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A preliminary phylogeny of Loasaceae subfam. Loasoideae (Angiospermae: Cornales) based on *trnL*_(UAA) sequence data, with consequences for systematics and historical biogeography

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Abstract

The phylogeny of Loasaceae subfam. Loasoideae is investigated with sequences of the chloroplast *trnL*_(UAA) intron, all genera and infrageneric entities are included in the analysis. Loasaceae subfam. Loasoideae is monophyletic, and the two most speciose, and monophyletic, clades (which account for approximately 90% of the species total) are *Nasa* and the so-called Southern Andean Loasas (*Blumenbachia*, *Caiophora*, *Loasa* s.str., *Scyphanthus*), but the phylogeny of the remainder is not completely resolved. The data underscore a basal position for *Chichicaste*, *Huidobria*, *Kissenia*, and *Klaprothieae* (*Xylopodia*, *Klaprothia*, *Plakothira*). High bootstrap support values confirm the monophyly both of *Klaprothieae* and *Presliophytum* (when expanded to include *Loasa* ser. *Malesherbioideae*). *Aosa* and *Blumenbachia* are not resolved as monophyletic, but have clear morphological apomorphies. Within *Nasa*, “*N. ser. Saccatae*” is paraphyletic, and “*N. ser. Carunculatae*” is polyphyletic. However, the *N. triphylla* group in “*N. ser. Saccatae*” is a well-supported monophyletic group, as is *N. ser. Grandiflorae*. “*Loasa*” in its traditional circumscription is paraphyletic, but *Loasa* s.str. (*L. ser. Macrospermae*, *L. ser. Deserticolae*, *L. ser. Floribundae*) is monophyletic. The remainder of “*Loasa*” (*L. ser. Pinnatae*, *L. ser. Acaules*, *L. ser. Volubiles*) is probably closely allied to the essentially Patagonian-High Andean group comprising also *Scyphanthus* and *Caiophora*. These findings are congruent with morphology and phytogeography. *Nasa* seems to have undergone its primary radiation at moderate elevations (1500–2500 m) in the Andes of northern Peru (Amotape-Huancabamba Zone) and subsequently diversified into high elevations (above 4000 m) of the tropical Central Andes. South Andean Loasas appear to have undergone their primary diversification in the southern temperate and mediterranean regions of Chile and Argentina, with a subsequent northwards expansion of *Caiophora* into the high elevations of the tropical Andes. Hummingbird pollination has evolved independently from melittophily in High Andean clades of *Nasa* and *Caiophora*.

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Introduction

Loasaceae are medium-sized (ca. 300 spp.) and largely Neotropical plant family whose precise relationships among angiosperms have been controversially discussed.

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The last few years have brought enormous progress in this field, and the Loasaceae have been shown to be closely allied to Hydrangeaceae and firmly nested in Cornales (Hempel et al. 1995; Moody and Hufford 2000). Morphological studies confirm this placement, and the similarity between some groups in Loasaceae and some groups in Hydrangeaceae (e.g. *Deutzia* Thunb., *Jamesia* Torr. & Gray, and *Philadelphus* L.) is indeed striking in varied character complexes such as seed morphology, flower morphology, indument morphology, phytochemistry, and leaf morphology (Weigend, 2004).

The subdivision of Loasaceae has also been controversially discussed (Davis and Thompson 1967; Poston and Thompson 1977; Weigend 1997; Moody and Hufford 2000). The mainly North American subfamilies Gronovioideae (*Cevallia* Lag., *Fuertesia* Urb., *Gronovia* L.), Mentzelioideae (*Eucnide* Zucch., *Mentzelia* L., *Schismocarpus* Blake), and Petalonychoideae (*Petalonyx* A.Gray) have been extensively studied, but these make up only about 1/3 of the family's species total. The subfamily that is by far the largest (over 200 spp.) and most diverse (morphologically, ecologically, and phytochemically), the Loasoideae, has been the subject of very few detailed studies, and these have usually been limited to the few commonly cultivated representatives, such as *Blumenbachia insignis* Schrad., *B. hieronymi* Urb., *Caiophora lateritia* Klotzsch, and *Nasa triphylla* (Juss.) Weigend subsp. *triphylla*.

Until recently, the only comprehensive study available was the "Monographia Loasacearum" (Urban and Gilg 1900) which was based nearly exclusively on herbarium material much of which was very poorly preserved. This study recognized a total of seven genera in Loasoideae (*Blumenbachia* Schrad., *Caiophora* C.Presl, *Kissenia* Endl., *Klaprothia* Kunth, *Loasa* Adans., *Sclerothrix* C.Presl, *Scyphanthus* D.Don), with "*Loasa*" accounting for more than half of the species (83 of 153). The genera were grouped into three tribes, Kissenieae (*Kissenia*; 2 spp.), Klaprothieae (*Klaprothia*, *Sclerothrix*; 2 spp.), and Loaseae (*Blumenbachia*, *Caiophora*, "*Loasa*", *Scyphanthus*; 149 spp.). "*Loasa*" was circumscribed exclusively by the presence of fruits opening with apical valves, a truly plesiomorphic character also found in Mentzelioideae and outside of Loasaceae in the putative sistergroup Hydrangeaceae. Urban and Gilg (1900) wrote detailed studies on many aspects of morphology and studied Loasaceae with enormous accuracy, but in their subsequent classification they made little use of the numerous characters observed.

At species level, their decisions have been widely criticized as being too narrow (Darlington 1934; Sleumer 1956), and the generic concepts have also been challenged: *Sclerothrix* was reduced to synonymy under *Klaprothia* (Poston and Nowicke 1990), and *Huidobria* Gay, which Urban and Gilg (1900) had treated as a

subgenus in "*Loasa*", was re-instated at genus rank (Grau 1997). However, because no detailed new studies are available, the treatments of Urban and Gilg (1900) have been largely followed, and only *Schismocarpus* Blake (subfam. Mentzelioideae) from southern Mexico and *Plakothira* Florence (subfam. Loasoideae, tribe Klaprothieae) from the Marquesas Islands in Polynesia have been described since the "Monographia Loasacearum".

Weigend (1997) made an attempt to arrive at a more natural classification of Loasaceae subfam. Loasoideae (Table 1) considering a wide range of morphological traits and character polarity. This study led to the segregation from "*Loasa*" of a total of four genera:

1. *Nasa* Weigend (short for "North Andean Loasas"; Urban and Gilg's groups *Loasa* ser. *Grandiflorae*, L. ser. *Saccatae*, L. ser. *Carunculatae*, L. ser. *Alatae*), for by now approx. 100 spp. mainly from the northern and central Andes;
2. *Aosa* Weigend, for the Brazilian and Hispaniolan representatives of "*Loasa*" (Urban and Gilg's groups L. ser. *Corymbosae*, L. ser. *Parviflorae*, L. ser. *Pusillae*);
3. *Presliophytum* (Urban and Gilg) Weigend (Urban & Gilg's *Loasa* subg. *Presliophytum*);
4. *Chichicaste* Weigend, for *Loasa grandis* Standl. (described after Urban & Gilg's studies).

Another systematic addition was the description of *Xylopodia* Weigend belonging to the Klaprothieae (discovered in northern Peru in 1997), and *Caiophora* was redefined by removing the two sections *Angulatae* and *Gripidea* to *Blumenbachia* (Weigend 1997).

The Loaseae were informally segregated into two "grades": "Lower Loaseae", with a number of small genera characterized by relatively simple and upright flowers without thigmonastic stamens (*Chichicaste*, *Huidobria*, *Presliophytum*); and Higher Loaseae, comprising genera with more complex and usually pendulous flowers with thigmonastic stamens (*Aosa*, *Blumenbachia*, *Caiophora*, "*Loasa*", *Nasa*, *Scyphanthus*). The Higher Loaseae divide into three clearly monophyletic assemblages: *Aosa*, *Nasa*, and a complex informally called South Andean Loasas comprising *Blumenbachia*, *Caiophora*, "*Loasa*", and *Scyphanthus*. Currently, South Andean Loasas remain largely unresolved, since they show reticulate patterns of variation in many characters and have not been studied in detail. *Caiophora* has recently been subdivided into species groups to make the large genus more manageable (Weigend and Ackermann 2003). "*Loasa*" still contains one highly divergent entity, L. ser. *Malesherbioideae* (Table 1), which lacks the derived characters of *Loasa* s.str. and rather appears to be closely allied to *Presliophytum*. It is here treated separately (both species of L. ser. *Malesherbioideae* were available for analysis).

Table 1. Synopsis of the classification of Loasaceae subfamily Loasoideae, modified from Weigend (1997).

Loasaceae subfam. Loasoideae	Genus	Infrageneric entity	Acronym	Species total	Species studied	Distribution
Tribe Klaprothieae	<i>Klaprothia</i>	—		2	2	C & S America
	<i>Plakothira</i>	—		3	1	Marquesas Islands
	<i>Xylopodia</i>	—		1	1	N Peru
Tribe Loaseae, “Lower Loaseae”	<i>Kissenia</i>	—		2	1	Africa
	<i>Chichicaste</i>	—		1	1	Panama & Costa Rica
	<i>Huidobria</i>	—		2	2	N Chile
	<i>Presliophytum</i>	—		3	2	Peru
	“ <i>Loasa</i> ”	ser. <i>Malesherbioideae</i> ^a	LoMa	2	2	N Chile
	<i>Aosa</i>	ser. <i>Corymbosae</i>	AoCo	1	—	Brazil
Tribe Loaseae, Higher Loaseae		ser. <i>Pusillae</i>	AoPu	2	1	Brazil
		ser. <i>Parviflorae</i>	AoPa	4	2	Brazil & Hispaniola
	<i>Nasa</i>	ser. <i>Saccatae</i>	NaSc	29 (?)	11	Cordillera Mexico–Bolivia
		ser. <i>Carunculatae</i>	NaCa	4	2	Peru & S Ecuador
		ser. <i>Alatae</i>	NaAl	26 (?)	7	Colombia–Bolivia
		ser. <i>Grandiflorae</i>	NaGr	38 (?)	7	N Colombia to Peru
		<i>N. venezuelensis</i> group ^b		3	1	N Colombia & NW Venezuela
	“ <i>Loasa</i> ”	ser. <i>Acaules</i>	LoAc	1	1	Chile & Argentina (Andes)
		ser. <i>Deserticolae</i>	LoDe	2	2	N & C Chile (Costa)
		ser. <i>Floribundae</i>	LoFl	4	3	N & C Chile (Costa)
		ser. <i>Loasa</i>	LoLo	2	—	S Chile & Argentina
		ser. <i>Macrospermae</i>	LoMc	8 (?)	7	C & N Chile, Peru (Costa)
		ser. <i>Volubiles</i>	LoVo	4	2	S Chile
		ser. <i>Pinnatae</i>	LoPi	20 (?)	4	Chile & Argentina (Andes)
	<i>Blumenbachia</i>	sect. <i>Blumenbachia</i>	BlBl	4	2	Argentina–S Brazil
		sect. <i>Gripidea</i>	BlGr	3	1	S Brazil
		sect. <i>Angulatae</i>	BlAn	3 (4?)	3	S Chile & Argentina
	<i>Scyphanthus</i>	—		2	1	C Chile
	<i>Caiohpora</i> ^c	<i>C. arechvaletae</i> group	CaAr	1	—	S Brazil–Uruguay
		<i>C. carduiifolia</i> group	CaCa	5	—	Peru
		<i>C. chuquitensis</i> group	CaCh	8	2	Peru–Argentina
		<i>C. cirsiiifolia</i> group	CaCi	2	1	N Chile–Peru
		<i>C. clavata</i> group	CaCl	5	—	S Bolivia–N Argentina
	<i>C. contorta</i> group	CaCo	2	—	Ecuador–Peru	
	<i>C. coronata</i> group	CaCr	2	—	Peru–Chile	
	<i>C. lateritia</i> group	CaLa	8	1	Peru–Argentina	
	<i>C. nivalis</i> group	CaNi	2	—	Argentina	
	<i>C. pterosperma</i> group	CaPt	2	1	Peru	
	<i>C. rosulata</i> group	CaRo	1	—	Peru–Argentina	

?not fully revised, numerous undescribed species.

