

Nectar, Floral Morphology and Pollination Syndrome in Loasaceae subfam. Loasoideae (Cornales)

MARKUS ACKERMANN* and MAXIMILIAN WEIGEND

Institut für Biologie der Freien Universität Berlin, Systematische Botanik und Pflanzengeographie,
Altensteinstraße 6, 14195 Berlin, Germany

Received: 21 February 2006 Returned for revision: 20 March 2006 Accepted: 19 May 2006 Published electronically: 4 July 2006

• **Background and Aims** Loasaceae subfam. Loasoideae are mostly distributed in South America (sea level to over 4500 m) with a wide range of animals documented as pollinators. The aim was to investigate correlations between nectar parameters, flower morphology, pollination syndrome and phylogeny.

• **Methods** Nectar was collected from 29 species from seven genera in the subfamily. Concentration and volumes were measured and the amount of sugar calculated. Correlations of nectar data were plotted on a ternary graph and nectar characteristics compared with flower visitors, floral morphology and phylogenetic data.

• **Key Results** Sugar concentrations are generally higher than reported for most plant families in the literature. The species investigated can be roughly grouped as follows. Group I: plants with approx. 1.5–(3.5) μL nectar with (40–)60–80% sugar and 0.19–2 mg sugar flower⁻¹; with small, white, star-shaped corollas, pollinated by short-tongued bees. Groups II, III and IV: plants with mostly orange, balloon-, saucer-, bowl- or bell-shaped corollas. Group II: plants with approx. 9–14 μL nectar with 40–60% sugar and 4–10 mg sugar flower⁻¹; mostly visited by long-tongued bees and/or hummingbirds. Group III: plants with 40–100 μL nectar with 30–40% sugar and 14–36 mg sugar flower⁻¹, mostly visited by hummingbirds. Group IV: geoflorous plants with 80–90 μL with 10–15% sugar and 8.5–12 mg sugar flower⁻¹, presumably visited by small mammals. Groups II and III include species visited by bees and/or hummingbirds.

• **Conclusions** Pollinator switches from short-tongued bees via long-tongued bees to hummingbirds appear to have taken place repeatedly in the genera *Nasa*, *Loasa* and *Caiophora*. Changes in nectar amount and concentration appear to evolve rapidly with little phylogenetic constraint.

Key words: Nectar, pollination, *Caiophora*, *Loasa*, *Nasa*, Loasaceae, short-tongued bees, long-tongued bees, Colletidae, Apidae, Anthophoridae, rodents, ornithophily.

INTRODUCTION

Nectar production and composition are understood to be crucial factors influencing flower visitation and consequently pollinator preferences for particular plant species (e.g. Baker and Baker, 1982; Endress, 1994). Certain pollinator species show a distinct preference for particular nectar types (e.g. Baker, 1975; Bolten and Feinsinger, 1978; Bolten *et al.*, 1979; Baker and Baker, 1983; Heyneman, 1983; Zimmermann, 1983; Blem *et al.*, 2000; McDade and Weeks, 2004). Thus, there is general agreement, that sugar concentration in hummingbird-pollinated flowers is generally lower (20–26% sugar; Hainsworth and Wolf, 1972; Baker, 1975; Cruden *et al.*, 1983) than in insect-pollinated flowers (>30% sugar; rarely up to 80%). Further differences have been reported between ‘lowland hummingbird nectar’ and ‘highland hummingbird nectar’, with highland nectar less concentrated and hence less viscous, but present in higher volumes such that these flowers present roughly the same caloric value (Hainsworth and Wolf, 1972; Baker, 1975). Heinrich and Raven (1972) and Forcone *et al.* (1997) argue that energetic reward for pollinators in habitats with low temperatures is higher than in areas with high temperatures. Cruden *et al.* (1983) claim that the nectar volume of flowers pollinated by ‘large bees’ (e.g. *Bombus*,

Xylocopa, *Centris*) has to be significantly higher than that of flowers pollinated by ‘small bees’ (e.g. *Colletes*, *Apis*). Because bee pollination seems to phylogenetically precede hummingbird pollination in most plant groups [e.g. Scrophulariaceae: tribe Antirrhineae (Elisens and Freeman, 1988; Ghebrehiwet *et al.*, 2000); *Penstemon* (Wilson *et al.*, 2006); *Mimulus* (Fishman *et al.*, 2002; Beardsley *et al.*, 2003); Gesneriaceae: tribe Sinningieae (Perret *et al.*, 2001, 2003)], scientists have variously addressed the question as to how the transition from ‘typical’ bee nectar to ‘typical’ hummingbird nectar took place. Bolten and Feinsinger (1978) argue that the relatively low sugar concentration in hummingbird nectar is not due to a preference for lower sugar concentrations by hummingbirds, but rather serves to render the flowers less attractive to bees. One crucial problem of many of the data sets published on the relationship between nectar and pollination is the comparison of nectar and pollinator data including taxa from distantly related plant groups. Differentiating between adaptive responses and possible phylogenetic constraints is thus difficult. There have been two major studies attempting to elucidate the evolution of nectar characteristics and pollination syndromes within presumably monophyletic plant groups, albeit without an explicit phylogenetic framework. The study on Scrophulariaceae (now Plantaginaceae) Tribe Antirrhineae (20 North American species; Elisens and Freeman, 1988)

* For correspondence. E-mail ackermal@zedat.fu-berlin.de

concentrated on sugar composition, i.e. the relative percentages of different sugars in the nectar, while giving no data on absolute nectar volumes, sugar concentrations or absolute sugar amounts. The study on Gesneriaceae Tribe Sinningieae (45 Neotropical species; Perret *et al.*, 2001) provides data on sugar concentration, and sugar composition, but not on overall nectar production. Both studies show correlations between pollination syndromes and nectar composition, but in neither case is an explicit correlation of pollination syndrome to quantitative nectar features clarified.

The present study intends to compare nectar and pollination syndromes in Loasaceae subfam. Loasoideae, a monophyletic, largely Neotropical plant group of approx. 200 species, with its centre of diversity in the Central Andes (Weigend, 2004a) and with considerable variability in their floral morphology and pollination biology (Urban, 1886, 1889, 1892; Urban and Gilg, 1900; Brown and Kaul, 1981; Weigend, 2004a; Weigend *et al.*, 2004). Representatives of this group are found in many different ecosystems ranging from tropical to temperate rainforests, from coastal lomas formations in the Atacama desert up to 4500 m in the Andes. The phylogeny of this group has been largely clarified (Hufford *et al.*, 2003, 2005; Weigend *et al.*, 2004). Taxa of subfamily Loasoideae share a complex floral morphology: the heterochlamydeous, polyandrous flowers have a highly differentiated androecium with antesealous stamens modified into staminodial complexes alternating with antepetalous fascicles of (10–28) fertile stamens (Urban, 1886; Weigend, 2004a, Weigend and Gottschling, 2006). The staminodial complexes typically consist of two free, inner staminodia, and three outer, fused staminodia forming the so-called nectar scale. All flowers of Loasoideae are primarily nectar flowers, and pollen presentation is typically triggered by the manipulation of the nectar scale during nectar extraction by the flower visitor (Schlindwein, 2000). Nectar is secreted from the receptacle through antesealous, inframarginal stomata into the nectar scales, where nectar is stored (Urban, 1886, 1892; Weigend and Rodriguez, 2003; Weigend, 2004b). The nectar is thus hidden from the flower visitor and only accessible through the opening between the apex of the floral scale and the free staminodia, by manipulating the floral scale and tilting it outwards. This functional floral morphology has been described as ‘tilt-revolver flower’ (Weigend and Gottschling, 2006). While this general pattern is fairly universal in Loasoideae, there are major differences in the size and coloration of the overall flower and also in the shape and size of the nectar scales (Weigend *et al.*, 1998, 2003, 2004; Dostert and Weigend, 1999; Rodriguez and Weigend, 1999; Weigend, 2000a, b, 2001, 2004b; Weigend and Rodriguez, 2002, 2003; Weigend and Ackermann, 2003; Weigend and Gottschling, 2006). In some taxa the opening of the much larger floral scales is widened and nectar can be accessed without moving the floral scale. This flower type has recently been described as ‘funnel-revolver flower’ (Weigend, 2004b). Tilt-revolver flowers are characterized by producing very small amounts of very viscous nectar

from very small nectaries (Weigend and Rodriguez, 2003), whereas funnel-revolver flowers produce larger amounts of less viscous nectar from much larger nectaries (Weigend, 2004b). Some functional morphological aspects have thus been clarified, but both pollination data and nectar analysis are still scarce for the family.

Pollinator observations have been published for 29 species (from eight genera: *Aosa*, *Blumenbachia*, *Caiophora*, *Eucnide*, *Loasa*, *Mentzelia*, *Nasa* and *Scyphanthus*) from the USA, Chile, Argentina and Brazil (Linsley and Hurd, 1959; Thompson and Ernst, 1967; Brown and Kaul, 1981; Keeler, 1981; Arroyo *et al.*, 1982; Stiles and Freeman, 1993; Harter, 1995; Harter *et al.*, 1995; Schlindwein, 1995, 2000; Wittmann and Schlindwein, 1995; Forcone *et al.*, 1997; Schlindwein and Wittmann, 1997; Cocucci and Sérsic, 1998; Medan *et al.*, 2002; Villagrán *et al.*, 2003; Sargent and Otto, 2004; Troncoso and Vargas, 2004), and the reports include various groups of bees (long-tongued bees: Anthophoridae, Apidae, Megachilidae, Mellitidae; short-tongued bees: Colletidae, Halictidae), wasps (Ichneumonidae), flies (Syrphidae), moths (Sphingidae), hummingbirds (Trochilidae), passerines (Emberizidae, Tyrannidae) and small mammals (Muridae), i.e. a considerable range of very different pollinator groups. However, a large proportion of the taxa in Loasoideae are apparently primarily visited by short-tongued bees of a particular group (Colletidae; see Wittmann and Schlindwein, 1995; Weigend, 2004a; Weigend *et al.*, 2004). Ornithophilous taxa in *Nasa* and *Caiophora* can be shown to represent derived and largely high Andean clades in originally melittophilous genera from intermediate elevations (Weigend *et al.*, 2004; Weigend and Gottschling, 2006). Nectar analyses had so far been published for only three species of Loasoideae from Argentina and Costa Rica (Stiles and Freeman, 1993; Forcone *et al.*, 1997; Cocucci and Sérsic, 1998). However, Loasaceae subfam. Loasoideae have their centre of diversity, both in terms of taxic richness and morphology, in the Central Andes (Weigend, 2000b, 2002, 2004a–c), and no data sets on either pollinators or nectar have been published from that region.

