

# MONOPHYLY OF THE CONVULVACEAE AND CIRCUMSCRIPTION OF THEIR MAJOR LINEAGES BASED ON DNA SEQUENCES OF MULTIPLE CHLOROPLAST LOCI<sup>1</sup>

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Convolvulaceae, a large family of worldwide distribution, exhibit a rich diversity of morphological characteristics and ecological habitats. Previous efforts to systematize this diversity without a cladistic phylogenetic framework have disagreed on the circumscription of the family as well as tribal composition and relationship. In order to circumscribe the family and assess the relationships among its major lineages, a broad data set was constructed containing representatives of all ten recognized tribes of Convolvulaceae plus representatives of putatively related families within Asteridae. This is done by using four chloroplast regions: *rbcL*, *atpB*, *psbE-J* operon, and *trnL-trnF* intron/spacer. The results indicate that Convolvulaceae are monophyletic and sister to Solanaceae. Two of the three groups that have been proposed previously as separate families, *Cuscuta* and *Dichondreae*, are nested within the Convolvulaceae in this analysis, and the third, *Humbertia*, is the sister to all other members of the family. The exact position of *Cuscuta* could not be ascertained, but some alternatives were rejected with confidence. The study identified several distinct monophyletic groups, some of which correspond to earlier taxonomic treatments. Close relationships of tribes Hildebrandtieae with Cresseae and Ipomoeae with Argyreieae (forming Echinoconieae) were confirmed. The polyphyly of Merremieae, Convolvuleae, Poraneae, and Erycibeae is first identified in this study.

**Key words:** *atpB*; chloroplastDNA; cladistic analysis; Convolvulaceae; *Cuscuta*; *Humbertia*; phylogeny; *psbE-J* operon; *rbcL*; *trnL-F*.

Convolvulaceae are a large family, comprising approximately 50–60 genera with some 1600–1700 species (Mabberley, 1987), exhibiting a rich diversity of morphological characteristics and occupying a broad range of ecological habitats. More than one-third of the species are included in two major genera, *Ipomoea* and *Convolvulus* (Cronquist, 1988). Convolvulaceae are distributed throughout the world, but are primarily tropical, with many genera endemic to individual continents. Although the family is best known in temperate regions for its weedy representatives (e.g., *Calystegia*, *Convolvulus*), many tropical species are valuable ornamentals, medicinals, and food crops. The sweet potato, *Ipomoea batatas*, is the world's second most important root crop ( $>128 \times 10^9$  kg/yr; Simpson and Ogorzaly, 1995). The record of microfossils attributed to the family is known as far back as the Eocene (~40–45 million years ago [mya]), but without accompanying macrofossils.

A traditional placement for Convolvulaceae is in the order Solanales (sensu Cronquist, 1988; Dahlgren, 1989; Thorne, 1992; APG, 1998) along with the Solanaceae. Takhtajan (1997), however, segregates this family into its own order, Convolvales. Current knowledge of Convolvulaceae relationships relies largely on the work of Choisy (1845), Bentham and Hooker (1873), Hallier (1893), Peter (1891, 1897), Rob-

erty (1952, 1964), and Austin (1973, 1998b). Typical members of the family are annual or perennial vines, with milky sap, internal (intraxylary) phloem, alternate, simple to lobed leaves, and actinomorphic, perfect, hypogynous flowers. The corolla is sympetalous, often large and showy; stamens are epipetalous. The gynoecium is composed of two united carpels, unlobed, forming a two-locular, superior ovary, with 2–4 ovules.

Uncertainties exist regarding both delimitation of the family and intrafamilial (tribal) relationships. The precise delimitation has been controversial due to three lineages that do not share some of the abovementioned characters, but seem clearly allied with Convolvulaceae. These are *Humbertia* (monotypic), *Cuscuta* (parasites with ca. 150 spp.) and *Dichondreae* (*Dichondra*, *Falkia*, and *Nephrophyllum*, together with ca. 15 spp.).

The Malagasy endemic *Humbertia madagascariensis* was first described by Lamarck (1786). *Humbertia* is a large tree with some unusual characteristics for Convolvulaceae (tree habit, zygomorphic flowers, numerous ovules per carpel, absence of intraxylary phloem), which has given rise to several different interpretations of its appropriate systematic placement. For example, Baillon (1891) placed this genus in Solanaceae based on an indefinite number of ovules. This is characteristic of some Solanaceae, and it is not encountered in any other Convolvulaceae. However, many other early authors recognized its closer relationship with Convolvulaceae, placing the genus in one of the existing tribes of the family (Argyreieae, Convolvuleae, or Erycibeae). Pichon (1947) segregated *Humbertia* into its own family, a decision based mainly on the absence of internal phloem and the zygomorphic nature of its flowers. This special status of the genus has been reflected in many subsequent classifications, which assigned *Humbertia* to the subfamily Humbertioidae within the Convolvulaceae. All of these classifications were based on very few fragmented

<sup>1</sup> Manuscript received 15 January 2002; revision accepted 3 May 2002.

The authors thank D. F. Austin, M. W. Chase, A. L. Denton, Th. Deroin, R. Neyland, J. D. Palmer, G. W. Staples, H. Thornton, and S. J. Wagstaff as well as the curators of A, GH, H, K, MO, P, US, and WTU for supplying plant material; and Pat Reeves, Sean Graham, Dan Austin, and Rick Miller for critical comments on the manuscript. This work was supported by the Karling Graduate Student Research Award from the Botanical Society of America, the Research Award for Graduate Students from the American Society of Plant Taxonomists to S. Stefanovic, and the NSF Doctoral Dissertation Improvement grant DEB-0073396 to R. G. Olmstead for S. Stefanovic.

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herbarium specimens, and this species, collected last in the early 18th century, was thought to be extinct (Pichon, 1947). After examination of the newly collected material from southeastern Madagascar (Humbert, 1948), both Austin (1973) and Derooin (1992) have suggested that the two Old World genera, *Humbertia* and *Erycibe*, together with the three American genera, *Maripa*, *Dicranostyles*, and *Lysiostyles*, form a natural group, tribe Erycibeae.

The genus *Cuscuta* (dodder) is another problematical and controversial group. Members of this cosmopolitan genus are stem parasites with little or no chlorophyll. They have twining, slender, pale stems, with reduced, scale-like leaves, no roots, and are attached to the host by haustoria. In addition, *Cuscuta* does not have internal phloem and its embryo is without well-differentiated cotyledons. Even though vegetative characters are altered in association with its eccentric mode of life, *Cuscuta* floral morphology is quite similar to that of the Convolvulaceae, and the clear association with this family was recognized early on. Most classifications recognize a separate tribe or subfamily within the family for this genus. However, some students of the family (e.g., Roberty, 1952, 1964; Austin, 1973) adopted Dumortier's (1829) view that the genus should be recognized as a separate family. This opinion is reflected also in some major synoptic works on flowering plants (e.g., Cronquist, 1988; Takhtajan, 1997) that accept *Cuscuta* on the family level. Subdivisions within the genus were proposed by Engelmann (1859; adopted by Yuncker, 1932), based primarily on the morphology of styles and stigmas, which show considerable diversity.

Tribe Dichondreae, unlike the rest of Convolvulaceae, has deeply two- or four-lobed ovaries with the lobes united at the base and with gynobasic styles. This peculiarity was recognized from the earliest treatments of the family, but the importance given to these features, and their taxonomic implications, differed greatly, ranging from tribe to family (Dichondraceae; Dumortier, 1829).

The early schemes of classification for the family were based mainly on one or a few characters. Among the most influential ones are those of Peter (1891), based primarily upon fruit type, and Hallier (1893; adopted by Peter, 1897), which uses pollen surface texture as well as fruit and stylar characters. Hallier divided the family into two groups: Echinoconiae, with spiny pollen surface, and Psiloconiae, with smooth pollen. The major outlines of Hallier's classification were subsequently adopted by many authors (but see Roberty, 1952, 1964), with modifications concerning mainly the taxonomic levels. Different systems of classifications as well as points of conflict and congruence among them are summarized by Austin (1973, modified 1998b), who proposed a phylogenetic scheme based mainly on chromosome numbers. That classification, being the most recent comprehensive one, is used in this study.

