

SYSTEMATICS OF *IPOMOEA* SUBGENUS *QUAMOCLIT* (CONVOLVULACEAE) BASED ON ITS SEQUENCE DATA AND A BAYESIAN PHYLOGENETIC ANALYSIS¹

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A Bayesian phylogenetic analysis of 36 *Ipomoea* species using sequence data from the internal transcribed spacer region was compared with classification schemes based on traditional methods and a previously published cpDNA restriction fragment length polymorphism (RFLP) study. These molecular studies support a diversity of groups that were circumscribed on the basis of phenetic principles and agree generally with the results from cpDNA RFLP analyses. The congruence between the phylogenetic hypotheses based on new molecular data and the understanding of relationships developed in earlier studies indicate that these classifications may reflect evolutionary history. Two large clades of species, with one including sections *Tricolores*, *Calonyction*, and *Pharbitis* and the other including sections *Mina* and *Leptocallis*, were identified. Furthermore, morphologically distinct groups of *Ipomoea* species received support from the DNA sequence data. Indices of convergence for the Markov chain Monte Carlo (MCMC) in the Bayesian phylogenetic analysis were evaluated. A limited range of posterior probabilities for each node in the trees from a set of five MCMC samples provides a useful index of convergence. Bayesian node support values were generally higher than bootstrap values from a maximum parsimony analysis. This is consistent with the notion that these measures of support estimate different qualities of the data.

Key words: Bayesian phylogenetic analysis; Convolvulaceae; *Ipomoea*; ITS; Markov chain Monte Carlo; molecular phylogenetics; morning glory.

Ipomoea is an exceptionally large and diverse genus in the Convolvulaceae, comprising over 600 species in strict and traditional concepts of the group (Austin and Huáman, 1996) or up to 1000 species in recent phylogenetic conceptions of the group (*Ipomoea* and its segregates; e.g., *Argyreia*, *Turbina*, *Astripomoea*, *Stictocardia*, *Lepistemon*, and *Rivea* pro synonymo; Wilkin, 1999; Manos et al., 2001; Miller et al., 2002). Most *Ipomoea* occur in tropical and subtropical climates throughout the world, but representative elements of the genus are in all known biomes (McDonald, 1991; Wilkin, 1999).

Infrageneric classifications of *Ipomoea* were provided by Choisy (1845), Hallier (1893a, b), and House (1908a). Relationships among Old World *Ipomoea* species were further refined by van Oostroom (1953), who recognized seven infrageneric taxa in his studies on Asian species. Borrowing liberally from van Oostroom's concepts, Verdcourt (1957, 1963) recognized eight infrageneric taxa in his treatment of African species. American *Ipomoea* have received more attention than those of the Old World (e.g., House, 1908a; Matuda, 1963; Standley and Williams, 1970; Austin, 1975a, b, 1979, 1997; McPherson, 1979; McDonald, 1982, 1991; Austin and

Huáman, 1996). Austin and colleagues have provided the most recent and comprehensive treatment of American *Ipomoea*, recognizing three subgenera within the genus: *Eriospermum* (Hallier) Verdcourt ex Austin, *Ipomoea*, and *Quamoclit* (Moench) Clarke (Austin and Huáman, 1996; Austin, 1997; Austin and Bianchini, 1998). The treatments of Austin are similar in most respects to those of McDonald (1991) on Mexican *Ipomoea* species. It is important to point out, however, that both McDonald and Austin have encouraged caution with their systems and have consistently recognized the enormous challenges presented by the study of this group of plants (e.g., McDonald, 1991; Austin and Huáman, 1996). This provides one of the motivations for the research program presented here of turning to additional sources of phylogenetic data to attempt to better understand the relationships among morning glories.

The present study focuses on species of the subgenus *Quamoclit*, as well as the species of section *Pharbitis* (formerly aligned in subgenus *Ipomoea*, Table 1). The nested relationship of *Pharbitis* species within subgenus *Quamoclit* has been established on the basis of DNA sequence data (Miller et al., 1999) and is supported by morphological evidence (Wilkin, 1999). There also are indications of this relationship in a chloroplast DNA RFLP study of McDonald and Mabry (1992), though this result does not hold for all species of section *Pharbitis* included in their study. The *Quamoclit* group (subgenus *Quamoclit*, plus section *Pharbitis*) forms a well-supported clade (Miller et al., 1999) within the clade /*Astripomoeinae* (Stefanovic et al., 2003) based on both separate and combined analyses of internal transcribed spacers (ITS) and *waxy* sequence data.

The species of the *Quamoclit* group are largely restricted to the neotropics (McDonald, 1991; Austin and Huáman, 1996). A number of infrageneric taxa within this species group have been the subject of various revisional studies, including sec-

¹ Manuscript received 31 October 2003; revision accepted 8 April 2004.

We thank E. Jones from PSM lab for technical assistance. We greatly appreciate the notes and translations of Hallier's papers by A. Forche. We thank M. Rausher, K. Keeler, A. Iacchetti, T. Mendelson, J. Kniskern, P. Tiffin, J. Hille Ris Lambers, M. Vallejo-Marin, E. Simms, A. Denton, R. Searles, C. Welch, and R. Jarret for kindly contributing to R. E. M.'s seed collection. Seed of *Ipomoea nil* was generously provided by Jill Parsons of the Royal Botanical Gardens, Kew. Comments on the manuscript by B. Crother, S. Stefanovic, and an anonymous reviewer are greatly appreciated. R. E. M. acknowledges postdoctoral support provided by NSF DEB 9707223 to M. Rausher, as well as support from Louisiana Board of Regents LEQSF(2003–06)-RD-A-23 and a Southeastern Louisiana University Faculty Development Grant. P. S. M. was supported in part by NSF DEB 9707945.

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TABLE 1. Treatment according to Austin and colleagues (Austin and Huáman, 1996; Austin, 1997; Austin and Bianchini, 1998) of *Ipomoea* subgenus *Quamoclit* and of section *Pharbitis*. Type species for infrageneric groups are indicated in boldface type.