^aHere removed from *Loasa* based on molecular and morphological data.

^bNot formalized, see Weigend (1997).

^cInformal classification, see Weigend and Ackermann (2003).

Some molecular data have already been published (*matK*: Moody and Hufford 2000; *matK* and *trnL-trnF*: Hufford et al. 2003). While the principal results of these studies are largely congruent with the re-classification provided by Weigend (1997), we here aim at providing a more complete understanding of subfamily Loasoideae and especially its largest groups (*Nasa*, South Andean Loasas). The present study, using sequences from the chloroplast *trnL*_(UAA) intron, addresses three primary aims: Firstly, molecular delimitation of the generic entities recognized in Weigend (1997), and relationships among them; secondly, identification of the lineages within the poorly understood South Andean Loasas; thirdly, recognition of infrageneric groupings in “*Loasa*” and *Nasa*.

Material and methods

The plant material used in this study largely came from our own field collections over the past 10 years. Species were identified on the basis of all available literature, and are vouchered in herbaria as indicated in Table 2. A few taxa were only available from herbarium collections, samples of these were taken from specimens in the herbaria B, LPB, M, and MO.

A total of 77 species assigned to Loasaceae have been investigated (Table 2). Furthermore, 3 sequences from *Deutzia* and *Philadelphus* (Hydrangeaceae) were used for the user-specified outgroup comparison, they were treated as monophyletic in the molecular analysis. Only a small sample of species of *Caiophora* was included, since these showed minimal sequence divergence, and no internal resolution could be obtained with the marker chosen.

DNA extraction, PCR, purification and sequencing followed standard protocols, which are described in detail in Gottschling and Hilger (2001). Primers used for amplification and sequencing of the *trnL*_(UAA) intron were those of Taberlet et al. (1991). The sequences were manually aligned using Se-Al v2.0a72 (Rambaut 2001). The complete data matrix is available in NEXUS format on request.

Phylogenetic calculations were run on a Macintosh computer with the help of PAUP* 4.0b1 (Swofford 1998). Parsimony trees were generated using heuristic searches, with gaps considered as informative based on the frequent occurrence of deletions and insertions in the *trnL* intron. The poly A region in the R-loop of the *trnL* secondary structure (Kuhse et al. 1990) was excluded since it follows no phylogenetic pattern in Loasaceae. A bootstrap analysis (criterion=parsimony, BS) was estimated based on 350 replicates (addseq=random, nreps=10, MaxTrees=1000). A PUZZLE analysis (criterion=likelihood, P) was performed with 1000

quartet puzzling steps. Likelihood settings from the best-fit model were determined using the AIC criterion in Modeltest 3.06 (Posada and Crandall 1998) (Fig. 1).

The *trnL* phylogeny is used as the basis for a hypothetical phylogeny of Loasoideae with possible morphological apomorphies mapped onto a cladogram (Fig. 7). The corresponding morphological characters are illustrated in Figs. 2–6.

Results

The aligned *trnL* data set was 536 bp in length. Of these sites, 113 (22%) were parsimony-informative (1.5 per taxon). The heuristic search found 13,284 most parsimonious trees, for which a strict consensus tree was computed (Fig. 1; $L = 293$, $CI = 0.78$, $RI = 0.91$). In the tree, bootstrap support values (BS; criterion=parsimony) and PUZZLE support values (P; criterion=likelihood, calculated with the best-fit model: GTR + G model) are indicated.

Within the monophyletic Loasaceae subfam. Loasoideae (63 BS), the analysis of *trnL* sequences results in a basal polytomy involving eight unresolved single species, two minor clades (Klaprothieae, 90 BS; and *Presliophytum* including “*Loasa*” *longiseta* from *L.* ser. *Malesherbioideae*, 84 BS), and two major and species-rich clades (South Andean Loasas, 72 BS, comprising *Blumenbachia*, *Scyphanthus*, *Caiophora*, and the majority of “*Loasa*”; and *Nasa*, 88 BS, 91 P).

Within Klaprothieae, *Xylopodia* is sister to a clade comprising *Klaprothia* and *Plakothira* (monophyletic: 99 BS, 67 P), and PUZZLE analysis further indicates that *Klaprothia* may be paraphyletic with respect to *Plakothira* (*K. mentzeloides* as sister to *K. fasciculata* and *Plakothira parviflora*: 62 P). The two species of *Presliophytum* are retrieved as sister taxa (85 BS, 55 P) and appear to be closely allied to “*Loasa*” *longiseta* (84 BS) as well as to “*L.*” *malesherbioides* as a second species of *L.* ser. *Malesherbioideae*. The latter relationship receives support from the PUZZLE analysis (54 P).

The species remaining unresolved in the basal polytomy represent *Huidobria*, *Chichicaste*, and *Kissenia* from the “Lower Loasoideae”, and *Aosa* from the Higher Loaseae. Neither *Aosa* (3 of 7 species sampled) nor *Huidobria* (2 of 2) are retrieved as monophyletic, but their distinctness from “*Loasa*” is evident. The same is true for monotypical *Chichicaste*.

Nasa is well supported (88 BS, 91 P) and falls into two clades comprising: (1) *N. carunculata* (Urb. & Gilg) Weigend (*N.* ser. “*Carunculatae*”) and some species of *N.* ser. “*Saccatae*” (moderately supported: 63 BS); (2) the remainder of *N.* ser. “*Carunculatae*” and *N.* ser. “*Saccatae*”, all of *N.* ser. *Grandiflorae* and *N.* ser. *Alatae*, and the *N. venezuelensis* group (99 BS). Within

Table 2. List of species and vouchers

DNA No.	Species	Classification	Collector/collection No. (herbarium)	Country of origin	GenBank Acc. No. (<i>trnL</i>)
1376	<i>Aosa rostrata</i> (Urb. & Gilg) Weigend	AoPu	Salino 3042 (M)	Brazil	AY285677
1289	<i>Aosa rupestris</i> (Gardner) Weigend	AoPa	Weigend 7138 (BSB, M)	Brazil	AY285678
1387	<i>Aosa plumierii</i> (Urb.) Weigend	AoPa	R.A. & E.S. Howard 9398 (B)	Hispaniola	AY388479
590*	<i>Blumenbachia espinheira</i> Gay	BlAn	Weigend et al. 6816 (BRCO, BSB, M)	Argentina	AY285679
1384	<i>Blumenbachia exalata</i> Weigend	BlGr	Sehnm 3993 (B)	Argentina	AY285680
592*	<i>Blumenbachia insignis</i> Schrad.	BIBl	Weigend, s.n. (M)	Argentina	AY285681
1383	<i>Blumenbachia latifolia</i> Cambess.	BIBl	Schwabe s.n. anno 1958 (B)	Argentina	AY285682
591*	<i>Blumenbachia prietea</i> Gay	BlAn	Weigend et al. 6823 (BRCO, BSB, M)	Argentina	AY285683
1385	<i>Blumenbachia sylvestris</i> Poepp.	BlAn	Weigend et al. 6807 (BRCO, BSB, M)	Argentina	AY285684
1342	<i>Caiophora andina</i> Urb. & Gilg	CaCh	Ackermann 360 (BSB, HUSA, M, USM)	Peru	AY285685
1341	<i>Caiophora chuquitensis</i> (Meyen) Urb. & Gilg	CaCh	M. & K. Weigend 2000/70 (M, NY, USM)	Peru	AY285686
1345	<i>Caiophora cirsiifolia</i> C.Presl	CaCi	Weigend et al. 5022 (BSB, HUT, M, USM)	Peru	AY285687
1389	<i>Caiophora nivalis</i> Lillo	CaNi	Coccucci s.n. (CORD)	Argentina	AY388480
1357	<i>Caiophora madrequisa</i> (Killip)	CaLa	M. & K. Weigend 2000/191 (M, NY, USM)	Peru	AY285688
1356	<i>Caiophora</i> cf. <i>Pterosperma</i> (Ruiz & Pav. ex G.Don) Urb. & Gilg	CaPt	Weigend et al. 5188 (BSB, HUT, M, USM)	Peru	AY285689
1472	<i>Chichicaste grandis</i> (Standl.) Weigend	“Lower Loaseae”	Croat 13381 (MO)	Panama	AY388482
1293	<i>Deutzia discolor</i> Hemsley	Hydrangeaceae	Weigend 5615 (B 045-13-87-10/274, BSB)	China	AY285690
1292	<i>Deutzia rubens</i> Rehder	Hydrangeaceae	Weigend 5613 (B 103-26-74-80/1, BSB)	China	AY285691
1325	<i>Huidobria chilensis</i> Gay	“Lower Loaseae”	Ackermann 482 (BSB, CONC, M, SGO)	Chile	AY285692
1327	<i>Huidobria fruticosa</i> Phil.	“Lower Loaseae”	Dillon 8034 (F, M)	Chile	AY285693
1364	<i>Kissenia capensis</i> Endl.	“Lower Loaseae”	Greuter 2167 (B)	South Africa	AY285694
1348	<i>Klaprothia fasciculata</i> (C.Presl) Poston	Klaprothieae	Weigend et al. 5362 (BSB, HUT, M, USM)	Peru	AY285695
1349	<i>Klaprothia mentzelioides</i> Kunth	Klaprothieae	Henning & Schneider 276 (BSB, HUT, M, USM)	Peru	AY285696
1340	<i>Loasa acerifolia</i> Domb.	LoMc	Weigend et al. 6848 (BRCO, BSB, M, NY)	Chile	AY285697
1355	“ <i>Loasa</i> ” <i>asterias</i> Dusen	LoPi	Weigend et al. 6984 (BRCO, BSB, M, NY)	Argentina	AY285698
596*	“ <i>Loasa</i> ” <i>bergii</i> Poepp.	LoPi	Weigend et al. 6846 (BRCO, BSB, M, NY)	Argentina	AY285699
1464	<i>Loasa elongata</i> Hook. & Arn.	LoDe	Ackermann 514 (BSB, M, SGO)	Chile	AY388483
1354	“ <i>Loasa</i> ” <i>filicifolia</i> Poepp.	LoPi	Weigend et al. 6880 (BRCO, BSB, M, NY)	Argentina	AY285700
1393	<i>Loasa floribunda</i> Phil.	LoFl	Weigend et al. 5937 (BSB, M)	Chile	AY388484
598*	“ <i>Loasa</i> ” <i>gayana</i> Urb. & Gilg	LoVo	Weigend et al. 7057 (BSB, M)	Chile	AY285701
1323	<i>Loasa heterophylla</i> Hook. & Arn.	LoMc	Weigend et al. 5920 (BSB, HUT, M, USM)	Chile	AY285702
1390	<i>Loasa illapelina</i> Phil.	LoFl	Ackermann 519 (BSB, M, SGO)	Chile	AY388485
1394	<i>Loasa insons</i> Poepp.	LoMc	Weigend et al. 5913 (BSB, HUT, M, USM)	Chile	AY388486
1391	<i>Loasa</i> cf. <i>insons</i> Poepp.	LoMc	Weigend et al. 5922 (BSB, M)	Chile	AY388487
1392	<i>Loasa intricata</i> Gay	LoMc	Weigend et al. 5934 (BSB, HUT, M, USM)	Chile	AY388488
1350	“ <i>Loasa</i> ” <i>lateritia</i> Gill. ex Arn.	LoAc	Werdermann 1342 (M)	Chile	AY285703
1367	“ <i>Loasa</i> ” <i>longiseta</i> Phil.	“Lower Loaseae”	Ehrhardt s.n. (M)	Chile	AY285704
1328	“ <i>Loasa</i> ” <i>malesherbioides</i> Phil.	“Lower Loaseae”	Wagenknecht 18509 (M)	Chile	AY285705
1466	“ <i>Loasa</i> ” <i>micrantha</i> Poepp.	LoVo	Grau s.n. (M)	Chile	AY388489
597*	“ <i>Loasa</i> ” <i>nana</i> Phil.	LoPi	Weigend et al. 7080 (BRCO, BSB, M, NY)	Argentina	AY285706
1339	<i>Loasa nitida</i> Desr.	LoMc	Weigend et al. 7346 (BSB, HUT, M, USM)	Peru	AY285707
1386	<i>Loasa pallida</i> Gill. ex Arn.	LoFl	Ricardi et al., 886 (B)	Chile	AY388490