Recent treatments have focused on either a taxonomic subset of the family (Austin, 1979, 1998a; Austin and Staples, 1980; Robertson, 1982; Lejoly and Lisowski, 1986; Staples, 1987, 1990; Derooin, 1992; Demissew and Austin, 1996; Miller, Rausher, and Manos, 1999; Wilkin, 1999; Manos, Miller, and Wilkin, 2001), a geographic area (Austin, 1973; Austin and Staples, 1991; McDonald and Mabry, 1992; Austin and Huáman, 1996), or were based on very few characters (Sengupta, 1972; Carlquist and Hanson, 1991). One exception to this is a cladistic analysis of the family by Austin (1998b) based on morphology, which resulted in "an initial hypothesis

of relationships within the Convolvulaceae." This treatment included all 55 traditionally recognized genera and used 128 characters. The characters included in the study ranged from habit, vegetative morphology and anatomy, reproductive structures, and embryo features, to chromosome numbers. Although very useful and information-rich, Austin's study lacked an in-depth evaluation of the ramifications and robustness of the results. To date, Convolvulaceae have not been the subject of broad molecular phylogenetic work.

A molecular phylogenetic approach could help resolve long-standing controversies and nurture a greater understanding of the evolutionary processes that have shaped Convolvulaceae. Previous limited molecular data for the family suggested the Convolvulaceae are closely related to Solanaceae (e.g., Olmstead and Palmer, 1992; Olmstead et al., 1992, 1993; Soltis et al., 1997; Savolainen et al., 2000). The geographical distribution of these two families (Convolvulaceae cosmopolitan and well developed in New and Old World; Solanaceae primarily New World) as well as absence of an obvious, unique and unreversed morphological synapomorphy for the Convolvulaceae *sensu lato* (s.l.), might indicate an older origin of Convolvulaceae, possibly as a paraphyletic group related to the monophyletic Solanaceae.

The research on Convolvulaceae was undertaken with several goals in mind: (1) to test the monophyly of Convolvulaceae; (2) to circumscribe major lineages within the family and to help place taxa that are placed ambiguously in present classifications; (3) to develop a well-supported phylogenetic hypothesis of Convolvulaceae at the tribal and generic level; (4) to ascertain the position of *Cuscuta*, the only parasitic genus associated with Convolvulaceae; (5) to investigate the scenarios of morphological character evolution within the family; (6) to develop, in conjunction with a reevaluation of traditional taxonomic characters, a comprehensive, phylogeny-based classification; and (7) to investigate the molecular processes of chloroplast genome evolution.

The present study focuses mainly on the first three goals: the monophyly of the family and circumscription of their major lineages, as well as relationships among tribes and genera that constitute the family. It is based on DNA sequence information from four single-copy chloroplast loci, the *rbcL* and *atpB* genes, the *psbE-J* gene operon, and the fragment containing the *trnL* intron, the 3' *trnL* exon, and the intergenic spacer between this exon and *trnF* gene (*trnL-F* region). Trees derived from *rbcL* and *atpB* sequences are well documented in their utility for phylogenetic inference (e.g., Chase et al., 1993, and references therein; Savolainen et al., 2000). A large data set exists for these genes for angiosperms, in general, and for Asterideae, in particular. This allows us to assess the monophyly of the family and the genera it comprises with confidence and to determine appropriate outgroups. Although some studies based on *rbcL* and/or *atpB* have addressed phylogeny at the inter- and intragenic levels, those genes alone are usually too conservative to resolve phylogenetic relationships at these lower taxonomic levels. It has been shown that noncoding chloroplast regions, e.g., *trnL-F* region, evolve faster than coding regions and contain enough information to resolve relationship among closely related taxa (Gielly and Taberlet, 1994; McDade and Moody, 1999). On the other hand, the *psbE-J* operon, the most conserved of the chloroplast regions used here (Graham and Olmstead, 2000), was chosen to help resolve the backbone relationships (i.e., deeper nodes) with more confidence.

## MATERIALS AND METHODS

A total of 112 species were used in this study. Taxa, source, voucher, and accession numbers have been archived at the Botanical Society of America website (<http://ajbsupp.botany.org/v89/>). Austin's classification (1973, modified 1998b) is followed here, because it is widely used and represents the most recent comprehensive work at the family level. The 102 green Convolvulaceae taxa, on which our analyses are principally based, include genera from all nine traditionally recognized tribes and 46 of 54 recognized nonparasitic genera. Efforts were made to sample two or more representatives of each genus for all except very small genera. Some analyses included seven *Cuscuta* species as well. Four additional genera, placed in synonymy in Austin's system (marked by an asterisk in the supplementary data; <http://ajbsupp.botany.org/v89/>), are included also. Relying on previously published molecular systematic studies of the Asterideae (e.g., Olmstead and Palmer, 1992; Chase et al., 1993; Soltis et al., 1997) and our preliminary analyses, we selected two taxa spanning the root node of the Solanaceae, the sister family, as well as one additional species (*Montinia caryophyllacea*) belonging to the Asterideae as outgroups.

Total genomic DNA was isolated from herbarium specimens or silica-gel dried tissue (0.05–0.2 g), or from fresh (1–2 g) tissue by the modified 2× hexadecyltrimethylammonium bromide (CTAB) procedure (Rogers and Bendich, 1985; Doyle and Doyle, 1987) and purified using Qiagen mini-columns following protocols provided by the manufacturer. This cleaning procedure was found to be especially useful for the numerous herbarium specimens used in this study (supplementary data; <http://ajbsupp.botany.org/v89/>).

Double-stranded DNA fragments for the regions of interest were obtained by polymerase chain reaction (PCR) using the primers described by Olmstead et al. (1992) for the *rbcL* gene, by Hoot, Culham, and Crane (1995) for the *atpB* gene, by Graham and Olmstead (2000) for the *psbE-J* operon, and by Taberlet et al. (1991) for the *trnL-F* region. Some PCR products, mainly those involving the *Cuscuta* taxa, were cloned into the pCR2.1 vector (Invitrogen, Carlsbad, California, USA) and multiple clones were sequenced. Amplified products were cleaned using Qiagen (Valencia, California, USA) mini-columns. Cleaned products were then directly sequenced, including both strands to ensure accuracy, using the dRhodamine DNA sequencing kit (PE Applied Biosystem, Foster City, California, USA) on an Applied Biosystems 377 DNA Sequencer. Sequence data were edited and assembled using Sequencher (Gene Codes Corporation, Ann Arbor, Michigan, USA). The alignment was obtained manually using the EDIT option of the MUST package (Philippe, 1993).

The preliminary analysis using only *rbcL* and *atpB* sequences, designed to verify the monophyly of the Convolvulaceae and to find their closest and progressively more distant relatives, was conducted on a large 165-sequence set, comprising all major orders of the Asterideae (results not shown). Once the monophyly of Convolvulaceae (including *Humbertia* and *Cuscuta*) had been determined, detailed analyses of Convolvulaceae were performed using the data set of 112 taxa (See supplemental material at <http://ajbsupp.botany.org/v89/>) and sequences of all four chloroplast loci.

All of the sequenced regions used in this study occur in the haploid chloroplast genome. Their histories are thus linked (see Doyle, 1992; Moore, 1995), and there is no a priori reason to believe that four resulting gene trees will differ. However, their patterns of evolution might be different. For example, differences in rates of evolution and/or base composition have been suggested to lead to the incongruence among data sets (Bull et al., 1993). In order to check for significance of heterogeneity among these four sets of sequences, we conducted an incongruence length difference test (ILD test; Farris et al., 1994), which tests whether the original data partitions differ significantly from randomly shuffled partitions of the combined data set. All parsimony-uninformative characters were excluded before performing the ILD test (Cunningham, 1997).

The heuristic searches for most parsimonious (MP) trees were performed using PAUP\* (Swofford, 1999) with the MULTREES option on. Parsimony analyses of the data were conducted for each region separately (both with and without indels for the *atpB*, *psbE-J*, and *trnL-F* region) and in combination. All changes were weighted equally (Olmstead, Reeves, and Yen, 1998). Gaps in the *atpB*, *psbE-J*, and *trnL-F* data set were scored as missing and coded

as binary characters. In order to maximize the probability of discovering different islands of trees (Maddison, 1991), the analyses involved 1000 replicates with stepwise random taxon addition and tree bisection-reconnection (TBR) branch swapping. The maximum likelihood (ML) scores for all the equally parsimonious trees obtained were calculated using the HKY85 +  $\Gamma$  DNA substitution model (Hasegawa, Kishino, and Yano, 1985; indel characters excluded) with the following parameters: transition/transversion ratio of 1.0, base frequencies estimated from the data, and a discrete gamma rate distribution with four categories and an  $\alpha = 0.41$  (estimated from the MP trees).