The present paper intends to fill this gap and provide an overview of nectar composition in subfam. Loasoideae. Nectar composition was studied in cultivated plants under flower visitor exclusion. In *Caiophora* there are several taxonomically unresolved species complexes comprising closely allied species with differences in floral colour and size (Weigend and Ackermann, 2003). Multiple accessions from these groups, representing different floral morphologies, were studied to investigate possible differences in pollination and nectar composition. In Andean South America the main pollinator groups for Loasoideae are long-tongued and short-tongued bees and hummingbirds, with a single report of small mammals. Assuming that nectar composition correlates with pollinator taxon, a wide range of different nectar types would be expected. The observations on nectar composition are also compared with phylogenetic data compiled from published phylogenies (Weigend *et al.*, 2004; Hufford *et al.*, 2005; Weigend and Gottschling, 2006) to investigate whether

there have been multiple convergent changes of nectar composition in the evolution of subfam. Loasoideae. The aims are: (a) to clarify the characteristics of nectar produced by Loasoideae; (b) to provide flower visitor data for additional groups in Loasaceae subfam. Loasoideae from the full range of habitats from the Pacific coast to the high Andean region; (c) to correlate quantity and quality of nectar with overall floral morphology and flower visitors; and (d) to investigate a possible phylogenetic constraints versus adaptive responses on the basis of published systematic and phylogenetic data

MATERIALS AND METHODS

Plant material

Field studies were carried out in Argentina, Chile, Colombia, Ecuador, Peru and Venezuela, where pollinator observations were realized and habitat, growth habit and morphological data were obtained (approx. 200 collections of *Nasa*; approx. 200 collections of *Caiophora*; several collections of *Blumenbachia*, *Loasa*, *Presliophytum* and *Xylopodia*). Approx. 60 species of Loasaceae subfam. Loasoideae were brought into cultivation in the greenhouses at the Institut für Biologie, Freie Universität Berlin (February 2003 to December 2005). Seeds were sown into standard soil for seedlings and later potted into clay pots (potting soil: 2 parts mature leaf compost, 1 part peat). In winter (October–April) artificial light was used in the greenhouses (12 h, high pressure sodium lamps: Philips SON-T AGRO® 400 W). High Andean and south temperate taxa *Caiophora*, *Nasa dillonii*, *N. macrothyrsa* and *Loasa sclareifolia* were cultivated with night-time temperatures of 5–15 °C and daytime temperatures of 15–25 °C; all other species were grown at night-time temperatures of 18–20 °C and daytime temperatures of 20–25 °C. Cultivation in the greenhouses permitted nectar samples to be obtained under fairly standardized conditions eliminating possible effects of, for example, altitudinal differences, water stress, ambient air humidity (Corbet *et al.*, 1979a, b; Plowright, 1981; Bertsch, 1983; Zimmermann, 1988; Carroll *et al.*, 2001; Pacini *et al.*, 2003). The measurements obtained document the amount and composition of nectar produced in the absence of flower visitors. There are several lines of evidence that argue that the samples obtained from the plants cultivated for this research represent a good proxy to natural conditions:

- (a) Published nectar data based on samples collected in nature agree with the present analysis of closely allied species: The field data on *Caiophora coronata* (Cocucci and Sérsic, 1998) are similar to greenhouse data on the closely allied *C. pentlandii* (Table 1 and Fig. 1). Field data on *Nasa speciosa* (Stiles and Freeman, 1993) and *Caiophora nivalis* (investigated by A. Wertlen) are also close to data for allied taxa from the greenhouse.
- (b) The few measurements of nectar volume that were taken in nature [*Caiophora carduifolia* (3), 20.0–23.5 µL; *C. carduifolia* (4), 10.5–21.5 µL; *C. carduifolia* (5), 11.0–60.0 µL; *C. carduifolia* (6), 13.5–20.0 µL;

C. chuquitensis, 10–18.5 µL; *C. pentlandii* (2), 21.0–49.0 µL; *Nasa urens*, 0.2–2.0 µL] are close to those obtained from cultivation, but generally lower, probably due to pollinator visits (excluded in the greenhouse).

- (c) There is close agreement between floral morphology, pollinator spectra documented in the wild and nectar composition, so that there is no reason to believe that nectar data are grossly aberrant.
- (d) Kaczorowski *et al.* (2005) found that *Nicotiana* L. section *Alatae*, species with hummingbird-pollinated flowers show similar nectar composition under greenhouse conditions and in the field.
- (e) Several studies of nectar composition across a larger group of closely allied species used greenhouse experiments (Elisens and Freeman, 1988; Perret *et al.*, 2001).

Total nectar amount of individual flowers

The entire amount of nectar present in each flower was harvested by inserting micro-capillaries between the two staminodia and the floral scale (micro capillaries: 1- and 2-µL Microcaps; Drummond Scientific Co., Broomall, PA, USA; 5, 10 and 25-µL Duran Ringcaps; Hirschmann Laborgeräte, Eberstadt, Germany). Nectar was harvested twice from each floral scale within 5 min to obtain the full amount of nectar. Brix measurements were then made with a handheld refractometer (neoLab-Handrefraktometer Universal; 10–80 % Brix). Small amounts of nectar (mostly highly concentrated and therefore highly viscous as in all species of group I) was pipetted into 1 µL distilled water on the refractometer for measurements and the concentration was calculated for the original amount.

Nectar from 607 flowers (15.97 flowers mean per species, 14.23 s.d.) from 31 species (including three subspecies, 37 accessions in total) of seven genera (*Aosa*, *Blumenbachia*, *Caiophora*, *Loasa*, *Nasa*, *Presliophytum* and *Xylopodia*) was analysed, including multiple accessions of heterogeneous species complexes such as the *Caiophora cirsifolia*- and *C. carduifolia*-aggregates. A complete list of the accessions used for the nectar analysis including all authors of plant names is given in the Appendix. Multiple accessions of individual species are differentiated by Arabic numerals in brackets behind the species and name throughout the text and in the appendix. Nectar data were all taken during the first half of the staminate phase to ensure that the data are comparable. Sugar concentration (%) and nectar volume (µL) were measured and total sugar production (mg) calculated for the individual flowers. Mean values and standard deviations were calculated for all flowers of one accession. To visualize the correlation between the three data sets (total amount of nectar, total amount of sugar and sugar concentration) the percentage of each value (mean value) was calculated relative to the total amount of nectar data (µL nectar + % sugar + mg sugar = 100 %) and these data then plotted, with Sigmaplot (for windows vers. 8.0, SPSS Inc. 2002) in a ternary plot. This plot is here favoured over a two-dimensional plot, since it pulls the

TABLE 1. Nectar parameters (means \pm s.d.), pollinators, morphological pattern and elevational distribution of Loasaceae subfam. Loasoideae from South America