Table 2 (continued)

DNA No.	Species	Classification	Collector/collection No. (herbarium)	Country of origin	GenBank Acc. No. (<i>trnL</i>)
1388	<i>Loasa triloba</i> Juss.	LoMc	<i>Mahu</i> , 1412 (B)	Chile	AY388491
1465	<i>Loasa urmenetae</i> Phil.	LoDe	<i>Ackermann</i> , 491 (BSB, CONC, M, SGO)	Chile	AY388492
1285	<i>Mentzelia albescens</i> Griseb.	Mentzelioideae	<i>Weigend</i> et al., 6865 (BRCO, BSB, M, NY)	Argentina	AY285708
1286	<i>Mentzelia scabra</i> Kunth	Mentzelioideae	<i>Weigend</i> et al., 98/470 (F, HUT, M, USM)	Peru	AY285709
1375	<i>Nasa aeqatoriana</i> (Urb. & Gilg) Weigend	NaSc	<i>Weigend & Jaramillo</i> , 3937 (M, QCNE)	Ecuador	AY285710
1236	<i>Nasa carunculata</i> (Urb. & Gilg) Weigend	NaCa	<i>Weigend & Weigend</i> , 2000/363 (HUT, M, NY, USM)	Peru	AY285711
1335	<i>Nasa cymbopetala</i> (Urb. & Gilg) Weigend	NaGr	<i>Weigend</i> et al., 7458 (BSB, HUT, M, USM)	Peru	AY285712
1238	<i>Nasa driesslei</i> Weigend	NaAl	<i>Henning & Schneider</i> , 243 (BSB, HUT, M, USM)	Peru	AY285713
1242	<i>Nasa ferruginea</i> (Urb. & Gilg) Weigend	NaSc	<i>M. & K. Weigend</i> 2000/199 (HUT, M, NY, USM)	Peru	AY285714
1351	<i>Nasa herzogii</i> (Urb. & Gilg) Weigend	NaAl	<i>Müller</i> 6596 (LPB)	Bolivia	AY285715
1378	<i>Nasa hornii</i> (Weigend) Weigend	NaGr	<i>Weigend & Horn</i> 3815 (M, QCNE)	Ecuador	AY285716
1365	<i>Nasa humboldtiana</i> (Urb. & Gilg) Weigend subsp. <i>obliqua</i> Dostert & Weigend	NaSc	<i>Dostert</i> 98/154 (F, HUT, MSB, USM)	Peru	AY285717
1374	<i>Nasa insignis</i> Weigend	NaGr	<i>Dostert</i> 98/161 (F, HUT, M, USM)	Peru	AY285718
1377	<i>Nasa jungiaefolia</i> (Weigend) Weigend	NaGr	<i>Weigend & Horn</i> 3838 (QCNE, M)	Ecuador	AY285719
1232	<i>Nasa laxa</i> (Killip) Weigend	NaSc	<i>Weigend</i> et al. 98/547 (F, HUT, M, USM)	Peru	AY285720
1239	<i>Nasa lenta</i> (Urb. & Gilg) Weigend	NaAl	<i>Weigend</i> et al. 5446 (BSB, HUT, M, USM)	Peru	AY285721
1240	<i>Nasa loxensis</i> (Kunth) Weigend	NaAl	<i>Grant & Struwe</i> 4063 (BSB)	Ecuador	AY285722
1330	<i>Nasa macrantha</i> (Urb. & Gilg) Weigend	NaGr	<i>Weigend</i> et al. 2000/816 (HUT, M, NY, USM)	Peru	AY285723
1329	<i>Nasa macrothyrsa</i> (Urb. & Gilg) Weigend	NaCa	<i>Weigend</i> et al. 97/ <i>s.n.</i> (M, USM)	Peru	AY285724
1333	<i>Nasa magnifica</i> (Urb. & Gilg) Weigend	NaGr	<i>Weigend</i> et al. 97/468 (F, M, USM)	Peru	AY285725
1243	<i>Nasa poissoniana</i> (Urb. & Gilg) Weigend	NaSc	<i>M. & K. Weigend</i> 2000/208 (HUT, M, NY, USM)	Peru	AY285726
1237	<i>Nasa pteridophylla</i> Weigend subsp. <i>Pteridophylla</i>	NaSc	<i>Weigend</i> et al. 97/307 (F, M, HUT, USM)	Peru	AY285727
1244	<i>Nasa raimondii</i> (Standley & Barkley) Weigend	NaSc	<i>M. & K. Weigend</i> 2000/167 (M, NY, USM)	Peru	AY285728
1353	<i>Nasa ramirezii</i> (Weigend) Weigend	NaSc	<i>Weigend</i> 3523C (COL, M)	Colombia	AY285729
1373	<i>Nasa rubrastra</i> (Weigend) Weigend	NaAl	<i>Schwerdtfeger</i> 22207 (M)	Ecuador	AY285730
1235	<i>Nasa solata</i> (Killip) Weigend	NaAl	<i>Weigend & Dostert</i> 98/259 (M, USM)	Peru	AY285731
1380	<i>Nasa trianae</i> (Urb. & Gilg) Weigend	NaAl	<i>Weigend</i> 3610 (COL, M)	Colombia	AY285732
—	<i>Nasa triphylla</i> (Juss.) Weigend subsp. <i>triphylla</i> (= <i>Loasa triphylla</i> Juss.)	NaSc	<i>Erixon & Bremer</i> 42 (UPS)	Ecuador	AJ430868
1246	<i>Nasa urens</i> (Jacq.) Weigend	NaSc	<i>Weigend & Förther</i> 97/542 (F, M, USM)	Peru	AY285733
1231	<i>Nasa vargasii</i> (J.F.Macbr.) Weigend	NaSc	<i>M. & K. Weigend</i> 2000/289 (HUSA, M, NY, USM)	Peru	AY285734
1366	<i>Nasa venezuelensis</i> (Steyerf.) Weigend	NaVe	<i>Weigend</i> 3604 (COL, M)	Venezuela	AY285735
1337	<i>Nasa weberbaueri</i> (Urb. & Gilg) Weigend	NaGr	<i>Weigend & Dostert</i> 98/261 (F, HUT, M, USM)	Peru	AY285736
1291	<i>Philadelphus pekinensis</i> Ruprecht	Hydrangeaceae	<i>Weigend</i> 5614 (B 270-16-96-10, BSB)	China	AY285737
1290	<i>Plakothira parviflora</i> Florence	Klaprothieae	<i>Weigend s.n.</i> (BSB, M, NTBG 970008)	Marquesas Isl.	AY285738
1288	<i>Presliophytum arequipense</i> Weigend	“Lower Loaseae”	<i>Weigend & Förther</i> 97/848 (F, M, USM)	Peru	AY285739
1369	<i>Presliophytum heucheraefolium</i> (Killip) Weigend	“Lower Loaseae”	<i>Weigend</i> 7368 (BSB, HUT, M, USM)	Peru	AY285740
1467	<i>Scyphanthus elegans</i> D.Don	—	<i>Grau & Ehrhart</i> 2-093 (M)	Chile	AY285741
1287	<i>Xylopodia klaprothioides</i> Weigend	Klaprothieae	<i>Weigend</i> et al. 97/450 (F, M, USM)	Peru	AY285742

DNA numbers follow an internal numbering code of the Institut für Biologie, Systematische Botanik und Pflanzengeographie, Freie Universität Berlin. Abbreviations for infrageneric taxa see Table 1. *Nasa triphylla* subsp. *triphylla* sequence from Bremer et al. (2002, as *Loasa triphylla* Juss.).

