

## PHYLOGENY OF AMORPHEAE (FABACEAE: PAPILIONOIDEAE)<sup>1</sup>

MICHELLE MCMAHON<sup>2</sup> AND LARRY HUFFORD

School of Biological Sciences, Washington State University, Pullman, Washington 99164-4236 USA

The legume tribe Amorpheae comprises eight genera and 240 species with variable floral form. In this study, we inferred a phylogeny for Amorpheae using DNA sequence data from the plastid *trnK* intron, including *matK*, and the nuclear ribosomal ITS1, 5.8S, and ITS2. Our data resulted in a well-resolved phylogeny in which the tribe is divided into the daleoids (*Dalea*, *Marina*, and *Psorothamnus*), characterized by generally papilionaceous corollas, and the amorphoids (*Amorpha*, *Apoplanesia*, *Errazurizia*, *Eysenhardtia*, and *Parryella*), characterized by non-papilionaceous flowers. We found evidence for the paraphyly of *Psorothamnus* and for the monophyly of *Dalea* once *D. filiciformis* is transferred to monophyletic *Marina*. *Errazurizia rotundata* is more closely related to *Amorpha* than to the other errazurizias, and *Eysenhardtia* is supported to be monophyletic. The monotypic *Parryella* and *Apoplanesia* are placed within the amorphoids. Among Papilionoideae, *trnK/matK* sequence data provide strong evidence for the monophyly of Amorpheae and place Amorpheae as sister to the recently discovered dalbergioid clade.

**Key words:** Amorpheae; Fabaceae; floral evolution; ITS; Papilionoideae; phylogeny; *trnK/matK*.

The New World papilionoid tribe Amorpheae Borissova emend. Barneby comprise eight genera (approximately 240 species) and encompasses many variations on the pea flower, including variation in fusion among parts and numbers and positions of parts. Floral morphological variation has influenced intra-tribal taxonomy, but neither the morphology nor the taxonomy have been examined phylogenetically. In this study, we infer a phylogeny and discuss its relationship to traditional tribal taxonomy, based in part on floral form.

Floral diversity in Amorpheae is centered on the corolla and androecium. Among species, corollar variation includes differences in number (no petals, one petal, five petals), in fusion between petals, and in differentiation between petals. Androecia also vary in number and fusion of parts. Further variation includes the presence, in some species, of a unique organ arrangement such that the petals appear perched on the staminal column.

Understanding how these forms have evolved requires understanding relationships among lineages in Amorpheae. As a natural group, Amorpheae have only been recognized since Barneby's monograph of four of the genera (Barneby, 1977). Barneby presented an evolutionary hypothesis for the tribe based largely on characters assumed to be primitive, such as woody habit, chromosome number, and inflorescence structure. This attempt was the first and only to characterize rela-

tionships among the genera, but it is not explicitly phylogenetic. Understanding this diversification will also require evaluation of relationships among Amorpheae and other papilionoids. Inferring monophyly for Amorpheae and identifying its near relatives will provide valuable information on the ancestral Amorpheae floral form. Additionally, understanding the evolution of the various Amorpheae floral forms in the context of the papilionoid flower requires an evaluation of the position of Amorpheae in the legume family.

Several recent studies have addressed papilionoid phylogeny; some of these have included exemplar taxa from Amorpheae. Pennington et al. (2001) used data from the chloroplast *trnL* (UAA) intron to study relationships among "basal" papilionoids. Two taxa from Amorpheae were included and formed a sister pair with strong support (100% bootstrap value). Unfortunately, resolution among clades was poor; the Amorpheae clade formed part of a large polytomy that included all papilionoid taxa known or inferred to have a 50-kilobase (kb) inversion in the chloroplast (Pennington et al., 2001). A single taxon from Amorpheae, *Amorpha fruticosa*, was included in phylogenetic analyses using the chloroplast gene *rbcL* (Doyle, 1995; Doyle et al., 1997; Kajita et al., 2001). The most recent and most taxon-rich analysis using this gene (Kajita et al., 2001) places *Amorpha* in a large polytomy but with 0% bootstrap support. Previously published results using *rbcL* (Doyle et al., 1997) indicate *Amorpha* is sister to a clade containing Aeschynomeneae, Dalbergieae, and Adesmieae (together comprising the dalbergioid clade), but the support for this placement was also extremely weak (<50% jackknife support). In the study of Lavin et al. (2001), data from the chloroplast *trnK* intron (including the *matK* gene), alone and in combination with nonmolecular data, and data from the *trnL* intron indicate strong support for Amorpheae as sister to the dalbergioids (bootstrap values of 92% for *trnK/matK*, 92% for *trnK/matK* + nonmolecular, and 79% for *trnL*). However, the focus of the study was relationships among the Dalbergieae, Adesmieae, and Aeschynomeneae. Nine papilionoid outgroup sequences were selected from among hundreds, based on their relevance to the dalbergioids, but the placement of Amorpheae was not specifically addressed. In a study of the tribe Millettieae (Hu et al., 2000), species that may be

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<sup>2</sup> Present address: Evolution and Ecology, University of California, Davis, CA 95616 USA (e-mail: mmmcmahon@ucdavis.edu).

closely related to Amorpheae were sequenced for *trnK/matK* but no Amorpheae were included in their published results.

The goals of this study are to test for the monophyly of Amorpheae, to address the placement of Amorpheae in the phylogeny of the Papilionoideae, to infer phylogenetic relationships among Amorpheae, and to discuss floral evolution in the tribe.

## MATERIALS AND METHODS

**DNA sequence data**—The chloroplast *trnK* intron, including the *matK* gene, has been shown to be informative among papilionoid genera, tribes, and, in some cases, among species (Hu et al., 2000; Lavin et al., 2001). To complement the chloroplast DNA data, we selected the internal transcribed region of the nuclear ribosomal genes, which has also been shown to be informative among species in papilionoids (Wojciechowski et al., 1993, 1999).

Tissue was removed from plants in the field and dehydrated with silica gel or from dried herbarium specimens. Total DNA was isolated using a standard cetyltrimethylammonium bromide (CTAB) protocol (Doyle and Doyle, 1987). The *trnK/matK* region was amplified using the primers trnK3914F and psbAR or trnK2R and sequenced using at least six of the following primers: trnK3914F, matK710F, matK1470R, trnK2R, trnK290R, matK820R, matK840R, matK1221F, matK2025F, matK2340R (Appendix Table 1 for sequences and citations in Supplemental Data accompanying the online version of this article). Polymerase chain reaction (PCR) was conducted using the following profile: 4 min at 94°C, 35 cycles of 1 min at 94°C, 1 min at 44–48°C, and 3 min at 72°C, followed by a final extension of 5 min at 72°C. The internal transcribed spacer (ITS) region was amplified and sequenced using the primers Nnc18s10 and C26A using a “touchdown” profile: 4 min at 94°C, 5 cycles of 1 min at 94°C, 1 min at 52°C, and 2 min at 72°C, decreasing the annealing temperature by 1° each cycle, followed by 30 cycles using a 48°C annealing temperature, and ending with a final extension of 5 min at 72°C. Cycle sequencing reactions were exposed to 4 min at 94°C, 30 cycles of 1 min at 94°C, 1 min at 44°C (*trnK/matK*) or 50°C (ITS), and 2 min at 60°C.

Additional sequences outside of Amorpheae were acquired from GenBank and an aligned data set from M. Lavin (web site: <http://gemini.ocsc.montana.edu/~mlavin/data/dalbdad.htm>). GenBank accession numbers for all outgroups are listed in Hu et al. (2000) or Lavin et al. (2001).