Subgenus <i>Ipomoea</i>	
Section <i>Pharbitis</i> (Choisy) Griseb.	
Series <i>Pharbitis</i> (House) Austin	
<i>I. ampullacea</i> Fernald	
<i>I. neurocephala</i> Hallier* = <i>I. igualensis</i> Weatherby	
<i>I. mairetii</i> Choisy	
<i>I. purpurea</i> (L.) Roth	
Series <i>Heterophyllae</i> (House) Austin	
<i>I. hederacea</i> Jacq.	
<i>I. indica</i> (Burm.) Merr.	
<i>I. lindheimeri</i> A. Gray	
<i>I. nil</i> (L.) Roth	
<i>I. pubescens</i> Lam.	
Series <i>Tyrianthinae</i> (House) Austin	
<i>I. orizabensis</i> (Pell.) Led. Ex Steudl.	
<i>I. sescossiana</i> Baillon	
<i>I. stans</i> Cav.	
Subgenus <i>Quamoclit</i> (Moench) Clark	
Section <i>Calonyction</i> (Choisy) Griseb.	
<i>I. alba</i> L.	
<i>I. turbinata</i> Lag.	
<i>I. santillanii</i> O'Donell	
Section <i>Exogonium</i> (Choisy) Griseb.	
<i>I. dumetorum</i> Willd. Ex Roem. & Schult.	
<i>I. expansa</i> McDonald	
<i>I. purga</i> (Wender.)	
<i>I. seducta</i> House	
Section <i>Leptocallis</i> (G. Don) McDonald	
<i>I. chamelana</i> McDonald	
<i>I. tenuiloba</i> Torr.	
<i>I. ternifolia</i> Cav.	
Section <i>Mina</i> (Cerv.) Griseb.	
<i>I. coccinea</i> L.	
<i>I. funis</i> Schlecht. & Cham.	
<i>I. hastigera</i> H. B. K.	
<i>I. hederifolia</i> L.	
<i>I. lobata</i> (Cerv.) Thellung	
<i>I. lutea</i> Hemsl.	
<i>I. neei</i> (Spr.) O'Donell	
<i>I. quamoclit</i> L.	
Section <i>Tricolores</i> McDonald	
<i>I. cardiophylla</i> A. Gray	
<i>I. marginisepala</i> O'Donell	
<i>I. parasitica</i> (H. B. K.) G. Don	
<i>I. tricolor</i> Cav.	

tions *Calonyction* (Gunn, 1972), *Exogonium* (Austin, 1977; McDonald, 1987), *Leptocallis* (McDonald, 1995), *Mina* (O'Donell, 1959), and series *Tyrianthinae* of section *Pharbitis* (McDonald, 2001). The chloroplast DNA restriction fragment length polymorphism (RFLP) study of McDonald and Mabry (1992) included 31 New World species of *Ipomoea*, 22 of which were members of the *Quamoclit* group. Their study identified the morphologically distinct sections *Calonyction* and *Mina* as well-supported groups. They also found that three species of *Ipomoea* section *Tricolores* form a well-supported monophyletic group, although the placement of *I. parasitica* (section *Tricolores*) was problematic. Evidence was provided that series *Pharbitis* and series *Heterophyllae* of section *Pharbitis* are sister taxa, though not closely associated with species of series *Tyrianthinae* of this same section. Species of section *Exogonium* and series *Tyrianthinae* are highly variable (McDonald, 1987 and McDonald, 2001, respectively). The

three *Exogonium* species sampled by McDonald and Mabry (1992) do not form a distinct clade, but this might owe in part to a small sample of species in this group. This is also the case for four species of *Ipomoea* series *Tyrianthinae* included in their study.

The ultimate goal of the research presented here is to develop a well-resolved phylogeny for the morning glories in the Ipomoeae (Miller et al., 1999). The objectives of this specific study are to further examine relationships among the species of *Ipomoea* subgenus *Quamoclit* and section *Pharbitis*. To meet these objectives, we sampled species from sections within subgenus *Quamoclit* and from series within section *Pharbitis* and obtained intraspecific samples when possible. We examined the correspondence between a molecular data set constructed with ribosomal nuclear DNA sequences from the 5.8S gene and associated internal transcribed spacers (ITS region) and traditional classifications of this group (Table 1). In particular, we wanted to determine whether or not the sections and series recognized of *Quamoclit* are supported by these molecular data. The results of the molecular analyses based on the ITS region are compared to earlier molecular studies based on chloroplast DNA restriction site variation (McDonald and Mabry, 1992), as well as on other recent molecular phylogenetic results (Miller et al., 1999; Manos et al., 2001). In carrying out the analyses of the molecular data, a Bayesian phylogenetic analysis is emphasized (e.g., Huelsenbeck et al., 2002; Miller et al., 2002). An additional objective of this study was to further develop this approach for the analysis of phylogenetic data. In particular, methods to examine convergence of the Markov chain Monte Carlo (MCMC) sampling are emphasized.

MATERIALS AND METHODS

Taxon sampling—Thirty-six species of *Ipomoea* were investigated that represent five sections of subgenus *Quamoclit* and represent three series of section *Pharbitis* (that were formerly accommodated in subgenus *Ipomoea*) (Table 1). The sample represents approximately 30% of the species of subgenus *Quamoclit* (Austin and Huáman, 1996; Austin, 1997; Austin and Bianchini, 1998). In addition, multiple accessions for several individual species were included, resulting in a total of 68 samples for this investigation (Appendix 1; see Supplemental Data accompanying the online version of this article). Many of the plants used in this study were grown from the seed collection of J. A. M. (Appendix 1). The remaining plants were obtained by R. E. M. from various seed sources (Appendix 1), as well as one sample for this study provided by P. Wilkin (Royal Botanic Gardens, Kew). Vouchers specimens were deposited in one of the following: SLU, TEX (Appendix 1). Two species, *Ipomoea cairica* and *I. sepiaria*, were selected as outgroup taxa based on previous, higher-level analyses (Miller et al., 1999; Manos et al., 2001).

Molecular methods—Total genomic DNA was obtained using the DNeasy Plant mini kit (Qiagen, Valencia, California, USA) from live plants grown from seed in the Duke University Greenhouse. Extracted DNA is under the care of R. E. M. Molecular methods for PCR (polymerase chain reaction) and sequencing of the internal transcribed spacers of nuclear ribosomal DNA, or ITS region (ITS 1–5.8S–ITS 2), generally followed Miller et al. (1999). For the amplification of ITS, we substituted standard PCR components with an Advantage-GC cDNA PCR kit (Clontech, Palo Alto, California, USA), which permitted direct sequencing of PCR products.

Sequences—The sequence data from the ITS region (ITS 1–5.8S–ITS 2) included 58 new sequences and 10 previously published sequences (Miller et al., 1999; Manos et al., 2001) (Appendix 1; see Supplemental Data accompanying the online version of this article). The 68-taxon ITS data set was examined for identical sequences. The sequences for seven species included

identical sequences for some of the multiple accessions, in which case a single sequence was used to represent these identical sequences. This reduced data set included a total of 54 taxa. Sequences were aligned manually. Fourteen sites were excluded due to ambiguous alignment. Sequences are available from GenBank (accessions AY538275-AY538332), and the aligned Nexus data file is available from R. E. M. (www.selu.edu/Academics/Faculty/rickmiller) and from TreeBase (www.treebase.org).

Phylogenetic analyses—We adopted a general time-reversible model of DNA substitution with among-site rate variation drawn from a gamma distribution (GTR + Γ) for the analysis. This model was selected from a comparison of 56 models using the Akaike information criterion (Akaike, 1974) as implemented in Modeltest version 3.0 (Posada and Crandall, 1998). A Bayesian phylogenetic analysis was used to examine tree topology, support for clades, and to address specific questions about relationships (MrBayes version 2.0 software; Huelsenbeck and Ronquist, 2001). The posterior probabilities of the phylogenetic model were estimated as part of the Bayesian analyses using Markov chain Monte Carlo (MCMC) sampling with the Metropolis–Hastings–Green algorithm running four chains, three heated and one cold chain. The analysis used uniform prior distributions for the alpha-shape parameter of the gamma distribution (0–10), proportion of invariable sites (0–1), rate matrix parameters (1–100), and branch lengths (1–10). A flat prior was used for the topology and a Dirichlet distribution was used for the base frequencies. Unique random starting trees were used for each of 15 separate analyses (see Results). Every hundredth tree was sampled from the MCMC analysis to increase independence of samples.

