Major lineages of Loasaceae subfam. Loasoideae diversified during the Andean uplift

Rafael Acuña Castillo[a,c,*], Federico Luebert[a,d], Tilo Henning[b], Maximilian Weigend[a]

[a] Universität Bonn, Nees-Institut für Biodiversität der Pflanzen, Meckenheimer Allee 170, 53115 Bonn, Germany
[b] Freie Universität Berlin, Botanischer Garten Botanisches Museum, Königin-Luise-Straße 6–8, 14195 Berlin, Germany
[c] Universidad de Costa Rica, Escuela de Biología, Apdo. Postal: 11501-2060 San Pedro de Montes de Oca, Costa Rica
[d] Universidad de Chile, Departamento de Silvicultura y Conservación de la Naturaleza, Santiago, Chile

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ABSTRACT

The Loasoideae is the largest clade in the Loasaceae. This subfamily is widespread throughout the Neotropics and centered in the Andes, presenting an excellent opportunity to study diversification across much of temperate and mid to high-elevation areas of South America. Despite that, no studies have addressed the historical biogeography of the Loasoideae to date, leaving an important knowledge gap in this plant group. Here, we used four plastid markers (i.e., *trn*L–*trn*F, *mat*K, *trn*S–*trn*G, and *rps*16) and sequenced 170 accessions (134 ingroup taxa) to infer the phylogeny of Loasoideae. We then used this phylogeny as basis to estimate divergence times using an uncorrelated relaxed molecular clock approach and seven fossils as primary calibration points. We employed the Dispersal-Extinction-Cladogenesis (DEC) approach to reconstruct the ancestral ranges of the subfamily. Our results indicate that stem Loasoideae diverged from its sister group in the Late Cretaceous to Early Paleocene (ca. 83–62 Ma). The crown node of the whole clade goes back to the Middle Paleocene to Middle Eocene (ca. 60–45 Ma), corresponding to the earliest diversification events of the extant groups, prior to most of the Andean orogeny and roughly coinciding with the Paleocene-Eocene Thermal Maximum. On the other hand, the crown nodes of most genera appear to have originated in the Oligocene and Miocene (median ages: 28–10 Ma). The diversification of some extant lineages appears to have happened in parallel to Andean uplift pulses that seem to have had an effect on the orogeny and concomitant establishment of new habitats and latitudinal corridors. The most likely ancestral areas retrieved for crown Loasoideae, are the tropical Andes and Pacific arid coast. Most of the extant clades have remained restricted to their ancestral areas. Transoceanic Long Distance Dispersal appears to have been involved in the arrival of Loasoid ancestors to South America, and in the distribution of the small clades *Kissenia* in Africa and *Plakothira* on the Marquesas Archipelago. The results presented here suggest that the historical biogeography of the continental scale radiation of Loasoideae, follows the sequence and timing of the development of temperate and mid to high-elevation habitats across South America during the Tertiary.

1. Introduction

South America is one of the most diverse regions on Earth, three of the five most important centers of plant diversity are found here (Barthlott et al., 2007). Biotic exchanges between South America and Africa, North America, Australia and Antarctica, have had profound effects in the Neotropical flora and fauna since the Cretaceous, as shown by the fossil record (Wilf et al., 2013), and molecular phylogenetic studies (Antonelli and Sanmartín, 2011). At the continental scale, Andean uplift, and the environmental changes associated with it have been identified as major driving forces, especially after the Oligocene

(Luebert and Weigend, 2014). Parallel geological events include the change in drainage and rainfall patterns in the Amazon Basin, the aridification of the Atacama desert, the creation of a whole range of new mid to high-elevation habitats and the establishment of a South-North corridor for temperate plants. The spatio-temporal geodiversity of this mountain range is considered as an essential driver for the elevated biodiversity that currently inhabits the region (Mutke and Weigend, 2017). Although there is still controversy concerning the details of the timing of the Andean uplift (Barnes and Ehlers, 2009; Richardson et al., 2018), major trends, such as the general progression of the Andean orogeny from South to North and from West to East (Graham, 2009;

Gianni et al., 2016) are widely accepted. The historical biogeography of many plant groups seems to reflect this pattern (Hughes et al., 2013; Luebert and Weigend, 2014).

The Cornales represents one of the earliest unequivocal documented radiations of any extant clade of eudicots (89 Ma; Atkinson et al., 2018). This clade is sister to the remaining Asterids, which represent one of the major radiations of land organisms (Soltis et al., 2018). Extant Cornales are subcosmopolitan in distribution, with considerable diversity in the subtropical to temperate zones of the Northern Hemisphere (Christenhusz et al., 2017; Soltis et al., 2018). Loasaceae, the largest family of the order is thought to have diverged from its sister, Hydrangeaceae, during the Late Cretaceous (Xiang et al., 2011). The phylogenetic diversity of the Loasaceae subfamilies Mentzelioideae, Gronovioideae, and Petalonychoideae is centered in southwestern North America and Mexico, which is inferred as the likely ancestral area of the Loasaceae as a whole (Weigend, 1997, 2004; Hufford, 2004; Schenk et al., 2017). However, Loasaceae subfam. Loasoideae, the largest clade of the family, is widespread in Central and South America and particularly species rich from temperate Patagonia over Mediterranean Chile to the tropical Andes, being centered in the latter (Weigend, 2004; Mutke et al., 2014). Loasaceae subfam. Loasoideae thus presents an opportunity to study the diversification of a clade across much of temperate and mid to high-elevation areas of South America.

The historical biogeography of Loasaceae subfam. Loasoideae is still incompletely understood, in spite of published phylogenetic studies (Weigend et al., 2004; Hufford et al., 2005; Acuña et al., 2017). Weigend (1997) suggested that the common ancestor of Loasoideae and its sister group might have grown in Mexico and/or adjacent areas in the Late Cretaceous. Two age estimates have been reported previously for this clade. While Schenk and Hufford (2010) estimated the age of the crown group of Loasoideae around the Eocene-Oligocene (ca. 56–23 Ma), Strelin et al. (2017), focusing on the Argentinean *Caiophora* C.Presl., dated the most recent common ancestor (MRCA) of the living *Caiophora* to the Miocene-Pliocene boundary (17.64–4.37 Ma). Despite that, none of these studies have addressed the historical biogeography of the Loasaceae subfam. Loasoideae, the largest clade in the family, leaving an important gap in the knowledge of this plant group.