To infer the relative support for particular clades we used two resampling methods and decay analysis. The resampling methods, nonparametric bootstrapping (Felsenstein, 1985), and jackknifing (Farris et al., 1996; 36% of characters randomly deleted) were performed with 500 replicates, each with 20 replicates initiated with random order entry of taxa for starting trees and TBR branch swapping, but with MULTREES off (DeBry and Olmstead, 2000). Decay analysis (Bremer, 1988, 1994; Donoghue et al., 1992) was conducted using AutoDecay (Ericksson, 1998) in conjunction with PAUP\*. For each of the decay constraint trees a heuristic analysis with 20 replicates and TBR swapping was performed.

Alternative topologies, mainly designed to investigate the monophyly of traditionally defined genera and tribes, were constructed using MacClade (Maddison and Maddison, 1992), and their cost in parsimony was assessed by implementing the TOPOLOGICAL CONSTRAINTS function in PAUP\*. In order to compare support for the MP trees against alternative branching hypotheses and to assess whether there is substantial difference in length between those trees for a given data set, the one-tailed Shimodaira-Hasegawa nonparametric tests (SH tests; Shimodaira and Hasegawa, 1999; Goldman, Anderson, and Rodrigo, 2000) were conducted, using the same aforementioned substitution model and likelihood settings. To reduce the computational time, we used a resampling estimated log-likelihood (RELL) method (Kishino and Hasegawa, 1989) with 1000 bootstrap replicates. More than one MP tree is often inferred in phylogenetic analyses. Because the SH test requires bifurcating trees in order to assess properly the difference, the consensus of all MP trees cannot be used. The more MP trees there are, the more pairwise tests are possible to the point where it becomes time prohibitive. To minimize the number of tests, we chose two representative test trees from among the MP trees: one with the highest ML score and the other with the lowest ML score. Each of these two trees was tested against all best trees for a given alternative topology.

## RESULTS

**Sequences**—Characteristics of the sequenced regions as well as statistics of trees derived from each of four regions are summarized in Table 1. All *Cuscuta* taxa under investigation were readily amplifiable with the same set of primers used for other taxa and have open reading frames (ORF) for the *rbcL* and *atpB* genes, and *psbE-J* operon. Several clones for each species of *Cuscuta* were sequenced, but we found no evidence for the presence of pseudogenes. The same applies for the amplification of the noncoding *trnL-F* region. No significant heterogeneity in base composition was observed within any of these data matrices across all taxa (Table 1). Also, no significant difference was encountered among *Cuscuta* sequences alone. Due to the poor quality of the DNA extracted from the herbarium specimens, data for one or another of four regions are missing for ten species (see below). Seven of those are lacking sequences for only one gene, and three species are missing data for two regions. The *rbcL* gene is present for all taxa (Table 1). Aligned data files are available upon request from the first author.

Sequences of *rbcL* used here were 1403 bp in length (position 27–1429 in tobacco). No size variation was found in this portion of the *rbcL* gene and alignment among taxa posed no problem. However, two *rbcL* pseudogenes were detected,

TABLE 1. Summary descriptions for sequences included in individual and combined analyses of four chloroplast regions, *rbcL*, *atpB*, *psbE-J*, and *trnL-F*. df = degrees of freedom.

	<i>rbcL</i>	<i>atpB</i>	<i>psbE-J</i>	<i>trnL-F</i>	Combined data
Number of taxa included	112	108	104	111	112
Sequence characteristics:					
Length of sequenced region (range)	1320–1494 <sup>a</sup>	1464–1497	730–798	478–971	3992–4760
Aligned length	1403	1500	828	1244	4975
Number of gaps coded as binary characters	0	7	11	57	75
Variable sites	562	589	305	586	2042
Parsimony informative sites	358	428	179	374	1339
Pairwise uncorrected distances (range in %)	0.001–15.5	0–15.5	0–18.2	0.02–23.84	0.001–15.7
Mean AT content (%)	55	57	61	63	58
Base frequency homogeneity ( $\chi^2/df/P$ )	33.5/333/1.00	26.9/321/1.00	33.7/309/1.00	74.3/330/1.00	50.3/333/1.00

<sup>a</sup> The *rbcL* gene from *Cuscuta reflexa* has a C-terminal extension of 60 nucleotides as compared to the *rbcL* gene from tobacco.

one in *Ipomoea coccinea* (Olmstead and Palmer, 1994) and one in *Humbertia madagascariensis*. In order to incorporate these two sequences into the alignment, five frame-shift gaps were required (two in *Ipomoea* and three in *Humbertia*). Previous hybridization experiments showed that the pseudogene of *I. coccinea* resides in its mitochondrial genome and phylogenetic analysis suggested that this represents relatively recent duplication and subsequent transfer to the mitochondrion (Olmstead and Palmer, 1994). Approximately 250 base pairs (bp) are deleted from this pseudogene at the 3' end, along with the stop codon. The pseudogene for *H. madagascariensis*, the location of which is unknown, could not be amplified at the 3' end. This might imply that this portion is either missing or has a very divergent sequence, but we have no further evidence to bear on this. However, the phylogenetic analysis indicates a relatively recent duplication event as well (results not shown).

The *atpB* gene, like *rbcL*, is very conserved in its length across all angiosperms. However, the ORF for *atpB* was found to be missing at least two codons (position 163–168 in tobacco *atpB*) for all Convolvulaceae, including parasitic *Cuscuta*. The only exception to this is *Humbertia madagascariensis*, which has a full-length gene, as do all angiosperms other than Convolvulaceae (Savolainen et al., 2000). In the same region, some taxa are lacking 3–5 additional amino acids (*Dicranostyles*, *Maripa*, *Falkia*, *Metaporana*, *Petrogenia repens*, *Porana velutina*, *P. volubilis*, *Calycobolus nutans*). All but one *Cuscuta* species are missing only two amino acids in this region. Additional gaps are found for some *Cuscuta* species (subgenus *Grammica*) elsewhere in the *atpB* gene. Consequently, seven gaps were necessary to align this gene. These were coded as missing data and were appended to the data matrix as presence/absence characters. Sequences for *atpB* could not be obtained for four species, *Paralepistemon shirensis*, *Dichondra brachypoda*, *D. occidentalis*, and *Cordisepalum thorelii*.

Aligned sequences of the *psbE-J* operon used here were 828 bp in length with the individual sequences varying between 730 and 798 bp. Most of the length variation is found in three intergenic spacer (IGS) regions. The longest IGS, found between the *psbL* and *psbJ* genes (124 bp in tobacco) is the most variable one. This spacer accounts for seven parsimony-informative gaps. No size variation was found in the *psbF* and *psbI* genes. However, *psbE* and *psbL* genes exhibited some length variation within the open reading frame. The *psbE* gene has three independent occurrences of gaps with respect to tobacco, two found in green Convolvulaceae (*Humbertia* and *Jacquemontia*) and one in *Cuscuta* subgenus *Grammica*. Due to this

gap (deletion) in *Cuscuta* subgenus *Grammica*, the stop codon for *psbE* gene overlaps with the start codon for *psbF*. Altogether, 11 parsimony-informative gaps were introduced in the alignment and coded as presence/absence characters. In most angiosperms (except monocots) for which the sequences are known, the initiation codon of *psbL* has an edit site (Kudla et al., 1992; Bock et al., 1993; Graham and Olmstead, 2000). Editing of C to U is necessary to produce a functional translation initiation codon for this gene in these taxa. This edit site is found in most of the Convolvulaceae also, except in a subset of taxa in which the editing is not necessary. Sequences for eight taxa, *Turbina oblongata*, *Paralepistemon shirensis*, *Argyrea osyrensis*, *Seddera arabica*, *Dichondra brachypoda*, *Petrogenia repens*, *Dicranostyles villosus*, and *Cuscuta reflexa*, could not be obtained for this region.