The MCMC analysis starts at a random tree. The trees from the sample of interest are those within the stationary distribution. There is an initial burn-in period before the MCMC sampling is within the stationary distribution. To determine the burn-in period, both likelihood values and tree lengths were graphed against generation determining the number of generations at which these values reached a plateau.

One of the most important and difficult components of a Bayesian phylogenetic analysis is determining when MCMC analyses have run for enough generations for posterior probabilities to be sufficiently close to their true values, in other words, estimating convergence. Two approaches were adopted in this study to obtain estimates of convergence. In both cases, five separate analyses were carried out for a particular number of generations. Then the number of generations was increased for an additional set of five analyses, increasing the number of generations for sets of analyses until convergence was obtained. One approach used was a heuristic method. In this case, the index of convergence was when consistent results were obtained among five estimates of the parameters of the phylogenetic model. Specifically, we focused on tree topology and Bayesian posterior probabilities of individual clades. The indication of convergence used for posterior clade probabilities was when these values for all nodes of the five trees fell within a range of 3%. In addition, convergence of the parameters of the phylogenetic model were also evaluated more formally by adapting a method of Gelman et al. (1995); within-run variation was compared to between-run variation (details presented in Appendix 2; see Supplemental Data accompanying the online version of this article). An index of convergence is when the ratio of these two estimates of variation equal one. We examined log-likelihoods and tree lengths for this index of convergence. The analysis of the data consisted of constructing a 50% majority rule consensus tree from the concatenated set of trees from the final set of five analyses, once convergence had been met by the most conservative criterion.

Application of Bayesian analyses to phylogenetic systematics is still in the exploratory phase, and certain aspects of these analyses are still being evaluated (Huelsenbeck et al., 2002). Therefore, a separate parsimony analysis was compared to the Bayesian phylogenetic analyses. The parsimony analysis was carried out using weighted parsimony with a six-parameter weighting scheme based on the model of DNA substitution obtained from the Bayesian analyses (gt = 1.0, ct = 4.99, cg = 0.54, at = 1.26, ag = 2.82, ac = 1.71). Heuristic searches were used with 1000 random-addition replicates using MULPARS, TBR, and AMB options as implemented in PAUP* version 4.0b10 (Swofford, 2000). Branch support was estimated using bootstrap sam-

TABLE 2. Index of convergence of Bayesian phylogenetic analysis of *Ipomoea* subgenus *Quamoclit* using Markov chain Monte Carlo sampling for sets of five runs of varying length (in generations). *R* statistics were calculated for log likelihood of the phylogenetic model and tree length.

No. generations	<i>R</i> statistic	
	Log likelihood	Tree length
Three million	1.00108	1.00611
Five million	1.00023	1.00078
Seven million	1.00039	1.00214

pling with 1000 pseudoreplicates and 10 random-addition replicates with a full heuristic search.

RESULTS

Sequences—Fourteen ambiguous sites were removed from the final alignment of 698 nucleotide sites of the ITS data set of 54 taxa. Bayesian phylogenetic analyses were based on 280 site patterns and maximum parsimony analyses were based on 161 phylogenetically informative sites. There were no missing data. Sequences had a G/C bias of 0.61. The data exhibited a stationarity of base frequencies for all nucleotide sites ($P = 0.99$).

Bayesian phylogenetic analyses—Burn-in period—A graph of log likelihoods and tree lengths for each generation was used to identify the burn-in period. In a previous study, tree length was found to be one of the most variable parameters in Bayesian phylogenetic analyses (Miller et al., 2002). This was consistent with the analyses presented here. Log likelihoods reached a plateau at 10 000 generations, whereas tree lengths reached a plateau at 150 000 generations. Therefore, a burn-in period of 150 000 generations was used in the analyses.

Convergence—Sets of five analyses were used to evaluate convergence, running each set for 3, 5, and 7×10^6 (million) generations. The *R* statistic of Gelman et al. (1995) was close to one after three million generations for both likelihood values and tree length (Table 2). This index of convergence remained essentially unchanged over the range of runs examined here.

Among the set of three-million-generation analyses, the estimate of tree topology was slightly different for one analysis in comparison to the topologies of the other four analyses. Specifically, two clades united to form a weakly supported clade, a clade not found in the other 50% majority rule consensus trees. The estimate of tree topology converged on a single topology among the 5 five-million- and 5 seven-million-generation analyses.

Posterior clade probabilities were variable among the set of 5 three-million-generation analyses. Many of the nodes differed by as much as 6%, and three differed by over 30% (Fig. 1). Among the 5 five-million-generation analyses, the range of posterior probabilities were within 3% or less for all nodes. An additional set of 5 seven-million-generation analyses was obtained to ensure the narrow range of posterior clade probabilities detected among the set of five-million-generation runs was a consistent result. The range of posterior probabilities continued to be within 3% or less for all nodes for the latter set of analyses. Taken together, these results indicate that con-

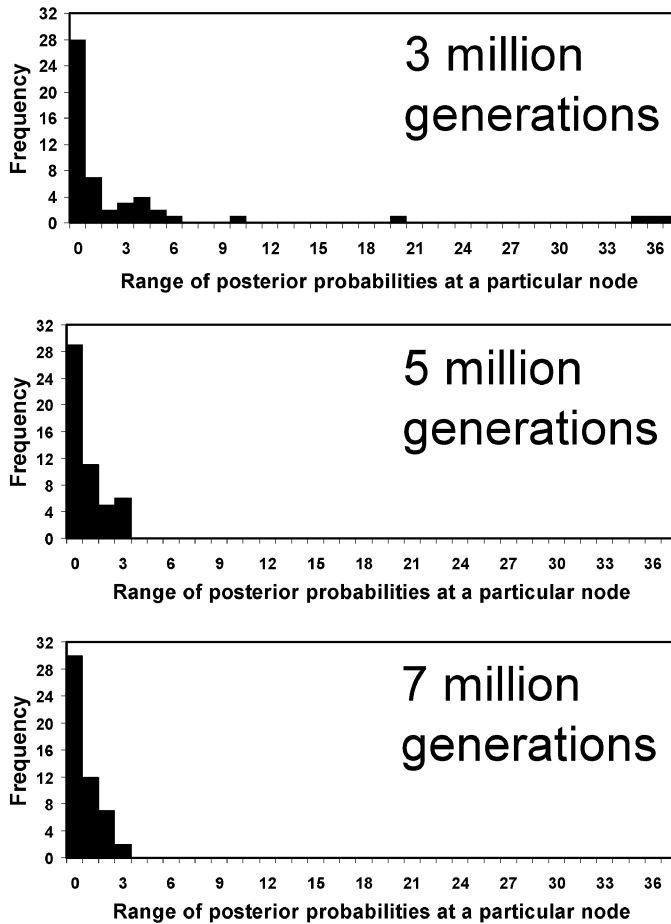


Fig. 1. The range of posterior clade probabilities for each node in the trees from sets of five runs for three million, five million, and seven million generations of Markov chain Monte Carlo sampling in a Bayesian phylogenetic analysis of ITS sequences for 36 *Ipomoea* species.

vergence had been met for these data by five million generations and certainly by seven million generations.

