The major clades of Loasaceae: phylogenetic analysis using the plastid *matK* and *trnL-trnF* regions[1]

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Phylogenetic analyses of Loasaceae that apply DNA sequence data from the plastid *trnL-trnF* region and *matK* gene in both maximum-parsimony and maximum-likelihood searches are presented. The results place subfamily Loasoideae as the sister of a subfamily Gronovioideae-*Mentzelia* clade. *Schismocarpus* is the sister of the Loasoideae-Gronovioideae-*Mentzelia* clade. The *Schismocarpus*-Loasoideae-Gronovioideae-*Mentzelia* clade is the sister of *Eucnide*. Several clades in Loasoideae receive strong support, providing insights on generic circumscription problems. Within *Mentzelia*, several major clades receive strong support, which clarifies relationships among previously circumscribed sections. Prior taxonomic and phylogenetic hypotheses are modeled using topology constraints in parsimony and likelihood analyses; tree lengths and likelihoods, respectively, are compared from constrained and unconstrained analyses to evaluate the relative support for various hypotheses. We use the Shimodaira-Hasegawa (SH) test to establish the significance of the differences between constrained and unconstrained topologies. The SH test rejects topologies based on hypotheses for (1) the placement of gronovioids as the sister of the rest of Loasaceae, (2) the monophyly of subfamily Mentzelioideae as well as Gronovioideae and Loasoideae, (3) the monophyly of *Loasa* sensu lato as circumscribed by Urban and Gilg, and (4) the monophyly of *Mentzelia torreyi* and *Mentzelia* sect. *Bartonia*.

Key words: Loasaceae; maximum likelihood; parsimony; phylogeny; Shimodaira-Hasegawa (SH) test; systematics.

Loasaceae were established as a family, consisting of the genera *Loasa* and *Mentzelia*, by de Jussieu (1804). Flowers of these two genera are choripetalous, polystemonous, and syncarpous, usually with several ovules. The morphological disparity of Loasaceae was heightened when Bartling (1825) included in the family *Gronovia*, in which flowers are haplostemonous and have only a single ovule. Reichenbach (1837) was the first to circumscribe genera in the three groups Gronovieae, Mentzelieae, and Blumenbachieae that would ultimately correspond to the modern subfamilies. Not all 19th-century systematists accepted Reichenbach's groups. For example, Baillon (1888) recognized only two series in the family, including Gronovieae, corresponding to Reichenbach's group of that name, and Loaseae, which combined Mentzelieae and Blumenbachieae of Reichenbach. In contrast to workers who have grouped gronovioid, mentzelioid, and loasoid taxa as Loasaceae, Endlicher (1841) and Weigend (1997; Weigend et al. 2000) segregated the gronovioids from Loasaceae as the family Gronoviaceae.

Gilg (1895, 1925) and especially Urban and Gilg (1900)

provided influential systematic treatments of Loasaceae in which the subfamilies Gronovioideae, Mentzelioideae, and Loasoideae were circumscribed in a manner that has been largely followed since those publications (including Weigend [1997] despite his elevation of Gronovioideae to the familial level). The monograph of Urban and Gilg (1900), a comprehensive taxonomic treatment of Loasaceae, presented revisions for genera and provided several new subgeneric groups. Since Urban and Gilg (1900), generic circumscriptions have been debated. Thompson and Ernst (1967) revised *Eucnide* and reduced *Sympetaleia* to synonymy within *Eucnide*. Various workers have questioned broad circumscriptions of the larger genera *Cajophora*, *Loasa*, and *Mentzelia*. 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) excluded sections *Angulatae* and *Bialatae* from *Cajophora* and placed them in *Blumenbachia*. Grau (1997) resurrected *Huidobria*, which had been included as a section of *Loasa* s.l. by Urban and Gilg (1900). Weigend (1997) segregated the new genera *Aosa*, *Chichicaste*, *Nasa*, and *Presliophytum* from *Loasa* s.l. Several authors have advocated segregating the genera *Acrolasia* and *Nuttallia* from *Mentzelia* (Rydberg, 1903; Davidson, 1916; Weber and Wittman, 2001). Brown (1971), Hempel (1995; Hempel and Jansen, 1996), and Weigend (1997) considered *Mentzelia* to be paraphyletic. Aside from the several segregate genera, only three new genera lying clearly outside of those circumscribed by Urban and Gilg (1900) have been described. The first of these, *Schismocarpus*, was described by Blake (1918) and allied with Mentzelioideae. The two other novel genera, *Plakothira* of Florence (1985) and *Xylopodia* of Weigend (1997), were relegated to Loasoideae.

These taxonomic studies have provided considerable insight

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into the distribution of character diversity among Loasaceae and clarified key issues in the systematics of the family. Circumscription problems and questions of evolutionary diversification, however, require an approach centered primarily on hypotheses of monophyletic groups and their relationships. Hufford (1988) first applied phylogenetic systematics to problems of monophyly in the Loasaceae; however, his analysis of morphological characters derived strictly from the literature resolved few relationships. Subsequent phylogenetic studies by Poston and Nowicke (1993) and Hempel (1995; Hempel and Jansen, 1996) were hampered by limited taxon sampling. Moody et al. (2001) considerably advanced our understanding of relationships in Loasaceae in a phylogenetic study that sampled taxa broadly within the family and applied analyses of DNA sequences of the plastid gene *matK* to examine the placement of gronovioids. The results of Moody et al. (2001) identified several major clades in Loasaceae, including the Gronovioideae-*Mentzelia* clade, and provided good support for many monophyletic groups.

Our phylogenetic analyses extend those of Moody et al. (2001) by adding DNA sequence data from the plastid *trnL-trnF* region (which includes the *trnL* intron and *trnL-trnF* intergenic spacer; Taberlet et al., 1991) to data from the *matK* gene and increasing taxon sampling within Loasaceae. Both maximum-parsimony and maximum-likelihood analyses are applied as optimality criteria for the selection of phylogenetic trees. We examine further the support for major clades of Loasaceae, considering especially Loasoideae and *Mentzelia*, and clarify where additional data are needed to resolve clades. We explicitly evaluate whether prior circumscriptions at the familial, subfamilial, generic, and subgeneric levels correspond to monophyletic groups and, if so, how well they are supported by our character data. Our approach is to model existing circumscriptions and hypotheses of evolutionary relationship as topologies, then search for the most parsimonious and most likely trees using these topologies as constraints. We compare the tree lengths and likelihoods, respectively, from constrained and unconstrained analyses as a means to evaluate the relative support, using our data, for various hypotheses. We use the Shimodaira-Hasegawa test (Shimodaira and Hasegawa, 1999) to establish the significance of the differences we find in constrained and unconstrained topologies from maximum-likelihood analyses.

MATERIALS AND METHODS

Taxon sampling—Outgroups were selected on the basis of 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). Nine outgroups were used for the analysis of *matK*; based on those results, three outgroups were applied for the analysis of the *trnL-trnF* and the combination of *matK* and *trnL-trnF*. Our sampling in Loasaceae aimed to encompass groups delimited in or suggested to be problematic by earlier taxonomic studies (Urban and Gilg, 1900; Gilg, 1925; Darlington, 1934; Daniels, 1970; Brown, 1971; Poston and Thompson, 1977; Poston and Nowicke, 1993; Hempel, 1995; Weigend, 1997). We intensified taxon sampling when possible in clades of the family found by Moody et al. (2001) to examine the composition of monophyletic groups. Our sampling includes representatives from most suprageneric entities recognized in Urban and Gilg's (1900) comprehensive monograph of Loasaceae. Among the genera recognized in modern treatments of the family, all were sampled except for those described only recently, including *Xylopodia*, which is known only from the type specimens, and the segregate genus *Chichicaste* (Weigend, 1997). We sampled broadly among the species recognized in the sections of

Mentzelia. For a list of taxa and accession sampled see the Supplementary Data accompanying the online version of this article.

DNA sequences—The *matK* sequences for several outgroups and Loasaceae were obtained from Moody et al. (2001). New *matK* sequences were obtained for an additional 32 Loasaceae. All *trnL-trnF* sequences used in the study were new. Total DNA was extracted from either herbarium or silica-dried specimens of leaves using a standard cetyltrimethylammonium bromide (CTAB) procedure (Doyle and Doyle, 1987). The polymerase chain reaction (PCR) mixes varied somewhat, although most used 20 mmol/L Tris-HCl pH 8.3, 50 mmol/L KCl, 1.5 mmol/L MgCl₂, 0.01% Tween-20, 150 μmol/L dNTPs, 0.5 μmol/L forward- and reverse-amplification primers, diluted DNA template (1:10–1:100, depending on extraction quality), and water to a total volume of 25 μL. The PCR primers for *matK* were *matK*-710F and *trnK*-2R (Johnson and Soltis, 1995) and for *trnL-trnF* were c and f (Taberlet et al., 1991). Sequencing primers were *matK*-710F, *trnK*-2R, *matK*-1713F, and *matK*-1848R (citations in Moody et al., 2001) and *trnL* c and f. Sequences were aligned manually in Se-Al (Rambaut, 1996). In selected regions of the aligned sequences, character homology was equivocal; hence, for the analysis one 6-bp region of *matK* and 20 short regions of *trnL-trnF* were deleted.

