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A PHYLOGENETIC ANALYSIS OF LOASACEAE SUBFAMILY LOASOIDEAE BASED ON PLASTID DNA SEQUENCES

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Questions of tribal and generic circumscriptions and relationships in Loasaceae subfamily Loasoideae are addressed in phylogenetic analyses that apply four plastid regions in parsimony and maximum likelihood analyses. As circumscribed in the influential monograph of Urban and Gilg, Loaseae are paraphyletic to the sister clades Klaprothieae (*Klaprothia*, *Plakothira*, and *Xylopodia*) and Kissenieae (*Kissenia*). This problem centers on the paraphyly of *Huidobria*: *Huidobria chilensis* is sister to Klaprothieae + Kissenieae, and *Huidobria fruticosa* is sister to all other Loasoideae. Parametric bootstrapping finds topologies that force the monophyly of *Huidobria* to be significantly different from the optimal topologies in which the genus is paraphyletic; however, Templeton and Shimodaira-Hasegawa tests did not distinguish between these phylogenetic alternatives. We recognize a strongly supported Loaseae *sensu stricto* (s.str.) as a clade consisting of *Nasa*, *Aosa*, *Chichicaste*, *Presliophytum*, *Blumenbachia*, *Cajophora*, *Loasa* sect. *Loasa*, and *Scyphanthus*. In Loaseae s.str., the monophyly of each of the following has strong support: (1) *Nasa*, (2) *Aosa* + *Chichicaste*, (3) *Presliophytum* + *Loasa malesherbioides*, and (4) a higher Loaseae clade that consists of *Blumenbachia*, *Cajophora*, *Scyphanthus*, and the *Loasa* complex (=sect. *Loasa*, excluding *L. malesherbioides*). *Blumenbachia*, *Cajophora* (including exemplars from sections *Bialatae* and *Bicallosae*), and *Scyphanthus* are independently monophyletic, and clades of the *Loasa* complex are mixed among them. The paraphyletic *Loasa* complex includes the following clades: (1) ser. Pinnatae, (2) ser. *Acaules* + *Volubile*, (3) ser. *Macrospermae*, placed as the sister of *Blumenbachia*, and (4) ser. *Acanthifolia* + *Floribundae* + *Deserticolae*, which includes the type for *Loasa* and is the group we recommend as the basis for a revised circumscription of *Loasa*.

Keywords: Loasaceae, phylogeny, plastid DNA, systematics.

Introduction

Loasaceae subfamily Loasoideae is primarily a New World group that has its greatest species richness in South America but also extends into southern Mesoamerica and the Caribbean Islands. Two genera are disjunct from the New World: *Kissenia* is in Africa and the adjacent Arabian peninsula, and *Plakothira* is on the Marquesas Islands. Loasoideae have long been considered to be a natural group because all have flowers in which the androecium is differentiated into fertile and nonfertile stamens (Gilg 1895, 1925; Urban and Gilg 1900). Recent molecular phylogenetic studies support the hypothesis that functional differentiation among stamens is synapomorphic for Loasoideae and have found additional DNA data that provide strong support for the monophyly of the subfamily (Moody et al. 2001; Hufford 2003; Hufford et al. 2003). Within Loasoideae, however, tribal and generic circumscriptions have raised concerns among systematists, and these taxonomic issues are the focus of our study.

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Gilg (1895, 1925; also Urban and Gilg 1900) recognized in Loasoideae the three tribes Kissenieae, Klaprothieae, and Loaseae (table 1). Kissenieae has been limited only to the Old World *Kissenia*, which consists of one or two species (Dandy 1926). Klaprothieae, which are found in northwestern South America, Mesoamerica, Hispaniola, and the Marquesas Islands, also consists of few species in *Klaprothia*, including *Sclerothrix* (two species; Poston and Nowicke 1990), *Plakothira* (three species; Florence 1997), and *Xylopodia* (one species; Weigend 1997*b*). The Gilg (1895, 1925) and Urban and Gilg (1900) concept of Loaseae contrasts with their treatment of the two other tribes; Loaseae encompasses greater geographic range, habitat diversity, morphological disparity, and much greater species richness. In contrast to the long-prevailing treatments by Gilg (1895, 1925) and Urban and Gilg (1900), Weigend (1997*b*; table 1) recognized only the two tribes Loaseae (including *Kissenia* of Kissenieae) and Klaprothieae. Weigend (1997*c*, p. 42) hypothesized that it was "unlikely that [Klaprothieae] arose within Loaseae" but also that they were "firmly connected to Loaseae via *Loasa* [*Aosa*] *plumieri*" [sic]. In contrast to the tribal revision proposed by Weigend (1997*b*), Hufford et al. (2003) advocated the retention of the three previously recognized tribes because they found strong support for an unexpected sister group relationship of Klaprothieae and *Kissenia* and placement of those two tribes as the sister of a weakly

Table 1

Two Contrasting Classifications of Loasaceae Subfamily Loasoideae Proposed by Urban and Gilg (1900) and Weigend (1997*b*)

Urban and Gilg 1900	Weigend 1997*b*
Loaseae:	Loaseae:
Loasa	*Loasa* (incl. *Cajophora* and *Scyphanthus*)
Scyphanthus	*Aosa* (*Loasa* segregate)
Blumenbachia	*Huidobria*
Cajophora	*Nasa* (*Loasa* segregate)
Kissenieae:	*Presliophytum* (*Loasa* segregate)
Kissenia	*Chichicaste* (*Loasa* segregate)
Klaprothieae:	*Blumenbachia*
Klaprothia	*Kissenia*
Sclerothrix	Klaprothieae:
	Klaprothia (incl. *Sclerothrix*)
	Plakothira
	Xylopodia

supported Loaseae. The limited support for Loaseae found by Hufford et al. (2003) raises the possibility of its paraphyly to both Klaprothieae and Kissenieae, which is a key question we seek to resolve in this study.

Several small genera and the larger, more broadly circumscribed *Cajophora* and *Loasa* were recognized in Loasoideae by Gilg (1895, 1925) and Urban and Gilg (1900). The broad circumscriptions of both *Cajophora* and *Loasa* have since been questioned. For example, Poston and Thompson (1977) suggested that *Cajophora sensu lato* (s.l.) was polyphyletic and hypothesized that *Cajophora* section *Bialatae* was more closely related to *Blumenbachia* than to other *Cajophora*. Weigend (1997*b*) excluded not only section *Bialatae* but also sections *Angulatae* and *Bicallosae* from *Cajophora* and suggested that they were more closely related to other genera of Loasoideae. Hufford et al. (2003) did not sample from *Cajophora* sections *Bialatae*, *Angulatae*, or *Bicallosae* but did find very strong support for the monophyly of sampled *Cajophora*, which included species of Weigend's (1997*b*) *Cajophora sensu stricto* (s.str.). The Weigend et al. (2004) phylogenetic analysis sampled exemplars of section *Angulatae* but did not find them allied with either *Cajophora* s.str. or *Blumenbachia*.

The monophyly of the broadly circumscribed *Loasa* of Urban and Gilg (1900) is also suspect. Hufford et al. (2003) rejected the monophyly of *Loasa*, using the Shimodaira-Hasegawa (SH) test (Shimodaira and Hasegawa 1999). Their most likely cladogram, based on DNA sequence data from the plastid regions *matK* and *trnL-trnF*, was significantly more likely than the best topology that was constrained to model Urban and Gilg's (1900) *Loasa* s.l. as a monophyletic group. Before the Hufford et al. (2003) phylogenetic study, Urban and Gilg's (1900) *Loasa* had been dismantled partially by recent workers. Grau (1997) resurrected *Huidobria*, which had been included as a section of *Loasa* s.l. by Urban and Gilg (1900) and Gilg (1925). Weigend (1997*b*) segregated the new genera *Aosa*, *Chichicaste*, *Nasa*, and *Presliophytum* from *Loasa* s.l. The phylogenetic results of Hufford et al. (2003) and Weigend et al. (2004) found strong support for the monophyly of *Nasa* and *Presliophytum*. Neither Hufford et al. (2003) nor Weigend et al. (2004) found support for the monophyly of *Huidobria*. Weigend et al. (2004) did not find support for the monophyly of *Aosa* (only one species had been sampled by Hufford et al. 2003). *Chichicaste* was not sampled by Hufford et al. (2003), and in the results of Weigend et al. (2004), it formed part of a large basal polytomy with numerous other clades. Important goals of our study are to test further the monophyly of both *Huidobria* and *Aosa* and to test for the sister group of *Chichicaste*.

