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Testing the Phylogenetic Position of a Parasitic Plant (*Cuscuta*, Convolvulaceae, Asteridae): Bayesian Inference and the Parametric Bootstrap on Data Drawn from Three Genomes

SAŠA STEFANOVIĆ[1] AND RICHARD G. OLMSTEAD

Department of Botany, University of Washington, Box 355325, Seattle, Washington 98195-5325, USA

Abstract.—Previous findings on structural rearrangements in the chloroplast genome of *Cuscuta* (dodder), the only parasitic genus in the morning-glory family, Convolvulaceae, were attributed to its parasitic life style, but without proper comparison to related nonparasitic members of the family. Before molecular evolutionary questions regarding genome evolution can be answered, the phylogenetic problems within the family need to be resolved. However, the phylogenetic position of parasitic angiosperms and their precise relationship to nonparasitic relatives are difficult to infer. Problems are encountered with both morphological and molecular evidence. Molecular data have been used in numerous studies to elucidate relationships of parasitic taxa, despite accelerated rates of sequence evolution. To address the question of the position of the genus *Cuscuta* within Convolvulaceae, we generated a new molecular data set consisting of mitochondrial (*atpA*) and nuclear (*RPB2*) genes, and analyzed these data together with an existing chloroplast data matrix (*rbcL*, *atpB*, *trnL-F*, and *psbE-J*), to which an additional chloroplast gene (*rpl2*) was added. This data set was analyzed with an array of phylogenetic methods, including Bayesian analysis, maximum likelihood, and maximum parsimony. Further exploration of data was done by using methods of phylogeny hypothesis testing. At least two nonparasitic lineages are shown to diverge within the Convolvulaceae before *Cuscuta*. However, the exact sister group of *Cuscuta* could not be ascertained, even though many alternatives were rejected with confidence. Caution is therefore warranted when interpreting the causes of molecular evolution in *Cuscuta*. Detailed comparisons with nonparasitic Convolvulaceae are necessary before firm conclusions can be reached regarding the effects of the parasitic mode of life on patterns of molecular evolution in *Cuscuta*. [Bayesian analysis; Convolvulaceae; cpDNA; *Cuscuta*; maximum likelihood; maximum parsimony; mitochondrial DNA; molecular systematics; parametric bootstrap; *RPB2*.]

Understanding the fascinating changes that have shaped the evolution of parasitic plants would be greatly facilitated, from both morphological and molecular standpoints, by detailed comparative studies with their closest nonparasitic relatives. However, the phylogenetic position of parasitic angiosperms and their precise relationship to autotrophic relatives are not easy to deduce (Nickrent et al., 1998). In general, problems are encountered with both morphological and molecular evidence. Parasitism is associated with extreme reduction and/or modification of vegetative structures, and convergence with other parasitic taxa is common. Both phenomena are encountered in *Cuscuta* (dodder; Convolvulaceae; asterids). The morphology of this parasitic genus is characterized by loss of roots, significant reduction of chlorophyll synthesis, almost complete reduction of leaves and cotyledons, and the evolution of haustoria, organs that enable these plants to connect to the hosts. Both *Cuscuta* and *Cassytha* (Lauraceae; magnoliids) are pale, twining, stem parasites, and provide an excellent example of convergent evolution in parasitic plants (Kuijt, 1969).

Molecular data have been used in numerous studies seeking to elucidate the relationships of parasitic taxa (e.g., Nickrent and Starr, 1994; Wolfe and dePamphilis, 1995, 1997; dePamphilis et al., 1997; Duff and Nickrent, 1997; Young et al., 1999). Despite this effort, the phylogenetic affiliation of many parasitic groups, especially the so-called "nonasterid holoparasites" (Balanophoraceae, Cynomoriaceae, and Cytinaceae), are not known (Nickrent et al., 1998). In many cases the chloroplast and nuclear genes typically used to deduce large-scale flowering plant relationships (e.g., Chase et al., 1993; Soltis et al., 1997) are lost, significantly altered, or evolving at greatly accelerated rates, making phylogenetic inferences extremely difficult. In recent progress, the holoparasitic family Hydnoraceae was placed as sister to Aristolochiaceae (Nickrent and Duff, 1996; Nickrent et al., 2002) and enigmatic *Rafflesia*, genus with the largest known flowers, was found to be a member of rosids, most closely related to the order Malpighiales (Barkman et al., 2004). In contrast to nonasterid holoparasites, the general position of most hemiparasites (e.g., *Cassytha*, Krameriaceae, Santalales) as well as the "asterid holoparasites" (e.g., Lennoaceae, Orobanchaceae in part) in the global angiosperm phylogeny is not in dispute. However, even in these cases the precise relationships to nonparasitic taxa remain uncertain. For example, there is little doubt, based both on reproductive morphology and molecular data, that the hemiparsitic genus *Cassytha* is closely associated with Lauraceae, but its placement, either as a sister-group to Lauraceae or nested deeper within Lauraceae, remains uncertain (Rohwer, 2000; Renner and Chanderbali, 2000). Likewise, the small holoparasitic family Lennoaceae was recognized early on, based on floral and pollen morphology, to be closely related to Boraginaceae. Preliminary results of molecular analyses indicated that this family is indeed related to the Boraginaceae subfamily Ehretioideae, but its closest relatives remain uncertain (Smith et al., 2000). In certain instances, however, the closest nonparasitic relatives of parasitic plants were ascertained with high support using molecular data. For example,

[1]Present address: Department of Biology, Indiana University, 1001 E. Third Street, Bloomington, IN 47405, USA; E-mail: sstefano@bio.indiana.edu

Lindenbergia, a nonparasitic genus, is clearly sister to the rest of Orobanchaceae s.l., which comprises both hemi- and holoparasitic species of the traditional Scrophulariaceae and Orobanchaceae (Young et al., 1999; Olmstead et al., 2001). Also, the parasitic species of Santalales are clearly nested within autotrophic members of the paraphyletic family Olacaceae (Nickrent and Duff, 1996; Nickrent et al., 1998).

The main source of difficulties for precise inference of relationships of parasitic angiosperms is the widespread homoplasy in molecular data due to accelerated rates of sequence evolution (reviewed in Nickrent et al., 1998), and associated analytical problems. Apparently, all three genomes in parasitic plants can be affected by this phenomenon (e.g., Colwell, 1994; Nickrent and Starr, 1994; Duff and Nickrent, 1997; Wolfe and dePamphilis, 1998). The relaxation of selection on genes involved in photosynthesis is one hypothesis for the increased nucleotide substitution rates in the chloroplast genome of parasitic plants (Wolfe and dePamphilis, 1998). However, it is not fully understood why the parasitic habit causes the acceleration observed in nuclear (Colwell, 1994; Nickrent and Starr, 1994; Neyland, 2000), and, to a lesser extent, mitochondrial genomes (Duff and Nickrent, 1997; Barkman et al., 2004), although small effective population size and molecular drive have been proposed as possible explanations (Nickrent and Starr, 1994; Nickrent et al., 1998).

The genus *Cuscuta*, consisting of some 160 to 170 species, is nearly cosmopolitan in distribution, and occurs in a wide range of habitats (Yuncker, 1932; Mabberley, 1987). Engelmann (1859) recognized three groups within *Cuscuta*, based primarily on the morphology of styles and stigma, which were assigned subgeneric ranks by Yuncker (1932). Subgenera *Cuscuta* and *Grammica* are characterized by two distinct styles, and are distinguishable by their stigma morphology (elongated or short and capitate, respectively). Subgenus *Monogyna* has a single style, partially or completely united, with capitate, conical, or ovate stigmas.

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, the morning-glory family, and the clear association with this family was recognized early on. Many classifications recognize a separate tribe (Choisy, 1845; Bentham and Hooker, 1873; Baillon, 1891; Hallier, 1893; Peter, 1897; Austin, 1998) or subfamily (Peter, 1891; Melchior, 1964) within Convolvulaceae for *Cuscuta*. However, some botanists (e.g., Roberty, 1952, 1964; Austin, 1973) adopted Dumortier's (1829) view that *Cuscuta* should be recognized as a separate family. This opinion is reflected in some major synoptic works on flowering plants (e.g., Cronquist, 1988; Takhtajan, 1997) that place *Cuscuta* in its own family, Cuscutaceae.

Cuscuta has been the focus of many scientific studies for several reasons. Many species are recognized to be pests on an array of important agricultural crops such as alfalfa, clover, beans, soy, cranberry, and, most importantly, members of the grass family. Infested crops now can be treated efficiently both by herbicides (Dawson, 1987, 1990) and by fungal bioagents (Bewick et al., 1987; Li, 1987). A substantial body of literature deals with the life history, ecology, and pest control of different dodder species (reviewed by Dawson et al., 1994, and references therein). Because this branch parasite is amenable to culture and direct experimental manipulation, it is also frequently used as a model system for developmental research, especially of haustorial initiation and formation (e.g., Dörr, 1987; Heide-Jørgensen, 1987; Lee and Lee, 1989; Subramaniam and Mahadevan, 1994).

In addition, *Cuscuta* has been the subject of extensive molecular analyses. Both hemiparasitic (e.g., *C. reflexa*) and holoparasitic (e.g., *C. europaea*) species occur in the genus. This diversity of photosynthetic ability among species prompted several physiological studies of photosynthetic enzymes and molecular evolutionary studies of the chloroplast genome. The results indicate that the hemiparasitic *C. reflexa* retains an affected, yet functional, chloroplast genome. This species retains most of the plastid genes generally found in autotrophic land plants, including both those involved in photosynthesis and 'house-keeping' functions (Haberhausen et al., 1992). However, putatively chlororespiratory (*ndh*) genes seem to be either altered to the point of becoming pseudogenes (*ndhB*) or are lost from the plastid genome (Haberhausen and Zetsche, 1994). The plastid genome of holoparasite *C. europaea* has sustained greater losses (Freyer et al., 1995), and the crude extract of this species shows no ribulose-1,5-bisphosphate carboxylase-oxygenase (Rubisco) activity nor light-dependent CO_2 fixation, but cpDNA retains an *rbcL* open reading frame (Machado and Zetsche, 1990). The findings of chloroplast genome structural rearrangements in *Cuscuta* were attributed to its parasitic life style, but without proper comparison to related nonparasitic members of the family. The phylogenetic problems within the family, especially the placement of *Cuscuta*, as well as the relative placement of hemi- and holoparasitic species within this genus, need to be resolved satisfactorily before many evolutionary questions can be answered.

Convolvulaceae have been the subject of only one broad molecular phylogenetic study (Stefanović et al., 2002). That study was based on sequences from four chloroplast loci—*rbcL*, *atpB*, *psbE-J* operon, and the *trnL-F* region—obtained from 112 taxa, including 109 species from all 10 traditionally recognized tribes (Austin, 1973; modified 1998) as well as three outgroups. Those results found that two groups that have been proposed as segregate families (Dumortier, 1829), *Cuscuta* and tribe Dichondreae, were nested within the Convolvulaceae. The exact position of *Cuscuta* could not be elucidated, however, mainly due to its highly divergent sequences. One alternative pertinent for circumscription of the family, the position of *Cuscuta* as sister to the rest of Convolvulaceae, was rejected. This result was further corroborated by the distribution of deletions in the *atpB* gene and *trn*L intron found in *Cuscuta* species as well as in all nonparasitic Convolvulaceae except *Humbertia*, which is sister to the rest of the family (Stefanović et al., 2002).

To address the question of the position of *Cuscuta* within Convolvulaceae, we generated a new molecular data set consisting of mitochondrial (*atpA*), nuclear (*RPB2*), and chloroplast (*rpl2*) genes, and analyzed these data together with the existing (Stefanović et al., 2002) chloroplast data matrix (*rbcL*, *atpB*, *trnL-F*, and *psbE-J*). The majority of data in this study are derived from organellar sequences. The chloroplast/mitochondrial-haplotype tree has a substantially higher probability of accurately inferring short internodes (e.g., those resulting from recent and/or rapid radiations) than does a nuclear gene tree due to more rapid coalescence time and lower subsequent substitution rates (Moore, 1995). The only nuclear data used in this study are derived from partial *RPB2* gene sequences. The product of this gene forms a part of the catalytic core of the RNA polymerase II. This protein is highly conserved across the angiosperms at the amino-acid level, but the nucleotide sequences are quite variable, which enables its use at the lower phylogenetic level (Denton et al., 1998; Oxelman and Bremer, 2000).

