

Phylogenetic relationships and floral evolution in the papilionoid legume clade Amorpheae

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McMahon, M. M. (University of California, Davis, One Shields Avenue, Davis, CA, 95616, U.S.A.; e-mail: mmmcmahon@ucdavis.edu). Phylogenetic relationships and floral evolution in the papilionoid legume clade Amorpheae. *Brittonia* 57: 397–411. 2005.—Amorpheae (Fabaceae: Papilionoideae) was first considered a natural group by Rupert Barneby in his illustrated monograph *Daleae Imagines*. Amorpheae currently comprise eight genera, ca. 250 spp., and extensive floral diversity, including loss of corolla and addition of a stemonozone. The Amorpheae and many of Barneby's subtribal groups are supported as monophyletic by previous phylogenetic analysis of nuclear ribosomal and chloroplast sequence data. However, some relationships remain unclear. A nuclear marker derived from a genomic study in *Medicago*, CNGC4, was sequenced in selected Amorpheae. This is one of the first applications of this marker for phylogenetic study. The new data confirm some relationships inferred using *trnK* and ITS, but also provide evidence for new arrangements. Combined data were used to explore several aspects of Barneby's taxonomic framework. The phylogeny, in concert with data on floral morphology, implies that simplification of the complex papilionoid flower has occurred several times in the history of the Amorpheae.

Key words: Amorpheae, Barneby, CNGC4, *Dalea*, floral evolution, Papilionoideae.

Amorpheae Borrissova emend. Barneby is a group of ca. 250 papilionoid legume species with much variation in floral form. Although nested within the Papilionoideae (Lavin et al., 2001; McMahon & Hufford, 2004; Wojciechowski et al., 2004) the group contains many members with flowers that depart from the standard papilionoid form. Aspects that vary include a unique structure (the "stemonozone", Fig. 1A, B; McMahon & Hufford, 2002), losses of structures such as petals and stamens, and the loss of petal attributes such as asymmetry and strong blade/claw distinctions (Fig. 1C, D; McMahon & Hufford, 2005). First considered a natural group by Rupert Barneby (1977), Amorpheae is natively distributed from southern Canada to northern Chile, with areas of highest diversity in the arid and semi-arid regions of Mexico, the Andes, and the prairies of North America. Although no explicit phylogenetic analysis

was conducted, Barneby presented a hypothesis of relationships among groups in Amorpheae (Fig. 2). This hypothesis was based on several informative morphological characters, some of which are consistent with the phylogeny based on DNA sequences, some of which are not.

Previous analyses resulted in very strong support for a clade of three genera (the daleoids: *Dalea*, *Marina*, *Psorothamnus*), sister to a clade of the remaining five genera (the amorphoids: *Errazurizia*, *Parryella*, *Eysenhardtia*, *Amorpha*, and *Apoplanesia*) (McMahon & Hufford, 2004). Furthermore, relationships among many lineages within the daleoids were strongly supported. However, within the amorphoids, the previously used markers (ITS and the *trnK* intron) varied little. These taxa are particularly interesting because they include several apparent simplifications from the complex papilionoid flower. Therefore, additional data

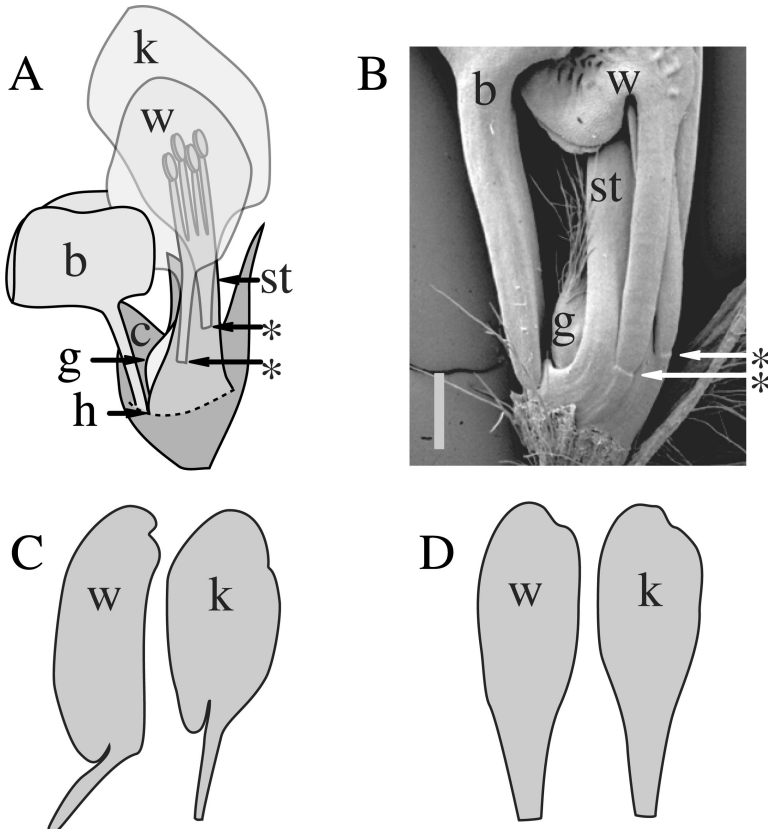


FIG. 1. Selected aspects of variation among flowers of Amorpeae. **A.** Schematic of a generalized member of *Marina* or *Dalea* showing the lateral petals attaching above the hypanthium (asterisks). The region between the petal insertion and the hypanthium is the stemonozone (McMahon & Hufford, 2002). **B.** Scanning electron micrograph of *Dalea pulchra* Gentry; proximal portion of bud near anthesis (calyx mostly removed), showing lateral petal attachment points. **C.** Line drawing of lateral petals, *Psorothamnus scoparius* (A. Gray) Rydb., showing difference between wing and keel, asymmetry within each petal, and distinction between claw (narrow proximal region) and blade (distal laminate region). **D.** Line drawing of lateral petals, *Errazurizia megacarpa* (S. Wats.) I. M. Johnst., showing similarity of wing to keel, near symmetry within petals, and gradual tapering of distal region to proximal region. [In all, distal is up and adaxial (with respect to the inflorescence axis) is to the left. b, banner petal; c, calyx; g, gynoecium; h, hypanthium (arrow points to rim); k, keel petal; st, stamens; w: wing petal. Scale bar (B) = 0.5 mm.]