Weigend (1997*b*) argued for a greatly modified circumscription of *Loasa*. His concept of the genus included part of Urban and Gilg's (1900) *Loasa* sect. *Loasa* (excluding the species segregated as *Aosa*, *Chichicaste*, and *Nasa*), *Cajophora* (excluding sections *Bialatae*, *Angulatae*, and *Bicallosae*), and *Scyphanthus*. This grouping is not consistent with any of the clades recovered by the phylogenetic analysis of Hufford et al. (2003) or Weigend et al. (2004). Although Hufford et al. (2003) did recover a monophyletic group that consisted of *Cajophora*, *Scyphanthus*, and elements of *Loasa*, some species of *Loasa* were more closely related to other clades. For example, *Loasa heterophylla* was found to be more closely related to *Blumenbachia* and *Loasa malesherbioides* more closely related to *Presliophytum* than to the *Loasa* s.str. + *Cajophora* + *Scyphanthus* clade (the latter result was also found by Weigend et al. 2004). We infer from those results that the relationships of Urban and Gilg's (1900) *Loasa* sect. *Loasa* (excluding the series segregated as *Aosa*, *Chichicaste*, and *Nasa*) require further investigation to uncover the composition of a monophyletic *Loasa*.

We address the issues raised above with the objective of providing a taxonomy of Loasoideae that reflects knowledge of monophyly and phylogenetic relationships. Many of these same issues were addressed recently in a phylogenetic analysis by Weigend et al. (2004), who applied data only from the plastid *trnL* intron, although Hufford et al. (2003) had already shown that this region alone was insufficiently variable to resolve the taxonomic problems of Loasoideae. We apply DNA sequence data not only from the *matK*, *trnL* intron, and *trnL-trnF* intergenic spacer regions that were used earlier by Hufford et al. (2003) for a broader phylogenetic analysis of Loasaceae but also from two additional plastid intergenic spacers, *rpl20-rps12* and *psbA-trnH*. We have also increased the sampling of critical taxa that bear on the important taxonomic problems of Loasoideae.

Material and Methods

DNA Sequences

DNA sequences for the plastid regions *matK*, *trnL-trnF*, *rpl20-rps12*, and *psbA-trnH* were used for phylogeny reconstructions. All *rpl20-rps12* and *psbA-trnH* and 20 *matK* and *trnL-trnF* sequences were generated as part of this investigation, whereas other *matK* and *trnL-trnF* sequences were from Moody et al. (2001) and Hufford et al. (2003) (GenBank accessions and voucher information for all sequences are in table 2). For the new sequences, total DNA was extracted from either herbarium or silica-dried specimens of leaves with a standard CTAB procedure (Doyle and Doyle 1987). PCR mixes varied but approximated the following: 2.5 μL of

Promega reaction buffer, 3 μL of 1.5 mM $MgCl_2$, 2.5 μL of both 0.5 μM forward- and reverse-amplification primers, 1.5 μL of 150 μM dNTPs, 0.25 μL of Taq polymerase, 4 μL of template, and water to bring the total volume to 50 μL.

The PCR primers for *matK* were *matK*-710F and *trnK*-2R (Johnson and Soltis 1995), and the sequencing primers were *matK*-710F, *trnK*-2R, *matK*-1713F, and *matK*-1848R (citations in Moody et al. 2001). For the other three markers, the same pairs of primers were used for PCR and cycle sequencing, respectively; these included the following: for *trnL-trnF*, c and f (Taberlet et al. 1991); for *rpl20-rps12*, rpl20 and 5′ rps12 (Hamilton 1999); and for *psbA-trnH*, psbA and trnH (GUG) (Hamilton 1999). Sequences were aligned manually in Se-Al (Rambaut 1996). In the *trnL-trnF* data set, 20 short regions could not be aligned unequivocally and were deleted from the analyses.

Taxon Sampling

Outgroup selection was based on results from earlier studies that placed Loasaceae as the sister of Hydrangeaceae in Cornales of the Asteridae (Xiang et al. 1993, 1998, 2002; Hempel et al. 1995; Olmstead et al. 2000; Hufford et al. 2001) and resolved relationships of major clades of Loasaceae outside of Loasoideae (Moody et al. 2001; Hufford et al. 2003). For the sampling of *matK*, *trnL-trnF*, and the combined data sets, several outgroups were applied, including Loasaceae, Hydrangeaceae, and Cornaceae. The Loasaceae outgroups included *Mentzelia albescens*, *Mentzelia nitens*, *Mentzelia oligosperma*, *Mentzelia torreyi*, *Cevallia sinuata*, and *Petalonyx linearis*, representing the *Mentzelia* + Gronovioideae clade that has been shown to be the sister of Loasoideae in the phylogenetic analyses of Moody et al. (2001) and Hufford et al. (2003). Clades found by Moody et al. (2001) and Hufford et al. (2003) at more basal nodes of Loasaceae were represented as outgroups by *Eucnide bartonioides* and *Schismocarpus pachypus*. Thus, eight outgroups from Loasaceae were applied in the phylogenetic analyses. Three outgroups outside of Loasaceae were selected, including *Fendlera rupicola* and *Hydrangea hirta*, representing Hydrangeaceae, and *Cornus florida* of the Cornaceae. Trees were rooted between *C. florida* and its sister clade. Phylogenetic analyses of the *rpl20-rps12* data used *Cevallia sinuata*, *Petalonyx linearis*, four species of *Mentzelia*, and *Eucnide aurea* as outgroups and were rooted between *Eucnide* and its sister clade. Phylogenetic analyses of the *psbA-trnH* data applied only *E. aurea* as an outgroup.

For Loasoideae, we have emphasized increasing the taxon sampling beyond that used by Moody et al. (2001) and Hufford et al. (2003), especially for taxa that will help to resolve problems of generic circumscription. *Xylopodia* and *Chichicaste*, which were not included by Moody et al. (2001) or Hufford et al. (2003), are included in our sampling. We have also sampled additional taxa of Weigend's (1997*b*) *Loasa* s.str. and *Cajophora* to test hypothesized relationships. A total of 46 and 45 accessions of Loasoideae were sampled, respectively, for *matK* (28 more than in Moody et al. 2001 and 14 more than in Hufford et al. 2003) and *trnL-trnF* (15 more than in Hufford et al. 2003); however, only 26 were sampled for *rpl20-rps12* and 20 for *psbA-trnH*. Taxon sampling for *matK* and *trnL-trnF* overlaps entirely except for the inclusion of *Loasa acanthifolia* in the former data set but not the latter.

Phylogenetic Analyses

Maximum parsimony (MP) analyses were conducted using PAUP*, version 4.0 (Swofford 2002). MP analyses were conducted on the independent *matK* (46 ingroup and 11 outgroup taxa), *trnL-trnF* (45 ingroup and 11 outgroup taxa), *rpl20-rps*12 (26 ingroup and seven outgroup taxa), and *psbA-trnH* (20 ingroup taxa and one outgroup taxon) data sets. Three combined marker data sets of 56 taxa were constructed, including (1) *matK* and *trnL-trnF*; (2) *matK*, *trnL-trnF*, *rpl20-rps12*, and *psbA-trnH* (=four-marker data set); and (3) a four-marker data set with phylogenetically informative insertions and deletions (indels) coded as presence/absence characters. For the 56-taxon combined data sets, *Loasa acanthifolia* was deleted from the *matK* data because it was not available for any other markers. In the four-marker data set, missing data were coded as "?." The missing data in the four-marker data set include entire sequences for taxa sampled for *matK* and *trnL-trnF* but not for *rpl20-rps12* and *psbA-trnH*. Because *E. bartonioides* was sampled as an outgroup for *matK* and *trnL-trnF* and *E. aurea* for *rpl20-rps12* and *psbA-trnH*, a composite *Eucnide* outgroup combining those sequences was used for the four-marker data set. The four-marker data set with phylogenetically informative indels is highly provisional because of the limited taxon sampling for *rpl20-rps12* and *psbA-trnH*.

MP analyses used tree bisection reconnection (TBR) branch swapping on topologies from 100 replicated searches (except for the four-marker data set with phylogenetically informative indels, for which the search was not replicated) in which taxon addition was randomized to begin each search. Character state transitions were equally weighted and unordered. Indels (gaps) were treated as missing data except in the four-marker data set with phylogenetically informative indels. Tree statistics and measures of homoplasy were calculated using PAUP*, with uninformative characters removed. Multiple most parsimonious trees were combined in PAUP* to construct strict consensus cladograms. Confidence in clades was assessed using the nonparametric bootstrap (Felsenstein 1985) implemented in PAUP*. For the independent data sets and the four-marker data set, 1000 pseudoreplicated heuristic searches were initiated with random taxon addition and branch-swapped using the TBR option, and the MAXTREES option was set at 100 to reduce computational time. The bootstrap analysis of the combined *matK* and *trnL-trnF* data was similar, except that the MAXTREES option was set at 10,000.