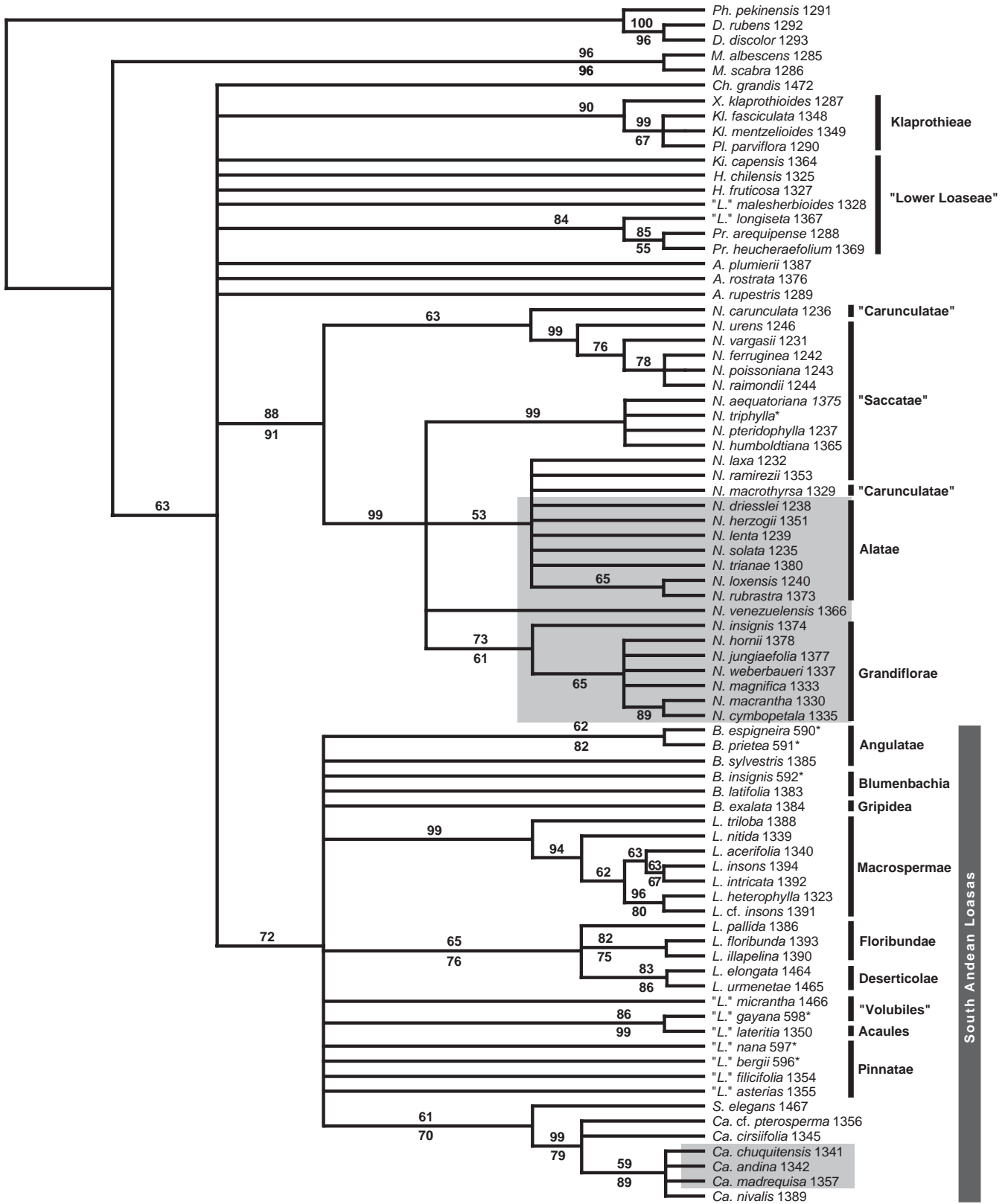


Fig. 1. Strict consensus tree of 13,284 most parsimonious trees ($L = 293$, $CI = 0.78$, $RI = 0.91$) of Loasoideae (with Mentzelioideae and Hydrangeaceae as outgroups), with bootstrap support values (above branches) and PUZZLE support values (below branches). Areas shaded in grey indicate the ornithophilous taxa (all other taxa in Loasoideae are melittophilous and/or autogamous).

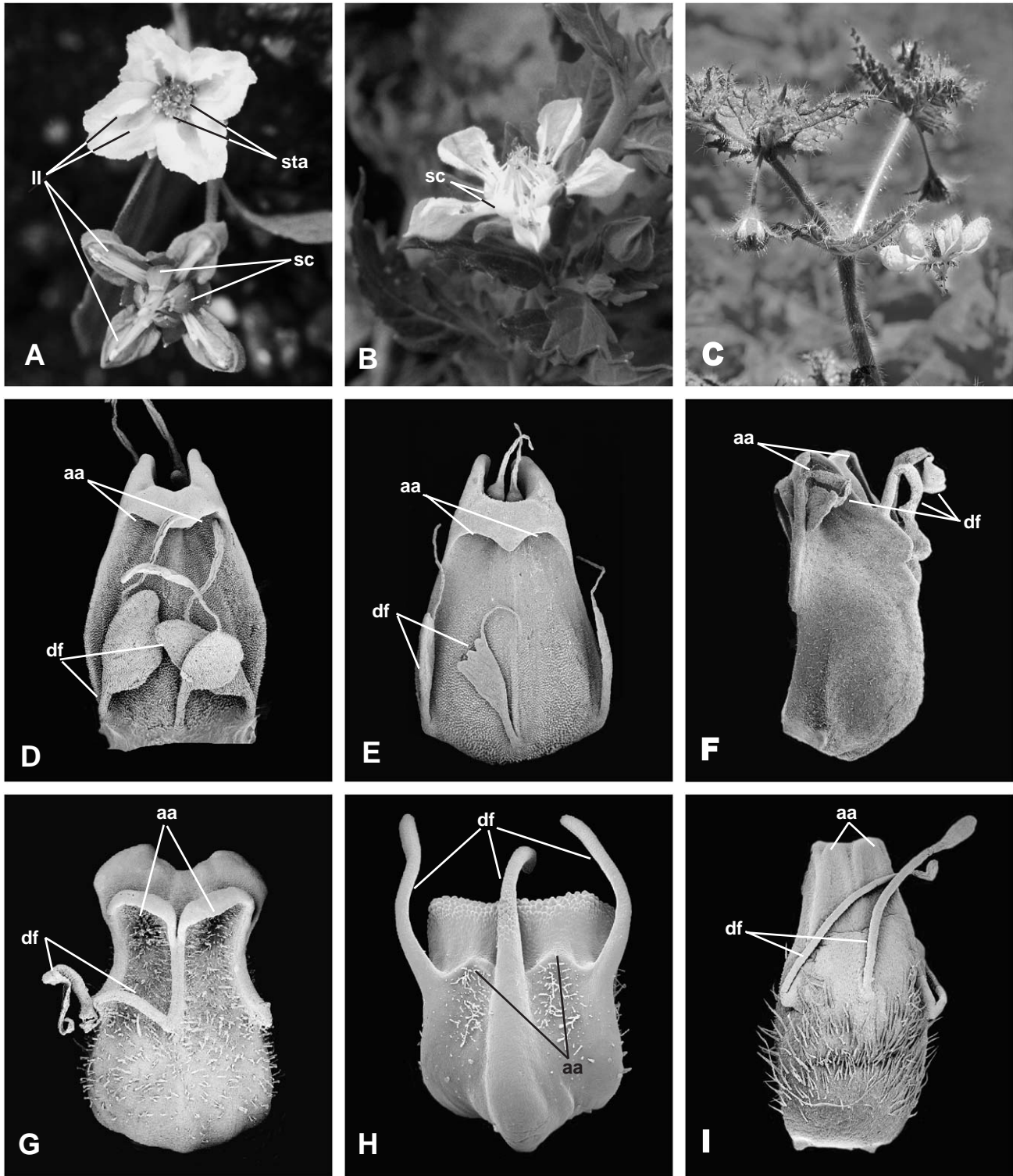


Fig. 2. Morphological characters in Loasoideae. (A) Flowers of *Xylopodia klaprothioides* (Weigend et al., 97/450C) and *Plakothira parviflora* (Weigend s.n.); note the apically free staminodes (sta) in *Plakothira* and the distinct floral scales (sc) in *Xylopodia*. Both species have longitudinal lamellae (ll) on their petals. (B) Erect flower of *Presliophytum incanum* (Weigend et al. 2000/695). (C) Pendulous flower of *Loasa insons* (LoMc, Weigend et al., 5913). (D) Floral scale of *Loasa acerifolia* (LoMc, Weigend et al., 6848) with double arch (aa) and flag-shaped dorsal filaments (df). (E) Floral scale of *Loasa nitida* (LoMc, Weigend et al., s.n.) with double arch (aa) and flag-shaped dorsal filaments (df). (F) Floral scale of “*Loasa*” *filicifolia* (LoPi, Weigend et al., 5880) with double arch (aa) and distally widened dorsal filaments (df). (G) Floral scale of *Caiophora pterosperma* (CaPt, Weigend & Dostert, 97/27) with double arch (aa) and distally widened dorsal filaments (df). (H) Young floral scale of *Caiophora canarinoides* (CaLa, Ackermann 402) with double arch (aa) and distally widened dorsal filaments (df). (I) Floral scale of “*Loasa*” *gayana* (LoVo, Weigend et al., 7057) with double arch (aa) and distally widened dorsal filaments (df).

