such as *L. bergii*, *C. nivalis*, or *C. coronata* than in others such as *C. chuquitensis* or *C. hibiscifolia* (Figure 3). Furthermore, in some species such as *L. acerifolia*, *B. insignis, B. silvestris*, and *C. coronata*, there is a reduction of the angle between the main axes of the nectar scale and the staminode, but there are also some species where this angle becomes larger during growth, such as *C. hibiscifolia* and *C. dumetorum*.

Comparisons of the strengths of ontogenetic allometry showed that, for the petals, the allometries tend to be weaker in *Caiophora* than in species in its allied genera (except for *C. lateritia*; Table1). In accordance with this impression, the permutation test found a statistically significant phylogenetic signal for the strength of allometry in petals ($p = 0.021$). Overall, species in *Caiophora* tend to undergo less change in petal shape per unit of increase in log-transformed centroid size than species in its allied genera (Table 1). For the staminode complex, the strength of ontogenetic allometry varies substantially and without any apparent relationship to phylogeny or pollinators (Table 1), and the permutation test found no significant phylogenetic signal ($p = 0.73$). The ontogenetic allometries of the hummimgbird-pollinated species, *C. hibiscifolia,* and *C. chuquitensis*, are among the weakest in this study, both for the petal and the staminode complex (Table 1).

TABLE 1 The strength of ontogenetic allometry (in units of Procrustes distance per unit of increase in log-transformed centroid size, corresponding to an increase by a factor of 2.718), and percentage of shape variation explained by size in each species

	Petal		Staminode complex	
Species	**Strength**	**% shape variation**	**Strength**	**% shape variation**
Blumenbachia insignis	0.259	54.22	0.227	24.98
Blumenbachia silvestris	0.291	62.84	0.168	18.02
Loasa acerifolia	0.208	49.21	0.349	50.06
Loasa bergii	0.266	41.71	0.432	33.45
Caiophora chuquitensis	0.151	46.01	0.085	9.38
Caiophora clavata	0.191	45.87	0.389	30.50
Caiophora coronata	0.166	35.60	0.515	34.48
Caiophora dumetorum	0.193	52.31	0.478	50.84
Caiophora hibiscifolia	0.104	42.11	0.185	45.52
Caiophora lateritia	0.235	51.67	0.168	19.68
Caiophora nivalis	0.137	16.67	0.599	35.15