**Taxon sampling**—Within Amorpheae, taxa were chosen for inclusion in the study using several related criteria. Using taxonomy as a guide, we selected species from all eight genera (Appendix Table 2 for voucher specimen information in Supplemental Data accompanying the online version of this article) and attempted to include representatives of subgenera when possible. A second criterion was our initial estimate of the phylogeny, based on preliminary DNA sequence data (McMahon and Hufford, 2000) and morphological data (Barneby, 1977). We used this information to increase sampling density for those genera suspected of being non-monophyletic (e.g., *Psorothamnus*). We also used our preliminary analyses to attempt to span the root of subclades or break up long branches (Graybeal, 1998). For example, if a single taxon was attached to a relatively deep node in our preliminary analyses, we attempted to include putative near-relatives of that taxon, particularly in the larger genera *Dalea* and *Marina*. A third criterion was to include all the major variations in floral morphology in the tribe. Our final analysis was conducted on 54 taxa from Amorpheae (13 of these contain only ITS sequences).

Taxa outside Amorpheae were included based on their position in published phylogenies. Amorpheae is nested in the “50 kb inversion clade” according to data from *rbcl* (Doyle, 1995) and the *trnL* intron (Pennington et al., 2001). We included all published *trnK/matK* sequences for papilionoid taxa outside the “50 kb inversion” clade and several published sequences from within the clade, concentrating on the dalbergioids (Lavin et al., 2001) and sampling more sparsely from tribes included in the *trnK/matK* study of Milletieae (Hu et al., 2000). Two different sets of outgroups were used. For our analysis in which we investigated the position of Amorpheae, we used 70 outgroups (with

a subset of the ingroup). For our analysis of ingroup relationships, we reduced the outgroups to four.

**Phylogenetic analyses**—Sequences were aligned manually using Se-AL (Rambaut, 1995). Regions that were difficult to align (all in noncoding regions, generally involving indels of varying lengths) were excluded from the analysis and gaps were treated as missing data. To evaluate sensitivity to alignment, sequences (all ingroup and four selected outgroups) were also aligned using Clustal X (Jeanmougin et al., 1998) with a variety of gap-opening costs (from five to 25), a gap extension cost of five, and transitions weighted equally with transversions. Parsimony searches were conducted using PAUP\* (Swofford, 2000) using 100 random addition sequence starting trees and tree bisection-reconnection (TBR) branch swapping. Bootstrap analyses (Felsenstein, 1985) were conducted to study support for clades. Resampling was repeated 500 times; for each data set, two random addition starting trees were swapped using tree bisection-reconnection, holding a maximum of 1000 trees within each replicate. For maximum-likelihood analyses, we first compared models of evolution using Modeltest (Posada and Crandall, 1998). We used the results of this algorithm in two ways. First, we allowed all parameters in the selected model, GTR+G+I, to be optimized; this search continued until 5000 rearrangements had been considered. Second, to allow a more thorough tree search, we used the parameter values estimated by Modeltest; this search ran to completion. For both maximum likelihood (ML) analyses, we reduced the data set to 38 taxa (from 58 for ITS and 44 for *trnK*), eliminating taxa that differed from another taxon by only a few autapomorphic changes.

To estimate the placement of Amorpheae in the Papilionoideae, we used 70 taxa outside and 20 taxa from within Amorpheae and conducted parsimony searches as above. We further explored the data in the following ways: First, in order to ensure that we were exploring treespace thoroughly and were not hampered by the large number of taxa, we reduced the taxa to 81 (from an original total of 90) by trimming taxa from each identified subclade. We also reduced it to 39, following the same procedure. To investigate the effect of codon, we downweighted third position sites (Swofford et al., 1996), and to evaluate sensitivity to transition bias, we applied a step matrix that made transversions twice as costly as transitions. To each, we applied the parsimony search parameters described.

Based on the results from our papilionoid analysis, we were confident that Amorpheae are monophyletic and that the number and selection of outgroups does not affect the rooting of Amorpheae. Therefore, to improve the efficiency of our searches within Amorpheae, we reduced the outgroups to four, and we included all Amorpheae sampled, 41 for *trnK/matK* and 54 for ITS/5.8S. We analyzed each data set separately and in combination, and we combined data sets using two methods. First, we included only the 40 ingroup taxa for which we had both genes fully sequenced. Second, we included all 55 Amorpheae taxa for which we had ITS or *trnK/matK*, or both, assigning missing data where needed.

Character evolution was studied using parsimony as implemented in MacClade 4.0 (Maddison and Maddison, 2000).

## RESULTS

**Placement of Amorpheae**—Alignment of the *trnK* intron, including the *matK* gene, resulted in a matrix of 90 taxa by 3318 base pairs (bp). Of these, 1114 were in the 5′ and 585 were in the 3′ ends of the intron, where unequal sequence termination and highly variable sequences proved difficult to align with certainty across the range of taxa. The *matK* gene provided 1619 aligned bp, of which 752 were parsimony informative for the 90 taxa.

Using only the *matK* gene, our search resulted in 792 most parsimonious trees of length 3346 on one island (Fig. 1). Amorpheae is strongly supported to be monophyletic, with a bootstrap value of 100%. In all of the most parsimonious trees, Amorpheae is placed as sister to the recently recognized dal-