Phylogenetic analyses—All phylogenetic analyses used PAUP* 4.0 (Swofford, 2002). Parsimony analyses were conducted independently on the *matK* and *trnL-trnF* data sets and on a data set in which *matK* and *trnL-trnF* sequences were combined. Heuristic searches included 1000 starting trees built by random taxon addition followed by tree bisection-reconnection (TBR) branch-swapping. All character state transitions were equally weighted and unordered. Indels were treated as missing data. 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. The robustness of clades was assessed using branch decay (Bremer, 1988; Donoghue et al., 1992) and bootstrap (Felsenstein, 1985) analyses. Decay analyses were implemented using AutoDecay (Eriksson, 1999) and PAUP* (Swofford, 2002). Bootstrap analyses were implemented in PAUP* using the heuristic search procedures described, including 500 pseudoreplicates for the analysis of the *trnL-trnF* data and 1000 pseudoreplicates for the analyses of the *matK* and combined data sets (maxtrees was set at 1000 for the independent data sets and 2000 for the combined data set).

Maximum-likelihood (ML) analyses were conducted only on the combined *matK* and *trnL-trnF* data. To reduce computational times, taxon sampling was reduced to two outgroup and 27 ingroup taxa. Taxa were sampled to represent the major clades found in the parsimony searches and to facilitate evaluating prior taxonomic and phylogenetic hypotheses. Modeltest (Posada and Crandall, 1998), which uses likelihood ratio tests to compare 56 alternative models for DNA substitution, was used to select a substitution model for the reduced taxon data set. Two tree topologies, one inferred using maximum parsimony (as specified above for the combined data set) and the other using neighbor joining, were used in Modeltest to determine the model that best fit the combined *matK* and *trnL-trnF* data. The model of DNA substitution selected by Modeltest, a submodel of the GTR + Γ model in which only one parameter was needed for transitions, was the same for both of the preliminary topologies. The ML analysis used was a heuristic search procedure that included a single starting tree built by random taxon addition followed by TBR branch-swapping. The robustness of the ML topology was assessed using bootstrap analysis, which was limited to 50 pseudoreplicates because of computational time. The bootstrap analysis used a heuristic procedure in PAUP* that included random taxon addition and TBR branch swapping.

Alternative topologies—Hypotheses of taxonomic groups and their interrelationships can be modelled as cladogram topologies. We designed five constraint topologies based on existing hypotheses of relationships; each constraint specified a few nodes to force the monophyly of selected groups of taxa. We applied these constraints to searches using the combined *matK* and *trnL-trnF* data under both parsimony and likelihood criteria. The parsimony analyses used the same 70 taxa as in the unconstrained parsimony analysis

of the combined data. We conducted full heuristic searches for the most parsimonious cladograms under each of the topology constraints (all analyses swapped to completion), permitting us to compare the lengths of constrained topologies to that of the most parsimonious topologies from the unconstrained analyses. Constrained ML searches used the same 29 taxa, model of DNA substitution, and search procedure as the unconstrained ML search described earlier.

We used the Shimodaira-Hasegawa (SH) test (Shimodaira and Hasegawa, 1999) to compare the results of our analyses to prior hypotheses. The SH test provides a statistical evaluation of the differences in likelihoods between trees of interest; we compared those trees that resulted from the constrained and unconstrained ML analyses. Some likelihood-based tests are compromised by the inclusion of both a priori and a posteriori hypotheses (e.g., the Kishino-Hasegawa test; Shimodaira and Hasegawa, 1999; Goldman et al., 2000). To address this problem, investigators have advocated as viable alternatives either parametric bootstrapping (Huelsenbeck et al., 1996) or the SH test (Shimodaira and Hasegawa, 1999; Goldman et al., 2000), which is less computationally intensive and more conservative (Buckley, 2002). The SH test adjusts the significance values as needed for multiple comparisons and conducts one-tailed tests that are appropriate when some of the trees are determined a posteriori (Shimodaira and Hasagawa, 1999; Goldman et al., 2000). The SH test is designed for multiple, simultaneous comparisons among trees and assumes that the true tree is included. The set of topologies that we compared included the ML tree from the unconstrained search, the most likely tree from each constrained ML search, and a set of trees based on a parsimony analysis of the 29 taxon data set. Trees from a parsimony analysis were included because of the possibility that the true tree might not be the ML tree from the unconstrained analysis. From the parsimony analysis, we included the most parsimonious tree (same topology as the ML tree) and all trees up to five steps longer (a total of 887 trees) in the SH test. It was logistically impractical to consider all possible topologies for the 29 taxon data set in the SH test; topologies up to five steps longer than the most parsimonious tree provided a broad range of trees that shared the well-supported branches found in our analyses, and we were able to include all trees up to five steps longer that were found. Shimodaira and Hasegawa (1999, p. 1115) suggested that "extremely unlikely topologies" not be included in the set of topologies compared in the SH test, and topologies more than five steps longer than the most parsimonious tree tended to have multiple branches that were inconsistent with existing ideas about relationships in Loasaceae. Earlier studies that applied the SH test have not included as large a set of topologies for simultaneous comparison (e.g., Buckley et al., 2001; Hahn, 2002; Leaché and Reeder, 2002; Silberman et al., 2002; Turmel et al., 2002). The SH test was implemented in PAUP*, using 1000 bootstrap replicates that applied the RELL method for resampling (Kishino et al., 1990) to establish a null distribution for the test statistic.

RESULTS

Parsimony analysis of matK—The aligned matrix included 77 taxa (68 Loasaceae) and 1700 characters, among which 959 were constant, 332 were variable but parsimony uninformative, and 409 were parsimony informative. The parsimony search did not swap to completion and was stopped after saving 28 000 trees of 1384 steps (consistency index [CI] = 0.58; retention index [RI] = 0.84; rescaled consistency index [RC] = 0.59; consensus cladogram in Fig. 1). The data provided very strong support (bootstrap [BS] ≥ 95%) for the monophyly of several major clades, including Loasoideae-Gronovioideae-*Mentzelia*, Loasoideae, *Presliophytum-Loasa-Blumenbachia-Cajophora-Scyphanthus*, *Presliophytum-Loasa maleshenbioides*, Klaprothieae, Gronovioideae-*Mentzelia*, Gronovioideae, and *Cevallia-Fuertesia-Gronovia*. Several genera also received a similar level of very strong support, including *Blumenbachia*, *Cajophora*, *Presliophytum*, *Nasa*, *Petalonyx*, *Mentzelia*, and *Eucnide*. Support was particularly

strong for "backbone" nodes of *Mentzelia* (BS ≥ 94%). The basal nodes of Loasaceae, including the *Eucnide* and *Schismocarpus* clades, received more modest support (BS = 78–82%).

Parsimony analysis of trnL-trnF region—The aligned matrix included 70 taxa (67 Loasaceae) and 915 characters, among which 600 were constant, 157 were variable but parsimony uninformative, and 158 were parsimony informative. The parsimony search did not swap to completion and was stopped after saving 20 000 trees of 498 steps (CI = 0.64; RI = 0.90; RC = 0.69; consensus cladogram in Fig. 2). The *trnL-trnF* results were largely consistent with those based on *matK*; exceptions are limited to nodes that have weak support (e.g., *Schismocarpus* was placed in the *trnL-trnF* results as the sister of the Gronovioideae-*Mentzelia* clade, but the result was not robust under bootstrap analysis [Fig. 2]). The monophyly of several genera, including *Eucnide*, *Petalonyx*, *Nasa*, *Presliophytum*, *Cajophora*, and *Blumenbachia*, received very strong support (BS ≥ 94%).

