DANIEL ET AL.: PHYLOGENY OF TETRAMERIUM LINEAGE

The “*Tetramerium* Lineage” (Acanthaceae: Acanthoideae: Justicieae): Delimitation and Infra-lineage Relationships Based on cp and nrITS Sequence Data

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ABSTRACT. We used DNA sequence data from five genic regions (nrITS; chloroplast *trnL-F*, *trnT-L*, *rps16*, *trnS-G*) to study phylogenetic relationships of the *Tetramerium* lineage (Acanthaceae: Justicieae). From a sample of 70 species (representing 25 genera) previously affiliated with the *Tetramerium* lineage, 68 are included therein. Our analyses excluded Papuasian *Calycacanthus* and Neotropical *Streblacanthus monospermus* from the *Tetramerium* lineage; however, two species described in *Justicia* (*J. gonzalezii* and *J. medranoi*) and a Malagasy species of uncertain generic affinities are nested within the lineage. A monophyletic *Tetramerium* lineage consists of 23 currently recognized genera with at least 168 species, more than 70% of which occur in the New World. Old World *Chlamydacardia* and *Clinacanthus* are serially sister to all other members of the lineage. Other Old World taxa consist of: *Ecbolium* clade (all sampled species of *Ecbolium* plus Malagasy *Populina richardii)*, *Megalochlamys* clade (*Megalochlamys, Trichaulax* and the unidentified Malagasy plant), and two isolated taxa (*Angkalanthus* and *Chorisochora*). All analyses strongly support monophyly of the New World *Tetramerium* lineage. The three basal clades of New World plants, all with nototribic flowers, are: the taxonomically heterogeneous but palynologically consistent *Mirandea* clade, the *Pachystachys* clade, and the South American *Anisacanthus* clade. The last two are sister taxa and are together sister to all other New World plants, referred to here as the core *Tetramerium* lineage. We recognize five clades within the core *Tetramerium* lineage related as follows: (*Henrya* clade (*Carlowrightia parviflora* clade (North American *Anisacanthus* clade (core *Carlowrightia* clade + *Tetramerium*)))). Macromorphological synapomorphies are unknown for the *Tetramerium* lineage and for many of its constituent clades. However, we propose sternotribic flowers as synapomorphic for the core *Tetramerium* lineage and flowers with the lower-central lobe of the corolla modified into a keel as synapomorphic for a lineage consisting of *Tetramerium* and the core *Carlowrightia* clade. Palynological characters provide putative synapomorphies for some clades (e.g., *Ecbolium* clade, *Mirandea* clade) and autapomorphies for several species (e.g., *Mexacanthus mcvaughii*, *Trichaulax mwasumbii*). An Old World origin is postulated for the *Tetramerium* lineage, and we posit a single dispersal event to

America and subsequent extensive radiation there, especially in arid zones of Mexico and adjacent regions. Taxonomic implications of our results are extensive. Notably, many traditionally recognized genera (e.g., *Anisacanthus*, *Carlowrightia*, *Mirandea*) are not monophyletic and emphasis on floral form often has been phylogenetically misleading (e.g., floral adaptations to pollination by hummingbirds have evolved at least eight times in the New World *Tetramerium* lineage).

KEYWORDS: Acanthaceae, biogeography, cp sequences, nrITS, phylogeny, pollen, *Tetramerium* lineage.

A group of morphologically similar and mostly New World (NW) genera, including *Tetramerium*, has been recognized for more than 20 years (e.g., Daniel 1983, 2000; Daniel and Chuang 1993). Phylogenetic analysis of DNA sequence data confirmed the existence of the *Tetramerium* lineage as a strongly supported clade within Justicieae that includes at least 13 genera from both the Old World (OW) and NW (McDade et al. 2000a). In terms of morphology, plants of the *Tetramerium* lineage share the synapomorphies of Justicieae as a whole and lack those of other closely related lineages of Justicieae. For example, with few exceptions as detailed herein, members of the *Tetramerium* lineage share the pollen morphology that is hypothesized to be synapomorphic for Justicieae as a whole and is thus symplesiomorphic for this lineage: 3-colporate, 6-pseudocolpate (i.e., with two pseudocolpi per mesocolpium), and with compound sculpturing of exine in the interapertural regions (Fig. 1). The compound sculpturing consists of a prominent series of muri and lumina that form a coarse reticulum within which finer sculpturing appears foveolate to microreticulate (Fig. 2A, B); we refer subsequently to this as “bireticulate” sculpturing of the interapertural exine (cf. Daniel 1998:219). In addition, members of the *Tetramerium* lineage share the androecial synapomorphy of having two stamens and no staminodes (Fig. 3A) with all but the basalmost lineage of Justicieae (i.e., the *Pseuderanthemum* lineage sensu McDade et al. 2000a). Moreover, members of this lineage lack the synapomorphies of both their sister group (i.e., ‘justicioids’: corolla rugulate and thecae unequally inserted on the filament, often not parallel, and appendaged in various ways, cf. Fig. 3A, B) and the lineage basal to the *Tetramerium* lineage (i.e., Isoglossinae sensu Kiel et al. 2006: monothecous stamens and ‘gürtelpollen’). Almost without exception, chromosome counts of plants belonging to this lineage are $n = 18$ (Daniel 2000, 2007), but this number is not unique to the *Tetramerium* lineage. Thus, although plants of the *Tetramerium* lineage can generally be recognized by a combination of traits that are phylogenetically informative at other levels (or in the case of chromosome number may be homoplastic), molecular data provide the only known synapomorphies for the *Tetramerium* lineage (McDade et al. 2000a).

Based on macromorphology, pollen, or chromosome numbers, McDade et al. (2000a) hypothesized that a number of taxa that were not included in their study were also part of the *Tetramerium* lineage, and subsequent work has identified a number of additional putative members (Table 1). The present study tests these hypotheses by expanding sampling to include representatives of these taxa. We also test here the validity of the many small genera known or hypothesized to belong to the *Tetramerium* lineage: most contain < five species and seven are unispecific (Table 1). More generally, among genera in the *Tetramerium* lineage with multiple species, some have a long history of taxonomic recognition, but delimitation of them based on morphological characters has been problematic (e.g., Daniel and Chuang 1993; Vollesen 1994); indeed, morphological synapomorphies have been identified for very few genera of the *Tetramerium* lineage (McDade et al. 2000a). With expanded sampling within traditionally recognized genera, phylogenetic analyses of molecular data permit evaluation of generic delimitations.

Variation in floral form is extensive in the *Tetramerium* lineage (Fig. 3) and has contributed to the recognition of numerous taxa therein, both at generic and infrageneric ranks. Analogous differences in shape, size, and color of corollas have been used to distinguish genera from one another (e.g., *Carlowrightia* from *Anisacanthus*; Daniel 1983) and to distinguish sections within a genus (e.g., *Tetramerium*; Daniel 1986). Because such variation is known or expected to be associated with adaptation for pollinators, these kinds of characters may (if pollinator relationships are evolutionarily conserved) or may not (if these relationships are plastic) mark clades. Phylogenetic analysis of the *Tetramerium* lineage based on molecular data permits examination of the evolutionary pattern of floral traits and pollinator relationships, and evaluation of the taxonomic utility of these traits.

In this study we focus on the *Tetramerium* lineage with taxon sampling expanded to include representatives of all genera known or inferred to be part of this lineage (Table 1) and with nearly 5kb of DNA sequence data representing both the nuclear and chloroplast (cp) genomes. Our goals were: (1) to delimit the *Tetramerium* lineage based on molecular and morphological traits and to identify its constituent genera; (2) to propose phylogenetic relationships within the lineage; (3) to test monophyly of traditionally recognized genera and of infrageneric taxa (where previously proposed); (4) to test validity of the unispecific genera (i.e., are they phylogenetically isolated or are they nested among species belonging to other genera?); (5) to test hypotheses of inter- and intra-generic relationships that have been proposed in earlier research on members of the *Tetramerium* lineage (Table 2); (6) to gain insights into the historical biogeography of the lineage; and (7) to examine patterns of character evolution, including those associated with pollinators.

MATERIALS AND METHODS

Taxon Sampling. We obtained sequences for 68 species representing all genera placed in or tentatively assigned to the *Tetramerium* lineage by McDade et al. (2000a) (Table 1). One of our goals was to test monophyly of the larger genera (i.e., *Anisacanthus*, *Carlowrightia*, *Ecbolium*, *Tetramerium*) and we thus attempted to include species that represent recognized subgeneric taxa, if any, and that span the range of morphological diversity and geographic distribution of each genus. Our Malagasy collections contributed species of *Ecbolium* as well as one unidentified species with morphological characters suggestive of the *Tetramerium* lineage. Preliminary results from a parallel project on relationships among all genera of Justicieae placed two of three species (data for the third not available) treated in *Justicia* by Henrickson and Hiriart (1988) in the *Tetramerium* lineage and we include these here (i.e., *J. gonzalezii* and *J. medranoi*). To determine whether all putative in-group taxa are actually part of a monophyletic *Tetramerium* lineage, we included members of the other major lineages of Justicieae (i.e., the *Pseuderanthemum* lineage, Isoglossinae, and justicioids (sensu McDade et al. 2000a; Kiel et al. 2006). Appendix 1 lists all taxa included here, along with voucher data and sources of sequences that were not newly generated for this project.

Molecular Methods. Fresh leaf material, leaf material dried in silica gel, or recently collected herbarium specimens were used as sources of DNA. Total genomic DNA was extracted using the modified CTAB method of Doyle and Doyle (1987) except that QIAGEN DNeasy™ kits for plant tissue were usually used for herbarium samples. Procedures for amplifying all of the genic regions used here have been described in detail elsewhere as follows: *trnL-F* (McDade and Moody 1999), *rps*16 and *trnS-G* (McDade et al. 2005), *trnT-L* (Kiel et al. 2006), and nrITS (McDade et al. 2000b). Two of the cp regions required additional optimization with regard to primers. For rps16, the primer pairs that we have optimized for Acanthaceae (i.e., ACA5'rps16: GAGGACARRATCCGTTGTGGAT andACA3'rps16: AGACGGCTCATTGGGATA) were usually used as sequencing primers, and were used for initial amplification when the 5' and 3' exon primers designed by Downie and Katz-Downie (1999) failed. Our preliminary results with *trnT-L* were in accord with the observation of Shaw et al. (2005) that Taberlet et al.'s (1991) "a" and "b" primers for the *trnT-L* spacer do not as reliably amplify plant DNAs as the other Taberlet primers. As reported by Kiel et al. (2006), we optimized a primer for Acanthaceae that is slightly internal to the "b" primer (ACA3'*trnT/L*: 5'TTTNTACTNAAACCCTTGAAT) and were able to amplify and sequence about half of the target templates with the primer pair "a" and ACA3'*trnT/L*. Cronn et al. (2002) designed an alternative for the 5', "a" primer; combining this with either of the 3' primers just mentioned enabled us to sequence all but two of the acanths attempted.

The PCR and sequencing strategy for nrITS described in our previous papers on acanth phylogenetics left many taxa missing all or portions of sequences. This is partly because of poly-C/G runs in both its1 and its2 that often result in premature termination of sequence reading. However, about 1/3 of our target taxa failed to amplify entirely using the "N-nc18S10" and "C26A" primers of Wen and Zimmer (1996). S.-H. Oh (pers. comm., Duke Univ.) has designed an alternative primer anchored in the 18S ('its9': TCGTAACAAGGTTTCCGTAGGTG) that worked well in combination with C26A and enabled us to obtain sequence for all but one of our target taxa. Still, owing to the poly-C/G runs, completing these sequences often required multiple sequencing reactions and use of the internal primer its2 (Baldwin et al. 1995) that is seated in the 5.8s gene.

To optimize sequencing results, PCR products were run on a 1% agarose gel for several hours, the dominant band was excised, and the template was purified using QIAGEN QIAQuick™ gel extraction kits. Sequences were generated on ABI or Beckman automated sequencers; for most samples, both strands were sequenced for verification and to complete the sequence. Electropherograms of all sequences were proofread manually. Overlapping portions were reconciled by reverse-complementing one, aligning the two, and double-checking any inconsistencies against the electropherograms; mismatches that could not be resolved were coded as uncertain.

Sequencing Strategy. Sequences for all five loci were not obtained for all taxa, in most cases by design. Our previous work has consistently shown that *trnL-F* and *rps16* vary little among close relatives; our strategy was therefore to sequence thse regions for representatives of all clades identified in our preliminary work, including at least one species of each genus putatively placed in the *Tetramerium* lineage. As new clades were revealed (e.g., species of *Carlowrightia* are placed in three clades, see below), we added additional sequences for these slowly evolving loci. Preliminary work with nrITS (our most variable locus, see results) showed little variation among North American *Anisacanthus* and we did not attempt to gather sequence data for the less variable cp loci for two of six taxa (Appendix 1). Excepting these two *Anisacanthus*, we attempted to obtain sequences for *trnT-L* and *trnS-G* for all target taxa and gathered data for all but two and one of these taxa, respectively (Table 3). Likewise, for nrITS, we gathered sequence data for all but a single target taxon.

Alignment and Analysis. Sequences for each DNA region were aligned separately by eye in SeqApp 1.9a169 (Gilbert 1992). Sequences from the cp regions were easily aligned across sampled taxa; some regions of nrITS were more difficult to align and we fine-tuned the alignment until further changes had no impact on topology or goodness of fit statistics (i.e., consistency and retention indices). As noted by a number of authors (e.g., McDade and Moody 1999; Shaw et al. 2005), the non-coding cp regions have a relatively high frequency of parsimony informative indels (i.e., length mutations). Five, eight, seven, and 19 indels were added to the *trnL-F*, *trnT-L*, *rps16,* and *trnS-G* data matrices, respectively, as presence/absence characters. Ten indels were also added to the nrITS data set. The indels scored were identified conservatively (i.e., with common 5’ and 3’ termini) and were parsimony informative (i.e., shared by two or more taxa). Table 3 compares the five DNA regions in terms of length, variability, number of taxa sequenced, and missing data.

For analysis, data matrices for the five DNA regions were prepared as Nexus files in MacClade version 4.06 (Maddison and Maddison 2000). We tested for congruence among genic regions sequenced using the partition homogeneity test (implemented in PAUP* [Swofford 2000] as the Incongruence Length Difference test; 200 replicates, 25 random addition sequences, maxtrees = 10,000), and by inspection of the bootstrap consensus trees from analyses of each genic region for strongly supported conflicting clades. The cp regions were either congruent by the ILD test or marginally so (i.e., $P<0.05$ but some of random data partitions yielded summed lengths $\leq$ the sum of tree lengths from the original partitions, and summed tree lengths from all random partitions < 1% longer than the original) and there were no resolved differences among trees resulting from analyses of individual data sets, much less strongly supported differences. As a result, data for all four cp regions were combined.

The ILD test indicated significant incongruity between the combined cp and nrITS data. As a result, we analyzed the cp and nuclear data separately, and compared the results to identify taxa that were placed differently by data from the two genomes. These taxa were omitted to yield a core data set of 65 in-group taxa. Analyses of the nuclear and cp data for this core data set differed in degree of resolution but in no case could data from one genome reject the more resolved topology supported by the other genome. The two data sets were therefore combined and the analysis of the core set of taxa was used as a framework for study of the phylogenetic placement of excluded taxa. These were added serially, and results from analyses of the nuclear and cp data were compared in an effort to place these taxa and to understand the nature of conflict between the cp and nuclear data.

All parsimony analyses used rigorous heuristic search strategies designed to find all islands of equally parsimonious trees (i.e., multiple random addition sequences, TBR branch swapping). For the core taxon set, the combined (i.e., cp + nrITS) character set was analyzed using Bayesian likelihood as implemented in MrBayes v3.0B4 (Huelsenbeck and Ronquist 2001; Huelsenbeck et al. 2001; Ronquist and Huelsenbeck 2003). The program was run with settings corresponding to a GTR model with gamma-distributed rate variation and proportion of invariant sites estimated by the program; a mixed model approach permitted the model of evolution to be optimized independently for data from the two genomes. Two replicates of three heated and one ‘cold’ chain were run for 3,000,000 generations, with trees saved every 100 generations. Bayesian posterior probability values for branches were determined by opening the tree file produced by MrBayes in PAUP, filtering to remove the pre-burn-in trees, and then constructing the majority rule consensus tree; this tree is the maximum a posteriori (MAP) tree.