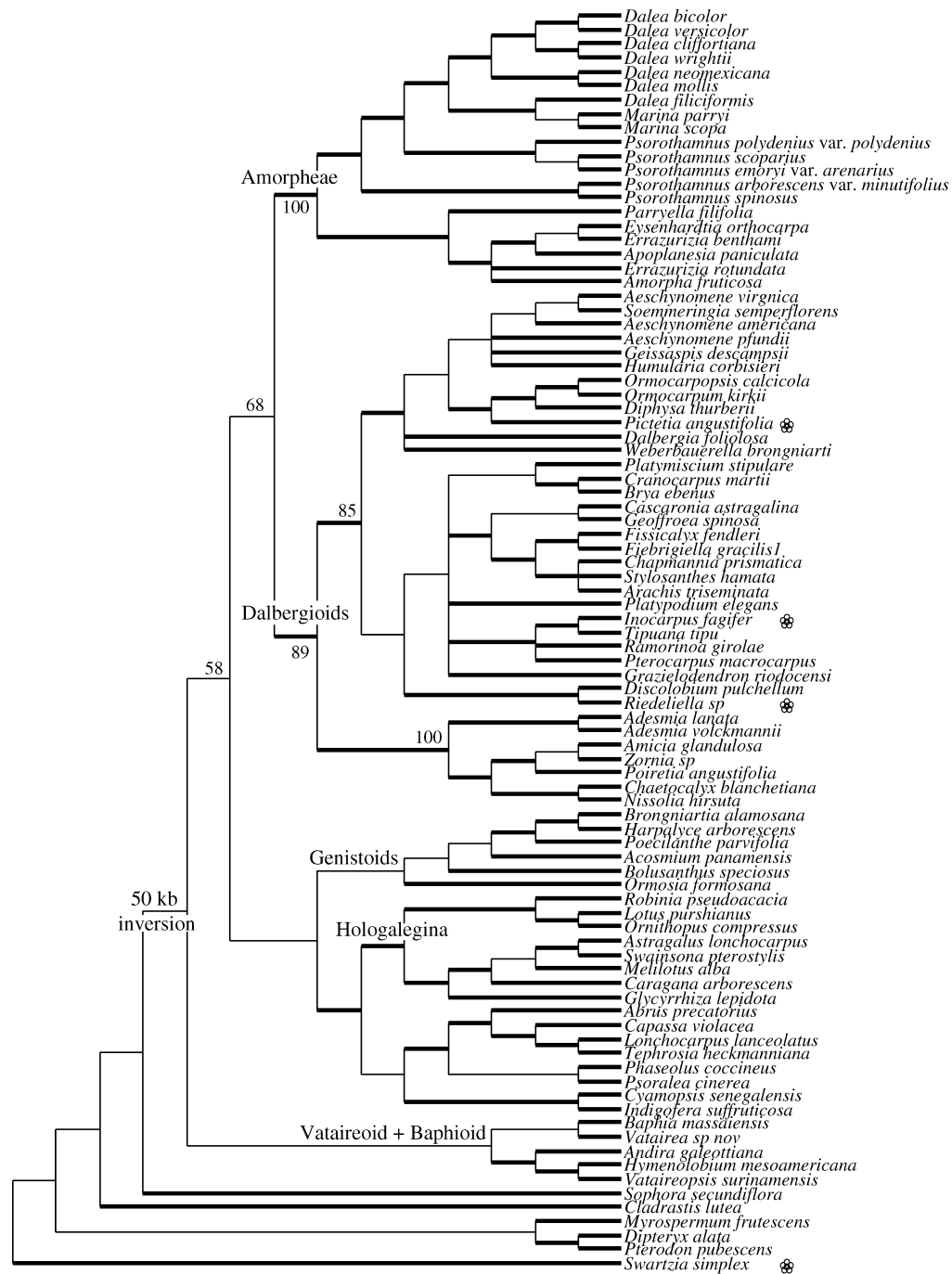


Fig. 1. Phylogeny of 20 Amorpheae and 70 outgroup Papilionoideae based on DNA sequence data from *matK*. The tree is a strict consensus of 792 most parsimonious trees, rooted using *Swartzia simplex*. Bootstrap values for the nodes relevant to placement of the Amorpheae are given, and branches indicated with narrow lines have bootstrap values <80%. Clades named in recent papilionoid phylogenetic analyses are labeled. Amorpheae is strongly supported to be monophyletic but is placed with weak support as sister to the dalbergioid clade. Taxa with flower symbols are those outside of Amorpheae that have been reported to have nonpapilionaceous flowers.

bergioid clade (Lavin et al., 2001). However, bootstrap support for this placement is weak. All branches ancestral to the node uniting Amorpheae and dalbergioids are also poorly supported by the data. When we downsized the taxon set, downweighted the third positions, or applied a step matrix weighting transversions more heavily than transitions (2 : 1), our main results did not change. In no case did we recover trees that placed

Amorpheae sister to any other group, and in all cases the dalbergioids and the Amorpheae were each strongly supported to be monophyletic.

**Phylogeny of Amorpheae**—We used 2162 characters from the 5' end of the intron and the *matK* gene, excluding the many gaps necessary to align the Amorpheae to the other pap-

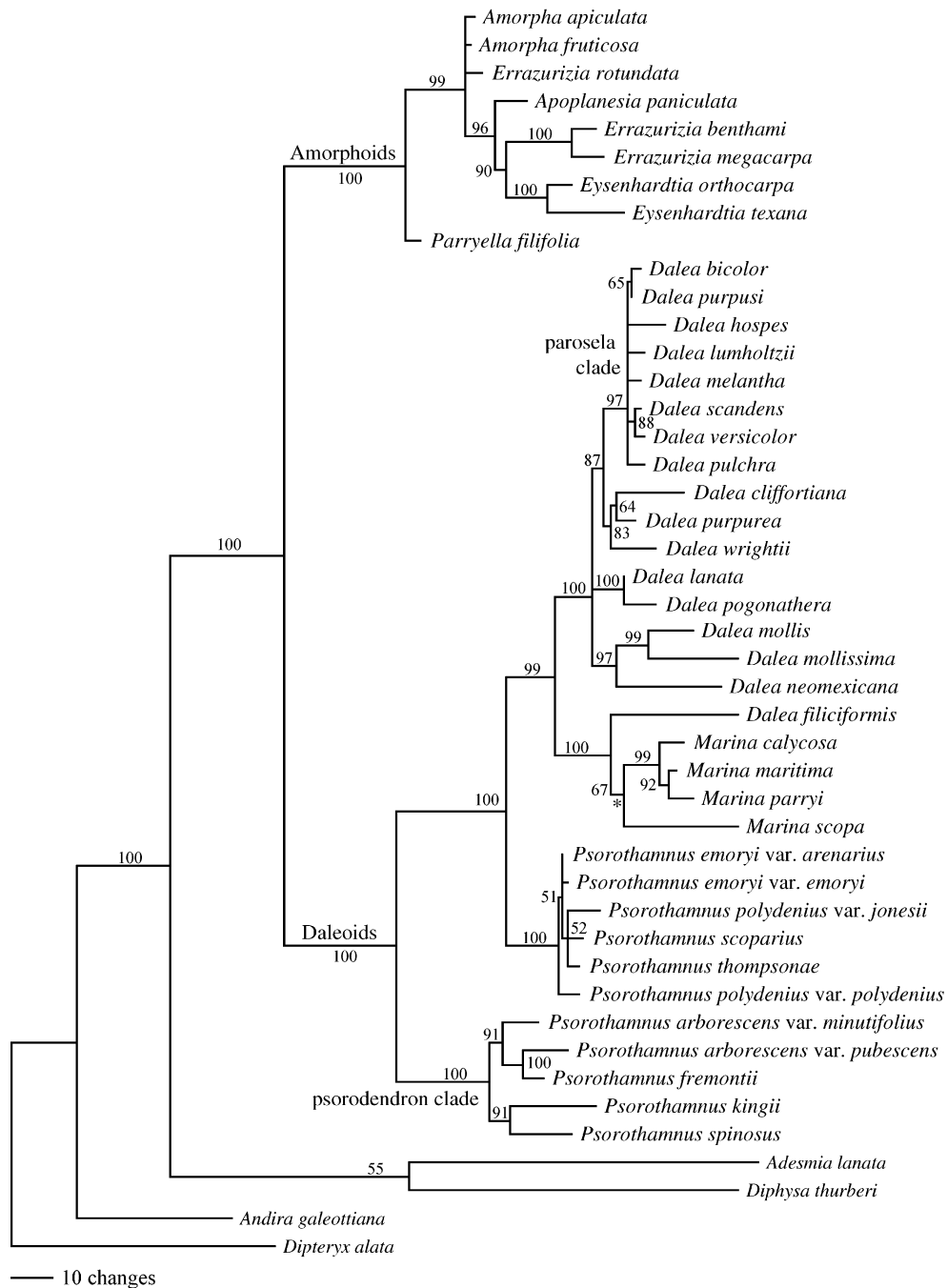


Fig. 2. Phylogram indicating approximate branch lengths in a strict consensus of 112 most parsimonious trees obtained using the 5' end of the *trnK* intron and the *matK* gene. Bootstrap values higher than 50% are given, and clades that are discussed in the text are labeled. Likelihood analysis produced an identical topology except that a zero-length branch was reconstructed at the base of *Marina* (at asterisk). Outgroups are indicated with a smaller font.

ilionoids and the difficult-to-align 3' end of the *trnK* intron. Of these 2162 characters, 328 were parsimony-informative for the 45 included taxa. Our parsimony search using *trnK/matK* found 112 trees of length 1006 on one island (Fig. 2). Likelihood analysis resulted in a topology nearly identical to the strict consensus of the parsimony trees (one branch that is poorly supported by parsimony bootstrap was found to be of zero length and collapsed by the ML analysis, Fig. 2). Alternative alignments created by Clustal X (Jeanmougin et al., 1998) resulted in the same strict consensus trees. Many of the

deep nodes are strongly supported by the data, as indicated by high parsimony bootstrap values. The high bootstrap values, in conjunction with the reconstructed branch lengths (Fig. 2) indicate that there are many synapomorphic changes for several clades. The monophyly of Amorpheae is again supported. The placement of the root is also strongly supported, as indicated by the bootstrap values of 100 for the clades on either side of the root. We will refer to these two clades as amorphoid and daleoid for ease of discussion. Within the amorphoids, sequence divergence provides data to resolve several clades,