Parsimony analysis of combined matK and trnL-trnF—The aligned matrix included 71 taxa (68 Loasaceae) and 2615 characters, among which 1747 were constant, 453 were variable but parsimony uninformative, and 415 were parsimony informative. The parsimony search, which swapped to completion, found 2795 trees of 1383 steps (CI = 0.75; RI = 0.93; RC = 0.79; consensus cladogram in Fig. 3, phylogram in Fig. 4). The results placed *Eucnide* as the sister of the rest of Loasaceae. *Schismocarpus* formed the sister of the Loasoideae-Gronovioideae-*Mentzelia* clade, which received strong support. Loasoideae were very strongly supported (BS = 100%). Within Loasoideae, the monophyly of several clades received very strong support, including *Presliophytum-Loasa-Cajophora-Scyphanthus-Blumenbachia* (BS = 98%), *Presliophytum-Loasa maleshenbioides* (BS = 98%), *Presliophytum* (BS = 100%), *Cajophora-Scypthanthus* (BS = 99%), *Cajophora* (BS = 100%), *Blumenbachia* (BS = 100%), *Nasa* (BS = 100%) and *Nasa chenopodiifolia-N. urens* (BS = 100%). There was very strong support for the monophyly of the Gronovioideae-*Mentzelia* clade (BS = 99%) and several of its constituent clades, including Gronovioideae (BS = 99%), *Petalonyx* (BS = 100%), *Cevallia-Fuertesia-Gronovia* (BS = 99%), *Fuertesia-Gronovia* (BS = 100%), and *Mentzelia* (BS = 100%). Within *Mentzelia*, *M. torreyi* (sect. *Micromentzelia*) was placed as the sister to a clade consisting of sects. *Mentzelia*, *Dendromentzelia*, *Trachyphytum*, *Bicuspidaria*, and *Bartonia* (BS = 100%). Within the clade of the latter sections, there was very strong support (BS = 100%) for the *Trachyphytum-Bicuspidaria-Bartonia* clade and its constituent *Bicuspidaria-Bartonia* clade. The individual sects. *Mentzelia*, *Trachyphytum*, and *Bartonia* were each very strongly supported (BS = 100%) as monophyletic. The monophyly of sect. *Bicuspidaria* was modestly supported (BS = 88%). Notably, no support was found for relationships within sect. *Bartonia*.

Maximum-likelihood analysis of combined matK and trnL-trnF—The ML search swapped to completion, and the topology had a log likelihood of −10 501.523. This topology (Fig. 5) was entirely consistent with the results of the parsimony analysis of the combined data set (Fig. 3). Parsimony analysis of the 29 taxon data set resulted in a single most parsimonious tree that had a topology identical to the ML tree.
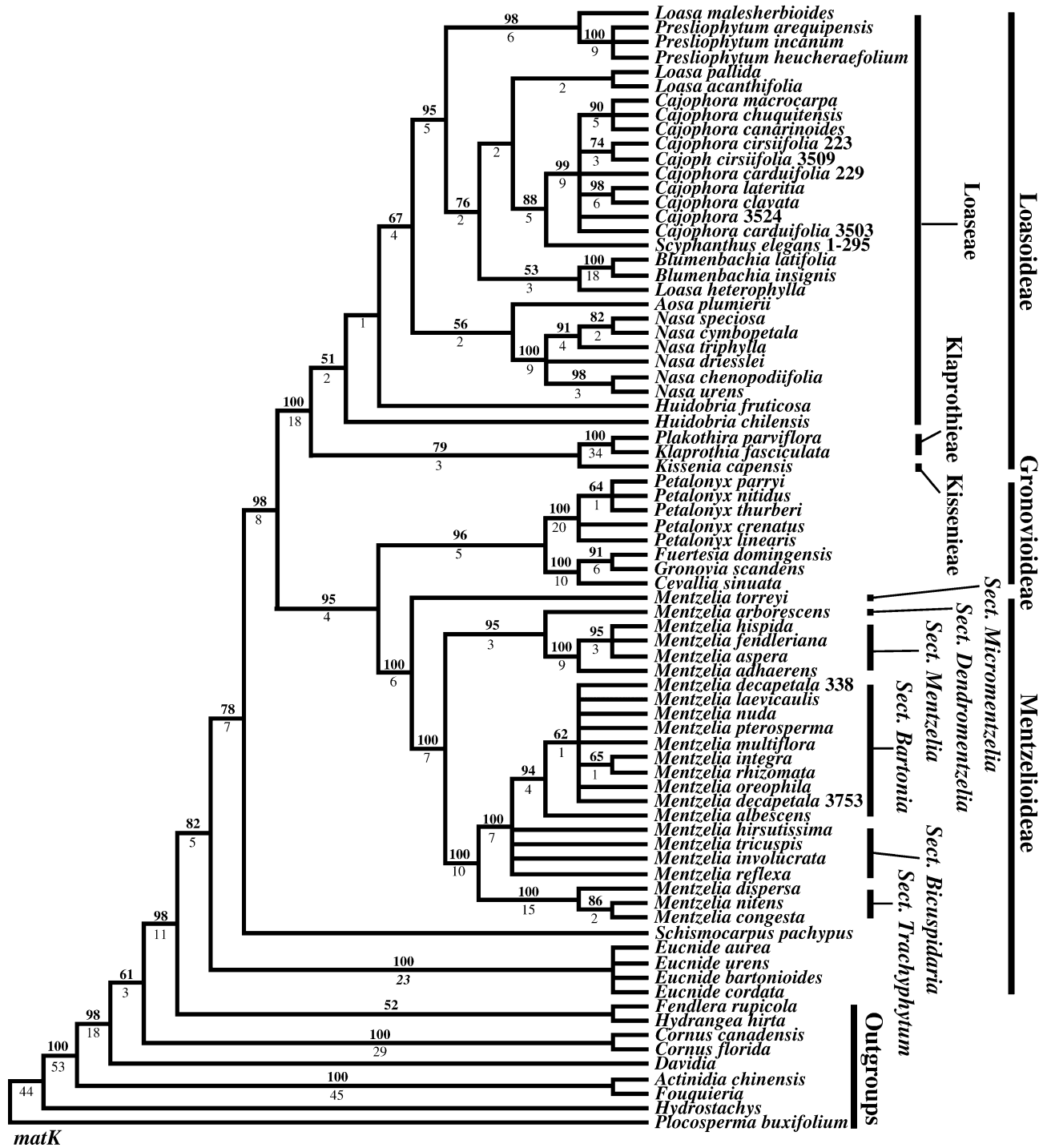
Fig. 1. Strict consensus of 28 000 trees from the parsimony search of the *matK* sequence data for Loasaceae (CI = 0.58; RI = 0.84; RC = 0.59). Numbers above clades are bootstrap percentages when 50% or above, and numbers below clades are branch decay values. The subfamilies of Loasaceae, tribes of subfamily Loasoideae, and sections of *Mentzelia* as circumscribed by Urban and Gilg (1900) are indicated. Numbers that follow taxon names refer to collection numbers provided in the Supplementary Data accompanying the online version of this article.

Alternative topologies—The five MP analyses conducted under topological constraints resulted in trees that were longer than the most parsimonious cladogram from the unconstrained parsimony analysis (Figs. 6–10; Table 1). Constraint topologies involving *Mentzelia* resulted in both the longest and shortest alternative trees. The constraint topology that forced *M.*