Hypothesis Tests

Hypotheses of taxonomic groups and their interrelationships can be modeled as cladogram topologies. The circumscription of *Huidobria* leads to an inference that *Huidobria chilensis* and *Huidobria fruticosa* are monophyletic, although this was not recovered in our results. We applied a topological constraint that forced the monophyly of *H. chilensis* and

Table 2

Sources of DNA and GenBank Numbers for *matK* and *trnL-trnF* Sequences

Taxon	Voucher	*matK*	*trnL-trnF*	*rpl20-rps12*	*psbA-trnH*
Aosa plumierii (Urb.) Weigend	Moody 47 (WS)	AF503319	AY254225		
A. rostrata (Urb.) Weigend	Anderson 9219 (F)	AY781437	AY781512		AY781458
A. rupestris (Gardner) Weigend	Chiappeta 307 (F)	AY781438	AY781513	AY781479	
Blumenbachia insignis Schrad.	Kittredge 1010 (M)	AY250186	AY254226	AY781480	AY781459
B. latifolia Cambess	Zandini et al. 2920 (MO)	AF503324	AY254227	AY781481	AY781460
Cajophora sp.	Hufford 3518 (WS)	AY781439	AY254228		
Cajophora sp.	Hufford 3524 (WS)	AY254056	AY254230		
Cajophora sp.	Hufford 3854 (WS)	AY781440	AY781514		
C. buraeavii Urb. & Gilg	Beck 12582 (M)	AY781441	AY781515		
C. canarinoides Urb. & Gilg	Wood 8056 (M)	AY254057	AY254231		
C. carduifolia Presl.	Hufford 3503 (WS)	AY254058	AY254234	AY781482	AY781461
C. chuquitensis Urb. & Gilg	Beck 17894 (MO)	AF503329	AY254233	AY781483	
C. cirsiifolia Presl.	Hufford 3509 (WS)	AY254059	AY254234	AY781484	AY781462
C. clavata Urb. & Gilg	Poelt s.n. 14.11.53 (M)	AY254060	AY254236		
C. coronata Hook. & Arn.	Deginani, Ciadella, and Bortiri 596 (MO)	AY781442	AY781516		
C. eichleri Urban	Hatschbach and Hatschbach 41572 (MO)	AY781443	AY781517		
C. hibiscifolia Urb. & Gilg	Stuessy 18058 (WU)	AY781444	AY781518		
C. hibiscifolia	Krapovick and Seijo 47730 (F)				AY781463
C. macrocarpa Urb. & Gilg	Krapovickas et al. 219667 (MO)	AF503326			
C. macrocarpa	Stuessy 18079 (WU)	AY781445	AY781519		
C. stenocarpa Urb. & Gilg	Weigend and Weigend 2000/211 (F)	AY781446	AY781520	AY781485	AY781464
Cevallia sinuata Lag.	Waterbrook 175 (WS)	AF503301	AY254237	AY781486	
Chichicaste grandis (Standl.) Weigend	Maas 7982 (F)	AY781447	AY781521	AY781487	AY781465
Cornus florida L.	Cagle 94 (WS)	AY254061	AY254238		
Eucnide aurea (A. Gray) Thompson & Ernst	Hufford 2634 (WS)			AY781488	AY781466
E. bartonioides Zucc.	Moody 41 (WS)	AF503316	AY254240		
Fendlera rupicola Engelm. & A. Gray	Hufford 541 (WS)	AY254063	AY254243		
Huidobria chilensis Gay	Munoz et al. 2749 (MO)	AF503317	AY254246	AY781489	AY781467
H. fruticosa Phil.	Dillon & Trujillo C. 8034 (M)	AY254064	AY254247	AY781490	AY781468
Hydrangea hirta Sieb.	Takasu s.n. 17.6.94 (WS)	AY254065	AY254248		
Kissenia capensis Endl.	Goldblatt and Manning 8718 (MO)	AF503333	AY254249	AY781491	AY781469
Klaprothia mentzelioides H. B. & K.	Dostert 98/48 (M)	AY781448	AY781522	AY781492	
Loasa acanthifolia Desr.	West 4675 (LA)	AF503323			
L. bergii Hieron.	Stuessy 18027 (WU)	AY781449	AY781523	AY781493	
L. elongata Hook. & Arn.	Hufford 3864 (WS)	AY781450	AY781524	AY781494	
L. filicifolia Poepp.	Stuessy et al. s. n. 28.2.2002 (WU)	AY781451	AY781525	AY781495	AY781470
L. gayana Urb. & Gilg	Zollitsch 121 (M)	AY781452	AY781526	AY781496	
L. heterophylla Hook. & Arn.	Grau and Ehrhart 94/547 (M)	AY254066	AY254250	AY781497	
L. lateritia Gill.	Marticorena-Mattei 930 (MO)	AY781453	AY781527		
L. malesherbioides R. A. Phil.	Kiesling et al. 7814 (MO)	AF503318	AY254251	AY781498	AY781471
L. nana Phil.	Stuessy et al. 18104 (WU)	AY781454	AY781528	AY781499	AY781472
L. pallida Gill.	Teillier and Pauchard 2510 (MO)	AF503322	AY254252	AY781500	AY781473
L. tricolor Ker.	Hufford 3877 (WS)	AY781455	AY781529	AY781501	
Mentzelia albescens (Gill) Griesb.	Schajorskoy s.n., 9-XII-1966 (M)	AY254068	AY254254	AY781502	
M. nitens Greene	Hufford 3609 (WS)	AY254084	AY254276	AY781503	
M. oligosperma Nutt. & Sims	Hufford 3757 (WS)	AY781456	AY781530	AY781504	
M. torreyi A. Gray	Taylor 4870 (MO)	AF503302	AY254279	AY781505	
Nasa chenopodiifolia (Desr.) Weigend	Weigend et al. 97/461 (M)	AY254086	AY254281	AY781506	AY781474
N. cymbopetala (Urb. & Gilg) Weigend	Hufford 3525 (WS)	AY254087	AY254282	AY781507	AY781475
N. driesslei Weigend	Hofreiter and Hofreiter 13/97 (M)	AY254088	AY254283		
N. triphylla (Juss.) Weigend	Almeda and Almeda 2605 (MO)	AF503321	AY254285		
N. urens (Jacq.) Weigend	Hufford 3839 (WS)	AY254089	AY254286	AY781508	AY781476
Petalonyx linearis Greene	Fishbein 3732 (WS)	AY254090	AY254288	AY781509	

Table 2

(*Continued*)

Taxon	Voucher	*matK*	*trnL-trnF*	*rpl20-rps12*	*psbA-trnH*
Plakothira parviflora Florence	NTBG 970008 (PTBG)	AF503331	AY254292		
Presliophytum arequipensis Weigend	Weigend and Fortner 97/848 (M)	AY254091	AY254293		
P. heucheraefolium (Killip) Weigend	Hufford 3515 (WS)	AY254092	AY254294	AY781510	AY781477
P. incanum (Graham) Weigend	Hufford 3498 (WS)	AY254093	AY254295	AY781511	AY781478
Schismocarpus pachypus Blake	Breedlove 50788 (CAS)	AF503313	AY254296		
Scyphanthus elegans D. Don	Zollner III83 (MO)	AF50334	AY254298		
Xylopodia klaprothioides Weigend	Weigend, Dostert, and Driessle 97/540 (F)	AY781457	AY781531		

H. fruticosa in MP and maximum likelihood (ML) analyses to compare the support for this suboptimal alternative with the most parsimonious and most likely topologies using the Templeton test (Templeton 1983), the SH test (Shimodaira and Hasegawa 1999; Goldman et al. 2000), and parametric bootstrapping (Huelsenbeck et al. 1996; Goldman et al. 2000). The Templeton test was conducted on results of analyses of the combined *matK* and *trnL-trnF* data set, whereas the SH test and parametric bootstrap were conducted on results of analyses of the four-marker data set.

For the Templeton test, we conducted a full heuristic search of the data as described above for the most parsimonious cladograms under the topology constraint. The Templeton test, as implemented in PAUP*, version 4.0 (Swofford 2002), was used to compare the length of all topologies from the constrained search with the length of the most parsimonious topologies from the unconstrained analysis.