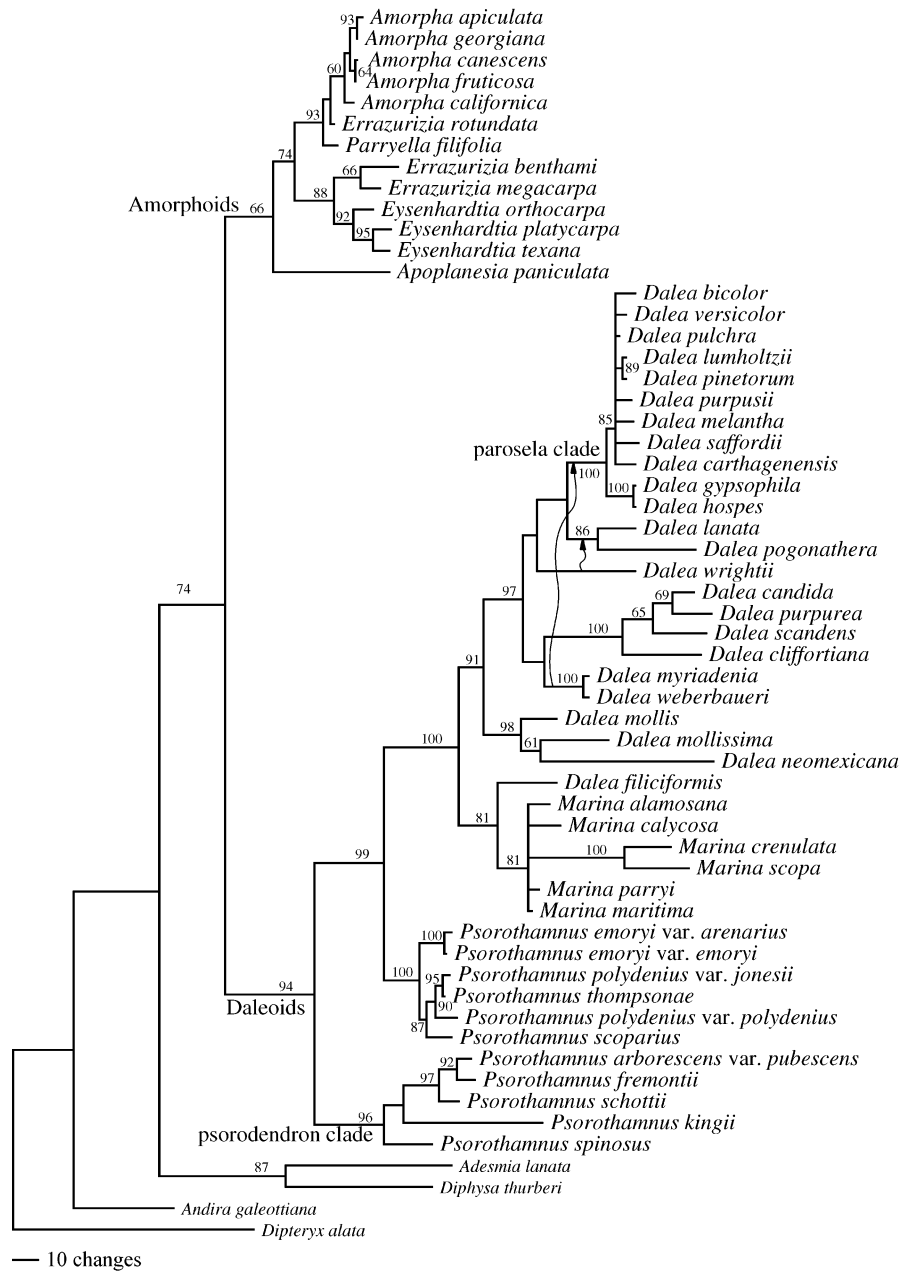


Fig. 3. Phylogram indicating approximate branch lengths in a strict consensus of 144 most parsimonious trees obtained using ITS/5.8S. Bootstrap values higher than 50% are given, and clades discussed in the text are labeled. Likelihood analysis produced a similar topology; exceptions are the placements of two branches within *Dalea*, indicated by curving lines. Outgroups are indicated with a smaller font.

except for the amorphas and *Errazurizia rotundata*. Within the daleoids, the data strongly support the paraphyly of *Psorothamnus* and the inclusion of *Dalea filiciformis* in the *Marina* clade. Some further resolution within these clades is achieved, but among some species of *Psorothamnus* and among many species of *Dalea* sequence divergence is minimal, resulting in short internodes and no support for resolution within these clades.

Sequences from ITS1, the 5.8S gene, and ITS2 resulted in 799 aligned base pairs, of which 658 were unambiguously aligned and used in the analysis. Of these, 329 were parsimony-informative for our data set of 54 Amorpheae and four outgroups. Because of the presence of many insertions and

deletions in the noncoding regions of these sequences, the alignments produced by Clustal X varied across the set of gap insertion costs that we used. However, we found that the alignments (and the trees inferred when using the alignments) were extremely sensitive to the taxon-addition sequence used to derive the alignment program's starting dendrogram. Therefore, we chose a more conservative approach and removed those sites for which we were unsure of homology. Our parsimony search resulted in 144 most parsimonious trees of length 1641 in one island (Fig. 3). Our likelihood search resulted in a topology that was very similar to the strict consensus of the most parsimonious trees, except for the placement of a few daleas (Fig. 3). This sequence region provided strongly supported res-

olution among many lineages. Poor resolution was obtained in the clade of daleas that contains most of the sampled members of the subgenus *Parosela* (labeled “parosela clade”; Fig. 3) as a result of very short internodes; i.e., ITS is not sufficiently variable to resolve relationships within this clade, perhaps indicating that the clade is too young for ITS to have accumulated many changes. The branches leading to *Amorpheae* and to the amorphoid clade, however, have significant branch lengths but low bootstrap support, indicating that the data are variable, but that there is conflict among the characters.

The consensus trees from ITS1/5.8S/ITS2 (Fig. 3) and from *trnK/matK* (Fig. 2) differ in resolution and in support, but their topologies are consistent with one exception: the relationships among the amorphoids. The ITS data weakly support *Apoplanesia paniculata* as sister to the rest, with *Parryella filifolia* as sister to *Errazurizia rotundata* + *Amorpha*. The *trnK/matK* data strongly support *Parryella filifolia* as sister to the rest, with *Apoplanesia* as sister to *Eysenhardtia* + the other two *errazurizias*.

Combining data sets and including only those taxa for which we had both gene regions sequenced, we found 62 most parsimonious trees of length 2544 on one island. When we included the 55 *Amorpheae* taxa and the four outgroups, assigning missing data where needed, we found 2016 trees of length 2675 on one island. The consensus tree from the smaller taxon set, i.e., only those taxa for which we have both genes sequenced, is a subtree of the consensus using the larger taxon set (Fig. 4), with one exception (*Dalea wrightii* is placed as sister to *D. lanata* + *D. pogonathera*; however, neither this placement nor the one obtained with all taxa is supported strongly by the data). Likelihood analysis of the smaller data set produced nearly the same tree as parsimony, but *Errazurizia rotundata* attaches closer to the root of the amorphoids (Fig. 4). The parsimony bootstrap values are nearly identical for trees inferred from the two taxon sets, implying that inclusion of the taxa for which we had only one gene did not impact inferences of support.

Combining *trnK/matK* and ITS results in a well-resolved tree in which support is generally higher than in either data set alone. The exception to this, as expected, is the base of the amorphoid clade, in which weak support and conflicting synapomorphies combine to produce no resolution.