For the final analysis, the trees from 5 seven-million-generation analyses were concatenated together (after removing 1500 trees for each burn-in) to result in a sample of 342 500 trees. In the analysis of the 342 500 trees, 1238 taxon bipartitions were identified. This large number of taxon bipartitions can be explained in part by multiple accessions of the same species, as well as by many closely related species being included in the analyses.

Bayesian posterior clade probabilities—The 50% majority rule consensus tree of the 342 500 trees provided estimates of posterior clade probabilities. Well-supported nodes (e.g., posterior probabilities >95%) were found throughout the topology for the *Ipomoea* taxa (Fig. 2). Over 50% of the clades received 100% support. Furthermore, over 60% of the clades were found in 95% or greater of the sampled trees. There also were numerous nodes without strong support, demonstrating the preliminary nature of these molecular data for certain regions of the topology. Twenty-two percent of the clades received less than 60% support, with two clades receiving as little as 23% support, the latter including terminal taxa on short branches (Fig. 3).

Maximum parsimony analyses—Weighted parsimony analysis recovered 192 most-parsimonious trees with 498 steps. The strict consensus tree with bootstrap support values indicates the parsimony analysis also resulted in a combination of well-resolved clades and poorly resolved clades (Fig. 4). For example, the maximum parsimony analysis resulted in four polytomies, including a large polytomy with 11 branches.

Comparison of phylogenetic methods—The strict consensus tree obtained from the maximum parsimony analysis (Fig. 4) and the 50% majority rule consensus of the 342 500 trees obtained from the Bayesian analysis (Fig. 2) recovered essentially the same topology. The Bayesian analysis of these data resolved a more structured tree (fewer polytomies). Furthermore, nodes with posterior clade probabilities of less than approximately 95% support were not supported in the maximum parsimony strict consensus tree (represented by solid dots in Fig. 2).

Bayesian posterior probabilities vs. bootstrap support—In general, posterior probabilities were greater than bootstrap values (Figs. 2, 4). For one node of 22 for which bootstrap values differed from posterior clade probabilities, the bootstrap value was greater than the posterior probability. In contrast, for 21 of the same comparisons posterior clade probabilities were greater than bootstrap values. Both bootstrap support and posterior probabilities were 100% for 10 nodes. Bootstrap values ranged from 63 to 99%, while the corresponding posterior probabilities were 100% for 15 nodes. However, the relationship between these two measures of support is not merely a scaling difference, as indicated in Fig. 5.

Phylogenetic relationships—Two major clades with posterior clade probabilities of 100% were identified from the Bayesian analyses (Fig. 2). Clade 1 (clade 2A–1 of Miller et al., 1999) unites sections *Mina* and *Leptocallis*, which was found in all 342 500 trees sampled in the Bayesian analysis (Fig. 2). In contrast, this node collapses to a polytomy in the strict consensus of 192 most-parsimonious trees (Fig. 4). Clade 2 (clade 2A–2 of Miller et al., 1999) includes the species from section *Pharbitis* with species from sections *Calonyction* and *Tricolores*. This clade also was identified in the maximum parsimony analysis (Fig. 4).

Species of sections *Calonyction*, *Mina*, and *Leptocallis* are all identified as monophyletic groups with at least 95% support in the Bayesian analyses (Fig. 2). The results from the maximum parsimony analysis are consistent with these findings (Fig. 4). Three species of section *Tricolores* form a clade with 100% support in both analyses, although species *I. parasitica* of section *Tricolores* does not join this clade in the analysis (Figs. 2, 4).

Species of series *Pharbitis* and series *Heterophyllae*, both of section *Pharbitis*, form a well-supported monophyletic group in both the Bayesian analyses (100% support) and maximum parsimony analyses (83% support) (Figs. 2, 4). Species of series *Heterophyllae* are identified as a clade derived from within subgenus *Pharbitis*, with the addition of *I. purpurea* (series *Pharbitis*) as a member of the *Heterophyllae* clade. In contrast, the three species of section *Pharbitis* series *Tyrianthinae* do not form a distinct clade, nor are they sister to the other *Pharbitis* species.

The species of section *Exogonium* do not form a clade, but two species pairs are identified in both analyses; *I. dumetorum*