This molecular data set, derived from all three plant genomes, is analyzed by three methods of character-based phylogenetic reconstruction: maximum likelihood (ML), maximum parsimony (MP), and Bayesian inference (BI). ML provides an objective way to incorporate important aspects of molecular evolution, such as unequal base frequencies, complex substitution models, and among-site rate variation. In addition, ML is a more consistent estimator of phylogenies (Felsenstein, 1981, 1988) and is less sensitive to the effects of rate variation (Huelsenbeck and Hillis, 1993; Huelsenbeck, 1995) than MP. However, taking advantage of complex models of DNA evolution imposes severe computational constraints, especially when a larger number of taxa is sampled (Sanderson and Kim, 2000). MP, on the other hand, is less impaired by large number of taxa (Hillis, 1996), but more realistic models of DNA sequence evolution are difficult to implement within this framework.

Bayesian phylogenetic inference (Larget and Simon, 1999; Huelsenbeck and Ronquist, 2001) is, like ML, a probabilistic method that relies on explicit models of sequence evolution. However, because it does not attempt to find the global optimum likelihood and uses Markov Chain Monte Carlo (MCMC) to estimate the posterior distribution of parameters (Huelsenbeck and Bollback, 2001), the BI method is, in comparison with ML, computationally much less intensive, and can be employed even with a relatively large number of taxa. An additional advantage of BI is that the interpretation of Bayesian posterior probabilities is considered to be straightforward, unlike the nonparametric bootstrap analyses, employed in both MP and ML contexts, where the relationship between bootstrap and statistical probability has been debated (e.g., Hillis and Bull, 1993). BI has recently been used successfully in addressing some of the most difficult phylogenetic problems such as the origin of land plants (Karol et al., 2001) and the early mammalian radiation (Murphy et al., 2001).

The main goal of this study is to narrow down the phylogenetic position of *Cuscuta*, the only parasitic genus associated with Convolvulaceae. In addition, we compare the performance of BI with ML and MP methods in the notoriously difficult task of assessing parasitic plant relationships. Finally, we explore an empirical implementation of the parametric bootstrap method for testing different alternative phylogenetic hypotheses using MP, which should be applicable to large data sets not only in Convolvulaceae, but also for sequence-based phylogenies in general.

Materials and Methods

Taxon Sampling

The 35 species included in this study (Appendix 1) represent a subset of taxa used by Stefanović et al. (2002) in their broad analysis of Convolvulaceae. Members of all but 1 out of 10 tribes sensu Austin (1973, modified 1998) were sampled. To represent the diversity of the family, efforts were made to include two or more species, selected to span the root node of each well supported clade, for all except very small clades circumscribed in our previous study. In addition, many species of unresolved relationships were sampled, especially those found within the "bifid style" clade (Stefanović et al., 2002). Some genera, found to be monophyletic and strongly supported, but exhibiting long branches and uncertain placement are represented by two species (e.g., *Jacquemontia*, *Erycibe*). *Cuscuta* is also represented by two species, *C. japonica* (subgenus *Monogyna*) and *C. europaea* (subgenus *Cuscuta*), chosen as place-holders for the genus, because they exemplify the morphological (united style versus bifid style) and physiological (hemiparasite versus holoparasite) diversity within the genus. These two species showed the least amount of sequence divergence compared to the photosynthetic members of the family, and their sequences were fully alignable throughout the *trnL-F* region, which was not the case with any member of the more highly divergent subgenus *Grammica*. Relying on previously published molecular systematic studies of the asterids (e.g., Olmstead and Palmer, 1992; Chase et al., 1993; Soltis et al., 1997) and Convolvulaceae (Stefanović et al., 2002), we selected two taxa (*Nicotiana tabacum* and *Schizanthus pinnatus*) spanning the root node of the Solanaceae, the sister family, as well as one additional species (*Montinia caryophyllacea*) belonging to the Solanales as outgroups.

The same species, and in many cases the same DNA isolate, was used to represent each taxon for each gene sequence whenever possible. However, certain gene regions that could not be obtained from a given species, due to the poor quality or lack of DNA, were sequenced from their respective closest relatives, as inferred from our previous analysis (Stefanović et al., 2002). Those taxa are labeled on trees by genus name only.

Sequence Data and Alignment

In addition to the DNA samples used in our previous study (Stefanović et al., 2002), total genomic DNA was isolated from herbarium specimens or silica-gel dried

tissue (0.05 to 0.2 g), or from fresh (1 to 2 g) tissue by the modified hexadecyltrimethylammonium bromide (CTAB) procedure (Rogers and Bendich, 1985; Doyle and Doyle, 1987) and purified using Qiagen columns following protocols provided by the manufacturer.

Double-stranded DNA fragments for the regions of interest were obtained by polymerase chain reaction (PCR) from total genomic DNA using the primers described by Olmstead et al. (1992) for *rbcL*, by Hoot et al. (1995) for *atpB*, by Graham and Olmstead (2000) for the *psbE-J* operon and *rpl2* gene, by Taberlet et al. (1991) for the *trnL-F* region, and by Davis et al. (1998) for the mitochondrial *atpA* gene. The nuclear *RPB2* gene was shown to be duplicated in Gentianales (*RPB2*-i and *RPB2*-d; Oxelman and Bremer, 2000), and current research indicates that the duplication is found in the euasterid I clade (sensu APG, 1998) and in Ericales (Oxelman et al., 2004). Although multiple copies of similar, paralogous sequences can confound phylogenetic interpretations, the lack of introns in the *RPB2*-d copy in euasterid I plants makes a priori homology assessment easy. Plant-specific *RPB2* primers P10F and P11aR (Denton et al., 1998) were used for initial amplifications of *RPB2*-d, targeting a region that corresponds to exons 18–24 in the *Arabidopsis thaliana RPB2* gene. Based on these initial sequences, two more Convolvulaceae-specific primers were designed and used for PCR and sequencing (Conv-f: 5′-GCCATYGCMTGTYAYTCRGG-3′; and Conv-r: 5′-CGCCCTTGTGAATCTTGTCATCCACC-3′). Some PCR products, mainly those involving the low-copy nuclear *RPB2* gene, were cloned (pCR2.1 vector; Invitrogen, Carlsbad, California, USA) and three to five clones were sequenced. Amplified PCR products were cleaned using Qiagen columns (Valencia, California, USA). Cleaned products were then directly sequenced, including both strands to ensure accuracy, using the BigDye Terminator cycle sequencing kit (PE Applied Biosystem, Foster City, California, USA) on an ABI 377 DNA automated sequencer (PE Applied Biosystem). Sequence data were edited and assembled using Sequencher 4.1 (Gene Codes Corporation, Ann Arbor, Michigan, USA). The alignments were obtained manually using the edit option of the MUST package (Philippe, 1993) or directly with PAUP* 4.0b10 (Swofford, 2002).

Phylogenetic Analyses

Bayesian inference.—We used MrBayes v2.01 (Huelsenbeck and Ronquist, 2001) to perform the Bayesian phylogenetic analyses. This software uses a Metropolis-coupled Markov chain Monte Carlo, or "$(MC)^3$," algorithm that runs several chains at the same time to permit a more thorough exploration of data space. Five separate runs were carried out, using the GTR+I+Γ model. The model parameters were treated as unknown variables with uniform prior probabilities and were estimated as part of the analyses together with tree topologies. We ran four chains simultaneously, three heated and one cold, enabling the random exchange of parameters between them, thereby minimizing the chance of being trapped in local optima. Each Markov chain was initiated from a random starting tree and run for 1×10^6 generations. For the first of the five runs, the sampling was done every 100th generation resulting in 10,000 samples. The remaining four runs were sampled every 50th generation for a total of 20,000 sample points per run. In order to determine whether and where stationarity was achieved, and to decide on the cut-off value ("burn-in," i.e., data points sampled before the chain reaches stationarity), the −log likelihood scores of each run were plotted against generation time. After discarding all samples preceding stationarity, the remaining data points were first analyzed separately for each run and than combined into a single file. Separate and combined files containing tree topologies were analyzed using PAUP* to compute the 50% majority rule consensus tree. The percentage of samples recovering any particular node represents the posterior probability of that node (Huelsenbeck and Ronquist, 2001). These values are considered to be the true probabilities of the nodes given the assumptions of the model of DNA sequence evolution (Rannala and Yang, 1996), and therefore the nodes receiving $\geq$0.95 could be considered statistically significantly supported.

Maximum likelihood.—The most complex model of nucleotide substitution currently available, the GTR+I+Γ model (Yang, 1994), was selected as the best-fit by ModelTest v3.04 (Posada and Crandall, 1998), by both the LR test and Akaike Information Criterion (AIC; Akaike, 1973). The gamma distribution was separated into four discrete rate classes. A heuristic analysis was done, using PAUP* (Swofford, 2002), with 20 replicates with stepwise random taxon addition, TBR branch swapping, and model parameters set to the values estimated by ModelTest v3.04. Because ML tree searches are computationally intensive, this procedure was conducted on the combined data set only.

To facilitate the nonparametric bootstrap analysis (Felsenstein, 1985) under the ML criterion, the topological constraint option in PAUP* was used to constrain certain taxonomic groupings that had been identified as monophyletic and strongly supported in our previous study (Stefanović et al., 2002). This approach effectively reduces the number of terminal taxa in the analysis while maintaining all of the sequence data, thereby enabling the optimal assessments of substitutions on the tree (Olmstead et al., 1992). Internal nodes on the tree, where branching patterns are critical to the questions addressed by this analysis, were left unconstrained. Fourteen nodes that were constrained in the bootstrap analysis are indicated by asterisks in Figure 3. ML bootstrap analysis incorporated 100 pseudoreplicates, SPR branch swapping, starting trees obtained by neighbor-joining (with uncorrected "*p*" distances), and the same DNA sequence parameters estimated by ModelTest v3.04 as in the original ML search.

Maximum parsimony.—For the parametric bootstrap analyses the heuristic searches for most parsimonious (MP) trees were performed using PAUP* (Swofford, 2002). Parsimony analyses of the data were conducted

for each region separately and in combination using 1000 replicates (Maddison, 1991) with stepwise random taxon addition and TBR branch swapping and multrees on.

Testing of Alternative Hypotheses

Shimodaira-Hasegawa test.—To compare alternative phylogenetic hypotheses statistically, the one-tailed Shimodaira-Hasegawa nonparametric tests (SH tests; Shimodaira and Hasegawa, 1999; Goldman et al., 2000) were conducted, using the aforementioned substitution model and likelihood settings. These tests are recommended for evaluation when the number of candidate trees is not very large (Shimodaira, 2002). The SH tests were conducted with PAUP* using 1000 bootstrap replicates and full parameter optimization of the model. In this fashion, a particular version of the SH test is implemented, referred to as the *posNPfcd* by Goldman et al. (2000). Using this approach, we tested the placement of *Cuscuta* by evaluating the ML topology against a set of topologies differing in the phylogenetic placement of this parasitic genus. All together, we tested seven alternative hypotheses against the optimal ML topology (depicted on Fig. 5).