## DISCUSSION

**Monophyly and phylogenetic placement of *Amorpheae***—Prior to the monograph by Barneby (1977), members of *Amorpheae* belonged to Daleae Hutch. (characterized by four lateral petals raised above the rim of the hypanthium) and to Psoraleae Benth. emend. Hutch. (characterized by petals inserted on the hypanthial rim) (Hutchinson, 1964). Barneby united the group based on the presence of several synapomorphies: epidermal glands throughout the plant body; dry, indehiscent fruits that are single-seeded; and terminal inflorescences. This last character, along with leaves that are mostly pinnate, separates the tribe from the mostly trifoliolate Psoraleae sensu stricto, now understood to be distantly related (Barneby, 1977; Stirton, 1981; Turner, 1986; Doyle et al., 1997).

The monophyly of *Amorpheae* is strongly supported by the *trnK/matK* sequence data, confirming Barneby’s hypothesis of *Amorpheae* as a natural group. This result is also consistent with previous molecular studies in which multiple taxa from *Amorpheae* were included (Hu et al., 2000; Lavin et al., 2001;

Pennington et al., 2001). The placement of *Amorpheae*, however, is not as strongly supported. The data indicate a sister relationship between *Amorpheae* and the recently circumscribed dalbergioid clade, as was found in the dalbergioid analysis (Lavin et al., 2001) and is being confirmed in a larger study of papilionoids (M. Wojciechowski, Arizona State University, and M. Lavin, Montana State University, personal communication).

When we added other taxa to challenge this placement, this relationship was maintained but with poor support (Fig. 1). We tentatively accept the placement, however, because the poor support is from a weak signal, not a strongly conflicting one. Branch lengths, in terms of inferred evolutionary changes in this gene region, are very short for most internodes along the “backbone” of the phylogeny.

Other sources of data may help to place *Amorpheae*, although it is clear that *rbcL* (Doyle et al., 1997), ITS (Lavin et al., 2001), and *trnL* (Lavin et al., 2001; Pennington et al., 2001) do not provide sufficient resolution to place *Amorpheae* with confidence. Additional taxa may help for those sources of data that may be, as currently assembled, too variable to address deeper nodes, such as the 5’ end of the *trnK* intron.

**Phylogeny of *Amorpheae***—Our results, parsimony and likelihood analyses alike, indicate a phylogeny for *Amorpheae* that is consistent with some previously circumscribed taxonomic groups and with some aspects of the general distribution of floral forms (Fig. 5). On one side of the root lie the daleoids (*Psorothamnus*, *Dalea*, and *Marina*), genera in which the papilionoid-appearing flower is prevalent. On the other side of the root lie the amorphoids (*Apoplanesia*, *Amorpha*, *Eysenhardtia*, *Errazurizia*, and *Parryella*), genera in which the papilionoid floral form is not seen.

**The daleoid clade**—*Psorothamnus* and *Dalea* as circumscribed by Barneby (1977) are each paraphyletic, whereas the monophyly of the sampled *Marina* is strongly supported. The two clades of *Psorothamnus* divide neatly along taxonomic lines. Barneby’s (1977) section *Psorothamnus* (Rydberg’s genus *Psorothamnus*), based on sessile or nearly sessile flowers, non-exserted pods, and non-spiny inflorescences, is the sister of *Dalea* + *Marina*. The clade that is sister to *Dalea* + *Marina* + *Psorothamnus* section *Psorothamnus* consists of stout shrubs or trees with spiny inflorescences and/or exerted pods. This group of five species corresponds to sections *Xylodalea* (Rydberg’s genus *Psorodendron*), *Capnodendron*, and *Winnemucca*; we will refer to this clade as the *Psorodendron* clade. These characters, as well as our DNA sequence data, indicate that *Psorothamnus* should be divided into two genera. A formal set of species transfers will be proposed after further taxonomic study.

The genera *Marina* and *Dalea* together form a strongly supported clade. This clade corresponds to Hutchinson’s (1964) conception of Daleaeae, which was characterized by the lateral petal attachments positioned above the rim of the hypanthium. However, we have found that some *Psorothamnus* (McMahon and Hufford, 2002) also have this condition, although the petals are attached much closer to the hypanthium than in *Dalea* or *Marina*. The *Psorothamnus* that share this trait are mostly in the clade that is sister to *Dalea* + *Marina*, with the exceptions of *Psorothamnus polydenius* (in the sister clade, but petals attach on the rim of the hypanthium) and *Psorothamnus*

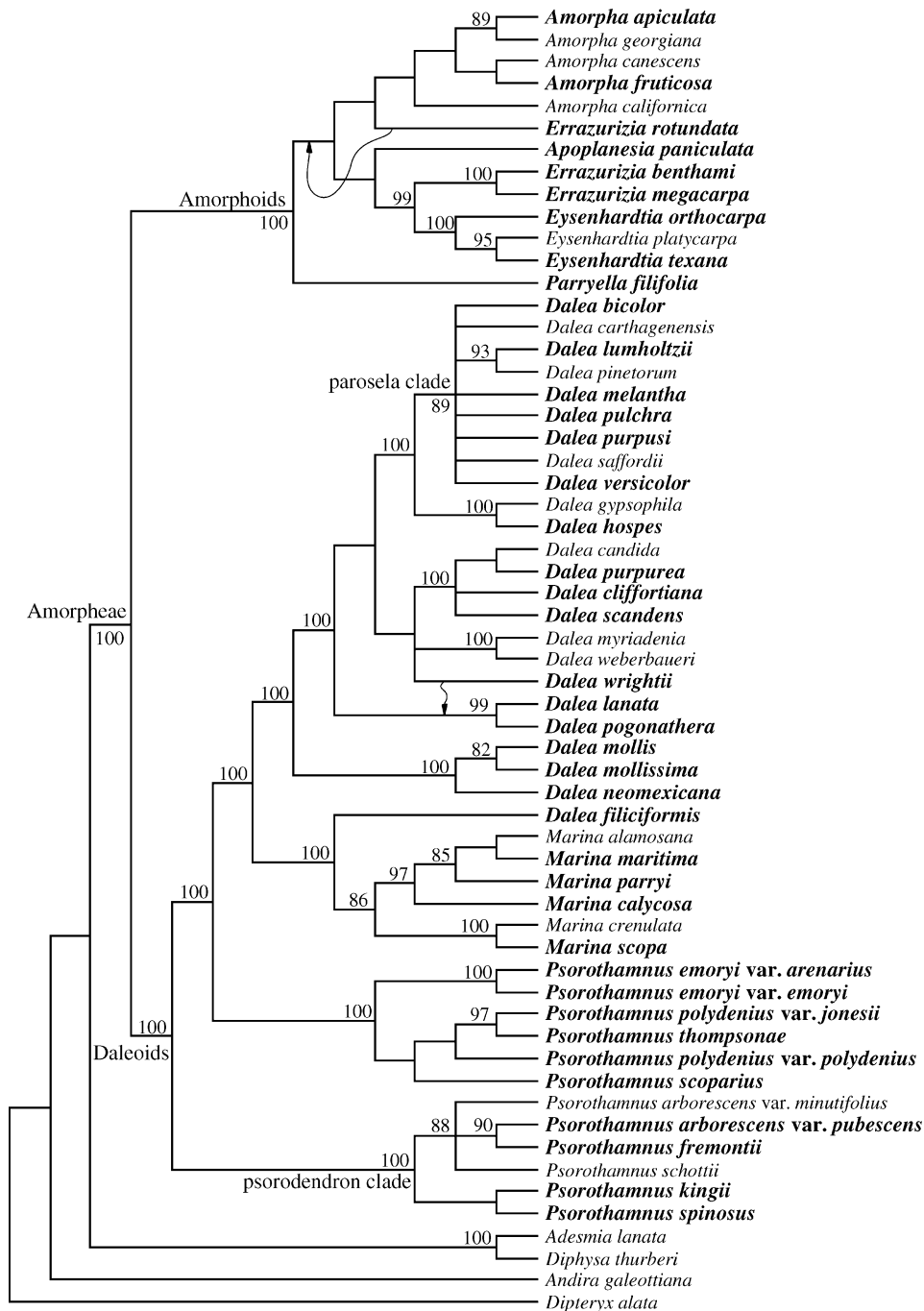


Fig. 4. Strict consensus of 2016 most parsimonious trees obtained by combining *trnK/matK* with ITS/5.8S. Taxa for which both regions were sequenced are in bold and larger font size; nonbolded taxa have missing data for one of the gene regions. Bootstrap values  $\geq 80\%$  are indicated. Likelihood analysis of taxa for which both regions were sequenced resulted in a nearly identical topology. *Errazurizia rotundata* was placed as indicated by the curving line in the maximum likelihood (ML) tree. Note that this is consistent with the results from ITS (Fig. 3), reflecting an alternative resolution of the polytomy involving *E. rotundata* and *Amorpha* species. *Dalea wrightii* was also placed differently by ML, as indicated by the curving line.