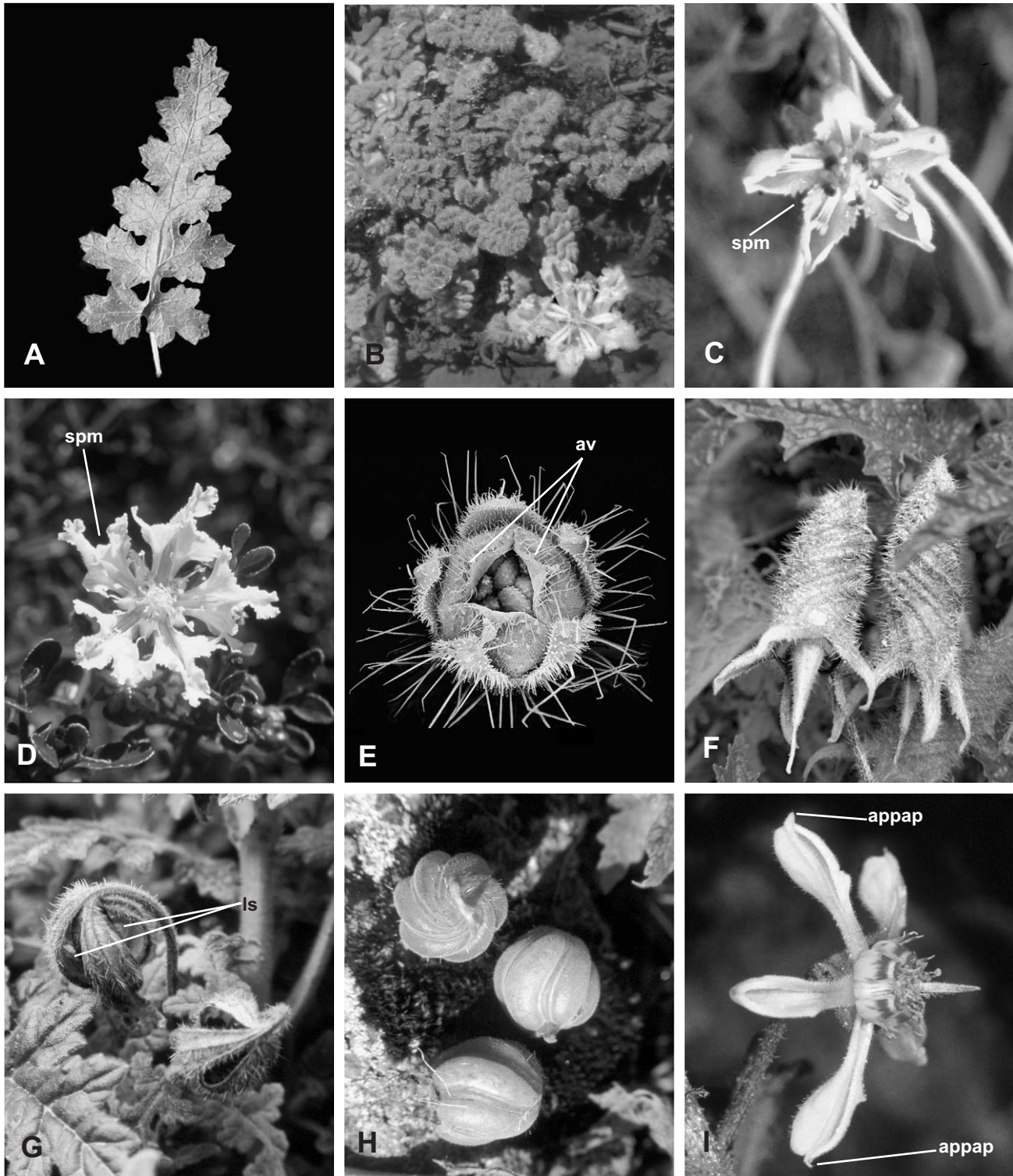


Fig. 3. Morphological characters in Loasoideae. (A) Shortly petiolate, deeply pinnatifid leaf of *Caiophora cirsiifolia* (CaCi, Weigend & Dostert, 97/194). (B) Shortly petiolate, deeply pinnatifid leaves of *Loasa nana* (LoPi, Weigend et al., 7080). (C) Serrate petal margin (spm) in the bee-pollinated flower of *Caiophora pterosperma* (CaPt, Weigend & Dostert, 97/27). (D) Serrate petal margin (spm) in “*Loasa*” *filicifolia* (LoPi, Weigend et al., 5880). (E) Capsule dehiscence with apical valves (av) in *Aosa rupestris* (Weigend, 7138). (F) Capsules twisted antidromously in *Caiophora carduifolia* (CaCa, Weigend et al., 5470). (G) Longitudinal dehiscence in capsules of *Caiophora scarlatina* (CaCh, M. & K. Weigend, 2000/108). (H) Capsules twisted anticlockwise in *Blumenbachia hieronymi* (BIBI, Weigend s.n.). (I) Hood-shaped, abruptly apiculate petals (appap) in *Blumenbachia catarinensis* (BIBI, Foto C. Schlindwein).

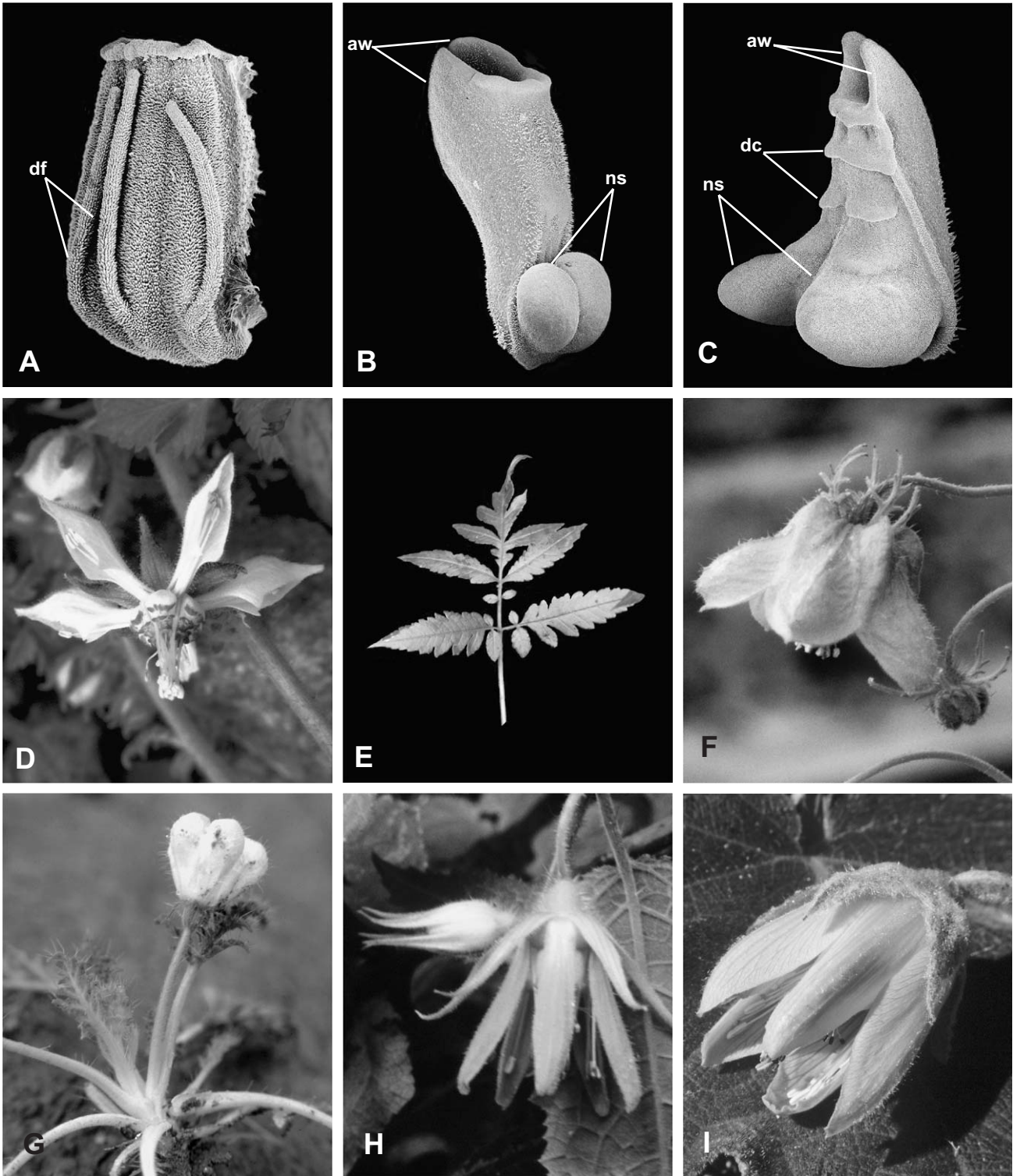


Fig. 4. Morphological characters in Loasoideae. (A) Floral scale of *Blumenbachia hieronymi* (BIBI, Weigend, s.n.) with basally inserted dorsal filaments (df). (B) Floral scale of *Nasa lenta* (NaAl, Weigend et al., 5446) with nectar sacs (ns) and apical wings (aw), dorsal filaments absent. (C) Floral scale of *Nasa urens* (NaSc, Weigend & Skrabal, 5889) with nectar sacs (ns), apical wings (aw), and dorsal calli (dc), dorsal filaments absent. (D) Pendulous flower of bee-pollinated *Nasa carunculata* (NaCa, Weigend et al., 5035). (E) Pinnate leaf of *Nasa aequatoriana* (*N. triphylla* group, NaSc, Weigend 3997). (F) Campanulate, hummingbird-pollinated flower of *Caiophora buraeavii* (CaLa, Kraus s.n., cultivated at Munich). (G) Campanulate, hummingbird-pollinated flower of *Caiophora rosulata* (CaCh, M. & K. Weigend, 2000/23). (H) Star-shaped, hummingbird-pollinated flower of *Nasa trianae* (NaAl, Weigend, 3610). (I) Campanulate, hummingbird-pollinated flower of *Nasa weberbaueri* (NaGr, Weigend & Dostert, 98/261).