The total alignment length for the *trnL-F* region is 1241 bp, while individual sequences varied from 478 to 971 bp in length. For the nonparasitic taxa, the sequences were aligned easily throughout the region despite the fact that numerous gaps had to be introduced in order to do so. All Convolvulaceae under investigation, except *Humbertia madagascariensis*, lack a large portion of the *trnL-F* intron (169 bp as compared to tobacco). This large deletion has a distribution pattern similar to the one described for *atpB*. In the context of the rooted phylogenetic hypothesis (Fig. 1, see below), the lack of these deletions underlines further the isolated basal position of *H. madagascariensis* within the family. Parasitic taxa also are found to be readily alignable for the intron. The spacer region, however, was almost entirely missing in *Cuscuta*, except in *C. japonica*. In addition, the small remaining part of the spacer showed great nucleotide divergence. Consequently, the spacer portion was excluded from the alignment for all *Cuscuta* sequences except *C. japonica*. Altogether, 173 gaps, 1–197 bp in length, were introduced to accommodate the alignment among all taxa. Of these, 57 were potentially parsimony informative. Information from this source was added to the data matrix as presence/absence characters. As noticed in previous studies dealing with *trnL-F* data (e.g., Gielly and Taberlet, 1994; McDade and Moody, 1999), the spacer region is evolving more rapidly than the *trnL* intron, both in terms of point mutations and length mutations. Sequences for the *trnL-F* could not be obtained for one species, *Cuscuta reflexa*.

The combined data matrix contains 112 terminal units and 4975 characters, including 1339 that are parsimony informative (Table 1).

**Unconstrained topologies and overall levels of support—**Because the ILD test indicated no significant heterogeneity

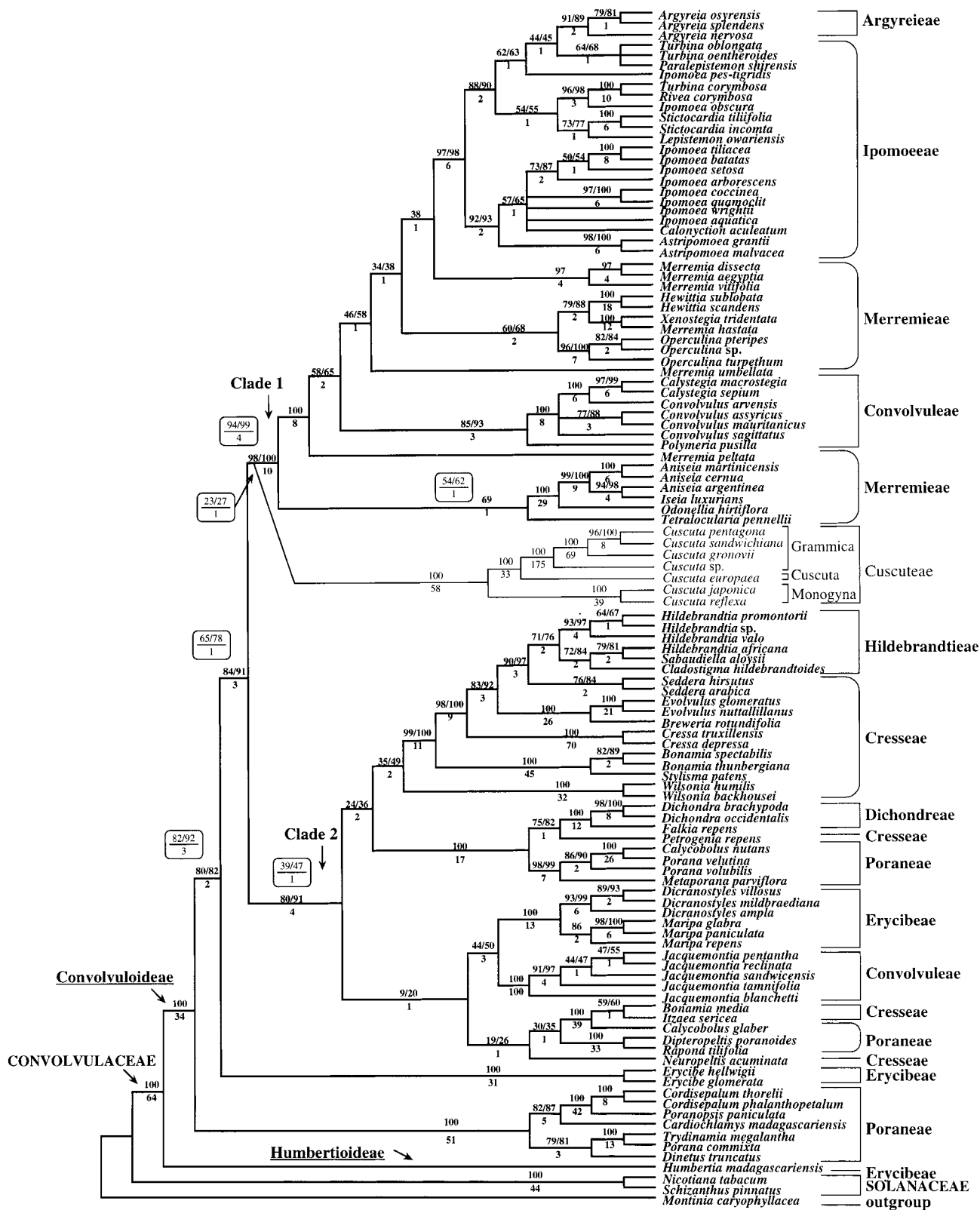


Fig. 1. The strict consensus of 54 equally parsimonious trees (L = 3366; CI = 0.62; RI = 0.79) from the total evidence analysis (i.e., *rbcL*, *atpB*, *psbE-J*, and *trnL-F* sequence) comprising genera from all traditionally recognized green Convolvulaceae tribes is shown in boldface type. The placement of the *Cuscuta* species when this parasitic genus is included in analysis (L = 4591; CI = 0.61; RI = 0.77) is shown appended to the tree in lighter type. The tree is rooted using three taxa belonging to closely related families as outgroups. Numbers above branches are bootstrap and jackknife values, respectively (when percentages are identical, only one number is shown). Numbers below branches are decay indices. Boxed numbers show bootstrap, jackknife, and decay (support when *Cuscuta* is included) for those nodes most affected by its inclusion. Classification by tribe based on Austin (1973, modified 1998b). Square brackets indicate monophyletic groups; rounded indicate paraphyletic groups. Two well-supported clades are indicated.

between the four individual data sets ( $P = 0.09$  with *Cuscuta*;  $P = 0.12$  without *Cuscuta*) and independent analyses, albeit quite unresolved, gave remarkably congruent results (results not shown), we combined all four matrices. Two sets of analyses were performed on this combined data matrix. One included only non-parasitic members of the family (105 taxa) and the other included green Convolvulaceae and seven parasitic species. The combined data analysis (i.e., all sequence data plus gaps) performed on green Convolvulaceae resulted in 54 MP trees, each 3366 steps in length, with a consistency index (CI) = 0.62/0.52 and a retention index (RI) = 0.79. Figure 1 presents the strict consensus (in boldface type).

According to our results, Convolvulaceae, including *Humbertia*, are found to be monophyletic, with Solanaceae as the sister group. Both of these results received very high overall support (Fig. 1). In addition to nucleotide substitutions, monophyly of the family is supported by three unambiguous insertions in the *trnL-F* region. The monotypic *Humbertia* forms an isolated lineage positioned as a sister group to the rest of Convolvulaceae. The branch supported by 100% bootstrap replicates (BS), 100% jackknife replicates (JK), and a decay index (DI) of 34 supports the clade comprising the remainder of the family. Furthermore, *H. madagascariensis* does not share the signature two amino-acid deletion in the *atpB* gene and 169 bp deletion in the *trnL* intron (Fig. 2, open circle), with the rest of the Convolvulaceae emphasizing the basal position of the genus. The next two lineages to diverge within the Convolvulaceae are two small clades, one comprising some members of Poraneae and the other genus *Erycibe*. Each of these two lineages is strongly supported as monophyletic. Their progressively more terminal placements on the MP trees are moderately supported (BS = 80%, JK = 82%, DI = 2, and BS = 84%, JK = 91%, DI = 3, respectively).

The rest of the family is split into two major clades (Fig. 1; clade 1, clade 2). The first clade includes tribes Argyreieae, Ipomoeae, Convolvuleae, and Merremieae and comprises the majority of species in the family (BS = 98%, JK = 100%, and DI = 10). Within clade 1 a moderately supported group consisting of four genera assigned to Merremieae (*Tetralocularia*, *Odonellia*, *Aniseia*, and *Iseia*) is found to diverge first, forming the sister group to the strongly supported rest of this clade. Here, Argyreieae and Ipomoeae, taxa with spiny pollen, are most closely related to each other. Convolvuleae (minus *Jacquemontia*) are moderately supported, and the relationship among the rest of the genera belonging to Merremieae is mainly unresolved.