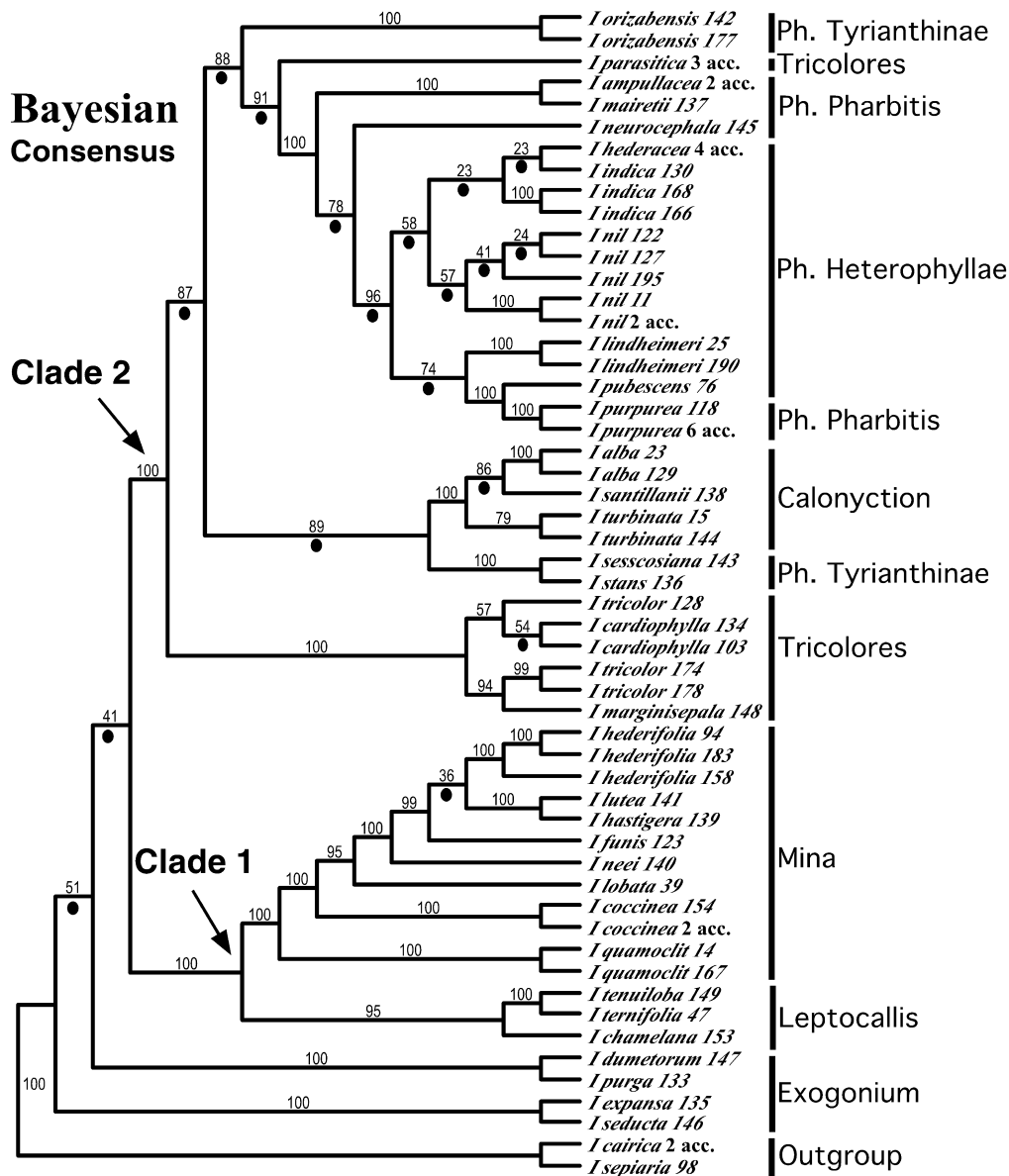


Fig. 2. Phylogenetic tree for 36 *Ipomoea* species based on ITS sequence data using a Bayesian analysis showing 50% majority rule consensus of 342,500 trees from Markov chain Monte Carlo sampling. Multiple accessions of the same species are identified by DNA number (see Table 2), except for taxa representing multiple accessions (acc.). Posterior clade probabilities (as percentages) are given above each node. Nodes not supported in the strict consensus in the maximum parsimony analysis (Fig. 4) are indicated with a solid circle. Abbreviation for section *Pharbitis* is Ph.

united with *I. purga*, and *I. expansa* united with *I. seducta* (Figs. 2, 4).

DISCUSSION

Phylogenetic relationships—Congruence was observed between our molecular results based on a ITS data set and classifications and treatments based on morphological analyses (McDonald, 1991; Austin and Huáman, 1996; Austin, 1997; Austin and Bianchini, 1998). These molecular results corroborate the results from previous molecular studies (Miller et al., 1999; Manos et al., 2001) and the systematic study of some of these same species based on chloroplast DNA RFLP variation (McDonald and Mabry, 1992). Congruence between these hypotheses and revisional treatments indicates that the

relationships developed in former studies may represent a classification that reflects evolutionary history. This is particularly true for species groups within *Ipomoea* that are easily recognized based on morphology, such as *Ipomoea* sections *Calonyction*, *Leptocallis*, *Mina*, and series *Pharbitis* and series *Heterophyllae* (section *Pharbitis*).

This study included taxa that were formerly aligned in two separate subgenera: *Ipomoea* and *Quamoclit* (Table 1). Support for these subgenera as distinct clades is not provided by the data. More specifically, species of section *Pharbitis* (subgenus *Ipomoea*) were nested within species of subgenus *Quamoclit*. This result was shown previously by Miller et al. (1999) with a broader sample of *Ipomoea* species for both ITS and *waxy* sequence data, as well as from a combined analysis. Wilkin (1999) also observed this same result based on a morpholog-

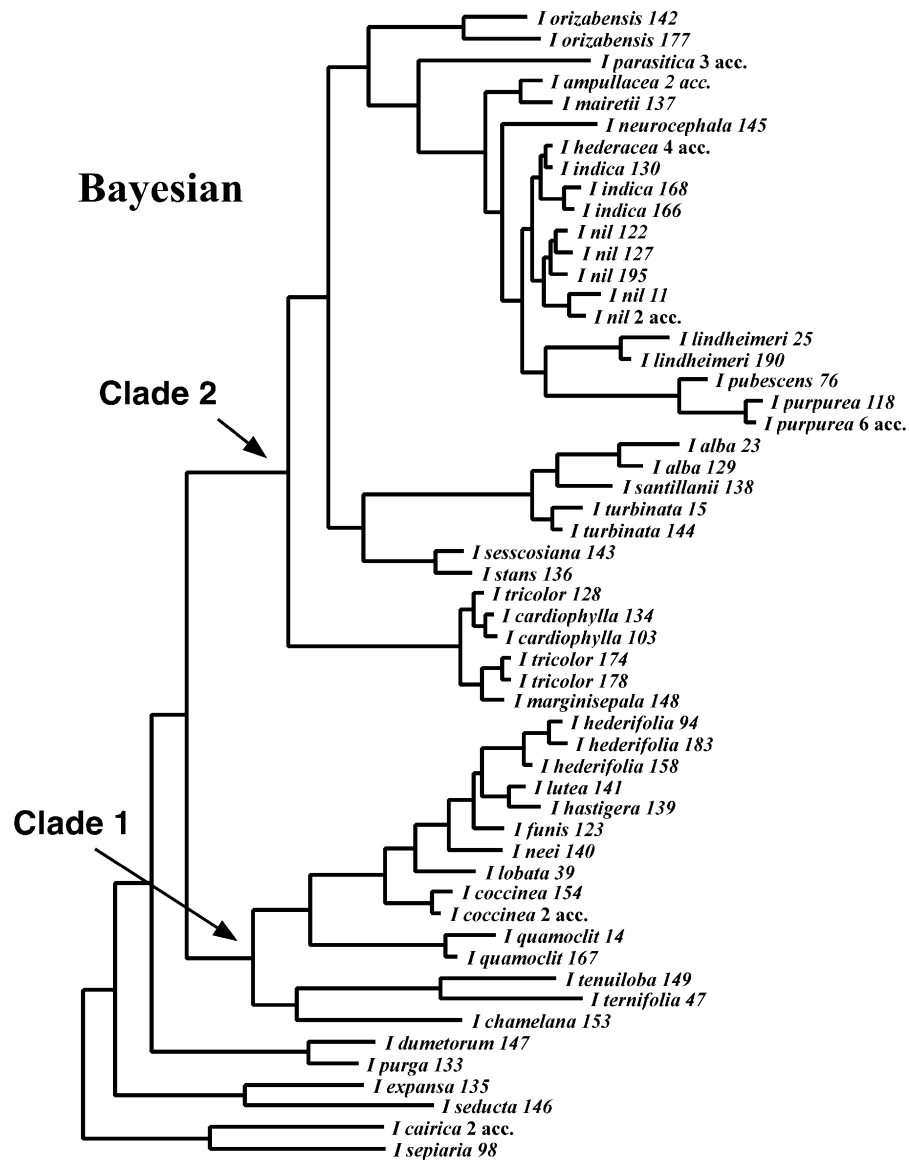


Fig. 3. Phylogenetic tree for 36 *Ipomoea* species based on ITS sequence data using a Bayesian analysis showing mean branch lengths. See Fig. 2 for additional details.

ical cladistic study of 142 *Ipomoea* species. The results of McDonald and Mabry (1992) do not support these two subgenera as distinct clades, but the specific nesting of *Pharbitis* species within subgenus *Quamoclit* was not indicated in their study. Within the *Quamoclit* group, two major clades were identified (Figs. 2, 3). The first clade (clade 1) includes sections *Mina* and *Leptocallis*. The second clade (clade 2) includes sections *Tricolores*, *Calonyction*, and *Pharbitis*. The *Quamoclit* group was identified as clade 2A in Miller et al. (1999); while clades 1 and 2 here were identified as clade 2A–1 and clade 2A–2, respectively, in the previous paper. Unfortunately, no obvious morphological features have been recognized that define the members of these two different groups.