reflexa to form a monophyletic group with accepted taxa of sect. *Bartonia* resulted in trees that were only two state changes longer than the most parsimonious cladograms (Fig. 9). In contrast, constraint topologies that forced *M. torreyi* to form a monophyletic group with accepted taxa of sect. *Bartonia* resulted in trees that were 36 state changes longer than the
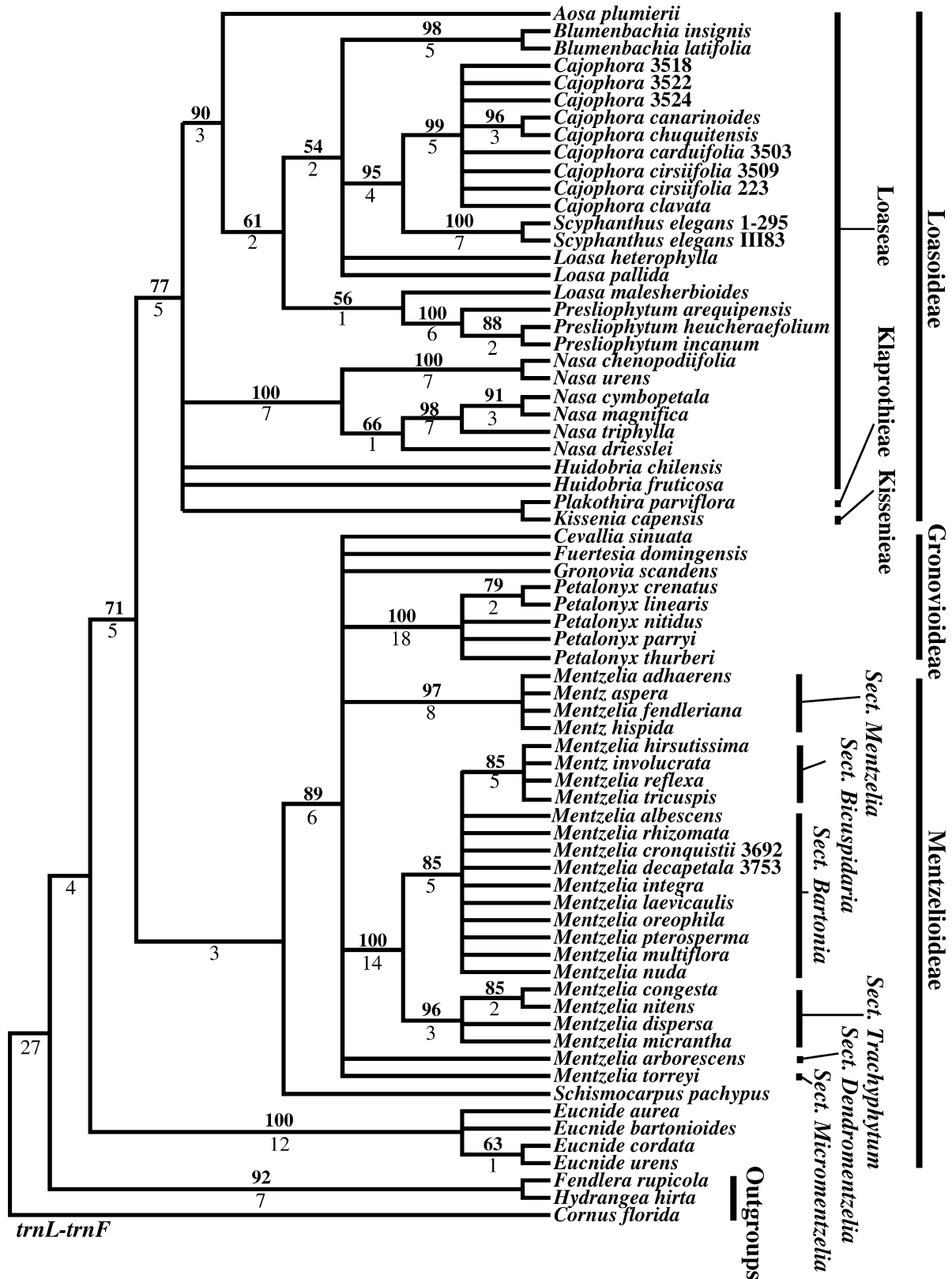
Fig. 2.   Strict consensus of 20 000 trees from the parsimony search of the *trnL-trnF* sequence data for Loasaceae (CI = 0.64; RI = 0.90; RC = 0.69). Numbers above clades are bootstrap percentages when 50% or above, and numbers below clades are branch decay values. The subfamilies of Loasaceae, tribes of subfamily Loasoideae, and sections of *Mentzelia* as circumscribed by Urban and Gilg (1900) are indicated. Numbers that follow taxon names refer to collection numbers provided in the Supplementary Data accompanying the online version of this article.
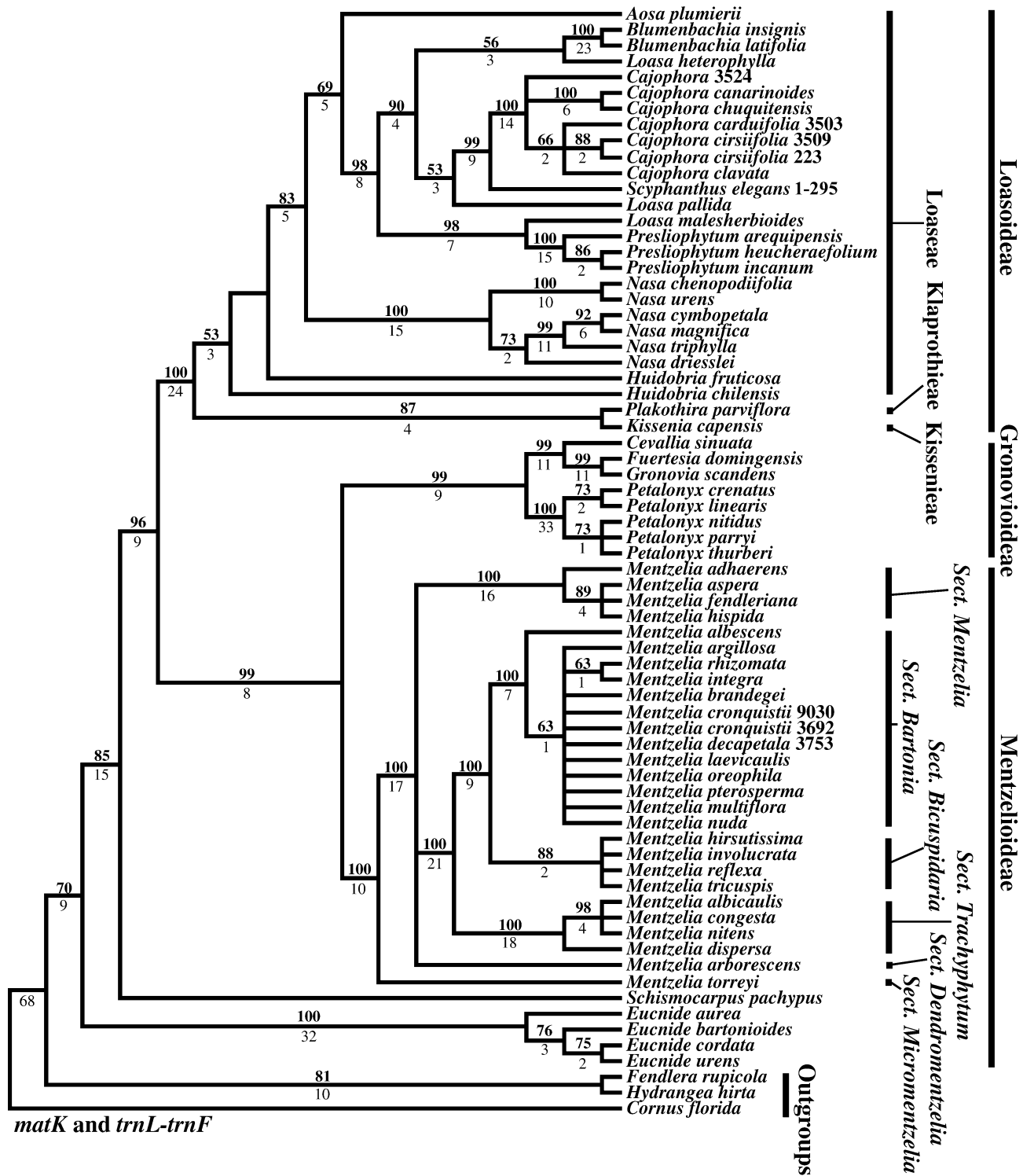
Fig. 3. Strict consensus of 2795 trees from the parsimony search of the combined *matK* and *trnL-trnF* sequence data for Loasaceae (CI = 0.75; RI = 0.93; RC = 0.79). Numbers above clades are bootstrap percentages when 50% or above, and numbers below clades are branch decay values. The subfamilies of Loasaceae, tribes of subfamily Loasoideae, and sections of *Mentzelia* as circumscribed by Urban and Gilg (1900) are indicated. Numbers that follow taxon names refer to collection numbers provided in the Supplementary Data accompanying the online version of this article.

most parsimonious cladograms (Fig. 10). The other three constrained topologies had similar costs. The constrained analyses that forced the monophyly of the broadly circumscribed *Loasa* (including taxa recognized here as the genera *Aosa*, *Huidobria*,