The second major clade comprises the remainder of the family (BS = 80%, JK = 91%, and DI = 4). Among the molecular characters that support this group unambiguously is the ACG to ATG transition responsible for the switch from the edited to nonedited f-Met codon in the *psbL* gene. The edited codon (ACG) is found in all other Convolvulaceae as well as in the sister family Solanaceae. A morphological character provides additional support for this clade. All genera grouped here, with the notable exception of *Jacquemontia*, have a more or less deeply divided style (Fig. 2), following, to a certain extent, the concept of Dicranostyleae and its derivatives from Hallier's (1893) phylogenetic scheme for the family. The backbone relationships within this "bifid style" clade are largely unresolved. For example, *Jacquemontia*, which exhibits the greatest sequence divergence of any nonparasitic Convolvulaceae, and the group consisting of *Maripa* and *Dicranostyles* do not reveal well-supported affiliation to any other group.

Nevertheless, several strongly supported clades are identified, including three composed entirely, or in part, of taxa historically assigned to three tribes. The monophyletic African tribe Hildebrandtieae is found within a clade comprising genera traditionally attributed to the Cresseae, and this relationship is strongly supported. Tribe Dichondreae shows a close alliance with some members of the Poraneae.

Analysis of a matrix that included *Cuscuta* species (112 taxa) recovered the same 54 equally parsimonious trees, with L = 4591, CI = 0.61, and RI = 0.77. This resulted in a strict consensus with a branching pattern identical to the one described above, but with the *Cuscuta* species inserted as the sister group to the clade consisting of tribes Argyreieae, Ipomoeae, Convolvuleae, and Merremieae (clade 1; Fig. 1). One MP tree, the one with the best ML score given our data set, is chosen to illustrate branch lengths (Fig. 2). This placement for *Cuscuta*, however, is poorly supported (BS = 23%, JK = 27%, DI = 1), and its exact position is considered unresolved. The support for nodes immediately surrounding the branching point of *Cuscuta* were affected (Fig. 1, boxed numbers), by the inclusion of the highly diverged, homoplastic sequences from *Cuscuta*. The majority of the nodes, however, were relatively unaffected by the inclusion of *Cuscuta*. The genus *Cuscuta* itself is one of the best supported groups in this analysis (two unambiguous deletions in the *trnL* intron; BS = 100%, JK = 100%, DI = 58). The relationships within *Cuscuta* are fully resolved and well supported. All three subgenera (sensu Yuncker, 1932) are found to be monophyletic, with subgenus *Monogyna* as the sister group to subgenus *Cuscuta* plus subgenus *Grammica* (Fig. 1).

**Alternative topologies**—All together, seven alternative hypotheses were tested concerning some particular relationships within the green Convolvulaceae (Table 2). Tribes Cresseae and Ipomoeae were reported previously as paraphyletic (Austin, 1998b; Manos, Miller, and Wilkin, 2001). Results presented here strongly agree with those findings and formal tests were not conducted. Four of our alternative topologies were designed to test possible monophyly of traditionally described tribes, which are first reported as polyphyletic in this study (Fig. 2, shaded boxes). Enforced monophyly of Merremieae, Convolvuleae, Erycibeeae, and Poraneae resulted in trees 14, 27, 49, and 83 steps longer, respectively. All four were rejected as significantly worse solutions by SH tests (Table 2). Three additional hypotheses were tested, aimed mainly to address the unexpected and/or poorly supported position of certain genera on the MP trees. First, the position of *Jacquemontia* is unexpected from a morphological viewpoint (see discussion). An alternative branching point (Fig. 2, pound sign) would be more consistent with the styler morphology and closer to its traditional placement. Second, Erycibeeae is not monophyletic predominantly due to the quite isolated position of *Humbertia*, traditionally assigned to this tribe. The possibility exists, however, that more narrowly circumscribed Erycibeeae (without *Humbertia*) may be a natural group. Third, most of Cresseae constitute a grade with monophyletic Hildebrandtieae nested within it (Fig. 1). The rest of this tribe is interspersed elsewhere on the tree. We wanted to determine the cost in parsimony and its significance for the alternative in which all of the genera attributed traditionally to the Cresseae and Hildebrandtieae were monophyletic. All three of these alternatives yielded trees five to eight steps longer than the MP trees, but

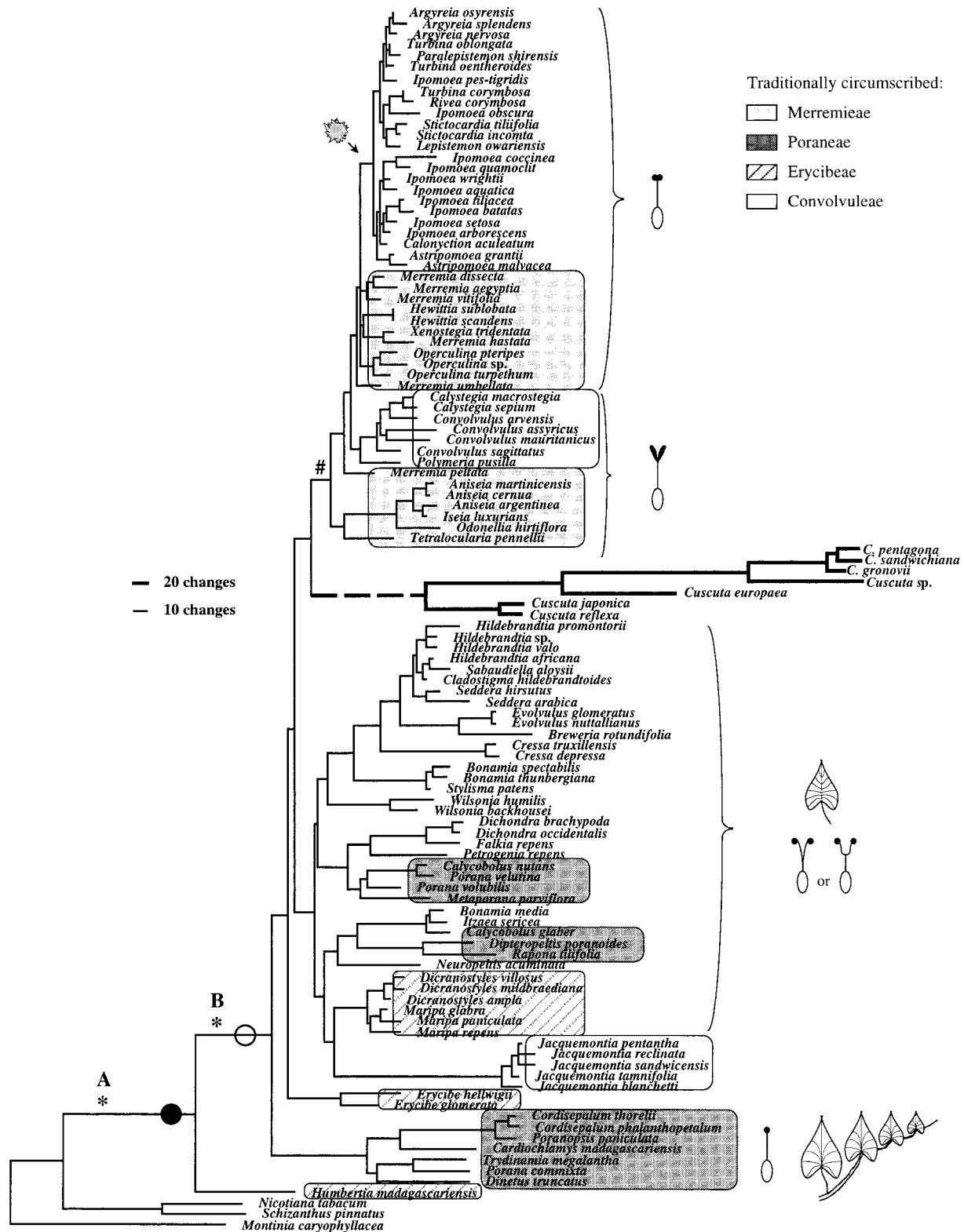


Fig. 2. One of 54 most parsimonious trees, the one with the highest maximum likelihood score of all MP trees ( $L = 4591$ ;  $CI = 0.61$ ;  $RI = 0.77$ ), from the total evidence analysis, including both parasitic and non-parasitic species of Convolvulaceae chosen to illustrate branch lengths. Branch lengths are drawn proportionally to the number of changes. Note the different scale for the *Cuscuta* lineage (in boldface type). Asterisks (A, B) depict alternative placements for the genus *Cuscuta* and the # sign depicts one alternative placement for *Jacquemontia* used in the SH tests. Shaded boxes depict four of the traditionally defined tribes tested for monophyly (compare with Table 2). Circles indicate inferred origin of some structural changes in the chloroplast genome (solid, *rpl2* intron deletion; open, 6 bp deletion in *atpB* gene and 169 bp deletion in *trnL* intron). Some important morphological characters are optimized onto the tree and indicated by illustration (see text for full explanation).