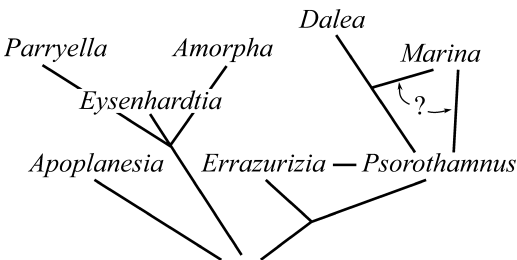


FIG. 2. Evolutionary relationships among members of Amorpeae as previously hypothesized, from Barneby's Figure 1 (Barneby, 1977: 13).

are required, ideally from a region that is independent from the ribosomal and chloroplast sequences used previously. Markers derived from genomic data in *Medicago truncatula* have been recently developed for comparative genome studies across crop legumes (Choi et al., 2004b). Application of these markers to phylogenetic questions is promising but not always straightforward (Scherson et al., 2005). Extending their application from *Medicago* to a group such as Amorpeae, diverged for perhaps tens of millions of years (Choi et al., 2004b), may help provide resolution within Amorpeae.

TABLE I
GENBANK ACCESSION NUMBERS FOR NEWLY GENERATED AND DOWNLOADED SEQUENCES

| Taxon | Collection | GenBank accession |
|---|---------------------------|-------------------|
| Sequences submitted: CNGC4 | | |
| <i>Amorpha apiculata</i> Wiggins | Fishbein 3745 (ARIZ) | DQ023320 |
| <i>Amorpha californica</i> Nutt. ex Torr. & A. Gray | Grable 6434 (WS) | DQ023322 |
| <i>Amorpha canescens</i> Pursh | McMahon 683 (WS) | DQ023323 |
| <i>Apoplanesia paniculata</i> C. Presl | Cabrera C. 5479 (ARIZ) | DQ023327 |
| <i>Errazurizia benthami</i> (Brandege) I. M. Johnst. | McMahon 438 (WS) | DQ023324 |
| <i>Errazurizia megacarpa</i> (S. Watson) I. M. Johnst. | Fishbein 3773 (WS) | DQ023321 |
| <i>Errazurizia rotundata</i> (Wooton) Barneby | McMahon 452 (WS) | DQ023325 |
| <i>Eysenhardtia orthocarpa</i> (A. Gray) S. Watson | Fishbein 3683 (ARIZ) | DQ023328 |
| <i>Eysenhardtia texana</i> Scheele | Van Devender 29V79 (ARIZ) | DQ023326 |
| <i>Parryella filifolia</i> Torr. & A. Gray ex A. Gray | Porter 8868 (ARIZ) | DQ023329 |
| <i>Psorothamnus fremontii</i> (Torr. & A. Gray) Barneby | McMahon 339 (ARIZ) | DQ023330 |
| <i>Psorothamnus kingii</i> (S. Watson) Barneby | McMahon 688 (ARIZ) | DQ023331 |
| <i>Psorothamnus schottii</i> (Torr.) Barneby | McMahon 150 (ARIZ) | DQ023332 |
| Taxon | Sequence region | GenBank accession |
| Sequences downloaded | | |
| <i>Amorpha fruticosa</i> | ITS | AFU59890 |
| <i>Amorpha fruticosa</i> | matK | AF270861 |
| <i>Apoplanesia paniculata</i> | ITS | AF187093 |
| <i>Apoplanesia paniculata</i> | matK | AF270860 |
| <i>Dalea pulchra</i> | matK | AY386860 |
| <i>Eysenhardtia orthocarpa</i> | matK | AY386909 |
| <i>Eysenhardtia</i> sp. Lavin 5052 | ITS | AF187096 |
| <i>Marina parryi</i> | matK | AY386859 |
| <i>Marina</i> sp. Lavin 5341 | ITS | AF187095 |
| <i>Medicago truncatula</i> | CNGC4 | BV164997 |
| <i>Medicago truncatula</i> | ITS | AF233339 |
| <i>Medicago truncatula</i> | matK | AF522109 |
| <i>Pisum sativum</i> | CNGC4 | BV165002 |
| <i>Pisum sativum</i> | ITS | AY143481 |
| <i>Pisum sativum</i> | matK | AY386961 |

For all other accession numbers, see McMahon and Hufford (2004).

It will also be of value in exploring the benefits of transferring data from comparative genomic analyses to phylogenetic systematics.

The goals of this paper are (1) to present a preliminary set of data from one genomic derived nuclear marker, CNGC4, alone and in combination with previously reported DNA sequence data, (2) to summarize current understanding of Amorpheae relationships, (3) to review patterns of floral evolution in Amorpheae, and (4) to compare these inferences to Barneby's hypotheses of floral evolution in the group.

Methods and Materials

The nuclear region identified in the genome-wide analysis of *Medicago truncatu-*

la as CNGC4 (cyclic nucleotide-regulated ion channel like protein) (Choi et al., 2004a) was sequenced for 13 Amorpheae (Table I). Although many markers will be examined in the future, this marker was selected to be first based on its potential for variation among species (Choi et al., 2004b; Scherson et al., 2005). Additionally, the primers uniformly produced a single PCR band and a clean sequencing chromatogram, consistent with the possibility that the region is single-copy in Amorpheae, although this is not yet confirmed. Taxa were chosen based on their placement in previous phylogenetic analyses, in which many relationships were resolved with strong support. However, relationships among key taxa that are involved in floral simplifica-

tions were not well resolved (McMahon & Hufford, 2004, 2005). Therefore, available members of *Eysenhardtia*, *Errazurizia*, *Amorpha*, *Parryella*, and *Apoplanesia* (the amorphoid clade) were selected for sequencing CNGC4. Three members of *Pso-rothamnus* were chosen to span the root of the daleoid clade (Graybeal, 1998; McMahon & Hufford, 2004) in an effort to resolve the position of the amorphoid root. Sequences from *Medicago truncatula* and *Pisum sativum* were obtained from GenBank (Table I) and included as outgroups. Additional ITS and *trnK* sequences from Amorpheae have been submitted to GenBank by other labs; these were downloaded in order to use all available data (Table I).