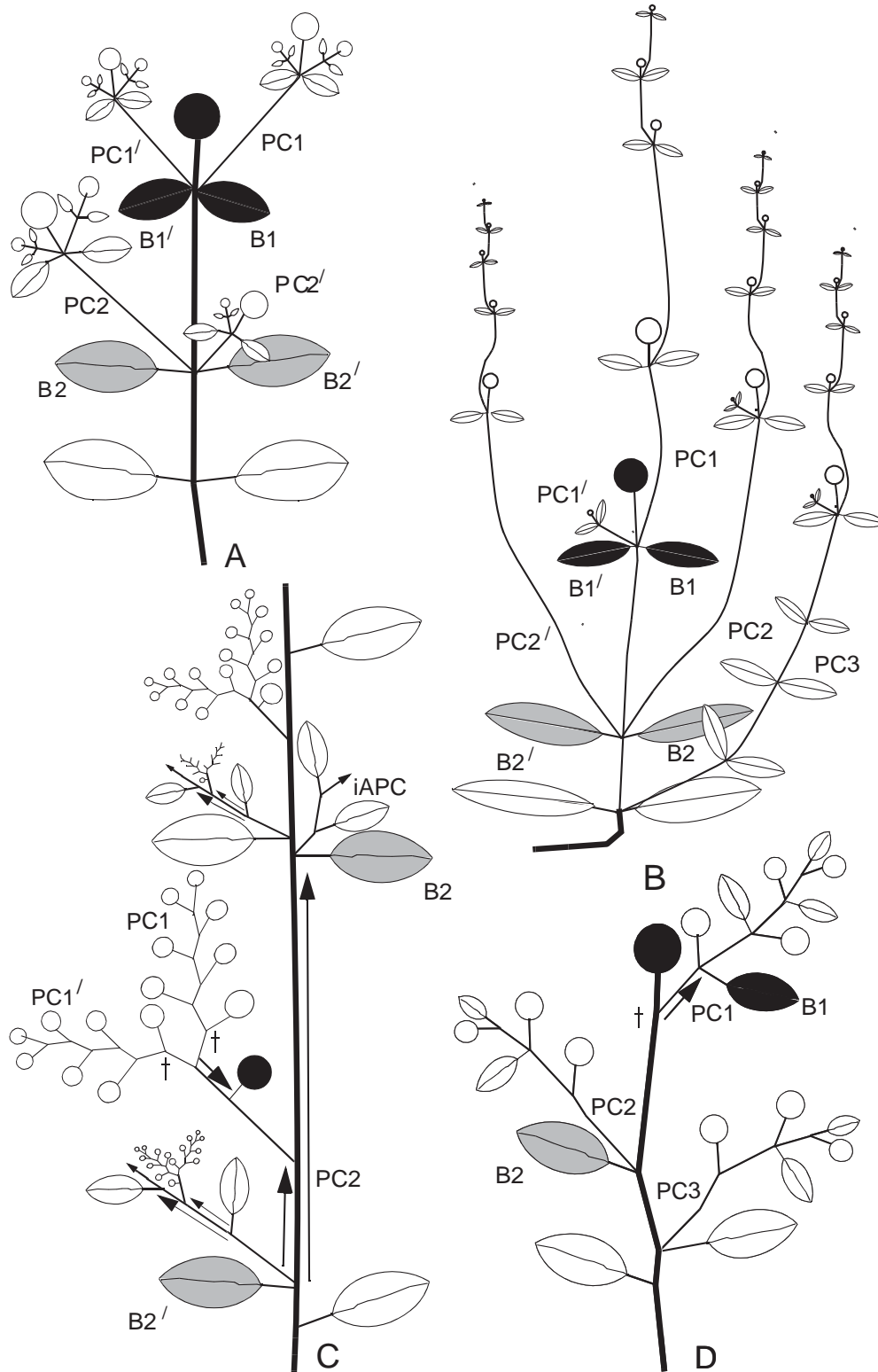


Fig. 5. Inflorescence architecture of Loasoideae (bracts and primary flower in distal dichasium black, next-lower bracts or pair of bracts grey; free arrows indicate metatopia). (A–B) Typical frondose inflorescences of *Loasa* s.str., *Caiophora* and *Scyphanthus* with non-recaulescent bracts (B, B') and asymmetrically dichasial (A) to monochasial (B) paraclades. (C) Inflorescence of *Aosa* (*A. plumerii*, AoPa), both the ebracteose (†) distal dichasium and one bract (B2) are metatopically displaced onto paraclade 2 (PC2), distal paraclades strictly monochasial. (D) Frondose inflorescence of *Nasa* (*N. picta*, NaSc), one distal paraclade absent (†), the other strictly monochasial with one metatopic bract per flower.

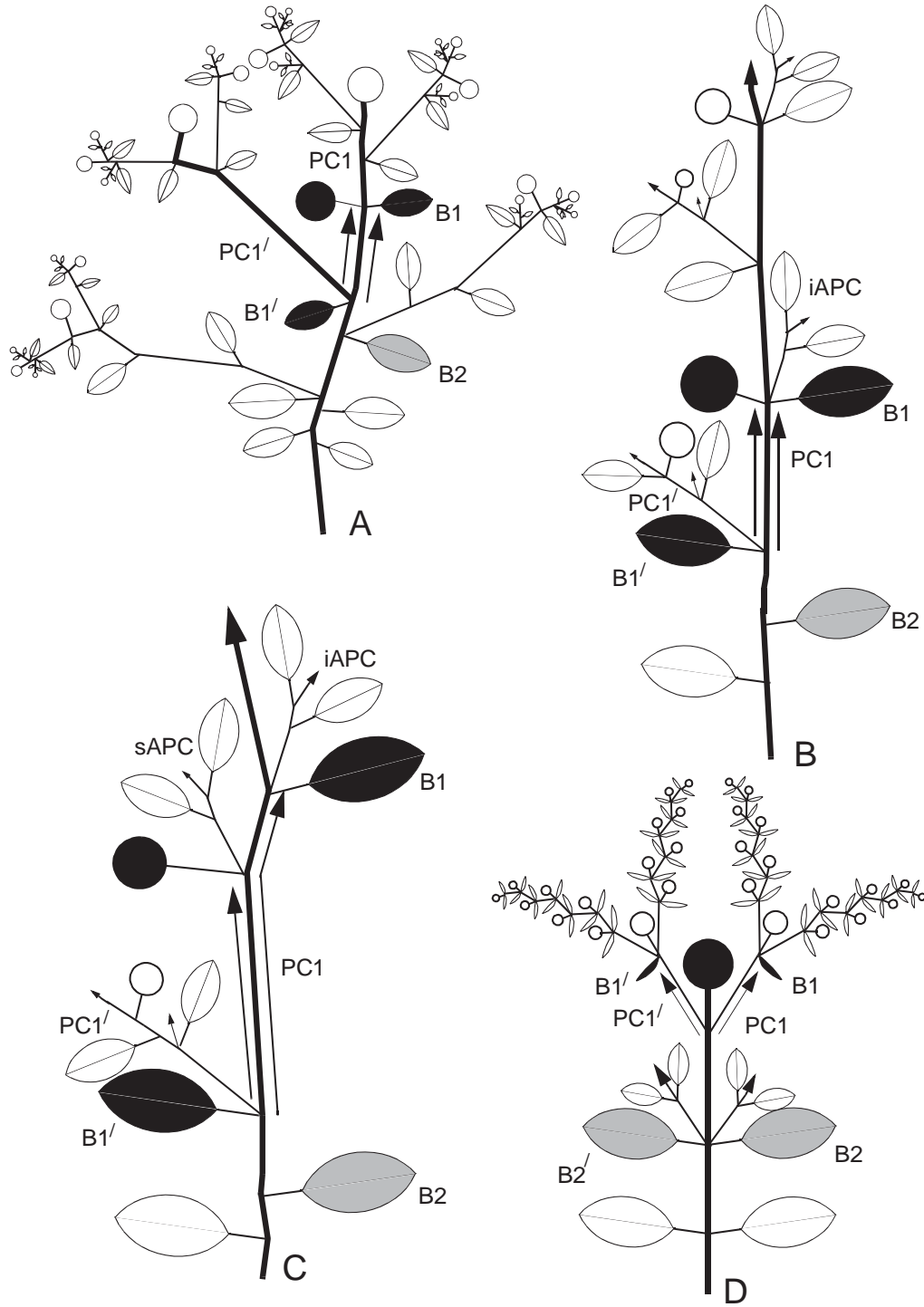


Fig. 6. Inflorescence architecture of Loasoideae (bracts and primary flower in distal dichasium black, next-lower bracts or pair of bracts grey; free arrows indicate metatopia). (A) Inflorescence of “*Loasa*” ser. *Malesherbioideae* (“*L.*” *longiseta*, LoMa), primary flower and one bract (B1) metatopic on one primary paraclade (PC1), PC1 moved into one line with primary axis. (B) Inflorescence of *Huidobria* (*H. chilensis*), as 6A, but with inferior accessory paraclades (iAPC). (C) Inflorescence of *Presliophytum* (*P. incanum*), as 6B, but with inferior and superior accessory paraclades (iAPC, sAPC). (D) Bracteose inflorescence of *Klaprothia mentzeloides*, distal paraclades once dichasial, second-order paraclades strictly monochasial, all bracts metatopic with their axillary products (if present).