Parametric bootstrap.—The likelihood ratio (LR) test is frequently used to distinguish between competing hypotheses. As long as the tested hypotheses are nested, i.e., special cases of one another, the LR approximates a χ^2 statistic (G statistics) with degrees of freedom (df) equal to the difference in the number of parameters in the two models. In a phylogenetic context, this test is implemented to evaluate different models of DNA substitution and/or molecular clock hypotheses. However, one important common goal in phylogenetics—evaluating and choosing between competing tree topologies—cannot be tested using the χ^2 approximation, because different topologies are not nested within each other, and consequently, the df $= 0$. The parametric bootstrap (PB) is shown to be a statistically sound method of evaluating different alternative topological hypotheses (Huelsenbeck et al., 1996; Swofford et al., 1996; Goldman et al., 2000). This procedure uses a simulation to generate the null distribution from which statistical significance is deduced, thereby avoiding the need to rely on χ^2 statistics. However, despite the demonstrated power of the PB test (Goldman et al., 2000), it has been used very rarely on larger data sets (e.g., Knowles, 2000; Fishbein et al., 2001; Zanis et al., 2002), mainly due to the enormous computational cost this approach involves when implemented in the ML context (up to 200-fold the initial ML estimation time per one PB analysis). This time is further multiplied by the number of different hypotheses that one might wish to evaluate, because each alternative hypothesis requires not only the new test statistics but also the new null distribution for differences between the optimal tree and the model tree to be generated. The implementation of the PB for testing different a posteriori topologies is equally valid under the MP criterion (Goldman et al., 2000). Under this criterion some well known biases in the data are difficult to accommodate (see above), but it offers the ability to perform statistically sound, and thorough, tests in significantly less time (Sanderson and Kim, 2000). To compare support for the optimal tree against alternative branching hypotheses designed to investigate the placement of the parasitic genus *Cuscuta*, and to assess the significance of the observed differences between those trees given our data set, a series of PB tests was conducted (depicted on Fig. 5).

The implemented parametric bootstrapping procedure is summarized by the flow chart in Fig. 1. The original combined data matrix was used to obtain the optimal (MP) tree (H_a), as described above. An alternative topology constraint was constructed using MacClade (Maddison and Maddison, 1992). The best tree given this constraint and the original data set (H_0) was assessed by implementing the topological constraints function in PAUP*. This analysis involved 100 replicates with stepwise random taxon addition and TBR branch swapping. The observed difference between H_0 and H_a ($\delta_{observed}$) represents the test statistic. In order to determine whether the $\delta_{observed}$ is significant, i.e., whether it is larger than expected under the null hypotheses, the null distribution of differences must be generated via simulated data matrices. We simulated 99 data matrices of the same size as the original one using Seq-Gen v1.2.4.1 (Rambault and Grassly, 1997). The GTR+I+Γ model was used to simulate data sets, with parameters, including its branch lengths, estimated from the original data matrix and using the null hypothesis (constraint) topology.

For each of the 99 simulated matrices two searches were performed: first without any constraints, producing the h_0 score, and the second with the constraint compatible with the original optimal (unconstrained) tree, resulting in h_a score. Those searches were done using the same strategy as on the original data set. The difference for each pair (i.e., h_{01}-h_{a1} through h_{099}-h_{a99}) was calculated. To these δ's, the original $\delta_{observed}$ is added (for a total of 100), and the histogram of the null distribution of δ's was generated to determine the rejection region. The hypothesis that the difference observed between the original optimal tree and the constraint topology was due to chance alone is tested by comparison with this null distribution directly. The significance level is calculated as the proportion of times that the $\delta_{observed}$ exceeds the values obtained in simulations (Huelsenbeck and Crandall, 1997; Goldman et al., 2000).

Because in parametric bootstrapping the simulated data sets are generated under the assumption that the particular null hypothesis (H_0) is correct, neither the model parameters nor the simulated matrices could be reused. Therefore, this procedure was repeated for each alternative position of *Cuscuta*.

RESULTS AND DISCUSSION

Sequences and Alignments

Characteristics of the sequenced regions as well as statistics of MP trees derived from each of the seven loci are summarized in Table 1. Descriptions of sequences

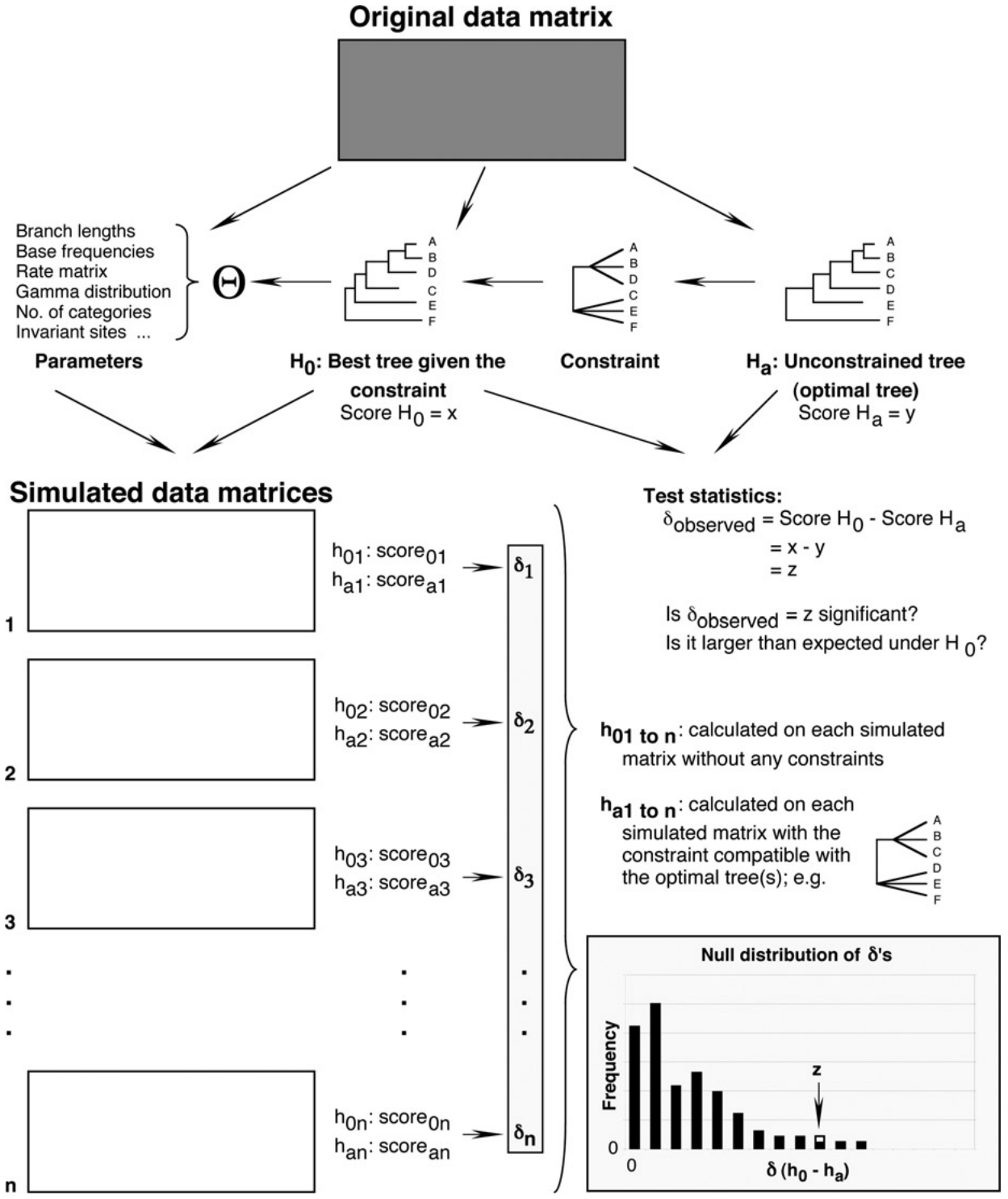

FIGURE 1. Parametric bootstrap implementation procedure flow chart (see Materials and Methods for full explanation). An example for testing the alternative tree topology is included.

derived from chloroplast genes used in this study that were published previously (i.e., *rbcL*, *atpB*, *psbE-J*, and *trnL-F*) are discussed in more detail in the original study (Stefanović et al., 2002). The alignments of three newly obtained genes, *rpl2* (chloroplast), *atpA* (mitochondrion), and partial *RPB2* (nucleus), were straightforward. All of these three protein coding regions exhibited some length variation, always within the open reading frame (ORF).

TABLE 1. Summary descriptions for sequences included in, and maximum parsimony trees derived from, individual and combined analyses.

	rbcL (cp)	*atpB* (cp)	*psbE-J* (cp)	*trnL-F* (cp)	*rpl2* (cp)	*atpA* (mt)	*RPB2* (nuc)	Combined data
Number of taxa included	35	35	35	35	35	35	33	35
Sequence characteristics								
Length of sequenced dregion (range)	1320–1434	1464–1497	730–798	478–971	611–614[a]	1284–1293	684–690	3992–4760
Aligned length	1461	1500	822	1193	617	1293	690	7576
Analyzed length[b]	1376	1452	714	788	560	1262	690	6842
Variable sites	358	413	186	382	236	84	275	1934
Parsimony informative sites	196	206	74	174	122	33	193	998
Pairwise uncorrected distances (range in %)	0.4–10.6	0.3–10.2	0–12.2	0.7–24.6	0.9–16.4	0–2.1	0.7–22.3	0.6–11.3
Mean AT content (%)	56	57	61	64	55	54	54	57
Base frequency homogeneity (χ^2/df/P)	9.96/102/1.0	8.72/102/1.0	6.01/102/1.0	19.97/102/1.0	8.9/102/1.0	8.12/102/1.0	33.77/96/1.0	10.07/102/1.0
Tree characteristics								
Number of trees	436	845	87	280	12	3823	53	1
Length	728	736	275	690	418	113	827	3850
CI/RI	0.63/0.58	0.7/0.63	0.79/0.7	0.76/0.61	0.74/0.67	0.81/0.74	0.5/0.43	0.66/0.56

CI = consistency index; cp = chloroplast; df = degrees of freedom; mt = mitochondrion; nuc = nucleus; RI = retention index.
[a]Excluding the length of *rpl2* intron found in all outgroups but not in Convolvulaceae.
[b]After exclusion of portions of alignments where substantial sequence data are missing (e.g., 5′ and 3′ termini, primer sites, and/or gaps).

Only one gap was needed to align sequences for all taxa for *atpA* and *RPB2* (deletions in *Cuscuta japonica* and *Erycibe glomerata*, respectively). The *rpl2* alignment required two gaps; one was an insertion in *Cuscuta japonica* and the other was an insertion in *Montinia* relative to all other sequences (but cannot be polarized). In addition, an intron usually found in the *rpl2* gene of angiosperms is deleted in all Convolvulaceae, including *Humbertia* and *Cuscuta*, representing a unique event within asterids and a synapomorphy for Convolvulaceae (Stefanović et al., 2002). Either because of incomplete sequences or gaps, the analyses involving sequence simulations may be biased owning to the presence of a large amount of missing information in the alignment. Thus, the portions of alignments where substantial sequence data are missing (e.g., 5′ and 3′ termini, and/or gaps) were excluded, resulting in a total analyzed length of 6842 bp (Table 1). No significant heterogeneity in base composition was observed within any of the separate matrices across all taxa (Table 1). Also, no significant difference in base composition was encountered among *Cuscuta* sequences alone. Due to the poor quality of the DNA extracted from herbarium specimens, sequences for the low copy nuclear *RPB2* gene could not be obtained for two species, *Humbertia madagascariensis* and *Wilsonia backhousei*. Alignments in Nexus format are available on request from the first author, and have been archived also at the *Systematic Biology* (http://ag.arizona.edu/systbiol/SSBWeb/) website.

Phylogenetic Analyses and Implications for the Placement of Cuscuta

The separate equally weighted MP analyses of seven DNA matrices, conducted to detect potential areas of strongly supported incongruence, gave remarkably similar results (results not shown). All analyses identified several well-supported monophyletic groups. Differences mainly involved the number of resolved nodes and their bootstrap support. Visual inspection of the resulting cladograms revealed no topological incongruences that were, at the same time, conflicting and well-supported by different data partitions. Because these independent analyses gave congruent results, albeit quite unresolved, we combined all seven matrices. The trees produced by combined analysis had better resolution and overall support relative to those produced by independent analyses. Therefore, we have based our discussion on the analyses of the combined data set.