Support for individual branches in the parsimony trees was evaluated using nonparametric bootstrap values (BS; Felsenstein 1985) and decay indices (DI; Bremer 1988; Donoghue et al. 1992). Bootstrap values are from at least 100 replicates with 10 random addition sequences and

TBR branch swapping. DIs for each branch were determined by using MacClade to prepare a batch file that directed PAUP* to find the shortest trees inconsistent with each of the branches resolved in the strict consensus of MP trees. The difference between the length of these trees and the globally shortest trees is the DI for the branch in question.

Alternative phylogenetic hypotheses (Table 2) were evaluated using MacClade to prepare trees reflecting these relationships. These were loaded into PAUP* as constraint trees and the program was asked to find the shortest trees consistent with the constraints. One of the resultant MP trees consistent with each constraint was randomly selected and compared to one randomly selected MP tree using Templeton's test (reported as z statistics). The same strategy was used to compare likelihood scores of trees reflecting alternative phylogenetic hypotheses with all likelihood parameters (except base frequencies for which empirical values were used) estimated using one randomly selected MP tree. These parameters were then used as the model to compare likelihood scores of the most likely tree to that of trees consistent with each of the alternative phylogenetic hypotheses using the Kishino-Hasegawa RELL test (K-H RELL) as implemented in PAUP*. Tests were one-tailed because an optimal tree was one of the trees being compared in each case (Felsenstein 2004:369).

RESULTS

Molecular Evolution.—Assessed as proportion of parsimony informative sites, nrITS was most variable and *trnL-F* least variable (Table 3). Even the most variable of the cp regions (*trnS-G*) was considerably less variable than nrITS (12% vs. 30% variable sites) although indels (24 vs. 10) added comparatively more to the utility of *trnS-G* than to nrITS. Based on our earlier work with a closely related lineage of Justicieae, Isoglossinae (Kiel et al. 2006), we expected *trnT-L* to be more variable than *rps16* but this was not the case (Table 3).

Phylogenetic Relationships.—Unexpectedly, results indicated that *Calycacanthus* K.

Schum. is not part of the *Tetramerium* lineage. *Calycacanthus* was placed with strong support with one of the out-group taxa, *Ptyssiglottis*; however, data sets assembled for this study do not include enough taxa to place *Calycacanthus* with confidence. One member of the NW genus *Streblacanthus*, *S. monospermus* was placed outside of the *Tetramerium* lineage (nrITS) or far from other species of *Streblacanthus* and embedded in a clade of otherwise OW taxa of the *Tetramerium* lineage (*trnS-G*). It proved impossible to obtain sequence data for this taxon for the other three cp loci.

Comparison of the tree topologies from separate analyses of the cp and nrITS data sets indicated that the following taxa were placed differently by data from the two genomes: *Fittonia albivenis*, *Gypsacanthus*, *Justicia gonzalezii*, *Mirandea sylvatica*, *Yeatesia platystegia*, and *Y. viridiflora*. When these taxa were omitted, there were four differences between the cp and nrITS results (clade names are as in Fig. 4). (1) The cp data place *Carlowrightia ecuadoriana* in an unresolved polytomy with North American (NA) *Anisacanthus*, core *Carlowrightia*, and *Tetramerium*, but these data cannot reject the placement of this species basal to the core *Carlowrightia* clade, as supported by the nrITS and combined data sets (Fig. 4; +1 step, z=-1.000, P=0.317). (2) The strict consensus of MP trees from the nrITS analysis does not support monophyly of the *Henrya* clade but some of the MP trees are of this topology. (3) The *Pachystachys* clade is not resolved in the strict consensus of MP trees from the cp analysis but trees with this topology are among the MP trees. (4) The cp data provide less resolution among OW clades than do the nrITS data, which place *Chorisochora* and *Angkalanthus* in the *Ecbolium* clade, and this clade sister to the *Megalochlamys* clade; however, the cp data cannot reject the ITS topology (+1 step; z=-0.229, P=0.818). Given the lack of evidence for strongly supported conflict between the data from the two genomes, we combined the data, and present the results of parsimony and Bayesian analyses of these data for the core taxa as Fig. 4. Note that this combined analysis omitted the six problematic taxa; these are placed tentatively in Fig. 4, as described below.

***Major Clades of the* Tetramerium *lineage.*—**The *Tetramerium* lineage is monophyletic with strong support (Bootstrap, BS=100; Decay Index, DI=15; Bayesian posterior probability, BPP=100). The OW genera *Chlamydocardia* and *Clinacanthus* are sequentially sister to all other members of the lineage with strong support (BS=96, DI=10, BPP=100). All other members of the *Tetramerium* lineage form a strongly supported clade (BS=100, DI=10, BPP=100). The remaining OW taxa are placed in two strongly supported clades, the *Ecbolium* and *Megalochlamys* clades, plus two unaligned taxa, *Angkalanthus* and *Chorisochora*. The *Megalochlamys* clade is sister to the NW *Tetramerium* lineage with strong support from Bayesian analysis (BPP=100) but little support from parsimony (BS=66, DI=0). New World members of the *Tetramerium* lineage are monophyletic (BS=94, DI=5, BPP=100) and are placed in eight strongly supported clades: (1) *Mirandea* clade (BS=100, DI=9, BPP=100), (2) *Pachystachys* clade (BS=87, DI=4, BPP=100) (*Schaueria calicotricha* is the basal member of the *Pachystachys* clade but this relationship is only weakly supported), (3) South American (SA) *Anisacanthus* clade (BS=99, DI=8, BPP=100), (4) *Henrya* clade (BS=93, DI=6, BPP=100), (5) *Carlowrightia parviflora* clade (BS=100, DI=10, BPP=99), (6) NA *Anisacanthus* clade (BS=100, DI=7, BPP=100), (7) core *Carlowrightia* clade (BS=98, DI=6, BPP=100), and (8) *Tetramerium* (BS=100, DI=32, BPP=100). There is strong support for monophyly of the last five of these clades together and for relationships among them as follows: (4 (5 (6 (7+8)))). We refer to this last group (i.e., clades 4-8) as the 'core *Tetramerium* lineage' (Fig. 4). There is especially strong support from Bayesian analysis for the basal position of the *Mirandea* clade (i.e., BPP=100 for monophyly of all other NW *Tetramerium* lineage taxa, but BS=64, DI=2), and for the sister relationship between the *Pachystachys* clade and the SA *Anisacanthus* clade (BPP=98, BS=58, DI=2).

***Relationships within Major Clades of the* Tetramerium *Lineage*.**—*ECBOLIUM* CLADE. All sampled species of *Ecbolium* are part of this clade as is Malagasy *Populina*. The Malagasy and African taxa are reciprocally monophyletic.

MEGALOCHLAMYS CLADE. An unidentified Malagasy plant (*Daniel et al. 10455*) is sister to African *Trichaulax* with strong support (BPP=100, BS=95, DI=6) in a small clade that also includes the single sampled species of African *Megalochlamys*.

MIRANDEA CLADE. Three of five sampled species of *Mirandea* are part of a clade that also includes *Justicia medranoi* and unispecific *Hoverdenia*. *Mirandea grisea* and *M. huastecensis* are sister taxa with strong support (BPP=100, BS=100, DI=8), but *M. nutans* is basal to *Hoverdenia* + *J. medranoi* (BPP=100, BS=100, DI=9) such that the *Mirandea* species placed here do not form a clade.

PACHYSTACHYS CLADE. The sampled species of *Pachystachys*, *Schaueria*, and *Streblacanthus* are together monophyletic; support for the inclusion of *S. calicotricha* in this clade is weak (BS=66, DI=2, BPP=77) but inclusion of *S. azaleiflora* is strongly supported (BS=87, DI=4, BPP=100). Monophyly of the sampled species of *Pachystachys* and *Streblacanthus* is strongly supported (BS=98, DI=8, BPP=100). The two sampled species of *Pachystachys* are sister taxa in all analyses but with little support. The three sampled species of *Streblacanthus* form a paraphyletic grade basal to *Pachystachys* with no support for resolution of relationships.

SOUTH AMERICAN (SA) *ANISACANTHUS* CLADE. The two sampled species of SA plants that have been treated in *Anisacanthus* are placed as sister taxa but with weak support from parsimony and no support from Bayesian analysis. *Yeatesia mabryi* and *Mirandea hyssopus* are sister taxa (BS=98, DI=3, BPP=100) and are also part of this strongly supported clade.

HENRYA CLADE. Two unispecific genera (*Chalarothyrsus* and *Aphanosperma*), *Henrya* (1 of 2 species sampled), and one of the 12 sampled species of *Carlowrightia* (*C. myriantha*), are together monophyletic but without strong support for relationships among them.

CARLOWRIGHTIA PARVIFLORA CLADE. Four of 12 sampled species of *Carlowrightia* plus the single sampled Central American (CA) species of *Anisacanthus* compose this clade. There is strong support from Bayesian analysis but weak support from parsimony for the basal position of *A. tetracaulis*, for monophyly of the four species of *Carlowrightia*, and for the sister relationship of *C. hapalocarpa* + *C. parviflora*.

NORTH AMERICAN (NA) *ANISACANTHUS* CLADE. All sampled NA (i.e., Mexican and southwestern USA) species of *Anisacanthus* plus unispecific *Mexacanthus* are together monophyletic. The sister group relationship between *A. junceus* and *Mexacanthus* is especially strongly supported by Bayesian analysis.

CORE *CARLOWRIGHTIA* CLADE. Seven of 12 sampled species of *Carlowrightia* are monophyletic and, except for the inclusion of *C. ecuadoriana* as the basal member, there is mostly strong support for relationships among them: (*C. neesiana* + *C. arizonica*) + (*C. linearifolia* (*C. serpyllifolia* (*C. texana* + *C. torreyana*))). The two accessions of *C. arizonica* are sister taxa.

TETRAMERIUM. All sampled species of *Tetramerium* are monophyletic with remarkably strong support (BS=100, DI=30, BPP=100). The trio of (*T. abditum* (*T. glandulosum* + *T. ochoterenae*)) is sister to *T. tenuissimum* + a polytomy that includes all other sampled members of *Tetramerium*. Both accessions of *T. nervosum*, one from Arizona and one from Peru, are part of this polytomy.

Taxa placed inconsistently by cp and nrITS data sets.— *MIRANDEA SYLVATICA*. This species is placed by the cp data as a single species lineage, sister to the core *Tetramerium* lineage; in contrast, the strict consensus of trees from the nrITS data collapses both the *Mirandea* and *Pachystachys* clades with *M. sylvatica* part of this polytomy. The nrITS data cannot reject the cp topology (+1 step, z=-0.200, P=0.842). As discussed below, *M. sylvatica* differs from other *Mirandea* in a number of ways and lacks the pollen synapomorphies that we propose for the *Mirandea* clade. We thus treat *M. sylvatica* among the several lineages of NW plants basal to the core *Tetramerium* lineage but of otherwise uncertain relationships (Fig. 4).

YEATESIA. The nrITS data were unable to place *Y. viridiflora* and *Y. platystegia* with confidence: parsimony analysis yielded >100,000 trees on three islands that place these taxa differently with respect to the *Mirandea*, SA *Anisacanthus*, and *Pachystachys* clades. In contrast, the cp (and combined) data place these two species in the *Mirandea* clade with strong support. One of the islands from the nrITS analysis is of trees with essentially this same topology such that the nrITS data cannot reject the cp result. The cp data strongly reject placement of the two problematic species of *Yeatesia* with *Y. mabryi* in the SA *Anisacanthus* clade (+25 steps, z=-4.226, P<0.0001). As discussed below, there is also morphological support for placement of *Y. viridiflora* and *Y. platystegia* with the *Mirandea* clade; we thus provisionally accept this result (Fig. 4C).

FITTONIA. Our sample of *F. albivenis* is placed by the cp data in a polytomy with the SA *Anisacanthus* and *Pachystachys* clades, with the *Mirandea* clade basal to this polytomy and the core *Tetramerium* lineage distal. NrITS data place this taxon sister to the core *Tetramerium* lineage but with major rearrangements of the taxa basal to these clades. In particular, the *Pachystachys* clade collapses except for the sister relationship between the two species of *Pachystachys*. It is possible that our accession of *Fittonia* is of hybrid origin but neither the cp nor nrITS results clearly point to candidate taxa. Given the difficulties in working with nrITS in some plants of the *Tetramerium* lineage, it is also possible that our sequence data for this plant might be chimeric or for a pseudogene and thus misleading. Until these ideas can be evaluated, we treat this species as among the basal clades of the NW *Tetramerium* lineage but of otherwise uncertain relationships (Fig. 4).

JUSTICIA GONZALEZII. The cp data provide strong support for placement of this taxon with *M. hyssopus* and *Y. mabryi* in the SA *Anisacanthus* clade. In contrast, the nrITS data place *J. gonzalezii* basal to a lineage composed of the three basal clades of the core *Tetramerium* lineage: (*Pachystachys* clade (SA *Anisacanthus* clade + *Mirandea* clade)). The nrITS data cannot reject the cp topology (+2 or 3 steps depending upon how precisely the relationship is constrained, $z < -0.7$, $P \geq 0.5$), but the cp data strongly reject the nrITS topology (+21 steps, $z=-4.0415$, $P<0.0001$). It is possible that *J. gonzalezii* has chloroplasts of a member of the SA *Anisacanthus* clade as a result of hybridization, but the nrITS data do not provide a strongly supported alternative placement and cannot reject the cp topology. We tentatively accept placement of *J. gonzalezii* with the SA *Anisacanthus* clade (Fig. 4B).

GYPSACANTHUS. The cp (and combined) data place *Gypsacanthus* between the *Henrya* clade and the remaining members of the NW *Tetramerium* lineage. In contrast, the nrITS data place *Gypsacanthus* sister to (core *Carlowrightia* + *Tetramerium*) and collapse the *Henrya* clade with all of these taxa part of a polytomy with the *Carlowrightia parviflora* clade, NA *Anisacanthus* clade, and (*Gypsacanthus* (core *Carlowrightia* + *Tetramerium*)). The cp data reject the nrITS topology (+12 steps, $z=-2.558$, $P=0.010$) but the nrITS data cannot reject the cp topology (+2 steps, $z=-0.500$, $P=0.804$). It is possible that the cp and nucleus of *Gypsacanthus* have different evolutionary histories but our results do not provide clear signal of these histories. We treat this genus as part of the 'core *Tetramerium* lineage' but of otherwise uncertain relationships (Fig. 4).

Tests of Monophyly of Currently Recognized Genera and Sections. Our data support monophyly of *Tetramerium*, *Pachystachys*, and *Ecbolium* (including *Populina*) and cannot reject monophyly of *Schaueria* and *Streblacanthus* (excluding *S. monospermus*), which are paraphyletic in our results. All other genera of which we sampled more than one species were polyphyletic and monophyly can be rejected, as reported in Table 2 and discussed below. Likewise, our data reject monophyly of several of Daniel's (1983, 1986) sections of *Carlowrightia* and *Tetramerium* (Table 2), as discussed below.

DISCUSSION

The present study expands that of McDade et al. (2000a) nearly four-fold in terms of taxon sampling from the *Tetramerium* lineage and includes representatives of all genera tentatively assigned to the lineage by those authors. Results reported here confirm monophyly of the *Tetramerium* lineage and inclusion of these additional taxa, with two exceptions. The unispecific Papuasian genus *Calycacanthus* appears consistent with taxa in this lineage based on macromorphological and palynological characters. However, Daniel (2000) documented a chromosome number of $n = 16$ for this plant, a number unknown among members of the *Tetramerium* lineage. Indeed, our sequence data place it elsewhere in Justicieae. We will return to this taxon in a future study with a more comprehensive sample of Acanthaceae. *Streblacanthus monospermus* is also excluded from the *Tetramerium* lineage by our data. This species is morphologically disparate compared to congeners; its traits and placement are discussed below in the context of the genus as a whole. On the other hand, our continuing family-wide studies suggest that three additional unispecific genera may be members of the *Tetramerium* lineage: *Ancistranthus* Lindau (Cuba), *Celerina* Benoist (Madagascar), *Ichthyostoma* Hedrén & Vollesen (tropical eastern Africa). We will seek to include these in future work.