TABLE 2. Comparison of unconstrained (most parsimonious) topologies with specific alternative hypotheses and results of the corresponding Shimodaira-Hasegawa tests.

Topology <sup>a</sup>	Length	No. of trees	Length penalty	<i>P</i> <sup>b</sup>	Rejected <sup>c</sup>
Unconstrained (MP) with green taxa only; Fig. 1, in bold	3366	54	—	—	Best
Merremieae monophyletic	3380	180	14	0.001–0.004	Yes
Poraneae monophyletic	3449	162	83	0.000	Yes
Convolvuleae monophyletic	3393	2268	27	0.000–0.002	Yes
<i>Jacquemontia</i> with clade containing Convolvuleae, Ipomoeae, Argyreieae, and Merremieae (#)	3374	54	8	0.038–0.161	No
Erycibeae monophyletic	3415	1188	49	0.000	Yes
Erycibeae, without <i>Humbertia</i> , monophyletic	3374	756	8	0.206–0.469	No
All the members of Cresseae associated with Hildebrandtieae	3371	540	5	0.617–0.956	No
Unconstrained (MP) with parasitic taxa included; Fig. 1	4591	54	—	—	Best
<i>Cuscuta</i> as sister to the rest of Convolvulaceae (A)	4626	54	35	0.000–0.003	Yes
<i>Humbertia</i> basal lineage, <i>Cuscuta</i> diverging next (B)	4598	54	7	0.225–0.442	No

<sup>a</sup> Alternative branching patterns for the genus *Cuscuta* (A, B) marked by asterisks in Fig. 2; that of *Jacquemontia* marked by #.

<sup>b</sup> *P* values, the confidence limit for rejection of the alternative topologies from the one-sided SH test; interval given for the range of *P* values across all tested trees.

<sup>c</sup> Yes, rejected as significantly worse topology by the SH test using RELL bootstrap (*P* < 0.05); No, not rejected by the SH test.

none were rejected as significantly different from the MP trees by SH test (Table 2).

Two other hypotheses concerning the parasitic taxa were tested. *Cuscuta* exhibits strong rate acceleration in chloroplast DNA evolution (Fig. 2), ostensibly due to relaxation of functional constraints on the chloroplast genome because of its parasitic habit (Wolfe and dePamphilis, 1998). Even though MP trees position it nested well within the Convolvulaceae, as a sister group to the clade composed of Argyreieae, Ipomoeae, Convolvuleae, and Merremieae, this placement is not well supported. Because of the long branch leading to the *Cuscuta* clade, caution is warranted when interpreting the relationships of this genus with the remainder of the family. Two specific alternative hypotheses, bearing the most importance for the circumscription of the family as a whole, were tested using constrained searches. The different points of attachment tested for constrained topologies are marked with asterisks in Fig. 2 and results are summarized in Table 2. Only one alternative position, *Cuscuta* as a sister group to the rest of the family, i.e., consistent with its recognition as a distinct family, was significantly worse than the MP trees according to the SH test. Constraining the *Cuscuta* clade to diverge within the family, as sister to all, except *Humbertia*, resulted in trees that were not significantly different from the tree depicted in Fig. 1.

## DISCUSSION

The present study is based on sequences from four chloroplast loci. Although the chloroplast genome behaves genetically as a single locus (Doyle, 1992), different parts of the genome are constrained by selection to be suitable for phylogenetic studies at different hierarchical levels. The cluster of four small genes linked in an operon (*psbE-J*) are part of the photosystem II complex and represent the most conservative component of our data (Graham and Olmstead, 2000). The *rbcL* and *atpB* genes, involved in photosynthesis and ATP synthesis, respectively, have been used widely in plant systematics where moderately slowly evolving sequences are needed (Olmstead and Palmer, 1994; Hoot, Culham, and Crane, 1995). The *trnL-F* region, on the other hand, is noncoding, under less intense selection, and thus evolves more rapidly. Because these four loci represent an amalgam of sites evolving at different

rates they should lend resolution and support both for older divergences (deeper nodes) and for more closely related taxa in the same analysis. The results from these sequences are quite well resolved as well as robust, including the high level of support along the spine of the tree. This represents the most complete molecular phylogenetic hypotheses for Convolvulaceae yet made.

**Circumscription of the family**—Convolvulaceae, a cosmopolitan family, are found to be sister to the Solanaceae, a mainly New World family, with 100% bootstrap support for each family and the clade containing both families (Fig. 1). Our sequence data support the single origin of the Convolvulaceae, even though there is no evident, unique, and unreversed morphological synapomorphy, to the best of our knowledge, for all members of the family. Two of the three groups that have been proposed as segregate families (Dumortier, 1829), *Cuscuta* and tribe Dichondreae, are nested within Convolvulaceae in the present analysis, and the third, *Humbertia*, is the sister group to the other members of the family.

Convolvulaceae monophyly is strongly supported also by a structural change in the chloroplast genome of this family. An intron usually found in the *rpl2* gene of angiosperms is deleted in all Convolvulaceae, including *Humbertia* and *Cuscuta* (S. Stefanović and R. G. Olmstead, unpublished data). In their survey of angiosperms, Downie et al. (1991) reported at least six independent losses of the *rpl2* intron, including in two representatives of green Convolvulaceae and one *Cuscuta* species. Outside of Convolvulaceae no other member of the Asterideae was found to lack this intron. Based on this observation, a more detailed examination was conducted with expanded sampling in Convolvulaceae and some putatively closely related families (S. Stefanović and R. G. Olmstead, unpublished data). We concluded that the *rpl2* intron deletion represents a unique event within Asterideae and a synapomorphy for Convolvulaceae (Fig. 2, solid circle).

***Humbertia***—This monotypic genus, endemic to Madagascar, is the sister group to the rest of Convolvulaceae. *Humbertia* is a tree with slightly zygomorphic flowers among otherwise predominantly herbaceous taxa with actinomorphic floral symmetry. This species retains several suspected ancestral features of Convolvulaceae. Baillon (1891) first noted the so-



lanaceous character of placentation, i.e., indefinite number of ovules (~40), and transferred this genus to the Solanaceae. Hallier (1893) kept *Humbertia* within Convolvulaceae (tribe Erycibeae) in spite of the specialized anatomical traits he described, such as the absence of internal phloem and secretory cells, both of which are characteristics of the Convolvulaceae. The most detailed anatomical study of the genus was done by Deroin (1992), who showed that secretory cells, typical for the family, are present in the corolla, androecium, and gynoecium of *H. madagascariensis*. Furthermore, Deroin pointed out that, with the exception of the absence of internal phloem, a condition also found in *Cuscuta*, there are no major anatomical differences between *Humbertia* and the other genera of Convolvulaceae, especially those belonging to the tribe Erycibeae.

The isolated position of *H. madagascariensis* on our trees is consistent with either segregating the genus in its own monotypic family, Humbertiaceae (Pichon, 1947), or keeping it within the Convolvulaceae as subfamily Humbertioidae (Roberty, 1952; Melchior, 1964). However, our results are not consistent with its placement in tribe Erycibeae (Hallier, 1893; Austin, 1973, 1998b; Deroin, 1992). Relying on strongly supported relationships inferred from chloroplast data, including the absence of deletions in the *atpB* gene and *trnL* intron that characterize the rest of family (Fig. 2), and taking into account well-documented similarities between *Humbertia* and the other genera of Convolvulaceae (Deroin, 1992), we retain it in the family and recognize two subfamilies: Humbertioidae and Convolvuloideae (Fig. 1). Retention in the family is further supported by presence of a derived chloroplast structural character, the *rpl2* intron deletion, shared with the rest of the family (S. Stefanović and R. G. Olmstead, unpublished data).