Leaf tissue was removed from dried herbarium specimens or from plants in the field and dehydrated with silica gel. Total DNA was isolated using a standard CTAB protocol (Doyle & Doyle, 1987). Fragments were amplified using the primers 5'-AGAGATGAGAATCAAGAGGAGGGA-TGCA-3' and 5'-CATGATGAAGAGCA-TTTCGTCCACTGGA-3' (Choi et al., 2004a) and the following profile: 15 min at 95°, 35 cycles of 1 min at 94°, 1 min at 52°, 1 (or 3) min at 72°, with a final extension of 5 min at 72°. Sequences were generated using the same primer pair.

Phylogenetic analysis was conducted on the data from CNGC4 alone and in combination with previously published sequences of Amorpheae (see McMahon & Hufford, 2004, for voucher information). The three data partitions differed in taxonomic coverage: 59 sequences were from ITS, 47 from *trnK*, and 13 from CNGC4. For combined analyses, missing data were added to complete the matrix (a supermatrix approach; Driskell et al., 2004). Combining data sets may not be appropriate when processes such as lineage sorting or introgression could cause different markers to have different histories (Maddison, 1997). However, this is generally difficult to detect unless there is strong and conflicting signal that cannot be explained by homoplasy. The three data sets were evaluated for evidence of strong conflicting signal by first pruning the data sets to only those taxa with all three genes, and then comparing

the trees obtained. No strongly supported relationships ($\geq 90\%$ bootstrap; Felsenstein, 1985) differed among the trees. Additionally, the partition homogeneity test (or incongruence length difference test, Farris et al., 1994) as implemented in PAUP* (Swofford, 2003) with 1000 replicates, failed to reject homogeneity ($P = 0.8220$). This method may be inappropriate as a test for combinability (Barker & Lutzoni, 2002), but the negative result, in concert with the lack of strong conflicting signal, provides justification for combining the data.

Parsimony and likelihood optimality criteria were used to compare phylogenetic trees, and support for relationships was assessed using the nonparametric bootstrap (Felsenstein, 1985). For the likelihood analyses, models of sequence evolution were compared using hierarchical likelihood ratio tests as calculated by Modeltest 3.0 (Posada & Crandall, 1998). The comparison favored a model in which transitions and transversions may have different evolutionary rates (the Hasegawa-Kishino-Yano model, HKY, Hasegawa et al., 1985) and this was the model used for the CNGC4 data. Model comparisons favored more complicated models for the ITS data and for the *trnK* data, notably the inclusion of rate heterogeneity across sites. A compromise model was selected for the combined data set in which separate rates were estimated for transitions and for transversions and variation in rates across sites was estimated using a gamma distribution with four discrete categories (Yang, 1994), the HKY Γ model. Likelihood analysis of the small data set (CNGC4 alone; 13 taxa) began with a random-taxon-addition starting tree, and parameter values were optimized during the search. Likelihood analysis of the larger combined data (*trnK*, ITS, and CNGC4; 62 ingroup and 2 outgroup taxa) was made more computationally tractable by starting with a reasonably good tree (the first most-parsimonious tree found in a parsimony analysis limited to 100 trees), estimating the HKY Γ parameter values on that tree, and using these values throughout the tree search.

To explore hypotheses of monophyly,

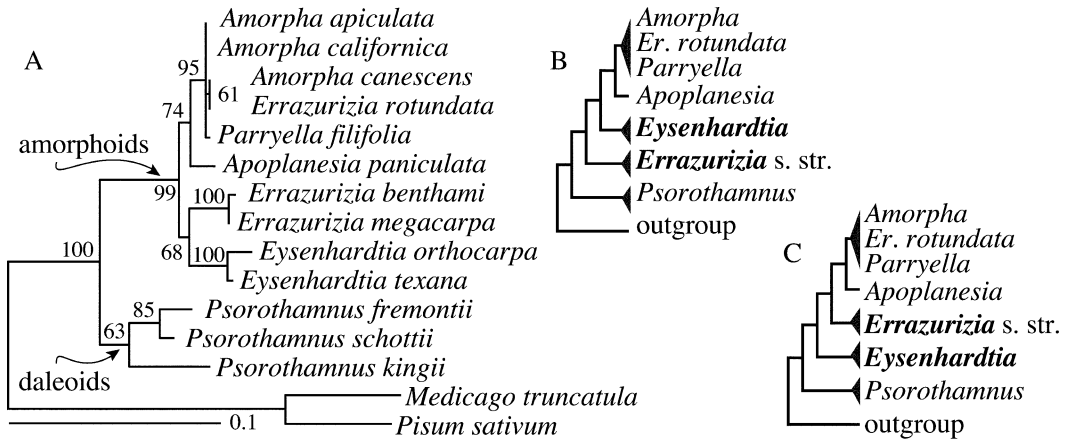


FIG. 3. Phylogenetic relationships inferred using the nuclear marker CNGC4. **A.** Maximum likelihood estimate of topology and branch lengths. Numbers above branches indicate likelihood bootstrap values. **B., C.** Parsimony analysis resulted in three equally parsimonious trees (EPTs), one of which matches the topology shown in A. The other two are depicted here, with some taxa removed. Note that the three EPTs include all possible relationships between *Eysenhardtia*, *Errazurizia*, and the rest of the sampled amorphoid taxa. Parsimony bootstrap analysis resulted in values nearly identical to the ML analysis except for the branch leading to *Psorothamnus* (90%) and the branch leading to *Eysenhardtia* + *Errazurizia* (43%).

analyses were conducted in which the following four clades were constrained to be monophyletic: *Dalea*, *Amorpha*, *Errazurizia*, and *Psorothamnus*. Optimal trees with and without each constraint were compared using parsimony and likelihood. To assess significance of difference in the likelihood values with and without the constraint, the set of trees that included the best trees with and without all of the constraints was subjected to an SH test (Shimodaira & Hasegawa, 1999) as implemented in PAUP* (Swofford, 2003) using the RELL approximation to estimate log likelihoods and 1000 bootstrap replicates to estimate the distribution of the test statistic.

Results

The nuclear marker CNGC4, sequenced for 13 Amorpheae, resulted in an aligned data set of 535 nucleotides. Aligning the ingroup to two outgroup sequences from GenBank (*Pisum sativum* and *Medicago truncatula*) required the addition of four gapped characters to the ingroup for a final matrix of 539 sites. The maximum pairwise divergence (uncorrected distance) between ingroup taxa was ca. 10% between *Psorothamnus kingii* and *Errazurizia benthami*. Two species of *Amorpha* had the same se-

quence, and this differed little from the sequences found in the third *Amorpha*, in *E. rotundata*, and in *Parryella filifolia*. All three species of *Psorothamnus* have a ~95 bp insertion relative to the outgroup and the remaining ingroup. Other than this large insertion, few gaps were necessary (c. 1%) to align the sequences. The sequences have been submitted to GenBank (Table I) and the aligned data set is available on the author's website (ginger.ucdavis.edu/mcmahon/data).