The *Tetramerium* lineage is well marked by molecular synapomorphies (including three length mutations), and it is remarkable that we know of no morphological synapomorphies that are shared by these plants. As set forth in the introduction, plants belonging to this lineage may be recognized by a number of traits that are synapomorphic at more inclusive levels (e.g., 3-colporate/6-pseudocolpate/bireticulate pollen; two stamens and no staminodes) or are symplesiomorphic compared to its sister group (i.e., 'justicioids'); a chromsome number of n = 18 is ubiquitous in the lineage but is infrequently reported among other Acanthaceae. Identifying non-molecular synapomorphies for the *Tetramerium* lineage is complicated by the fact that the two basal-most lineages are autapomorphic in a number of traits, as described below.

***Chlamydocardia*.** The basal clade of the *Tetramerium* lineage is the single sampled species of *Chlamydocardia*. Taxonomic uncertainties involving this poorly known genus of two (or up to four) species occurring in tropical Africa were noted by Heine (1966). The two species that have been confirmed for *Chlamydocardia*, *C. buettneri* (included here) and *C. subrhomboidea* Lindau, have conspicuous, spathulate to broadly obovate bracts and 6-colporate pollen with the mesocolpia covered with undulating ridges bearing papillae (Fig. 1K; Raj 1961; Scotland and Vollesen 2000); these palynological traits appear to be synapomorphic for the genus. The affinities of the other two species that have been treated in this genus (*C. lanciformis* Lindau, *C. nuda* C. B. Clarke) remain in doubt (Heine 1966). To further our understanding of the *Tetramerium* lineage, future taxonomic and phylogenetic work should include these.

***Clinacanthus* through NW *Tetramerium* Lineage.** All other sampled members of the *Tetramerium* lineage are monophyletic in our analyses with strong support. As for the lineage as a whole, we know of no morphological synapomorphies that mark this clade.

***Clinacanthus*.** This small genus (3 species) is the only exclusively Asian genus in the *Tetramerium* lineage. Pollen of *C. nutans* (Burm.f.) Lindau and *C. siamensis* (included here) is 3-colporate/6-pseudocolpate (Fig. 1B; rarely with some grains 4-colporate and 8-pseudocolpate fide Raj 1973), as is typical of the *Tetramerium* lineage. Further, pollen of *C. nutans* is bireticulate, again as typical of the *Tetramerium* lineage, but this trait has not been assessed for the remaining species. With regard to a number of other traits, including monothecous anthers and floral resupination, plants of this genus are unlike any others in the *Tetramerium* lineage although these traits are known among other Justicieae. These traits appear to be synapomorphic for *Clinacanthus* and have led to the misclassification of this genus in the past. *Clinacanthus* was treated by Lindau (1895) in Monotheciinae because of its monothecous anthers. It has also been included in Diclipterinae (e.g., Hu and Tsui 2002), likely because of its putatively resupinate flowers and monothecous anthers. Floral resupination was noted for both *C. nutans* (Bremekamp 1965) and *C. siamensis* (Bremekamp 1961). Other authors (e.g., Ridley 1923, Benoist 1935) have not noted this feature when describing plants of *Clinacanthus*. Examination of herbarium specimens of *C. nutans* (e.g., #4089E-1 from cultivated material, C) shows flowers that indeed appear to have the upper lip trilobed and the lower lip bilobed. However, this reversal in orientation of the lips is not the result of twisting of the corolla, as in other Justicieae with resupinate flowers (i.e., Diclipterinae, Daniel and McDade 2005), but is accomplished by a strong geniculation of the corolla tube (Fig. 3K). Similar bending (as opposed to twisting) of the corolla occurs in several species of *Strobilanthes* in Ruellieae (cf. Wood 1994; Bennett and Scotland 2003; T. Daniel, pers. obs.). Resupination has clearly been achieved via multiple mechanisms and at least some of these evolved more than once among acanths. Within the *Tetramerium* lineage, no other types of resupination are known.

Angkalanthus *through NW* Tetramerium *Lineage*. Sister to *Clinacanthus* is a very strongly supported clade that includes the vast majority of members of the *Tetramerium* lineage. Remarkably, we have discovered no morphological synapomorphies that support monophyly of this clade.

The remaining OW taxa are placed in two strongly supported clades, the *Ecbolium* and *Megalochlamys* clades, plus two unaligned taxa, *Angkalanthus* and *Chorisochora*. We discuss these clades in series below and then return to the topic of relationships among them.

Ecbolium *Clade*. The strongly supported *Ecbolium* clade includes all sampled species of *Ecbolium* and *Populina*. Vollesen (1989) recognized 22 species of *Ecbolium* occurring in tropical and southern Africa, Madagascar and the Comoros, southern Arabia, and southern to southeastern Asia; no infrageneric taxa were recognized. Species of *Ecbolium* are usually readily recognizable by their strobilate inflorescences with caducous bracts, flowers with elongate and cylindric corolla tubes, and narrow upper lips that are reduced in length and often recurved or arched. Most of these features are likely associated with pollination, but floral visitors remain undocumented for *Ecbolium*. Corolla color (i.e., usually yellow or greenish) may be derived as these colors do not occur among other OW plants of the *Tetramerium* lineage. Other traits that characterize these plants are symplesiomorphic including nototribic flowers (i.e., stamens proximate to the upper lip of the corolla and anthers opening toward the lower lip) and 3-colporate/6-pseudocolpate pollen (Fig. 1E) that is usually bireticulate, and/or homoplastic (e.g., *Trichaulax*, in the *Megalochlamys* clade, has a similarly reduced upper corolla lip).

Among the four sampled species of *Ecbolium*, Tanzanian *E. tanzaniense* is sister to widespread *E. viride*, confirming Vollesen's (1989) indication of an affinity between them. The other two species of *Ecbolium* in our sample are both from Madagascar and are sister taxa, again confirming Vollesen's (1989) suggestion of a close relationship. *Populina richardii* is sister to the two Malagasy species of *Ecbolium*. Although monophyly of *Ecbolium* cannot be rejected (+3 steps, z=-0.317, P=0.508), trees consistent with this constraint simply move *P. richardii* to immediately below *Ecbolium*.

Populina, a poorly known Malagasy genus of two species, was treated by Lindau (1895) in Isoglosseae: Isoglossinae, despite its not having "Gürtelpollen" like most other genera of the subtribe. Kiel et al. (2006) included *Populina richardii* in a study of relationships among Isoglossinae but this taxon was instead placed with strong support with the few members of the *Tetramerium* lineage that were included in that study as out-groups. The genus has never been adequately diagnosed and morphological synapomorphies that might characterize the two species remain unknown. Pollen of *P. perrieri* Benoist has been described as 3-colporate/6-pseudocolpate (Muller et al. 1989), the symplesiomorphic trait for the *Tetramerium* lineage, but pollen of *P. richardii* (included here) has not been studied. Our results indicate that *P. richardii* should be treated within *Ecbolium*. However, because diagnostic morphological characters for *Populina* are not established, the placement of *P. perrieri* remains uncertain.

Angkalanthus. The single species of *Angkalanthus* occurs on the Indian Ocean island of Socotra (Yemen) off the Horn of Africa (Vollesen 1994). Plants have pendulous inflorescences and orange-red corollas with the tube exceptionally expanded distally (Fig. 3I). These features are autapomorphic and undoubtedly play a role in pollination biology, details of which remain unknown. Other traits are symplesiomorphic (e.g., stamens proximate to the upper lip, 3-colporate/6-pseudocolpate pollen) or likely homoplastic (e.g., pedicellate flowers shared with *Chorisochora*; anthers held perpendicular to the filaments shared with *Megalochlamys* and *Trichaulax*). Stamens are proximate to the upper lip, but nototriby cannot be assumed owing to the perpendicular orientation of the anthers relative to the filaments; study of fresh material is necessary to assess direction of anther dehiscence and consequent location of pollen deposition on pollinators. We cannot identify synapomorphies to support (or refute) placement of this taxon basal to the *Ecbolium* clade (Fig. 4).

Chorisochora. Vollesen (1994) recognized three species of *Chorisochora*, one South African (sampled here: *C. transvaalensis*) and two from the island of Socotra (Yemen). The combination of flowers that are pedicellate and have stamens positioned most or less equidistant between the upper and lower lips seems to distinguish plants of this genus from other OW members of the *Tetramerium* lineage. However, strong evidence for monophyly of the genus is lacking because both characters may be homoplastic: *Angkalanthus* also has pedicellate flowers and the phylogenetic utility of staminal position is difficult to assess. *Megalochlamys* and *Trichaulax* apparently also have flowers with stamens positioned more or less equidistant between the upper and lower lip, but morphology and orientation of the lips varies among these taxa. Finally, it is clear that understanding of *Chorisochora* requires inclusion of the Socotran species in phylogenetic work.

Megalochlamys *Clade*. The single sampled species of *Megalochlamys*, *M. revoluta*, is the basal member of this clade that also includes *Trichaulax* and an unidentified Malagasy plant (*Daniel et al. 10455*). *Megalochlamys* consists of ten species from tropical and southern Africa and Arabia (Vollesen 1989); based on differences in seed ornamentation, Vollesen (1989) recognized three sections. The genus is marked by a number of palynological apomorphies, including reduced (often ± indistinct) colpi and absence of pseudocolpi in the mesocolpia (Fig. 1H; Furness 1989). Flowers of *Megalochlamys* have stamens positioned most or less equidistant between the upper and lower lip and anthers oriented perpendicular to the filaments. Although the pollen characters are synapomorphic for the genus, the floral characters are homoplastic (i.e., *Chorisochora* also has flowers with stamens positioned more or less equidistant between the upper and lower lip; *Angkalanthus* also has anthers held perpendicular to the filaments; both traits also occur in *Trichaulax* and may be synapomorphic for this genus plus *Megalochlamys*). Future work should include more species of *Megalochlamys* to test monophyly and Vollesen's (1989) infrageneric taxonomy. Based on clear palynological synapomorphies, we predict that species assigned to this genus will form a clade.

Prior to the work of Vollesen (1989), *Ecbolium* and *Megalochlamys* had been confused. Our results support Vollesen's recognition of two genera, and a sister relationship between the two is rejected by both parsimony and likelihood (Table 2). In fact, reproductive characters seem to clearly distinguish these two genera: *Megalochlamys* has floral bracts that are persistent (vs. usually caducous in *Ecbolium*), flowers that have mostly blue (vs. mostly greenish to yellowish) corollas with the tube usually shorter (vs. considerably longer) than the lips, stamens positioned more or less equidistant between the upper and lower lip (vs. stamens proximate to the upper lip) with straight (vs. curved) anthers that are perpendicular (vs. parallel) to the filaments, and indistinctly (vs. distinctly) colporate pollen with no (vs. six) pseudocolpi. Floral differences suggest that plants in these genera rely on different predominant pollinators.

Megalochlamys revoluta is sister to *Trichaulax* + an unidentified Malagasy plant (*Daniel et al. 10455*). Vollesen (1992) recognized a single species of *Trichaulax* from tropical eastern Africa based in part on the remarkable pollen grains (Furness 1992). Pollen of *Trichaulax* (Fig. 1L) is (4--) 5 (--6)-colporate; the colpi expand or bifurcate toward the poles; there are two or four pseudocolpi per mesocolpium with the two pseudocolpi (or when four, the corresponding pseudocolpi from each pair) usually fused toward one (forming pseudocolpal arcs) or both poles (forming pseudocolpal circles or ellipses); and the reticulum of the exine is coarser at the poles than elsewhere on grains. The combination of these characteristics appears to be unique in the family (Furness 1992). In contrast, pollen of *Megalochlamys* is 3-colporate and lacks pseudocolpi. Despite these differences in pollen morphology, our results support Vollesen's (1992) suggestion of a close relationship between the genus and *Megalochlamys*. These plants share numerous traits, among which stamens positioned more or less equidistant between the upper and lower lip and anthers held perpendicular to the filaments may be synapomorphic, although both are homoplastic (i.e., *Chorisochora* has stamens positioned similarly, and *Angkalanthus* shares the anther orientation character). The unidentified Malagasy plant, *Daniel et al. 10455*, has flowers with anthers borne parallel to the filaments and the 3-colporate/6-pseudocolpate/bireticulate pollen (Fig. 1D) that is plesiomorphic for the *Tetramerium* lineage as a whole. Position of the stamens relative to the corolla lips and direction of anther dehiscence are not known for this taxon. Thus, synapomorphies for the *Megalochlamys* clade remain to be identified, but the clade is notable for transitions to autapomorphic pollen types and homoplastic changes in orientation of anthers.

From the foregoing, it is clear that putatively synapomorphic traits are homoplastic among these four clades of OW plants. Caducous bracts may be an exception as this trait seems to occur only in *Chorisochora, Angkalanthus,* and *Ecbolium* (unknown in *Populina richardii* but *P. perrieri* appears to have caducous bracts). Our data provide very weak support for a clade including these taxa (Fig. 4; BS=58, DI=0, BPP<50). However, degree of persistence of bracts has not been widely studied or reported such that caution is warranted. We suspect that position of anthers relative to corolla lips and especially direction of dehiscence of anthers may be phylogenetically informative, but both are difficult to assess except from fresh materials and have not been explicitly studied in these OW plants. In sum, we can identify no non-molecular synapomorphies that unambiguously support resolution of relationships among these clades and thus treat them as unresolved (Fig. 4) while pointing to the need for more study. Vollesen (1994) has worked with most of these plants; he noted that they are all African representatives of Odontoneminae but did not infer degrees of relatedness except to predict a close relationship between *Trichaulax* and *Megalochlamys* as noted above.

It is likewise not possible to identify characters that support the sister relationship between the *Megalochlamys* clade and the NW *Tetramerium* lineage that is strongly supported by Bayesian analysis (Fig. 4). Notably, plants in the basal three lineages of the NW *Tetramerium* lineage have flowers with the stamens held proximate to the upper lip. This differs from the possibly synapomorphic trait for the *Megalochlamys* clade of having stamens positioned more or less equidistant between the upper and lower lip. Flowers of the three basal NW lineages are also nototribic (i.e., with anthers dehiscing toward the lower lip) but, as noted above, comparative data are lacking for many OW members of the *Tetramerium* lineage.

NW* Tetramerium *Lineage. All sampled NW plants are monophyletic with strong support. The common ancestor of this lineage shared a number of symplesiomorphic characters including 3-colporate/6-pseudocolpate/bireticulate pollen. Nototribic flowers also likely represent a symplesiomorphy for this lineage as plants of the three basal lineages share the trait and other floral patterns, if present among OW taxa, are likely autapomorphic. As discussed below, our results indicate that, in many cases, current taxonomy of plants in this lineage is not congruent with their phylogenetic relationships. Failure of gene trees to recover species phylogenies owing to biological processes other than divergent evolution (e.g., hybridization, cp capture) may explain some cases of incongruence. However, in a number of these, morphological characters do not provide strong support for current taxonomy and, in fact, instead support our phylogenetic results.

Mirandea *Clade*. Three of five sampled species of *Mirandea* are part of the basal lineage of NW plants that also includes unispecific *Hoverdenia* and a species described as *Justicia medranoi* by Henrickson and Hiriart (1988). Following examination of the results from separate analyses of the nrITS and cp data sets, we also accept placement of two species of *Yeatesia* (*Y. platystegia* and *Y. viridiflora*) in this clade (Fig. 4C). Although this is a taxonomically heterogeneous group, plants belonging to this clade share pollen with interapertural exine that is not bireticulate as a palynological synapomorphy (e.g., Fig. 2C—E). The lineage is also geographically cohesive, with most species occurring in arid habitats of central to northern Mexico (*Y. viridiflora* occurs in mesic habitats in the southeastern USA). Two morphologically similar species of the Chihuahuan Desert region, *M. grisea* and *M. huastecensis*, form a clade that is sister to the remaining members of the clade. *Yeatesia viridiflora*, *Y. platystegia*, and *Mirandea nutans* are, in series, phylogenetically basal to *Hoverdenia* + *J. medranoi*. These last two taxa share an additional palynological synapomorphy of relatively broad colpi (Fig. 1F, G). Pollen of *Y. platystegia* and *Y. viridiflora* greatly resembles one another, with colpi reduced and pseudocolpi indistinct (Fig. 1I; see also Hilsenbeck 1989: Figs. 3—8), and it is surprising that these are not sister taxa. Similar pollen that lacks pseudocolpi and has reduced to nearly indistinct colpi is characteristic of *Megalochlamys* in the OW (Furness 1989). Our results refute a close relationship between these genera (+29 steps, z=-3.387, P=0.0007), indicating instead that there has been parallel evolution of pollen traits in these two groups of plants.