Group	Species	No. (in Fig. 1)	Elevation (m)	PL (mm)	Corolla shape	FT	Pollinator	n	Nectar amount (μ L)	Concentration (%)	Sugar amount (mg)
I	<i>A. rupestris</i>	1	0–2500 (*–3000)	5	Star-shaped	T	Co!	16	0.75 \pm 0.64	51.25 \pm 17.05	0.34 \pm 0.22
	<i>B. hieronymi</i>	2		12	Star-shaped	T	Co! + Le! + Be! + Hu!	36	0.91 \pm 0.70	72.95 \pm 13.03	0.68 \pm 0.57
	<i>B. insignis</i>	3		15	Star-shaped	T	Co! + Meg! + Hal!	7	0.34 \pm 0.19	66.76 \pm 18.35	0.24 \pm 0.18
	<i>C. nivalis</i> ³	4		11	Star-shaped	T	Be	15	0.71 \pm 0.51	40.18 \pm 7.57	0.26 \pm 0.16
	<i>L. gayana</i>	5		16	Star-shaped	T	Be	16	0.74 \pm 0.65	63.55 \pm 15.27	0.41 \pm 0.25
	<i>N. moroensis</i>	6		14	Star-shaped	T	Co!	17	1.70 \pm 0.99	57.23 \pm 13.83	0.99 \pm 0.63
	<i>N. picta</i> *	7		15	Star-shaped	T	Co! + Bo!	6	0.55 \pm 0.28	83.00 \pm 4.10	0.46 \pm 0.24
	<i>N. poissoniana</i>	8		14	Star-shaped	T	Co	16	1.47 \pm 0.82	66.51 \pm 10.21	0.95 \pm 0.54
	<i>N. triphylla</i> ssp. <i>flavipes</i>	9		15	Star-shaped	T	Co	7	2.14 \pm 1.45	51.40 \pm 22.05	1.03 \pm 0.70
	<i>N. triphylla</i> ssp. <i>triphylla</i>	10		18	Star-shaped	T	Co	6	0.67 \pm 0.15	68.62 \pm 12.24	0.45 \pm 0.13
	<i>N. triphylla</i> spec. nov. ined.	11		15	Star-shaped	T	Co	6	1.62 \pm 0.70	62.30 \pm 8.17	0.98 \pm 0.40
	<i>N. urens</i>	12		18	Star-shaped	T	Co!	9	3.66 \pm 2.03	51.13 \pm 4.07	1.87 \pm 1.02
	<i>N. vargasii</i>	13		16	Star-shaped	T	Co!	16	1.04 \pm 1.23	65.94 \pm 11.84	0.68 \pm 0.79
	<i>P. arequipensis</i>	14		16	Star-shaped	T	Le! + Xy! + Hu!	1	1.20	16.30	0.20
	<i>X. klaprothioides</i> *	15		11	Star-shaped	T	Co!	18	0.28 \pm 0.19	69.67 \pm 7.90	0.19 \pm 0.13
II	<i>N. dyeri</i> ssp. <i>australis</i>	16	0–1500	20	Star-shaped	T	Co!	11	9.88 \pm 6.04	39.77 \pm 9.45	3.85 \pm 2.17
	<i>L. sclaireifolia</i>	17		18	Saucer-shaped	T	Co	11	9.36 \pm 1.28	62.16 \pm 5.77	5.79 \pm 0.77
	<i>P. incanum</i>	18	0–2500	17	Star-shaped	T	Co! + Le! + Be! + Hu!	22	14.34 \pm 4.60	55.50 \pm 7.05	7.94 \pm 2.58
	<i>C. cirsiifolia</i> (1)	19	2500–3500	19	Saucer-shaped	T	Ce! + Bo!	7	11.43 \pm 7.34	65.71 \pm 3.15	7.35 \pm 4.31
	<i>C. cirsiifolia</i> (2)	20		21	Bowl-shaped	T	Ce!	6	17.33 \pm 11.31	58.58 \pm 6.18	10.27 \pm 7.02
	<i>C. cirsiifolia</i> (3)	21		17	Bowl-shaped	T	Ce! + Bo!	29	11.97 \pm 6.51	44.14 \pm 9.52	4.97 \pm 2.30
	<i>C. grandiflora</i> (1)	22		18	Balloon-shaped	T	Hu! + Bo!	10	11.41 \pm 7.98	44.00 \pm 22.93	4.70 \pm 3.00
	<i>C. grandiflora</i> (2)	23		18	Balloon-shaped	T	Hu! + Bo!	11	11.36 \pm 4.65	49.68 \pm 18.05	5.30 \pm 2.25
	<i>C. lateritia</i>	24		20	Bowl-shaped	F	Hu	5	9.70 \pm 5.37	63.40 \pm 2.38	6.09 \pm 3.28
	III	<i>L. acanthifolia</i>	25	500–1000 (–1500)	18	Bowl-shaped	T	Bo! + Co!	6	46.83 \pm 17.57	54.00 \pm 9.52
<i>N. dillonii</i>		26		29	Bell-shaped	F	Hu?	40	53.18 \pm 23.35	31.40 \pm 6.28	17.10 \pm 8.98
<i>N. olmosiana</i>		27		25	Balloon-shaped	F	?	65	54.17 \pm 21.38	29.38 \pm 5.89	15.88 \pm 7.12
<i>P. heucheraefolium</i>		28		25	Star-shaped	T	Xy	17	50.06 \pm 13.38	38.06 \pm 8.04	19.71 \pm 9.05
<i>C. canarinoides</i>		29	(**2000–) 3000–4000	40	Bell-shaped	F	Hu!	14	50.32 \pm 24.02	41.73 \pm 12.97	21.15 \pm 11.75
<i>C. carduifolia</i> (1)		30		21	Bowl-shaped	T	Hu!	11	69.59 \pm 35.21	34.14 \pm 14.46	24.96 \pm 20.09
<i>C. carduifolia</i> (2)		31		23	Bowl-shaped	T	Hu!	7	75.86 \pm 32.18	49.00 \pm 7.30	35.91 \pm 12.75
<i>C. chuquitensis</i>		32		22	Balloon-shaped	F	Hu!	6	101.83 \pm 28.47	32.47 \pm 9.74	31.62 \pm 8.79
<i>C. cirsiifolia</i> (4)		33		30	Saucer-shaped	T	Ce! + Bo!	19	42.53 \pm 14.12	44.47 \pm 10.14	19.02 \pm 7.37
<i>C. cf. superba</i>		34		26	Balloon-shaped	F	Ce!	19	51.03 \pm 24.32	29.91 \pm 9.67	14.08 \pm 6.00
<i>C. cf. madrequisa</i>		35		18	Bell-shaped	F	Hu	8	45.44 \pm 22.03	41.19 \pm 8.62	17.90 \pm 7.56
<i>N. macrothyrsa</i> **		36		32	Star-shaped	T	Xy! + Co!	64	75.13 \pm 28.98	32.39 \pm 10.61	24.04 \pm 11.51
<i>N. speciosa</i> ²		37		55	Bell-shaped	F	Hu!	8	36.00	18.83	6.78
IV	<i>C. coronata</i> ¹	38	3500–4500	30	Bowl-shaped	F	Ma! + Be! + Hu! + Pa!		79.90 \pm 39.65	14.88	11.90
	<i>C. pentlandii</i> (1)	39		30	Bowl-shaped	F	Ma?	18	87.39 \pm 25.53	9.78 \pm 2.05	8.61 \pm 3.23
	<i>C. pentlandii</i> (2)	40		30	Bowl-shaped	F	Ma?	9	79.78 \pm 54.21	12.90 \pm 3.00	10.19 \pm 6.66

PL, petal length; FT, floral type (see also Fig. 3); n, number of investigated flowers. Groups: I, short-tongued bee-pollinated; II, long-tongued bee and hummingbird-pollinated; III, hummingbird-pollinated; IV, mammal-pollinated. Genus names: A., *Aosa*; B., *Blumenbachia*; C., *Caiophora*; L., *Loasa*; N., *Nasa*; P., *Presliophytum*; X., *Xylocopa*. Floral type: T, tilt-revolver flower *sensu* Weigend and Gottschling (2006); F, funnel-revolver flowers *sensu* Weigend (2004b). Pollinator: Be, bee; Bo, *Bombus*; Ce, *Centris*; Co, colletids; Hal, Halictidae; Hu, Hummingbird; Le, Lepidoptera; Ma, Mammals; Meg, Megachilidae; Pa, Passerines; Xy, *Xylocopa*. !, direct observation in the field; abbreviation + ?, doubtful; ?, unknown. Literature data: ¹, Cocucci and Sársic (1998); ², Stiles and Freeman (1993); ³, unpublished data from A. Wertlen, 2003. Asterisks next to species correspond to those next to elevation.

individual data sets apart much more clearly and is thus better suited to illustrate the divergence of nectar characteristics. Two data sets published elsewhere were included in the ternary plot (*Nasa speciosa* = as '*Loasa spectabilis*', Stiles and Freeman, 1993; *Caiophora coronata*, Cocucci and Sársic, 1998) and also the unpublished data set of *Caiophora nivalis*, analysed in

Argentina by Anna Wertlen (Institut für Biologie, Neurobiologie, Freie Universität Berlin, Germany).

Pollinator observations

Qualitative data on flower visitors were obtained in Peru and Ecuador (see Table 1 for observations and

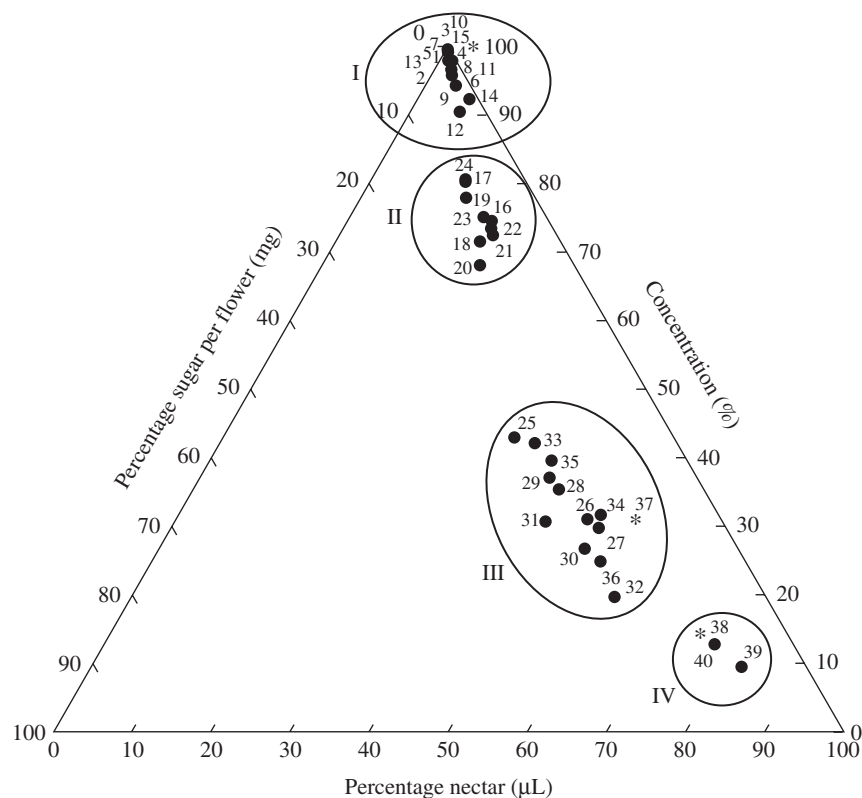


FIG. 1. Ternary plot illustrating the relationships between nectar production (NP), sugar concentration (SC) and sugar production (SP) of some species of Loasaceae subfam. Loasoideae (data set from Table 1 is plotted in percentages, numbers and groups I–IV in diagram correspond to numbering and grouping in Table 1); e.g. *Blumenbachia hieronymi*, no. 2 in Table 1: original data: 0.91 NP + 72.95 SC + 0.68 SP = 100 %, thus calculated percentages = 1.22 % (NP) + 97.87 % (SC) + 0.91 % (SP). Four groups are recognizable: (I) SC high, NP and SP low; (II) SC, NP and SP between groups I and III; (III) SC lower than in group II, NP and SP high; (IV) SC very low, NP high and SP lower than in group III. *, published data from Cocucci and Sérsic (1998), no. 38; Stiles and Freeman (1993), no. 37; and unpublished data from A. Wertlen (2003), no. 4.