*spinus* (in the *Psorodendron* clade, but the petals attach slightly above the rim of the hypanthium).

*Marina* is supported to be monophyletic. *Dalea* is also supported to be monophyletic, with the exception of *Dalea filiciformis*, which is placed as sister to *Marina*. This unique *Dalea* was segregated by Barneby (1977) into the monotypic subgenus *Psoropteris* and was proposed to be a primitive relic, linking *Dalea* to *Marina* and *Psorothamnus*. This species has

loose inflorescences of pedicellate flowers and a laterally compressed pod with two crescent-shaped rows of blister glands (Barneby, 1977), closely resembling conditions in *Marina*. Barneby did not include *D. filiciformis* in *Marina* because it differs from *Marina* in several diagnostic characteristics: chromosome number, ovule number, vestiture, and leaf surface features. The latter two are easily considered as synapomorphies of *Marina sensu stricto*. A single ovule was considered diag-

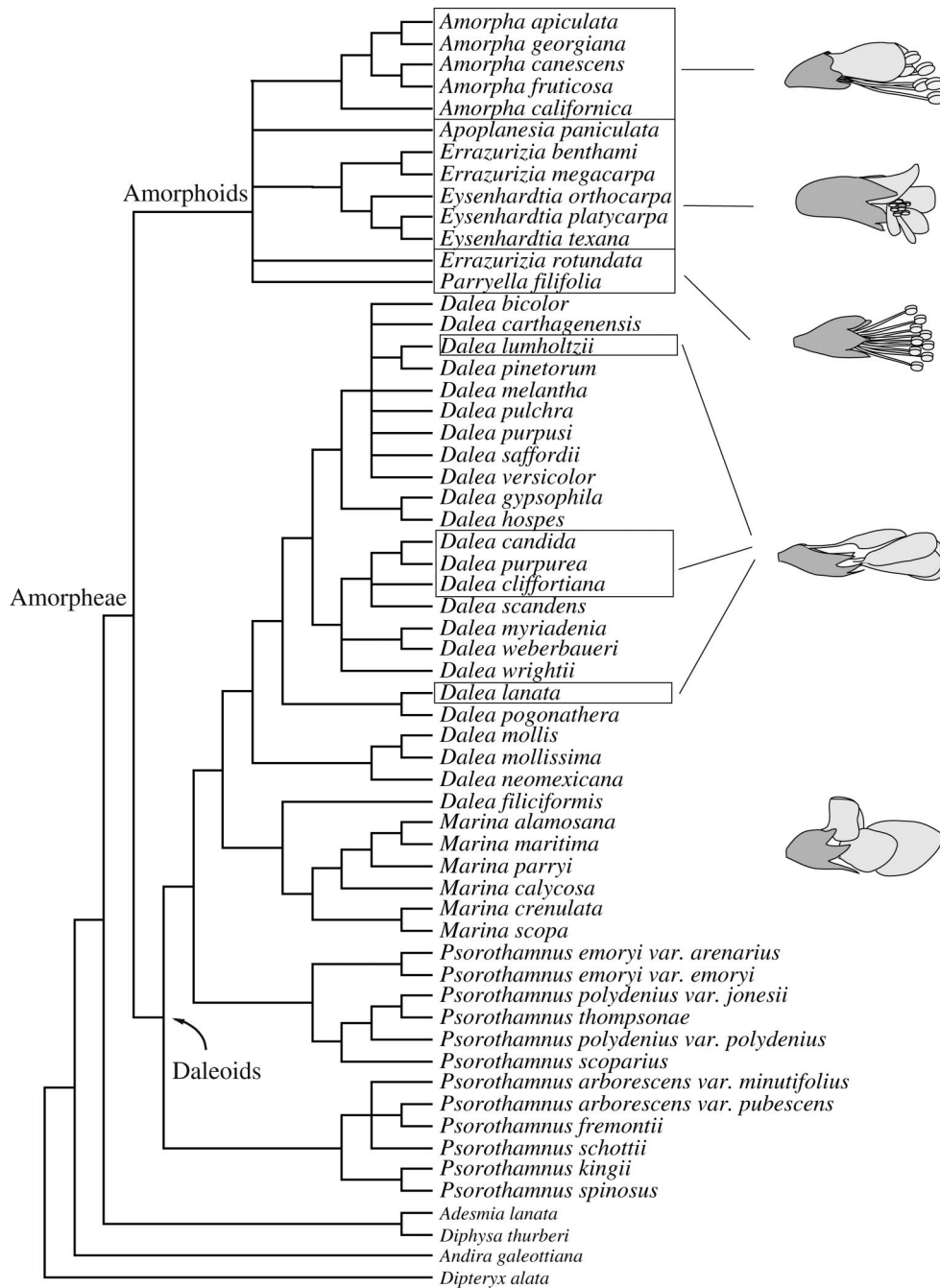


Fig. 5. Phylogeny of Amorpheae obtained using *trnK/matK* and ITS, indicating distribution of major corolla forms. Nodes at the base of the amorphoids have been collapsed to reflect uncertainty in our hypothesis. Taxa that are not included in boxes have a papilionaceous corolla, as in the lowermost floral diagram (note that this group also includes flowers that are not truly papilionaceous, because of the presence of the stemonozone).

nostic of marinas, but we have found several marinas that have two collateral ovules (as in the daleas), although one generally aborts prior to anthesis. Chromosome number is more difficult to accommodate because this placement of *D. filiciformis* would require homoplasy in chromosome numbers. Daleas have  $x = 8$  or  $x = 7$ ; all other Amorpheae have  $x = 10$  (Barneby, 1977). Our inferred phylogeny would require either an independent change from  $x = 10$  to  $x = 8$  in *D. filiciformis* or a return to  $x = 10$  in the ancestor of the marina clade. Alternatively, the count may be incorrect for *D. filiciformis*

and would be worth revisiting; the reported number is  $\pm 8$  chromosome pairs, whereas other counts in the groups were decisively reported as either  $x = 8$  or  $x = 7$  (Barneby, 1977).

Within *Dalea*, our inferred phylogeny corresponds to portions of Barneby's subgeneric classification. Subgenus *Theodora* is represented in our study by three species, placed together in a well-supported clade. These three, *Dalea mollis*, *D. mollissima*, and *D. neomexicana*, are similar low-herbaceous desert dwellers with  $x = 8$  (Barneby, 1977) and corollas loosely enclosing the androecium. Conversely, we found the



subgenera *Dalea* and *Parosela* to be not monophyletic. Although there are many polytomies in *Dalea*, all strongly supported clades include members of both subgenera, despite clear morphological differences. Subgenus *Dalea* is characterized by free petals and/or exerted androecia, whereas subgenus *Parosela* is characterized by having abaxial petals fused into a conventional papilionoid keel that encloses the androecium.