Nasa, and *Presliophytum* as well as *Loasa* s.l.) *fide* Urban and Gilg (1900) resulted in trees that were 15 state changes longer than the most parsimonious trees (Fig. 8). The constrained analysis that forced the monophyly of each of the three sub-
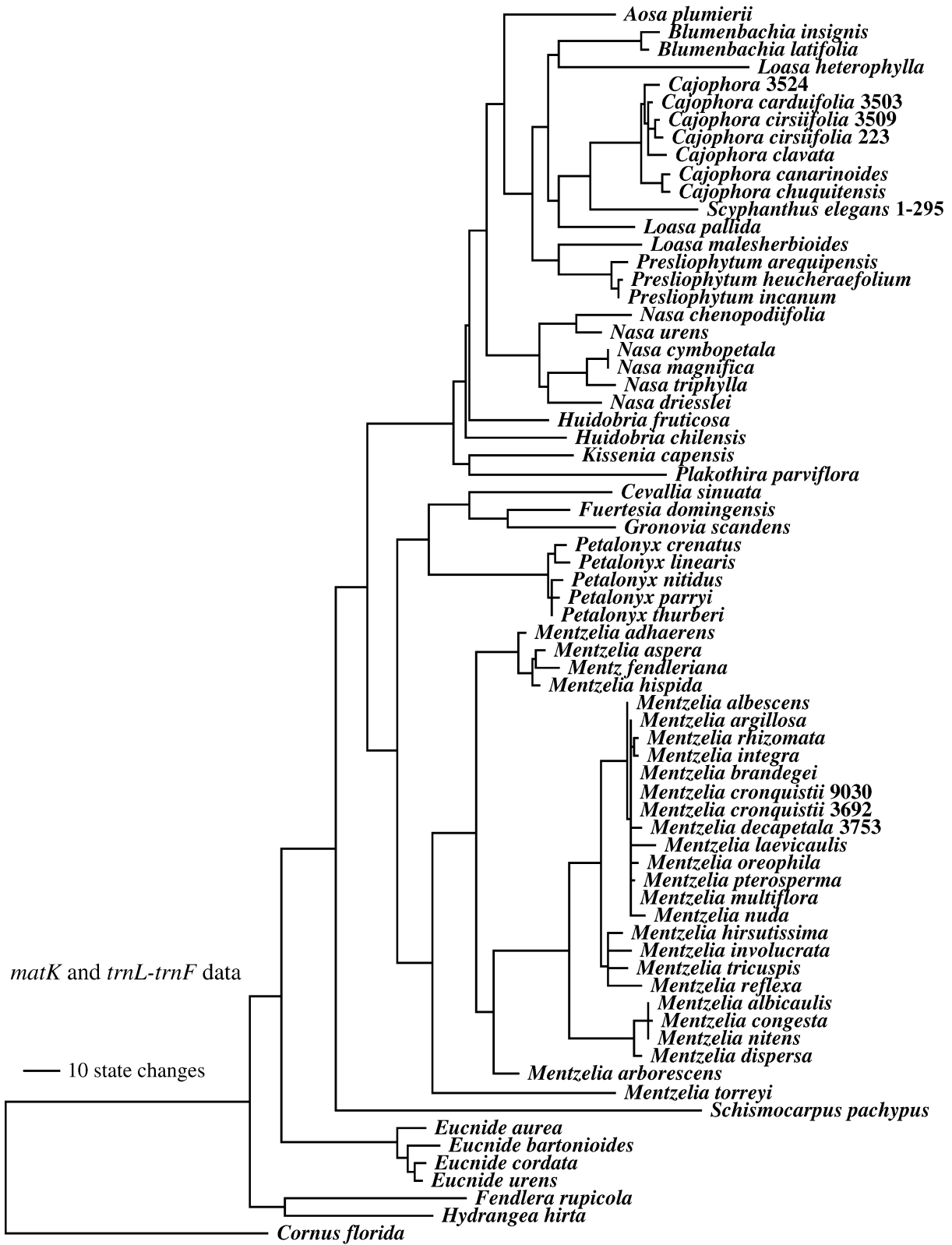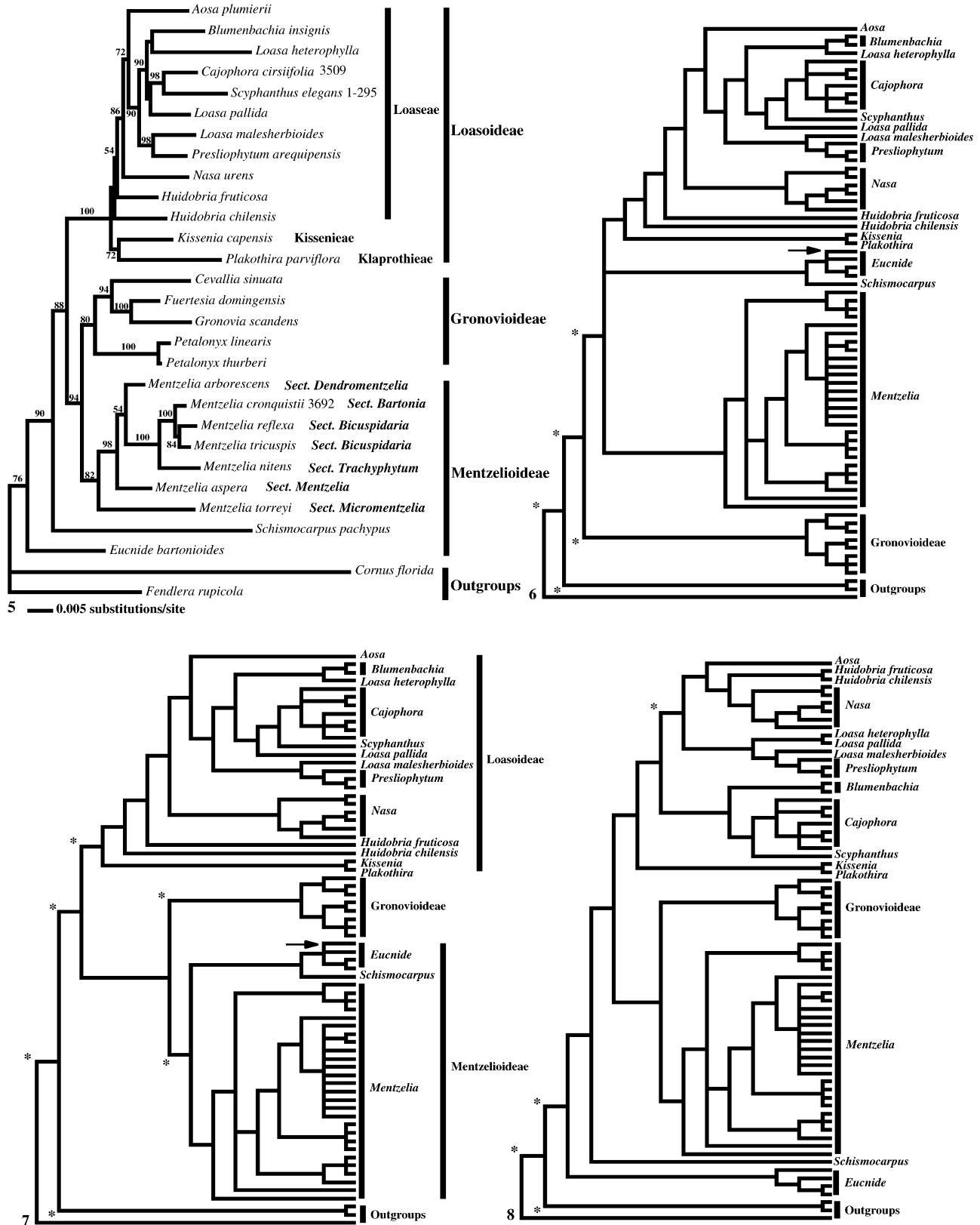
Fig. 4.   Phylogram of one most parsimonious tree of 1383 state changes from the parsimony search of the combined *matK* and *trnL-trnF* sequence data for Loasaceae. Numbers that follow taxon names refer to collection numbers provided in the Supplementary Data accompanying the online version of this article.

Figs. 5–8. Cladograms of Loasaceae. **5.** Best tree (−lnL = 10 501.523) from the maximum-likelihood search of the combined *matK* and *trnL-trnF* sequence data for Loasaceae. Numbers above clades are bootstrap percentages when 50% or above. Numbers that follow taxon names refer to collection numbers provided in the Supplementary Data accompanying the online version of this article. 6–8. Strict consensus cladograms from constrained parsimony analyses. Asterisks indicate nodes specified in topology constraints. Clades within selected genera on which the names of species have been deleted have the same relationships as shown in Fig. 3 (unless noted). **6.** Tree constrained to have gronovioids as the sister to the rest of Loasaceae (1401 character state changes). Arrow indicates

families Mentzelioideae, Gronovioideae, and Loasoideae as circumscribed by Urban and Gilg (1900) resulted in trees that were 16 state changes longer than the most parsimonious trees (*Schismocarpus* had not been described at the time of Urban and Gilg's [1900] monograph and was included in Mentzelioideae as suggested by Blake [1918]) (Fig. 7). The constrained analyses that forced the gronovioids (*Cevallia, Fuertesia, Gronovia,* and *Petalonyx*) to be the sister to the rest of the Loasaceae, modeling the segregation of Gronoviaceae from Loasaceae as advocated by Endlicher (1841) and Weigend (1997; Weigend et al., 2000), resulted in trees that were 18 state changes longer than the most parsimonious trees (Fig. 6).

Each constrained ML search resulted in trees (not shown) that were less likely than the most likely tree from the unconstrained ML analysis (Table 1; constrained ML trees not shown because topologies were the same as those from the corresponding constrained parsimony searches [Figs. 6–10] but with only the 29 taxa used in the unconstrained ML search [Fig. 5]). The SH test rejected the topologies constrained to force (1) the gronovioids to be the sister of the rest of Loasaceae, (2) the monophyly of Mentzelioideae, Gronovioideae, and Loasoideae as circumscribed by Urban and Gilg (1900), (3) the monophyly of *Loasa* s.l. as circumscribed by Urban and Gilg (1900), and (4) the monophyly of *Mentzelia torreyi* and *Mentzelia* sect. *Bartonia*. The SH test did not reject either the trees that resulted when *M. reflexa* was constrained to form a monophyletic group with *Mentzelia* sect. *Bartonia* or any of the trees derived from the unconstrained parsimony analysis.

DISCUSSION

Overview—The parsimony analyses of the separate *matK* and *trnL-trnF* data sets resulted in highly consistent topologies, although many clades recovered by both data sets differ in their level of character state support. The only inconsistency in the results of the analyses of the separate data sets was the placement of *Aosa*. The *matK* data placed *Aosa* as the sister of *Nasa* with only modest support; in contrast, the *trnL-trnF* data placed the taxon with strong support as the sister of a clade that consists of *Blumenbachia, Cajophora, Scyphanthus, Presliophytum,* and *Loasa. Aosa* was placed in the results of the combined analysis as it was in those from the *trnL-trnF* data alone, although support for its placement was diminished as would be expected given the conflict between the two data sets.