In this study, we reconstruct a time-calibrated phylogeny of the Loasaceae subfam. Loasoideae, estimate ancestral ranges and the biogeographic history of the group in order to address the following two questions: (i) When did main Loasoideae clades diverge from their closest extant relatives? (ii) Where did these clades originate and spread afterwards? We discuss the results in the light of important geologic events that show a spatio-temporal correspondence with the diversification of the main clades of Loasoideae and mention other elements of the flora with similar ecology, distribution, and ages.

2. Materials and methods

2.1. Taxon sampling

We sampled a total of 170 species of Cornales including 134 ingroup species, representing ca. 65% of the currently accepted species of Loasoideae. The remaining 36 outgroup taxa are representatives of other subfamilies of Loasaceae, Hydrangeaceae, Nyssaceae, Cornaceae, and Curtisiaceae. Voucher information is presented as Supplementary Material (Supplementary Table S1).

2.2. DNA Amplification, sequencing, and alignment

DNA was extracted using the CTAB method (Doyle and Doyle, 1987). Four plastid regions were amplified: *trn*L–*trn*F, *mat*K, the *trn*S–*trn*G intergenic spacers, and the *rps*16 intron. Although many sequences were newly generated for this study, we also included data from Acuña et al. (2017, 2018) and Henning et al. (2018). The *trn*L–*trn*F

sequence of *Aosa plumieri* (Urb.) Weigend was obtained from GenBank (Hufford et al., 2005). GenBank accession numbers are presented as Supplementary Material (Supplementary Table S1). The amplification, sequencing, and alignment protocols follow Acuña et al. (2017). The four plastid marker sequences were combined in a single matrix[1], divided in four partitions (one per marker).

2.3. Divergence time estimation

The molecular dataset was prepared in BEAUti 1.8.4 (Drummond et al., 2012). FindModel (http://hcv.lanl.gov/content/sequence/findmodel/findmodel.html), which implements Posada and Crandall's (1998). Modeltest, suggested GTR + Γ as the best-fit model for all four partitions. For each partition we chose an uncorrelated relaxed clock in order to allow clock rates to vary across the tree. We compared the divergence age estimates of chronograms, using Yule Process and Birth-Death tree priors. The largest difference in divergence time estimates for the ingroup (Loasaceae subfam. Loasoideae) was 1.17 Ma. We chose the results obtained with the Yule Process tree prior for further analyses, because less parameters are included than the Birth-Death model. We placed the following seven fossil calibration points for Cornales, using absolute ages (Ogg et al., 2016) corresponding to the youngest boundary of the geologic age to which the fossils have been assigned. To account for dating and identification uncertainties, we set each prior to lognormal distributions, with 1.0 as standard deviation, and a lognormal mean so that the median age fell within the time interval of the respective geological age assigned to the fossil.

1. The crown node of *Cornus* L. was set to a minimum age of 72 Ma (end of the Campanian, logMean: 0.9) based on recently described fossil fruits of *Cornus* cf. *piggae* from the Late Campanian (~73 Ma) of Vancouver Island, British Columbia, which have been confidently assigned to the extant Cornelian Cherry clade (Atkinson et al., 2016).
2. Within Nyssaceae, the split between *Davidia* Baill. and *Camptotheca* Decne. + *Nyssa* L. was set to a minimum age of 56 Ma (end of the Thanetian, logMean: 0.8), based on fruits and leaves of *Davidia antiqua* (Newberry) Manchester, from the Late Paleocene (57–55.5 Ma) of Dakota, Montana and Wyoming, USA (Manchester, 2002), displaying many diagnostic traits of the extant genus. Although older fossils putatively assigned to *Davidia* and *Nyssa* (dating as far back as the late Campanian ca. 72 Ma) have been reported (Serbet et al., 2004; Manchester et al., 2015), a recent morphological analysis by Atkinson (2017, 2018) revealed that these cannot be unequivocally assigned to any clade within crown Nyssaceae and are therefore not used in our analyses.
3. The stem node of Nyssaceae (sensu APG IV, 2016) was set to a minimum age of 86 Ma (end of the Coniacian, logMean: 0.4) based on the fossil fruits of *Obamacarpa edenensis* Atkinson, Stockey & Rothwell (Atkinson et al., 2018), from the Early Coniacian (89 Ma) of Vancouver Island, British Columbia. Although these cannot be assigned with certainty to any extant group of Cornales, morphological analyses place this genus as more closely related to Nyssaceae than to any other extant group (Atkinson, 2017, 2018).
4. The crown node of Jamesioideae (Hydrangeaceae) was set to a minimum age of 23 Ma (end of the Chattian, logMean: 0.9) based on fossil leaves of *Jamesia caplani* Axelrod from the Late Oligocene (28–23 Ma) of Colorado (Axelrod, 1987). Although it is not known if every specimen assigned to this name belongs to the same species, at least some show close resemblance to extant *Jamesia americana* Torr. & A.Gray leaves, indicating that the extant genera of the subfamily had already diverged by this time.

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[1] Aligned matrix with all markers, available in Mendeley data: either here https://data.mendeley.com/datasets/698gcrrb2z/1 or the link provided by Elsevier.

5. The crown node of *Hydrangea* L. was set to a minimum age of 41 Ma (end of Lutetian, logMean: 1.1) based on *Hydrangea knowltonii* Manchester from the Late Eocene (43–45 Ma) of Oregon (Manchester, 1994; Manchester et al., 2015). This species has dorsoventrally flattened seeds, a morphological trait shared only with *H. anomala* D.Don from Asia among extant Hydrangeaceae (Hufford, 1995). Although not included in our sampling, *H. anomala* was retrieved as sister to *Hydrangea* Sect. *Cornidia* by De Smet et al. (2015), which is represented in our study by *H. oerstedii* Briq. Potentially older (possibly Paleocene) fossils of *Hydrangea* from Washington were described by Mustoe (2002), but their actual age remains uncertain.