CNGC4 provides strong evidence for several clades among the small set of taxa sampled (Fig. 3). An amorphoid clade consisting of *Amorpha*, *Errazurizia*, *Eysenhardtia*, *Parryella*, and *Apoplanesia* is recovered with strong support [99% bootstrap (BS) in likelihood and parsimony analyses]. The branch leading to the daleoids, here represented by only three members of *Psorothamnus*, is weakly supported by likelihood BS but is supported with 90% by parsimony bootstrap. The pair of species from *Eysenhardtia* are supported as sisters with 100% BS, as are two of the three *Errazurizia* species, *E. benthami* + *E. megacarpa*. *Parryella filifolia* groups together with the *Amorpha* species and the third *Errazurizia*, *E. rotundata*, but the relationship among

them is not clear. This clade's sister is *Apoplanesia*, with moderate support. Poorly supported are the relationships among *Eysenhardtia*, *Errazurizia*, and the *Apoplanesia*—*Amorpha* clade. The ML analysis inferred a sister relationship between *Eysenhardtia* and *Errazurizia*, but with low support (68% BS). Parsimony analysis resulted in three equally parsimonious topologies reflecting the three possible resolutions of this node (Fig. 3A–C).

Combining these data with ITS and *trnK* for a larger sample of taxa resulted in 1080 MP trees and one ML tree (Fig. 4). The strict consensus MP tree has nearly the same topology as the ML tree except that several nodes with less than 60% BS (Fig. 4) are collapsed in the strict consensus tree (not shown). Additionally, in the MP strict consensus tree, *Eysenhardtia* is sister to the remaining amorphoid taxa (as depicted in Fig. 3C) whereas the ML tree places *Eysenhardtia* sister to *Errazurizia* s. str., together sister to *Apoplanesia* (Fig. 4). Species in *Psorothamnus* are strongly supported to be in two clades by substantial branch lengths and 100% BS (Fig. 4). All sampled members of *Dalea* form a clade except one: *Dalea filiciformis* is placed in or sister to *Marina*. Excluding *D. filiciformis*, *Marina* and *Dalea* are sisters, and the data support some relationships within each of these genera. *Errazurizia rotundata* is placed with *Parryella filifolia* with strong support (97% BS), and these are nested within or sister to the *Amorpha* clade (100% BS, Fig. 4).

Three of the four constrained analyses resulted in trees that were considerably longer (and significantly less likely) than the unconstrained trees (Table II). Forcing *Dalea* to be monophyletic (i.e., forcing *D. filiciformis* to group with the other daleas instead of with the marinas) resulted in an increase of 19 steps required to explain the tree using parsimony, and a likelihood value decrease of 57 ln likelihood units. The SH test assessed this difference as significant ($P = 0.0350$). Likewise, forcing the three *Errazurizia* species together was found to be significantly less likely ($P = 0.0010$) than the best tree in which *E. rotundata* is placed with *Parryella*. Constraining *Psorothamnus* to be monophyletic re-

sulted in the largest addition to the number of parsimony steps required (50) and the greatest decrease in likelihood value (106 lnL units) and was highly significant ($p < 0.0001$). Forcing *Amorpha* to be a clade had little effect; tree length remained the same (although fewer trees were recovered), and the log-likelihood changed insignificantly (Table II).

Discussion

Continuing research into the systematics of Papilionoideae confirms multiple independent simplifications of the papilionoid corolla. Although some lineages have retained an ancestral non-papilionoid flower form, others have had it and lost it (Pennington et al., 2000), contradicting inferences that non-papilionoid forms should be considered plesiomorphic (Mansano et al., 2004). In Amorpheae, the loss has occurred several times (McMahon & Hufford, 2004, 2005). The addition of data from a nuclear marker helps refine inferences of relationships within the amorphoid clade, in which corolla loss and dedifferentiation has occurred. The nuclear marker strongly supports the result that all species with zero or one petal are a clade. Data from ITS also support this, but weakly, and data from *trnK* disagree, although weakly (McMahon & Hufford, 2005). Therefore, the new analyses do not overturn previous conclusions regarding floral evolution and simplification in Amorpheae, but instead add evidence for more precise inferences.

To summarize recent phylogenetic research in Amorpheae, it is important to place it in the context of its taxonomic history, primarily the work of Rupert Barneby, to whom this symposium is dedicated.

RELATIONSHIPS AMONG AMORPHEAE

Rupert Barneby's classification of the Amorpheae revolutionized our understanding of this part of the legume tree of life. Prior to his treatment, workers had considered all glandular legumes with indehiscent single-seeded pods and simple basifixed hairs to belong together in Psoraleeae Benth. (with variations in rank and spelling) (Bentham, 1865; Taubert, 1894; Rydberg,