The best topologies found for the combined *matK* and *trnL-trnF* data sets using parsimony and likelihood analyses were entirely consistent. Many clades recovered in these analyses were very strongly supported. Less support was found for the most basal nodes of the family. Additional data need to be acquired to evaluate and presumably provide enhanced support for the placements of *Eucnide* and *Schismocarpus*. The poorest support for clades and, indeed, a lack of clade resolution, was found in some of the more terminal clades, especially in *Mentzelia*, in which we found little sequence divergence. The un-

resolved relationships among species in the sections of *Mentzelia* contrasts greatly to the very strong support for relationships among most of the sections. This pattern of support may reflect the antiquity of the major clades of *Mentzelia* in contrast to what may have been relatively recent taxon radiations in the sections, each of which is associated with arid provinces in western North America and Mexico that originated during the latter part of the Tertiary (Axelrod, 1950; Cronquist, 1978; Van Devender et al., 1987; Graham, 1993).

Splitting Loasaceae—Several authors have questioned the monophyly of Loasaceae. Most of these authors (Payer, 1857; Hirmer, 1916; Leins and Winhard, 1973; Ronse Decraene and Smets, 1987) suggested splitting taxa with centripetal androecial development (e.g., *Mentzelia*) from those that have centrifugal androecial development (e.g., *Cajophora* and *Nasa*). Such suggestions proved problematic when Hufford (1990) demonstrated that some Loasoideae, including *Cajophora* and *Nasa*, purported to have centrifugal androecia actually begin androecial development with a phase of centripetal initiation of stamen primordia. Dickson (1866) and Hufford (1990) demonstrated that androecia throughout Loasaceae s.l. (as circumscribed in Urban and Gilg, 1900) share common developmental attributes that could be expected if the family were monophyletic. Our results indicate that either diplostemony (characteristic of *Schismocarpus*) or polystemony in which stamen initiation is strictly centripetal (as in *Eucnide*) was plesiomorphic in Loasaceae; polystemonous androecia that have phases of centripetal and centrifugal initiation or strictly centrifugal initiation patterns are novelties within a monophyletic Loasaceae in Loasoideae. Although neglected by Payer (1857), Hirmer (1916), Leins and Winhard (1973), and Ronse Decraene and Smets (1987), who focused on differences in polystemonous androecia when suggesting that Loasaceae were not monophyletic, Gronovioideae have haplostemonous androecia, a derived simplification of androecial development within Loasaceae (Moody and Hufford, 2000).

Gronovioids have also been segregated from Loasaceae as their own family, Gronoviaceae (Endlicher, 1841; Weigend, 1997). Weigend (1997) placed Gronoviaceae as the sister of Loasaceae in a phylogenetic diagram. This placement is inconsistent with our results (e.g., their placement within Loasaceae is supported by two nodes that have bootstrap values greater than 95%). A constrained parsimony analysis that forced a sister group relationship between gronovioids and the rest of Loasaceae resulted in topologies that were 18 steps longer than the most parsimonious cladograms in the unconstrained analysis of *matK* and *trnL-trnF* sequences (Table 1). The ML topology constrained to place gronovioids as the sister to the rest of Loasaceae was rejected using the SH test ($P = 0.036$). Our results provide very strong support for the sister group relationship of Gronovioideae and *Mentzelia*.

Subfamilies—The three subfamilies of Loasaceae—Loasoideae, Mentzelioideae, and Gronovioideae—as circumscribed by Urban and Gilg (1900), have been modified little to date,

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the placement of *Eucnide aurea*, which shifted from a placement in the unconstrained results as the sister to other species of *Eucnide*. **7.** Tree constrained to force the monophyly of the subfamilies Mentzelioideae, Gronovioideae, and Loasoideae as circumscribed by Urban and Gilg (1900) (1399 character state changes). Arrow indicates the placement of *Eucnide aurea*, which shifted from a placement in the unconstrained results as the sister to other species of *Eucnide*. **8.** Tree constrained to force the monophyly of *Loasa* s.l. as circumscribed by Urban and Gilg (1900) (1398 character state changes).
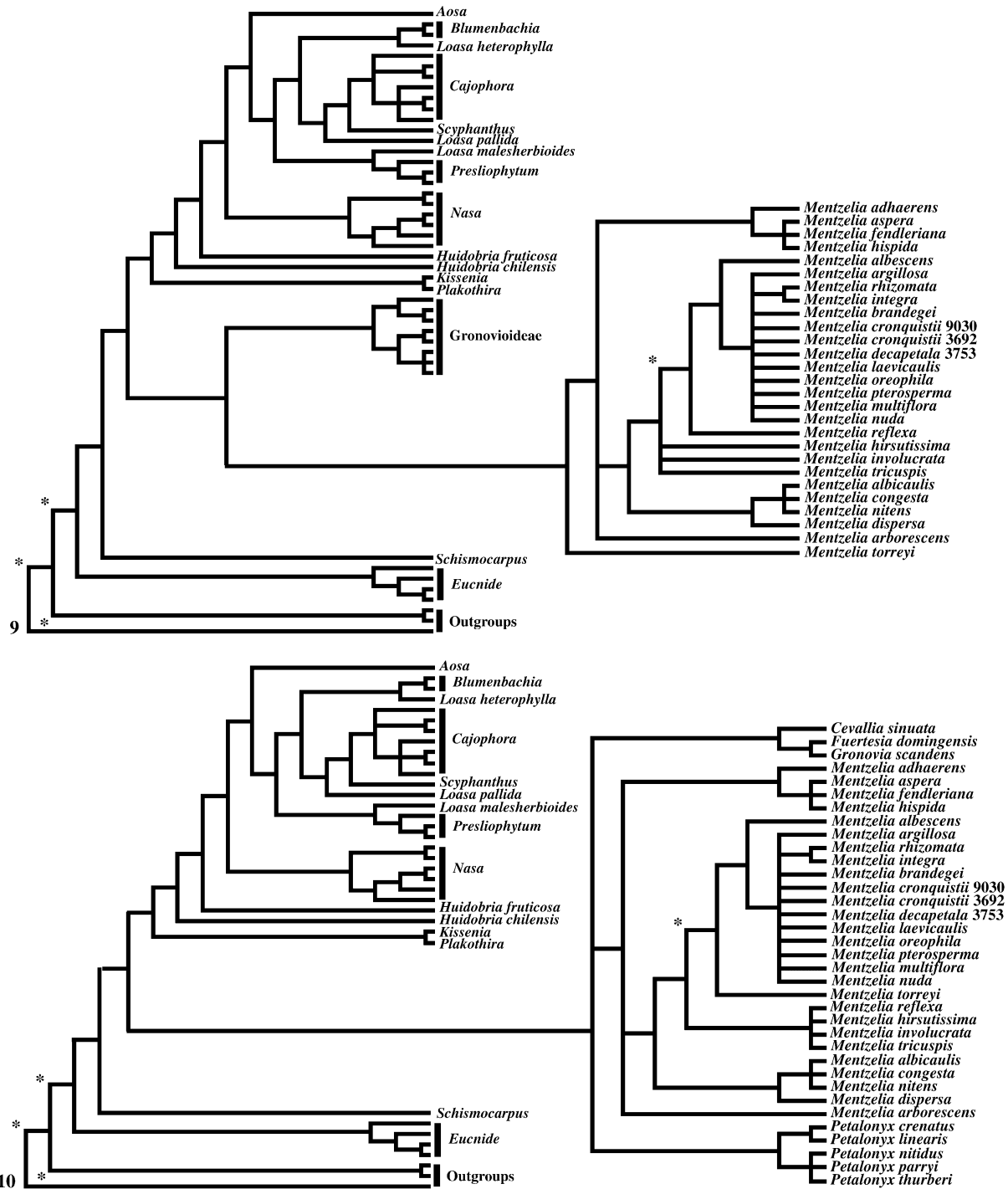
Figs. 9–10.   Strict consensus cladograms from constrained parsimony analyses. Asterisks indicate nodes specified in topology constraints. **9.** Tree constrained to force the monophyly of *Mentzelia reflexa* and *Mentzelia* sect. *Bartonia* (1385 character state changes). **10.** Tree constrained to force the monophyly of *Mentzelia torreyi* and *Mentzelia* sect. *Bartonia* (1419 character state changes).

and no major realignments or new subfamilies have been proposed. Mentzelioideae were expanded to incorporate *Schismocarpus* (Gilg, 1925), following the recommendation of Blake (1918). Loasoideae were expanded to include *Plakothira* (Florence, 1985) and *Xylopodia* (Weigend, 1997). Circumscriptions of the subfamilies have received only modest challenges (Ernst and Thompson, 1963), and most of these have

focused on Gronovioideae (Davis and Thompson, 1967; Poston and Nowicke, 1993). Hempel's (1995) phylogenetic analysis of Loasaceae did not recover a monophyletic Gronovioideae; however, she sampled only seven species in the family for the plastid genes *ndhF* and *rbcL*. In contrast to challenges to the circumscription of Gronovioideae, Weigend (1997) emphasized that gronovioids had several synapomorphies and ar-

TABLE 1.   Results of the phylogenetic analyses conducted using topology constraints. The Shimodaira-Hasegawa (SH) test was used to evaluate
the topologies that resulted from the constrained maximum-likelihood (ML) analyses of the 29-taxon data set. Using the SH test, we simulta-
neously compared the best tree from the unconstrained ML analysis to the five most likely topologies that resulted from the constrained ML
searches as well as to the single most parsimonious (MP) tree and all trees that differed from it by five or fewer steps that resulted from a
parsimony analysis of the 29-taxon data set. Specifications for the topology constraints listed below are shown in Figs. 6–10.