All five Bayesian analyses, each initiated from a random starting tree, converged on similar log-likelihood scores and reached stationarity at no later than 200,000 generations (Fig. 2). The initial 2000 samples from the first run, and 4000 from each of four more densely sampled runs, were discarded. In both cases the discarted samples accounted for 20% of the total sample points. The burn-in of data points accumulated before stationarity left a total of 72,000 combined samples. A majority-rule consensus of the 72,000 trees resulted in the phylogenetic hypothesis depicted in Figures 3 and 4. When analyzed separately, all five independent runs found essentially identical tree topologies and posterior probabilities (results not shown), indicating that the sample number was sufficient to permit the algorithm to converge on a global solution.

The relationships inferred through the BI analysis (Figs. 3 and 4) are topologically congruent with results derived from a data set with larger taxon sampling (Stefanović et al., 2002) under the MP criterion. According to the BI results *Humbertia* forms the sister to the rest of the family. The next two lineages to diverge within the Convolvulaceae are two small clades,

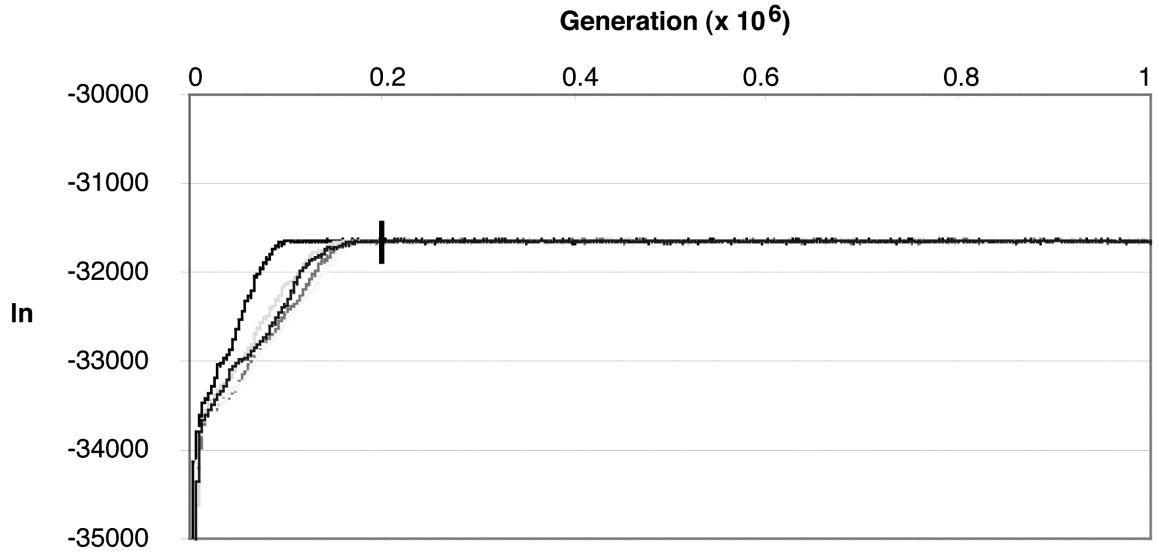

FIGURE 2. Burn-in plots of the combined data Bayesian analyses. The results of five independent runs are superimposed, illustrating that the log-likelihood scores converged on similar values. The vertical bar represents the cut-off point by which all five runs had reached stationarity. Data points sampled to the left of the bar were discarded. Those to the right were used to generate the 50% majority-rule consensus tree.

one comprising some members of Poraneae and the other the genus *Erycibe*. Monophyly of each of these two lineages is strongly supported, but their progressively more terminal placements on the optimal tree is not ($P = 0.5$). The rest of the family is split into two major clades (Fig. 3; clade 1, clade 2). Clade 1 includes tribes Argyreieae, Ipomoeeae, Convolvuleae, and Merremieae and comprises the majority of species in the family. Within this clade the relationships are largely resolved and well supported except for the relationships of Merremieae and Convolvuleae. Clade 2 consists mostly of taxa that have a more or less deeply divided style ("bifid style" clade) and includes several strongly supported subclades, but the backbone relationships are largely unresolved. Overall, 24 out of 32 nodes (75%) were supported with a significance level ≥95%. However, the position of *Cuscuta* as a sister-group of clade 1 on the optimal tree is not significantly supported ($P = 0.58$). The remaining 42% of bipartitions place *Cuscuta* either as sister-group to clade 2 (26%) or as sister to the large clade combining both clades 1 and 2 (16%).

Under the ML criterion, with the GTR+I+Γ model of sequence evolution, one optimal tree was obtained. This tree differed from the optimal BI solution only in branching patterns for two weakly supported nodes (Fig. 3; dotted lines). The position of *Cuscuta* is the same as in the BI tree, with similarly weak support (52% BS).

Taken overall, the combined data analysis, using two probabilistic methods, ML and BI, recovered highly congruent topologies (Figs. 3 and 4). The points of disagreement included only those nodes that have not received substantial support. The basal position of *Humbertia*, subdivision of the rest of the family into two major clades, the relationships within clade 1 as well as most of the relationships within clade 2, are all points of complete agreement. The results from the present analyses are also quite similar, both in inferred patterns of evolution and support, as well as lack of support in certain regions, with the MP analysis based on a larger taxon sampling and chloroplast data only (Stefanović et al., 2002). However, the overall support for the position of *Cuscuta* remains weak under all criteria, including BI, which has proved effective in resolving some other difficult phylogenetic issues such as the early mammalian radiation and land plant phylogeny (Murphy et al., 2001; Karol et al., 2001). The consensus places this parasitic genus in the general vicinity of clades 1 and 2, without further bearing on exact patterns among these three groups. The current evolutionary hypothesis for Convolvulaceae based on these different analyses is summarized in Figure 5.

Evaluation of Alternative Placements for Cuscuta

Traditional classifications, for the most part, ignored the question of *Cuscuta*'s precise relationships with its putative nonparasitic relatives, owing mainly to the lack of useful taxonomic characters. Even though the association with the Convolvulaceae was recognized, based on reproductive morphology, no attempts were made to propose a more detailed scheme of relationships between *Cuscuta* and nonparasitic members of the family. The approaches taken have usually fallen into two categories: (1) recognition of *Cuscuta* as a monotypic family, implying, in modern terms, a sister-group relationship to the rest of Convolvulaceae, or (2) placing *Cuscuta* within Convolvulaceae under various taxonomic ranks, but without bearing any further on its possible relationships. Examples of the former approach include classifications by Dumortier (1829) and Roberty (1952, 1964), which were subsequently followed by most major synoptic works on flowering plants (e.g., Cronquist, 1988; Takhtajan, 1997). The latter approach includes

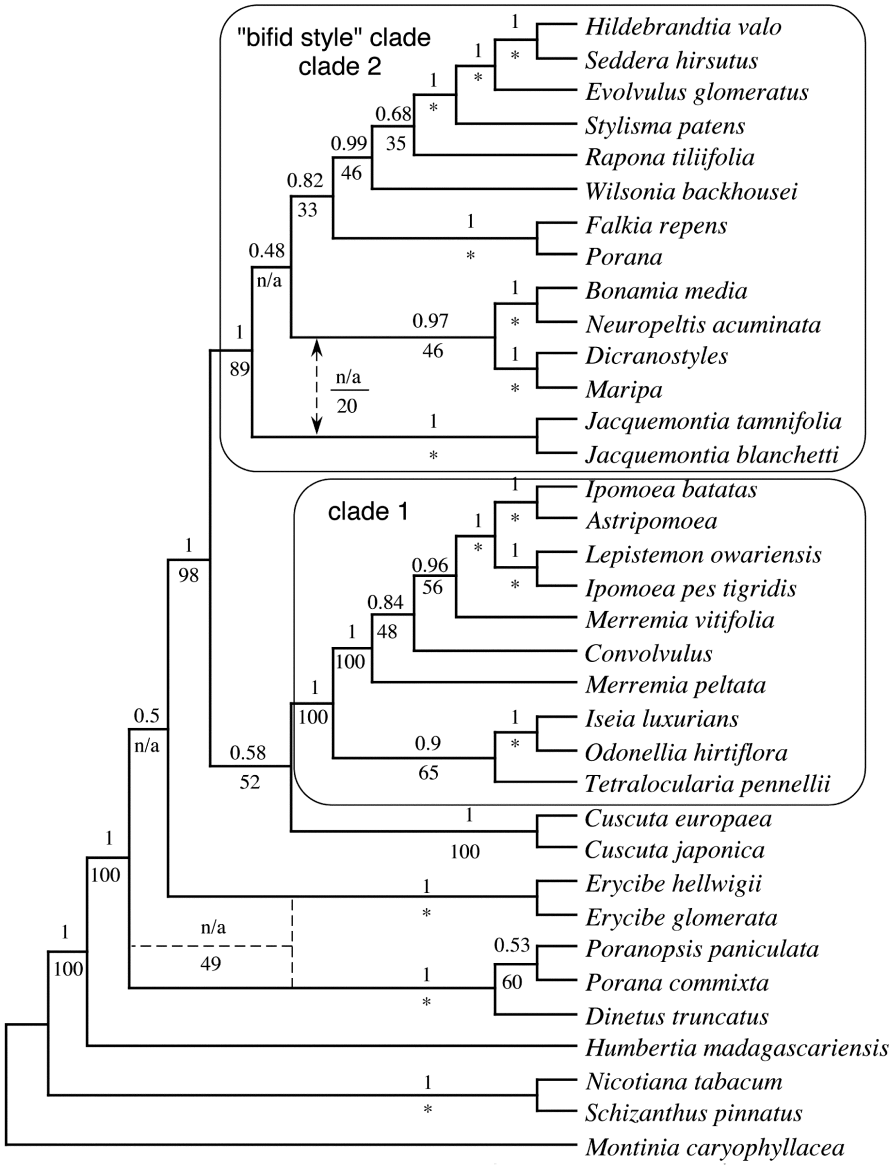

FIGURE 3. Phylogeny of Convolvulaceae reconstructed using a Bayesian phylogenetic approach and combined data matrix. Dotted lines depict the only topological differences found in the ML analysis ($-\ln L = 31{,}604.51$) with the same data set. Numbers above branches are the Bayesian posterior probabilities; numbers below branches represent percent support in nonparametric ML bootstrap analysis. Asterisks indicate nodes constrained in the ML bootstrapping. The tree is rooted using three taxa belonging to closely related families as outgroups (*Nicotiana*, *Schizanthus*, and *Montinia*).

recognition of *Cuscuta* as tribe Cuscuteae (Choisy, 1845; Bentham and Hooker, 1873; Baillon, 1891; Hallier, 1893; Peter, 1897; Austin, 1998) or as subfamily Cuscutoideae (Peter, 1891; Melchior, 1964). An exception to this is a study by Austin (1973). Austin system also segregated *Cuscuta* as a separate family, but a close association with tribe Dichondreae is implicit from his phylogenetic scheme based mainly on chromosome numbers (his Fig. 33). This *Cuscuta*-Dichondreae connection was supported by some unique shared fruit features as well as similarities in embryo morphology.