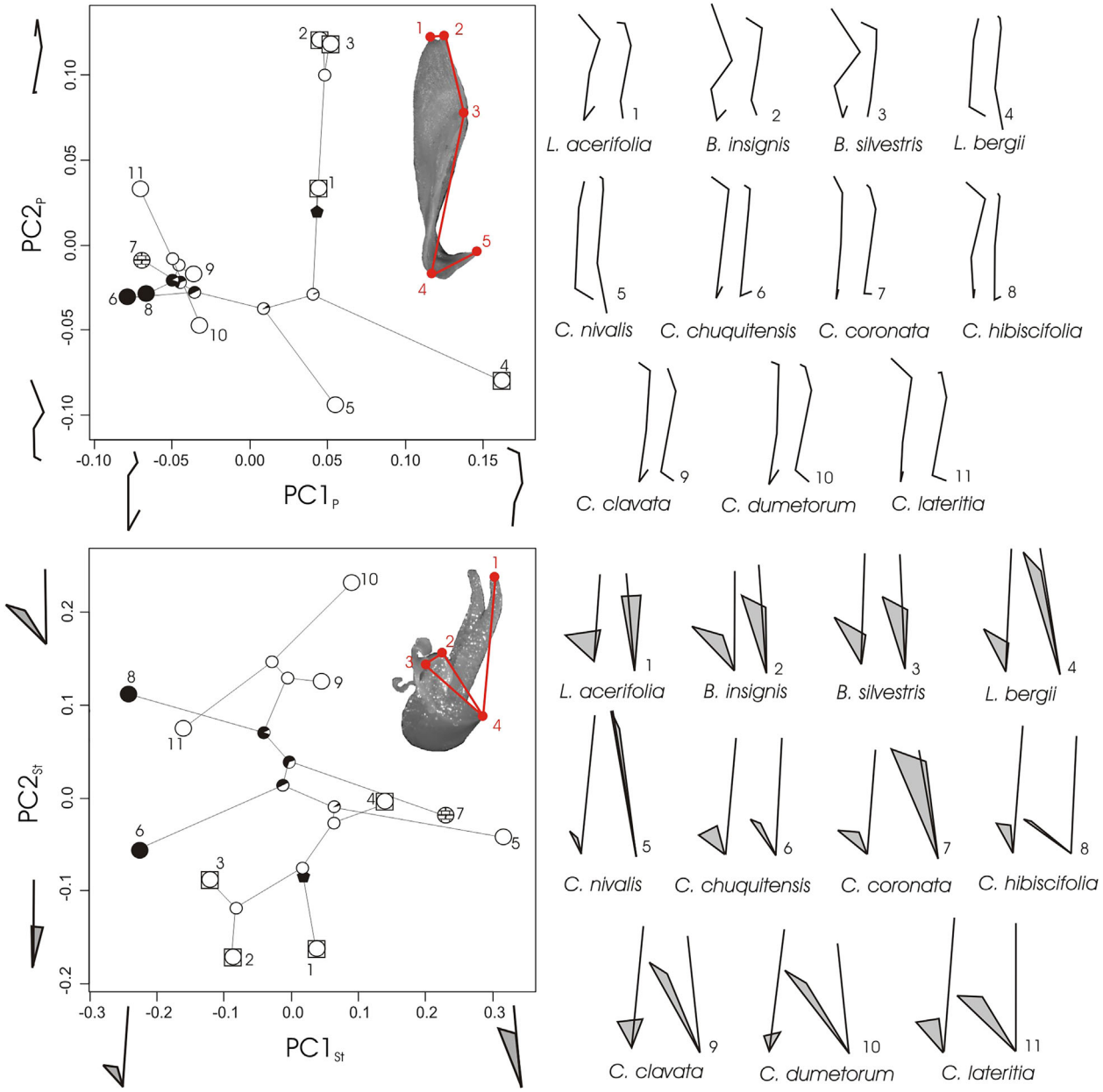

FIGURE 3 Ontogenetic allometries of the individual taxa and the allometric spaces of the petal (top) and of the staminode complex (bottom) in the 11 studied species. A photograph of the corresponding flower structure was added to each plot, showing the selected landmarks. The ontogenetic allometries of each species are represented next to the corresponding allometric space. Development proceeds from left to right in each representation. Species in *Loasa* and *Blumenbachia* are indicated with a square. Pollination modes are represented: white, bee pollination; black, hummingbird pollination; bricks, small rodent pollination. The posterior probability of pollination modes is represented on each node. This information was obtained from Strelin et al. (2017). The root of the phylogeny is indicated with a polygon

3.2 | Allometric spaces

For the petals, the first two PCs accounted for 44.17% and 37.75% of the total variance among allometric vectors of the 11 taxa, whereas for the staminode complex, the first two PCs took up 67.02% and 30.44% of the total variance. Two dimensions were therefore sufficient to summarize more than 80% of the variation among the allometric vectors for the petal and nearly all the variation for the staminode complex.

For the petals, the PC1 primarily represents variation between allometries for which the straightening of the base is weaker (Figure 3, negative values of $PC1_P$) or stronger (positive values of $PC1_P$). This change is combined with differential expansion of the portion of the petal between landmarks 3 and 4 versus the two adjacent regions (between landmarks 2 and 3, 4 and 5; in favor of the middle portion for negative values of $PC1_P$, in favor of the adjacent regions for positive values of $PC1_P$). The PC2 for petals mainly

represents variation in the disproportionate growth of the two distal parts of the petal, with some allometries featuring a greater relative contraction of the distal-most portion between landmarks 1 and 2 (Figure 3, positive values of $PC2_P$) and others with a weaker relative contraction (negative values of $PC2_P$, appearing as a relative expansion in the diagram). For the staminode complex, the PC1 represents variation in the degree to which the nectar scale overgrows the staminodes, which is only relatively weak in the allometries of some taxa (Figure 3, negative values of $PC1_{St}$) but very pronounced for the allometries of other taxa (positive values of $PC1_{St}$). The PC2 for the staminode complex stands mainly for variation in the allometric changes of the angle at which the staminodes emerge from the nectar scale: this angle narrows during growth for allometries corresponding to negative values of $PC2_{St}$, whereas the angle widens for allometries with positive values of $PC2_{St}$.