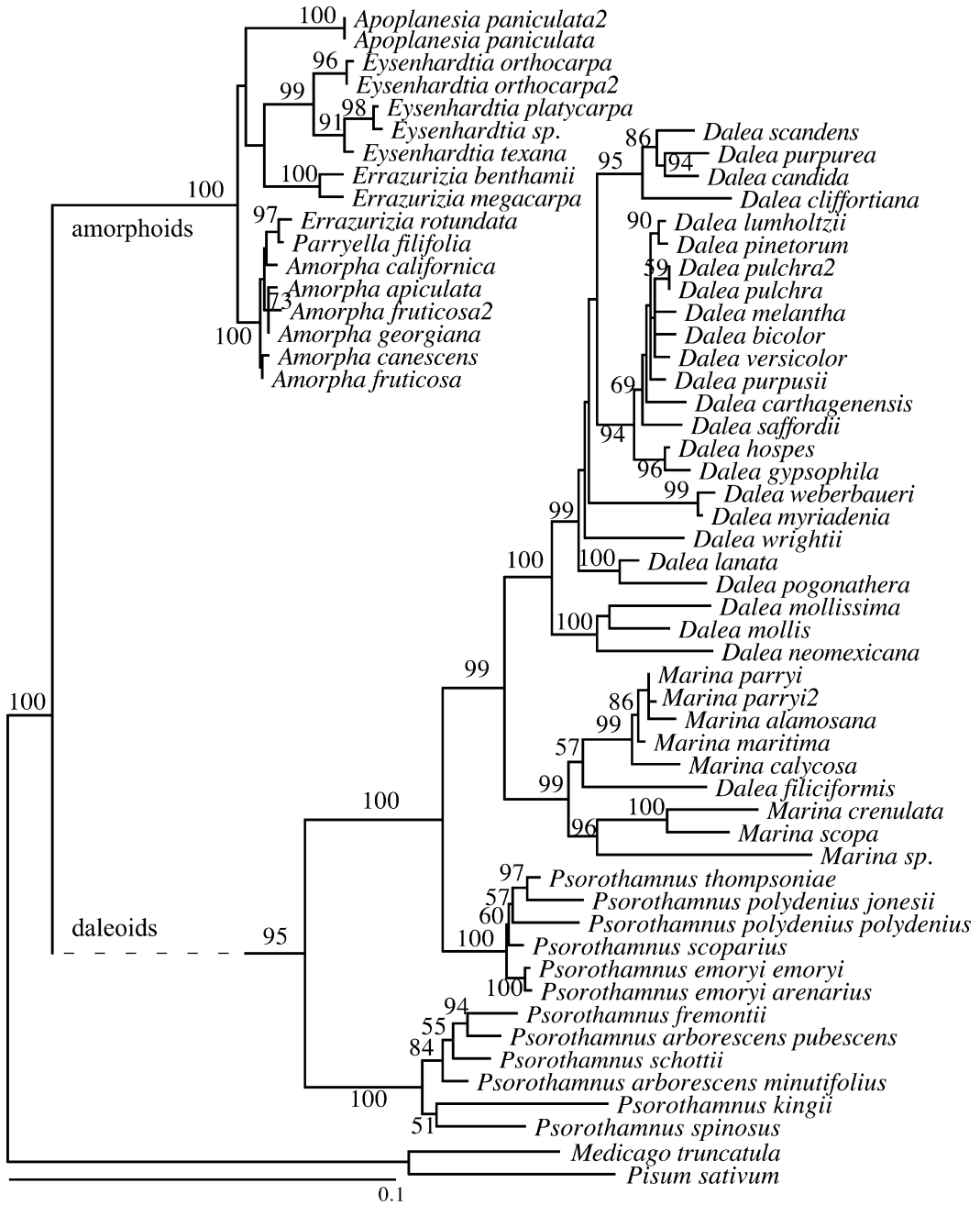


FIG. 4. Phylogenetic relationships among Amorpheae based on *trnK*, ITS, and CNGC4 DNA sequence data. Topology and branch lengths represent one of the two maximum likelihood trees obtained using an HKYT model of sequence evolution. Numbers above branches indicate parsimony bootstrap values greater than 50%. The dashed line leading to the daleoid clade is inserted for graphical convenience; the inferred branch length is given by the solid portion only.

TABLE II
RESULTS FROM UNCONSTRAINED AND CONSTRAINED PARSIMONY AND LIKELIHOOD ANALYSES

| Constraint | MP trees | Steps | Dif. (steps) | ML trees | ln L | Dif. (lnL) | P |
|---------------------|----------|-------|--------------|----------|---------------|------------|-------------------|
| None | 1080 | 2893 | | 3 | -22,082.43954 | | |
| <i>Amorpha</i> | 90 | 2893 | 0 | 2 | -22,084.56305 | -2.12351 | 0.8490 |
| <i>Dalea</i> | 1080 | 2912 | 19 | 2 | -22,139.70861 | -57.26907 | 0.0350 |
| <i>Errazurizia</i> | 360 | 2921 | 28 | 3 | -22,181.34296 | -98.90342 | 0.0010 |
| <i>Psorothamnus</i> | 1620 | 2943 | 50 | 2 | -22,189.02309 | -106.58355 | <0.0001 |

Significance value for difference in likelihood score was assessed using the SH test. Boldface text indicates significance at the 5% level.

1919, 1920, 1928a, 1928b; Isely, 1962). Hutchinson (1964) first suggested splitting Bentham's Psoraleae into two tribes, Daleae and Psoralieae, based on a single character, the position of the petals. In Hutchinson's Daleae, petals are perched above the rim of the hypanthium, appearing to attach to the fused staminal column (thereby the term "epistemonous," Barneby, 1977), whereas all petals of Psoralieae insert on the hypanthial rim. Several inconsistencies arose from this arrangement, including falsely placing in Psoralieae the (then) monotypic *Marina*, whose petals are perched just as in *Dalea*. Furthermore, Rydberg's *Psorothamnus* and *Psorodendron* (which have sessile petals) and the genus *Dalea* (which have epistemonous petals), had always been associated based on characteristics of the pod, glands, habit, and foliage (Munz, 1959; Shreve & Wiggins, 1964, Wiggins, 1980). Indeed, most species of *Psorothamnus* and *Psorodendron* were originally described as members of *Dalea*. The separation of taxa based solely on petal position, therefore, proved unsatisfying.

Barneby's (1977) re-evaluation of the group provided new characters on which to base tribal classification. Inflorescence architecture became primary in delimiting two newly circumscribed tribes: Amorpheae Borrissova emend. Barneby, in which inflorescences are terminal, and Psoraleae s. str., in which inflorescences are axillary. Furthermore, in Psoraleae s. str., leaves are usually trifoliolate whereas leaves in Amorpheae are mostly pinnate. This led Barneby to suggest a relationship between Psoraleae and Trifolieae, far separated from Amorpheae. Delimitation of these two tribes and the phylogenetic distance between them has

been further supported by additional characters, for example, gland anatomy and development (Turner, 1986), root nodules (Corby, 1981), pollen morphology (Ferguson & Skvarla, 1981; Ferguson, 1990), and aspects of flower development (Tucker & Stirton, 1991; McMahan & Hufford, 2002; Prenner, 2004). Molecular studies have also supported the monophyly of the two tribes and their separation on the legume tree. In studies that include more than one species of Amorpheae, they form a clade with high support and are often placed as sister to the dalbergioid clade by *trnK/matK* (Lavin et al., 2001; McMahan & Hufford, 2004; Wojciechowski et al., 2004), and *trnL* (Lavin et al., 2001; but note polytomy in Pennington et al., 2001). However, support levels for a dalbergioid + Amorpheae clade appear to depend on sampling; bootstrap values were either 92% (Lavin et al., 2001), 73%–78% (Wojciechowski et al., 2004), or 68% BS (McMahan & Hufford, 2004) using the same sequencing region but different sets of taxa. On the other hand, ITS places Amorpheae with the Brongiartieae (Hu et al., 2002) or in a basal polytomy (Lavin et al., 2001). Although the precise placement of Amorpheae is not yet clearly established, molecular data place Psoraleae phylogenetically quite distant, not close to *Trifolium* as Rydberg (1928a) and Barneby (1977) had suggested, but nested within the millettoid-phaseoloid clade (Hu et al., 2000; Kajita et al., 2001; Wojciechowski et al., 2004).