The *Mirandea* clade is notable for diversity of corolla morphology. The species of *Mirandea* that are placed here have relatively small, blue-purple corollas (Fig. 3D). Visitors to *M. grisea* and *M. huastecensis* include small- to medium-sized bees (T. Daniel and M. Manktelow, pers. obs.). The two species of *Yeatesia* have white to pinkish to lavender corollas with ± elongate, slender tubes suggesting adaptation for lepidopteran pollination. *Hoverdenia* has relatively large yellow corollas with a crateriform to saccate throat, maroon tinged bracts, and a large calyx (Fig. 3E). Its pollinators remain to be identified. *Justicia medranoi* has red or orange-red flowers likely adapted for pollination by hummingbirds. Clearly, this small clade shows considerable divergent evolution associated with adaptation for pollinators.

The *Mirandea* clade is also notable for taxonomic heterogeneity. Relationships for unispecific *Hoverdenia* have not previously been proposed and, although these plants have many highly distinctive and no doubt autapomorphic characters (e.g., large calyx, bract coloration, corolla form), placement of the genus in the *Mirandea* clade does not, to our knowledge, conflict with other putative synapomorphies. Indeed, a potential additional synapomorphy for *J. medranoi* and *Hoverdenia* is the possession of distinctive, geniculate trichomes on some vegetative organs (see Daniel and Acosta 2003: 63, B in Fig.; similar trichomes have been observed on *Tetramerium carranzae* (Daniel 2003a: 44, Fig. 5b).

It is at first surprising that two species of *Justicia* are placed in the *Tetramerium* lineage by our data (i.e., *J. medranoi*, *Mirandea* clade; *J. gonzalezii,* SA *Anisacanthus* clade). However, in treating these (plus *J. zopilotensis* Henr. & Hiriart) in *Justicia*, Henrickson and Hiriart (1988) acknowledged that the plants have some traits more at home in the *Tetramerium* lineage, and also that treating them in *Justicia* was likely a 'holding action.' These authors did not expect the three taxa to be close relatives and noted that pollen of *J. medranoi* is unlike that of the other two. Our results confirm Henrickson and Hiriart's (1988) conjecture in that at least *J. gonzalezii* and *J. medranoi* are not closely related; further, pollen morphology corroborates placement of *J. medranoi* in the *Mirandea* clade by our data. Interestingly, our observations do not confirm the presence of a rugula in *J. medranoi* as was noted by Henrickson and Hiriart (1988); the presence of a rugula would suggest that placement of the plant in *Justicia* was correct (see McDade et al. 2000a).

Inclusion of three species of *Mirandea* and two of *Yeatesia* adds to the taxonomic diversity of the *Mirandea* clade. Daniel (2003a, 2003b) treated *Mirandea* as comprising six species that share relatively small, nototribic flowers, and styles that are recurved just proximal to the stigma. These characteristics are likely associated with floral visitation by bees and/or flies (Daniel 1982; T. Daniel and M. Manktelow, pers. obs.) and none is clearly synapomorphic. Our results place two of five sampled species of *Mirandea* outside of the *Mirandea* clade and strongly reject monophyly of the genus (Table 2). These results are not especially surprising because both *M. sylvatica* and *M. hyssopus* have pollen with the bireticulate interapertural sculpturing (Fig. 1C; Daniel 2003a) that is symplesiomorphic for the entire *Tetramerium* lineage rather than the non-bireticulate sculpturing that we posit as synapomorphic for the *Mirandea* clade. *Mirandea hyssopus* (placed by our results in the SA *Aniscanthus* clade, see below) is similar to its three congeners in the *Mirandea* clade in floral characters, as well as habitat and range (i.e., plants occur in the same arid region of Querétaro and Hidalgo as *M. nutans*). However, these floral characters appear to reflect parallel adaptation to shared pollinators rather than evolutionary history. In contrast, *M. sylvatica* is unique in the genus in several macromorphological characters (yellow vs. bluish to purplish corollas; large, long-petiolate leaves vs. small, short-petiolate leaves) and occurs in rain forests of southern Mexico (vs. arid regions farther north).

Two of the three species of *Yeatesia* are placed by our analysis in the *Mirandea* clade, whereas the third, *Y. mabryi* is placed in the SA *Anisacanthus* clade. As for *Mirandea*, these results are not surprising in that *Y. mabryi* lacks the pollen trait that we posit as synapomorphic for the *Mirandea* clade and has instead pollen with the bireticulate interapertural sculpturing that is symplesiomorphic for the entire *Tetramerium* lineage. Species treated by Hilsenbeck (1989) in *Yeatesia* share quaternate spikes with large, imbricate bracts; corollas with a ± elongate and slender tube; and capsules with slight separation of the septae/retinacula from the inner wall at maturity. In circumscribing *Yeatesia*, Hilsenbeck (1989) noted the difference in pollen morphology between *Y. mabryi* and the other two species, and also that the former species differs by its larger, blue to purple (vs. white to pinkish or lavender) corollas with a longer tube (Fig. 3H), and larger seeds. Our data strongly reject monophyly of *Yeatesia* (Table 2) and indicate that the differences noted by Hilsenbeck (1989) are phylogenetically significant.

Hilsenbeck (1989) considered *Tetramerium* (in which *Y. platystegia* was originally described) and *Dicliptera* (into which *Y. viridiflora* was transferred by Long [1970]) to be close relatives of *Yeatesia* among NW Odontoneminae, and the OW genus *Ecbolium* to be its closest relative. Indeed, he noted that in characters of the inflorescence, corolla, androecium, and seeds

Ecbolium and *Yeatesia* do not differ significantly. *Dicliptera* has been shown to belong to a different lineage of Justicieae (see McDade et al. 2000a) and no species of *Yeatesia* is particularly closely related to either *Tetramerium* or *Ecbolium.* As monophyly of *Yeatesia* is resoundingly rejected by our data (Table 2), so too would be constraining this polyphyletic assemblage as a whole to a close relationship with *Ecbolium* or *Tetramerium.*

NW* Tetramerium *Lineage Distal to the* Mirandea *Clade. All members of the *Tetramerium* lineage distal to the *Mirandea* clade are monophyletic with especially strong support from Bayesian analysis. We have been unable to identify morphological synapomorphies that corroborate this clade.

***SA* Anisacanthus *Clade*.** As just discussed, the Mexican species *Mirandea hyssopus* and *Yeatesia mabryi* are part of this clade that also includes the two sampled species of SA *Anisacanthus*. Following examination of the results from separate analyses of the nrITS and cp data sets, we also accept placement of Mexican *Justicia gonzalezii* in this clade. *Mirandea hyssopus* is sister to *J. gonzalezii*, *Yeatesia mabryi* is sister to these two, and the two SA *Anisacanthus* are weakly supported as sister taxa. These plants retain the symplesiomorphic characters of nototribic flowers and 3-colporate/6-pseudocolpate/bireticulate pollen (Fig. 1A). The two SA species of *Anisacanthus* are likely pollinated by hummingbirds. The three Mexican taxa in this clade have conspicuously different floral forms, apparently reflecting specialization for pollination by hummingbirds (*J. gonzalezii*), lepidopterans (*Y. mabryi*; Fig. 3H), and bees/flies (*M. hyssopus*). The pattern of relationships among species suggests shifts in pollinators for *M. hyssopus* and *Y. mabryi*.

As for the *Mirandea* clade, the SA *Anisacanthus* clade is taxonomically heterogeneous. However, as discussed above, both *Mirandea hyssopus* and *Yeatesia mabryi* are distinctive in the genera in which they have been treated and it is not surprising that these genera are not monophyletic. *Anisacanthus* includes species that occur in three regions: South America (i.e., Argentina, Bolivia, Brazil, Guyana, Paraguay, Suriname, Venezuela), Central America (i.e., El Salvador to northern Costa Rica) and North America (i.e., Mexico to southwestern USA). Flowers of these species are superficially similar in that all are likely adapted for hummingbird pollination. However, SA taxa have nototribic flowers whereas the NA and CA species have sternotribic flowers (i.e., with the stamens proximate to the lower lip and opening toward the upper lip). Thus, it is not especially surprising that *Anisacanthus* is not monophyletic in our analyses. Interestingly, Mexican *Justicia gonzalezii*, here placed in the SA *Anisacanthus* clade, was originally described in *Anisacanthus*, but was referred to *Justicia* (Henrickson and Hiriart 1988) largely because of its nototribic flowers. In terms of corolla and pollen morphology (cf. Henrickson and Hiriart 1988: Figs. 1, 2, 5) this species is at home in the *Tetramerium* lineage as a close relative of *Anisacanthus* from South America. Based on these same traits, the third species treated in *Justicia* by Henrickson and Hiriart (1988), *J. zopilotensis*, likely also belongs to the SA *Anisacanthus* clade in the *Tetramerium* lineage rather than to *Justicia*.

Our data suggest that the SA *Anisacanthus* clade originated in South America and that, after a dispersal event to Mexico, the lineage diversified morphologically in response to selection by different pollinators. As discussed below, this is one of several clades within which significant disjunctions between South and North America seem to have taken place.

Pachystachys *Clade*. This clade includes all sampled species of *Pachystachys*, *Schaueria*, and *Streblacanthus*. *Pachystachys* is weakly support as monophyletic and the three species of *Streblacanthus* form a paraphyletic grade below *Pachystachys* although monophyly cannot be rejected. The nrITS data place the two sampled species of *Schaueria* as sister taxa but this relationship is not supported by the cp data. Plants in the *Pachystachys* clade are principally SA. These plants retain the symplesiomorphic characters of nototribic flowers and, so far as is known, 3-colporate/6-pseudocolpate/bireticulate pollen. We cannot identify non-molecular synapomorphies for this clade, but Hilsenbeck and Marshall (1983) noted similarities between *Schaueria* and *Streblacanthus* and suggested that these genera may not be separable. Also, one species of *Schaueria*, *S. parviflora* (Leonard) T.F. Daniel, was originally described in *Streblacanthus*, and at least one species of *Pachystachys*, *P. badiospica* Wassh., resembles *Streblacanthus* in both inflorescence and fruit characters. The only unambiguous characters that distinguish these last two genera pertain to the corolla and are undoubtedly related to different pollination syndromes. It is thus not surprising that species of these three genera form a clade in our results.

Like the other clades of the NW *Tetramerium* lineage discussed above, the *Pachystachys* clade includes species that are apparently adapted for different pollinators, although floral visitors have not, to our knowledge, been documented for *Schaueria* or *Streblacanthus*. Based on their form and size, we hypothesize that flowers of the former are pollinated by hummingbirds whereas those of the latter are likely adapted for lepidopterans. All but one species of *Pachystachys* have flowers with red corollas that have been noted (or assumed) to be visited and likely pollinated by hummingbirds (Wasshausen 1986). *Pachystachys lutea* has yellow bracts, white corollas, and green anthers but is likely pollinated by hummingbirds as well. The pattern of relationships among the taxa included here suggests that hummingbird pollination gave rise to lepidopteran pollination in this clade, although care is warranted given weak support for many aspects of relationships within this clade.

The *Pachystachys* clade is taxonomically diverse but the congeneric species placed here are adjacent in our phylogenetic results when not monophyletic, and no species of these genera is placed elsewhere. Further, monophyly of the paraphyletic genera *Schaueria* and *Streblacanthus* cannot be rejected by our data (Table 2). About 20 species are currently recognized in *Schaueria*, but no revision of the genus has been undertaken since 1847. *Schaueria* is not well collected and the actual number of species might be considerably fewer.

Our results included the commonly cultivated Brazilian species *S. calycotricha* and *S. azaleiflora* from Bolivia. Most species of *Schaueria* have white to yellow corollas subtended by filiform bracts, bracteoles, and calyx lobes. The filiform morphology of the latter structures might represent a morphological synapomorphy for the genus. However, some species (e.g., *S. azaleiflora*) deviate from these characteristics and the genus merits considerable additional study. Most species occur in Brazil and adjacent nations, but an amphitropical disjunct (*S. parviflora*) occurs in Mexico and Guatemala, and an amphiatlantic disjunct has been described from western tropical Africa (*S. populifolia* C.B. Clarke). Given our results for the *Tetramerium* lineage as a whole, it would be remarkable if this western African species is indeed a *Schaueria*.

Three of four included species of *Streblacanthus* form a paraphyletic grade basal to the two sampled species of *Pachystachys*. The fourth *Streblacanthus*, *S. monospermus* was excluded from the *Tetramerium* lineage by our data; these plants differ from the other species of *Streblacanthus* in a number of morphological features and our results regarding this species are not surprising. *Streblacanthus monospermus* has a four-lobed calyx (vs. 5-lobed in other species); a subsalverform corolla with a subactinomorphic limb (vs. strongly zygomorphic limb, Fig. 3L); anthers with thecae superposed, the distal theca fertile, and the proximal theca sterile and much reduced in size (vs. anthers with equally to subequally inserted, equally sized, fertile thecae); and seeds with a swollen margin (vs. seeds with undifferentiated margin) (Daniel 2001). The resemblance of flowers of the phylogenetically unrelated *S. monospermus* to those of other species of *Streblacanthus* (i.e., possession of a slender, elongate corolla tube) likely results from convergence due to adaptation for similar (lepidopteran?) pollinators, rather than from recent common ancestry.

Wasshausen (1986) recognized 12 species in *Pachystachys*; these are native to lowland rain forests of South America and the West Indies. Wasshausen (1986) did not recognize infrageneric taxa or hypothesize close relatives of *Pachystachys*. The large, imbricate bracts and large, infundibular corollas adapted for hummingbird pollination permit ready recognition of *Pachystachys.* These traits are synapomorphic within the *Pachystachys* clade but are homoplastic in any broader phylogenetic context.

Pachystachys *Clade + SA* Anisacanthus *Clade.* There is weak support from parsimony but stronger support from Bayesian analysis for a sister relationship between these two clades (BS=58, DI=2, BPP=98). We know of no morphological characters that support or contradict this relationship.

***Species Incertae Sedis:* Fittonia albivenis *and* Mirandea sylvatica.** Our data indicate that these taxa are among the basal clades of the NW *Tetramerium* lineage but do not support more precise placement. Both *Fittonia albivenis* and *Mirandea sylvatica* retain the symplesiomorphic traits of nototribic flowers and 3-colporate/6-pseudocolpate/bireticulate pollen (Figs. 1C, 2B), and they lack morphological traits that would clearly link them to any of our clades. Notably, as discussed above, *Mirandea sylvatica* lacks the pollen synapomorphies that we posit for the *Mirandea* clade and is also unlike other members of the genus in several traits. *Fittonia* is a genus of two species of low, perennial herbs that occur primarily in moist forests of Andean South America (Brummitt 1980). Numerous forms have been cultivated for more than a century. Among members of the *Tetramerium* lineage, *Fittonia* is distinctive by its habit and conspicuous white to red foliar venation. Other potential morphological synapomorphies for the genus are not obvious, and floral visitors remain undocumented. Based on the form, small size, and yellowish coloration of the corolla, bees or flies are potential pollinators.

***Core* Tetramerium *Lineage*.** The remaining members of the *Tetramerium* lineage, most of which are NA in distribution, are monophyletic with very strong support. These plants share sternotribic flowers that are further modified in just two taxa so far as is known: *Gypsacanthus* and *Mexacanthus* have pleurotribic flowers (i.e., with filaments positioned between the upper and lower lips, and with anthers opening toward each other). We propose that sternotribic flowers are synapomorphic for the core *Tetramerium* lineage.