Appendix for dates and localities), observation times typically ranged from 60 to 90 min per species and location and were performed in clear weather only (typically between 0900–1200 h and 1600–1800 h). Hymenopteran flower visitors were captured and determined by D. Wittmann (Institut für Landwirtschaftliche Zoologie und Bienenkunde der Universität Bonn, Germany) and C. Schlindwein (Universidade Federal de Pernambuco, Departamento de Botânica, Brazil), where the insects are also deposited. Determination to species was usually not possible, but the taxonomy of the visitors is given to family rank. Hummingbird observations were noted in the field book as means of documentation without identification to species.

Correlates of nectar production

Floral morphology, elevational distribution and pollinator observations are summarized in Table 1 to permit a direct comparison of nectar composition to the other data sets. Figure 2 provides a consensus diagram of Loasaceae subfam. Loasoideae based on various published phylogenies (Weigend *et al.*, 2004; Hufford *et al.*, 2005; Weigend and Gottschling, 2006) where each species analysed is assigned to a 'nectar group' and gross floral morphology (based on Weigend, 2004b; Weigend and Gottschling, 2006).

RESULTS

Total nectar amount

Table 1 summarizes the data on nectar quantities, concentrations and sugar amounts. The amounts of nectar secreted per flower range from 0.3 to 100 µL, the concentrations from 10–83 % and the total amounts of sugar provided per flower from 0.19 to 36 mg. These widely variable data can be roughly grouped into four classes (correlation of the data visualized in Fig. 1 in the form of a ternary plot). The amounts of nectar are discontinuously distributed and measurements mostly fall into the following ranges: group I, 0.3–1.5(–3.5) µL (1.19 µL mean, 0.87 s.d.); group II, 9–14(–17) µL (11.86 µL mean, 2.54 s.d.); groups III and IV, 40–100 µL (III, 57.84 µL mean, 17.99 s.d.; IV, 82.36 µL mean, 4.36 s.d.). Roughly the same groups are retrieved from sugar concentration [group I, (40–)60–80 % (59.12 % mean, 15.89 s.d.); group II, 40–60(–65) % (53.66 % mean, 9.57 s.d.)] but those taxa with 50–100 µL of nectar per flower fall into two subgroups with widely different concentrations [group III, 30–40(–55) % (36.69 % mean, 9.35 s.d.); group IV, 10–15 % (12.52 % mean, 2.57 s.d.)]. These patterns are reflected in the overall amount of sugar offered by the flowers, which falls into group I, 0.19–2 mg (0.65 mg mean, 0.46 s.d.); group II, 4–10 mg (6.25 mg mean,

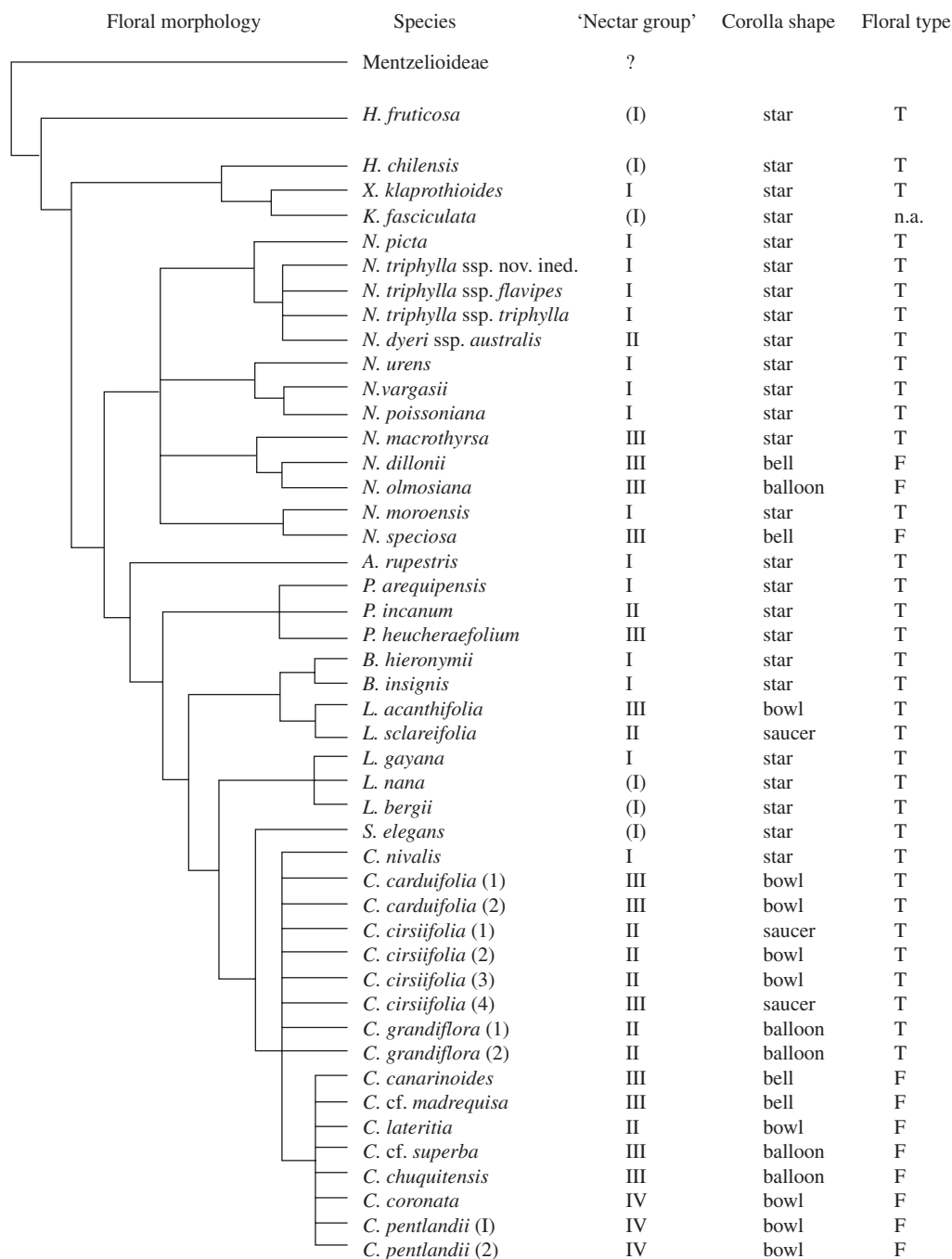


FIG. 2. Consensus phylogeny of Loasaceae subfam. Loasoideae (based on Weigend *et al.*, 2004; Hufford *et al.*, 2005; Weigend and Gottschling, 2006) with nectar groups, corolla shape (corresponding to Table 1 and Fig. 3) and flower type (T, tilt-revolver flowers, F, funnel-revolver flowers; see also Table 1). Clades with <50 bs in these three studies collapsed. Genus names: *A.*, *Aosa*; *B.*, *Blumenbachia*; *C.*, *Caiophora*; *H.*, *Huidobria*; *K.*, *Klaprothia*; *L.*, *Loasa*; *N.*, *Nasa*; *P.*, *Presliophytum*; *S.*, *Scyphanthus*; *X.*, *Xylopodia*. Nectar groups I–IV (see Table 1 and Fig. 1): ?, many *Mentzelia* species with pollen flowers and no nectar, representative data on nectariferous taxa not available; (I), tiny amounts of highly viscose nectar observed, but no measurements available. n.a., not applicable.

1.97 s.d.); group III, 14–36 mg (22.23 mg mean, 6.51 s.d.); and group IV, 8.5–12 mg (9.40 mg mean, 1.12 s.d.).

Altitudinal distribution, floral morphology and pollinators

The four nectar groups retrieved from nectar data roughly correspond to the morphological and ecological

data. The corolla shapes as here defined are illustrated in Fig. 3. Floral display depends on both flower shape and petal length (Fig. 3 and Table 1; mean value and s.d. for petal lengths: group I, 14.07 ± 3.28 ; group II, 18.67 ± 1.41 ; group III, 28.00 ± 10.11 ; group IV, 30.00). Bowl-shaped flowers have a much larger floral display than star-shaped flowers with the same petal length. Petal

size in combination with corolla shape is therefore here used as a proxy for display size. In general terms there is more nectar in (a) more closed flower types (versus more open), and flowers with (b) larger (versus smaller) petals. Also, highly concentrated nectar in small amounts (group I) is found only in tilt-revolver flowers and very dilute nectar in huge amounts (group IV) only in funnel-revolver flowers. Group II nectar is more often found in tilt-revolver flowers than in funnel-revolver flowers (six versus three taxa) and group III nectar is found roughly as often in tilt-revolver as in funnel-revolver flowers (six versus seven taxa).

Group I: mainly low-elevation plants (mostly <2500 m) with relatively small, typically white, star-shaped flowers [petals approx. (5–)12–18 mm long; Table 1 and Fig. 3A–D]. Flowers of this group are predominantly visited and pollinated by short-tongued bees, mostly colletid bees. Only *Nasa picta* and *Xylopodia klaprothioides* range into higher elevations.

Group II: mid-elevation plants (2500–3500 m) often with larger, more closed, mostly orange or red flowers (petals approx. 17–21 mm long; Table 1 and Fig. 3E, F, bell-, balloon-, saucer- or bowl-shaped). Both long-tongued bees and hummingbirds have been documented as flower visitors of that group. The only taxa which are aberrant in pollinator visitor (colletid bees) and elevational distribution (0–1500 m) for this group are *Loasa sclareifolia*, *N. dyeri* ssp. *australis* and *Presliophytum incanum*.