**The amorphoid clade**—The amorphoid clade is less well resolved than the daleoid clade, for two reasons. First, among species of *Amorpha*, there is very little sequence divergence in either ITS or *trnK/matK*. This genus, currently considered to have 15 species (Wilbur, 1975), is distributed through the American prairies into the southeastern United States and northern Baja California. Circumscription of species has been difficult, largely because there are few characters to distinguish them; many characters used by Rydberg (1919) to segregate the group into 23 species were later found to be variable within species across ranges and across seasons (Wilbur, 1975). Poor morphological differentiation coincides with poor molecular differentiation for the markers we have used and may indicate that *Amorpha* species evolved recently, although further sampling within *Amorpha* would be required to establish this. Alternatively, gene flow may be homogenizing the species, but this seems less likely. For example, one species pair that share identical sequences for both *trnK/matK* and ITS are of widely separated taxa: *A. apiculata* is restricted to a mountain range in northern Baja California, and its sister, *A. georgiana*, lives on the coastal plain of the southeastern United States. Also poorly distinguished from the amorphas is the morphologically unique *Errazurizia rotundata*. This plant combines the lack of petals as in *Parryella* (occasionally the single petal of *Amorpha*) with the densely glandular, low tortuous woody habit, and large glandular pod of many *Psorothamnus*, and the pollen of *Parryella* (Barneby, 1977). Tentatively placed with the amorphas in the parsimony analysis, the single occasional banner petal may be significant. This enigmatic plant is only known from a few small populations on the Little Colorado River in northern Arizona. Investigating the possibility of a cross-generic hybrid origin for *E. rotundata*, as tentatively suggested by Barneby (1977), would be valuable.

The second reason for poor resolution among amorphoids is the conflict between *trnK/matK* and ITS in the placement of the monotypic genera *Apoplanesia* and *Parryella*. In the ITS tree, *Apoplanesia* is positioned as sister to the rest of the amorphoids, although it has many autapomorphic changes and is placed without strong support (Fig. 3). In the *trnK/matK* tree, *Parryella* is placed as sister to the other amorphoids with strong support (Fig. 2). The issue is one of rooting the amorphoids; unrooted, they are identical hypotheses, varying in resolution. Combining the data collapses all nodes near the root of the amorphoids, as expected by observing the differences between data sets. For the *trnK/matK* data set, *Parryella* is on a relatively long branch (split by the rooting of the amorphoids). For the ITS data set, *Apoplanesia* is on a long branch. Unfortunately, no extant candidate taxa exist that would allow us to break these longer branches. More dense taxon sampling in *Amorpha* would help only if it served to identify additional synapomorphies shared with *Parryella*, an unlikely find using these two molecular markers. Adding more eysenhardtias and the one other *Errazurizia*, from Chile, may help if they share synapomorphies with *Apoplanesia*.

The two gene regions could also have different histories with respect to either *Apoplanesia* or *Parryella*. If the ITS tree is the correct organismal phylogeny, *Apoplanesia* as sister to the rest of the amorphoids would require either the retention of multiple chloroplast lineages through several speciation events or a lateral transfer from the ancestor of *Errazurizia sensu stricto* + *Eysenhardtia*. *Errazurizias* live in northern Mexico and Chile, eysenhardtias are distributed throughout Mexico into the southwestern United States, and *Apoplanesia* lives in southern Mexico through Central America. These overlapping distributions imply that a chloroplast capture may have been possible, but beyond that we cannot infer. Similar arguments pertain to *Parryella*, as it lives near *Errazurizia rotundata* in northern Arizona, and amorphas occur throughout most of the United States. Therefore, we cannot rule out the hypothesis that the gene regions differ because they have different histories. However, we require additional evidence to distinguish between this case and that of simply not having enough molecular data to identify the root of the amorphoid clade.

**Floral evolution**—Although inferring the evolutionary history of floral forms requires detailed morphological work to establish homologies, we can draw general evolutionary conclusions from the phylogenetic distribution of floral forms. Floral diversity arose in the Amorpheae along two themes. These themes each dominate a major clade: amorphoids vary in corolla merosity and have less differentiation among corolla parts than the ancestral form; daleoids vary primarily in the presence and extent of a stemonozone (a novel corolla-androecium synorganization; McMahon and Hufford, 2002). Flowers in *Amorpha* have a single petal and monadelphous stamens, fused in a sheath that is open on the adaxial side. The monotypic *Parryella* has no petals and a monadelphous androecium fused in an entire tube. *Eysenhardtia* and the monotypic *Apoplanesia* have five poorly differentiated petals held open, revealing the androecium. Three of four *Errazurizia* species have flowers similar to *Eysenhardtia*, but the fourth, *Errazurizia rotundata* (Wootton) Barneby, has no petals, although it is also reported, rarely, to have one. Most species of the three remaining genera, *Marina*, *Dalea*, and *Psorothamnus*, have flowers with more standard papilionaceous corollas (a banner, two wing petals, and two keel petals, marginally fused to enclose the androecium). Within this group, however, is a unique organ arrangement: the petals of *Marina*, *Dalea*, and some *Psorothamnus* appear perched on the staminal column.

**Ancestral floral form**—To discuss the evolution of floral form in Amorpheae, we must first discuss the ancestral state for the clade. To do this, we can compare the distribution of general floral form to the phylogeny obtained for Amorpheae and the large set of outgroups (Fig. 1). For this comparison, we will take a broad view and consider flowers either “papilionaceous” or “nonpapilionaceous,” although this greatly simplifies papilionoid floral diversity. Nevertheless, “papilionaceous” flowers have features in common: five petals differentiated into keels, wings and banner, a hypanthium, and an androecium that is commonly connate (but not adnate to the corolla, as in the daleoid stemonozone). Using this definition, among Amorpheae, only some *Psorothamnus* are papilionaceous (McMahon and Hufford, 2002), but almost all of the outgroups are. A few exceptions occur in the dalbergioids:

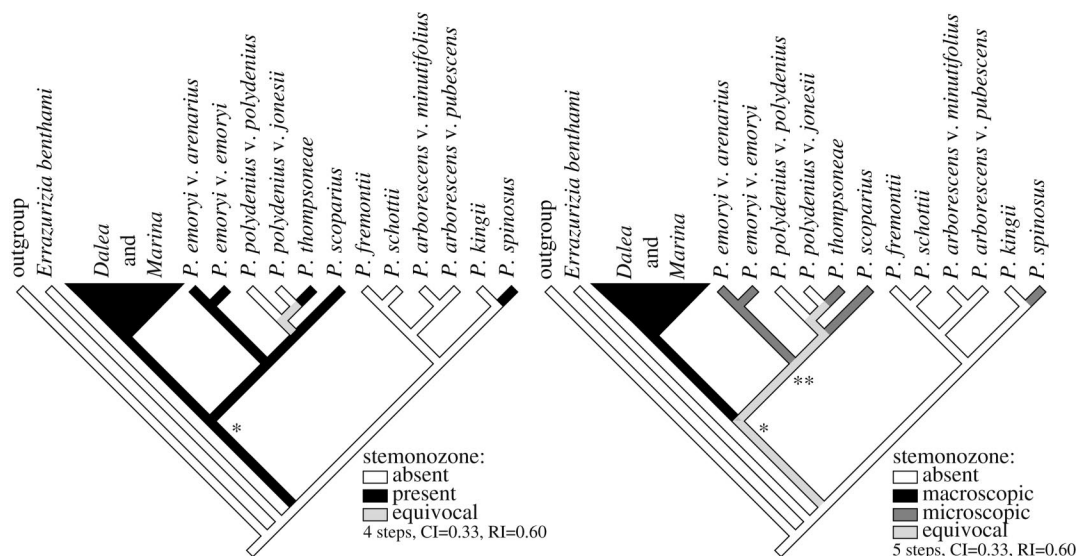


Fig. 6. Reconstructions of the evolutionary changes in the stemonozone. We include a generic outgroup without a stemonozone for purposes of illustration (see text for discussion of papilionoid ancestral character states). On the left, the stemonozone has been considered homologous and treated as a single character state, regardless of size. At least two gains are required. The ancestor of *Dalea*, *Marina*, and some *Psorothamnus* (asterisk) is reconstructed as having a stemonozone. On the right, we have delineated microscopic and macroscopic versions of the daleoid stemonozone. Each of the seven most parsimonious reconstructions include multiple gains and losses. In this scenario, the ancestor indicated by the single asterisk could have had any of the three states. The ancestor indicated by the double asterisk could have had no stemonozone or a microscopic stemonozone.