As expected, *Cuscuta* exhibits strong rate acceleration in chloroplast and, to a somewhat lesser extent, in mitochondrial and nuclear DNA evolution (Nickrent et al., 1998; results shown only in aggregate, Fig. 4). Even though optimal trees from all three methods of phylogenetic inference place *Cuscuta* nested well within the Convolvulaceae, its precise placement is not supported. Therefore, an in-depth analysis of the relationship of this genus with the remainder of the family is warranted. All together, seven hypotheses were tested concerning possible placements of *Cuscuta* using two different approaches: Shimodaira-Hasegawa test (Shimodaira and Hasegawa, 1999; Goldman et al., 2000) under the ML criterion and parametric bootstrapping (Swofford et al., 1996; Huelsenbeck and Crandall, 1997; Goldman et al.,

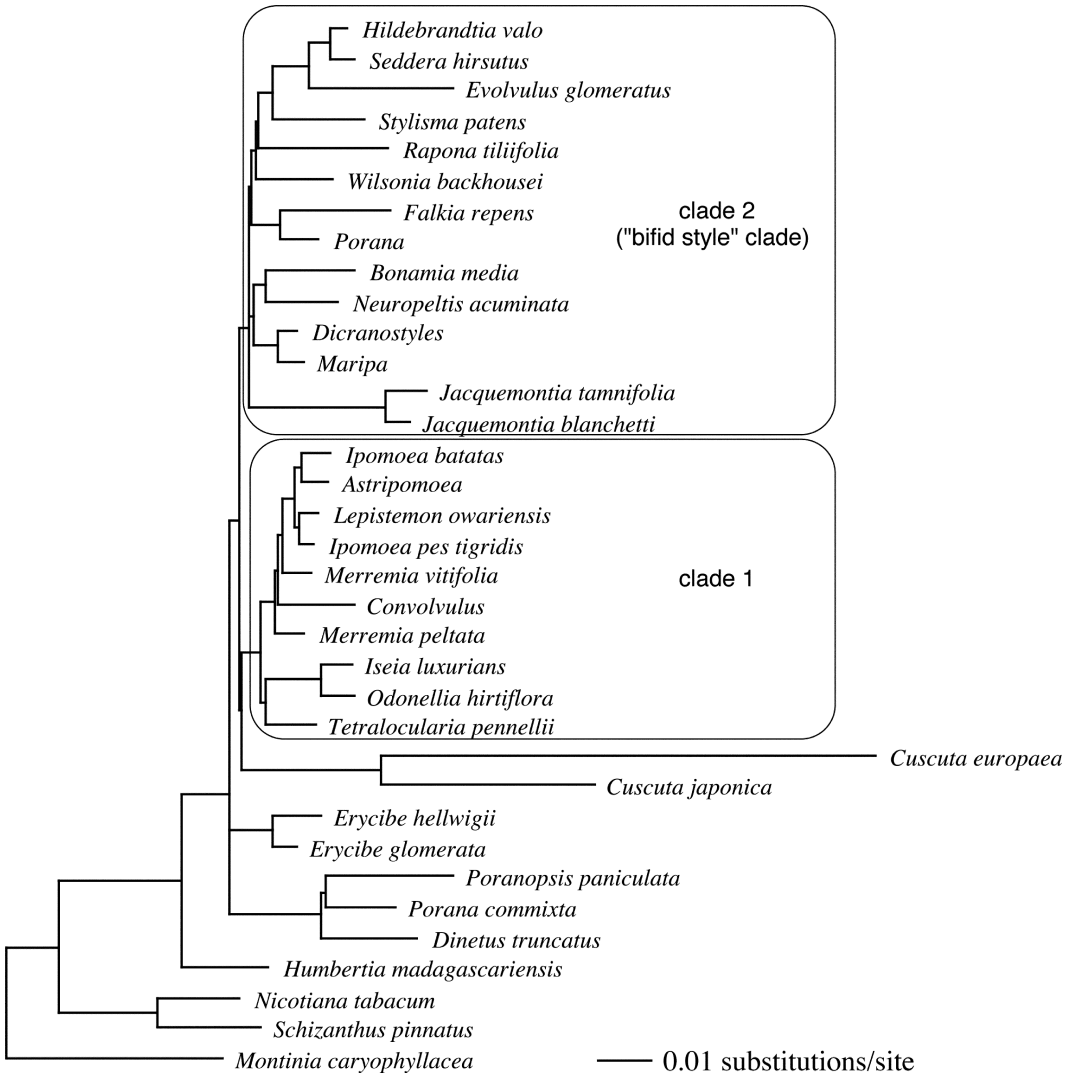

FIGURE 4. Inferred branch lengths on the Bayesian tree depicted in Figure 3. Branch lengths are mean values and are proportional to the number of substitution per site.

2000) under the MP criterion. The different tested points of attachment for constrained topologies are marked in Figure 5, and results are summarized in Table 2.

One alternative topology was designed to test the relationship proposed implicitly by Austin (1973). According to this hypothesis *Cuscuta* would be more closely related to *Falkia*, the place-holder for tribe Dichondreae in the present study (Fig. 5, no. 1). The SH test found significant difference in likelihood between this topology and the ML tree. Similarly, the PB test detected a significant difference. The observed length difference of 25 steps was far beyond the greatest difference (16 steps) observed in any simulated data sets ($P < 0.01$), rejecting the hypothesis of a closer *Cuscuta*-Dichondreae connection. It is possible, though unlikely, that *Cuscuta* could be closely related to some other members of the "bifid clade." The formal tests, however, were not conducted, because the backbone relationships in this clade were not resolved.

The other six tests conducted were designed to explore the limits of significance for alternative hypotheses and to help narrow down the possible placements for *Cuscuta* (Fig. 5, nos. 2 to 7). Clade 1 contains approximately 2/3 of all species belonging to Convolvulaceae, and the backbone relationships within that clade are resolved and well supported. We wanted to determine the cost in log-likelihood and parsimony and its significance for two alternatives in which *Cuscuta* would be nested within clade 1 (Fig. 5, nos. 2 and 3). Both SH and PB tests detected a significant difference (Table 2), suggesting that the hypotheses of closer association of *Cuscuta* with either the clade containing Argyreieae, Ipomoeeae, Convolvuleae, and some Merremieae or the clade containing the rest of Merremieae can be rejected. Even though the

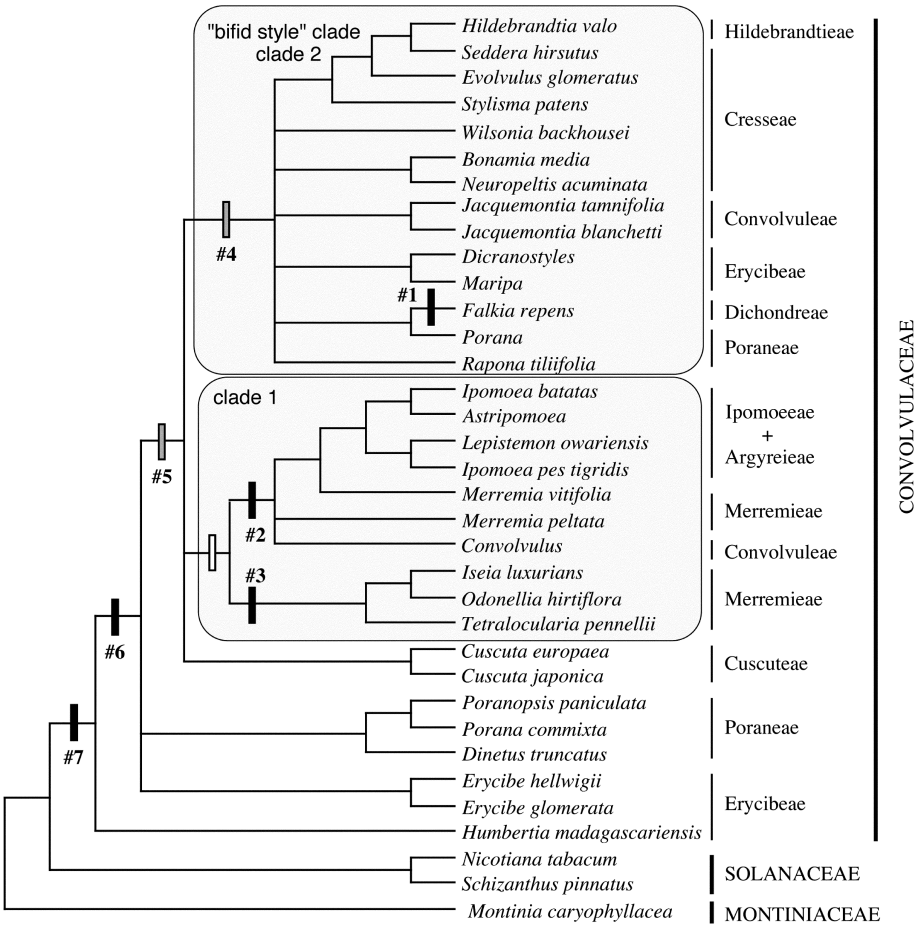

FIGURE 5. The summary evolutionary hypothesis for Convolvulaceae derived from the present study of combined sequence data from all three plant genomes analyzed with a range of analytical methods, and a previously published molecular systematic study of the family (Stefanović et al., 2002). Open bar depicts the inferred position of *Cuscuta* on the optimal trees. Bars numbered 1 to 7 depict alternative placements for the genus *Cuscuta* used in the Shimodaira-Hasegawa and parametric bootstrap tests. Shaded bars depict alternative branching points that were not rejected by these tests, whereas solid bars depict those that were found significantly different by the SH and PB tests (compare with Table 2). Classification by tribe based on Austin (1973, modified 1998).

current consensus of evolutionary hypotheses for Convolvulaceae based on different analyses places *Cuscuta* effectively in a polytomy with clades 1 and 2 (Fig. 5), we deemed it important to test this placement formally as well. Not surprisingly, neither the topology with *Cuscuta* as a sister group to clade 2, nor with *Cuscuta* as the sister group to clades 1 and 2 together (Fig. 5; nos. 4 and 5, respectively) could be rejected with either SH or PB tests

TABLE 2. Results of Shimodaira-Hasegawa (SH) and parametric bootstrap (PB) tests for comparison of alternative phylogenetic hypotheses.

	Shimodaira-Hasegawa test			Parametric bootstrap test			
Hypothesis[a]	$-\ln L$	$\delta - \ln L$	P	Length	δ length	P	Rejected[b]
ML tree	31,604.51	—	—	—	—	—	Best
MP tree	—	—	—	3850	—	—	Best
Cuscuta as sister to Dichondreae (no. 1)	31,676.65	72.14	<0.01	3875	25	<0.01	Yes
Cuscuta as sister to clade containing Convolvuleae, Ipomoeeae, Argyreieae, and some Merremieae (no. 2)	31,639.69	35.18	<0.01	3863	13	<0.01	Yes
Cuscuta as sister to clade containing the rest of Merremieae (no. 3)	31,638.95	34.46	<0.01	3864	14	<0.01	Yes
Cuscuta as sister to the "bifid style" clade (no. 4)	31,605.28	0.77	0.35	3853	3	0.34	No
Cuscuta as sister to clades 1 and 2 together (no. 5)	31,605.89	1.38	0.22	3855	5	0.13	No
Humbertia basal lineage, *Cuscuta* diverging next (no. 6)	31,619.64	15.14	0.025	3860	10	0.03	Yes
Cuscuta as basal lineage, compatible with its recognition at family level (no. 7)	31,682.54	78.03	<0.01	3886	36	<0.01	Yes

[a] Alternative branching patterns for the genus *Cuscuta* (nos. 1–7) are depicted in Figure 5.

[b] Yes, hypothesis rejected as significantly different by the SH (using 1000 bootstrap replicates with full parameter optimization of the GTR+I+Γ model) and PB (implemented under maximum parsimony criterion) tests ($P < 0.05$); No, not rejected by the SH and PB tests.

(Table 2). Finally, two alternative hypotheses, bearing the most importance for the circumscription of the family as a whole, were also tested. Both of these alternative positions, *Cuscuta* diverging within the family, as sister to all, except *Humbertia* (Fig. 5; no. 6), and *Cuscuta* as a sister group to the rest of the family, i.e., consistent with its recognition as a distinct family (Fig. 5; no. 7), were found to be significantly worse than the optimal trees according to the SH and PB tests.