Clade 1—Within clade 1, strong support was found for sections *Mina* and *Leptocallis* as monophyletic groups. Specifically, the *Mina* clade received 100% and 82% support and the *Leptocallis* clade received 95% and 75% support from Bayes-

ian and maximum parsimony analyses, respectively. Species of section *Mina* have long been recognized as a distinct group of morning glories and at times have received generic status (e.g., McPherson, 1979). These species can be clearly defined on the basis of various synapomorphic features: corollas ornithophilous; yellow-, orange-, or red-pigmented; tubes narrow (3–5 mm in diameter) the limb often flaring abruptly, style and stamens exserted, sepals bearing a single, fleshy subterminal appendage; and capsules four-locular (O'Donnell, 1959; McDonald, 1987, 1993). Species of section *Leptocallis* also usually possess a distinctive morphology: pedately dissected laminae (McDonald, 1995). Another important result from the analysis presented here was obtaining good resolution among the species within section *Mina*. Only one node of 10 was not well supported (i.e., one clade with 36% support). It is interesting to note that there are approximately 18 red-flowered species within the *Quamoclit* group (House, 1908a; MacBride, 1959; Kearney and Peebles, 1960; Matuda, 1963; Radford et

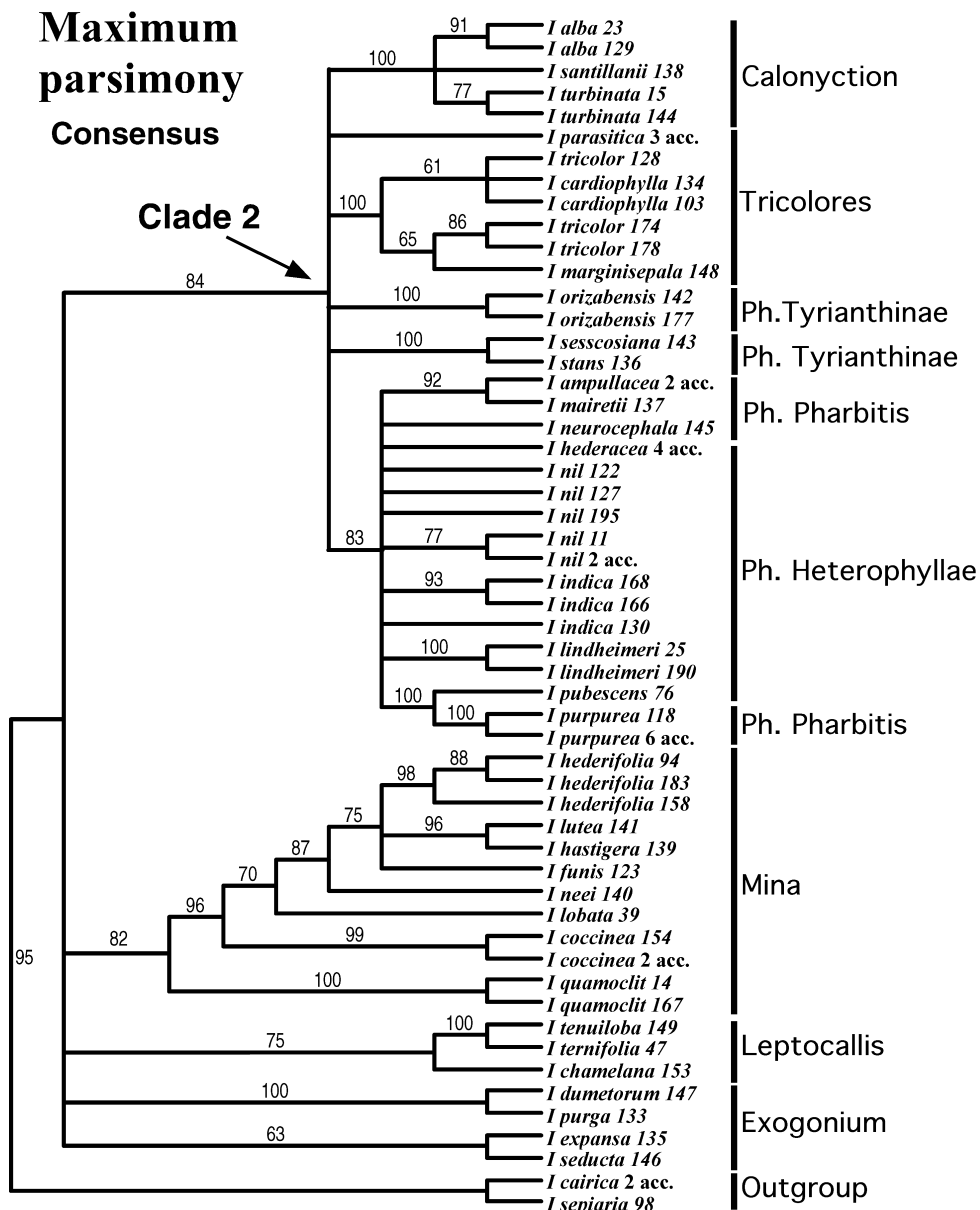


Fig. 4. Phylogenetic tree for 36 *Ipomoea* species based on ITS sequence data using a maximum parsimony analysis showing a strict consensus of 192 most-parsimonious trees. See Fig. 2 for additional details.

al., 1968; Standley and Williams, 1970; Adams, 1972; Gunn, 1972; Austin, 1975b, 1982; Correll and Johnston, 1979; McPherson, 1980; Austin and Cavalcante, 1982; McDonald, 1982, 1987, 1993, 1994, 1995; Eckenwalder, 1989; Wagner et al., 1990; Wilkin, 1995). Of these, 12 are members of section *Mina*. Zufall and Rausher (2004) recently demonstrated that red flowers among morning glory species is due to unique genetic changes in the anthocyanin biosynthetic pathway. Included in their study was the characterization of the genetic basis of red flowers in *Ipomoea quamoclit*, a member of section *Mina*. The results demonstrate that *I. quamoclit* produces pelargonidin-based anthocyanin pigments and that two genetic changes can account for red flowers in this species. One change is down regulation of transcription leading to decreased expression of one of the protein-coding genes, *flavonid*

3'-hydroxylase (f3'h). In addition, substrate specificity of dihydroflavonol reductase (DFR) for the precursor dihydrokaempferol leads to the production of pelargonidin-based anthocyanin pigments and also could account for red flowers in *I. quamoclit*. It is not clear, however, what the order of these two mutations was in the evolution of red flowers in this species. This line of investigation, coupled with the well-resolved relationships among the *Mina* species, could be used to determine whether the production of red flowers in section *Mina* is due to these specific mutations, as well as to determine the possible order of the origin of these mutations.