6. The stem node of *Philadelphus* L. + *Carpenteria* Torr. (the latter found to be nested in the former by Guo et al., 2013) was set to a minimum age of 23 Ma (end of the Chattian, logMean: 0.9) based on leaf fossils of *Philadelphus creedensis* Axelrod from the Late Oligocene (28–23 Ma) of Colorado (Axelrod, 1987). According to Axelrod (1987), leaf morphology resembles that of the extant *Philadelphus microphyllus* A.Gray.

7. The crown node of Klaprothieae was set to 28 Ma (end of the Rupelian, logMean: 2.7) based on amber preserved structures of *Klaprothiopsis dyscrita* Poinar, Weigend & T.Henning, from the Dominican Republic. The Dominican amber fossils have not been dated precisely and could have ages between 45 and 15 Ma (Poinar et al., 2015). Although *K. dyscrita* lacks many traits found in extant Loasoideae, it bears a closer resemblance to extant *Plakothira* Florence and *Klaprothia* Kunth than to their closest living relative, *Xylopodia* Weigend.

The fossil *Tylerianthus crossmanensis* Gandolfo, Nixon & Crepet has been cited as representing one of the oldest Cornalean taxa (e.g., Manchester et al., 2015; Soltis et al., 2018) due to its Turonian-Coniacian age (88.5–90.4 Ma) and putative Hydrangeaceous affinities (Gandolfo et al., 1998). Despite that, the age and phylogenetic assignment of this fossil remain equivocal (Friis et al., 2011; Atkinson et al., 2018), thus this fossil was not included in our analyses. A single secondary calibration point was placed at the crown node of Cornales based on the result of Magallón et al. (2015). We set this prior with a normal distribution, a mean of 104.6 Ma and standard deviation of 5.45.

The partitioned dataset was run in BEAST 1.8.4 (Drummond et al., 2012) on the CIPRES Science Gateway 3.3 (Miller et al., 2010). The Markov Chain Monte Carlo was set to 200 million generations sampling every 10,000 generations. We discarded 10% of the trees as burn-in. The effective size sample (ESS) and plot likelihoods were examined in Tracer 1.6 (Rambaut and Drummond, 2014) [ESS was > 200 for all parameters, except the prior (ESS = 130), most recent common ancestor (*Philadelphus creedensis*, ESS = 159), matk.ucld.mean (ESS = 186), matk.meanRate (ESS = 170) and speciation (ESS = 123)]. TreeAnnotator 1.8.4 (Drummond et al., 2012) was used to obtain a maximum clade credibility tree with median ages.

In order to assess the influence that alternative calibrations could have in our analyses, we explored three calibration schemes: (a) including all calibration points; (b) excluding the dubious in-group fossil *Klaprothiopsis dyscrita*; and, (c) including only the secondary calibration and the fossils *Cornus* cf. *piggae*, *Davidia antiqua*, and *Hydrangea knowltonii*, whose affinities are considered as unequivocal. Unless otherwise stated, the age ranges obtained in our analyses and cited in the discussion correspond to 95% highest posterior density intervals (HPDI). Chronograms were prepared using the R package 'phyloch' (Heibl, 2013).

2.4. Ancestral area reconstruction

We compiled distribution data from specimens deposited in herbaria in the Americas and Europe (see Acknowledgements), with additional data from the literature (Sleumer, 1955; Pérez-Moreau and Crespo, 1988; Noguera-Savelli, 2012; Slanis et al., 2016), and GBIF

(2017, with doubtfully identified specimens excluded). The ancestral area reconstruction was performed using the Dispersal-Extinction-Cladogenesis (DEC) approach described by Ree and Smith (2008) as implemented in the R-package 'BioGeoBEARS' 0.2.1 (Matzke, 2013). We defined eight geographic areas: (A) Central America and the Caribbean (including tropical Mexico and the lowlands of extreme northwestern Colombia), (B) Tropical Andes (Andes north of the Bolivian Orocline), (C) Arid Pacific (deserts of western Peru and northern Chile), (D) Altiplano (Central Andes between the Bolivian Orocline and ca. 30°S), (E) Eastern South America (Eastern Brazil, Pampas and Chaco, including the Sierras de Córdoba), (F) Southern South America (South of the Atacama desert and the Pampas), (G) Africa (including the Arabian Peninsula), and (H) Marquesas Islands.

We ran analyses with two alternative biogeographic scenarios employing the chronogram obtained using the calibration scheme (a). The first biogeographical scenario included no dispersal constraints between areas and a maximum of three areas per node (the maximum number of areas occupied by the most widely distributed extant species of Loasoideae). The second scenario included a dispersal-constrained scenario where the adjacency matrix was modified manually so that dispersal was allowed only between adjacent areas, even when separated by the sea (i.e., including combinations AE, AG, AH, BH, CH, EG, FG, and FH). In order to allow additional ancestral reconstructions under these dispersal constraints, the maximum number of areas per node was set to four. R scripts are available upon request.

3. Results

3.1. Phylogenetic relationships

Loasoideae is retrieved as monophyletic with high support [Posterior Probability (PP) = 1.0], and sister to the also highly supported *Mentzelia* L. + Gronovioideae + Petalonychoideae clade (PP = 1.0, Supplementary Fig. S2). With very few exceptions, the backbone of the phylogeny is well resolved with good to very good support (PP ≥ 0.9) across most nodes. The position of early branching *Kissenia* R.Br. ex Endl and *Huidobria* Gay is not clearly resolved and the monophyly of *Huidobria* has no support. Conversely, Klaprothieae is highly supported as monophyletic (PP = 1.0), with the Andean relic genus *Xylopodia* sister to the other genera. The 'Core Loaseae' constitutes a highly supported clade (PP = 1.0) including all the remaining genera of the subfamily, with *Nasa* Weigend highly supported (PP = 1.0) and retrieved as sister to all other genera. In this latter clade the generic relationships are all highly supported (PP = 1.0) and *Aosa* Weigend is found as sister to *Presliophytum* (Urb. & Gilg) Weigend and the South Andean Loasas. The latter are retrieved in three highly supported clades: (a) *Blumenbachia* Schrad., (PP = 1.0); (b) *Loasa* Adans. (PP = 1.0, as redefined in Acuña et al., 2017); and, (c) '*Caiophora* clade' (PP = 1.0, *Caiophora*, *Grausa* Weigend & R.H. Acuña, *Pinnasa* Weigend & R.H. Acuña and *Scyphanthus* Sweet). Relationships between these three clades are not resolved. In the '*Caiophora* clade' relationships are retrieved as (*Grausa* + (*Pinnasa* + (*Scyphanthus* + *Caiophora*))).