Barneby's entirely new concept for the membership of Amorpheae, therefore, has been supported by new data and new analytical techniques. The monophyly of Amorpheae sensu Barneby is consistently

supported regardless of molecular marker, optimality criterion, or choice of outgroup (Lavin et al., 2001; McMahon & Hufford, 2004; Wojciechowski et al., 2004). Also, within the tribe, many of Barneby's groupings are strongly supported by sequence data, but some refinements to his classification are required. The current best understanding of relationships within Amorpheae is presented in Figure 5, in which the cladogram was restricted to include only those hypothesized clades that received strong support from parsimony bootstrap and were present in the maximum likelihood tree. Clearly, much concords with Barneby's ideas, but do the few areas of discordance make sense?

According to data from nuclear ribosomal DNA and from the chloroplast *trnK* intron (including the *matK* gene), the nine species in *Psorothamnus* fall into two clades. These clades correspond neatly with Rydberg's original ideas of these taxa: (1) *Psorothamnus* Rydb., comprising genericitypus *P. emoryi* (A. Gray) Rydb., along with *P. scoparius* (A. Gray) Rydb., *P. polydenius* (Torr.) Rydberg, and their (now) synonyms, to which should be added *P. thompsonae* (Vail) S. B. Welsh & Atwood; and (2) *Psorodendron* Rydb., comprising genericitypus *P. johnsoni* (Torrey) Rydb. (= *P. fremontii* (S. Wats.) Rydb., fide Barneby, 1977), *P. kingii* (S. Wats.) Rydb., *P. arborescens* (Torr.) Rydb., *P. schottii* (Torr.) Rydb, and *P. spinosum* (A. Gray) Rydb., along with their (now) synonyms. Fruit and inflorescence characters support the paraphyly of *Psorothamnus* and the recognition of *Psorodendron*. Following additional morphological and taxonomic research, formal resurrection of *Psorodendron* will be published.

Barneby's expansion of *Marina* and the transfer thereto of dalean species with straight calyx trichomes, 10 chromosomes, and leaflets with epidermal lineoles is well supported by the molecular data, to the extent that it has been sampled. The inclusion of *Dalea filiciformis* is not outrageous, but requires further scrutiny. The leaflets of this species resemble the "typical" marina leaflet, and the pod has glands arranged in two concentric crescents, just like the pods of

Marina section *Marina*. The chromosome count for *D. filiciformis* is $2n = \pm 8$ which is elsewhere found only in a few daleas. Considering the leaflet, pod, and DNA characters, a formal transfer of *D. filiciformis* to *Marina* is supported, pending further investigation into the chromosome number.

Errazurizia, as circumscribed by Barneby (1962), has four species, three of which have been sequenced, and two of these form a clade ("*Errazurizia* s. str."). These two species are shrubs from around the Gulf of California and have flowers that, like *Eysenhardtia*, lack the wing/keel distinction, and are consequently only mildly zygomorphic. Morphologically very similar to one of these is the only amorphoid taxon in South America, the highly disjunct *Errazurizia multifoliolata* (Clos) I. M. Johnston. from northern Chile, which has not yet been included in the molecular analyses. The third sampled taxon, *E. rotundata* (Wootton) Barneby, has unusual morphology and the molecular data strongly support its placement away from the other two (the likelihood of it being placed with the others is nearly 100 lnL units worse). This Colorado Plateau shrub was first assigned to the genus *Parryella* as *P. rotundata* Wootton because it shares with *P. filifolia* Torr. & A. Gray ex A. Gray, the only other member of *Parryella*, a complete lack of corolla. Pod characteristics and habit caused Barneby to align *P. rotundata* with *Errazurizia*, but this was presented with uncertainty (Barneby, 1977), and its reassignment to the *Parryella* + *Amorpha* group is not a surprise. Pollen morphology agrees with these conclusions (Guinet & Ferguson, 1989). The evidence is mounting that *Parryella* and *E. rotundata* belong together, but whether they are nested within or are sister to *Amorpha* is not yet clear and awaits better sampling and more quickly evolving markers.

The largest genus, *Dalea*, contains at least 171 spp. (Barneby, 1977, 1980, 1981, 1988, 1990; Estrada-C. et al., 2004), ca. 2/3 of the entire tribe. Molecular data have been sampled the poorest in this genus (only 24 species, ~14%), but a few conclusions can be drawn. The members of subg. *Theodora* that were included in the study are the only sequenced species that have