Clade 2—Within clade 2 100% support was observed for section *Calonyction* based on both methods of analysis (Bayesian and parsimony). McDonald and Mabry (1992) also

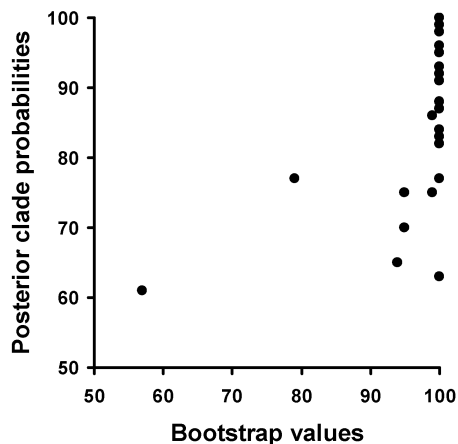


Fig. 5. Plot of posterior clade probabilities (as percentages) from the Bayesian analysis of 36 *Ipomoea* species and bootstrap values from the maximum parsimony analysis.

obtained support (80%) for the monophyly of these three species (*I. muricata* (L.) Jacq. = *I. turbinata* Lag.). The species of section *Calonyction* as presently defined are morphologically unique (Austin and Huáman, 1996; Austin, 1997). *Ipomoea alba* is typical of species in this taxon (Verdcourt, 1957; Austin, 1997), having a perennial habit, twining vines, stems armed with numerous herbaceous warts, sepals unequal bearing a long thick awn, fruit with greatly enlarged pedicels, corollas white, salverform, and fragrant, stigmas exserted, capsules two-celled, four-seeded, and seeds large (10–12 mm long) (Gunn, 1972; Austin, 1997). Within section *Calonyction*, *I. alba* and *I. santillanii* were found to be sister species, with *I. turbinata* sister to these taxa. Both *I. alba* and *I. santillanii* are white-flowered taxa, while *I. turbinata* is a smaller-flowered, autogamous species with pigmented flowers. Whether the white flowers of *I. alba* and *I. santillanii* represent independent loss-of-function mutations resulting in white flowers remains to be determined.

According to Austin (1997), *Ipomoea* section *Pharbitis* includes three series: *Heterophyllae*, *Pharbitis*, and *Tyrianthinae*. Section *Pharbitis* is distinguished on the basis of foliose, hispid sepals (McDonald and Mabry, 1992). While there is no support for the monophyly of all three series within section *Pharbitis*, there is strong support (100% from Bayesian analyses and 83% support from maximum parsimony analyses) for a clade composed of species that present a three-part gynoeceum—those of series *Pharbitis* and series *Heterophyllae* (section *Pharbitis*) (McPherson, 1979; Manos et al., 2001). Separation of species in series *Pharbitis* from those of series *Heterophyllae* is well supported in the Bayesian analyses with the *Heterophyllae* clade receiving 96% support. However, *Ipomoea purpurea*, traditionally placed within series *Pharbitis*, may belong to series *Heterophyllae*. The similarities between *I. purpurea* and species of series *Heterophyllae* have been noted previously (Austin et al., 2001), though considering this species a member of series *Heterophyllae* has not been suggested previously. Although this conclusion conflicts with cladistic analyses of section *Pharbitis* based on morphological criteria (Austin et al., 2001), we note that independent data using *waxy* sequences identifies a clade of series *Heterophyllae* species with 100% bootstrap support from a maximum parsimony

analysis that includes *I. purpurea* within this clade (Miller et al., 1999).

Ipomoea hederacea and *I. nil* are two annual species that may be regarded as variants of a singular species (Austin, 1975b). Morphological distinctions between these two species are based on subtle differences in sepal shape. From the analysis presented here, *I. hederacea* and *I. nil* appear to be closely related to *I. indica*. The resolution among these species is not clear based on these data. These species display a variety of mating systems: *I. hederacea* is largely a selfing species with almost no anther–stigma separation; *I. nil* is self-compatible with notable anther–stigma separation (and therefore probably has a mixed mating system); while *I. indica* is self-incompatible (Martin, 1970; R. Miller, Southeastern Louisiana University, unpublished data). Another set of closely related species with contrasting mating systems include *I. purpurea* and *I. pubescens*. *Ipomoea purpurea* is a self-compatible species with a mixed mating system (Rauscher and Fry, 1993), while *I. pubescens* appears to set seed in the bud (R. Miller, Southeastern Louisiana University, unpublished data). It also is noteworthy that the species of *Ipomoea* series *Tyrianthinae* (section *Pharbitis*) are not part of the clade that unites series *Pharbitis* and series *Heterophyllae*. In fact, species from series *Tyrianthinae* do not form a monophyletic group in our analysis. This is consistent with earlier findings by McDonald and Mabry (1992) and Austin and Huáman (1996), who note that series *Tyrianthinae* is a heterogeneous group of species (McDonald and Mabry, 1992). Nevertheless, McDonald (2001) recently regarded the *Tyrianthinae* complex as a monophyletic group based on morphological synapomorphies. These contrasting views of the relationships of these species within subgenus *Quamoclit* demands resolution with greater sampling of species of *Tyrianthinae*, as well as additional sources of phylogenetic data.

Three of the four species of section *Tricolores* included in this study form a well-supported clade. *Ipomoea tricolor* is a widely distributed species with relatively large flowers, while *I. cardiophylla* and *I. marginisepala* are small-flowered species (discussed in detail in McDonald, 1982). *Ipomoea tricolor* is a predominately outcrossing species, while *I. cardiophylla* and *I. marginisepala* are selfing species. Furthermore, *I. tricolor* is a widely distributed Mexican species, while *I. cardiophylla* is distributed in the northern Chihuahuan desert region and *I. marginisepala* is found in the Argentine desert. These two disjunct desert species may represent two independent origins of selfing species from a widespread species with a mixed mating system (McDonald, 1982), as suggested by the molecular phylogenetic results presented here. The fourth species of section *Tricolores* included in this study, *Ipomoea parasitica*, is not part of this clade. The Bayesian analysis suggested *I. orizabensis* (section *Pharbitis* series *Tyrianthinae*) is sister to *I. parasitica* (section *Tricolores*), which is then sister to other species of section *Pharbitis*, while the parsimony analysis showed *I. parasitica* as part of a polytomy. McDonald and Mabry (1992) obtained two different results for the placement of *I. parasitica* with chloroplast DNA RFLP data depending on the particular analysis used. In contrast, morphological evidence would suggest *I. parasitica* is closely related to species of either section *Tricolores* or section *Calonyction*. *Ipomoea parasitica* shares the tricolored corollas (blue throat, white limb, and yellow throat) of the other *Tricolores* species (McDonald, 1982) and the highly distinctive muricate stems of *Ipomoea* section *Calonyction*.