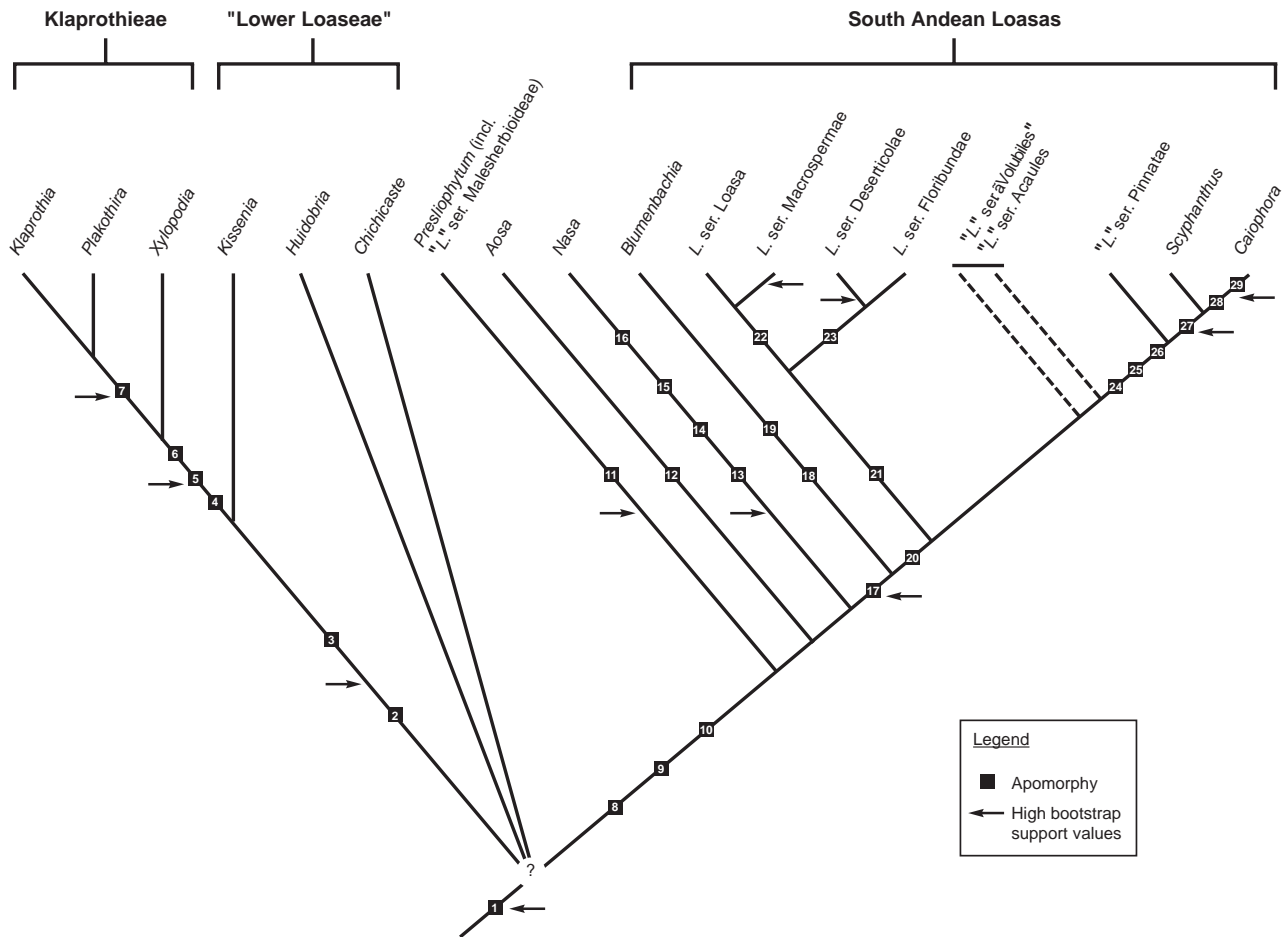


Fig. 7. Annotated cladogram of Loasoideae (for symbols see legend). (1) Stamines united into antesealous complexes (vs. absent or free). (2) Fruit wall strongly lignified (vs. not or weakly sclerified). (3) Inflorescences bracteose (vs. frondose or ebracteose). (4) Petals with longitudinal lamellae (Fig. 2A; vs. longitudinal lamellae absent). (5) Flowers tetramerous (Fig. 2A; vs. pentamerous). (6) Leaves opposite throughout (vs. alternate above). (7) Stamines of floral scale free in distal part, apically lobed (vs. united to top, and entire). (8) Floral scales always of 3 united stamines (vs. number variable or greater than 3). (9) Stamens thigmonastic (vs. with autonomous movement). (10) Flower scales strongly contrasting in colour (vs. white, yellow or greenish and more or less the same colour as petals). (11) Inflorescence with pronounced con- and recaulescence (Fig. 6; vs. metatopia absent or leading to different structure). (12) Inflorescences ebracteose (vs. bracteose or frondose). (13) Nectar scales with dorsal sacs (Fig. 4B and C; vs. sacs absent). (14) Nectar scales with well-developed apical wings (Fig. 4B and C; vs. wings absent or very short). (15) Each flower on paraclades with individual bract (Fig. 5D; vs. ebracteose or with 2 bracts). (16) $2n = 28$ (vs. $2n = 12, 24, 26$). (17) Metatopia in the inflorescence absent (or marginal recaulescence of bracts—Fig. 5A, B; vs. recaulescence with bracts moved to next flower). (18) Fruits twisted anticlockwise and with longitudinal dehiscence (Fig. 3H; vs. straight or twisted antidromously). (19) Petals apiculate (Fig. 3I; vs. petals acuminate). (20) Nectar scales with double arch on back (Figs. 2D–I; vs. double arch absent). (21) Complex hetero-oligomeric iridoids present (tricoloriside type, Weigend et al. 2000; vs. absent). (22) Dorsal filaments flag-shaped (Figs. 2D and E; vs. filiform or dorsoventrally flattened). (23) Thyroids basitonic, with alternate paraclades (vs. acrotonic and/or with opposite paraclades). (24) Leaves pinnatifid (to pinnate, bipinnate or bipinnatisect—Figs. 3A and B). (25) Leaves shortly petiolate (petiole less than 1/2 as long as lamina—Figs. 3A and B; vs. petiole equal to or longer than lamina). (26) Petal margins serrate (Figs. 3C and D; vs. margin entire). (27) Fruits with longitudinal sutures (Fig. 3G; vs. capsule opening with apical valves only). (28) 10-hydroxy-oleoside dimethyl ester present (vs. absent, Weigend et al. 2000). (29) Reduction of chromosome number ($2n = 14, 16$ vs. $2n = 24, 26$).

the second group, four more or less well-supported groups can be distinguished, of which the precise relationships are unresolved: (2a) *N. venezuelensis* (Steyerm.) Weigend, the only species of the *N. venezuelensis* group analyzed; (2b) the *N. triphylla* group (99 BS); (2c) *N. ser. Alatae* and a part of *N. ser. "Saccatae"*

(only low bootstrap support: 53 BS); (2d) *N. ser. Grandiflorae* (73 BS, 61 P).

The South Andean Loasas clade contains *Blumenbachia*, "*Loasa*" (excl. ser. *Malesherbioideae*), *Scyphanthus*, and *Caiophora*. *Blumenbachia* is weakly supported as monophyletic in the PUZZLE analysis

(50 P), but not in the bootstrap analysis, except for two of the taxa in *Blumenbachia* sect. *Angulatae* (63 BS, 84 P). In the parsimony tree, all species of “*Loasa*” ser. *Pinnatae* (“*L.*” *bergii*, “*L.*” *filicifolia*, “*L.*” *nana*, “*L.*” *asterias*) and one species of “*L.*” ser. “*Volubiles*” (“*L.*” *micrantha*) are found as a basal polytomy, with “*L.*” *gayana* (“*L.*” ser. “*Volubiles*”) and “*L.*” *lateritia* (“*L.*” ser. *Acaules*) retrieved as sister taxa (86 BS, 99 P). However, all taxa sampled of “*L.*” ser. *Pinnatae*, “*L.*” ser. *Acaules*, “*L.*” ser. “*Volubiles*”, *Scyphanthus*, and *Caiophora* constitute a monophyletic group in the PUZZLE analysis (67 P). *Scyphanthus* and *Caiophora* are invariably retrieved as sister taxa (61 BS, 70 P). There was little sequence variation in *Caiophora*. *Loasa* ser. *Macrospermae*, which was broadly sampled, is retrieved as a well-supported monophyletic group (99 BS), and the two closely allied series *Floribundae* (*L. illapelina*, *L. floribunda*, *L. pallida*; 53 P) and *Deserticolae* (*L. urmenetae*, *L. elongata*; 83 BS, 86 P) are identified as another monophylum (65 BS, 76 P).

Discussion

Systematics and plausibility of the molecular analyses

The *trnL* data confirm the conclusions of Weigend (1997) in showing the polyphyly of “*Loasa*” sensu Urban and Gilg (1900) and in confirming the monophyly of the segregate genera *Nasa* and *Presliophytum*, of the re-defined genus *Caiophora* with the exclusion of two sections of *Blumenbachia* (*B.* sect. *Angulatae*, *B.* sect. *Gripidea*), and of tribe Klaprothieae. “*Loasa*” remains paraphyletic (also indicated in Weigend 1997), even after the exclusion of “*L.*” ser. *Malesherbioideae*. These results are congruent with the data of Hufford et al. (2003), which also underscore the naturalness of *Caiophora*, *Presliophytum*, and *Nasa*, and the justification for the segregation of *Huidobria* and *Aosa* from “*Loasa*”. While the recent morphological classification of Weigend (1997) is thus largely vindicated, the infrageneric groups (i.e., sections and series) of the much older Urban and Gilg (1900) classification are also largely retrieved in *Loasa* s.str. (*L.* ser. *Deserticolae*, *L.* ser. *Macrospermae*, *L.* ser. *Floribundae*).

Klaprothieae (with *Xylopodia* as sister to *Klaprothia* and *Plakothira*) is identified as monophyletic by apomorphic characters such as longitudinal lamellae on the petals, tetramerous flowers (Figs. 2A and 7: characters 4, 5, 6), and strictly opposite, usually entire leaves (Fig. 7: character 7). The position of *Xylopodia* is crucial to understanding the evolution of Loasoideae flowers: It has antesealous staminodes united into nectar scales, whereas the other Klaprothieae have

distally free staminodes (Fig. 2A). Since all other Loasoideae have floral scales (and in all other subfamilies of Loaseae staminodes are either free or absent) an apomorphic reversion, and not a retained ancestral character, is most parsimonious for *Klaprothia* and *Plakothira* in this respect. Hufford et al. (2003) retrieve the African genus *Kissenia* as sistergroup to Klaprothieae (not resolved in our analysis), which renders further support for this hypothesis, since *Kissenia* also has staminodes united into a distinct nectar scale. The close relationship between Neotropical *Klaprothia*, including *K. (Sclerothrix) fasciculata* (C.Presl) Poston as suggested by Poston and Nowicke (1990), and the Marquesas Islands endemic *Plakothira* is reflected in the phylogeny, and *Plakothira* may indeed have arisen from epizoochorous ancestors (*Klaprothia mentzeloides* has tardily dehiscent, burr-like capsules), with subsequent modifications due to the island environment (loss of dispersal mechanism, island woodiness). Although its position remains unresolved in the present analysis, *Kissenia* probably is the sistergroup of Klaprothieae (Hufford et al. 2003), since morphological characters such as strongly lignified fruits and bracteose inflorescences (Fig. 6D) can be regarded as synapomorphic (Fig. 7: characters 2 and 3).

Another aspect that is entirely congruent between the data presented here and those published by Hufford et al. (2003) is that the two species of *Huidobria* are not retrieved as monophyletic. They are essentially held together by the fact that the number of staminodes in the antesealous groups is not fixed, but the same is true for Klaprothieae (whereas all other Loaseae have the apomorphic, fixed number of three staminodes; Fig. 7: character 8), indicating the plesiomorphic condition of this character state. Furthermore, both *Huidobria* species have a peculiar inflorescence morphology (Fig. 6D) which is, however, very similar to that found in *Presliophytum*. Grau (1997) discusses the profound differences between the two species of *Huidobria* regarding seed, leaf, and flower morphology. On balance, the available data suggest that the two species may indeed represent two only distantly related lineages.

The term “Lower Loaseae” was informally introduced for *Chichicaste*, *Huidobria*, *Kissenia*, *Presliophytum* and “*Loasa*” ser. *Malesherbioideae* (without indication of any close relationship), as a working concept to name the taxa lacking both thigmonastic stamens and colored floral scales (Weigend 1997). However, green-house experiments by the present authors have recently shown that *Presliophytum* (*P. heucheraefolium*, *P. incanum*) does indeed show thigmonastic stamens, and at least one species (*L. malesherbioides*) has colored nectar scales. By definition, *Presliophytum* and ser. *Malesherbioideae* would thus have to be placed into Higher Loaseae, although molecular resolution is satisfactory neither in

this analysis nor in Hufford et al. (2003). An expansion of *Presliophytum* s.l. to include “*L.*” ser. *Malesherbioideae*, as was suggested in Hufford et al. (2003), is probably the sensible taxonomical consequence. *Presliophytum* s.l. is then held together by its peculiar inflorescence morphology with extreme metatopia (Figs. 6A, C and 7: character 11), leaf and seed characters.

The remaining taxa of Loasoideae (*Aosa*, *Blumenbachia*, *Caiophora*, “*Loasa*” in a narrow sense, *Nasa*, and *Scyphanthus*) were united into Higher Loaseae, which are likely monophyletic based on apomorphies such as thigmonastic stamens and colored floral scales (Weigend 1997; Fig. 7: characters 9 and 10). The molecular results do not contradict this view if we include *Presliophytum* in this clade. The small Brazilian-Hispaniolan genus *Aosa* (6 spp.) is not retrieved as a clade, but shows various morphological characters (e.g., characteristic ebracteose inflorescences: Figs. 5C and 7: character 12) which render its monophyly likely.