***Genus Incertae Sedis:* Gypsacanthus**. Our data place this unispecific genus in the core *Tetramerium* lineage but without further resolution of its relationships. *Gypsacanthus nelsonii* is endemic to arid regions of southern Mexico. It differs from other genera of the *Tetramerium* lineage by several morphological attributes, including bracts, bracteoles, and calyx paler green than subtending foliage; flowers pleurotribic (Fig. 3C); and the relatively small (6.5--11 mm long), cream-colored corollas, with recurved to recoiled lobes of the lower lip. These characteristics likely relate to pollination biology; although pollinators are undocumented for plants of *G. nelsonii*, floral traits are consistent with pollination by small bees or flies.

Henrya *Clade.* A lineage composed of two unispecific genera (i.e., *Aphanosperma*, *Chalarothyrsus*), *Henrya* (one of two species sampled), and one of 12 sampled species of *Carlowrightia* is strongly supported as monophyletic and as sister to a clade composed of all other members of the core *Tetramerium* lineage (except *Gypsacanthus* just discussed). Relationships among these taxa are not further resolved by Bayesian analysis and are resolved with very weak support by parsimony. All are restricted to Mexico, except for one species of *Henrya* that ranges from the southwestern USA to Costa Rica, and *Carlowrightia myriantha* whose distribution extends into northernmost Belize. We can identify no morphological synapomorphies for the clade as a whole but *Aphanosperma* and *Chalarothyrsus* have seeds permanently retained in the capsules, as described below. Three of the four members of this clade have white to yellowish corollas with colored nectar guides on the upper lip, and are either known (e.g., Daniel 1990) or assumed to be pollinated by insects (especially bees and flies). In contrast, *Chalarothyrsus* has red, funnelform corollas with exserted stamens suggesting hummingbird pollination.

Like some other clades of the NW *Tetramerium* lineage, the *Henrya* clade is taxonomically heterogeneous but only *Carlowrightia myriantha* has putative congeners placed elsewhere by our results. Interestingly, in treating *Carlowrightia*, Daniel (1983, 1988b) placed all but three species into five sections; two of the problematic species are placed by our results in the *Henrya* clade: *C. myriantha* and *C. sinaloensis* (=*Aphanosperma sinaloensis*). Daniel (1988b) noted that *C. myriantha* does not conform to any of the recognized sections. The species is consistent with *Carlowrightia* based on floral morphology but our results suggest that these traits may be symplesiomorphic for the entire core *Tetramerium* lineage. Plants of *C. sinaloensis* were subsequently treated as unispecific *Aphanosperma* on the basis of the highly unusual fruits within which seeds are permanently retained (Daniel 1988a). The fruits are unique among Acanthaceae in shape, pubescence, and in progressive movement during maturation. Despite marked differences in floral morphology noted above, *Chalarothyrsus* shares permanent retention of seeds in the fruit. Seed retention in the capsule valves is otherwise unknown in the *Tetramerium* lineage, and possibly in the family, and we propose it as synapomorphic for these two genera. Our data do not resolve these as sister taxa but cannot refute this relationship (Table 2).

The single species of *Chalarothyrsus* occurs in western and southern Mexico. In addition to its remarkable fruits, these plants have flowers with four dithecous stamens, a trait otherwise unknown in the *Tetramerium* lineage. Based primarily on stamen number, Daniel (1999) treated the genus as a relative of *Spathacanthus* Baill., a genus placed in the basal clade of Justicieae, the *Pseuderanthemum* lineage (McDade et al. 2000a). Our results indicate that *Chalarothyrsus* has four stamens via reversal rather than retention of a symplesiomorphy (see McDade et al. 2000a:449).

Daniel (1990) recognized two species of *Henrya* from North and Central America. The genus can be characterized by at least two morphological synapomorphies that are either unique (i.e., partial coalescence of the bracteoles into a distinctive involucre) or uncommon (i.e., pollen with exceptionally broad colpi; Fig. 1M) in the *Tetramerium* lineage, and each species has additional autapomorphies (Daniel 1990). *Henrya* has been included within *Tetramerium* (e.g., Lindau 1895), but Happ (1937) and Daniel (1990) recognized it as distinct from, but closely related to, that genus. Both genera have relatively small capsules (4—12 mm long) in which the septa (with attached retinacula) separate slightly from the inner wall of the mature capsules. Daniel (1986) suggested that limited success from artificial hybridizations between *H. insularis* and one of four species of *Tetramerium* was consistent with a close relationship between *Tetramerium* and *Henrya*. Our results reject placement of *Henrya* with *Tetramerium* (Table 2). Floral similarities may be symplesiomorphies reflecting shared bee and fly pollinators (Daniel 1990). Slight separation of retinacula from mature capsule walls appears to have had independent origins in each genus.

Core* Tetramerium *Lineage Distal to the* Henrya *Clade. The remaining four clades of the core *Tetramerium* lineage are strongly supported as monophyletic and sister to the *Henrya* clade. Corroborating morphological synapomorphies have not been identified for this clade.

Carlowrightia parviflora *Clade.* There is strong support for monophyly of this clade that includes four of 12 sampled species of *Carlowrightia* and the only sampled CA species (of two described) of *Anisacanthus*. Once again, this clade includes plants with large red corollas adapted for hummingbird pollination (*A. tetracaulis*) and a group of species with smaller blue to purple (to whitish) corollas adapted for pollination by small to medium-sized insects. Because *A. tetracaulis* has sternotribic flowers, as do members of the core *Anisacanthus* clade, the placement of this species outside of the NA *Anisacanthus* clade is unexpected; this result is discussed below, in the section on that clade.

Morphological synapomorphies for the clade of four species of *Carlowrightia* (*C. hapalocarpa*, *C. huicholiana*, *C. mcvaughii*, and *C. parviflora*) that are sister to *A. tetracaulis* in our analysis have not been discovered. This is a taxonomically heterogeneous sample in that the four species have been placed in three sections of the genus. *Carlowrightia mcvaughii* was treated as the sole member of sect. *Thyrsiformes* based in part on the expanded thyrsiform inflorescence (Daniel 1983). In describing *C. huicholiana*, Daniel (1988b) noted that plants of this species are most similar to *Carlowrightia mcvaughii*. However, he placed the species in sect. *Tuberculosperma* based on its dimorphic seeds (see Fig. 4 in Daniel 1988b). The other species of sect. *Tuberculosperma* included here, *C. ecuadoriana,* is placed by our analysis in the core *Carlowrightia* clade; placement of these two as sister taxa is rejected by our data (Table 2: monophyly of sect. *Tuberculosperma*). It is remarkable that our data suggest that the unusual dimorphic seeds have evolved in parallel in these plants. *Carlowrightia hapalocarpa* and *C. parviflora* are sister taxa; both were placed by Daniel (1983) in sect. *Carlowrightia* and they share bisulcate stems as a possible morphological synapomorphy. The other member of sect. *Carlowrightia* included in our analysis, *C. linearifolia*, is placed in the core *Carlowrightia* clade (see below); monophyly of this section is rejected by our data (Table 2). In classifying this last species, Daniel (1983) noted that it lacks a number of traits that otherwise characterize the section, including bisulcate stems. As noted elsewhere, traits other than floral morphology may be better indicators of phylogenetic history among NW members of the *Tetramerium* lineage.

***NA* Anisacanthus *(Core* Carlowrightia + Tetramerium*) Clade*.** Monophyly of a lineage composed of the remaining three clades of the core *Tetramerium* lineage is strongly supported by our molecular data but morphological synapomorphies remain to be identified.

NA* Anisacanthus *Clade. All sampled NA (i.e., Mexican and southwestern USA) species of *Anisacanthus* plus unispecific *Mexacanthus* are together monophyletic. All species in this clade are either known or presumed to be pollinated by hummingbirds (e.g., Daniel 1984; Holmqvist et al. 2005) for which their flowers show several adaptations in common (Grant and Grant 1968). Inclusion of *Mexacanthus* in this clade is unexpected (our data cannot reject monophyly of NA *Aniscanthus* exclusive of *Mexacanthus* [Table 2] but trees consistent with this constraint simply place *Mexacanthus* basal to species of *Anisacanthus*). Daniel (1981) recognized a single species in this genus of shrubs endemic to dry forests in western Mexico and noted a number of distinctive characters including: 2-colporate, 4-pseudocolpate pollen (Fig. 1J); bi-colored corollas with an exceptionally short tube and elongate, linear lobes; and pleurotribic flowers with stamens held at an angle of ca. 60° above the lower lip (Fig. 3G). The floral macromorphological traits are likely related to pollination as Holmqvist et al. (2005) reported that flowers of *Mexacanthus* appear to be pollinated by carpenter bees in addition to hummingbirds. These authors inferred pollen transfer on the wings of visiting bees. *Mexacanthus* also shares with the NA species of *Anisacanthus* that have been studied to date a nectar barrier: a transverse obstruction at the mouth of the tube above the nectar (M. Manktelow, in prep.). Pollinators must push the style downward into a slit in the barrier in order to access nectar. We propose this trait as a synapomorphy for this clade; the structure is lacking in species of *Carlowrightia* and *Tetramerium* that were examined by Manktelow (ms in prep.), but the corolla of CA *A. tetracaulis* apparently has a similar structure and merits further investigation.

The close relationship among NA species of *Anisacanthus* is also apparent by the degree of interfertility between several pairs of these species (Daniel 1984). Artificial hybridizations among the largely allopatric *A. linearis*, *A. thurberi*, and *A. quadrifidus* (as *A. wrightii*) demonstrated fertility levels similar to self-pollinated controls (Daniel 1984). No crosses have been attempted between *Mexacanthus* and species of NA *Anisacanthus* or between any members of this clade and species from Central America or South America that have been treated as *Anisacanthus*.

Our results provide a new perspective on relationships of plants heretofore treated in *Anisacanthus* in that phylogenetic relationships mirror geography, with the NA (6 of 8 species sampled), CA (1 of 2 species included), and SA (2 of 6 species sampled) species placed in distinct clades. Monophyly of *Anisacanthus* is rejected (Table 2) and our results also do not corroborate Leonard's (1950) assertion that CA *A. tetracaulis* is related to SA *A. caducifolius* (Griseb.) Lindau (= *A. boliviensis* (Nees) Wassh.) (+36 steps, z=-4.648, P<0.0001). Clearly, floral morphological similarities are homoplastic. In fact, SA species are readily distinguished from NA and CA species by their nototribic flowers. Although CA and NA species share sternotribic flowers, our data also reject placement of CA *A. tetracaulis* with the NA *Anisacanthus* clade (+18 steps, z=-2.846, P=0.0044).

Anisacanthus has been closely allied with *Carlowrightia* (e.g., Hagen 1941; Daniel 1983) from which it differs by its taller plants; larger, funnelform, red and orange (vs. smaller, non-funnelform, white, yellow, blue or pink) corollas (Fig. 3F), and larger capsules and seeds. Many of these distinctions appear to correlate with bird pollination of *Anisacanthus* vs. insect pollination of *Carlowrightia*. Daniel (1983) suggested that *Carlowrightia* and *Anisacanthus* likely shared a common ancestor and diverged primarily due to adaptations for different pollinators. Because our results indicate that both *Anisacanthus* and *Carlowrightia* are polyphyletic, the sister relationship proposed by Daniel (1983) is not supported. However, CA *A. tetracaulis* is sister to a clade that includes four Mexican *Carlowrightia* and the NA *Anisacanthus* clade (including *Mexacanthus*) is sister to a lineage composed of core *Carlowrightia* + *Tetramerium*. Although a few species of *Tetramerium* are hummingbird-pollinated, the common ancestor shared by the core *Carlowrightia* + *Tetramerium* clade was likely insect pollinated, consistent with Daniel's (1983) hypothesis that divergence of NA *Anisacanthus* from its sister group reflected adaptations to different pollinators.

Tetramerium ***and the Core*** **Carlowrightia** ***Clade.*** These two clades are strongly supported as sister taxa by our molecular data. Most species in this lineage have flowers with the lower-central lobe of the corolla markedly folded to form a u- or v-shaped keel that partially encloses the stamens and style during anthesis (Fig. 3J). We propose the keel as synapormorphic for the clade. This hypothesis is complicated by the fact that a few species of both the core *Carlowrightia* clade (see below) and *Tetramerium* (e.g., *T. ochoterenae*) have flowers that lack this modification. A number of other plants in the core *Tetramerium* lineage (i.e., species of *Carlowrightia* in clades other than the core *Carlowrightia* clade, *Henrya insularis*, and *Aphanosperma sinaloensis*) have similar flowers, but also lack the keel. Flowers of plants of some species in the *Tetramerium* and core *Carlowrightia* clade have been further modified to achieve the legume-like 'pseudopapilionaceous' morphology described by Daniel (1983, 1986): the corolla tube is shorter than the limb, the lateral lobes of the lower lip spread laterally from the keel and are wing-like, and the two lobes of the upper lip are nearly completely fused, held ± perpendicular to the tube, and marked with nectar guides and a colored 'eye.'

Core **Carlowrightia** ***Clade.*** Seven of 12 sampled species of *Carlowrightia* form a clade that is sister to *Tetramerium*. Morphological synapomorphies for this clade are not apparent. Within the core *Carlowrightia* clade, SA *C. ecuadoriana* (sect. *Tuberculosperma*; Daniel and Wasshausen 1993) is sister to a strongly supported clade of NA species that occur in Mexico and adjacent regions of the USA and Central America. Among the six northern species, *C. texana* is sister to *C. torreyana* with strong support. These species were both treated in sect. *Mexicanae* by Daniel (1983), who noted their morphological similarities, largely sympatric distributions, and ability to hybridize both in nature and under artificial conditions.

Carlowrightia serpyllifolia (sect. *Pseudopapilionaceae*) is sister to *C. texana* + *C. torreyana*, and *C. linearifolia* (sect. *Carlowrightia*) is sister to these three taxa, with strong support for all of these relationships. Sister to these four taxa is a clade composed of *C. neesiana* basal to two accessions of *C. arizonica* (sect. *Pseudopapilionaceae*). *Carlowrightia neesiana* and *C. arizonica* share many features such that plants of these species can be difficult to distinguish (Daniel 1983 in which *C. neesiana* was treated as *C. glandulosa* B.L. Rob. & Greenm.).

As for *Anisacanthus*, our results provide a new perspective on plants heretofore treated in *Carlowrightia*. Daniel (1983) suggested that the genus was likely monophyletic based on morphological and geographical cohesiveness, and the relatively high degree of interfertility among 10 species in four sections used in artificial hybridizations (contrasted with failure of intergeneric crosses involving *Carlowrightia* and species of *Tetramerium* and *Anisacanthus*). Instead, our results reject monophyly of Carlowrightia (Table 2) and place the 12 sampled species in three distinct clades, two of which comprise species placed in multiple sections. Our results permit evaluation of Daniel's (1983) four sections of *Carlowrightia* that contain more than a single species (sect. *Thyrsiformes* consists of a single polymorphic species, *C. mcvaughii*, that was placed by our results in the *Henrya* clade as discussed above, and *C. myriantha* was not assigned to a section).

Section *Pseudopapilionaceae* consists of five species that occur from the southwestern USA to northwestern Costa Rica. All have pseudopapilionaceous corollas with the keeled lower-central lobe (Fig. 3J) that we posit as a synapomorphy for the lineage composed of the core *Carlowrightia* clade + *Tetramerium*. Three species belonging to this section were included in our analysis. Monophyly is rejected by likelihood but cannot be rejected by parsimony (Table 2); all three are placed in the core *Carlowrightia* clade.

Plants of the seven species belonging to sect. *Mexicanae* are low, prostrate to erect herbs, and most have corollas with the lower-central lobe modified into a keel. The two sampled species are sister taxa in our analysis and are part of the core *Carlowrightia* clade as expected based on their keeled corollas. In this context, it is interesting that Daniel's (1983) data from artificial hybridization studies indicated that *C. serpyllifolia* of sect. *Pseudopapilionaceae* was interfertile with species of sect. *Mexicanae.* This last species is sister to *C. texana* + *C. torreyana* in our results, suggesting that their interfertility is explained by phylogenetic relationship.