The Shimodaira-Hasegawa test (Shimodaira and Hasegawa, 1999) is a statistically appropriate nonparametric method for comparing a posteriori tree topologies (Goldman et al., 2000). The parametric bootstrap (Swofford et al., 1996; Huelsenbeck and Crandall, 1997; Goldman et al., 2000), although shown to be more powerful than the SH test, given that the assumptions of sequence evolution are not significantly violated (Goldman et al., 2000; Buckley, 2002), is used much less frequently (Knowles, 2000; Fishbein et al., 2001; Zanis et al., 2002) due, in part, to the much greater computational time involved. When implemented under the ML criterion, a number of shortcuts can be employed to lower the computational cost: (1) reduction in number of terminals, either by complete exclusion of taxa from analyses or by keeping all taxa, but constraining a number of nodes, which effectively reduces the number of terminals; (2) avoiding more time-consuming repeated random addition of taxa by using a neighbor-joining starting tree; and/or (3) implementing faster, but less thorough, branch swapping algorithms such as SPR or NNI. However, these shortcuts, especially when combined, present risk of missing the global optima. Using less than optimal trees, whatever the optimality criterion might be, to calculate the differences for simulated data matrices (δ's) will potentially result in a biased null distribution of δ's from which the P value is calculated. In addition, even when the shortcuts are employed, the computational time might still be prohibitive with ML, rendering the implementation of tests impractical (Buckley, 2002), especially if more than one alternative needs to be tested.

The maximum parsimony framework for parametric bootstrapping is an equally suitable approach to generate the null distribution of δ's (Goldman et al., 2000) and this distribution can be used to determine the significance of the observed cost in parsimony for the alternative. Comparatively few statistical assessments of alternative hypotheses using MP have been published (e.g., Ruedi et al., 1998; Carlini et al., 2000; Zanis et al., 2002). The advantages of the MP approach with parametric bootstrapping is twofold: (1) the computational time is greatly reduced enabling the testing of multiple hypotheses, and, more importantly, (2) the reasonably extensive measures can be taken to explore the tree space more thoroughly in search for global optima, thereby preventing potential biases in P value calculations and known tendency of PB test to generate type 1 errors (Buckley, 2002). In order to minimize the bias and to err on the conservative side (by failing to reject H_0), at minimum the same effort should be invested in exploration of simulated data matrices as was invested in searches involving the original matrix. In that case, however, the utility of the PB test would be severely restricted because of computational burden imposed by repeated ML topological searches in conjunction with ever increasing size of molecular data sets (Buckley, 2002). Hence, with moderate to large data sets (>30 terminals), multiple, yet thorough PB tests can be done under the MP criterion, but not under ML. One disadvantage of this approach is that some well-known characteristics of DNA sequence evolution cannot be readily incorporated within an MP framework. Also, the MP criterion will not be suitable to test certain types of hypotheses, notably those involving branch lengths, where the MP approach was demonstrated as underestimating branch lengths (Saitou, 1989). It will be on a case-by-case basis that researchers will evaluate the costs and benefits of different approaches, and decide which optimality criterion might be more suitable for any particular study.

For all seven hypotheses tested in the present study, the results from SH and PB tests were in agreement not only regarding the conclusions drawn from them, i.e., rejecting or failing to reject the null hypothesis, but also in P values associated with the rejection region (Table 2). The PB is putatively more powerful than the SH test for discrimination among different alternative hypotheses (Goldman et al., 2000), and our results are consistent with this idea. However, the extent of the differences between probabilities estimated by our tests was much smaller than in other studies implementing SH and PB tests (e.g., Goldman et al., 2000; Fishbein et al., 2001).

Phylogenetic Relationships of Nonparasitic Convolvulaceae

Besides helping to narrow down the phylogenetic position of *Cuscuta*, the present study also provides additional support for the relationships among nonparasitic taxa of Convolvulaceae. This is most evident with respect to the "bifid style" clade. This clade comprises genera that have a more or less deeply divided style, generally following the concept of Dicranostyleae proposed by Hallier (1893). The "bifid style" clade was first explicitly identified by Stefanović et al. (2002), but support for its monophyly was weak (39% with *Cuscuta* species included in analysis; 80% without *Cuscuta*). The present study, with additional sequence data, offers further evidence, not only for the monophyly of that clade ($P = 1$ from BI; 89% bootstrap support with ML), but also its composition. The inclusion of *Jacquemontia* in the "bifid style" clade was one of the most surprising results of molecular phylogenetic study of Convolvulaceae. This genus is traditionally regarded as a member of tribe Convolvuleae due to its undivided, filiform style with elongated stigmas. Even though the defining morphological character, divided style, is not present in *Jacquemontia*, this genus was found to share a unique synapomorphy with the rest of the "bifid style" clade, reversion to a nonedited start codon for the *psb*L gene (Stefanović et al., 2002). This condition is not found

anywhere else in Convolvulaceae and its closest relatives. Given the unresolved relationships within the "bifid style" clade, one possible evolutionary scenario is that *Jacquemontia* is the sister group to the rest of the taxa with divided styles. This would account for a single origin for each of these two characters. The BI analysis lends some support to this scenario, resolving *Jacquemontia* as the sister-group to the rest of the "bifid style" clade (Fig. 3). However, this relationship on the optimal tree is not well supported, and was not recovered using other methods of reconstruction. Also, the present study offers confirmation and further support for polyphyly of Erycibeae, Poraneae, and Merremieae, as well as close relationships of tribes Hildebrandtieae with Cresseae, Ipomoeeae with Argyreieae, and Dichondreae with some members of Poraneae (Fig. 5).

CONCLUSIONS

In terms of both the quantity of DNA sequence data and range of analytical methods, this study represents an intensive effort to estimate the phylogenetic position of a relatively small clade of parasitic plants. The inability to recover the exact position of *Cuscuta* with confidence even with relatively large amounts of data exemplifies the magnitude of the problem in inferring phylogenetic relationships of parasitic taxa. However, this approach did help to narrow down the position of *Cuscuta* and to reject with confidence a number of alternative hypotheses. Bayesian analysis, used with success to resolve other difficult phylogenetic problems, offered no more insight for *Cuscuta* placement than did maximum likelihood and maximum parsimony. However, the majority-rule consensus tree derived from the Bayesian analysis was very similar to the best phylogeny inferred by maximum likelihood analysis, both in terms of inferred topology and support, while requiring significantly less computational time. Significant computational time saving was achieved also by implementing the parametric bootstrap under the maximum parsimony criterion to test a series of alternative topologies.

The taxonomic implications of this study will have positive impacts on comparative studies of *Cuscuta*, which presently use the currently available classification as a framework. It is important for future comparative studies, especially those on chloroplast genome evolution, to recognize that the differences observed in different *Cuscuta* species are not attributable solely to *Cuscuta*'s parasitic mode of life and that a significant proportion of those changes could be better explained as a plesiomorphic condition within the family, i.e., conditions shared with other members of the Convolvulaceae.

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REFERENCES

Akaike, H. 1973. Information theory as an extension of the maximum likelihood principle. Pages 267–281 *in* Second International Symposium on Information Theory (B. N. Petrov and F. Csaki, eds.). Akademiai Kiado, Budapest.

Angiosperm Phylogeny Group (APG). 1998. An ordinal classification for the families of flowering plants. Ann. Mo. Bot. Gard. 85:531–553.

Austin, D. F. 1973. The American Erycibeae (Convolvulaceae): *Maripa*, *Dicranostyles*, and *Lysiostyles* I. Systematics. Ann. Mo. Bot. Gard. 60:306–412.

Austin, D. F. 1998. Parallel and convergent evolution in the Convolvulaceae. Pages 201–234 *in* Biodiversity and taxonomy of tropical flowering plants (P. Mathews and M. Sivadasan, eds.). Mentor Books, Culicut, India.

Baillon, H. 1891. Convolvulacées. Pages 305–331 *in* Histoire des plantes, Librairie Hachett and Cie., Paris, France.

Barkman, T. J, S.-H. Lim, K. M. Salleh, and J. Nais. 2004. Mitochondrial DNA sequences reveal the photosynthetic relatives of *Rafflesia*, the world's largest flower. Proc. Natl. Acad. Sci. U.S.A. 101:787–792.

Bentham, G., and J. D. Hooker. 1873. Genera plantarum 2:865–881.

Bewick, T. A., L. K. Binning, W. R. Stevenson, and J. Stewart. 1987. A mycoherbicide for control of swamp dodder (*Cuscuta gronovii* Willd.), Cuscutaceae. Pages 93–104 *in* Parasitic flowering plants (H. C. Weber and W. Forstreuter, eds.). Philipps-Universität, Marburg, Germany.

Bömmer, D., G. Haberhausen, and K. Zetsche. 1993. A large deletion in the plastid DNA of the holoparasitic flowering plant *Cuscuta reflexa* concerning two ribosomal proteins (*rpl2*, *rpl23*), one transfer RNA (*trn*I) and an ORF 2280 homologue. Curr. Genet. 24:171–176.

Buckley, T. R. 2002. Model misspecification and probablistic tests of topology: Evidence from empirical data sets. Syst. Biol. 51:509–523.

Carlini, B. B., K. S. Reece, and J. E. Graves. 2000. Actin gene family evolution and the phylogeny of coleoid caphalopods (Mollusca: Cephalopoda). Mol. Biol. Evol. 17:1353–1370.

Chase, M. W., D. E. Soltis, R. G. Olmstead, D. Morgan, D. H. Les, B. D. Mishler, M. R. Duvall, R. Price, H. G. Hills, Y.-L. Qui, K. A. Kron, J. H. Rettig, E. Conti, J. D. Palmer, J. R. Manhart, K. J. Sytsma, H. J. Michaels, W. J. Kress, K. G. Karol, W. D. Clark, M. Hedren, B. S. Gaut, R. K. Jansen, K.-J. Kim, C. F. Wimpee, J. F. Smith, G. R. Furnier, S. H. Strauss, Q.-Y. Xiang, G. M. Plunkett, P. S. Soltis, S. E. Williams, P. A. Gadek, C. J. Quinn, L. E. Eguiarte, E. Golenberg, G. H. Learn, S. W. Graham, S. C. H. Barrett, S. Dayanandan, and V. A. Albert. 1993. Phylogenetics of seed plants: An analysis of nucleotide sequences from the plastid gene *rbcL*. Ann. Mo. Bot. Gard. 80:528–580.

Choisy, J. D. 1845. Convolvulaceae. *In* A. DeCandole, Prodromus 9:323–465.

Colwell, A. E. 1994. Genome evolution in a non-photosynthetic plant, *Conopholis americana*. Ph.D. dissertation, Washington University, St. Louis, MO, USA.

Cronquist, A. 1988. The evolution and classification of flowering plants. The New York Botanical Garden, Bronx, NY, USA.

Davis, J. I., M. P. Simmons, D. W. Stevenson, and J. F. Wendel. 1998. Data decisiveness, data quality, and incongruence in phylogenetic analysis: An example from the monocotyledons using mitochondrial *atpA* sequences. Syst. Biol. 47:282–310.

Dawson, J. H. 1987. *Cuscuta* (Convolvulaceae) and its control. Pages 137–149 *in* Parasitic flowering plants (H. C. Weber and W. Forstreuter, eds.). Philipps-Universität, Marburg, Germany.

Dawson, J. H. 1990. Dodder (*Cuscuta* spp.) control with dinitroaniline herbicides in alfalfa (*Medicago sativa*). Weed Techn. 4:341–348.

Dawson, J. H., L. J. Musselman, P. Wolswinkel, and I. Dörr. 1994. Biology and control of *Cuscuta*. Rev. Weed Sci. 6:265–317.