Group III: mostly high elevation plants (3000–4000 m) and a few species from low elevations (500–1500 m), with some of the largest flowers in the subfamily, flowers are largely closed and orange, red or rarely yellow (petals up to 55 mm long; Table 1 and Fig. 3G–I, L, bell-, bowl- or balloon-shaped). Only exceptions are *Nasa macrothyrsa* and *Presliophytum heucheraefolium* from lower elevations with white, star-shaped flowers. Hummingbirds are likely to be the most important flower visitors of this group, but long-tongued bees (*Centris*, *Bombus* and *Xylocopa*) have also been observed, often on the same plant species.

Group IV: only two decumbent high elevation taxa (>3500 m) with large petals (approx. 30 mm long; Table 1 and Fig. 3J, K), either white or orange-red bowl-shaped flowers (similar to types also found in group III). While it has not been possible to document flower visitors in the field, there is one publication indicating that small rodents may be the principal pollinators for one of the two taxa (*C. coronata*; Cocucci and S ersic, 1998).

DISCUSSION

Overall nectar and sugar production in relation to pollination syndrome

Sugar concentrations here reported are generally higher than most literature data (both for bee- and hummingbird-pollinated flowers; Baker, 1975; Bolten and Feinsinger, 1978; Bolten *et al.*, 1979; Pyke and Waser, 1981; Cruden *et al.*, 1983; Heyneman, 1983; Forcone *et al.*, 1997; Galetto *et al.*, 1998; Bernardello *et al.*, 2000; Blem *et al.*,

2000; Chalcoff *et al.*, 2006; Wilson *et al.*, 2006) and this may be an idiosyncratic phenomenon of Loasaceae. Group III may be predominantly hummingbird-pollinated, but the hummingbird-pollinated taxa studied here have unusually high sugar concentrations in the nectar (Table 1 and Fig. 1, nos 29, 31, 33 and 35, 30–55% as compared with the ‘typical’ 20–26%; Baker, 1975; Cruden *et al.*, 1983). Heinrich and Raven (1972) and Forcone *et al.* (1997) argue that ‘highland hummingbird nectar’ should be less viscous and less concentrated, but this is apparently not true in Loasaceae. Higher than typical sugar concentrations in flowers pollinated by hummingbirds have also been found for hummingbird nectar by Kaczorowski *et al.* (2005) in *Nicotiana* sect. *Alatae*. This might be due to the high Andean habitat and the therefore high energy requirements of the birds: Heinrich and Raven (1972) and Forcone *et al.* (1997) argue that, in low temperatures, energetic rewards for hummingbirds must be higher than in high temperatures. Higher concentrations in this group of taxa may also be due to the fact that the flowers of at least some of the taxa concerned [e.g. *Caiophora* cf. *superba*, *C. cirsiifolia* (4), *Loasa acanthifolia*, *Nasa macrothyrsa* and *Presliophytum heucheraefolium*] are often also visited by long-tongued bees and there may be no reason for the plant to exclude them as flower visitors (Bolten and Feinsinger, 1978). Interestingly, there is a single data set from an ornithophilous species of *Nasa* from Costa Rica (*N. speciosa*; Stiles and Freeman, 1993) which has the typical, relatively low sugar concentration of hummingbird nectar (Heinrich and Raven, 1972; Forcone *et al.*, 1997).

Pollination, nectar and elevation

Comparing the four groups defined above, it becomes apparent that there is a trend towards higher nectar volume and higher total amount of sugar per flower at increasing elevations, i.e. bird- and mammal-pollinated taxa are largely high-Andean (groups III and IV), whereas the taxa pollinated by short-tongued bees are found at low and intermediate elevations (group I). At elevations above approx. 3500 m only the two genera *Nasa* and *Caiophora* are present in the Andes, and both with species where hummingbird pollination predominates among the taxa.

Evolution of nectar characteristics and pollination syndromes

Figure 2 shows a phylogeny of Loasoideae together with the assignment of terminal taxa to nectar group and gross floral morphology. It appears that group I nectar represents the plesiomorphic condition and this agrees with the previously published hypothesis that pollination by short short-tongued bees (especially colletid bees) is the plesiomorphic condition in the subfamily (Weigend *et al.*, 2004; Weigend and Gottschling, 2006). Evolution towards higher amounts of more dilute nectar appears to have happened several times: (a) at least twice in *Nasa* (in the *Nasa triphylla* group and at least once in the

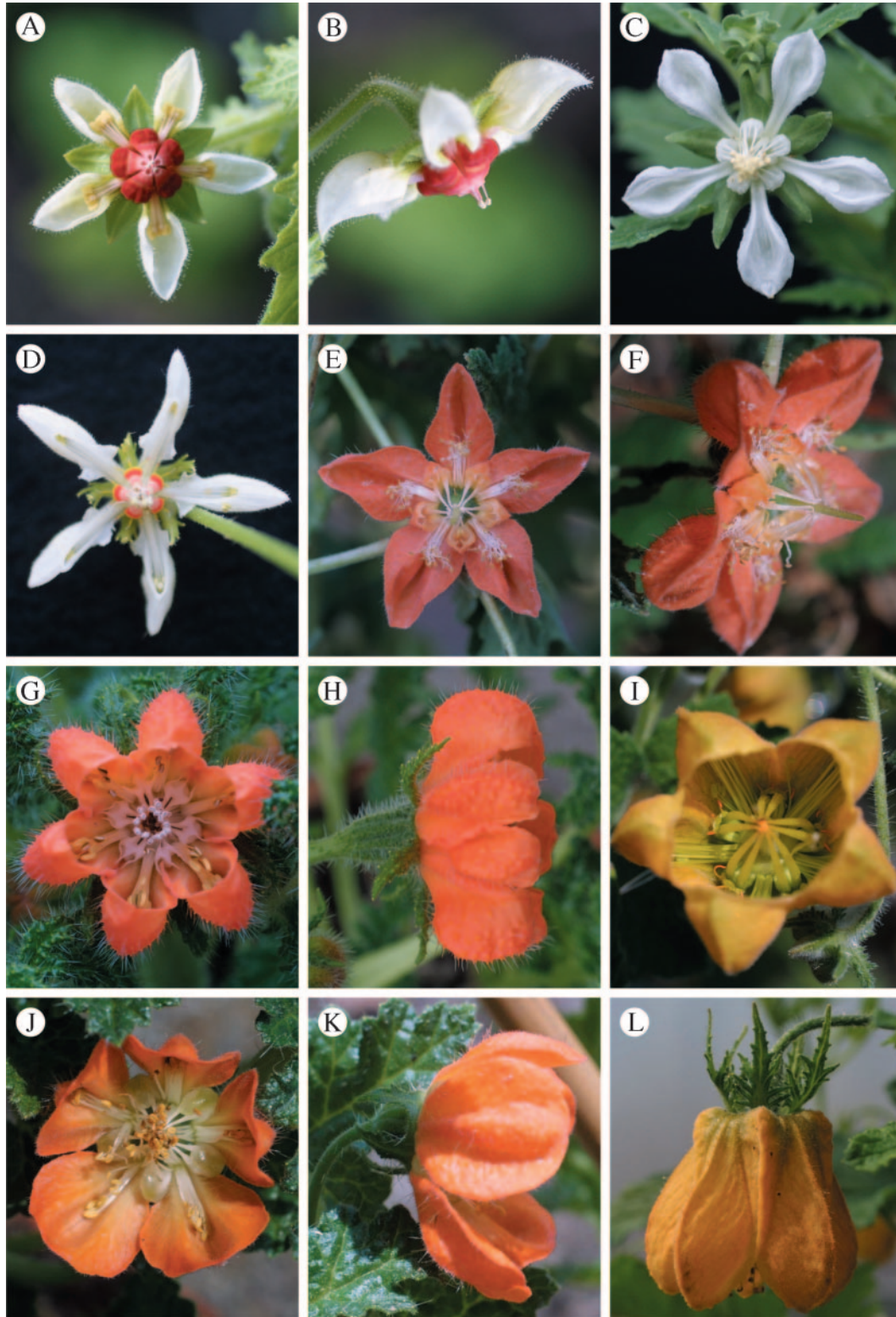


FIG. 3. Flower morphology in Loasaceae subfam. Loasoideae. (A–F) Tilt-revolver flowers: (A–D) star-shaped flowers (A, B, *Nasa moroensis*; C, *Presliophytum incanum*; D, *Blumenbachia insignis*); (E, F) saucer-shaped flower of *Caiophora cirsiifolia* (1). (G–L) Funnel-revolver flowers (G, H, balloon-shaped-flower of *Caiophora chuquitensis*; I, L, bell-shaped flower of *Caiophora canarinoides*; J, K, bowl-shaped flower of *Caiophora pentlandii*).

N. macrothyrsa–*N. speciosa* clade); (b) in *Presliophytum*; (c) in the *Blumenbachia*–*Loasa acanthifolia* clade; and (d) at least once in the *Loasa gayana*–*Caiophora* clade.

The transitions towards more dilute nectar took place without a transition towards funnel-revolver flowers in the *Blumenbachia*–*Loasa acanthifolia* clade and *Presliophytum*. It seems to be phylogenetically correlated with the transition from tilt-revolver flowers to funnel-revolver flowers in the *Loasa gayana*–*Caiophora* clade and in the *Nasa macrothyrsa*–*N. speciosa* clade. The nectar and pollinator data here presented show that the repeated morphological transformations of Loasoideae flowers from small, bee-pollinated flowers to large, bird-pollinated flowers (Weigend *et al.*, 2004; Weigend and Gottschling, 2006; see also corolla shapes and petal lengths in Table 1) were likely preceded by changes in nectar composition. The evolution of a different nectar type ('nectar group') as a means of recruiting different pollinators seems to be a rapid process in relative terms. There are considerable differences in nectar production between closely allied taxa with morphologically very similar flowers (*Nasa triphylla* group, *C. carduiifolia* complex, *C. cirsiifolia* complex). Vastly different forms of nectar production can apparently evolve with relative ease and nectar production (in terms of both absolute amounts and concentration) appears to evolve more rapidly than functional floral morphology in Loasaceae subfam. Loasoideae.