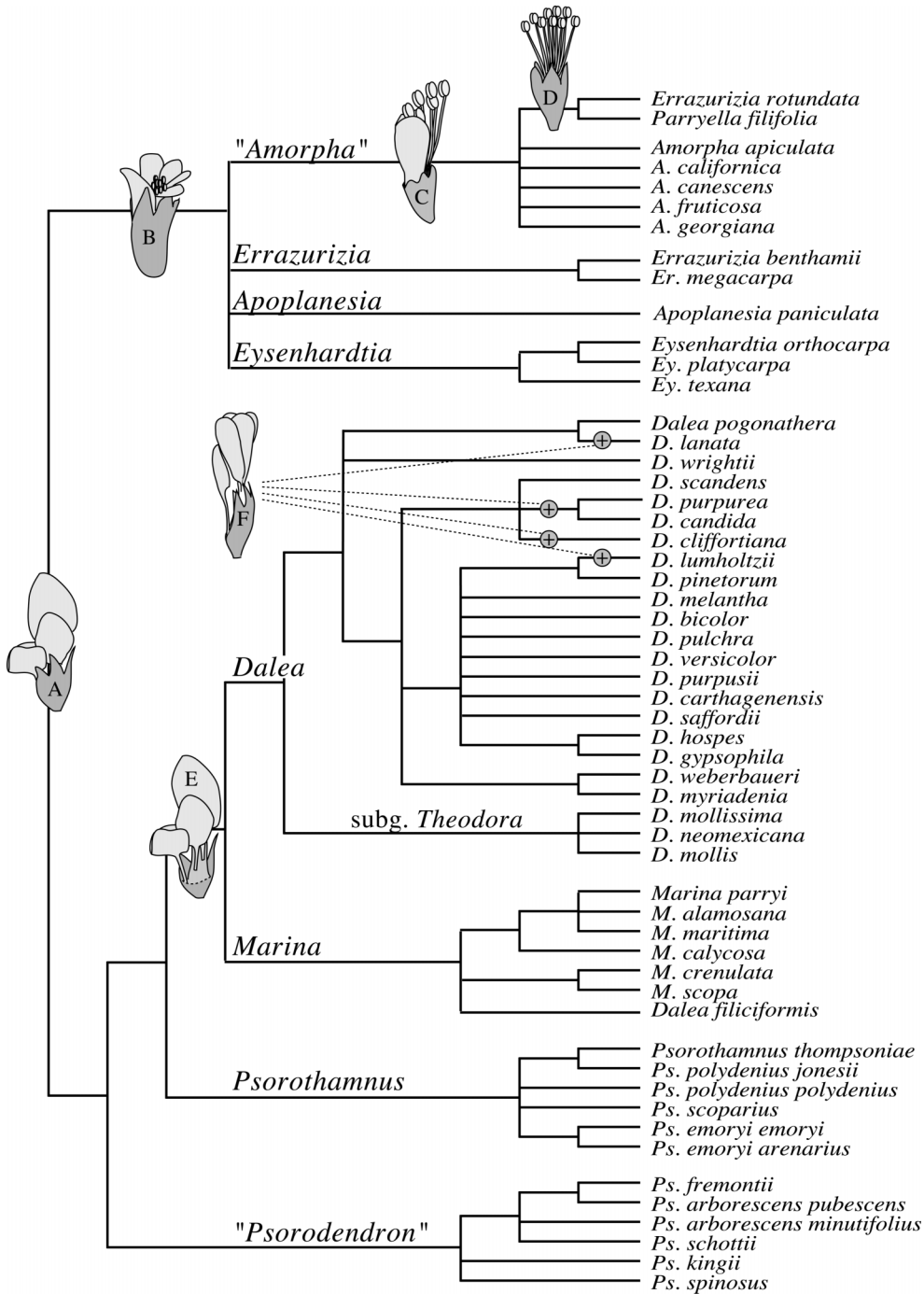


FIG. 5. Estimate of the phylogeny of Amorpheae, showing major events in flower evolution. Only those branches found in all most parsimonious trees and supported by >90% in the bootstrap analysis have been included. Clades that correspond to genera are indicated on branches and evolutionary changes in floral form are indicated by floral drawings. The ancestral flower, based on outgroup analysis (McMahon & Hufford, 2004), was papilionoid (A). Flowers with exposed androecia and non-differentiated petals evolved in the amorphoid clade (B). Loss of four petals (C) and loss of the banner petal (D) occurred in *Amorpha* and *E. rotundata* +

chromosome counts of $2n = 8$, and their monophyly is supported by sequence data (100% BS, Fig. 4). Further, they are strongly supported to be sister to the rest of *Dalea*, in which $2n = 7$ (all other Amorpheae have $2n = 10$, with the possible exception of *D. filiciformis*), in full agreement with Barneby's hypothesis. The rest of the *Dalea* species in the study fall in two subgenera in Barneby's taxonomy, subg. *Dalea* and subg. *Parosela* (Cav.) Barneby (treated as a separate genus by Rydberg, 1919, 1920). Most *Dalea* species are relatively similar in having standard papilionaceous corollas, except for the lateral petals inserting above the hypanthium. These species occupy the largest subgenus, *Parosela*. The second largest is subg. *Dalea*, which includes the American prairie clovers and other daleas with flowers that show little differentiation between wing and keel petals and in which the androecium is exposed at anthesis. According to the phylogenetic analyses, the sampled members of subg. *Dalea*, indicated in Figure 5, are scattered within subg. *Parosela*, and there is no support for mutually exclusive subgenera *Dalea* and *Parosela*.

MORPHOLOGICAL EVOLUTION IN AMORPHEAE

Rupert Barneby reorganized the members of Amorpheae based on insightful and extensive observation of morphology. What do the new, slightly modified hypotheses of relationship have to say about these characters? And what else have we learned about morphology of Amorpheae in the interim?

Amorpheae includes trees, shrubs, and herbs, with compound, unifoliolate, or simple leaves, and members live in mesic woodlands, grasslands, tropical deciduous forests, and alpine habitats. Yet, the most remarkable morphological evolution in Amorpheae has occurred in the flower. Petal number varies from five to zero; Barneby

suggested that the order of evolution was from five to smaller numbers, which is consistent with the phylogeny (Fig. 5). Current sequence data indicate that *Amorpha* (1 petal), *Parryella* (0 petals), and *Errazurizia rotundata* (0 petals) form a clade (Figs. 4, 5), implying that their loss of petals is evolutionarily related. The ancestral condition was probably five petals (McMahon & Hufford, 2004, 2005), but it is not clear from the phylogeny if the evolutionary events happened in a simple progression: five petals changed to one petal in the ancestor of the "amorpha" clade, changing again to zero petals in a lineage leading to *Parryella* and *E. rotundata*. If the phylogeny in Figure 4 is correct, and these two species are nested within *Amorpha*, then this seems the most plausible scenario. However, each of the three markers used in the molecular phylogenetic analyses (CNGC4, ITS, and *trnK*) root this clade differently, with different consequences for the inference of petal number evolution (Fig. 3A; McMahon & Hufford, 2005). Two species of *Dalea* have also lost petals (*D. urceolata* Greene and *D. confusa* (Rydb.) Barneby var. *hexandra* Barneby). Although they have not been sequenced, there is little doubt that they belong in *Dalea*, and therefore represent at least one other evolutionary loss of petals.