For the SH test, ML searches in PAUP*, version 4.0 (Swofford 2002), with and without *Huidobria* constrained to be monophyletic were started using a neighbor-joining tree with parameter values estimated under an HKY + Γ (Yang 1994; Hasegawa et al. 1995) model. This model was selected as a balance between computational efficiency and model efficacy. The best method for choosing an evolutionary model for use in likelihood-based phylogenetics is not yet clear (Minin et al. 2003), but the inclusion of across-site rate heterogeneity can be quite important (Lemmon and Moriarty 2004). Computational efficiency of the searches was enhanced by using the parameter values estimated on the neighbor-joining starting tree instead of simultaneously optimizing all parameters. The SH test was implemented in PAUP*, version 4.0 (Swofford 2002), to compare the best ML tree without constraint with the best ML tree under the constraint.

Because of the conservatism of the Templeton and SH tests and the sensitivity of the latter to the presence of multiple trees and to the quality of the trees (Shimodaira and Hasegawa 1999; Goldman et al. 2000; Buckley 2002), we also conducted a modified likelihood parametric bootstrap (Huelsenbeck et al. 1996; Goldman et al. 2000) to compare the two ML trees. The parametric bootstrap calls for the generation of data that are similar to what we would expect if the null hypothesis were true; in this case, the null hypothesis is the less optimal tree, the tree for which *Huidobria* was constrained to be monophyletic. Therefore, we estimated branch lengths and parameter values under the most parameter-rich model feasible, GTR + Γ + I, on the tree obtained using the constraint. These values were used to simulate 500 data sets with Seq-Gen, version 1.3 (Rambaut and Grassly 1997). Each of these data sets was subjected to the same parsimony analyses as above, and the distribution of parsimony tree score differences (tree length with constraint − tree length without constraint) was compared with our observed difference.

Results

Sizes of the aligned matrices and results of MP analyses of each independent data set are provided (table 3). The MP analysis of the *matK* (fig. 1*A*) and *psbA-trnH* (fig. 1*D*) data sets swapped to completion, but analyses of *trnL-trnF* (fig. 1*B*) and *rpl20-rps12* (fig. 1*C*) were stopped because of memory limitations after 20,000 equally parsimonious trees had been found. Results from analyses of the independent data sets are largely consistent, although the *matK* and *trnL-trnF* data (fig. 1*A*, 1*B*), for which we had the most extensive taxon sampling, produce most parsimonious trees that have two conflicts. (1) Both *matK* and *trnL-trnF* place *Chichicaste* among clades of *Aosa*, but the former places it as the sister of *Aosa rostrata* and the latter as the sister of *Aosa plumierii*; each of these alternative placements has moderate support. (2) *Cajophora eichleri* is placed in a well-supported, monophyletic *Cajophora* as the sister of *Cajophora stenocarpa* by the *matK* data but outside of the well-supported, monophyletic *Cajophora* in a polytomy with other higher Loaseae by the *trnL-trnF* data.

Analyses of the combined data sets swapped to completion (table 3) and provided consistent results (figs. 2, 3). The four-marker data sets (fig. 2*B*; fig. 3) resolve more clades than did the analysis of combined *matK* and *trnL-trnF* data (fig. 2*A*). For example, the former resolves the placement of *Loasa heterophylla* + *Loasa tricolor* as the sister of *Blumenbachia*; *C. stenocarpa* as the sister of a clade consisting of *Cajophora* sects. *Pentamerae*, *Dolichocarpae*, *Platypetalae*, and *Pleiomerae*; and *Scyphanthus* as the sister of *Cajophora*; however, we note that these instances of greater resolution receive little support.

The MP analyses of the combined data sets resolve a well-supported higher Loaseae clade that includes *Blumenbachia*, *Cajophora*, *Loasa* sect. *Loasa* (except *Loasa malesherbioides*),

Table 3

Summary of the Phylogenetic Data Sets and Results of the Parsimony Analyses of Each

Data set	Total taxa	Total characters	Parsimony informative characters	Search swapped to completion	Equally shortest trees	Length of the shortest trees (state changes)	CI (excluding uninformative characters)	RI	Rescaled CI
matK	57	1587	262	Yes	24	859	0.6183	0.8108	0.6098
trnL-trnF	56	863	150	No	20,000	478	0.6474	0.8300	0.6390
rpl20-rps12	33	782	79	No	20,000	325	0.7410	0.8500	0.7198
psbA-trnH	21	464	50	Yes	159	132	0.8025	0.8667	0.7795
matK + *trnL-trnF*	56	2444	410	Yes	144	1342	0.6251	0.8138	0.6149
matK + *trnL-trnF* + *rpl*20-*rps*12 + *psbA-trnH*	56	3690	583	Yes	3474	1737	0.6524	0.8195	0.6398
matK + *trnL-trnF* + *rpl*20-*rps*12 + *psbA-trnH*+ insertions and deletions	56	3708	557	Yes	764	1764	0.6527	0.8204	0.6391

Note. In the data set in which all four markers were combined, taxa for which *rpl20-rps12* and/or *psbA-trnH* were not available were coded with placeholder sequences consisting entirely of missing data (coded as "?"). CI = consistency index; RI = retention index.

and *Scyphanthus* (fig. 2). Sampled *Blumenbachia* and *Cajophora* are independent monophyletic groups. *Loasa* sect. *Loasa* are paraphyletic (all taxa labeled as *Loasa* in the figures are part of Urban and Gilg's [1900] sect. *Loasa*). Higher Loaseae are the sister of *Presliophytum* + *L. malesherbioides* (fig. 2). *Chichicaste* is nested in a strongly supported *Aosa* clade, and the inclusion of indel characters indicates that *Chichicaste grandis* is more closely related to *A. rostrata* than to other sampled species of *Aosa* (fig. 3*A*). Strong support is also found for the monophyly of sampled *Nasa*, and the inclusion of indel characters provides additional resolution of relationships in this genus (fig. 3*B*). *Nasa*, *Aosa*, *Presliophytum*, *L. malesherbioides*, and higher Loaseae form a monophyletic group designated here as Loaseae s.str. (fig. 2). Sister of Loaseae s.str. is a clade that consists of sampled Klaprothieae (*Klaprothia*, *Plakothira*, and *Xylopodia*), *Kissenia* (Kissenieae), and *Huidobria chilensis*. *Huidobria fruticosa* is the sister to the rest of Loasoideae. On the basis of the Templeton test, there was no significant difference ($P \geq 0.26$; tree length difference of three steps) in the lengths of most parsimonious cladograms from analyses of the four-marker data set between unconstrained topologies and those constrained to force the monophyly of *H. chilensis* and *H. fruticosa*. The SH test also failed to support a significant difference between the ML trees obtained with and without the constraint ($P = 0.24$). The parametric bootstrap, however, indicated that there was a significant difference between the trees with and without the constraints (a difference of three steps was found in only two out of 500 data sets; $P = 0.006$).

Discussion

Higher Loaseae

We define as higher Loaseae a clade that includes *Blumenbachia*, *Cajophora*, *Scyphanthus*, and members of several series of Urban and Gilg's (1900) *Loasa* sect. *Loasa*, including ser. *Acanthifoliae*, *Floribundae*, *Macrospermae*, *Acaules*, *Deserticolae*, *Pinnatae*, and *Volubile* (this group has been called the southern Andean loasas by Weigend [1997*b*]). The loss of recaulescent bracts in inflorescences may be a synapomorphy for this clade (Weigend et al. 2004). Most higher Loaseae have opposite leaves, but this leaf arrangement may be a symplesiomorphy shared also with *Loasa malesherbioides* (opposite leaves of Klaprothieae can be hypothesized to be an independent origin). Most higher Loaseae also have flowers in which the staminodial scales have a pair of prominent arches on the abaxial surface and seeds in which cells of the seed coat form deep reticulations (the anticlinal walls of these cells are highly protrusive; Hufford 1988), but these attributes are notably absent in *Blumenbachia* and *Cajophora* sect. *Bicallosae* (Weigend 1997*b*).