Section *Carlowrightia* consists of five species occurring from the southern USA to central Mexico. It is characterized by subactinomorphic corollas, subspherical capsules, and bisulcate stems except that this last character is lacking in *C. linearifolia*. Indeed, our results place *C. hapalocarpa* + *C. parviflora* as sister taxa in the *Carlowrightia parviflora* clade, whereas *C. linearifolia* is part of the core *Carlowrightia* clade. It appears that absence of bisulcate stems in this last taxon better indicates its affinities than the floral and fruit characters that were used to include this species in sect. *Carlowrightia* by Daniel (1983). Monophyly of this section is rejected (Table 2) but redefining the section based on bisulcate stems (and reassessing placement of *C. linearifolia*) yields a phylogenetically cohesive taxon. A single species of sect. *Carlowrightia*, *C. hapalocarpa*, was used in hybridization experiments (Daniel 1983) and results showed a low degree of interfertility with seven species in each of the other three sections studied. As *C. hapalocarpa* was also the only species of the *Carlowrightia parviflora* clade included in Daniel's (1983) crossing studies, these data offer limited insight into our phylogenetic results.

Section *Tuberculosperma* consists of five species that share several floral and fruit characteristics. Notably, the heteromorphic seeds (see Daniel 1988b) of this section are unique in the *Tetramerium* lineage and would seem to constitute a morphological synapomorphy. Our results reject monophyly of this section (Table 2), placing *C. ecuadoriana* in the core *Carlowrightia* clade and *C. huicholiana* in the *Carlowrightia parviflora* clade. If correct, these results indicate that the remarkable seed heteromorphism shared by these taxa has evolved independently at least twice. Our results merit verification using additional accessions of DNA from these species and inclusion of other species placed by Daniel (1983, 1988b; Daniel and Wasshausen 1993) in this section.

Corollas with the central lobe of the lower lip modified into a keel, the proposed synapomorphy for the core *Carlowrightia* + *Tetramerium* clade, occur in taxa placed in both of the strongly supported subclades of core *Carlowrightia* but are lacking in *C. linearifolia* and *C. ecuadoriana*. If the seven unsampled species of sections *Pseudopapilionaceae* and *Mexicanae* are placed here, as we expect based on floral morphology, these reversals become numerically less noteworthy. However, the basal position of *C. ecuadoriana* is especially problematic for the hypothesis that the common ancestor of the core *Carlowrightia* clade had keeled corollas, and it is notable that molecular data only weakly support placement of this species within the core *Carlowrightia* clade (BS=77, DI=2, BPP=80).

Because we obtained good resolution among species of *Carlowrightia* using these loci, adding additional species of this genus has merit. Notably, Argentinian *C. sulcata* (Nees) Ezcurra has been aligned with several other genera including *Justicia* (as *Dianthera*, *Jacobinia*, and *Siphonoglossa*), *Ecbolium*, and *Yeatesia* (Hilsenbeck 1989; Ezcurra 1994). DNA from our sample of *C. sulcata* (*Venturi 7328*) was apparently too degraded for amplification. Based on some anomalous morphological characters for *Carlowrightia* (e.g., dense terminal spikes, corolla tube longer than lips, short filaments) we suspect that it might be unrelated to other species in the genus.

Tetramerium. With 29 species, *Tetramerium* is the largest genus of the *Tetramerium* lineage. Its geographic range extends from the southwestern USA to Bolivia, but most species (19) are restricted to Mexico, where they occur in arid to semiarid habitats. Daniel (1986) distinguished the genus from other Mexican Odontoneminae by its inflorescence of usually densely bracteate, quaternate, and unbranched spikes; bracts relatively large and conspicuous; and capsules having the septa with attached retinacula separating slightly from the inner wall at maturity. Daniel (1986) recognized three sections based primarily on differences in characters related to pollination: *Tetramerium* (23 species) with relatively small, white to yellow to blue, pseudopapilionaceous flowers that are pollinated by bees, flies, and small butterflies; *Torreyella* (4 species) with larger, red to yellow, funnelform flowers that are visited and likely pollinated by hummingbirds and large butterflies; and *Siphonanthus* (1 species) with subsalverform (i.e., erect and with a narrow, cylindric corolla tube), white to yellowish flowers that are visited by lepidopterans (Manktelow and Holmquist, pers. obs). The lack of pseudopapilionaceous flowers in species belonging to sections *Torreyella* and *Siphonanthus* thus appears to reflect adaptation for pollinators other than small insects.

Our analysis included 12 accessions of 11 species (we sampled both Mexican and Peruvian plants of *T. nervosum*); these represent all of Daniel’s (1986) sections and cover the distributional range of the genus as a whole. *Tetramerium* is monophyletic with remarkably strong support and two subclades are likewise strongly supported. The subclade of *T. abditum* (*T. glandulosum* + *T. ochoterenae*) roughly corresponds to the Mexican species of sect. *Torreyella*. Although *T. glandulosum* was tentatively treated by Daniel (1986) in sect. *Tetramerium*, he noted that it was more similar to species of sect. *Torreyella* in several features. *Tetramerium sagasteguianum*, the other sampled member of sect. *Torreyella* is placed in the second subclade and, as a consequence, monophyly of this section is rejected by our data (Table 2). Our results indicate that the traits that define this section are homoplastic such that hummingbird-pollinated flowers have evolved at least twice in *Tetramerium*, once in the common ancestor of Mexican sect. *Torreyella* and once in the ancestor of SA *T. sagasteguianum*.

The second subclade includes *T. tenuissimum* sister to the remaining seven species but with no resolution of relationships among them. This subclade is taxonomically diverse, comprising species assigned to all three of Daniel's (1986) sections (i.e., five species in sect. *Tetramerium*, *T. sagasteguianum* [sect. *Torreyella*], and *T. peruvianum* [sect. *Siphonanthus*]). It is also geographically diverse in that it includes both NA and SA species. In some MP trees, the two accessions of *T. nervosum* are sister taxa but this relationship is not strongly supported. As all members of sect. *Tetramerium* (except *T. glandulosum* which was placed there only tentatively and, as noted above, shares numerous traits with sect. *Torreyella*) are in this subclade, our phylogenetic results are largely congruent with Daniel's (1986) treatment. The hummingbird-adapted flowers of *T. sagasteguianum,* and the lepidopteran-adapted flowers of *T. peruvianum* (the sole species of sect. *Siphonanthus*) have apparently evolved from the relatively small, pseudopapilionaceous flowers characteristic of sect. *Tetramerium*.

In the context of remarkably strong support for monophyly of *Tetramerium*, and of the three basal branches, the lack of resolution among members of the second subclade is notable. Indeed, mean pairwise distance between species in this subclade is only 1.1%. Our results are consistent with the idea that these species diverged both recently and rapidly, but might also be explained by hybridization. Indeed, putative natural hybrids were noted by Daniel (1986), and one of these was synthesized by crossing experiments in a greenhouse. Further, artificial hybridizations among eight species of sect. *Tetramerium* were successful to varying degrees (e.g., 48% of crosses between all pairs of the eight species set some seed; see Daniel 1986 for details).

Biogeography. We posit an OW origin for the *Tetramerium* lineage: all of the basal clades in the lineage are either African or Asian (Fig. 5) and an OW origin seems likely for Justicieae as a whole and for each of its constituent lineages, including that basal to the *Tetramerium* lineage (i.e., Isoglossinae; McDade et al. 2000a, Kiel et al. 2006) as well as its sister group (i.e., justicioids: McDade et al. 2000a). The basalmost clade consists of an African species and an African origin for the lineage is likely with dispersal eastward (to eastern Asia) and westward (to the NW). At the continental scale, our results suggest two dispersal events from the African/Arabian region to southern and eastern Asia, one of the common ancestor of species of *Clinacanthus* (restricted to Asia) and at least one to account for three species of *Ecbolium* (two endemic) that occur in India. As the NW members of the *Tetramerium* lineage are monophyletic, we posit a single dispersal event to the NW. In the unlikely event that the African species described as *Schaueria populifolia* pertains to this genus, an additional cross-Atlantic dispersal event would be required. As discussed by McDade et al. (2005), fossil evidence as well as dates estimated from calibration of molecular evolution place the origin of Acanthaceae too recent for the current range of the *Tetramerium* lineage to be explained by the break-up of Gondwanaland.

Among OW lineages, our results indicate at least two dispersal events from Africa to Madagascar. Taxon sampling to date of species in the *Ecbolium* clade yields results consistent with the hypothesis of a single dispersal event to Madagascar from Africa followed by diversification to yield the seven Malagasy species of *Ecbolium* and at least one of two species of *Populina*, but requires testing with addition of the unsampled taxa. One of the Malagasy species of *Ecbolium* also occurs on the Comoros, presumably via dispersal. A second dispersal event from Africa to Madagascar is required for the Malagasy taxon represented by *Daniel et al. 10455* of the *Megalochlamys* clade. Also interesting among OW clades is the presence of a number of these plants on Socotra: unispecific *Angkalanthus* and two species of *Chorisochora* (the third is South African). Vollesen (1994) noted that the disjunct distribution between

Socotra and southern Africa for *Chorisochora* is not unique, and he cited several examples of taxa disjunct between the regions. On the other hand, Thiv et al. (2006) rejected a Socotra-southern Africa relationship for species of *Aerva* (Amaranthaceae).

The geographic range of the common ancestor of the NW *Tetramerium* lineage is optimized by parsimony as NA (Fig. 5). The common ancestor of the *Pachystachys* clade + SA *Anisacanthus* clade dispersed to South America and radiated there notably to yield the estimated 32 species of the *Pachystachys* clade. One dispersal event from South America to North America is hypothesized for the common ancestor of the Mexican members of the SA *Anisacanthus* clade. Secondarily, there have been at least two dispersal events north to south, one of the ancestor of *Carlowrightia ecuadoriana* and one (or more) of the ancestor(s) of the SA members of *Tetramerium*. Unfortunately, our data do not resolve relationships within the clade that includes *T. nervosum* such that whether *T. nervosum* is monophyletic or paraphyletic, with the other SA species of *Tetramerium* derived from Peruvian *T. nervosum*, remains to be determined. Range extensions from North America into Central America of several clades (e.g., CA *Anisacanthus*) and, in some cases, populations of single, wide-ranging species (e.g., *Carlowrightia arizonica*, *Tetramerium nervosum*, *Henrya insularis*) have also occurred.

Habitats. Most species of the *Tetramerium* lineage occur in semi-arid to arid habitats; indeed several clades from both the OW (e.g., *Ecbolium*) and the NW (e.g., core *Carlowrightia*, NA *Anisacanthus*, *Tetramerium*, and *Mirandea*) have apparently radiated in regions of dry forest, thornscrub, and desert. It is therefore notable that the basalmost clades of the lineage consist of species from tropical Africa and Asia that apparently occur in moist to wet forests. Many other Justicieae also occur in moist to wet habitats. Most taxa in the large clade that is sister to *Clinacanthus* occur in arid habitats, such that the common ancestor of this clade, which includes most African and all taxa from the NW, is hypothesized to have been xerophytic. Among NW taxa, only *Streblacanthus*, *Pachystachys*, *Schaueria*, *Fittonia*, *Yeatesia viridiflora*, and *Mirandea sylvatica* occur in mesic to wet environments. The first three genera are in the *Pachystachys* clade, suggesting that the common ancestor this clade was likely adapted to moist/wet habitats. Relationships of *Fittonia albivenis* and *Mirandea sylvatica* are uncertain but our analyses place them close to the *Pachystachys* clade suggesting that habitat may map without homplasy among SA members of the *Tetramerium* lineage. Among NW plants, there has been profuse radiation of taxa in the arid and semi-arid regions of Mexico. For example, 13 species treated as *Carlowrightia* occur in the Chihuahuan Desert region of north-central Mexico (Daniel 1983) and 20 species of *Tetramerium* are found in regions of tropical deciduous forest throughout Mexico (Daniel 1986). In sum, our phylogenetic results for the *Tetramerium* lineage suggest an early shift from relatively wet to dry habitats in the OW, with one or a few apparent reversals to mesic or wet environments. It is remarkable that habitat seems to be more strongly conserved evolutionarily in these plants than are many morphological characters.

Species Richness. There is a marked difference in current species richness between the OW (ca. 45 species) and the NW (ca. 125 species). We suggest that the presence of hummingbirds, efficient pollinators that do not occur in the OW, may have been a stimulus for speciation among NW Acanthaceae. Many NW clades include taxa of diverse floral forms that attract a corresponding diversity of known or inferred pollinators. Indeed, our data indicate that shifts to hummingbird pollination have occurred at least eight times among NW members of the *Tetramerium* lineage (Fig. 5). The most extensive radiation within the *Tetramerium* lineage has occurred in southern North America (i.e., Mexico and adjacent regions of the USA and northern Central America) where at least 75 species (60% of the NW species) currently occur, many of which are endemic to this region including both traditionally recognized genera (i.e., *Aphanosperma*, *Chalarothyrsus*, *Gypsacanthus*, *Hoverdenia*, *Mexacanthus*, *Mirandea*) and major clades identified here (i.e., *Henrya* clade, *Mirandea* clade, NA *Anisacanthus* clade). Explanations for this radiation of taxa in southern North America are not obvious. The region offers a complex mosaic of semi-arid to xeric habitats (e.g., tropical dry forest, grasslands, thornscrub, deserts), but expansive and diverse arid regions also occur in South America, Africa, Madagascar, and India. These latter regions harbor many fewer species of the *Tetramerium* lineage. Other examples of extensive radiation among Acanthaceae in limited geographic regions are known (e.g., *Hypoestes* Sol. ex R. Br. of the Diclipterinae, with some 90 of about 150 species endemic to Madagascar) and also poorly understood.

Character Evolution. A chromosome number of *n*=18 predominates throughout the *Tetramerium* lineage and has been recorded for taxa in both the OW and the NW, with 52 of 53 species for which data are available having this number. A caveat is that chromosome numbers have been reported for only one OW genus (*Ecbolium*). To determine the ancestral chromosome number of the *Tetramerium* lineage, counts for additional OW genera should be obtained. Further, it is uncertain whether *n*=18 represents a synapomorphy for the *Tetramerium* lineage or for a larger clade, or is homoplastic. This number occurs elsewhere among Justicieae (e.g., Isoglossinae and a few species of *Justicia*). Within the *Tetramerium* lineage, exceptions to *n*=18 do not seem to be phylogenetically informative: some individuals of *Carlowrightia pectinata* have *n*=17 (Daniel 2000) and some *Yeatesia mabryi* are tetraploid with *n*=36 (Daniel et al. 1990). Counts of *n*=27 in *Pachystachys spicata* (Ruiz & Pav.) Wassh. (Daniel and Chuang 1998) and *Fittonia albivenis* (as *F. verschaffeltii* E. Coem. var. *pearcei* Nichols.; Grant 1955) might suggest a relationship between these commonly cultivated plants, but counts of *n*=18 have been obtained for other species of *Pachystachys* and for other individuals of *F. albivenis*.

Sculptural elements of pollen (e.g., aperture number and type) have been documented for all genera of the *Tetramerium* lineage known to us (e.g., Furness 1989, 1992; Daniel 1998). Tricolporate/6-pseudocolpate/bireticulate pollen is shared by most of the constituent genera; this pollen type is hypothesized to be synapomorphic for Justicieae as a whole and is thus symplesiomorphic for the *Tetramerium* lineage. Evolutionary shifts from this pollen type are mostly autapomorphic (e.g., *Chlamydocardia*, *Clinacanthus*, *Mexacanthus*, *Trichaulax*). As described above, we hypothesize that non-bireticulate pollen (e.g., with exine singly and inconspicuously reticulate) is a synapomorphy for the *Mirandea* clade, with further modification (i.e., broad colpi) in *Hoverdenia* + *Justicia medranoi*. Pollen of OW *Megalochlamys* is similar to that of the NW *Yeatesia* species that are members of the *Mirandea* clade; these similarities have apparently evolved in parallel. It is interesting that most of the sculptural diversity of pollen is encountered among OW genera, with at least four of the eight OW genera having species with highly distinctive pollen. Our results indicate that most OW clades originated before those in the NW and pollen diversity may reflect this greater antiquity. Pollen structure, which has been little studied among taxa of the *Tetramerium* lineage, might offer additional characters and merits attention.