3.2. Biogeographical analyses

All calibration schemes yielded the same topologies in all moderately to well-supported clades (PP > 0.9) (Fig. 1, Supplementary Fig. S2). Divergence time estimates for major nodes in Loasoideae were similar, independent of the specific calibration scheme (Table 1, Supplementary Fig. S2), although the ages obtained with calibration scheme (c) tended to be higher[2]. Median ages for the stem node of

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[2] .tre files of the time calibrated maximum clade credibility trees available in Mendeley data: either here https://data.mendeley.com/datasets/698gcrrb2z/1 or the link provided by Elsevier.
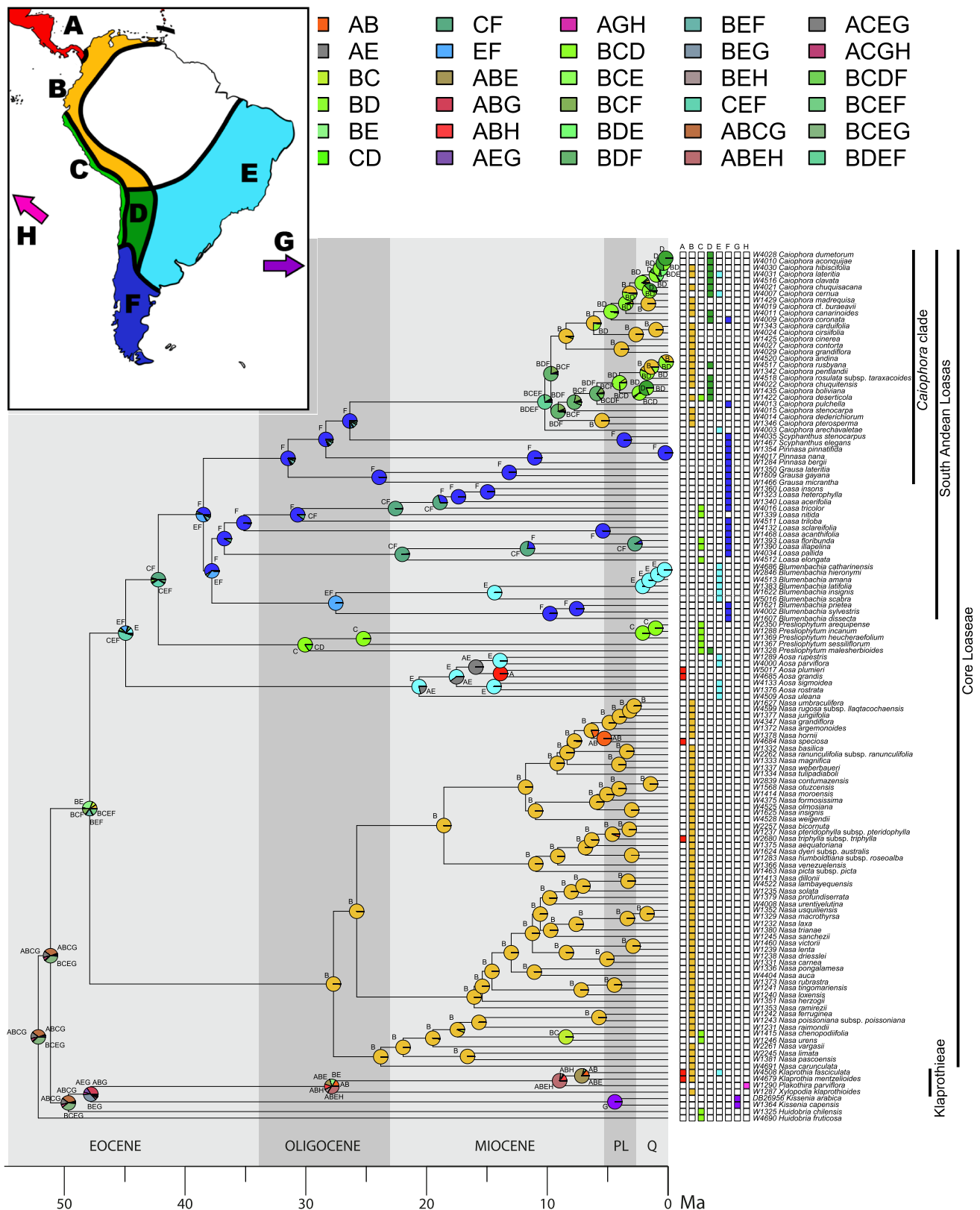
Fig. 1. Divergence time estimates for the phylogeny of Loasoideae using calibration scheme (a) for the dispersal-constrained DEC analysis, with a maximum of 4 areas per node. Colored grid to the right of the tips indicate the distribution assigned to the species. Color codes correspond to the areas indicated on the maps and legends. The Pie charts at the nodes depict relative probabilities of areas as estimated from the DEC analyses. The letters next to the pies indicate areas with relative probabilities > 0.2. Only area combinations with relative probabilities > 0.05 are included in the legend. Scale bar in millions of years before present (Ma). PL = Pliocene, Q = Quaternary. Map outline based on Rivas-Martínez et al. (2011).

Table 1
Comparison between the divergence time estimates in Millions of years before present (Ma) for the major nodes of the Loasaceae subfam. Loasoideae phylogeny under different calibration schemes with seven (a), six (b) and three (c) primary calibration points and estimates from other studies. Numbers in parentheses refer to 95% highest posterior density intervals, except for Schenk and Hufford (2010) who reported 95% Confidence Intervals. See the Material and Methods sections for the details on each calibration scheme.