Generic circumscriptions in higher Loaseae have been contentious, and this has been especially true for Urban and Gilg's (1900) *Cajophora*. Poston and Thompson (1977) suggested that *Cajophora* s.l. was polyphyletic and hypothesized that *Cajophora* section *Bialatae* was more closely related to *Blumenbachia* than to other *Cajophora*. Weigend (1997*b*) excluded sections *Angulatae* and *Bialatae* from *Cajophora*, placing them in *Blumenbachia*. Weigend et al. (2004) sampled three exemplars from sect. *Angulatae*, *Cajophora* [*Blumenbachia*] *espigneira*, *Cajophora* [*Blumenbachia*] *prietea*, and *Cajophora* [*Blumenbachia*] *sylvestris* but found support for the monophyly of neither *Blumenbachia* s.str. + sect. *Angulatae* nor even sampled sect. *Angulatae*. Weigend et al. (2004) did not sample from the sect. *Bialatae* of Urban and Gilg (1900); instead, Weigend et al. (2004) included only the more recently circumscribed *Blumenbachia exalata* Weigend. We sampled only *Cajophora eichleri* from sect. *Bialatae*, which was placed, in contrast to the suggestions of Poston and Thompson (1977) and Weigend (1997*b*), as part of a monophyletic *Cajophora*. Weigend (1997*b*) further hypothesized that section *Bicallosae* was not closely related to *Cajophora* s.str., and we note that he emphasized *Cajophora archavaletae* in this proposed realignment. We have not

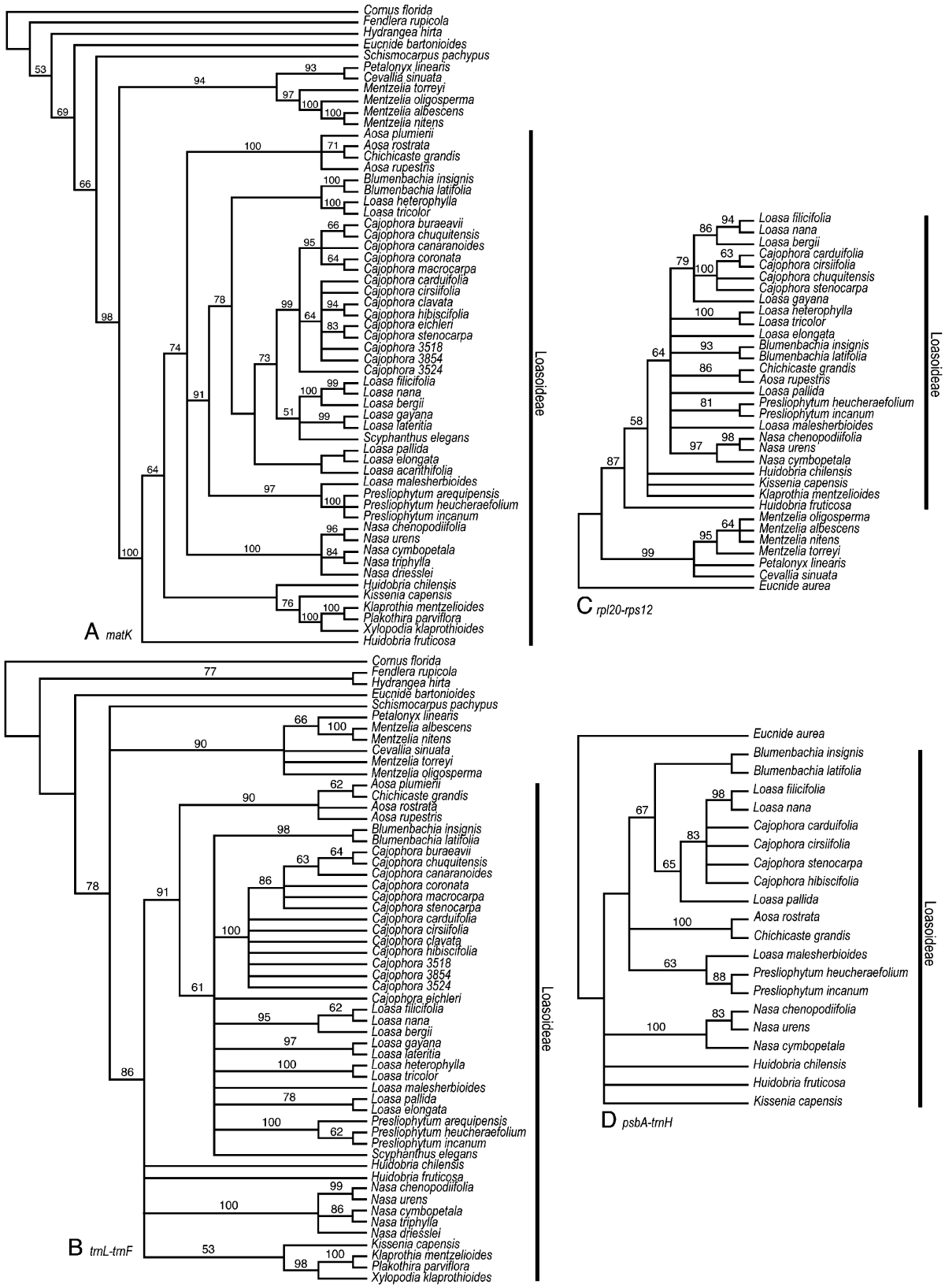

Fig. 1 Strict consensus cladograms of Loasoideae based on parsimony analyses of the independent plastid data sets. All sampled *Loasa* are part of Urban and Gilg's (1900) section *Loasa*. Numbers above clades are bootstrap proportions when above 50%. *A*, *matK*. *B*, *trnL-trnF*. *C*, *rpl20-rps12*. *D*, *psbA-trnH*.

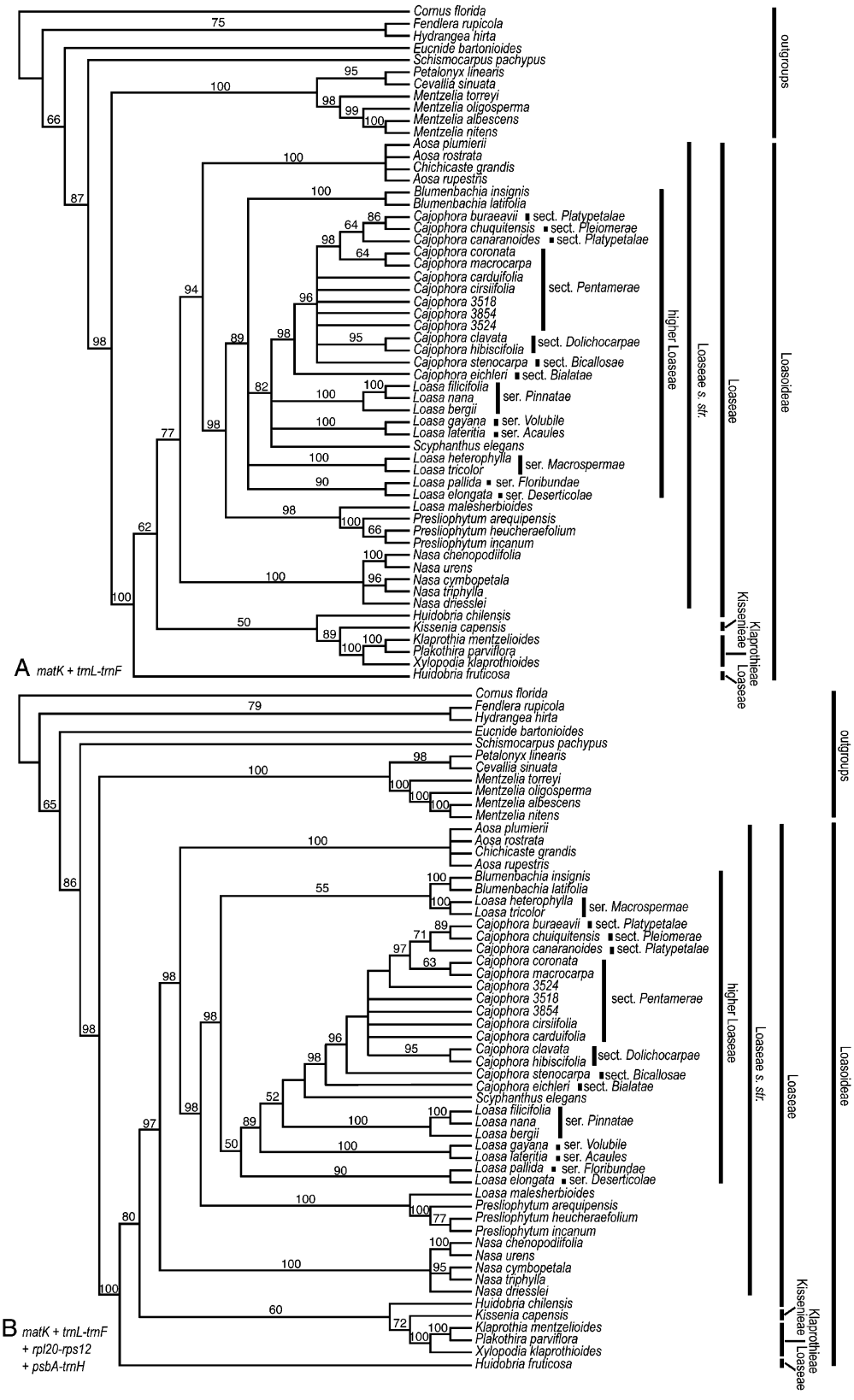

Fig. 2 Strict consensus cladograms of Loasoideae based on parsimony analyses of combinations of the plastid data sets. All sampled *Loasa* are part of Urban and Gilg's (1900) section *Loasa*. Numbers above clades are bootstrap proportions when above 50%. *A*, *matK* and *trnL-trnF*. *B*, *matK*, *trnL-trnF*, *rpl20-rps12*, and *psbA-trnH*.