ACKNOWLEDGEMENTS

We would like to express our sincere gratitude to M. Achatz, P. Beckers, G. Brokamp, N. Cusimano, G. Fröhlich, S. Grossmann, T. Henning, P. Kramer, B. Nordt, N. Nürk, N. Poser, E. Scherer, C. Schneider, C. Schwarzer and A. Tais (Berlin, Germany) for obtaining nectar from flowers and measuring sugar concentration. We would like to thank Natalie Cusimano (Berlin, Germany) for help with calculating the ternary graphs with Sigma Plot, Anna Flüge (Berlin, Germany) for the data set from *Caiophora nivalis*, as well as Natalie Hempel de Ibarra (Berlin, Germany) for methodological advice and helpful discussions. We thank A. Cano and M. I. La Torre (Lima, Peru), Eric Rodriguez (Trujillo, Peru), N. Salinas (Cuzco, Peru), G. Vobis and C. Ezcurra (Bariloche, Argentina), H. Förther (München, Germany), N. Dostert, T. Henning, D. Kollehn, O. Mohr, C. Schwarzer and K. Weigend (Berlin, Germany) for help in the field and collecting seeds. We thank D. Wittmann (Institut für Landwirtschaftliche Zoologie und Bienenkunde der Universität Bonn, Germany) and C. Schlindwein (Universidade Federal de Pernambuco, Departamento de Botânica, Brazil) for identifying the captured hymenopteran pollinators. The funds kindly provided by the following institutions at various stages of the project are gratefully acknowledged: Studienstiftung des Deutschen Volkes (1992–1997), Deutscher Akademischer Austauschdienst (1999–2000), Lewis B. and Dorothy Cullman Laboratory for Molecular Systematics Studies

at the New York Botanical Garden (1999–2000), Deutsche Forschungsgemeinschaft (Grant-nr. WE 2330/1, 2001–2003), botconsult GmbH (1999–present).

LITERATURE CITED

- Arroyo M, Primack R, Armesto J. 1982. Community studies in pollination ecology in the high temperate Andes of Central Chile. I. Pollination mechanisms and altitudinal variation. *American Journal of Botany* 69: 82–97.
- Baker HG. 1975. Sugar concentrations in nectars from hummingbird flowers. *Biotropica* 7: 37–41.
- Baker HG, Baker I. 1982. Chemical constituents of nectar in relation to pollination mechanisms and phylogeny. In: Nitecki HM, ed. *Biochemical aspects of evolutionary biology*. Chicago, IL: Chicago University Press, 131–171.
- Baker HG, Baker I. 1983. Floral nectar sugar constituents in relation to pollinator type. In: Jones CE, Little RJ, eds. *Handbook of experimental pollination biology*. New York, NY: Van Nostrand Reinhold, 117–141.
- Beardsley PM, Yen A, Olmstead RO. 2003. AFLP phylogeny of *Mimulus* section *Erythranthe* and the evolution of hummingbird pollination. *Evolution* 57: 1397–1410.
- Bernardello G, Galetto L, Anderson GJ. 2000. Floral nectary structure and nectar chemical composition of some species from Robinson Crusoe Island (Chile). *Canadian Journal of Botany* 78: 862–872.
- Bertsch A. 1983. Nectar production of *Epilobium angustifolium* L. at different air humidities: nectar sugar in individual flowers and the optimal foraging theory. *Oecologia* 59: 40–48.
- Blem CR, Blem LB, Felix J, van Gelder J. 2000. Rufous hummingbird sucrose preference: precision of selection varies with concentration. *Condor* 102: 235–238.
- Bolten AB, Feinsinger P. 1978. Why do hummingbird flowers secrete dilute nectar? *Biotropica* 10: 307–308.
- Bolten AB, Feinsinger P, Baker HG, Baker I. 1979. On the calculation of sugar concentration in flower nectar. *Oecologia* 41: 301–304.
- Brown DK, Kaul RB. 1981. Floral structure and mechanism in Loasaceae. *American Journal of Botany* 68: 361–372.
- Carroll AB, Pallardy SG, Galen C. 2001. Drought stress, plant water status, and floral trait expression in fireweed, *Epilobium angustifolium* (Onagraceae). *American Journal of Botany* 88: 438–446.
- Chalcoff VR, Aizen MA, Galetto L. 2006. Nectar concentration and composition of 26 species from the temperate forest of South America. *Annals of Botany* 97: 413–421.
- Cocucci AA, Sérsc AN. 1998. Evidence of rodent pollination in *Cajophora coronata* (Loasaceae). *Plant Systematics and Evolution* 211: 113–128.
- Corbet SA, Unwin DM, Prys-Jones OE. 1979a. Humidity, nectar and insect visits to flowers, with special reference to *Crataegus*, *Tilia* and *Echium*. *Ecological Entomology* 4: 9–22.
- Corbet SA, Willmer PG, Beament JWL, Unwin DM, Prys-Jones OE. 1979b. Post-secretory determinants of sugar concentration in nectar. *Plant, Cell and Environment* 2: 293–308.
- Cruden RW, Herman SM, Petterson S. 1983. Patterns of nectar production and plant–pollination coevolution. In: Bentley B, Elias T, eds. *The biology of nectaries*. New York, NY: Columbia University Press, 80–125.
- Dostert N, Weigend M. 1999. A synopsis of the *Nasa triphylla* complex (Loasaceae), including some new species and subspecies. *Harvard Papers in Botany* 4: 439–467.
- Elisens WJ, Freeman CE. 1988. Floral nectar sugar composition and pollinator type among New World genera in tribe Antirrhineae (Scrophulariaceae). *American Journal of Botany* 75: 971–978.
- Endress PK. 1994. *Diversity and evolutionary biology of tropical flowers*. Cambridge: Cambridge University Press.
- Fishman L, Kelly AJ, Willis JH. 2002. Minor quantitative trait loci underlie floral traits associated with mating system divergence in *Mimulus*. *Evolution* 56: 2138–2155.
- Forcone A, Galetto L, Bernardello G. 1997. Floral nectar chemical composition of some species from Patagonia. *Biochemical Systematics and Ecology* 25: 395–402.