Node	Calibration schemes			Schenk and Hufford (2010)	Xiang et al. (2011)	Strelin et al. (2017)
	(a)	(b)	(c)			
Stem Loasoideae	72.24 (62.11–83.29)	72.48 (62.10–83.42)	76.71 (67.73–86.35)	ca. 65 (44–75)	46.69–47.35	–
Crown Loasoideae	52.08 (44.78–59.73)	52.29 (45.38–59.90)	54.73 (48.26–61.89)	ca. 44 (24–65)	19.98–23.39	–
Crown Klaprothieae	27.81 (21.72–34.73)	28.49 (22.01–35.74)	29.60 (23.01–36.80)	ca. 20 (9–30)	–	–
Crown 'Core Loaseae'	47.82 (41.30–54.78)	47.89 (41.78–54.90)	50.09 (44.25–56.49)	ca. 40 (19–49)	19.98–23.39	–
Crown *Nasa*	27.67 (23.12–32.49)	27.84 (23.14–32.48)	28.94 (24.60–33.53)	–	–	–
Crown *Aosa*	20.59 (16.10–25.93)	20.61 (15.65–25.63)	21.38 (16.84–26.58)	ca. 10.5 (2–18)	–	–
Crown *Presliophytum*	30.01 (22.23–38.27)	30.15 (22.20–38.32)	31.48 (23.48–39.50)	ca. 21 (9–30)	–	–
Crown SAL	38.42 (33.49–44.19)	38.38 (33.68–44.11)	40.14 (35.43–45.24)	ca. 28 (13–40)	–	26.97 (13.74–41.00)
Crown *Blumenbachia*	27.48 (20.72–34.22)	27.43 (20.82–34.35)	28.78 (22.07–35.69)	–	–	11.83 (3.29–22.18)
Crown *Loasa*	36.69 (31.34–41.84)	36.66 (31.91–42.08)	38.33 (33.58–43.30)	–	–	20.76 (9.01–35.39)
Crown 'Caiophora clade'	31.42 (26.52–36.46)	31.35 (26.54–36.28)	32.74 (28.14–37.59)	ca. 22.5 (10–35)	–	20.09 (8.27–31.97)
Crown *Grausa*	23.89 (18.91–29.05)	23.88 (19.06–29.14)	24.90 (19.82–29.99)	–	–	–
Crown *Pinnasa*	11.02 (7.00–15.55)	11.06 (6.92–15.47)	11.48 (7.30–16.07)	–	–	4.81 (0.59–10.71)
Scyphanthus-Caiophora divergence	26.31 (21.48–31.32)	26.17 (21.60–31.19)	27.41 (22.67–33.36)	–	–	–
Crown *Caiophora*	10.18 (7.98–12.66)	10.20 (7.97–12.59)	10.67 (8.40–13.23)	–	–	10.43 (4.37–17.64)

Table 2
Results of the Dispersal Extinction Cladogenesis (DEC) analyses for the major nodes of the Loasaceae subfam. Loasoideae phylogeny. The log-likelihood of each analysis is indicated in the table header. The numbers that appear in the first column correspond to the respective node number assigned by BioGeoBEARS. The capital letters in the second and third column correspond to the ancestral areas at the respective node (as defined in materials and methods) arranged in decreasing order, with their relative probabilities in parenthesis. Only those areas with relative probabilities ≥ 0.05 are shown. A) Central America and the Caribbean, B) tropical Andes, C) Pacific arid coast, D) Altiplano, E) eastern South America, F) southern South America, G) Africa, H) Marquesas Islands.

Node	Dispersal unconstrained, three areas maximum LnL = −209.78	Dispersal constrained, four areas maximum LnL = −200.54
Crown Loasoideae (135)	BCG (0.47); BCE (0.20); BC (0.08); BCF (0.07)	BCEG (0.36); ABCG (0.33); ACEG (0.13); ACGH (0.07)
Crown Klaprothieae (140)	B (0.57); BH (0.15); ABH (0.10); AB (0.08)	ABEH (0.34); AB (0.16); ABE (0.15); BE (0.13); ABH (0.12); BEH (0.08)
Crown 'Core Loaseae' (143)	BCE (0.31); BE (0.19); BC (0.15); BCF (0.10)	BE (0.30); BEF (0.16); BCEF (0.16); BCF (0.14); B (0.09); BC (0.09);
Crown *Nasa* (144)	B (0.98)	B (0.99)
Crown *Aosa* (201)	E (0.69); AE (0.29)	E (0.79); AE (0.21)
Crown *Presliophytum* (208)	C (0.89); CD (0.08)	C (0.85); CD (0.15)
Crown SAL (212)	EF (0.39); F (0.35); CF (0.18)	F (0.43); EF (0.34); BEF (0.09); CF (0.05)
Crown *Blumenbachia* (214)	EF (0.97)	EF (1.00)
Crown *Loasa* (222)	F (0.77); CF (0.22)	F (0.91); CF (0.08)
Crown 'Caiophora clade' (233)	F (0.94)	F (0.82); BEF (0.08)
Crown *Grausa* (234)	F (1.00)	F (1.00)
Crown *Pinnasa* (237)	F (1.00)	F (1.00)
Crown *Scyphanthus* (240)	F (1.00)	F (1.00)
Crown *Caiophora* (241)	BEF (0.88); BE (0.07)	BDEF (0.73); BCEF (0.19)

Loasoideae ranged between 72 and 77 Ma, placing it around the late Campanian, while the crown node of Loasoideae was dated to a median age between 52 and 55 Ma (Ypresian). Crown node ages of most genera fell into the Oligocene to Miocene (31–10 Ma). Only the crown node of *Loasa* was retrieved as older (37–38 Ma, Eocene).