Fig. 3 Clades from the parsimony analysis of the provisional four-marker data set with phylogenetically informative insertions and deletions in which enhanced support was found, in comparison with the four-marker data set, in which insertions and deletions were coded as missing data. Numbers above clades are bootstrap proportions when above 50%. *A*, *Aosa* + *Chichicaste* clade. *B*, *Nasa*. *C*, *Presliophytum*.

sampled *C. archavaletae*, but it and *Cajophora stenocarpa* were circumscribed as sect. *Bicallosae* by Urban and Gilg (1900). Our results support the placement of *C. stenocarpa* and thus at least part of sect. *Bicallosae* in *Cajophora*. Given our results and those of Weigend et al. (2004) that have failed to find support for the monophyly of various sections of *Cajophora* as circumscribed by Urban and Gilg (1900), we suggest that greater attention needs to be given to testing the monophyly of sections, including *Angulatae* and *Bicallosae*, while also testing further the evolutionary relationships among these species that share some morphological features with *Blumenbachia*. Thus, at this point, we conclude from our results and those of Weigend et al. (2004) that (1) there is strong support for the monophyly of *Cajophora*, including elements of sects. *Bialatae* and *Bicallosae* and (2) members of sect. *Angulatae* fall outside of *Cajophora* but not necessarily with *Blumenbachia*. Few clades among core *Cajophora* have strong support, but some optimal trees indicate that sect. *Pentamerae* is paraphyletic to all *Cajophora* except sect. *Bialatae*. There is strong support for a sister group relationship between elements of sect. *Pentamerae* (*Cajophora coronata* and *Cajophora macrocarpa*) and a clade consisting of species from sects. *Platypetalae* and *Pleiomerae*. Sampled members of sect. *Platypetalae* are paraphyletic to *Cajophora chuquitensis* of sect. *Pleiomerae*.

We discuss ser. *Acanthifoliae*, *Floribundae*, *Macrospermae*, *Acaules*, *Deserticolae*, *Pinnatae*, and *Volubile* of *Loasa* sect. *Loasa* as the *Loasa* complex. Following the dismantling of the Gilg (1895, 1925; also Urban and Gilg 1900) *Loasa* s.l. by Grau (1997) and Weigend (1997*b*), the latter author and Müller et al. (1999) advocated a *Loasa* s.str. equal to the *Loasa* complex, but this is not a monophyletic group based on our results or those of Weigend et al. (2004). Our results show that *Blumenbachia*, *Cajophora*, and *Scyphanthus* are independently monophyletic and that clades of the *Loasa* complex are mixed among them (fig. 2). Next, we compare further the hypotheses of Weigend (1997*b*) and Müller et al. (1999) with our preliminary results.

They suggested that *Loasa* ser. *Acaules*, *Deserticolae*, *Pinnatae*, and *Volubile* were allied by seed and other characters. Our results and those of Weigend et al. (2004) support the monophyly of *Loasa lateritia* (ser. *Acaules*) and *Loasa gayana* (ser. *Volubile*). The four-marker data set (fig. 2*B*) provides limited phylogenetic signal indicating that ser. *Pinnatae* is more closely related to *Cajophora* + *Scyphanthus* than to *Acaules*, *Volubile*, or *Deserticolae*, as would be inferred from the alliance hypothesized by Weigend (1997*b*) and Müller et al. (1999). Significantly, our results indicate that ser. *Acaules*, *Pinnatae*, and *Volubile* are more closely related to *Scyphanthus* and *Cajophora* than to other *Loasa* s.str.

Müller et al. (1999) and Weigend et al. (2000) suggested that ser. *Floribundae* and *Macrospermae* have synapomorphic hetero-oligomeric iridoids. Neither our analyses nor those of Weigend et al. (2004) found support for the monophyly of *Floribundae* and *Macrospermae* hypothesized by Müller et al. (1999) and Weigend et al. (2000). In our sampling, we find weak support, based on our four-marker data set, for the monophyly of *Macrospermae* and *Blumenbachia*, which has not been previously hypothesized. This result adds further complexity to the hypothesized relationships of *Blumenbachia*, which has been linked to some of the more anomalous species of *Cajophora* by Poston and Thompson (1977) and Weigend (1997*b*).

An alliance of ser. *Floribundae* and *Macrospermae* with *Acanthifoliae* was also suggested by Müller et al. (1999) and Weigend et al. (2000). We have only *matK* data for *Loasa acanthifolia*, representing *Acanthifolia*, but those data indicate that it is most closely related to *Loasa pallida* (ser. *Floribundae*) + *Loasa elongata* (ser. *Deserticolae*), which is consistent with the Weigend et al. (2004) results, in which ser. *Floribundae* and *Deserticolae* were monophyletic.

Thus, the *Loasa* s.str. of Weigend (1997*b*) and Müller et al. (1999) is paraphyletic. Considerable additional taxon sampling among higher Loaseae will be necessary to resolve the constituent clades of this group, to hypothesize robust sister group relationships, and to provide a revised circumscription of *Loasa* based on monophyly. Our current results indicate that a monophyletic *Loasa*, centered on the type species *L. acanthifolia*, will include Urban and Gilg's ser. *Acanthifoliae*, *Deserticolae*, and *Floribundae*.

Aosa *Paraphyly*

Although Weigend et al. (2004) sampled *Chichicaste* and three species of *Aosa* for the *trnL* intron, which is part of the *trnL-trnF* region sampled here and by Hufford et al. (2003), they did not recover support for either the monophyly of *Aosa* or a clade consisting of *Aosa* and *Chichicaste*. We find very strong support for the monophyly of *Aosa* + *Chichicaste* and some support for the paraphyly of *Aosa*; however, *matK* and *trnL-trnF* conflict in their phylogenetic signal for the placement of *Chichicaste*. With only three species of *Aosa* sampled for these two markers (only one species sampled for *rpl20-rps12* and *psbA-trnH*), the *matK* data place *Chichicaste* as the sister of *Aosa rostrata* and *trnL-trnF* data place *Chichicaste* as the sister of *Aosa plumierii*; each of these alternative placements in the independent analyses of *matK* and *trnL-trnF* has moderate support. Indel characters provided additional support for the monophyly of *Aosa* and *Chichicaste*, including notably a four-nucleotide deletion in

the *trnL-trnF* region shared by all sampled species of both genera. Although taxon sampling was incomplete for the *psbA-trnH* marker, two unique indels (a three-nucleotide insertion and a three-nucleotide deletion) were shared by *A. rostrata* and *Chichicaste grandis* (fig. 3*A*), and in phylogenetic conflict with those indels was a unique nine-base insertion shared by *A. plumierii* and *C. grandis*. It will be important to add sequences for additional specimens of *Chichicaste* and other species of *Aosa* to our data to test further the placements found in our results.

Both *Aosa* and *Chichicaste* were segregated by Weigend (1997*b*) from the broadly circumscribed *Loasa* of Urban and Gilg (1900). Weigend's (1997*a*, 1997*b*) *Aosa*, which was limited to Brazil and Hispaniola, consists of six species distinguished by shared ebracteate inflorescences and a putatively unique tuberculate seed surface. *Chichicaste* lacks these potential apomorphies of *Aosa*; it has bracteate inflorescences and a reticulate seed surface that lacks notable tuberculae (*fide* Weigend 1997*b*). If our results reflect the true phylogenetic placement for *Chichicaste*, i.e., somewhere within *Aosa*, then its inflorescence and seed morphology states would be reversals. Weigend (1997*b*, p. 173) argued for the recognition of *Chichicaste* largely because it was ecologically aberrant as a member of lowland tropical rain forest, and he suggested it was "highly isolated in the subfamily." Such proposals of taxic "isolation" are vague, and their implications are unclear; however, our analyses of DNA sequence data do not find that *Chichicaste* has a long-branch relative to the aosas; thus, we argue, it is not "phylogenetically isolated." Although *Chichicaste* may be found in moister habitats than other Loasoideae, we note that *A. plumierii*, *A. rostrata*, and *Aosa parviflora* are also found in relatively moist, forested environments compared with most other Loasoideae. If our results are correct, then we recommend broadening the circumscription of *Aosa* to include the states of *Chichicaste*, which we propose be placed in synonymy with the former genus.