Denton, A. L., B. L. McConaughy, and B. D. Hall. 1998. Usefulness of RNA polymerase II coding sequence for estimation of green plant phylogeny. Mol. Biol. Evol. 15:1082–1085.

dePamphilis, C. W, N. D. Young, and A. D. Wolfe. 1997. Evolution of plastid gene *rps*2 in a lineage of hemiparasitic and holoparasitic

plants: Many losses of photosynthesis and complex patterns of rate variation. Proc. Natl. Acad. Sci. U.S.A. 94:7367–7372.
Dörr, I. 1987. The haustorium of *Cuscuta*—new staructural results. Pages 163–170 *in* Parasitic flowering plants (H. C. Weber and W. Forstreuter, eds.). Philipps-Universität, Marburg, Germany.
Doyle, J. J., and J. L. Doyle. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem. Bull. 19:11–15.
Duff, R. J., and D. L. Nickrent. 1997. Characterization of mitochondrial small-subunit ribosomal RNAs from holoparasitic plants. J. Mol. Evol. 45:631–639.
Dumortier, B.-C. 1829. Analyse des plantes. Tournay, Paris, France.
Engelmann, G. 1895. Systematic arrangement of the species of the genus Cuscuta with critical remarks on old species and descriptions of new ones. Trans. Acad. Sci., St. Louis 1:453–523.
Felsenstein, J. 1978. Case in which parsimony or compatibility methods will be positively misleading. Syst. Zool. 27:401–410.
Felsenstein, J. 1981. Evolutionary trees from DNA sequences: A maximum likelihood approach. J. Mol. Evol. 17:368–376.
Felsenstein, J. 1985. Confidence limits on phylogenies: An approach using the bootstrap. Evolution 39:783–791.
Felsenstein, J. 1988. Phylogenies from molecular sequences: Inference and reliability. Annu. Rev. Genet. 22:521–565.
Fishbein, M., C. Hibsch-Jetter, D. E. Soltis, and L. Hufford. 2001. Phylogeny of Saxifragales (angiosperms, eudicots): Analysis of a rapid, ancient radiation. Syst. Biol. 50:817–847.
Freyer, R., K. Neckermann, R. M. Maier, and H. Kössel. 1995. Structural and functional analysisof plastid genomes from parasitic plants: Loss of an intron within the genus *Cuscuta*. Curr. Genet. 27:580–586.
Goldman, N., J. P. Anderson, and A. G. Rodrigo. 2000. Likelihood-based tests of topologies in phylogenetics. Syst. Biol. 49:652–670.
Graham, S. W., and R. G. Olmstead. 2000. Utility of 17 chloroplast genes for inferring the phylogeny of the basal angiosperms. Am. J. Bot. 87:1712–1730.
Haberhausen, G., K. Valentin, and K. Zetsche. 1992. Organization and sequence of photosynthetic genes from the plastid genome of the holoparasitic flowering plant *Cuscuta reflexa*. Mol. Gen. Genet. 232:154–161.
Haberhausen, G., and K. Zetsche. 1994. Functional loss of all *ndh* genes in an otherwise relatively unaltered plastid genome of the holoparasitic flowering plant *Cuscuta reflexa*. Plant Mol. Biol. 24:217–222.
Hallier, H. 1893. Versuch einer natürlichen Gleiderung der Convolvulaceen auf morphologischer und anatomischer Grundlage. Bot. Jahrb. Syst. 16:453–591.
Heide-Jørgensesn, H. S. 1987. Changes in cuticle structure during development and attachment of the upper haustorium of *Cuscuta* L., *Cassytha* L., and *Viscum* L. Pages 319–334 *in* Parasitic flowering plants (H. C. Weber and W. Forstreuter, eds.). Philipps-Universität, Marburg, Germany.
Hendy, M. D., and D. Penny. 1989. A framework for the quantitative study of evolutionary trees. Syst. Zool. 38:297–309.
Hillis, D. M. 1996. Inferring complex phylogenies. Nature 383:130–131.
Hillis, D. M., and J. J. Bull. 1993. An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. Syst. Biol. 42:182–192.
Hoot, S. B., A. Culham, and P. R. Crane. 1995. The utility of *atpB* gene sequences in phylogenetic relationships: Comparison with *rbcL* and 18S ribosomal DNA sequences in Lardizabalaceae. Ann. Mo. Bot. Gard. 82:194–207.
Huelsenbeck, J. P. 1995. Performance of phylogenetic methods in simulations. Syst. Biol. 44:17–48.
Huelsenbeck, J. P., and J. P. Bollback. 2001. Empirical and hierarchical Bayesian estimation of ancestral states. Sysyt. Biol. 50:351–366.
Huelsenbeck, J. P., and K. A. Crandall. 1997. Phylogeny estimation and hypothesis testing using maximum likelihood. Annu. Rev. Ecol. Syst. 28:437–466.
Huelsenbeck, J. P., and D. M. Hillis. 1993. Success of phylogenetic methods in the four-taxon case. Syst. Biol. 42:247–264.
Huelsenbeck, J. P., D. M. Hillis, and R. Jones. 1996. Parametric bootstrapping in molecular phylogenies: Application and performance. Pages 19–45 *in* Molecular zoology: Advances, strategies, and protocols (J. D. Ferris and S. R. Palumbi, eds.). Wiley, New York, NY, USA.
Huelsenbeck, J. P., and F. Ronquist. 2001. MrBayes: Bayesian inference of phylogeny. Biometrics 17:754–755.
Karol, K. G., R. M. McCourt, M. T. Cimino, and C. F. Delwiche. 2001. The closest living relatives of land plants. Science 294:2351–2353.
Knowles, L. L. 2000. Tests of Pleistocene speciation in montane grasshoppers (genus *Melanoplus*) from the Sky Islands of western North America. Evolution 54:1337–1348.
Kuijt, J. 1969. The biology of parasitic flowering plants. University of California Press, Berkeley, CA, USA.
Larget, B. and D. L. Simon. 1999. Markov chain Monte Carlo algorithms for the Bayesian analysis of phylogenetic trees. Mol. Biol. Evol. 16:750–759.
Lee, K. B., and C. D. Lee. 1989. The structure and development of the haustorium in *Cuscuta australis*. *Canadian J. Bot*. 67:2975–2982.
Li, Y. 1987. Parasitism and integrated control of dodder of soybean. Pages 497–500 *in* Parasitic flowering plants (H. C. Weber and W. Forstreuter, eds.). Philipps-Universität, Marburg, Germany.
Mabberley, D. J. 1987. The plant book. Cambridge university press, Cambridge, UK.
Machado, M. A., and K. Zetsche. 1990. A structural, functional, and molecular analysis of plastids of the holoparasites *Cuscuta reflexa* and *Cuscuta europaea*. Planta 181:91–96.
Maddison, D. R. 1991. The discovery and importance of multiple islands of most-parsimonious trees. *Syst. Zool.* 40:315–328.
Maddison, W. P., and D. R. Maddison. 1997. MacClade: Analysis of phylogeny and character evolution, version 3.07. Sinauer, Sunderland, MA, USA.
Melchior, H. 1964. Engler's Syllabus der Pflanzenfamilien 12:427–429.
Moore, W. S. 1995. Inferring phylogenies from mtDNA variation: Mitochondrial-gene trees versus nuclear-gene trees. Evolution 49:718–726.
Murphy, W. J., E. Eizirik, S. J. O'Brien, O. Madsen, M. Scally, C. J. Douady, E. Teeling, O. A. Ryder, M. J. Stanhope, W. W de Jong, and M. S. Springer. 2001. Resolution of the early placental mammal radiation using Beayesian phylogenetics. Science 294:2348–2351.
Neyland, R. 2001. A phylogeny inferred from large ribosomal subunit (26S) rDNA sequences suggests that *Cuscuta* is a derived member of Convolvulaceae. Brittonia 53:108–115.
Nickrent, D. L., R. J. Duff, A. E. Colwell, A. D. Wolfe, N. D. Young, K. E. Steiner, and C. W. dePamphilis. 1998. Molecular phylogenetic and evolutionary studies of parasitic plants. Pages 211–241 *in* Molecular Systematics of Plants II: DNA sequencing (D. E. Soltis, P. S. Soltis, and J. J. Doyle, eds.). Kluwer, Boston, MA, USA.
Nickrent, D. L., and E. M. Starr. 1994. High rates of nucleotide substitution in nuclear small-subunit (18S) rRNA from holoparasitic flowering plants. J. Mol. Evol. 39:62–70.
Nickrent, D. L., A. Blarer, Y.-L. Qiu, D. E. Soltis, P. S. Soltis, and M. Zanis. 2002. Molecular data place Hydnoraceae with Aristolochiaceae. Am. J. Bot. 89:1809–1817.
Nickrent, D. L., and R. J. Duff. 1996. Molecular studies of parasitic plants using ribosomal RNA. Pages 28–52 *in* Advances in parasitic plant research (Moreno et al., eds.), Dirección General de Investigación Agraria, Servicio de Publicaciones y Divulgación, Córdoba, Spain.
Olmstead, R. G., C. W. dePamphilis, A. D. Wolfe, N. D. Young, W. J. Elisons, and P. A. Reeves. 2001. Disintegration of the Scrophulariaceae. Am. J. Bot. 88:348–361.
Olmstead, R. G, H. J. Michaels, K. M. Scott, and J. D. Palmer. 1992. Monophyly of the Asteridae and identification of their major lineages inferred from DNA sequences of *rbcL*. Ann. Mo. Bot. Gard. 79:249–265.
Olmstead, R. G., and J. D. Palmer. 1992. A chloroplast DNA phylogeny of the Solanaceae: Subfamilial relationships and characters evolution. Ann. Mo. Bot. Gard. 79:346–360.
Oxelman, B., and B. Bremer. 2000. Discovery of paralogous nuclear gene sequences coding for the second-largest subunit of RNA polymerase II (*RPB2*) and their phylogenetic utility in Gentianales of the asterids. Mol. Biol. Evol. 17:1131–1145.
Oxelman, B., N. Yoshikawa, B. L. McConaughy, J. Luo, A. L. Denton, and B. D. Hall. 2004. RPB2 gene phylogeny in flowering plants, with particular emphasis on asterids. Mol. Phylogenet. Evol. In press.
Peter, A. 1891. Convolvulaceae. *In* (A. Engler and K. Prantl, eds.), Die Natürlichen Pflanzenfamilien 4:1–40.