***Cuscuta***—Many classifications have accommodated *Cuscuta*'s unique form and parasitic lifestyle by segregating it into a separate family (Dumortier, 1829), even though its close alliance with Convolvulaceae was generally recognized and accepted (Cronquist, 1988; Takhtajan, 1997; Austin, 1998b). According to chloroplastDNA sequences, *Cuscuta* is nested within the family (Fig. 1). At least one lineage, *Humbertia*, diverges within the family before *Cuscuta*. This corroborates the tentative conclusion of Neyland (2001) based on partial 26S rDNA sequences with limited sampling in Convolvulaceae. However, the relationship of *Cuscuta* within the subfamily Convolvuloideae is not clear. The genus is found on the MP trees as a sister group to the clade comprising Convolvuleae, Ipomoeae, Argyreieae, and Merremieae, but this relationship is not strongly supported. This is mainly due to the *Cuscuta* sequences being highly divergent. The anticipated acceleration in the rate of sequence evolution for *Cuscuta* chloroplast DNA sequences is evident from Fig. 2, as depicted by the long branches on the phylogram within *Cuscuta* (note different scale for the *Cuscuta* lineage). In addition, even though this study is based on a large data set (about 5000 aligned bp), this amount of data may still not be sufficient to result in a robust hypothesis for the position of *Cuscuta* given the magnitude of this problem. This is clearly the case for some other poorly supported relationships where long branches cannot be invoked. Given the extensive sampling included here, it is unlikely that further sampling within Convolvulaceae (a widely used strategy for breaking long branches) would be sufficient to solve this issue. We believe, however, that additional sources of data should be taken into account before a firm conclusion on the position of *Cuscuta* can be drawn. For example,

trees derived from the mitochondrial *coxI* gene intron show less acceleration in the rate of nucleotide substitution for *Cuscuta* and could be of great value for resolving its position within Convolvulaceae (McNeal, Croom, and dePamphilis, 1999).

The main goal of this paper is the circumscription of the family and major lineages within the family as well as relationships among the green Convolvulaceae. The phylogenetic position of *Cuscuta* and its precise relationship to nonparasitic relatives will be addressed more definitively elsewhere. However, two possible alternative positions for *Cuscuta*, pertinent for the circumscription of the family, have been tested in this study (Fig. 2, asterisks). The position as sister to the rest of Convolvulaceae was found to be significantly worse. This is further corroborated by the distribution of deletions in *atpB* gene and *trnL* intron also found in *Cuscuta* species (Fig. 2, open circle). The other hypothesis, *Cuscuta* as a sister group to the subfamily Convolvuloideae, could not be rejected by the SH test (Table 2).

Ambiguities regarding the position of *Cuscuta* within the family contrast with the completely resolved and well-supported relationships within the genus. Some preliminary conclusions can be drawn, although the sampling of *Cuscuta* spp. in our data is not extensive. Cytological, anatomical, and morphological characters indicate the presence of three well-defined groups in the genus. Our results identify three lineages that are consistent with the subgenera proposed by Engelmann (1859) and Yuncker (1932). The subgenus with united styles, *Monogyne*, is sister to the rest of the genus. The bifid stylar structure provides a uniting character for subgenera *Cuscuta* and *Grammica*. These two infrageneric taxa can be distinguished by elongated and non-elongated stigmas, respectively.

**Major lineages within the Convolvulaceae—Poraneae**—The tribe Poraneae was first circumscribed by Hallier (1893) based mainly on a combination of two characters: the accrescent calyx (sepals equally or unequally enlarge as the fruit matures) and utricle (indehiscent, one-seeded fruit with papery pericarp). Many authors have subsequently adopted this tribal concept, with minor changes in circumscription (e.g., Peter, 1897; Melchior, 1964; Austin, 1973). The only major departure from Hallier's concept was proposed by Roberty (1952, 1964), but this system has been controversial and regarded as highly artificial. The most recent comprehensive examination of this tribe was done by Staples (1987, 1990). However, Poraneae, in any of proposed delimitations, is not monophyletic. The MP trees enforcing monophyly of the traditionally defined Poraneae were 83 steps longer than the MP trees and were strongly rejected by the SH test (Table 2).

Tribe Poraneae comprises three distant groups. The first two have deeply divided styles and pinnate leaf venation and are found within the "bifid style" clade. Most of these species are tightly associated with the monophyletic tribe Dichondreae, whereas the exact relationships for the rest (e.g., *Rapona*, *Dipteropeltis*) is not ascertained. The third group consists of *Cordisepalum*, *Cardiochlamys* and some segregates of *Porana* s.l. (*Poranopsis*, *Trydinamia*, *Dinetus*) and is sister to the rest of subfamily Convolvuloideae (Fig. 1). All members of this clade have a single, undivided style. Besides this character, which sets it apart from the "bifid style" clade members, this group is supported by palmate leaf venation and foliaceous, sessile bracts (Fig. 2), characters not found in other taxa assigned previously to Poraneae. Staples (1987) was the first to point

out the importance of these two morphological characters, style morphology and leaf venation, for the systematics of tribe Poraneae. The division of Poraneae using these characters is implicit in Staples' treatment of the tribe (his Fig. 13). However, his phylogeny, based on 17 morphological characters, was conducted with the premise of a single origin for the tribe and consequently could not infer the polyphyly of Poraneae. The genus *Porana* also is not monophyletic. This genus is defined, within the tribe, by having all five sepals equally accrescent. The genus, however, splits along the same morphological lines as the tribe, with the type species, *P. volubilis*, found in the "bifid style" clade (Fig. 2).

Taking all of these observations into account, it seems apparent that the accrescent sepals arose more than once. The convergent nature of this character is evident not only from its distribution with respect to a polyphyletic Poraneae, but also because it is found in several other unrelated genera such as *Aniseia*, *Operculina*, *Stictocardia*, and tribe Hildebrandtieae. Moreover, the number of sepals that becomes enlarged as well as the extent of enlargement varies across the taxa (see also Austin, 1998b). This is an example of analogous structures having the same biological function—adaptation to wind dispersal. In some cases, e.g., genus *Neuropeltis*, the same dispersal function is facilitated by enlargement of the flower-subtending bracts rather than the sepals themselves.

*Erycibae*—As circumscribed traditionally (e.g., Austin, 1973), this tribe should include *Erycibe*, *Humbertia*, *Maripa*, *Dicranostyles*, and *Lysiosyles* (Table 2). The last genus is not represented in our study, nevertheless, the well-supported, isolated position of *Humbertia* (see above) and the lack of evidence for a close relationship between *Erycibe* (South-East Asia, North Australia) and *Dicranostyles* plus *Maripa* (lowlands of Central and northern South America) renders this assemblage of taxa polyphyletic. This finding is further supported by stelar character distribution: *Humbertia* has one undivided style. Most species of *Dicranostyles* and *Maripa* have divided styles, and are found in the "bifid style" clade. This is also the predicted position for the unsampled genus endemic to Guyanas and Venezuela, *Lysiosyles*, which is morphologically and distributionally close to *Dicranostyles* (Austin, 1973). The style in *Erycibe* is absent and its conical stigmas are sessile. It is possible, though unlikely, that the tribe Erycibae, in a narrow sense (i.e., without *Humbertia*), could be found to be monophyletic once more characters and *Lysiosyles* are examined. This hypothesis could not be rejected by the SH test even with present data (Table 2). The absence of a style in *Erycibe* leaves the interpretation of this character open. If its isolated position, suggested by the present analyses, is confirmed, that would imply the complete reduction of an undivided ancestral style; if Erycibae sensu stricto (s.s.) were found to be monophyletic, nested in the "bifid style" clade, the reduction of a divided style would be a more parsimonious explanation.

*Dichondreae*—Tribe Dichondreae, the third group frequently segregated from the Convolvulaceae, is found to be a well-supported, natural group positioned within the family (Fig. 1). It occurs in the "bifid style" clade, as a sister group to *Petrogenia repens*, and further, the clade including some taxa with divided style assigned traditionally to Poraneae. The close relationship between Dichondreae and Poraneae was first suggested by Austin (1973), in his phylogenetic scheme based

primarily on cytology. This assemblage is strongly supported although its exact relationships within the "bifid style" clade are not resolved. The possible morphological synapomorphy for this clade is the utriculate fruit. The same fruit type is shared also with some additional Poraneae genera with divided style (e.g., *Rapona*, *Dipteropeltis*), but the precise relationships of these genera are not clear. Given our results, the indehiscent, one-seeded fruit with papery pericarp is inferred to evolve at least three times independently within Convolvulaceae: in the basal Poraneae clade with undivided style, in the clade comprising Dichondreae plus Poraneae with bifid styles, as well as in some *Hildebrandtia* species.