- Galetto L, Bernardello G, Sosa CA. 1998. The relationship between floral nectar composition and visitors in *Lycium* (Solanaceae) from Argentina and Chile: what does it reflect? *Flora* **193**: 303–314.
- Ghebrehiwet M, Bremer B, Thulin M. 2000. Phylogeny of the tribe Antirrhineae (Scrophulariaceae) based on morphological and ndhF sequence data. *Plant Systematics and Evolution* **220**: 223–239.
- Hainsworth FR, Wolf LL. 1972. Energetics of nectar extraction in small, high altitude, tropical hummingbird, *Selasphorus flammula*. *Journal of Comparative Physiology* **80**: 377–387.
- Harter B. 1995. *Blütenökologie einiger von Bienen und Kolibris bestäubter Cajophora-Arten (Loasaceae)*. Unpublished thesis, University of Tübingen, Germany.
- Harter B, Schlindwein C, Wittmann D. 1995. Bienen und Kolibris als Bestäuber von Blüten der Gattung *Cajophora* (Loasaceae). *Apidologie* **26**: 356–357.
- Heinrich B, Raven PH. 1972. Energetics and pollination ecology. *Science* **176**: 597–602.
- Heyneman AJ. 1983. Optimal sugar concentration of floral nectars—dependence on sugar intake efficiency and foraging costs. *Oecologia* **60**: 198–213.
- Hufford L, McMahon MM, Sherwood AM, Reeves G, Chase MW. 2003. The major clades of Loasaceae: phylogenetic analysis using the plastid matK and trnL-trnF regions. *American Journal of Botany* **90**: 1215–1228.
- Hufford L, McMahon MM, O'Quinn R, Poston MS. 2005. A phylogenetic analysis of Loasaceae subfamily Loasoideae based on plastid DNA sequences. *International Journal of Plant Sciences* **166**: 289–300.
- Kaczorowski RL, Gardener MC, Holtsford TP. 2005. Nectar traits in *Nicotiana* section Alatae (Solanaceae) in relation to floral traits, pollinators, and mating system. *American Journal of Botany* **92**: 1270–1283.
- Keeler KH. 1981. The nectaries of *Mentzelia nuda*: from pollinator attraction to seed protection. *American Journal of Botany* **68**: 295–299.
- Linsley EG, Hurd PD. 1959. Ethological observations on some bees of southeastern Arizona and New Mexico. *Entomology News* **70**: 63–68.
- McDade LA, Weeks JA. 2004. Nectar in hummingbird-pollinated neotropical plants. I. Patterns of production and variability in 12 species. *Biotropica* **36**: 196–215.
- Medan D, Montaldo NH, Devoto M, Mantese A, Vasellati V, Roitman GG, et al. 2002. Plant–pollinator relationships at two altitudes in the Andes of Mendoza, Argentina. *Arctic, Antarctic and Alpine Research* **34**: 233–241.
- Pacini E, Nepi M, Vesprini JL. 2003. Nectar biodiversity: a short review. *Systematics and Evolution* **238**: 7–21.
- Perret M, Chautems A, Spichiger R, Peixoto M, Savolainen V. 2001. Nectar sugar composition related to pollination syndromes in Sinningieae (Gesneriaceae). *Annals of Botany* **87**: 267–273.
- Perret M, Chautems A, Spichiger R, Kite G, Savolainen V. 2003. Systematics and evolution of tribe Sinningieae (Gesneriaceae): evidence from phylogenetic analyses of six plastid DNA regions and nuclear NCPGS1. *American Journal of Botany* **90**: 445–460.
- Plowright RC. 1981. Nectar production in the boreal forest lily *Clintonia borealis*. *Canadian Journal of Botany* **59**: 156–160.
- Pyke GH, Waser NM. 1981. The production of diluted nectar by hummingbird and honeyeater flowers. *Biotropica* **13**: 260–270.
- Rodríguez E, Weigend M. 1999. *Nasa umbraculifera* (Loasaceae: Loasoideae), una nueva especie con hojas peltadas para el Perú. *Arnaldia* **6**: 49–56.
- Sargent RD, Otto SP. 2004. A phylogenetic analysis of pollination mode and the evolution of dichogamy in angiosperms. *Evolutionary Ecology Research* **6**: 1183–1199.
- Schlindwein C. 1995. *Wildbienen und ihre Trachtpflanzen in einer südbrasilianischen Buschlandschaft: Fallstudie Guaritas, Bestäubung bei Kakteen und Loasaceen*. Eberhard-Karls-Universität Tübingen, Fakultät für Biologie. Ulrich E. Grauer Verlag, Tübingen, Germany.
- Schlindwein C. 2000. Verhaltensanpassungen oligolektischer Bienen an synchrone und an kontinuierliche Pollenpräsentation. In: Breckle SW, Schweizer B, Arndt U, eds. *Ergebnisse weltweiter ökologischer Forschung*. Stuttgart, Germany: Verlag Günter Heimbach, 235–250.
- Schlindwein C, Wittmann D. 1997. Micro-foraging routes of *Bicolletes pampeana* (Colletidae) and bee-induced pollen presentation in *Cajophora arechavaletae* (Loasaceae). *Botanica Acta* **110**: 177–183.
- Stiles FG, Freeman CE. 1993. Patterns in floral nectar characteristics of some bird-visited plant species from Costa Rica. *Biotropica* **25**: 191–205.
- Thompson HJ, Ernst WR. 1967. Floral biology and systematics of *Eucnide* (Loasaceae). *Journal of the Arnold Arboretum* **48**: 56–88.
- Troncoso AJ, Vargas RR. 2004. Efecto del vecindario floral sobre la tasa de visitas por insectos a *Loasa triloba* Domb. ex A.J. Juss. y *Loasa tricolor* Ker-Gawl en la Reserva Nacional de Río Clarillo, Región Metropolitana, Chile. *Chloris Chilensis* **7** (1). <http://www.chlorischile.cl/loasa/Loasaalejandra.htm> (24 March 2006).
- Urban I. 1886. Die Bestäubungseinrichtungen der Loasaceen. *Jahrbuch des Botanischen Gartens zu Berlin* **4**: 364–388.
- Urban I. 1889. Loasaceae. In: Martius CFP, ed. *Flora Brasiliensis* **13** (3). München, Germany: Verlag Beck F, Fleischer F, 205–222.
- Urban I. 1892. Blüten- und Fruchtbau der Loasaceen. *Berichte der Deutschen Botanischen Gesellschaft* **10**: 259–265.
- Urban I, Gilg W. 1900. *Monographia Loasacearum. Nova Acta Academiae Caesareae Leopoldino-Carolinae Germanicae Naturae Curiosorum* **76**: 1–368.
- Villagrán C, Romo M, Castro V. 2003. Etnobotánica del Sur de los Andes de la Primera Región de Chile: un Enlace entre las Culturas Altiplánicas y las de Quebradas Altas del Loa Superior. *Chungara Revista de Antropología Chilena* **35**: 73–124.
- Weigend M. 2000a. A revision of the Peruvian species of *Nasa* ser. Alatae in Peru. *Nordic Journal of Botany* **20**: 15–32.
- Weigend M. 2000b. Loasaceae. In: Andersson L, Harling G, eds. *Flora of Ecuador* **132**. Stockholm, Sweden: University of Göteborg and the Section for Botany, Riksmuseum, 1–92.
- Weigend M. 2001. Loasaceae. In: Bernal R, Forero E, eds. *Flora de Colombia* **22**. Santa Fé de Bogotá: Instituto de Ciencias Naturales, 1–100.
- Weigend M. 2002. Observations on the biogeography of the Amotape-Huancabamba Zone in northern Peru. In: Young K, Ulloa UC, Luteyn JL, Knapp S, eds. *Plant Evolution and Endemism in Andean South America—Botanical Review* **68**: 38–54.
- Weigend M. 2004a. Loasaceae. In: Kubitzki K, Bayer C, eds. *The families and genera of the vascular plants*. Vol. 6. Köln, Germany: Springer Verlag, 239–254.
- Weigend M. 2004b. Four new species of *Nasa* ser. Alatae (Loasaceae) in the Amotape-Huancabamba Zone of Peru. *Novon* **14**: 134–146.
- Weigend M. 2004c. Additional observations on the biogeography of the Amotape-Huancabamba Zone in Northern Peru—defining the south-eastern limits. *Revista Peruana de Biología* **11**: 127–134.
- Weigend M, Ackermann M. 2003. Los nombres antiguos en el género *Cajophora* (Loasáceas subfam. Loasoídeas) y una clasificación infragenérica preliminar. *Arnaldia* **10**: 75–94.
- Weigend M, Gottschling M. 2006. Evolution of funnel-revolver flowers and ornithophily in *Nasa* (Loasaceae). *Plant Biology* **8**: 120–142.
- Weigend M, Rodríguez E. 2002. Las especies arbustivas de *Nasa* Ser. Grandiflorae en el Norte de Perú, con la descripción de una especie nueva de la Abra de Barro Negro (Callacalla). *Arnaldia* **9**: 7–20.
- Weigend M, Rodríguez E. 2003. A revision of the the *Nasa stuebeliana* group [*Nasa* ser. Saccatae (Urb. and Gilg) Weigend, Loasaceae] with notes on morphology, ecology, and distribution. *Botanische Jahrbücher für Systematik, Pflanzengeschichte und Pflanzengeographie* **124**: 345–382.
- Weigend M, Henning T, Schneider C. 2003. Notes on the systematics, morphology, distribution and pollination of *Nasa* Ser. Carunculatae (Loasaceae subfam. Loasoideae). *Systematic Botany* **29**: 765–781.
- Weigend M, Gottschling M, Hoot S, Ackermann M. 2004. A preliminary phylogeny of Loasaceae subfam. Loasoideae (Angiospermae: Cornales) based on trnL(UAA) sequence data and its relation to systematics and historical biogeography. *Organisms, Diversity and Evolution* **4**: 73–90.
- Weigend M, Rodríguez E, Dostert N. 1998. *Nasa insignis* y *Nasa glandulosissima*, dos especies nuevas de *Nasa* con hojas peltadas. *Arnaldia* **5**: 151–157.

- Wilson P, Castellanos MC, Wolfe A, Thomson JD. 2006.** Shifts between bee- and bird-pollination among *Penstemon*. In: Waser N, Ollerton J, eds. *Plant-pollinator interactions: from specialization to generalization*. Chicago, IL: University of Chicago Press, 47–68. www.csun.edu/biology/grad/faculty/wilsonpdfs/chapter.pdf (24 March 2006).
- Wittmann D, Schlindwein C. 1995.** Melittophilous plants, their pollen and flower visiting bees in southern Brazil. 1. Loasaceae. *Biociências* 3 (2): 19–34.
- Zimmermann M. 1983.** Plant reproduction and optimal foraging: experimental nectar manipulations in *Delphinium nelsonii*. *Oikos* 41: 57–63.
- Zimmermann M. 1988.** Nectar production, flowering phenology, and strategies for pollination. In: Doust JI, Doust LL, eds. *Plant reproductive ecology, patterns and strategies*. Oxford: Oxford University Press, 157–178.

APPENDIX 1

Voucher data (multiple accessions of species are differentiated by Arabic numeral in brackets in list and throughout the text)

Aosa rupestris (Hook.) Weigend—cultivated from seeds collected in Bahía, Brazil by S. Vogel, Vienna, M. Weigend 7138 (B, M, BM).

Blumenbachia hieronymi Urb.—cultivated plants from Botanical Garden Berlin-Dahlem, 27 September 2004, M. Ackermann 601 (BSB).

Blumenbachia insignis Schrad.—cultivated plants from Botanical Garden Berlin-Dahlem, 27 September 2004, M. Weigend 7475 (BSB).

Caiophora canarinoides (Lenné & C.Koch) Urb. & Gilg—Peru, Depto. Puno, Prov. Sandía, road from Cuyocuyo passing Banos de Cuyocuyo, old Inca trail, 14°28'S, 69°32'W, 3550 m, 25 September 2002, M. Ackermann 395 (BSB, HUSA, M, USM).

Caiophora carduifolia C.Presl (1)—Peru, Depto. Apurímac, Prov. Andahuaylas, road from Abancay to Andahuaylas, 86 km, 13°41'S, 73°8'W, 3700 m 16 February 2000, M. and K. Weigend 2000/326 (HUSA, NY).

Caiophora carduifolia C.Presl (2)—Peru, Depto. Cuzco, Prov. Paucartambo, SE from Cuzco, from Saylla to Paucartambo, village of Huancarani, 13°30'S, 071°38'W, 3880 m, 17 September 2002, M. Ackermann & N. Salinas, 333 (BSB, HUSA, M, USM).

Caiophora carduifolia C.Presl (3)—Peru, Depto. Cuzco, Prov. Calca, road from Calca to Lares, after Rancal, 13°12'S, 71°56'W, 4310 m, 11 September 2002, M. Ackermann *et al.* 554 (BSB, HUSA, M).