Taxa that fall outside of clade 1 or clade 2 include elements of *Ipomoea* section *Exogonium*. The species belonging to *Exogonium* have traditionally included morning glories with red flowers, salverform corollas, and exerted stamens and stigmas (House, 1908b). There has been a dramatic redefinition of which species should be included in the section (Austin, 1977; McDonald, 1987). The instability of this taxon points to the difficulty in morphologically defining the group. For example, the species Austin and Huáman (1996) indicate belong to subgenus *Quamoelit* section *Exogonium* were placed in three different species assemblages by McPherson (1979) (his Purga group, Thurberi group, and Tyrianthina group). Wilkin (1999) also found that section *Exogonium* was not monophyletic based on morphological data. According to our results, *Ipomoea* section *Exogonium* does not form a monophyletic group, but rather a basal grade relative to clades 1 and 2. Within *Exogonium*, two informal groups have been identified, the purgoid and suffultoid complexes (McDonald, 1987). There is no correspondence between these groups and the results presented here, supporting McDonald's (1987) statement that these morphological distinctions may best represent ecological adaptations rather than phylogenetic relationship. *Ipomoea seducta* and *I. expansa* were recognized as sister taxa in this study, a result consistent with McDonald and Mabry (1992). Section *Exogonium*, as it is currently defined, includes 20 species with striking floral diversity (McDonald, 1987). This includes species with various floral shapes as well as the complete spectrum of corolla colors found within morning glories. Well-targeted sampling within this group could easily lay the foundation for studies of the evolution of floral form (e.g., Armbruster et al., 1994, 2002; Goldblatt et al., 1995; Barrett et al., 1996).

Bayesian phylogenetic analyses—The application of Bayesian analyses to phylogenetic studies is still being developed (Huelsenbeck et al., 2001, 2002; Lewis, 2001). Important progress has been made in the application of this method to phylogenetic and evolutionary studies (e.g., Huelsenbeck et al., 2000; Buckley et al., 2002; Huelsenbeck and Imennov, 2002; Leache and Reeder, 2002; Miller et al., 2002), but outstanding questions remain. One parameter to estimate in carrying out a Bayesian phylogenetic analysis is the burn-in period. This is the initial phase of MCMC sampling between the random starting point and when the sampling is within the stationary distribution of tree space. A widely used approach to determine the burn-in period is to plot likelihood values vs. generations of the search and determine when the likelihood value reaches a plateau and does not increase (e.g., Huelsenbeck and Bollback, 2001; Huelsenbeck and Imennov, 2002; Leache and Reeder, 2002). Results from this study suggest that monitoring tree length provides a more conservative estimate of the burn-in period than monitoring likelihood values. Specifically, in an earlier Bayesian phylogenetic analysis, tree length was one of the most variable parameters in the phylogenetic model (Miller et al., 2002). In the analysis presented here, this was confirmed where an order of magnitude difference in the burn-in period is suggested by these different parameters (10 000 generations for likelihood values vs. 150 000 generations for tree length).

Convergence of Markov chain Monte Carlo—A difficult question to address in a Bayesian phylogenetic analysis is determining how many generations to run the analysis (Lewis,

2001; Huelsenbeck et al., 2002; Miller et al., 2002). This is a different question from establishing the burn-in period and relates to obtaining estimates of all the parameters in the phylogenetic model that are sufficiently close to the true estimates. Comparisons among Bayesian posterior probabilities for individual clades from different analyses have been used to establish whether or not an adequate sample has been obtained or whether results are consistent among separate analyses. To evaluate this question, a simple approach is to plot the posterior clade probabilities of one analysis against another and look for consistency between the results (e.g., Huelsenbeck et al., 2001; Huelsenbeck and Imennov, 2002; Leache and Reeder, 2002). An alternative to this approach requires plotting the correlation between posterior probabilities for pairs of analyses with increasing generations of MCMC sampling and looking for a plateau in the correlation estimates (Miller et al., 2002). However, it is important to remember that the objective of evaluating convergence is to obtain consistent results among sets of analyses. The index of convergence proposed here, a narrow range of values among sets of five separate analyses, is perhaps the most conservative approach proposed to date. This method provides assurance that the final analysis has been run for an adequate length of time for the results to provide a reliable representation of the parameter estimates. Furthermore, applying the criteria of accepting a narrow range of values among sets of analyses would result in a plot of the posterior probabilities of clade support of one analysis against another that would be essentially a straight line (e.g., $r = 0.9996$ using the results presented here for two 7-million generation runs). For the data presented here, a MCMC sampling of five million generations was required to obtain consistent posterior clade probabilities (a range of 3% for all nodes). We included an additional set of longer seven-million generation analyses. This additional set of runs was informative, but if further studies demonstrate that a narrow range of posterior clade probabilities provides a robust indicator of convergence, then the additional seven-million runs would not be necessary. It also is noteworthy that we would have been content with three million generations if we had used Gelman's *R* statistic as applied here as an indicator of convergence, with the potential of reporting variable and possibly misleading estimates of posterior clade probabilities.

Comparison of phylogenetic methods—Phylogenetic analysis of the ITS region involving 54 samples of 36 *Ipomoea* species resulted in a phylogenetic hypothesis that included both well-resolved clades and clades with weak support (Figs. 2, 4). For some taxa, these results provide an excellent statement of relationships, although particular regions of the tree are likely to require additional phylogenetic data to develop a complete well-resolved hypothesis. This combination of well-resolved and poorly-resolved relationships is most apparent in the results produced by the maximum parsimony analysis, as indicated by numerous clades with support well over 70%, as well as recovering four polytomies (Fig. 4). Two of the four polytomies include closely related species that have only a few base-pair differences among the ITS sequences (e.g., *Ipomoea hederacea*, *I. nil*, *I. indica* and *I. tricolor*, *I. cardiophylla*, *I. marginispala*) (Fig. 3). Therefore, part of the varying resolution within the cladograms stems from the different levels of sampling within *Ipomoea* subgenus *Quamoelit*.

Comparing the different levels of support for particular nodes of the Bayesian analyses and the parsimony analyses highlights

differences between measures of support provided by posterior clade probabilities (Fig. 2) and bootstrap values (Fig. 4). Discussion of these two measures of node support is a burning issue in the systematics literature (e.g., Huelsenbeck et al., 2002; Suzuki et al., 2002; Wilcox et al., 2002; Alfaro et al., 2003; Cummings et al., 2003; Douady et al., 2003; Holder and Lewis, 2003). A general trend is that posterior clade probabilities are usually higher than bootstrap values (e.g., Leache and Reeder, 2002; Miller et al., 2002; Soltis et al., 2002). Simulation studies generally support the accuracy of posterior probabilities (Wilcox et al., 2002; Alfaro et al., 2003), although tendencies toward over-credibility of posterior clade probabilities have been identified (Suzuki et al., 2002; Alfaro et al., 2003; Cummings et al., 2003; Douady et al., 2003). It is important to recognize that bootstrap values and Bayesian posterior probabilities of node support measure two different processes (Alfaro et al., 2003). Bayesian posterior probabilities determine the strength of the data in supporting particular nodes, whereas bootstrap values indicate areas where additional data is needed to resolve relationships. Therefore, one should not expect these measures of support to be equal in value.

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