*Hildebrandtieae and Cresseae*—Tribe Hildebrandtieae is characterized by anatomically and/or functionally unisexual flowers, two free styles, and accrescent sepals in female flowers. Dioecism is unique here in the family. Hallier (1893) and Austin (1973) suggested a connection between Hildebrandtieae and genera from tribe Cresseae (sensu Austin), which share with Hildebrandtieae branched or free styles. Phylogenetic analyses by Demissew and Austin (1996) and Austin (1998b) confirmed this relationship between tribes Hildebrandtieae and Cresseae. They showed that a monophyletic Hildebrandtieae are nested within a paraphyletic Cresseae, with *Cladostigma* as a sister group to *Hildebrandtia*. Given the position of *Cladostigma hildebrandtoides* in our MP trees, it appears that this genus, characterized by the absence of anthers in female flowers and sepals clawed at the base, should be placed in synonymy with *Hildebrandtia*. *Sabaudiella* has been shown previously to share a number of characters with *Hildebrandtia*, and its inclusion in *Hildebrandtia* has been suggested (Demissew and Austin, 1996). Within *Hildebrandtia* defined in this broad sense, two well-supported groups emerged, one comprising all species from mainland Africa and Arabia, monophyly of which is highlighted by presence of two-locular ovaries and capsular fruits, and a second comprising species from Madagascar characterized by unilocular ovaries and utricular fruits. The east African genus *Seddera*, some species of which also have accrescent sepals, is confirmed to be closest to Hildebrandtieae of the genera in the Cresseae grade. The clade comprising tribe Hildebrandtieae and the majority of genera placed in tribe Cresseae is well resolved, well supported, and in agreement with previous, morphology based, findings. However, two genera belonging historically to Cresseae, *Itzaea* and *Neuropeltis*, as well as one species of *Bonamia*, are found elsewhere on the tree. Constraining these taxa with the rest of tribes Cresseae and Hildebrandtieae was not rejected by SH test (Table 2).

*Jacquemontia*—Five species have been sampled to encompass the diversity of this large genus (~120 spp.) with predominantly New World distribution. This genus, characterized by stellate trichomes and eight-valvate capsules, is found to be monophyletic, quite distinct molecularly, and very well supported (Fig. 1). However, the position of *Jacquemontia* inferred from our MP trees is one of the most perplexing results of this study. This genus, traditionally placed in tribe Convolvuleae due to its undivided, filiform style with elongated stigmas, is found within the "bifid style" clade. As mentioned before, the backbone relationships are not well resolved within this clade, and the sister group to *Jacquemontia* remains uncertain. Even though the defining morphological character, divided style, is not present in *Jacquemontia*, all five sampled

species share a unique synapomorphy with the rest of the “bifid style” clade, reversion to a nonedited start codon for the *psbL* gene. This condition is not found anywhere else in Convolvulaceae and its closest relatives.

Given the unresolved relationships within the “bifid style” clade, it is possible to reconcile these two important characters. A scenario in which *Jacquemontia* would be found as the sister group to the rest of taxa with divided style would account for a single origin for each of these two characters. This precise branching pattern, compatible with our MP trees, was not formally tested. Alternatively, the placement of *Jacquemontia* as a sister group to the clade comprising Convolvuleae, Ipomoeae, Argyreiae, and Merremiae (Fig. 2, # sign) would be more compliant with a traditional morphological viewpoint. This alternative topology could not be rejected by the SH test (Table 2). It would, however, imply at least two independent changes with respect to the start codon in the *psbL* gene. Enforcing the monophyly of Convolvuleae as circumscribed historically resulted in the trees 27 steps longer than the MP trees and was rejected by the SH test (Table 2).

*Merremiae, Convolvuleae, Ipomoeae, and Argyreiae*—The members of these four tribes together form a well-supported group. A clade composed of some Merremiae (*Aniseia, Iseia, Odonellia, and Tetralocularia*), most of which have unequally enlarged sepals and elongated stigmas, is a sister group to the well-supported remainder of the clade. The monotypic South American genus *Iseia* is nested within *Aniseia*. *Iseia* was recognized by O’Donnell (1953) as a genus separate from *Aniseia* due to several morphological characters (e.g., indehiscent fruit, subequal sepals, globose stigma) all of which appear to be autapomorphies for its single species, *I. luxurians*. Therefore, *Iseia* should be included in genus *Aniseia* as its fourth species. *Odonellia* is found to be closely associated with *Aniseia* s.l., as predicted by Robertson (1982) when he segregated two *Jacquemontia* species in this new genus, based on simple rather than stellate trichomes and some additional, mainly palynological, differences.

The well-supported remainder of the taxa includes most of the species within Convolvulaceae. Many genera in this group are notoriously difficult to delimit (Wilson, 1960; Austin, 1975). This morphological homogeneity is reflected in the similarity of molecular data (note branch lengths in Fig. 2). Both are probably due to a rapid and/or recent radiation. More rapidly evolving sequence data are necessary to confidently resolve the exact relationships among these taxa. Nevertheless, within this clade two groups received good support: (1) tribe Convolvuleae pro parte, excluding *Jacquemontia*, and (2) a clade comprising all taxa with spiny pollen (see symbol on Fig. 2), Echinoconieae sensu Hallier. The former group consists of *Calystegia* and *Convolvulus*, both cosmopolitan in their distribution, and the Australian endemic *Polymeria*. *Calystegia*, albeit well-defined morphologically by pantoporate pollen and supported as a monophyletic group, is nested within the bigger genus, *Convolvulus*, and should be included in it.

The second group corresponds entirely to subfamily Echinoconieae as defined by Hallier (1893), based on pollen morphology. Two major clades, both well supported, are resolved within the spiny-pollen group. One includes the small African genus *Astripomoea* and its sister group, a predominantly New World group of *Ipomoea* species. *Calonyction aculeatum*, recognized by previous authors as distinct but placed in synonymy with *Ipomoea* in Austin’s system, belongs in this clade.

The other group consists primarily of Old World genera classified traditionally in Ipomoeae such as *Lepistemon, Stictocardia, Paralepistemon, and Turbina* as well as some Old World *Ipomoea* species. Tribe Argyreiae is nested within the traditional Ipomoeae. All of the results regarding Ipomoeae and Argyreiae presented here are in agreement with two more detailed studies focusing on the “spiny pollen” clade (Miller, Rausher, and Manos, 1999; Manos, Miller, and Wilkin, 2001). These studies, sampling more taxa and using more rapidly evolving nuclear sequences (ITS and *waxy*) offered a well-resolved and supported phylogeny for the “spiny pollen” Convolvulaceae. The paraphyly of tribe Ipomoeae and genus *Argyreia* as well as polyphyly of *Ipomoea* and *Turbina* are reported and discussed in more detail in those studies. All of those results are further corroborated by our chloroplastDNA sequence data.

Merremiae were treated initially as an informal assemblage of taxa, the “merremioids,” and were formally recognized only recently (Austin, 1982). This reflects the lack of defining morphological characters for this group. In fact, the “merremioids” were defined as lacking some characters used to circumscribe other tribes (e.g., spiny pollen), rather than by their own putative synapomorphies. The hypothesis of a monophyletic Merremiae was rejected as significantly worse by the SH test, given our data. Members of the “spiny pollen” clade also have globose stigmas and appear to be nested within a grade consisting of taxa with globose stigmas assigned traditionally to tribe Merremiae (Fig. 2). We are able to resolve some clusters of species within this grade (e.g., *Xenostegia* and *Hewittia*, some *Merremia* species), but the relationships among them are largely unresolved.

**Conclusions**—Our analyses have shown that Convolvulaceae are monophyletic, including taxa that have been proposed as segregate families, such as *Humbertia* and *Cuscuta*. Within the family, *Humbertia* is sister to all others. To accommodate its position in the family and distinctive morphology, we recognized it as subfamily Humbertoideae (Pichon) Roberty. The parasitic genus *Cuscuta* clearly belongs within the family. Its exact position is not recovered, but some alternatives can be rejected with confidence. We have identified several distinct monophyletic groups, some of which correspond to earlier classifications and some of which do not. Close relationships of tribes Hildebrandtieae with Cresseae and Ipomoeae with Argyreiae (Echinoconieae) were suggested by previous workers and are confirmed here. The polyphyly of Poraneae, the putative basal position of some genera from this tribe, and the association of the remaining genera with Dichondreae are first reported here, as well as the polyphyly of Merremiae, Convolvulae, and Erycibeae.

The information provided here identifies some areas in need of future study. More data, including both more characters and more taxa, are required before a well-resolved phylogenetic hypothesis for the “bifid style” clade and the position of *Jacquemontia* can be offered. Also, there is a solid indication that some genera (e.g., *Calycobolus, Bonamia*) are polyphyletic, and it is clear that more species belonging to these genera need to be sampled.

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