Another characteristic of several members of Amorpheae is the placement of the four lateral petals: sessile, i.e., on the rim of a hypanthium, as is common in legumes, or epistemonous, i.e., above the hypanthial rim on tissue that resembles staminal column. Barneby suggested that this condition is derived in *Dalea* and *Marina*, either in concert or independently if *Marina* is more closely related to *Psorothamnus* (Fig. 2). The phylogeny supports the former hypothesis because *Marina* and *Dalea* form a strongly supported clade, and all members

←

FIG. 5. continued

P. filifolia, respectively. Gain of a stemonozone occurred in the ancestor of *Dalea* + *Marina* (E). Losses of lateral petal differentiation in the paraphyletic subg. *Dalea* are indicated by circles and dashed lines (F). All species in the *Dalea* clade that are neither subg. *Theodora* nor subg. *Dalea* are in subg. *Parosela*. Ingroup taxa downloaded from GenBank that were redundant or unidentified (e.g., "*Marina* sp.") were eliminated.

of these genera have easily observed epistemonous petal placement. However, several species of *Psoralea* also have a very short region below the insertion of the lateral petals, difficult to see with a hand lens but apparent under scanning electron microscopy (McMahon & Hufford, 2002). Further analysis has found this “minute stemozone” in *Errazurizia megacarpa* and yet absent in *E. benthami* (McMahon & Hufford, 2005). This condition is unknown in *Eysenhardtia* and *Apoplanesia*, inapplicable in the “amorpha” clade, and awaits further investigation within the probable sister group to Amorpheae, the dalbergioid clade (Lavin et al., 2001). Therefore, all that can be concluded currently is that epistemonous petals serve as a synapomorphy for the *Dalea* + *Marina* clade, and the minute stemozone is possibly homoplasious (McMahon & Hufford, 2004, 2005).

Differentiation of the corolla into three types of petals (a banner, a pair each of wing and keel petals) is a relatively standard condition in Papilionoideae (Polhill, 1981; Endress, 1994). However, in Amorpheae, several taxa have non- or weakly-differentiated wing and keel petals. *Errazurizia* s. str., *Eysenhardtia*, and *Apoplanesia* all show poor lateral petal differentiation, and this is consistent with their phylogenetic placement together in the amorphoid clade (Fig. 5). Phylogenetically independent from these cases are those in *Dalea*. In Barneby’s treatment of the genus, the two largest subgenera are distinguishable, in part, because of petal differentiation: *Dalea* subg. *Dalea* has wings and keels that are quite similar whereas subg. *Parosela* has the standard three types of petals. Molecular phylogenetic analysis, however, does not concord with the subgenera. Poor resolution and insufficient taxon sampling within *Dalea* limits precise claims, but there is evidence for at least three separate cases of dedifferentiation in *Dalea*: (1) *D. lanata* Spreng., (2) *D. cliffortiana* Willd., *D. purpurea* Vent., *D. candida* Michx. ex Willd. (but note that the related *D. scandens* (Mill.) R. T. Clausen has 3 types), and (3) *D. lumholtzii* Robins. & Fern. (Fig. 5).

What did Barneby conclude about the

evolution of flowers in Amorpheae? He was rather circumspect, but he suggested something startling: a secondary derivation of the papilionoid flower. He speculated that this was the case because of two things: (1) the presence of the epistemonous petals, being a somewhat subtle but fundamental departure from standard papilionoid forms and indeed rare among angiosperms, and (2) the hypothetical phylogeny derived in part from polarizing characters such as wood, inflorescence structure, chromosome number, and androecial exposure. However, additional data and explicit phylogenetic analysis, as well as a perhaps better understanding of the relationship of Amorpheae to the rest of Papilionoideae, lead to the conclusion that the floral simplifications seen in the amorphoids and in several *daleae* as well as the epistemonous petals are all derived conditions. Indeed, it is not the case that the complex papilionoid flower has evolved more than once in Amorpheae; rather, simplifications have occurred.

Several species that were originally placed in the tribes Sophoreae and Swartzieae (Polhill, 1981) have atypical flowers (Polhill, 1981; Mansano et al., 2004). However, it is becoming clear with each additional phylogenetic study that many of these are not closely related, for example, the several that are scattered now in the dalbergioid clade (Pennington et al., 2000, 2001; Lavin et al., 2001; Wojciechowski et al., 2004). Therefore, an important question in legume diversification is the source of independent origins of simplified flowers, of which several examples are seen in Amorpheae.

NUCLEAR CNGC4

Plant phylogenetic systematics relies heavily on molecular data, particularly from chloroplast and nuclear ribosomal regions, but these regions are often insufficiently variable to resolve relationships among species. Therefore, discovering and testing low-copy markers from the nuclear genome (e.g., Bailey et al., 2004) is an appealing approach to add data and corroborate previously used markers, none of which are without problems (Álvarez & Wendel,

2003; Bailey et al., 2003; Wolfe & Randle, 2004). The nuclear marker CNGC4 holds promise for Amorpheae. CNGC4, when analyzed alone, shows clear signal for several clades that were also inferred using ITS and *trnK/matK*, providing corroboration. However, all three markers show weak but disparate signal regarding the root of the amorphoids. Additional taxon sampling for CNGC4, as well as the application of new nuclear markers, will likely be needed to resolve this issue.

CONCLUSIONS

Phylogenetic analysis is largely in agreement with Barneby's thorough taxonomic revision of Amorpheae. Amorpheae is monophyletic and only distantly related to the taxa with which they were previously grouped (Psoraleae s. str.). *Psorothamnus* should be split into the original Rydberg genera, *Psorothamnus* and *Psorodendron*. Monotypic *Parryella* and *E. rotundata* may be better considered as members of *Amorpha*, and *D. filiciformis* is more closely related to *Marina*. Plants in Amorpheae either have typical papilionoid flowers (*Psorothamnus* only) or have flowers that are atypical in several different ways, some of which have apparently evolved more than once.

Barneby's insights into Amorpheae were borne of years of field observations, beginning with his mid-1960's trips to Mexico (Crace, 2004). Having North American *Astragalus* well in hand, he turned to *Dalea*, about which the biographer D. Crace writes "the way in which *Dalea* most seductively resembled *Astragalus* was not as a legume but as a taxonomic knot to be untied." (Crace, 2004: 71). It is fortunate for students of legumes, New World floristics, and floral diversification that Rupert Barneby was intrigued in this manner.

Acknowledgments

I thank Martin Wojciechowski and Aaron Liston for the invitation to participate in the symposium honoring the life work of Rupert Barneby; Larry Hufford for collaboration on previous work in this group; two anonymous reviewers for helpful com-

ments; and the following herbaria for their generous loans: ARIZ, MO, NY, RSA/POM, UC/JEPS.

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