some or all species of *Riedeliella*, *Etaballia*, and *Inocarpus* (Polhill, 1981), and *Pictetia* (Beyra-M. and Lavin, 1999). However, they are scattered and apparently derived within the dalbergioid clade (Fig. 1 and Lavin et al., 2001). Some members of the tribe Sophoreae (approximately 69 of a few hundred species) and all members of Swartzieae (approximately 184 species) also possess nonpapilionaceous corollas, but these taxa are not known to be closely related to Amorpheae (Fig. 1). If we consider papilionaceousness to be a character with two states, parsimony analysis on the tree confirms that the nonpapilionaceous state arose separately in all taxa indicated. This is a simplified view of nonpapilionaceous flowers, which can be structurally quite dissimilar. However, if papilionaceousness is in some way homologous, we can infer that the ancestors of the dalbergioid clade, the genistoid clade and its sister, and the 50-kb inversion clade (Fig. 1) all had papilionaceous flowers. Therefore, we infer that the Amorpheae did as well.

**Stemonozone evolution**—Members of *Dalea* and *Marina* and some members of *Psorothamnus* share a novel corolla-androecium synorganization in which the petals are perched above the rim of the hypanthium, appearing to attach on the staminal column (Barneby, 1977; McMahan and Hufford, 2002). We refer to the region below the insertion point of the petals as the “stemonozone” (McMahan and Hufford, 2002). The height of the stemonozone varies tremendously, from very short in some *Psorothamnus* to extensive in the prairie clover daleas (*Dalea* subgenus *Dalea* section *Kuhnistera*). Because our sampling is not exhaustive (all *Psorothamnus*, but only six out of 38 *Marina* and 24 of approximately 164 *Dalea* species were included), we cannot yet precisely map stemonozone height onto the phylogeny and fully describe its evolutionary changes. However, with the current phylogeny, we can address whether the various manifestations of stemonozone are homologous among Amorpheae.

Homology is used here to mean similarity by descent (Lankester, 1870; Donoghue, 1992; Hufford, 1996), and we can infer homology if we can establish both similarity and descent. To do this, we ascertain whether a trait is consistent with structural criteria (e.g., Remane, 1952), establishing similarity, and with phylogenetic criteria, establishing the possibility of descent from a common ancestor (e.g., Patterson, 1988). Based on morphological data, the stemonozones in several taxa were found to be structurally similar (McMahan and Hufford, 2002). However, mapping the stemonozone onto the phylogeny (Fig. 6), we find poor support for a single origin of the stemonozone. *Dalea* and *Marina*, in which the stemonozone is present as a structure visible to the unaided eye, share a common ancestor and therefore likely share the stemonozone by descent. The distribution of the stemonozone in *Psorothamnus*, however, is much more complex. There is a minute stemonozone, not visible by the unaided eye but structurally similar to the larger versions, in some members of the clade that is sister to *Dalea* + *Marina*: *P. scoparius*, *P. thompsonae*, and *P. emoryi* (McMahan and Hufford, 2002). However, it is lacking in both varieties of *P. polydenius*. In the second clade of *Psorothamnus*, all but *P. spinosus* lack a stemonozone. If the stemonozone is phylogenetically homologous, i.e., it was inherited in common from the daleoid ancestor, our phylogenetic hypothesis requires the additional inference of three or four losses, depending on whether *P. kingii* and *P. spinosus* are sisters or form a grade. Alternatively, *P. thompsonae* may have regained the trait, and/or *P. spinosus* may have evolved the trait independently. The most parsimonious mapping of the character requires four steps; these mappings include a single origin of the stemonozone only if *P. kingii* and *P. spinosus* form a grade, as weakly supported by the ITS data (Fig. 3). However, the *matK* data and the combined analysis place *P. kingii* sister to *P. spinosus* (Fig. 4). Therefore, we tentatively conclude that the daleoid stemonozone (at least

in its minute form) has arisen at least twice and has been lost up to two times (Fig. 6).

**Corolla evolution**—*Marina* and *Dalea* have nonpapilionaceous flowers, as defined earlier, because of the presence of the unusual stemonozone. However, the corollas of all *marinas* and most *daleas* are papilionaceous, i.e., they consist of five differentiated petals. The suggestion that *Marina* and *Dalea* may reflect a secondary derivation of the papilionaceous corolla (Barneby, 1977) was based in part on the presence of the stemonozone. It was also based on the idea that the woody species such as *Apoplanesia* and *Eysenhardtia*, with their non-differentiated petals held open (i.e., nonpapilionaceous corollas), reflected the ancestral condition in the tribe (Barneby, 1977). Our data do not support this hypothesis. We inferred earlier that the ancestral state for the tribe was papilionaceousness, and we also infer that the root of the daleoids was papilionaceous (Fig. 6). Therefore, the corollas in *Dalea* and *Marina* can be considered phylogenetically homologous to corollas outside of Amorpheae, and we conclude that they do not represent a reinvention of the papilionaceous corolla. This is consistent with our finding that the daleoid corolla is structurally homologous to other papilionoids (McMahon and Hufford, 2002).

The amorphoids form a clade in which the nonpapilionaceous corolla is derived and takes several forms (one petal, zero petals, five similar petals not enclosing the reproductive organs). Lack of resolution at the root of the amorphoids limits our ability to make precise inferences of the number and direction of trait changes, but it is clear that several changes occurred in this relatively small and molecularly poorly differentiated clade.

Although the daleoids are dominated by papilionaceous corollas and the presence of a stemonozone, there are exceptions. Members of *Dalea* subgenus *Dalea*, as circumscribed by Barneby (1977), have corollas that are not papilionaceous: a banner is present, but the four lateral petals are highly similar, unfused, and do not enclose the reproductive organs. Our data do not support this subgenus as monophyletic, and these cases are phylogenetically separate from the undifferentiated corollas in the amorphoid clade. Therefore, corolla differentiation has been lost in the Amorpheae at least three times (Fig. 5).

**Conclusion**—Traditional taxonomy in the tribe Amorpheae has been corroborated, in part, by our sequence data from *trnK/matK* and ITS/5.8S. For those cases of disagreement, there are sound reasons to accept the results from the molecular data. *Errazurizia rotundata*, lacking a corolla, is phylogenetically separated by our data from its corolla-bearing congeners. Although it is consistently placed with the amorphas as sister or as part of a polytomy, our data result in low support for these branches. Therefore, we cannot yet exclude the possibility that it belongs with *Parryella filifolia*, with which it shares floral form; indeed, the basionym for *E. rotundata* is *Parryella rotundata* Wooton, indicating its similarity. *Dalea* and *Marina* are strongly supported to be monophyletic with the exception *Dalea filiciformis*; this species is robustly placed by our data as sister to *Marina*, with which it shares many morphological features. Molecular data provide evidence for paraphyly of *Psorothamnus*, and division of the genus into two monophyletic genera will fall along previous taxonomic lines. We have also confirmed the monophyly of Amorpheae and further confirm its placement as sister to the dalbergioid clade.

This allowed us to infer that an ancestral papilionaceous floral form gave rise to tremendous diversity in the Amorpheae. Although many morphological traits corroborate clades that our molecular data resolved, we also have strong evidence for homoplasy in other traits, leading to the general conclusion of very active floral evolution in this relatively small tribe of legumes.

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