Peter, A. 1897. Convolvulaceae. *In* (A. Engler and K. Prantl, eds.), Die Natürlichen Pflanzenfamilien 4:375–377 (Appendix).
Philippe, H. 1993. MUST: A computer package of management utilities for sequences and trees. Nucleic Acids Res. 21:5264–5272.
Posada, D., and K. A. Crandall. 1998. ModelTest: Testing the model of DNA substitution. Bioinformatics 14:817–818.
Rambault, A., and N. C. Grassly. 1997. Seq-Gen: An application of Monte Carlo simulation of DNA sequence evolution along phylogenetic trees. Comput. Appl. Biosci. 13:235–238.
Rannala, B., and Z. Yang. 1996. Probability distribution of molecular evolutionary trees: A new method of phylogenetic inference. J. Mol. Evol. 43:304–311.
Renner, S. S., and A. S. Chanderbali. 2000. What is the relationship among Hernandiaceae, Lauraceae, and Monimiaceae, and why is this question so difficult to answer? Int. J. Plant Sci. 161:S109–S119.
Roberty, G. 1952. Genera Convolvulacearum. Candollea 14:11–60.
Roberty, G. 1964. Les genres des Convolvulacées (esquisse). Boissiera 10:129–156.
Rogers, S. O., and A. J. Bendich. 1985. Extraction of DNA from milligram amounts of fresh, herbarium and mummified plant tissues. Plant Mol. Biol. 5:69–76.
Rohwer, J. G. 2000. Toward a phylogenetic classification of the Lauraceae: Evidence from *mat*K sequences. Syst. Biol. 25:60–71.
Ruedi, M., M. Auberson, and V. Savolainen. 1998. Biogeography of Sulawesian shrews: Testing for their origin ith a parametric bootstrap on molecular data. Mol. Phylogenet. Evol. 9:567–571.
Saitou, N. 1989. A theoretical study of the underestimation of branch lengths by the maximum parsimony principle. Syst. Zool. 38:1–6.
Sanderson, M. J., and J. Kim. 2000. Parametric Phylogenies? Syst. Biol. 49:817–829.
Shimodaira, H. 2002. An approximately unbiased test of phylogenetic tree selection. Syst. Biol. 51:492–508.
Shimodaira, H., and M. Hasegawa. 1999. Multiple comparisons of log-likelihoods with applications to phylogenetic inferrence. Mol. Biol. Evol. 16:1114–1116.
Smith, R. A., D. M. Ferguson, T. J. Barkman, and C. W. dePamphilis. 2000. Molecular phylogenetic evidence for the origin of Lennoaceae: A case of adelphoparasitism in the angiosperms? Am. J. Bot. S87: 158.
Soltis, D. E., P. S. Soltis, D. L. Nickrent, L. A. Johnson, W. J. Hahn, S. B. Hoot, J. A. Sweere, R. K. Kusoff, K. A. Kron, M. W. Chase, S. M. Swenson, E. A. Zimmer, S.-M. Chaw, L. J. Gillespie, W. J. Kress, and K. J. Sytsma. 1997. Angiosperm phylogeny inferred from 18S ribosomal DNA sequences. Ann. Mo. Bot. Gard. 84:1–49.
Stefanović, S., L. Krueger, and R. G. Olmstead. 2002. Monophyly of the Convolvulaceae and circumscription of their major lineages based on DNA sequences of multiple chloroplast loci. Am. J. Bot. 89:1510–1522.
Subramaniam, K., and S. Mahadevan. 1994. The cDNA sequence of cytochrome b5 associated with cytokinin-induced haustoria formation in *Cuscuta reflexa*. Gene 149:375–376.
Swofford, D. L. 1999, 2002. PAUP*. Phylogenetic analysis using parsimony (*and other methods), version 4.0b2a. Sinauer, Sunderland, MA.
Swofford, D. L., G. J. Olsen, P. J. Waddell, and D. M. Hillis. 1996. Phylogenetic inference. Pages 407–514 *in* Molecular systematics, 2nd ed. (D. M. Hillis, C. Moritz, and B. K. Mable, eds.). Sinauer, Sunderland, MA.
Taberlet, P., L. Gielly, G. Pautou, and J. Bouvet. 1991. Universal primers for amplification of the noncoding regions of chloroplast DNA. Plant Mol. Biol. 17:1105–1109.
Takhtajan, A. 1997. Diversity and classification of flowering plants. Columbia University Press, New York, NY, USA.
Wolfe, A. D., and C. W. dePamphilis. 1995. Systematic implications of relaxed functional constraints on the RUBISCO large subunit in parasitic plants of the Scrophulariaceae and Orobanchaceae. Am. J. Bot. S82:6.
Wolfe, A. D., and C. W. dePamphilis. 1997. Alternate paths of evolution for the photosynthetic gene *rbcL* in four nonphotosynthetic species of *Orobanche*. Plant Mol. Biol. 33:965–977.
Wolfe, A. D., and C. W. dePamphilis. 1998. The effect of relaxed functional constraints on the photosynthetic gene *rbcL* in photosynthetic and nonphotosynthetic parasitic plants. Mol. Biol. Evol. 15:1243–1258.
Yang, Z. 1994. Estimating the pattern of nucleotide substitution. J. Mol. Evol. 39:105–111.
Young, N. D., K. E. Steiner, and C. W. dePamphilis. 1999. The evolution of parasitism in Scrophulariaceae/Orobanchaceae: Plastid gene sequences refute an evolutionary transition series. Ann. Mo. Bot. Gard. 86:876–893.
Yuncker, T. G. 1932. The genus *Cuscuta*. Memoirs of the Torrey Botanical Club 18:113–331.
Zanis, M. J., D. E. Soltis, P. S. Soltis, S. Mathews, and M. J. Donoghue. 2002. The root of the angiosperms revisited. Proc. Natl. Acad. Sci. U.S.A. 99:6848–6853.

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APPENDIX 1. Taxa, source of plant material from which DNA was extracted, and GenBank accession number for all the sequences used in this study. For the sequences reported in this paper for the first time, the GenBank number is followed an asterisk. Herbarium specimens were used except where indicated otherwise (FM = fresh material; SG = silica-gel dried). Classification by tribe based on Austin (1973, modified 1998b). MBG = Missouri Botanical Garden; RBGE = Royal Botanic Garden Edinburgh; RN = Ray Neyland; RGO = Richard G. Olmstead; SS = Saša Stefanović, SRPIS = USDA-Southern Regional Plant Introduction Station.

FAMILY Tribe Species	Voucher, source, or literature citations	GenBank accession number						
		rbcL	*atpB*	*psbE-J*	*trnL-F*	*rpl2*	*atpA*	*RPB2*
CONVOLVULACEAE Juss.								
Convolvuleae (Choisy) Choisy								
Convolvulus assyricus Gryseb.	RBGE 19950150; Turkey [SG]	AY100995	AY100786	AY100890	AY101104	AY596748	AY596678	—
Convolvulus mauritanicus Boiss.	Cultivated, MBG 912594; USA [FM]	—	—	—	—	—	—	AY596715
Jacquemontia blanchetti Moric.	*Nee 48736*, NY; Bolivia	AY101039	AY100828	AY100931	AY101148	AY596763	AY596693	AY596729
Jacquemontia tamnifolia (L.) Griseb.	RN 390; USA [FM]	AY101037	AY100826	AY100929	AY101146	AY596762	AY596692	AY596728
Cresseae Benth. & Hook.								
Bonamia media (R. Br.) Hall. f.	*PERTH 05373239*, UWA; Australia	AY101030	AY100819	AY100922	AY101139	AY596759	AY596689	AY596725
Evolvulus glomeratus Nees & Mart.	*Waimea 85P531*, MO; Brasil	AY101012	AY100803	AY100906	AY101121	AY596754	AY596684	AY596721
Neuropeltis acuminata (P. B.) Benth.	*Harder et al. 3346*, MO; Ghana	AY101033	AY100822	AY100925	AY101142	AY596760	AY596690	AY596726
Seddera hirsutus Hall. f.	*Luke et al. TPR 569*, US; Kenya	AY101010	AY100801	AY100905	AY101119	AY596753	AY596683	AY596720
Stylisma patens (Desr.) T. Myint	*Zomlefer 692*, FLAS; USA	AY101019	AY100810	AY100913	AY101128	AY596755	AY596685	AY596722
Wilsonia backhousei Hook.	*RGO 99-190;* WTU; Australia	AY101021	AY100812	AY100915	AY101130	AY596756	AY596686	—
Cuscuteae Choisy								
Cuscuta europaea L.	*Alanko 94416*, H; Finland [FM]	AY101060	AY100848	AY100951	AY101169	AY596771	AY596701	AY596737
Cuscuta japonica Choisy	*Hashimoto 853*, WTU; Japan	AY101061	AY100849	AY100947	AY101170	AY596707	AY596702	AY596738
Dichondrae (Choisy) Choisy								
Falkia repens L. f.	*RGO 99-11, WTU*; South Africa	AY101024	AY100813	AY100917	AY10133	AY596757	AY596687	AY596723
Erycibeae (Endl.) Hall. f.								
Dicranostyles ampla Ducke	*Marimon BS-031*, US; Brasil	AY101042	AY100831	AY100933	AY101151	AY596764	AY596694	—
Dicranostyles mildbraediana Pilger	*Nee 38892*, NY; Bolivia	—	—	—	—	—	—	AY596730
Erycibe glomerata Bl.	*Church et al. 1421*, A; Indonesia	AY101048	AY100837	AY100939	AY101157	AY596767	AY596697	AY596733
Erycibe hellwigii Prain	*Takeuchi 7132*, A; Papua New Guinea	AY101047	AY100836	AY100938	AY101156	AY596766	AY596696	AY596732
Humbertia madagascariensis Lam.	*McPherson 14267*, MO, P; Madagascar	AY101062	AY100850	AY100948	AY101171	AY596772	AY596703	—
Maripa glabra Choisy	*Morri & Pennington 18142*, P; French Guiana	—	—	—	—	—	—	AY596731
Maripa repens Rusby	*van Dulman & Matapi 124*, FAU; Colombia	AY101045	AY100834	AY100936	AY101154	AY596765	AY596695	—
Hildebrandtieae Peter								
Hildebrandtia valo Deroin	*McPherson & Pigeon 14964*, MO; Madagascar	AY101004	AY100795	AY100899	AY101113	AY596752	AY596682	AY596719
Ipomoeeae Hall. f.								
Astripomoea grantii (Rendle) Verdc.	*Koyombo 1000*, MO; Tanzania	AY100964	AY100755	AY100862	AY101073	AY596743	—	—
Astripomoea malvacea (Klotzsch) A. D. J. Meeuse	*Koyombo 6971*, A; Tanzania	—	—	—	—	—	AY596673	AY596710
Ipomoea batatas (L.) Lam.	*SS 00-20, WTU*; Costa Rica [SG]	AY100962	AY100753	AY100860	AY101071	AY596742	AY596672	AY596709
Ipomoea pes-tigridis L.	SRPIS-549258; Australia [FM]	AY100978	AY100769	AY100873	AY101087	AY596745	AY596675	AY596712
Lepistemon owariensis (P. Beauv.) Hall. f.	*Harder & Schmidt 3013*, MO; Zambia	AY100969	AY100760	AY100867	AY101078	AY596744	AY596674	AY596711
Merremieae D. Austin								
Iseia luxurians (Moric.) O'Donell	*Krapovickas & Cristóbal 14446*, P; Argentina	AY101001	AY100792	AY100896	AY101110	AY596749	AY596679	AY596716
Merremia peltata ((L.) Merr.	*Ambriansyah & Arifin AA190*, A; Indonesia	AY100990	AY100781	AY100885	AY101099	AY596747	AY596677	AY596714
Merremia vitifolia (Burm. f.) Hall. f.	*Maxwell 97-372*, NY; Thailand	AY100981	AY100772	AY100876	AY101090	AY596746	AY596676	AY596713
Odonellia hirtiflora (Martens & Galeotti) K. Robertson	*Croat 12751*, MO; Peru	AY101002	AY100793	AY100897	AY101111	AY596750	AY596680	AY596717
Tetralocularia pennellii O'Donell	*Smith & Shuhler 402*, US; Peru	AY101003	AY100794	AY100898	AY101112	AY596751	AY596681	AY596718
Poraneae Hall. f.								
Dinetus truncatus (Kurz) Staples	*Staples et al. 425*, A; Thailand	AY101053	AY100841	AY100944	AY101162	AY596769	AY596699	AY596735
Porana commixta Staples	*Wilson & Rowe 967*, A; Australia	AY101055	AY100843	AY100946	AY101164	AY596770	AY596700	AY596736
Porana velutina (Mart. & Gal.) Hall. f.	*Seigler et al. 13063*, MO; Mexico	—	—	—	—	—	AY596688	AY596724
Porana volubilis Burm. f.	*Staples 429*, A; Burma	AY101028	AY100817	AY100920	AY101137	AY596758	—	—
Poranaopsis paniculata (Roxb.) Roberty	*Acevedo-Rodriguez 9293*, NY; Puerto Rico	AY101051	AY100839	AY100942	AY101160	AY596768	AY596698	AY596734
Rapona tiliifolia (Bak.) Verdc.	*Door 4167*, MO; Madagascar	AY101035	AY100824	AY100927	AY101144	AY596761	AY596691	AY596727
SOLANACEAE Juss.								
Nicotiana tabacum L.	Cultivated, UWGH; USA [FM]	Z00044	Z00044	Z00044	Z00044	Z00044	AY596704	AY596739
Schizanthus pinnatus Ruiz & Pav.	*Lester 0224/66*, COLO	U08619	AY100851	AY100949	AY101172	AY596773	AY596705	AY596740
MONTINIACEAE Nikai								
Montinia caryophyllacea Thunb.	RGO 94-01, WTU	L11194	AY100852	AY100950	AY101173	AY596708	AY596706	AY596741