The unconstrained DEC analysis had a LnL = −209.78 (Supplementary Fig. S3), while the constrained analysis had a LnL = −200.54 (Fig. 1, Table 2). The results of both analyses differ mostly in the deeper nodes of the phylogeny (Table 2). Due to the difference in likelihood, we will focus on the dispersal-constrained analysis. The two most likely ancestral areas for the crown node of Loasoideae included the combinations of the tropical Andes + arid Pacific + Africa and either Central America or eastern South America (Fig. 1). The most likely ancestral area for crown Klaprothieae includes all the areas where the clade is currently distributed: Central America + tropical Andes + eastern South America + Marquesas islands, while tropical Andes + eastern South America was retrieved as the most likely ancestral area for the crown 'Core Loaseae'. The most likely ancestral area of the crown group *Nasa* corresponds to the tropical Andes, with two dispersal events into Central America and one into the Pacific arid coast. On the other hand, the remaining 'Core Loaseae' showed arid Pacific + eastern South America + southern

South America as the most likely ancestral area combination. For crown *Aosa,* eastern South America is the most likely ancestral area with a dispersal event into Central America, while for *Presliophytum* it is the Pacific arid coast. Crown South Andean Loasas (SAL) has two area combinations as most likely: southern South America and eastern + southern South America. The most likely ancestral area for *Blumenbachia* is eastern + southern South America, while southern South America is the area obtained for crown *Loasa* (with two identified dispersal events into the Pacific arid coast) and the MRCA of the *Caiophora* clade as well as of *Grausa*, *Pinnasa*, and *Scyphanthus*. Finally, the most likely ancestral area combination for *Caiophora* is tropical Andes + Altiplano + eastern + southern South America, coinciding with most of the areas where this taxon is distributed nowadays.

4. Discussion

In this study, we present the most comprehensive phylogeny of Loasaceae subfam. Loasoideae to date. This phylogeny is the most densely sampled both in terms of taxon and character sampling. We obtained high support values for most of the retrieved clades. At the same time, our estimations of the historical biogeography of the whole clade provide an exemplary scenario of the diversification of a mostly

South American mid to high-elevation plant group across several different biomes, in which the timing of their development and colonization can be compared to other plant groups as documented in recent studies.

4.1. Phylogenetic relationships

The relationships of Loasoideae as obtained in this study, including the sister group relationship between Loasoideae and the *Mentzelia* L. + Gronovioideae + Petalonychoideae clade, are mostly in agreement with published evidence (Weigend et al., 2004; Hufford et al., 2005; Acuña et al., 2017). The 'Core Loaseae,' the largest clade in the subfamily, characterized by nectar scales formed by three fused staminodia, was retrieved as monophyletic, corroborating the findings of Hufford et al. (2005) and Acuña et al. (2017). The topology of the SAL clade, as here retrieved, is essentially that of Acuña et al. (2017), which provided the most in-depth phylogenetic study on this clade to date.

The uncertain relationships of *Huidobria*, *Kissenia*, and Klaprothieae have remained since early phylogenetic reconstructions in this clade (Weigend et al., 2004; Hufford et al., 2005). Although access to new molecular techniques may improve the resolution of the deepest nodes of the Loasoideae, the divergence events that originated among these taxa seem to have resulted from ancient rapid radiations, as can be inferred from our time calibrated phylogenetic reconstructions. Ancient rapid radiations represent a major challenge for phylogenetic analyses due to the small amount of data that may support the divergence among lineages and the great potential to misinterpret phylogenetic information given the deep time-frame involved (Whitfield and Lockhart, 2007).

4.2. Biogeographical analyses

Our divergence time estimates for Loasoideae are considerably older than those previously published for this group (compare Table 1 with Schenk and Hufford, 2010; Xiang et al., 2011; Strelin et al., 2017). Only the estimates of the crown node age of *Caiophora* by Strelin et al. (2017) are largely congruent with our results, although our 95% HPDI is considerably narrower (Table 1). Albeit several of the fossils used for calibration were also employed in previous studies (Schenk and Hufford, 2010; Xiang et al., 2011), we also included calibration points based on recently described, unequivocal fossils from early Cornales that were not considered in previous studies (*Cornus* cf. *piggae*, and *Obamacarpa edenensis* Atkinson et al., 2016, 2018). Taxonomic sampling density and dissimilar evolutionary rates can bias molecular clock results (Linder et al., 2005; Soares and Schrago, 2015), while herbs tend to have higher rates of molecular evolution than woody species (Smith and Donoghue, 2008). In this study the sampling of Loasoideae is considerably expanded (134 species) compared to Schenk and Hufford (2010: 19 taxa), Xiang et al. (2011: five taxa), and Strelin et al. (2017: 31 taxa). At the same time, the first two studies included a higher proportion of woody species than ours.

The fossil record suggests that there was considerable biotic exchange between North and South America during late Cretaceous and Paleocene (Wilf et al., 2013). This is underscored by the fossil record of angiosperms (Stull et al., 2012), dinosaurs, and Therian mammals (Wilf et al., 2013). Divergence times of the stem Loasoideae (Table 1) from its North American sister group fall within this time frame. Right into the early Eocene, even southern South America was covered extensively by mesic tropical to subtropical forests (Wilf et al., 2013), biomes where Loasaceae are essentially absent today (Weigend, 2004). The Andean chain likely had only reached a small fraction of its current elevation (Graham, 2009), but drier habitats may have been present along the western margin of the continent (Hartley et al., 2005). Around the Paleocene-Eocene Thermal Maximum (PETM: 55 Ma, Zachos et al., 2008) there was a well-documented rapid increase in floristic diversity (Jaramillo et al., 2010) and the main clades of crown Loasoideae started

to diverge around (or shortly after) the same period. The deepest nodes – i.e. the divergence of the Atacama taxa *Huidobria chilensis* and *H. fruticosa*, African *Kissenia* and both stem Klaprothieae and 'Core Loaseae'– go back to ca. 57–40 Ma (mostly early Eocene).