Huidobria *Paraphyly*

Huidobria was first described by Gay (1846) and included only *Huidobria chilensis*. A second species, *Huidobria fruticosa*, was described subsequently by Philippi (1855). Bentham and Hooker (1867) included *Huidobria* in *Loasa*, and Gilg (1895) combined it with *Loasa* as section *Huidobria*. Grau (1997) resurrected *Huidobria*, and Weigend (1997*b*) placed the genus as the sister to the rest of Loaseae in his hypothetical phylogeny of Loasoideae. Hufford et al. (2003) did not find support for the monophyly of *H. chilensis* and *H. fruticosa* but noted that the basal nodes of Loasoideae, which included these two species as well as Klaprothieae and Kissenieae, had very little support. Our results resolve *H. fruticosa* as the sister of the rest of Loasoideae and place *H. chilensis* as the sister of *Kissenia* + Klaprothieae, and none of our markers independently provide support for *Huidobria* monophyly. The conservative Templeton and SH tests find, however, no significant difference in tree length between our optimal cladograms and those constrained to force the monophyly of *H. chilensis* and *H. fruticosa*. The more sensitive parametric bootstrap indicates that our data significantly favor *Huidobria* paraphyly. We interpret these results with caution, however, because the parametric bootstrap is known to be sensitive to model choice and is prone to Type I errors under certain circumstances (Buckley 2002). These results emphasize that the *Huidobria* of Philippi (1855) and Grau (1997) remain problematic but lead us to suggest that further data will be necessary to test adequately its monophyly.

Both species of *Huidobria* have haploid chromosome numbers of n=18, which Grau (1997) used to support their exclusion from *Loasa*. The base chromosome numbers for Loasoideae are x=6, 7. If all Loasoideae were not initially polyploids with n=18, then polyploidy must have evolved independently in *H. chilensis* and *H. fruticosa* if our results reflect their true evolutionary relationships. Although a haploid chromosome number of n=18 is uncommon among Loasoideae for which chromosome numbers have been counted, it occurs also in *Loasa triloba* (Grau 1988) of the higher Loaseae; thus, there is evidence that n=18 has evolved independently more than once in the subfamily.

Although Grau (1997) and Weigend (1997*b*) have argued that *H. chilensis* and *H. fruticosa* display great similarity, putatively reflecting their monophyly, their shared attributes may be plesiomorphies. *Huidobria chilensis* and *H. fruticosa* have notable differences. The two species differ, for example, in the range of stamen numbers typically included in the development of scales. *Huidobria fruticosa* can include up to seven stamens and *H. chilensis* up to five stamens, according to Grau (1997). Both *H. chilensis* and *H. fruticosa* have staminodial scales composed of variable numbers of constituent stamens, in contrast to other Loaseae, in which scales appear highly canalized to consist of three stamens. We hypothesize that having more than three stamens compose the staminodial scales and having regular variation in the number of constituent stamens are plesiomorphic for Loasoideae; thus, the higher number of constituent stamens in scales and the variation in number are symplesiomorphies shared by *H. chilensis* and *H. fruticosa* rather than synapomorphies indicative of monophyly. The two species differ in the forms of their seeds and seed coat sculpturing. Although seeds of the two species are of approximately the same length, those of *H. fruticosa* are narrower and have a seed coat that has prominent longitudinal ridges with few cross walls (weakly reticulate) compared with those of *H. chilensis*, which are broader and have a more reticulate seed coat sculpture (as illustrated by Grau 1997; a specimen sampled by Hufford 1988 had a largely smooth seed coat). *Huidobria chilensis* and *H. fruticosa* also differ considerably in leaf morphology. *Huidobria fruticosa* has more or less ovate leaves generally reminiscent of those of *Kissenia* and *Presliophytum*; in contrast, *H. chilensis* has linear leaves that are unusual for Loasaceae.

The placement of *H. chilensis* as the sister of *Kissenia* + Klaprothieae is not supported by obvious morphological synapomorphies. *Kissenia* is found in habitats very similar to those of the huidobrias, and these taxa have common vegetative features, including leaf form shared by *Kissenia* and *H. fruticosa*; but *Kissenia* has diverged substantially in floral, fruit, and seed character states that often can be synapomorphic in Loasoideae. For example, *Kissenia* has shifted from the dehiscent fruits that are plesiomorphic for Loasoideae to

indehiscent fruits, and this has been accompanied by changes in sepal and seed morphology. The staminodial scales of *Kissenia* are simpler than those of most other Loaseae, including *H. chilensis* and *H. fruticosa*, lacking free parts of the constituent stamens and not forming a neck (Hufford 2003). Indeed, in terms of androecial form, especially of the staminodes, *Kissenia* and Klaprothieae have novelties that are not found among the rest of the Loasoideae.

Loaseae Paraphyly and Tribal Circumscriptions

In Loasoideae, Urban and Gilg (1900) delimited taxon-depauperate genera on the basis of unique characters and taxon-rich genera based largely on the relative absence of unique characters. They approached tribal circumscriptions in the same manner (Urban and Gilg 1900). Their Kissenieae (table 1), consisting only of *Kissenia*, is well delimited by the unique staminodial scales of flowers and winglike persistent sepals on the indehiscent fruits. Similarly, their Klaprothieae (table 1), consisting initially of *Klaprothia* and *Sclerothrix* (the latter was reduced to synonymy with *Klaprothia* by Poston and Nowicke 1990), was delimited by flowers that had a tetramerous perianth and staminodes in the outer whorl of the androecium that were entirely separate or unified only at the base. Although the discovery of *Plakothira perlmanii* (Florence 1997) and *Xylopodia* (Weigend 1997*b*) has modified our understanding of the diversity of staminodial scales in Klaprothieae, the tribe remains well delimited on the basis of morphological attributes (Weigend 1997*b*). The Gilg (1895, 1925; also Urban and Gilg 1900) circumscription of a taxon-rich Loaseae emphasized what we can now recognize as symplesiomorphies (at least at the level of Loasoideae), including pentamerous flowers, uniloculate fruits, and dimorphic staminodes, including staminodial scales composed of highly unified stamens in the outer whorl of the androecium and free staminodes in the inner whorl of the androecium.

Weigend (1997*b*; table 1) submerged Kissenieae in Loaseae, calling attention to the potential paraphyly of the latter. Our results, however, indicate that Loaseae are paraphyletic to both Kissenieae and Klaprothieae. The circumscription of Loaseae could be broadened to include genera of Kissenieae and Klaprothieae, but this would make Loaseae the same as Loasoideae. To retain Loaseae as a valuable taxon, we delimit a monophyletic group (figs. 2*B*, 3) identified as Loaseae s.str. Our Loaseae s.str. excludes *H. chilensis* and *H. fruticosa*. We propose that further phylogenetic studies are needed to explore the relationships of the huidobrias, but the circumscription of additional tribes might be warranted to capture phylogenetic knowledge in a revised classification of Loasoideae.

Conclusion

Our results contribute to resolving questions of tribal and generic circumscription and evolutionary relationships in Loasoideae. We find support for the monophyly of both Kissenieae and Klaprothieae as circumscribed by Urban and Gilg (1900) and, moreover, support for the novel result of a Kissenieae + Klaprothieae + *Huidobria chilensis* clade. Our results are notably inconsistent with the proposal by Weigend (1997*c*, p. 42) that Klaprothieae are "firmly connected" to Loaseae via *Aosa plumierii*. The monophyly of *Huidobria* requires further testing, and this will require additional phylogenetic data. If the phylogenetic analyses of our combined data sets provide accurate phylogenetic signal, then *Huidobria* is paraphyletic and *Huidobria fruticosa* is sister to all other Loasoideae. This scenario renders paraphyletic the Loaseae of Urban and Gilg (1900) and Weigend (1997*b*). On the basis of those results, we call attention to a Loaseae s.str. clade that consists of *Aosa*, *Blumenbachia*, *Cajophora*, *Chichicaste*, *Loasa*, *Nasa*, *Presliophytum*, and *Scyphanthus*. Inferences of monophyly and sister group relationships remain problematic in the well-supported clade, consisting of *Blumenbachia*, *Cajophora*, *Loasa* sect. *Loasa* (except *Loasa malesherbioides*), and *Scyphanthus*, designated here as the higher Loaseae, despite the contribution of sequences from the plastid intergenic spacers *rpl20-rps12* and *psbA-trnH*. The application of more informative markers to resolve relationships among lineages of higher Loaseae, as well as in *Nasa* and the *Aosa* + *Chichicaste* clade, remains a critical need. Although its circumscription has been controversial, we recover support for a monophyletic *Cajophora* that includes representatives of sections *Bialatae* and *Bicallosae*, contrary to the suggestion of Weigend (1997*b*). The monophyly, as well as the relationships, of the anomalous sections *Angulatae* and *Bialatae*, allied variously to *Cajophora* and *Blumenbachia*, require further investigation. Our results call attention to the paraphyly of *Loasa* s.str. as circumscribed by Weigend (1997*b*), and we have identified provisionally several independent lineages of the *Loasa* complex that may warrant recognition as segregate genera following additional phylogenetic sampling.