Nasa is resolved as a well-supported monophyletic group in the present analysis as well as in Hufford et al. (2003, albeit with a smaller taxon sampling), and this is congruent with various unique morphological characters (presence of dorsal sacs and apical wings on the nectar scales: Figs. 4B and C; inflorescence morphology: Fig. 5D; karyology: $2n = 28$; Fig. 7: characters 13–16). The internal phylogeny of *Nasa* is partly resolved, with a basal dichotomy of a core clade comprising taxa of all five infrageneric groups and a smaller clade (*N. urens* group), but none of these clades are held together by any obvious morphological character. The *N. urens* group comprises a range of morphologically rather heterogenous species from *Nasa* ser. “*Saccatae*” (*N. picta*, *N. chenopodiifolia*, *N. urens*, *N. vargasii*), the morphologically coherent *N. poissoniana* group (*N. ferruginea*, *N. poissoniana*, *N. raimondii*), and *N. carunculata*, the type species of *N.* ser. “*Carunculatae*”. *Nasa carunculata* has been considered as closely allied to *N. macrothyrsa*, primarily because of its strikingly similar habit (strongly branched shrubs; Weigend et al. 2003), but *N. macrothyrsa* is nested in the core clade, thus *N.* ser. “*Carunculatae*” appears to be polyphyletic. The paraphyly of *Nasa* ser. “*Saccatae*”, previously postulated by Weigend and Rodriguez (2003), is again evident, involving at least three lineages. *Nasa* ser. “*Saccatae*” was established by Urban and Gilg (1900) on the basis of annual habit, and flowers with spreading petals and contrastingly colored nectar scales (Fig. 4D), but these character states are clearly plesiomorphic for Higher Loaseae, since they are found in nearly all lineages (*Loasa*: Fig. 2C, *Caiophora*: Fig. 3C, *Blumenbachia*: Fig. 3I). Within the well-supported core clade in *Nasa*, two groups are strongly supported as monophyletic which are also well-circumscribed morphologically: the *N. triphylla* group from *N.* ser. “*Saccatae*”, which has deeply divided (trifoliolate to pinnate) leaves (Fig. 4E; Dostert and Weigend 1999); and *N.* ser.

Grandiflorae, a mostly High Andean lineage with erect wings on the floral scales and subcircular to peltate foliage leaves (Weigend and Rodriguez 2002).

The South Andean Loasas sensu Weigend (1997) are retrieved with moderate support. They include nearly all the pre-1997 genera of Loaseae, namely *Blumenbachia*, *Caiophora*, “*Loasa*”, and *Scyphanthus*. The group is morphologically readily circumscribed on the basis of the absence of metatopia in its inflorescences (Figs. 5A, B and 7: character 17), whereas at least the bracts are metatopic in all other Loasaceae (indicating the plesiomorphic condition). All South Andean Loasas except *Blumenbachia* have a distinct double arch on the back of each nectar scale (Figs. 2D–I), and this uniqueness suggests the monophyly of the corresponding group (Fig. 7: character 20). *Blumenbachia* has been redefined by removing *B.* sect. *Angulatae* and *B.* sect. *Gripidea* from *Caiophora* (Weigend 1997), and the distinctness from *Caiophora* of these two groups is clearly confirmed by molecular data from the present study. Like *Caiophora*, *Blumenbachia* has fruits with longitudinal dehiscence (Fig. 3H), but this character appears to be convergent, since the two groups differ profoundly in other aspects of morphology (e.g. scale morphology: Fig. 4A, petal morphology: Fig. 3I). Moreover, apart from the opening mode, actual fruit morphology is also quite different: In *Caiophora*, capsules are straight, and twisted antidromously if twisted at all (Fig. 3F), whereas those of *Blumenbachia* are always twisted, and twisted anticlockwise only (Figs. 3H and 7: character 18). The monophyly of *Blumenbachia* sensu Weigend (1997) is weakly supported in the quartet puzzling analysis (but not with the parsimony criterion). Hufford et al. (2003) did not address this problem, since they only included two species of sect. *Blumenbachia* (i.e., *Blumenbachia* sensu Urban and Gilg 1900) in their analysis. However, all species of *Blumenbachia* share abruptly apiculate petals (Figs. 3I and 7: character 19), identical nectar scales (Fig. 4A), and capsules twisted anticlockwise (Fig. 3H), and are therefore likely to represent a monophyletic group in spite of the lack of molecular resolution. A sistergroup relationship between *Blumenbachia* and the remainder of the South Andean Loasas is plausible.

Within South Andean Loasas, two monophyletic clades with annual species of *Loasa* are clearly retrieved in the molecular tree: *L.* ser. *Macrospermae*, with extremely large and smooth seeds and very conspicuous, flag-shaped dorsal threads on their nectar scales (Figs. 2D, E and 7: character 22), and another clade comprising *L.* ser. *Floribundae* and *L.* ser. *Deserticolae*. The latter two groups show apomorphies in floral morphology (Urban and Gilg 1900) and have identical inflorescences (basitonic thyrsoids with alternate paraclasses, unique in Loasoideae; Fig. 7: character 23). Phytochemistry further indicates that all three series

(*Deserticolae*, *Floribundae*, *Macrospermae*) probably share exclusive common ancestry, since they are the only groups containing a particular type of complex hetero-oligomeric iridoids (e.g., tricoloriside; Weigend et al. 2000; Fig. 7: character 21). *Loasa* ser. *Loasa*, the type section of the genus with only two species (*L. acanthifolia* Desr., *L. sclareifolia* Juss.), is morphologically closely allied to *L. ser. Macrospermae* and shares its two most striking characters (see above). *Loasa acanthifolia* was sequenced, but the *trnL* sequence has a very long deletion (more than 250 bp in the alignment, representing helix 8 of the *trnL* secondary structure; Kuhse et al. 1990). Because its inclusion thus eliminates many informative alignment positions, it had to be removed from the analysis. However, the close relationship between *L. ser. Loasa* and *L. ser. Macrospermae* is evident, thus the group comprising four series (*Deserticolae*, *Floribundae*, *Loasa*, *Macrospermae*) likely represents *Loasa* s.str. in the very narrowest sense.

The exact placement of “*L.*” ser. *Pinnatae*, “*L.*” ser. “*Volubiles*”, and “*L.*” ser. *Acaules* remains unresolved in the bootstrap analysis, but the quartet puzzling analysis indicates a sistergroup relationship with the (well-supported) *Caiophora*/*Scyphanthus* clade, which is congruent with two unique morphological characters: the vast majority of species in these groups have deeply pinnatifid, very shortly petiolate leaves (Figs. 3A, B and 7: characters 24 and 25). In addition, “*L.*” ser. *Pinnatae*, *Caiophora*, and *Scyphanthus* share the predominance of serrate petal margins (Figs. 3C, D and 7: character 26), the latter being entire in all other groups. *Scyphanthus* contains two annual herbs from the mediterranean climate in Chile, whereas *Caiophora* is a High Andean taxon containing over 50 species and ranging from Central Argentina into southern Ecuador, with a single annual species in Uruguay and SE Brazil. *Caiophora* and *Scyphanthus* were also retrieved as sister taxa in the analyses of Moody and Hufford (2000) and Hufford et al. (2003), and they are evidently closely allied since they share apomorphic characters. The fruits of *Scyphanthus* and *Caiophora* open both with apical valves and with longitudinal sutures (synapomorphic; Fig. 7: character 27; in the derived taxa of *Caiophora* the capsule apex remains coherent: Fig. 3G), whereas other Loasoideae typically have fruits opening with apical valves only (Fig. 3E). The monophyly of *Caiophora* sensu Weigend (1997) is based on karyology ($2n = 14, 16$) and phytochemistry (10-hydroxyoleoside-dimethyl ester; Weigend et al. 2000; Fig. 7: character 28), and is confirmed by the present analysis.

Historical biogeography

Some aspects of historical biogeography can be addressed on the basis of the data presented here, but

the timing of divergence events appears to be impossible from the data available (see Table 1 for distribution areas). “*Huidobria*” (N Chile), *Klaprothia* (Bolivia to Mexico), *Xylopodia* (N Peru: Amotape-Huancabamba Zone; Weigend, 2000), *Kissenia* (Africa), *Presliophytum* (NW Chile, W Peru), *Plakothira* (Marquesas Islands in Polynesia), and *Aosa* (Brasil and Hispaniola) are geographically widespread in the tropical regions primarily of South America, but they are completely absent from higher elevations and the temperate and mediterranean regions. They are found with a series of evident paleoendemics in the coastal deserts of western South America (*Presliophytum* and *Huidobria*), the rain forests of Central America (*Chichicaste*), in Africa (*Kissenia*), and Brazil (*Aosa*). Conversely, High Andean and southern temperate habitats have only been colonized by two, species-rich groups: the South Andean Loasas and *Nasa*.

Nasa is restricted to the American Cordillera, and the limits of its distribution coincide rather precisely with the limits of the tropical region (southern limit: Department Santa Cruz in Bolivia, northern limit: Province Chiapas in Mexico). *Nasa* ser. *Grandiflorae* is the only genuinely High Andean group (2500–4500 m). This monophylum is apparently derived from plants growing at lower elevations in the (paraphyletic) remainder of *Nasa*, such as the only coastal species (200–1000 m; *N. urens* (Jacq.) Weigend), various species from moderate elevations in inner-Andean valleys (<2500 m; *N. poissoniana*, *N. vargasii*), or cloud forest taxa (2000–3000 m; *N. triphylla* group, *N. laxa* (Killip) Weigend, *N. ramirezii* (Weigend) Weigend, *N. ser. Alatae*). The Amotape-Huancabamba Zone in N Peru and adjacent Ecuador seems to be the primary centre of diversification of *Nasa*, and ascent into the higher elevations as well as ecological diversification may have taken place largely in this region (Weigend 2002).

Within the South Andean Loasas, it is also possible to trace geographical patterns: all species of *Loasa* s.str. (i.e. *L. ser. Loasa*, *L. ser. Macrospermae*, *L. ser. Deserticolae*, *L. ser. Floribundae*) are endemic to the mediterranean region or coastal desert of Chile, with only few species extending into similar habitats in Argentina or Peru; *Blumenbachia* is nearly exclusively south-east South American and is found in a wide arch from Brazil to Chile, with the morphologically most primitive group (*B. sect. Angulatae*) endemic to the southern Andes. The other groups of “*Loasa*” (“*L.*” ser. *Acaules*, “*L.*” ser. “*Volubiles*”, “*L.*” ser. *Pinnatae*) are largely Patagonian. *Scyphanthus* is restricted to the mediterranean region of Chile. *Caiophora* is widespread at elevations above 3500 m in the Andes (Argentinian Andes to Ecuador), but has its morphologically most primitive taxa (i.e., those most similar to *L. ser. Pinnatae* and *Scyphanthus*) in SW Brazil and Uruguay (*C. arechavaletae*), the Argentinian Andes (*C. pulchella*, *C. nivalis*), and at moderate elevations of the Peruvian

Andes (*C. pterosperma* group). This distribution pattern indicates a primary radiation of the South Andean Loasas in the southern temperate and mediterranean zones. Subsequent dispersal and diversification, essentially of *Caiophora*, led to the current distribution patterns. Within South Andean Loasas, only *Caiophora* has High Andean groups, and it seems to have colonized the High Andean region from the south temperate zone.

In the colonization of tropical High Andean habitats by *Caiophora* and *Nasa*, two independent, alternative routes have thus been explored: *Caiophora* took the southern track by adaptation to temperate climate, whereas *Nasa* took the direct route with progressive adaptation to colder climates in the central Andes. In both groups, the widely open, contrastingly colored flowers typical for Loasoideae with melittophily (Figs. 2C, 3C, D, I and 4D) are ancestral, and they independently developed into superficially similar, much larger, orange or red, hummingbird-pollinated flowers (Figs. 1 and 4F–I) in their High Andean representatives only.

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