Our results indicate that floral morphology is extremely vagile evolutionarily in the *Tetramerium* lineage such that, with few exceptions, traits associated with flowers are poor indicators of phylogenetic relationships. Exceptions include the shift from nototribic to sternotribic flowers in the common ancestor of the core *Tetramerium* lineage and the evolution (with subsequent modifications) of keeled corollas in the common ancestor of the core *Carlowrightia* clade + *Tetramerium*. A subsequent paper will present a more detailed examination of pollination biology among NW members of the *Tetramerium* lineage and here we note that, as mentioned above, hummingbird pollination has evolved at least eight times (Fig. 5). It is notable that the shift to sternotribic flowers seems not to have constrained evolution of other floral traits, as there is marked variation in corolla color, size, shape and orientation of the lobes among plants with both nototribic and sternotribic flowers. The exception is that flowers in which the lower-central lobe of the corolla is keeled occur only in plants with sternotribic flowers; in fact, sternotriby would seem to be a prerequisite for the evolution of pseudopapilionaceous flowers as having the stamens (and style) positioned adjacent to the lower lip may be a precondition for modification of the lower lip to sheath these structures.

Taxonomic Implications of Phylogenetic Results. The *Tetramerium* lineage has not been recognized in comprehensive classifications of Acanthaceae. Lindau (1895, 1904) treated these genera in diverse tribes: Asystasieae (*Chalarothyrsus*, *Henrya* [as *Solenoruellia*]), Graptophylleae (*Anisacanthus*, *Carlowrightia*, *Chlamydocardia*, *Pachystachys*), Isoglosseae (*Fittonia*, *Populina*, *Yeatesia* [as *Gatesia*]), and Odontonemeae (*Angkalanthus*, *Clinacanthus*, *Ecbolium*, *Hoverdenia*, *Schaueria*, *Streblacanthus*, and *Tetramerium* [including *Henrya*]); possible relatives of *Megalochlamys* were discussed by Lindau (1899), but the genus was not placed in a tribe. In a revision of infrafamilial classification of Acanthaceae, Bremekamp (1965) combined “typical representatives” of several of Lindau’s tribes into a single taxon, Justicieae: Odontoneminae, but he did not list the generic composition of the taxon. Although genera of the *Tetramerium* lineage would be included in Bremekamp’s Odontoneminae, they differ from his circumscription of that subtribe by having an androecium (Fig. 3A) of only two stamens and no staminodes (vs. four stamens or two stamens + two staminodes). In the most recent infrafamilial classification of Acanthaceae (Scotland and Vollesen 2000), all genera of the *Tetramerium* lineage were included in Ruellieae: Justiciinae (= Justicieae of McDade et al. 2000a); these authors did not attempt further resolution among members of this large taxon. Once we have completed a reexamination of Justicieae as a whole, we intend to recognize the *Tetramerium* lineage as a taxon.

At the generic level, especially among NW members of the *Tetramerium* lineage, our results show remarkably little correspondence to existing taxonomic treatments: many genera are not monophyletic and many unispecific genera have heretofore unsuspected relatives. Notable in this context is that Daniel (1983, 1986; Daniel and Chuang 1993) has repeatedly pointed out that diagnostic differences among many genera of the NW *Tetramerium* lineage are subtle at best and that similarities in floral form among taxa often reflect similar floral visitors rather than systematic affinity. Indeed, a consistent theme of our results is that floral morphological traits that have often been used to delimit genera can be extremely vagile evolutionarily. In the case of *Anisacanthus*, for example, our results reveal parallel evolution of floral traits associated with hummingbird pollination (e.g., large, red to orange, funnelform corollas that lack nectar guides); the arguably more subtle traits of orientation of the stamens and style (i.e., flowers of SA *Anisacanthus* species are nototribic whereas those of NA and CA *Anisacanthus* are sternotribic) and the nectar barrier that we propose as a synapomorphy for the NA *Anisacanthus* clade corroborate our phylogenetic results. Likewise, with regard to *Mirandea* and *Yeatesia*, pollen morphology supports our results that place species of each of these genera in different clades. With additional studies as noted herein, a redelimitation of genera will be necessary for the *Tetramerium* lineage.

Hilsenbeck (1989) noted that, of ca. 250 genera in Acanthaceae, 120 are unispecific. He suggested that additional studies would ally many of these to larger genera. Our results permit evaluation of the seven unispecific genera of the *Tetramerium* lineage, with mixed results. Consistent with their taxonomic treatment, OW *Angkalanthus* and NW *Gypsacanthus* are phylogenetically isolated from plants placed in other genera. Assessing the status of *Trichaulax* will require sampling more species of *Megalochlamys* and identification of *Daniel et al. 10455*. Recognition of *Hoverdenia* would require a number of nomenclatural changes for plants in the *Mirandea* clade, but our results indicate that a number of such changes are required regardless of the disposition of *Hoverdenia*. *Chalarothyrsus* and *Aphanosperma* are both part of the heterogeneous *Henrya* clade which merits additional study but likely will require nomenclatural changes. In this case, if *Henrya* is monophyletic (as morphological data strongly suggest is the case), *Chalarothyrsus* and *Aphanosperma* could continue to be recognized provided that *Carlowrightia myriantha* is assigned to one of these or treated as a new genus.

Recognition of *Mexacanthus* renders the NA *Anisacanthus* clade paraphyletic. Finally, our results suggest that *Mirandea sylvatica* may merit treatment as a unispecific genus.

Our results suggest a number of avenues for future research. Some of our more unexpected results, as well as relationships of plants placed differently by nuclear versus cp data, should be tested with data from a low copy nuclear gene. Many clades could be fruitfully studied to gather additional morphological data to test our results and to better understand character evolution. A ‘trait-based’ approach could also be taken (e.g., it would be useful to compare the development and anatomy of hummingbird adapted flowers representing the hypothesized eight evolutionary origins of the trait. Our results have also delimited the context in which biogeography can be examined (e.g., the pattern of north-south dispersal events within *Tetramerium* and of Africa-Madagascar dispersal events within *Ecbolium* [including *Populina*]). Finally, several clades comprise species that are remarkably different in floral morphology and likely pollinator relationships. These are ideal targets for field and experimental studies designed to elucidate the evolutionary mechanisms that have yielded such distinctive changes among close relatives.

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TABLE 1. Genera, species richness and geographic distribution of genera of the *Tetramerium* lineage. Total number of species per genus is followed in parentheses by number sampled here. Table reflects results reported here in that Paleotropical *Calycacanthus* (1 species) and New World *Streblacanthus monospermus* are excluded whereas a Malagasy plant of undetermined genus (represented by *Daniel et al. 10455*) and two Mexican species currently treated in *Justicia* (*J. gonzalezii* and *J. medranoi*) are included (see text for full explanation). The African species described as *Schaueria populifolia* is excluded from the species count for the otherwise Neotropical genus *Schaueria*.

Genera	No. of Species (no. sampled)	Geographic Distribution
Old World		
Angkalanthus Balf. f.	1 (1)	Yemen (Socotra)
Chlamydocardia Lindau	2 (1)	Western (--central) tropical Africa
Chorisochora Vollesen	3 (1)	South Africa, Yemen (Socotra)
Clinacanthus Nees	3 (1)	Southeastern Asia and Malesia
Ecbolium Kurz	22 (4)	Eastern and southern Africa, Madagascar, Comoros, southern Arabia, India to Malaysia
Megalochlamys Lindau	10 (1)	Eastern and southern Africa, southern Arabian Peninsula
Populina Baill.	2 (1)	Madagascar
Trichaulax Vollesen	1 (1)	Eastern Africa
Total Old World (includes *Daniel et al. 10455*)	45 (12)	
New World		
Anisacanthus Nees	16 (9)	North America, Central America, South America
Aphanosperma T.F. Daniel	1 (1)	Mexico

Carlowrightia A. Gray	26 (12)	North America, Central America, South America
Chalarothyrsus Lindau	1 (1)	Mexico
Fittonia Coem.	2 (1)	Western South America
Gypsacanthus E.J. Lott, V. Jaram. & Rzed.	1 (1)	Mexico
Henrya Nees ex Benth.	2 (1)	North America, Central America
Hoverdenia Nees	1 (1)	Mexico
Mexacanthus T.F. Daniel	1 (1)	Mexico
Mirandea Rzed.	6 (5)	Mexico
Pachystachys Nees	12 (2)	West Indies, South America
Schaueria Nees	±17 (2)	Mexico, South America
Streblacanthus Kuntze	3 (3)	Southern Central America, South America
Tetramerium Nees	29 (11)	North America, Central America, South America
Yeatesia Small	3 (3)	North America (southeastern USA and northeastern Mexico)
Total New World (includes two species currently treated in *Justicia*)	123 (56)	
Total *Tetramerium* Lineage	168 (68)	

TABLE 2. Hypotheses regarding clades and relationships among them. As indicated, monophyly of *Ecbolium* (including *Populina*), *Pachystachys*, and *Tetramerium* is supported by the maximum parsimony (MP) and Bayesian results. Other hypotheses were contrasted with the MP result using the parsimony-based Templeton test (z value) and likelihood based Kishino-Hasegawa RELL test (K-H RELL). For parsimony, we report the difference between the MP trees and those consistent with the contstraint (percent difference is calculated relative to lengths of the MP trees); for likelihood, we report the difference between the –ln likelihood score of the most likely versus the constrained topologies.

Hypothesis	Results of Test
Ecbolium (excluding *Populina*) is monophyletic	Cannot Reject (+3 steps, 0.11%, z=-1.00, P=0.317; Diff –ln L=9.804, K-H RELL, P=0.132)
Megalochlamys and *Ecbolium* are sister taxa	Reject (+12 steps, 0.44%, z=-2.683, P=0.0073; Diff –ln L=37.489, K-H RELL, P=0.003)
Chalarothyrsus and *Aphanosperma* are sister taxa	Cannot Reject (+4 steps, 0.14%, z = 0.206, P=0.344; Diff –ln L=8.702, K-H RELL, P=0.113)
Henrya and *Tetramerium* comprise a clade	Reject (+28 steps, 1.03%, z=-4.042, P<0.0001; Diff –ln L=124.950, K-H RELL, P<0.001)
North American *Anisacanthus* (excluding *Mexacanthus*) is monophyletic	Cannot Reject (+4 steps, 0.15%, z=0.317, P=0.454; Diff –ln L=18.716, K-H RELL, P=0.081)

Recognized genera in the OW (*Ecbolium*) and NW (*Anisacanthus*, *Carlowrightia*, *Mirandea*, *Pachystachys*, *Schaueria*, *Streblacanthus*, *Tetramerium*, *Yeatesia*) are monophyletic	Accept (MP and Bayesian trees): *Ecbolium* (including *Populina*), *Pachystachys, Tetramerium* Cannot Reject: *Schaueria* (+6 steps, 0.22%, z=0.221, P=0.308; Diff –ln L=15.381, P=0.204); *Streblacanthus* (excluding *S. monospermum*) (+1 step, 0.04%, z=-0.301, P=0.763; Diff –ln L=3.015, K-H RELL, P=0.346) Reject: *Anisacanthus* (including *Mexacanthus*) (+48 steps, 1.76%, z= -5.908, P<0.0001; Diff –ln L=276.197, K-H RELL, P<0.001); *Carlowrightia* (+47 steps, 1.72%,z=05.409, P<0.0001; Diff –ln L=212.458, K-H RELL, P<0.001) *Mirandea* (+39 steps, 1.43%, z=-5.077, P<0.0001; Diff –ln L=183.153, K-H RELL, P<0.001); *Yeatesia* (+36 steps, 1.32%, z=-4.356, P<0.0001; Diff –ln L=157.042, K-H RELL, P<0.001)
Sections of *Carlowrightia* (Daniel 1983) are monophyletic	Accept (MP and Bayesian trees): *Mexicanae* MP/ML disagree: *Pseudopapilionaceae* (+8 steps, 0.33%, z=-1.886, P<0.0593; Diff –ln L=36.839, P=0.012) Reject: *Carlowrightia* (+30 steps, 1.1%, z=-4.629, P<0.0001; Diff –ln L=152.148, K-H RELL, P<0.001); *Tuberculosperma* (+24 steps, +1.0%, z=-3.531, P=0.0003; Diff –ln L=84.779, K-H RELL, P<0.001)

TABLE 3. Characteristics of five DNA regions used here. Aligned length (a) includes all taxa sequenced for each locus; the remaining statistics are for members of the in-group only. Missing data are reported as (h) number of total of 68 in-group taxa (because of low sequence divergence between close relatives, we did not seek to obtain *trnL-trnF* and *rps16* sequences for all taxa; for two species of North American *Anisacanthus*, only nr-ITS was sequenced, see Fig. 4) and as (i) percent of sites excluding taxa that were not sequenced for that locus. Statistics for the *trnL-trnF* region include the *trnL-trnF* spacer, the 3' *trnL* exon, and the *trnL* intron; those for the nr-ITS region include 25 and 28 bp of the 18S and 26S ribosomal genes, respectively, that flank ITS1 and ITS2, plus the 5.8S gene.

	trnL-trnF region	*trnT-trnL* spacer	*rps16* intron	*trnS-trnG* spacer	nr-ITS region
a. Aligned length	1024	866	959	992	838
b. Variable sites (proportion)	187 (0.182)	192 (0.222)	219 (0.228)	254 (0.256)	485 (0.579)
c. Parsimony informative sites (proportion)	66 (0.064)	68 (0.079)	90 (0.094)	120 (0.121)	251 (0.299)
d. Parsimony informative indels	5	8	7	19	10
e. Pairwise distances (range)	0.0-9.9%	0.0-7.0%	0.1-9.9%	0.0-5.8%	0.6-23.5%
f. Consistency index	0.838	0.846	0.789	0.782	0.567
g. Retention index	0.852	0.857	0.787	0.865	0.639
h. Missing data (# taxa of 68 in-group taxa)	26	4	14	3	1
i. Missing data (not including taxa for which entire sequence is missing)	12.9%	3.2%	10.3%	3.9%	13.0%

APPENDIX 1. Taxa, Genbank accession numbers (*trnS-G*, *trnT-L, rps16*, *trnL-F*, nrITS; -- = sequence not obtained), sources of plant materials from which DNA was extracted for sequencing, and lineage and clade where placed by the analyses presented here (i.e., as depicted in Fig. 4). To facilitate data location, taxa are listed in alphabetical order by genus and species. When plants in cultivation were used, we provide information on native range in parentheses. Abbreviations for herbaria follow Holmgren et al. (1990). [NB: xxxxx = genbank accesscion numbers in progress]