Constraint	Length of MP trees from constrained parsimony search of 71-taxon data set (+ steps longer than unconstrained MP tree)	−Log likelihood of best trees from constrained ML search of 29-taxon data set (difference in log likelihood from best unconstrained ML tree)	SH test result
Gronovioids sister to rest of Loasaceae (sensu Weigend, 1997)	1401 (+18)	10 566.180 (64.657)	Rejected ($P = .036$)
Monophyly of Urban and Gilg's (1900) subfamilies	1399 (+16)	10 564.648 (63.125)	Rejected ($P = .043$)
Monophyly of *Loasa* sensu Urban and Gilg (1900)	1398 (+15)	10 588.480 (86.957)	Rejected ($P = .009$)
Monophyly of *Mentzelia reflexa* and *Mentzelia* sect. *Bartonia* (sensu Darlington, 1934)	1385 (+2)	10 507.595 (6.072)	Not rejected ($P = .991$)
Monophyly of *Mentzelia torreyi* and *Mentzelia* sect. *Bartonia* (sensu Darlington, 1934)	1419 (+36)	10 640.068 (138.545)	Rejected ($P < .0005$)

gued for their monophyly. Moody et al. (2001) used *matK*
sequences from a broad sampling of Loasaceae in phyloge-
netic analyses that provided strong support for the monophyly
of Gronovioideae. Although our *trnL-trnF* data alone provided
little information on Gronovioideae aside from strong support
for the monophyly of *Petalonyx* (Fig. 2), the addition of these
data to the *matK* sequences have provided increased support
for the Moody et al. (2001) results as assessed by decay and
bootstrap analyses.

Although our results provide support for the monophyly of
Loasoideae and Gronovioideae of Urban and Gilg (1900) and
Gilg (1925), they are clearly incongruent with their concept
of Mentzelioideae. No morphological synapomorphies have
been suggested previously for Mentzelioideae, and some au-
thors have hypothesized that they are paraphyletic to Loasoi-
deae and/or Gronovioideae (Brown, 1971; Hufford, 1988).
Scant attention has been given to the circumscription of Ment-
zelioideae, perhaps because it lacks the notable morphological
elaborations of Loasoideae flowers and simplifications of
Gronovioideae flowers. Urban and Gilg (1900) considered the
subfamily to consist of the genera *Eucnide*, *Mentzelia*, and
Sympetaleia. Thompson and Ernst (1967) reduced *Sympetal-
eia*, a group of three species centered largely in Baja Califor-
nia, to a section of *Eucnide*. In our results, *Eucnide* sensu
Thompson and Ernst (1967) is strongly supported as mono-
phyletic for the species sampled, which includes *E. aurea*, a
representative of sect. *Sympetaleia*, placed as the sister to the
rest of the genus. Blake's (1918) alliance of *Schismocarpus*
with Mentzelioideae was challenged by Ernst and Thompson
(1963, p. 141), who suggested that the genus was "discordant
in the Mentzelioideae." The Moody et al. (2001) results failed
to recover a monophyletic Mentzelioideae. Their results,
which are replicated in our analyses of the combined *matK*
and *trnL-trnF* data set, placed *Eucnide* as the sister to the rest
of Loasaceae and *Schismocarpus* at the next internal node. As
noted earlier, *Mentzelia* was placed as the sister of Grono-
vioideae.

We examined the Urban and Gilg (1900) and Gilg (1925)
approach to the circumscription of three subfamilies in Loas-
aceae using topology constraints. Our constraint topologies

forced not only the monophyly of Loasoideae (including *Plak-
othira*) and Gronovioideae, which had been recovered in our
best trees from the parsimony and ML analyses, but also Ment-
zelioideae (including *Schismocarpus*). Our constrained parsi-
mony cladograms were 16 steps longer than the most parsi-
monious trees from unconstrained analysis of *matK* and *trnL-
trnF* sequences. The ML topology constrained to force the
monophyly of the three subfamilies was rejected under the SH
test ($P = 0.043$).

To achieve a subfamilial classification of Loasaceae based
on monophyletic groups, we suggest that Loasoideae and
Gronovioideae be maintained as circumscribed by Gilg (1925,
except with the addition of *Xylopodia* and *Plakothira* to Loa-
soideae) but that the circumscription of Mentzelioideae should
be revised. We recommend that Mentzelioideae be restricted
only to *Mentzelia*. To accommodate *Eucnide* and *Schismocar-
pus* in a revised subfamilial classification, we recommend that
each be included in its own subfamily.

Loasoideae—The monophyly of Loasoideae, which are
characterized by complex staminodes, has been unchallenged
since Urban and Gilg's (1900) monograph of the family. Our
results provide very strong support for the monophyly of Loa-
soideae. Phylogenetic relationships found within Loasoideae
are consistent with those of Moody et al. (2001), although our
addition of *trnL-trnF* characters to the *matK* sequence data has
resulted in greater resolution and better support for clades of
the subfamily. Our greater taxon sampling permitted us to
evaluate earlier systematic treatments and evolutionary hy-
potheses that were not examined by Moody et al. (2001).

Urban and Gilg (1900) and Gilg (1925) recognized in Loa-
soideae the three tribes Klaprothieae, Kissenieae, and Loaseae.
Weigend (1997) retained Klaprothieae and Loaseae but argued
that *Kissenia*, the only genus of Kissenieae, evolved among
Loaseae; thus, he reduced Kissenieae to synonymy in Loaseae.
Our results support the monophyly of Loaseae and Klapro-
thieae; however, we found strong support for a sister group
relationship of Klaprothieae and *Kissenia*, which has not been
previously suggested. Our results place the Klaprothieae-*Kis-
senia* clade as the sister to the rest of Loasoideae, a group

equivalent to the Loaseae of Urban and Gilg (1900) and Gilg (1925). We suggest that the three tribes Klaprothieae, Kissenieae, and Loaseae be maintained in Loasoideae. We emphasize the caveat, however, that little support was found for the two most basal nodes of Loaseae (Fig. 3). The weak nodes at the base of the Loaseae raise the possibility that the tribe could be paraphyletic to the Klaprothieae-*Kissenia* clade. Such arrangements should be tested as additional phylogenetic data become available.

Within Loaseae, Weigend (1997) distinguished "lower Loaseae" from "higher Loaseae." In his summary phylogenetic diagram, his "lower Loaseae" was a grade that consisted of *Huidobria*, *Presliophytum*, *Kissenia*, *Chichicaste* (= *Loasa grandis*), and *Loasa malesherbioides*. Weigend's putatively monophyletic "higher Loaseae" included *Aosa*, *Nasa*, *Loasa* sensu stricto (s.str.), *Scyphanthus*, *Cajophora* s.str., *Cajophora* sect. *Angulatae*, *Cajophora* sect. *Bialatae*, and *Blumenbachia*. Not all of these groups were sampled for our analyses (either unavailable or the specimens sampled had degraded DNA), but our results did not recover either Weigend's "lower Loaseae" grade or "higher Loaseae" clade. For example, our results placed both *Presliophytum* and *Loasa malesherbioides* in what would correspond to Weigend's "higher Loaseae."

The monophyly of *Loasa* as circumscribed broadly by Urban and Gilg (1900) has been recognized as problematic (Grau, 1997; Weigend, 1997) and was not supported by our data. When we forced the monophyly of *Loasa* s.l., resulting cladograms were 15 steps longer than the unconstrained most parsimonious cladograms, and the constrained ML tree was rejected by the SH test ($P = 0.009$; Table 1). Recently, Weigend (1997) argued for a circumscription of *Loasa* s.str. that would include only Urban and Gilg's (1900) *Loasa* sect. *Loasa* series *Acanthifoliae*, *Macrospermae*, *Floribundae*, *Pinnatae*, *Volubiles*, *Acaules*, and *Deserticolae*. Weigend (1997) segregated various portions of Urban and Gilg's *Loasa* s.l. as new genera, including: *Loasa* sect. *Presliophytum* as *Presliophytum*; *Loasa* sect. *Loasa* series *Grandiflorae*, *Alatae*, *Saccatae*, *Carunculatae*, and the *L. venezuelensis* group as *Nasa*; *Loasa* sect. *Loasa* series *Pusillae*, *Corymbosae*, and *Parviflorae* as *Aosa*; and *Loasa grandis* as *Chichicaste*. Grau (1997) resurrected *Huidobria* for Urban and Gilg's (1900) *Loasa* sect. *Huidobria*. We did not find support for the monophyly of *Huidobria*; however, the nodes at which these two species diverged had little support. Two characters may support the monophyly of the genus: chromosome numbers of $2n = 36$ and the morphology of the staminodial scales of flowers (Grau, 1997). Our results provide very strong support for the monophyly of *Nasa* and *Presliophytum* and are consistent with the recognition of *Aosa*. *Chichicaste* was not available to sample for this study.