Projecting the phylogeny into the allometric spaces shows the patterns of evolutionary divergence of ontogenetic allometries (Figure 3). For both floral structures, the allometries of the different taxa occur in regions of the allometric space that are more or less separated between species of *Caiophora* and its allied genera. In the allometric space for the petals, all *Caiophora* species, except for the early diverging *C. nivalis*, are clustered together in a limited region toward the lower-left of the plot in Figure 3, suggesting that they share fairly similar and phylogenetically derived petal allometries. In accordance with this, the permutation test indicated a statistically significant phylogenetic signal ($p < 0.0001$). By contrast, for the staminode complex, ontogenetic allometries diverged markedly among the species of *Caiophora*, with substantial divergence even among closely related species, whereas the variation among the remaining taxa is fairly limited. The permutation test found no significant phylogenetic signal for the allometries of the staminode complex ($p = 0.20$). Intriguingly, the two hummingbird-pollinated species included in this study (species 6 and 8 in Figure 3) occupy extreme positions in both allometric spaces.

4 | DISCUSSION

This study explored the evolution of ontogenetic allometry in flowers of 11 Loasoideae species. It extends an earlier study reporting that ontogenetic scaling is not a sufficient explanation to account for the range of floral shapes in this group (Strelin, Benitez-Vieyra, Fornoni, et al., 2016), raising the questions whether and how ontogenetic allometries themselves evolve. As a tool to address these questions, we have used allometric spaces, a method that has long been used in traditional morphometrics, but not yet in the context of geometric morphometrics. Exploring the structure of allometric spaces and the distribution of taxa within them provides insight into the evolution of ontogenies in the Loasoideae and suggests possible adaptive connections to pollination systems.

The analysis of allometric spaces indicated that the first two dimensions accounted for more than 80% and 90% of the total variation in ontogenetic allometries for the two floral structures under study. For 11 taxa, the number of dimensions of the allometric space is ten (one degree of freedom is lost for the overall mean), as it would for any other phenotypic spaces, including morphospaces. That just two of the available ten dimensions account for the vast majority of variation suggests that evolutionary changes in allometric trajectories were concentrated mainly in a few morphological features of the two floral structures. Accordingly, two-dimensional plots (Figure 3) provide a mostly complete picture of the allometric space and thus the evolution of ontogenetic allometries.

The diversification of *Caiophora* involved the colonization of new regions in the allometric spaces both for the petal and the staminode complex (Figure 3). Whereas the area of the petal allometric space colonized by *Caiophora* is smaller than that occupied by species in its allied genera, this is not the case for the allometric space of the staminode complex, where the area occupied by *Caiophora* is comparatively larger (Figure 3). In the allometric space for petals, ancestral state estimation yielded a scenario where many terminal branches were relatively short (i.e., there were relatively small changes in allometries), whereas the internal nodes tended to be clearly separated (Figure 3). By contrast, in the allometric space for the staminode complex, internal branches tended to be shorter than terminal branches, so that even closely related species can drastically diverge from each other (Figure 3). This pattern is in agreement with the statistically significant phylogenetic signal for petal allometry (both in the strength and type of growth related shape changes) and with the lack of a significant phylogenetic signal for the allometry of the staminode complex. While the petal can be considered a unitary structure, the staminode complex of Loasoideae is modular as it is composed of two separate but coupled subunits: the nectar scale and the staminode (Hufford, 2003). The lack of phylogenetic signal in the allometry of this structure may relate to its modularity, which may render its development and thus ontogenetic allometry more evolutionarily labile than the unitary petal (Diggle, 2014).

Inspection of the allometric spaces for both structures suggests that the variation of ontogenetic allometries is linked to the mode of pollination. For both allometric spaces, the two hummingbird-pollinated species (*C. hibiscifolia* and *C. chuquitensis*) are close to each other, but in an extreme position in relation to the other taxa, and the presumably partly hummingbird-pollinated *C. lateritia* is also nearby (Figure 3). The rodent-pollinated *C. coronata* is in near-extreme positions in both allometric spaces, close to the hummingbird-pollinated taxa for the petals and far from them

for the staminode complex (Figure 3). Mature flowers in hummingbird-pollinated *Caiophora* species tend to present narrow corollas (with petals bent at their bases) and staminode complexes with the staminodes protruding noticeably from the nectar scale opening, when compared to bee and small rodent pollinated species (Figures 2b and 2c; Strelin, Benitez-Vieyra, Ackermann, et al., 2016). The narrow corollas and the elongated staminode tips guide the hummingbird beak toward the nectar containers, ensuring the contact of the hummingbird head with fertile flower structures (Figure 2c). Flowers of the rodent-pollinated *C. coronata* have a partially open corolla, with staminodes almost enclosed by the nectar scale (Figure 2d; Cocucci & Sérsic, 1998).