Caiophora carduifolia C.Presl (4)—Peru, Depto. Cuzco, Prov. Urubamba, road from Urubamba to Quillabamba, between Ollantaytambo and Abra Malaga, 13°12'S, 72°17'W, approx. 3500 m, 12 September 2002, M. Ackermann and D. Kollehn 288 (BSB, HUSA, M, USM, NY, F).

Caiophora carduifolia C.Presl (5)—Peru, Depto. Cuzco, Prov. Cuzco, SE from Cuzco, from Saylla on small road to the ruins of Tipon, 13°34'S, 71°47'W, 3440 m, 17 September 2002, M. Ackermann and N. Salinas 329 (BSB, HUSA, M).

Caiophora carduifolia C.Presl (6)—Peru, Depto. Cuzco, Prov. Cuzco, road from San Jeronimo to Huacoto (small street to the east), fields near Huacoto, 13°30'S, 71°51'W, 4130 m, 13 September 2002, M. Ackermann and N. Salinas 296 (BSB, HUSA, M, USM, NY, F).

Caiophora cf. madrequisa Killip—Peru, Depto. Puno, Prov. Paucartambo, road from Paucartambo to Tres Cruces, Parque Nacional Manu, 13°10'S, 71°36'W, 3050 m, 18 September 2002, M. Ackermann 356 (BSB, HUSA, M, NY, USM).

Caiophora cf. superba Phil.—Peru, Depto. Moquegua, Prov. General Sanchez Cerro, between Puwina and Omate, last road bends before the descent to Omate, near Charijon, approx. 3000 m, 21 May 2003, M. Weigend *et al.* 7761 (BSB).

Caiophora chuquitensis (Meyen) Urb. & Gilg (1)—Peru, Depto. Cuzco, Prov. Calca, road from Calca to Lares, after Rancal, 13°10'S, 71°57'W, 4000 m, 11 September 2002, M. Ackermann *et al.* 274 (BSB, HUSA, M).

Caiophora cirsiifolia C.Presl (1)—Peru, Depto. Arequipa, Prov. Arequipa, environment of Chiquata, east from Arequipa, 16°24'S, 71°22'W, 3100 m, 1 October 2002, M. Ackermann *et al.* 420 (BSB, HUSA, M, USM, NY, F).

Caiophora cirsiifolia C.Presl (2)—Peru, Depto. Lima, Prov. Yauyos, Road from Yauyos to Jauja, after Tomas, 12°17'S, 75°48'W, approx. 2300 m, 7 October 2002, M. Weigend *et al.* 7260 (BSB, HUSA, USM, M, NY).

Caiophora cirsiifolia C.Presl (3)—Peru, Depto. Cajamarca, Prov. Santa Cruz, La Florida, above Montesecco, 1200–1500 m, 5 May 2003, M. Weigend *et al.* 7559 (BSB).

Caiophora cirsiifolia C.Presl (4)—Peru, Depto. Ancash, Prov. Huarez, Rio Grande/Rio Chaccan, towards Pariacoto, 18L 0200571 UTM 8942645, 2999m, 16 May 2003, M. Weigend *et al.* 7697 (BSB, USM).

Caiophora grandiflora (G.Don) Weigend & Mark. Ackermann (1)—Peru, Depto. Cajamarca, Prov. San Miguel, road San Miguel to Tongad (Sta. Rosa—Hualgayoc), 6°46'S, 78°38'W, 3986 m, 2 May 2003, M. Weigend *et al.* 7509 (BSB, USM).

Caiophora grandiflora (G.Don) Weigend & Mark. Ackermann (2)—Peru, Depto. Cajamarca, Prov. Hualgayoc, 6°48'S, 78°57'W, 3600 m, 2 May 2003, M. Weigend *et al.* 7510 (BSB, USM).

Caiophora lateritia Benth.—cultivated plants from Botanical Garden Berlin Dahlem, 1 August 2004, M. Ackermann 603 (BSB).

Caiophora nivalis Lillo, Argentina, Prov. Mendoza, Vallecitos. 2826 m, 32°58'S 69°21'W, 8–18 January 2003, A. A. Cocucci *et al.* 2219 (CORD).

Caiophora pentlandii (Paxton) G.Don ex Loudon (1)—Peru, Depto. Puno, Prov. Melgar, road from Sicuani to Nunoa, approx. 3 km before Nunoa, 14°31'S, 70°37'W, 4000 m, 20 September 2002, M. Ackermann 360 (BSB, F, HUSA, M, NY, USM).

Caiophora pentlandii (Paxton) G.Don ex Loudon (2)—Peru, Depto. Puno, Prov. Puno, road from Puno to Juliaca, Ruins of Sillustani, 15°43'S, 70°9'W, 3880 m, 23 September 2002, M. Ackermann 366 (BSB, F, HUSA, M, NY, USM).

Loasa acanthifolia Desr. var. *albomaculata* Gunckel—Argentina, Prov. Neuquen, Depto. Aluminé, road N of Lago Quillén towards Lago Hui Hui, 39°22'S, 71°14'W, 1050 m, 17 January 2002, M. Weigend *et al.* 6925 (BRCO, BSB, M).

Loasa gayana Urb. & Gilg—Chile, X. Región, Los Lagos, road entre Lagos and Osorno, 25 km E of Osorno, approx. 2 km N of road, entrance to Fundo Los Pellines, 40°35'S, 72°50'W, 132 m, 3 February 2002, M. Weigend *et al.* 7057 (B, M, NY).

Loasa sclareifolia Juss.- Chile, VIII Región del Bío Bío, Prov. de Ñuble, east of San Fabián de Alico, orig. collection J. Grau, July 2005, M. Weigend 8183 (BSB, M).

Nasa dillonii Weigend—Peru, Depto. Cajamarca, Prov. Santa Cruz: La Florida, above Montesecco, 1200–1500 m, 5 May 2003, M. Weigend *et al.* 7556 (B, USM).

Nasa dyeri (Urb. & Gilg) Weigend ssp. *australis* Dostert & Weigend—Peru, Depto. Amazonas, Prov. Bagua, trail from La

Peca to El Arenal, just above El Arenal, 1200 m, April 1998, N. Dostert 98/80 (M, USM).

Nasa macrothyrsa (Urb. & Gilg) Weigend—Peru, Depto. Cajamarca, Prov. San Miguel, one of the last road bends before San Miguel, 7°0'S, 78°51'W, 2517 m, 30 April 2003, M. Weigend *et al.* 7471 (BSB, HUT, USM).

Nasa moroensis Weigend—Peru, Depto. Ancash, Prov. Huaylas, Rio Grande/Río Chacchan, 2143 m, 16 May 2003, M. Weigend *et al.* 7694 (BSB, HUT, M, USM).

Nasa olmosiana (J.F. Macbr.) Weigend—Depto. Cajamarca, Prov. Santa Cruz, road from Monte Seco to Espinal, close to turn off to La Florida, 600–800 m. 7 March 1998 to 9 May 1998, Nicolas Dostert 98/163-C (BSB, M).

Nasa picta (Hook.f.) Weigend—Peru. Depto. Cajamarca. Prov. Chota, Huambos, 93 km from Chota on road Huambos, Llama Chiclayo, 2300 m. 14 May 1998, M. Weigend and N. Dostert 98/158 (M, USM).

Nasa poissoniana (Urb. & Gilg) Weigend—Peru, Depto. La Libertad, Prov. Pataz, road Buldibuyo to Tayabamba, 8°07'S, 77°23'W, 3163 m, 24 April 2004, M. Weigend and Ch. Schwarzer 8007 (B, USM).

Nasa triphylla (Juss.) Weigend ssp. *flavipes* Weigend & Dostert—Peru, Depto. Piura, Prov. Huancabamba, due west of town, 1700–1900 m, May 1998, M. Weigend and N. Dostert 98/203 (M, USM).

Nasa triphylla (Juss.) Weigend ssp. *triphylla*—cultivated plants from Botanical Garden Berlin Dahlem, 1 August 2004, M. Ackermann 602 (BSB).

Nasa triphylla (Juss.) Weigend ssp. nov. ined.—Peru, Depto. La Libertad, Prov. Sanchez Carrion, road Huamachuco to Chagual—Pataz, after Chugay and between Molino Viejo and Aricapampa, 7°48'S, 77°41'W, 2389 m, 20 April 2004, M. Weigend & Ch. Schwarzer 7913 (B, USM).

Nasa urens (Jaq.) Weigend—Peru, Depto. Lima, Prov. Yauyos, road from Quilmana to Panamericana, 122 km on Panamericana, Lomas de Quilmana, 12°57'S, 76°26'W, approx. 320 m, 8 October 2002, M. Weigend *et al.* 7327 (BSB, HUSA, USM, M, NY).

Nasa Vargasii (Macbr.) Weigend—Peru, Depto. Huánuco. Prov. Ambo. Road from Huánuco to Cerro de Pasco, 27.3 km from Ambo. 2300 m. 10°11'S, 76°10'W, 3 April 2001, M. Weigend *et al.* 5463 (HUT, B, M, USM).

Presliophytum arequipensis Weigend—Peru, Depto. Moquegua, Moquegua on road to Torata, 1855 m, 13 October 1997, M. Weigend and H. Förther 97/848 (M, USM).

Presliophytum heucheraefolium (Killip) Weigend—Peru, Depto. Ancash, Prov. Huaylas, Rio Grande/Río Chacchan, 18L 0181840 UTM 8941740, 16 May 2003, M. Weigend *et al.* 7691 (BSB, USM).

Presliophytum incanum (Graham) Weigend—Peru, Depto. Lima, Prov. Huarochiri, Matucana, 2400 m, M. Weigend and N. Dostert 97/12 (M, USM).

Xylopodia klaprothioides Weigend—Peru, Depto. Cajamarca, Prov. Contumazá, road Contumaza to Chilete, first road bend after highest point of pass. 2900 m. April 1997, M. Weigend *et al.* 97/450 (M, USM).