The lineages of Loasoideae found in the western coastal deserts of South America each have an idiosyncratic history. Two of the earliest diverging groups of Loasoideae are found in the Atacama desert: *Huidobria fruticosa* and *H. chilensis*. These two clades diverged from the remainder of Loasoideae at 44.78–59.73 Ma and 42.45–56.91 Ma, respectively, suggesting the presence of arid environments in western South America during the Eocene. These results are in line with Hartley et al., (2005) who suggested that the Atacama has been semi-arid at least since the Mesozoic (150 Ma). *Presliophytum* diverged from its sister group 36.47–43.24 Ma (Late Eocene) and this was followed by a South-North progression with the two Atacama lineages diverging during the Oligocene and the three closely allied Peruvian taxa only diverging from each other in the Pliocene to Pleistocene (3.83–0.78 Ma). These arid-Pacific lineages seem to represent ancient and isolated taxa. The stem age interval of *Presliophytum* overlaps with the that of one of *Heliotropium* L. sect *Cochranea* (Heliotropiaceae; 32.1–40.2 Ma: Luebert et al., 2011). This finding contrasts with the more recent stem ages of other important Atacama groups such as *Oxalis* L. lineages *Carnosae* + *Giganteae* (Oxalidaceae; 14.35–27.44 Ma: Heibl and Renner, 2012), the *Mathewsia* Hook. & Arn. + *Schizopetalon* Sims clade (Brassicaceae; 10.35–20.77 Ma: Salariato et al., 2016), and *Nolana* L.f. (Solanaceae; Pliocene-Middle Miocene: Dillon et al., 2009). The single dispersal event of *Nasa* into the arid Pacific (*Nasa chenopodiifolia* (Desr.) Weigend and *N. urens* (Jacq.) Weigend 5.23–12.05 Ma) appears to have originated from the tropical Andes, overlapping marginally with the inferred age of origin of the Lomas formations in the Early Pliocene (Eichler and Londoño, 2013).

Deserticolous, African *Kissenia* diverged from its closest living relatives at 40.07–55.31 Ma. Even if this clade arrived in Africa at such early ages, long-distance dispersal (LDD) must be invoked for the intercontinental dispersal, as previously suggested for *Fagonia* L. (Zygophyllaceae; Beier et al., 2004), *Thamnosma* Torr. & Frem. (Rutaceae; Thiv et al., 2011) and *Turnera* L. (Turneraceae; Thulin et al., 2012) respectively. Our divergence age estimates between both species of *Kissenia* (1.96–7.48 Ma: Late Miocene-Pliocene), agree broadly with those of African elements showing similar distributions (Pokorny et al., 2015). Some of these taxa are thought to have dispersed via an arid corridor across east Africa during Pliocene-Pleistocene (Bellstedt et al., 2012).

The crown node age of Andean 'Core Loaseae', was dated to the mid-Eocene (41.30–54.78 Ma), predating both rapid (ca. 10 Ma: Gregory-Wodzicki, 2000), and gradual (ca. 40 Ma: Barnes and Ehlers, 2009) estimates of Andean uplift. The topography of South America during that time was dramatically different from today: The "Incaic II" deformation would have caused uplift of the Western Cordillera by the late Eocene (Taylor, 1991; Gregory-Wodzicki, 2000), but the highlands were neither extensive nor continuous, as indicated by marine incursions into western Amazonia (Hoorn et al., 2010) and the presence of low-elevation paleofloras in regions that today lie thousands of meters above sea level (Graham, 2009). By the end of the Eocene, the dense forests that covered southern South America (Patagonia) were replaced by more open habitats (Dunn et al., 2015) probably allowing the range of Core-Loaseae to expand. We retrieve two distinct geographical clades in Core-Loaseae, the tropical Andean *Nasa* and its mostly South Andean sister group (Fig. 1).

Considerable diversification took place in the Southern Andes between the late Eocene and Middle Oligocene, including the divergence of the bulk of the lineages from Mediterranean Chile. Crown *Loasa* is ancient (31.34–41.84 Ma), and reaches its highest diversity in Mediterranean Chile. Heibl and Renner (2012) consider this area as a refuge for *Oxalis*, but for *Loasa* it acts as a source (i.e., an area where new lineages originated and expanded into new habitats and regions):

two independent dispersals into the arid Pacific (Fig. 1) took place (15.8–28.7 Ma) largely preceding the advent of hyperaridity (Mid-Miocene: Houston and Hartley, 2003). Floristic interchange between Mediterranean Chile and the arid Pacific coast has been reviewed for related species in e.g., Southern Tecophilaeaceae, *Tropaeolum* L. sect. *Chilensia* (Tropaeolaceae), and *Chaetanthera* Ruiz & Pav. (Asteraceae) (Luebert, 2011). However, it is uncertain if the dispersal of those taxa was in a northwards direction, like observed in *Loasa*. Andean orogeny pulses, starting ca. 22 Ma, as well as the late Miocene global climatic cooling trend (Ogg et al., 2016) could have triggered diversification in the high Andean/Patagonian clades *Blumenbachia* sect. *Angulatae* (crown age 6.11–14.11 Ma), *Caiophora* (7.98–12.66 Ma), *Pinnasa* (7.00–15.55 Ma), and the divergence of the *Grausa lateritia* (Gillies ex Arn.) Weigend & R.H.Acuña lineage (7.83–18.49 Ma). Similar crown ages, coinciding with South Andean orogeny, have been found in other families, namely: The subclades of Calyceraceae (ca. 12–15 Ma: Denham et al., 2016), *Puya* Molina (Bromeliaceae; 10 Ma: Givnish et al., 2011), *Azorella* Lam. sect. *Laretia* (Apiaceae; 7.49–18.35 Ma: Nicolas and Plunkett, 2014), the Austral Clade of Brassicaceae tribe Eudemeae (6.07–12.89 Ma: Salariato et al., 2016), and the *Oxalis* lineage *Palmatifoliae* (5.91–19.6 Ma: Heibl and Renner, 2012). Conversely, the diversification of the more northerly distributed, mostly high-Andean *Caiophora,* only started in the late Miocene, after the uplift pulses in the Central Andes at ca. 13–10 Ma (Gregory-Wodzicki, 2000; Graham, 2009), which likely facilitated the northward expansion and diversification in this and other groups with south temperate origin (Luebert and Weigend, 2014).