Abiotic environmental factors may also play a role in the evolution of ontogenetic allometries. In the allometric space of the petals, the *Caiophora* species were separated from the species of the allied genera in a region that corresponds to allometries involving an incomplete unfolding of the corolla, maintaining a clear angle at the petal base (Figure 3). By contrast, there was no such separation in the allometric space of the staminode complex (Figure 3).Whereas *Loasa* and *Blumenbachia* are lowland lineages (<1,000 m.a.s.l.), most of the *Caiophora* species are associated to the Andes and grow at intermediate (1,500–3,000 m.a.s.l.) to high elevations (>3,000 m.a.s.l.) (Ackermann & Weigend, 2006). When compared to lowland lineages, *Caiophora* plants therefore experience greater exposure to UV-B radiation. It has been demonstrated that UV-B radiation plays an important role in the evolution of protective floral forms in alpine plants, since UV-B is detrimental for male and female plant fitness (Wang, Meng, Yang, & Duan, 2010). Protective floral forms evolving at high altitudes include down-facing flowers (Wang et al., 2010), and tubular corollas (Zhang, Yang, & Duan, 2014). The retention of partially bud-like corollas in *Caiophora*, independently of the pollination system, may convey protection to fertile flower structures from UV-B damage.

The strength of ontogenetic allometry for both floral structures varied extensively among the species included in this study (Table 1). Evolutionary changes in the strength of ontogenetic allometry can produce heterochronic changes (Alberch, Gould, Oster, & Wake, 1979; Klingenberg, 1998). The evolution of weaker ontogenetic allometry can result in paedomorphic, or underdeveloped, shape features, since it involves attainment of reproductive maturity (flower anthesis in our study) with a shape that corresponds to earlier developmental stages in the ancestor. Conversely, comparatively stronger ontogenetic allometries can give rise to peramorphic, or overdeveloped, shape features. Note that evolutionary changes in the initial shape, the size at maturity and the direction of the allometric trajectory can complicate these relationships. Although we did not find a clear match between the strength of ontogenetic allometries and pollination systems, ontogenetic allometries for both the petal and the staminode complex are weak in the two hummingbird-pollinated species (*C. hibiscifolia* and *C. chuquitensis*). This may suggest concerted evolution of paedomorphic features in both floral structures of hummingbird-pollinated species (Table 1). Interestingly, evolution of hummingbird- pollinated flowers from a presumably bee-pollinated ancestor via paedomorphosis was already reported for *Delphinium* (Ranunculaceae) by Guerrant (1982). Such consistent variation in the strengths of the allometries of both flower organs was not seen in all taxa. For instance, ontogenetic allometry in *C. coronata* was relatively weak for the petals and strong for the staminode complex (Table 1). As ontogenetic allometries of petals in *Caiophora* tend to be weaker than in *Loasa* and *Blumenbachia* species (Table 1), the relation of petal allometry with phylogeny or altitude holds for the strength of allometry just as it does for the allometric space. For the staminode complex, by contrast, *Loasa* and *Blumenbachia* have allometries of intermediate strengths, whereas species of *Caiophora* have both the strongest and weakest allometries (Table 1), suggesting evolution of allometries from intermediate ancestors to both extremes in *Caiophora*.

The relationships of ecological factors such as pollination mode and altitude with the position of taxa in allometric spaces are reminiscent of the results of previous studies in animals, where patterns of ontogenetic allometry were found to relate to diet or habitat (Frédérich & Vandewalle, 2011; Urošević et al., 2013; Wilson, 2013; Wilson & Sánchez-Villagra, 2010). Direct comparisons are difficult, however, because the methods for characterizing allometric spaces used in this study differ slightly from those in the previous studies. Most previous studies of allometric spaces were based on traditional morphometric traits (Gerber et al., 2008; Klingenberg & Froese, 1991; Klingenberg & Spence, 1993; Wilson, 2013; Wilson & Sánchez-Villagra, 2010). There are differences even to the rare studies using geometric morphometrics, because they used computations that normalized allometric vectors to unit length (focusing on the angles between vectors), so that the strength of allometry was not included as a component of variation (Frédérich & Vandewalle, 2011; Urošević et al., 2013). A full comparison of methods is beyond the scope of this paper, and will be presented elsewhere. Nevertheless, it is clear that the use of allometric spaces in the framework of geometric morphometrics provides powerful analytical tools for the comparative analysis of ontogenetic allometry, which is still a largely unexplored area of evolutionary developmental biology.

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SUPPORTING INFORMATION

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