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Literature Cited

Bentham G, JD Hooker 1867 Genera plantarum. Vol 1, pt 3. Reeve, London.

Buckley TR 2002 Model misspecification and probabilistic tests of topology: evidence from empirical data sets. Syst Biol 51:509–523.

Dandy JE 1926 Notes on *Kissenia* and the geographical distribution of the Loasaceae. Kew Bull 1926:174–180.

Doyle JJ, JL Doyle 1987 A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem Bull 19:11–15.

Felsenstein J 1985 Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39:783–791.

Florence J 1997 New species of *Plakothira* (Loasaceae), *Melicope* (Rutaceae), and *Apetahia* (Campanulaceae) from the Marquesas Islands. Allertonia 7:238–253.

Gay C 1846 Historia física y política de Chile. Botanica 2. Gay, Paris.

Gilg E 1895 Loasaceae. Pages 100–121 *in* A Engler, K Prantl, eds. Die natürlichen Pflanzenfamilien. Vol 3, pt 6a. Engelmann, Leipzig.

——— 1925 Loasaceae. Pages 522–543 *in* A Engler, K Prantl, eds. Die natürlichen Pflanzenfamilien. 2nd ed. Vol 21. Engelmann, Leipzig.

Goldman N, JP Anderson, AG Rodrigo 2000 Likelihood-based tests of topologies in phylogenetics. Syst Biol 49:652–670.

Grau J 1988 Chromosomenzahlen chilenischer Loasaceae. Mitt Bot Staatsamml Münch 27:7–14.

——— 1997 *Huidobria*, eine isolierte Gattung der Loasaceae aus Chile. Sendtnera 4:77–93.

Hamilton MB 1999 Four primer pairs for the amplification of chloroplast intergenic regions with intraspecific variation. Mol Ecol 8:521–523.

Hasegawa M, H Kishino, T Yano 1995 Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. J Mol Evol 21:160–174.

Hempel A, PA Reeves, RG Olmstead, RK Jansen 1995 Implications of *rbc*L sequence data for higher order relationships of the Loasaceae and the anomalous aquatic plant *Hydrostachys* (Hydrostachyaceae). Plant Syst Evol 194:25–37.

Huelsenbeck JP, DM Hillis, R Nielsen 1996 A likelihood ratio test of monophyly. Syst Biol 45:546–558.

Hufford L 1988 Seed coat morphology of *Eucnide* and other Loasaceae. Syst Bot 13:154–167.

——— 2003 Homology and developmental transformation: models for the origins of the staminodes of Loasaceae subfamily Loasoideae. Int J Plant Sci 164(suppl):S409–S439.

Hufford L, M McMahon, AM Sherwood, G Reeves, MW Chase 2003 The major clades of Loasaceae: phylogenetic analysis using the plastid *matK* and *trnL-trnF* regions. Am J Bot 90:1215–1228.

Hufford L, ML Moody, DE Soltis 2001 A phylogenetic analysis of Hydrangeaceae based on sequences of the plastid gene *matK* and their combination with *rbcL* and morphological data. Int J Plant Sci 162:835–846.

Johnson LA, DE Soltis 1995 Phylogenetic inference in Saxifragaceae sensu stricto and *Gilia* (Polemoniaceae) using *matK* sequences. Ann Mo Bot Gard 82:149–175.

Lemmon AR, EC Moriarty 2004 The importance of proper model assumption in Bayesian phylogenetics. Syst Biol 53:265–277.

Minin V, Z Abdo, P Joyce, J Sullivan 2003 Performance-based selection of likelihood models for phylogeny estimation. Syst Biol 52:674–683.

Moody ML, L Hufford, DE Soltis, PS Soltis 2001 Phylogenetic relationships of Loasaceae subfamily Gronovioideae inferred from *matK* and ITS sequence data. Am J Bot 88:326–336.

Müller A, JK Kufer, KG Dietl, SA Reiter, J Grau, M Weigend 1999 Iridoid glucosides: chemotaxonomic markers in Loasoideae. Phytochemistry 52:67–78.

Olmstead RG, KJ Kim, RK Jansen, SJ Wagstaff 2000 The phylogeny of Asteridae sensu lato based on chloroplast *ndhF* gene sequences. Mol Phylogenet Evol 16:96–112.

Philippi A 1855 *Huidobria fruticosa*. Anales Univ Chile 13:219.

Poston ME, JW Nowicke 1990 A reevaluation of *Klaprothia* and *Sclerothrix* (Loasaceae: Klaprothieae). Syst Bot 15:671–678.

Poston ME, HJ Thompson 1977 Cytotaxonomic observations in Loasaceae subfamily Loasoideae. Syst Bot 2:28–35.

Rambaut A 1996 Se-Al: sequence alignment editor, version 1. http://evolve.zoo.ox.ac.uk/software.html.

Rambaut A, NC Grassly 1997 Seq-Gen: an application for the Monte Carlo simulation of DNA sequence evolution along phylogenetic trees. Comput Appl Biosci 13:235–238.

Shimodaira H, M Hasegawa 1999 Multiple comparisons of log-likelihoods with applications to phylogenetic inference. Mol Biol Evol 16:1114–1116.

Swofford DL 2002 PAUP*: phylogenetic analysis using parsimony (*and other methods), version 4.0. Sinauer, Sunderland, MA.

Taberlet P, L Gielly, G Pautou, J Bouvet 1991 Universal primers for the amplification of three non-coding regions of chloroplast DNA. Plant Mol Biol 17:1105–1109.

Templeton AR 1983 Phylogenetic inference from restriction endonuclease cleavage site maps with particular reference to the evolution of humans and the apes. Evolution 37:221–244.

Urban I, E Gilg 1900 Monographia Loasacearum. Nova Acta Leopold 76:1–370.

Weigend M 1997*a* Loasoideae in eastern South America and on Hispaniola: names, types and a key. Sendtnera 4:207–220.

——— 1997*b* *Nasa* and the conquest of South America. PhD diss. Ludwig-Maximilians-University, Munich.

——— 1997*c* Some aspects of the biogeography, morphology and systematics of Loasoideae in northern South America. Pages 37–50 *in* R Valencia, H Balslev, eds. Estudios sobre diversidad y ecologia de plantas. Pontificia Universidad Católica del Ecuador, Quito.

Weigend M, M Gottschling, S Hoot, M Ackermann 2004 A preliminary phylogeny of Loasaceae subfam. Loasoideae (Angiospermae: Cornales) based on $trnL_{(UAA)}$ sequence data, with consequences for systematics and historical biogeography. Org Divers Evol 4: 73–90.

Weigend M, J Kufer, AA Müller 2000 Phytochemistry and the systematics and ecology of Loasaceae and Gronoviaceae (Loasales). Am J Bot 87:1202–1210.

Xiang QY, ML Moody, DE Soltis, CZ Fan, PS Soltis 2002 Relationships within Cornales and circumscriptions of Cornaceae: *matK* and *rbcL* sequence data and effects of outgroups and long branches. Mol Phylogenet Evol 24:35–57.

Xiang QY, DE Soltis, DR Morgan, PS Soltis 1993 Phylogenetic relationships of *Cornus sensu lato* and putative relatives inferred from *rbcL* sequence data. Ann Mo Bot Gard 80:723–734.

Xiang QY, DE Soltis, PS Soltis 1998 Phylogenetic relationships of Cornaceae and close relatives inferred form *mat*K and *rbcL* sequences. Am J Bot 85:285–297.

Yang Z 1994 Maximum likelihood phylogenetic estimation from DNA sequences with variable rates over sites. J Mol Evol 39: 306–314.