Weigend (1997) argued that *L. malesherbioides* should also be segregated from *Loasa*, although he made no formal taxonomic change. Our results, which strongly support the placement of *L. malesherbioides* as the sister of *Presliophytum*, are consistent with his suggestion. Given the robust placement of *L. malesherbioides* in our results, a sensible option might be to transfer the species to *Presliophytum*, expanding slightly the circumscription of this genus.

Loasa acanthifolia, the type species for *Loasa*, was included in our parsimony analysis of the *matK* data (this DNA did not amplify for *trnL-trnF*) and placed with *L. pallida* as the sister of *Cajophora* and *Scyphanthus*. A similar placement for *L. pallida* was found in the parsimony and ML analyses of the combined *matK* and *trnL-trnF* data set. The only other member of Weigend's (1997) *Loasa* s.str. sampled for our analyses was *L. heterophylla*, which was one of the 10 species placed by Urban and Gilg (1900) in series *Macrospermae*. Although the placement of *L. heterophylla* was shown under bootstrap and decay analyses to have only weak support in the parsimony results, its separation from *L. pallida* indicates that Weigend's *Loasa* s.str. warrants further revisionary and phylogenetic study.

Urban and Gilg's (1900) broad circumscription of *Cajophora* has also been of concern to systematists. Poston and Thompson (1977) suggested that *Cajophora* sect. *Bialatae* was more closely related to *Blumenbachia* than to other *Cajophora*. Weigend (1997) excluded both sects. *Angulatae* and *Bialatae* from *Cajophora* and placed them in *Blumenbachia*. Material of *Cajophora* sects. *Angulatae* and *Bialatae* was not available to sample for this investigation. Our results provided very strong support for the monophyly of the sampled *Cajophora*, a set of species that corresponds to the limited boundaries of Weigend's (1997) *Cajophora* s.str. Within *Cajophora* s.str. our sampling was limited, but our results indicated that Urban and Gilg's (1900) sect. *Orthocarpae* (including the sampled species *C. carduifolia*, *C. cirsiifolia*, and *C. chuquitensis*) was paraphyletic to both sects. *Dolichocarpae* (represented by *C. clavata*) and *Platypetalae* (represented by *C. canarinoides*). A sister group relationship of the sampled *Cajophora* to the monotypic genus *Scyphanthus* was very strongly supported.

Mentzelia—Our results contributed substantially toward understanding the monophyly of *Mentzelia* as well as its major clades and their relationships. Brown (1971) considered *Mentzelia* to be paraphyletic to the rest of Loasaceae. Hempel (1995; Hempel and Jansen, 1996), and Weigend (1997) considered *Mentzelia* to be paraphyletic to *Eucnide*. This phylogenetic concept was not supported by our results, which showed the sister clade relationship of *Mentzelia* to Gronovioideae to be very strongly supported. We found very strong support for the monophyly of *Mentzelia* as traditionally circumscribed (e.g., by Urban and Gilg [1900] and Gilg [1925]). The monophyly of the traditional *Mentzelia* and the pattern of relationships displayed among its clades weigh against the recognition of the segregates *Acrolasia* Presl and *Nuttallia* Raf. (e.g., Rydberg, 1903; Davidson, 1916; Weber and Wittmann, 2001), which would render paraphyletic a more narrowly circumscribed *Mentzelia*.

The most recent comprehensive revision of *Mentzelia* by Darlington (1934) divided the genus in the four sects. *Mentzelia*, *Bartonia*, *Trachyphytum*, and *Bicuspidaria*; this contrasts with Urban and Gilg's (1900) earlier recognition of seven sections. The difference between these two treatments lies particularly in the placements of *M. arborescens*, *M. reflexa*, and *M. torreyi*. Urban and Gilg (1900) placed each of these species in its own monotypic section: *M. arborescens* in sect. *Dendromentzelia*, *M. reflexa* in sect. *Octopetaleia*, and *M. torreyi* in sect. *Micromentzelia*.

Darlington (1934) combined sect. *Dendromentzelia* with sect. *Mentzelia*, placing *M. arborescens* in the latter. Thompson and Lewis (1955) followed the Darlington treatment of *M. arborescens*, including it in sect. *Mentzelia*; however, Ernst and Thompson (1963) treated it as sect. *Dendromentzelia*. Our results provided modest support for the placement of *M. arborescens* outside of sect. *Mentzelia* s.str., which is consistent with Urban and Gilg's (1900) placement of the species in its

own section. Our parsimony analyses of the combined *matK* and *trnL-trnF* data found some trees in which *M. arborescens* was placed as the sister of sect. *Mentzelia* s.str. and others in which it was placed as the sister of the sects. *Bartonia-Bicupidaria-Trachyphytum* clade. The ML analysis was consistent with the latter set of parsimony trees, but as with the parsimony results the *M. arborescens* node had weak support. Thus, although the placement of *M. arborescens* remains equivocal, our results are consistent with Urban and Gilg's distinction between sects. *Dendromentzelia* and *Mentzelia*.

Darlington (1934) also collapsed Urban and Gilg's (1900) sects. *Octopetaleia* and *Micromentzelia*, placing both *M. reflexa* and *M. torreyi* in sect. *Bartonia*. Thompson (1963) suggested that Darlington's treatment of these two species might have been ''inadvertent'' and noted that both ''are clearly more similar to species of other sections'' (p. 17). Contrary to Darlington's treatment, our results provided very strong support for the exclusion of *M. torreyi* from sect. *Bartonia*. When we used constraint topologies to force *M. torreyi* to form a monophyletic group with the species of sect. *Bartonia* the resulting topologies were 36 steps longer than the most parsimonious constrained trees, and the SH test rejected this placement as inconsistent with our data ($P < 0.0005$). In contrast, the constrained analyses were less conclusive in regard to the placement of *M. reflexa*. Constrained analyses in which *M. reflexa* was forced to be monophyletic with sect. *Bartonia* resulted in trees that were only two steps longer than the most parsimonious cladograms from unconstrained analyses, and the SH test did not reject similarly constrained ML topologies ($P = 0.991$). The results of the constrained analyses for *M. reflexa* may be more a consequence of the low sequence divergence (short branch length and decay value = 2 in analyses using the combined *matK* and *trnL-trnF* data) for sect. *Bicuspidaria* than a problem inherent solely to *M. reflexa*. Our results found strong support for the monophyly sect. *Bicuspidaria*, including *M. reflexa*, which is consistent with Daniels's (1970) revision of the section.

Conclusions—Our results provide additional support for the major clades of Loasaceae found by Moody et al. (2001). We test key prior hypotheses using the approach of Shimodaira and Hasegawa (1999). These tests reject the placement of gronovioids as the sister of the rest of Loasaceae; in contrast to the strongly supported placement of gronovioids as the sister of *Mentzelia* in our results. We are able to reject the best topologies that modeled the subfamilial taxonomy of Urban and Gilg (1900) and Gilg (1925), making Mentzelioideae as well as Gronovioideae and Loasoideae monophyletic. Our results provide very strong support for the monophyly of Gronovioideae and Loasoideae and demonstrate the paraphyly of Mentzelioideae. We recommend that Mentzelioideae be restricted only to *Mentzelia* and new subfamilies described to accommodate *Eucnide* and *Schismocarpus*. The SH test rejects the best topologies in which we force the monophyly of *Loasa* s.l. as circumscribed by Urban and Gilg (1900) and Gilg (1925). Our results demonstrate the paraphyly of *Loasa* s.l. and provide support for several new genera proposed by Weigend (1997). Additional taxon sampling is needed to examine support for *Loasa* s.str. as circumscribed by Weigend (1997). Among our novel results is the placement of *Kissenia* as the sister of Klaprothieae. The *Kissenia*-Klaprothieae clade forms the sister of Loaseae, providing support for the three tribes recognized by Urban and Gilg (1900) in Loasoideae. Addi-

tional phylogenetically informative data are needed to examine support for the basal nodes of Loasoideae and to resolve relationships among the more terminal branches (e.g., within *Cajophora*). Similarly, more data are needed to resolve relationships among species within sections of *Mentzelia*. The lack of resolution within sections of *Mentzelia* contrasts with the very well supported clades that correspond to the sections of Urban and Gilg (1900).

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