Nasa is retrieved as essentially Tropical Andean, with ancestors that likely inhabited the moderate relief areas resulting from the Incaic II phase of Andean uplift that preceded the crown age of this genus (23.12–32.49 Ma). This is broadly contemporary with ages of crown American *Hedyosmum* Sw., (Chloranthaceae; ca. Late Oligocene-Early Eocene: Zhang et al., 2011), and Rubiaceae tribe Cinchoneae (22.9–35.1 Ma: Antonelli et al., 2009). These groups reach their highest diversity in the middle elevations of the Andes (1000–3000 m, Todzia, 1988; Andersson, 1995, Mutke et al., 2014). However, the crown ages of other mid-elevation Andean radiations [*Centropogon* C.Presl and allies (Campanulaceae), *Ceroxylon* Bonpl. (Arecaceae), *Fuchsia* L. sect. *Fuchsia* (Onagraceae), Gesneriaceae tribe Episcieae, *Vasconcellea* A.St.-Hil. (Caricaceae)] are generally lower (ca. 4–23 Ma, Luebert and Weigend, 2014; Lagomarsino et al., 2016; Sanín et al., 2016). Accordingly, it has been suggested that the radiations of these clades were influenced by more recent uplift events (Berry et al., 2004; Carvalho and Renner, 2012; Lagomarsino et al., 2016; Sanín et al., 2016). The diversification of the genus *Nasa* throughout the last ca. 25 Ma may have resulted from the continuously increasing topographic and climatic complexity, being likely influenced by orogenic pulses in the tropical Andes that peaked at ca. 23 Ma and ca. 12 Ma. (Antonelli et al. 2009; Hoorn et al., 2010; Poulsen et al., 2010).

Aosa diversified in eastern South America prior to the Middle-Miocene. Its crown age (16.10–25.93 Ma) agrees broadly with the crown age of other clades with eastern South American origin such as the tribe Sinningieae (Gesneriaceae; 15.0–28.1 Ma: Perret et al., 2013), *Syagrus* Mart. (Arecaceae; 14.99–24.95 Ma: Meerow et al., 2014), *Attalea* Kunth (Arecaceae; ca. Early Miocene-Late Oligocene: Freitas et al., 2016), *Ficus* L. sect. *Pharmacosycea* (Moraceae; 13.9–27.0 Ma: Machado et al., 2018), and *Amorimia* W.R.Anderson (Malpighiaceae; 15.71–29.11 Ma: Almeida et al., 2018). An overall increase of the aridity in South America has been linked to the radiation of some of these clades (Perret et al., 2013; Almeida et al., 2018). We hypothesize that the MRCA of the Central American-Caribbean *Aosa grandis* (Standl.) R.H.Acuña & Weigend, and *A. plumieri* (Urb.) Weigend arrived via LDD from eastern South America. Our age estimates for their divergence from their sister group (12.13–19.78 Ma) post-date the submersion of the hypothetical GAARlandia (Greater Antilles-Aves Ridge) ca. 33 Ma (Iturralde-Vinent and MacPhee, 1999), but could have overlapped with

an early closure of the Central American Seaway 13–15 Ma (Montes et al., 2015).

Blumenbachia includes one Andean and two extra-Andean clades. Its crown age overlaps broadly with that of Calyceraceae (22–36.1 Ma: Denham et al., 2016), a group with similar distribution. The divergence between eastern and western lineages of *Blumenbachia* (20.72–34.22 Ma) appears to have resulted from vicariance (Fig. 1). However, this divergence predates that of taxa with similar disjunctions such as those of *Butia* Becc. and *Jubaea* Kunth (Arecaceae; 8.87–21.39 Ma: Meerow et al., 2014), *Myrceugenia* O. Berg (Myrtaceae; 8.86–21.67 Ma: Murillo et al., 2016), and *Fuchsia* sect. *Quelusia* (Onagraceae; ca. 13 Ma: Berry et al., 2004). For these clades, it has been suggested that the major orogenic events in southern South America (22–8 Ma: Giambiagi et al., 2016; Gianni et al., 2016), the establishment of the rainshadow effect in the region (ca. 16 Ma: Gianni et al., 2016) and the first Paranense Marine Transgression (15–13 Ma: Hernández et al., 2005) may have caused vicariance (Murillo et al., 2016). The latter two events correspond to the time of divergence (95% HPDI 9.53–19.96 Ma), and could have shaped the ranges of the two extra-Andean sections of *Blumenbachia*.

The data here presented shows that the historical biogeography of Loasaceae subfam. Loasoideae closely follows the known sequence and chronology of the development of temperate and mid to high-elevation habitats in South America. Early diversification falls into the PETM and largely predates the bulk of Andean orogeny. The historical biogeography of the clade implies that adaptations to aridity arose early in the evolution of the group and most of the early diversification seems to have taken place in semi-arid and temperate climates at low to moderate elevations. The long-lasting presence of Loasaceae subfam. Loasoideae in arid habitats, potentially since the early Eocene, makes this clade an interesting example of evolution associated with the dry biomes of the continent (in particular along the Pacific slope), as is the case for the legume genera *Amicia* Kunth, *Coursetia* DC., *Cyathostegia* (Benth.) Schery, *Mimosa* L., and *Poissonia* Baill from the seasonally dry Andean forests (Särkinen et al., 2012). Later diverging groups colonized more mesic and higher elevation habitats. Since the Oligocene, the diversification of these clades appears to have taken place mostly in parallel with the major Andean uplift pulses, especially in the Andean genera such as *Nasa*. As the Andes gradually reached higher elevations, these became both the source of new habitats and a latitudinal corridor (Luebert and Weigend, 2014) for Loasoideae. The high-Andean clades of *Nasa* started to diversify in the late-Miocene, when the predominantly Andean genus *Caiophora* also experienced considerable northward expansion and diversification. A further expansion of the sampling and the use of more highly-resolving molecular tools would help to resolve Pliocene and Quaternary nodes. The Quaternary glacial and interglacial cycles may then be reflected in speciation and extinction patterns of Andean Loasoideae, but our data is not sufficiently resolved at shallow levels to address this issue yet.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ympev.2019.106616 and https://data.mendeley.com/datasets/698gcrrb2z/1.

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