Angkalanthus oligophylla Balf.; xxxxx, xxxxx, xxxxx, xxxxx, xxxxx; Yemen (Socotra), *Miller M10292* (UPS); isolated lineage. *Anisacanthus andersonii* T. F. Daniel; --, --, --, --, xxxxx; Mexico, *Manktelow 743*; North American *Anisacanthus* clade. *Anisacanthus boliviensis (*Nees) Wassh.; xxxxx, xxxxx, xxxxx, xxxxx, xxxxx; Bolivia, *Wood & Serrano 14841* (CAS); South American *Anisacanthus* clade. *Anisacanthus brasiliensis* Lindau; xxxxx, xxxxx, xxxxx, xxxxx, xxxxx; Brazil, *Silva 2333* (US); South American *Anisacanthus* clade. *Anisacanthus junceus* Hemsl.; xxxxx, xxxxx, --, --, xxxxx; Mexico, *Manktelow 720* (UPS); North American *Anisacanthus* clade. *Anisacanthus linearis* (Hagen) Henr. & E. J. Lott; xxxxx, xxxxx, xxxxx, xxxxx, xxxxx; Mexico, *Louie s.n.* (CAS); North American *Anisacanthus* clade. *Anisacanthus puberulus* (Hagen) Henr. & E. J. Lott; xxxxx, xxxxx, --, --, AF289778; cultivated, University of Arizona campus (native to southwestern USA and northern Mexico), *McDade 1179* (ARIZ); North American *Anisacanthus* clade. *Anisacanthus quadrifidus var. wrightii* (Torr.) Henr.; --, --, --, --, xxxxx; Mexico, *Manktelow 688* (UPS); North American *Anisacanthus* clade. *Anisacanthus tetracaulis* Leonard; xxxxx, xxxxx, xxxxx, --, xxxxx; El Salvador, *J. Tucker 629* (CAS); *Carlowrightia parviflora* clade. *Anisacanthus thurberi* A. Gray; xxxxx, xxxxx, xxxxx, --, --; USA, *Jenkins 05-007* (ARIZ); North American *Anisacanthus* clade. *Anisacanthus thurberi* A. Gray; --, --, --, AF063122, AF169846; USA, *Van Devender 88-150* (ARIZ); North American *Anisacanthus* clade. *Aphanosperma sinaloensis* (Leonard & Gentry) T. F. Daniel; xxxxx, xxxxx, xxxxx, xxxxx, xxxxx; cultivated, San Francisco Conservatory of Flowers (native to northwestern Mexico), *Daniel 4070cv* (CAS); *Henrya* clade. *Brachystephanus africanus* S. Moore; DQ372491, DQ372446, xxxxx, --, DQ372469; Tanzania, *Luke et al. 6704* (US); out-group. *Calycacanthus magnusianus* K. Schum.; cultivated, Royal Botanic Gardens, Sydney (native to Papuasia), *Daniel 10072* (CAS); not part of *Tetramerium* lineage, to be treated subsequently. *Carlowrightia arizonica* A. Gray; xxxxx, xxxxx, xxxxx, AF063123, AF169845; USA, *Jenkins 89-24* (ARIZ); core *Carlowrightia* clade. *Carlowrightia arizonica* A. Gray; xxxxx, xxxxx, --, --, xxxxx; Mexico, *Manktelow 734* (UPS); core *Carlowrightia* clade. *Carlowrightia ecuadoriana* T. F. Daniel & Wassh.; --, xxxxx, xxxxx, xxxxx, xxxxx; Ecuador, *Harling & Anderson 24842* (UPS); core *Carlowrightia* clade. *Carlowrightia hapalocarpa* Robinson & Greenm.; xxxxx, xxxxx, xxxxx, xxxxx, xxxxx; Mexico, *Manktelow 715* (UPS); *Carlowrightia parviflora* clade. *Carlowrightia huicholiana* T. F. Daniel; xxxxx, xxxxx, xxxxx, --, xxxxx; Mexico, *Bauml & Voss 1896* (CAS); *Carlowrightia parviflora* clade. *Carlowrightia linearifolia* Lindau; xxxxx, xxxxx, xxxxx, xxxxx, --; Mexico, *Manktelow 722* (UPS); core *Carlowrightia* clade. *Carlowrightia mcvaughii* T. F. Daniel; xxxxx, xxxxx, --, xxxxx, xxxxx; Mexico, *Daniel 5262* (CAS); *Carlowrightia parviflora* clade. *Carlowrightia myriantha* Standl.; xxxxx, xxxxx, xxxxx, xxxxx, xxxxx; Belize, *Daniel 8267* (CAS); *Henrya* clade. *Carlowrightia neesiana* (Schauer ex Ness) T. F. Daniel; xxxxx, xxxxx, xxxxx, xxxxx, xxxxx; Mexico, *Manktelow 708* (UPS); core *Carlowrightia* clade. *Carlowrightia parviflora* (Buckley) Wassh.; xxxxx, xxxxx, xxxxx, --, xxxxx; Mexico, *Manktelow 704* (UPS); *Carlowrightia parviflora* clade. *Carlowrightia serpyllifolia* A. Gray; xxxxx, xxxxx, xxxxx, --, xxxxx; Mexico, *Manktelow 694* (UPS); core *Carlowrightia* clade. *Carlowrightia texana* Henr. & T. F. Daniel; xxxxx, xxxxx, xxxxx, xxxxx, xxxxx; Mexico, *Manktelow 700* (UPS); core *Carlowrightia* clade. *Carlowrightia torreyana* Wassh.; xxxxx, xxxxx, xxxxx, --, xxxxx; Mexico, Manktelow *690* (UPS); core *Carlowrightia* clade. *Chalarothyrsus amplexicaulis* Lindau; xxxxx, xxxxx, xxxxx, AF289740, AF289780; cultivated, San Francisco Conservatory of Flowers (native to western Mexico), *Daniel & Bartholomew 4842cv* (CAS); *Henrya* clade. *Chlamydocardia buettneri* Lindau; xxxxx, xxxxx, xxxxx, xxxxx, xxxxx; cultivated, National Botanic Garden of Belgium (native to Cameroun, Gabon, Ivory Coast and Nigeria), Accession No. 95-0034-44 (BR); isolated lineage. *Chorisochora transvaalensis* (A. Meeuse) Vollesen; xxxxx, xxxxx, xxxxx, xxxxx, xxxxx; South Africa, *Daniel 9379* (CAS); isolated lineage. *Clinacanthus siamensis* Bremek.; xxxxx, xxxxx, xxxxx, xxxxx, xxxxx; cultivated, National Botanic Garden of Belgium (native to Thailand), Accession No. 1979-0344 (BR); isolated lineage. *Ecbolium madagascariense* Vollesen; xxxxx, xxxxx, --, --, xxxxx; Madagascar, *Daniel et al. 10412*

(PH); *Ecbolium* clade. *Ecbolium syringifolium* (Vahl) Vollesen; DQ372480, DQ372435, xxxxx, AF289743, AF289786; Madagascar, *Daniel & Butterwick 6733* (CAS); *Ecbolium* clade. *Ecbolium tanzaniense* Vollesen; xxxxx, xxxxx, xxxxx, --, xxxxx; Tanzania, *Bidgood et al. 567* (CAS); *Ecbolium* clade. *Ecbolium viride* (Forssk.) Alston; xxxxx, xxxxx, xxxxx, --, xxxxx; Ethiopia, *Friis & Vollesen 5050* (CAS); *Ecbolium* clade. *Fittonia albivenis* (Lindl. ex Veitch) Brummitt; xxxxx, xxxxx, xxxxx, AF289741, AF289781; cultivated, Duke University greenhouse (native to western South America), *McDade 1178* (DUKE); incertae sedis. *Gypsacanthus nelsonii* E. J. Lott, V. Jaram. & Rzed. ; xxxxx, xxxxx, xxxxx, AF289739, AF289779; Mexico, *Daniel 8357* (CAS); incertae sedis. *Henrya insularis* Nees ex Benth; xxxxx, xxxxx, xxxxx, AF063125, AF169843; Mexico, *Jenkins 89-432* (ARIZ); *Henrya* lineage. *Hoverdenia speciosa* Nees; xxxxx, xxxxx, xxxxx, AF289738, AF289777; Mexico, *Daniel & Baker 3739* (CAS); *Mirandea* clade. *Justicia adhatoda* L.; xxxxx, xxxxx, DQ059214, AF289734, AF2897731; cultivated, University of Arizona (native to southern Asia), *Barr 60-393* (ARIZ); out-group. *Justicia gonzalezii* (Greenm.) Henr. & P. Hiriart; xxxxx, xxxxx, xxxxx, xxxxx, xxxxx; Mexico, *Daniel & Baker 3779* (CAS); South American *Anisacanthus* clade. *Justicia medranoi* Henr. & P. Hiriart; xxxxx, xxxxx, xxxxx, --, xxxxx; Mexico, *Daniel and Baker 3742* (CAS); *Mirandea* clade. Madagascar 10455; xxxxx, xxxxx, xxxxx, xxxxx, xxxxx; Madagascar, *Daniel et al. 10455* (CAS); *Megalochlamys clade. Megalochlamys revoluta* (Lindau) Vollesen; xxxxx, xxxxx, xxxxx, xxxxx, xxxxx; South Africa, *McDade & Balkwill 1264* (J); *Megalochlamys* clade. *Mexacanthus mcvaughii* T.F.Daniel; xxxxx, xxxxx, xxxxx, xxxxx, xxxxx; Mexico, *Van-Devender 94-23* (CAS); North American *Anisacanthus* clade. *Mirandea grisea* Rzed.; xxxxx, xxxxx, xxxxx, --, AF289783; Mexico, *Daniel & Baker 3717* (CAS); *Mirandea* clade. *Mirandea huastecensis* T. F. Daniel; xxxxx, xxxxx, xxxxx, xxxxx, xxxxx; Mexico, *Manktelow 706* (UPS); *Mirandea clade. Mirandea hyssopus* (Nees) T. F. Daniel; xxxxx, xxxxx, xxxxx, xxxxx, xxxxx; Mexico, *Diaz B. & Carranza 7498* (CAS); South American *Anisacanthus* clade. *Mirandea nutans* (Nees) T. F. Daniel; xxxxx, xxxxx, xxxxx, --, xxxxx; Mexico, *Rzedowski 53366* (IEB); *Mirandea* clade. *Mirandea sylvatica* Acosta C.; xxxxx, xxxxx, xxxxx, xxxxx, xxxxx; Mexico, *Breedlove & Almeda 57872* (CAS); incertae sedis. *Odontonema tubaeforme* (Bertol.) Kuntze; DQ059297, DQ372462, DQ059215, AF063127, AF169748; cultivated, Duke University greenhouse (native to Mexico and Central America); *McDade 1182* (ARIZ); out-group. *Pachystachys coccinea* Nees; xxxxx, xxxxx, --, xxxxx, xxxxx; French Guiana, *Gustafsson 330* (NY); *Pachystachys* clade. *Pachystachys lutea* Nees; ; xxxxx, xxxxx, xxxxx, AF063128, AF169844; cultivated, Duke University greenhouse (native to Peru), *McDade 1181* (DUKE); *Pachystachys* clade. *Populina richardii* Baill.; xxxxx, xxxxx, xxxxx, xxxxx, xxxxx; Madagascar, *Kerardren 1671* (P); *Ecbolium* clade. *Ptyssiglottis pubisepala* (Lindau) B.Hansen; DQ372483, DQ372438, --, AF289744, AF289787; Papua New Guinea, *Daniel 6630* (CAS); out-group. *Schaueria azaleiflora* Rusby; xxxxx, xxxxx, xxxxx, --, xxxxx; Bolivia, *J. Wood 12593* (CAS); *Pachystachys* clade. *S. calicotricha* (Link & Otto) Nees; xxxxx, xxxxx, xxxxx, xxxxx, AF289782; cultivated, garden in Homestead, Florida (native to Brazil), *Foote s.n.* (CAS); *Pachystachys* clade. *Streblacanthus cordatus* Lindau; xxxxx, xxxxx, xxxxx, AF289742, AF289784; Panama, *Daniel et al. 8203* (CAS); *Pachystachys* clade. *Streblacanthus dubiosus* (Lindau) V. M. Baum; xxxxx, --, xxxxx, xxxxx, xxxxx; Bolivia, *Daniel 10174* (CAS); *Pachystachys* clade. *Streblacanthus monospermus* Kuntze; Costa Rica, *Daniel et al. 6230* (CAS); not clearly part of *Tetramerium* lineage, to be treated subsequently. *Streblacanthus roseus* (Radlk.) B. L. Burtt; xxxxx, --, --, xxxxx, AF289785; cultivated, San Francisco Conservatory of Flowers (native to Peru), *Daniel s.n.* (CAS); *Pachystachys* clade. *Tetramerium abditum* (Brandegee) T. F. Daniel; xxxxx, xxxxx, xxxxx, --, xxxxx; Mexico, *Manktelow 727* (UPS); *Tetramerium. Tetramerium glandulosum* Oerst.; xxxxx, xxxxx, --, xxxxx, xxxxx; Mexico, *Van Devender 93-1457* (ARIZ); *Tetramerium. Tetramerium nemorum* Brandegee*;* xxxxx, xxxxx, xxxxx, --, xxxxx; Honduras, *Daniel 9569* (CAS); *Tetramerium. Tetramerium nervosum* Nees; xxxxx, xxxxx, --, AF063133, AF169847; USA, *McDade & Jenkins 1154* (ARIZ); *Tetramerium. Tetramerium nervosum* Nees; xxxxx, --, --, xxxxx, xxxxx; Peru, *Jenkins 00-135* (ARIZ); *Tetramerium. Tetramerium ochoterenae* (Miranda) T.F. Daniel; xxxxx, xxxxx, xxxxx, --, xxxxx; Mexico, *Gonzales Q. 3631* (DS); *Tetramerium. Tetramerium peruvianum* (Lindau) T. F. Daniel; xxxxx, xxxxx, xxxxx, xxxxx, xxxxx; Peru, *Jenkins 00-142* (ARIZ); *Tetramerium. Tetramerium sagasteguianum* T. F. Daniel; xxxxx, xxxxx, xxxxx, xxxxx, xxxxx; Peru, *Jenkins 00-113* (ARIZ); *Tetramerium. Tetramerium tenuissimum* Rose; xxxxx, xxxxx, xxxxx, --, xxxxx; Mexico, *Manktelow 730* (UPS); *Tetramerium. Tetramerium wasshausenii* T. F. Daniel; DQ372481, DQ372436, --, --, DQ372465; Peru, *Jenkins 00-170* (ARIZ); *Tetramerium. Tetramerium yaquianum* T. F. Daniel; xxxxx, xxxxx, xxxxx, --, xxxxx; Mexico, *Manktelow 733* (UPS); *Tetramerium. Tetramerium zeta* T. F. Daniel; xxxxx, xxxxx, --, --, xxxxx; Peru, *Jenkins 00-155* (ARIZ); *Tetramerium. Trichaulax mwasumbii* Vollesen; xxxxx, xxxxx, xxxxx, xxxxx, xxxxx; Tanzania, *Mwasumbi 14238* (CAS); *Megalochlamys* clade. *Yeatesia mabryi* Hilsenb.; xxxxx, xxxxx, xxxxx, xxxxx, xxxxx; Mexico, *Daniel & Baker 3698* (CAS); South American *Aniscanthus* clade. *Yeatesia platystegia* Small; xxxxx, xxxxx, xxxxx, xxxxx, xxxxx; cultivated, Starr Nursery in Tucson, Arizona (native to southern USA and northern Mexico), *McDade 1187* (ARIZ); *Mirandea clade. Yeatesia viridiflora* Small; xxxxx, xxxxx, --, --, xxxxx; USA, *Boufford & Wood 23871* (CAS); *Mirandea clade.*

FIGURE LEGENDS

FIG. 1. Diversity of pollen in the *Tetramerium* lineage. A. *Anisacanthus boliviensis* (*Daniel & Wood 10155*), 3-colporate and 6-pseudoclopate, apertural view. B. *Clinacanthus nutans* (*Maxwell 90-244*), 3-colporate and 6-pseudoclopate, interapertural view. C. *Mirandea sylvatica* (*Wendt et al. 4104*), 3-colporate and 6-pseudoclopate with adjacent pseudocolpi in each mesocolpium fused toward poles thereby forming pseudocolpal ellipses, interapertural view. D. Undetermined genus from Madagascar (*Daniel et al. 10455*), 3-colporate and 6-pseudoclopate, apertural view. E. *Ecbolium madagascariensis* (*Daniel et al. 10412*), 3-colporate and 6-pseudoclopate, polar view. F. *Justicia medranoi* (*Daniel & Baker 3742*), 3-colporate and 6-pseudocolpate with broad colpi, apertural view. G. *Hoverdenia speciosa* (*Rzedwoski 36934*), 3-colporate and 6-pseudocolpate with broad colpi, apertural view. H. *Megalochlamys violacea* (*Thulin et al. 9692*), 3-colporate with faint colpi, interapertural view. I. *Yeatesia viridiflora* (*Boufford & Wood 23871*), 3-colporate, interapertural view. J. *Mexacanthus mcvaughii* (*McVaugh 23016*), 2-colporate and 4-pseudocolpate, interapertural view. K. *Chlamydocardia buttneri* (*Taylor 3312*; image provided by R. Scotland, originally published in Kew Bulletin 55:558. 2000), 6-colporate and faintly 12-pseudocolpate, apertural view. L. *Trichaulax mwasumbii* (*Mwasumbi 14238*), 5-colporate with 4 pseudocolpi per mesocolpium, subapertural view. M. *Henrya insularis* (*Daniel & Bartholomew 4731*), 3-colporate and 6-pseudocolpate with broad colpi, interapertural view. Scales = 10 µm.

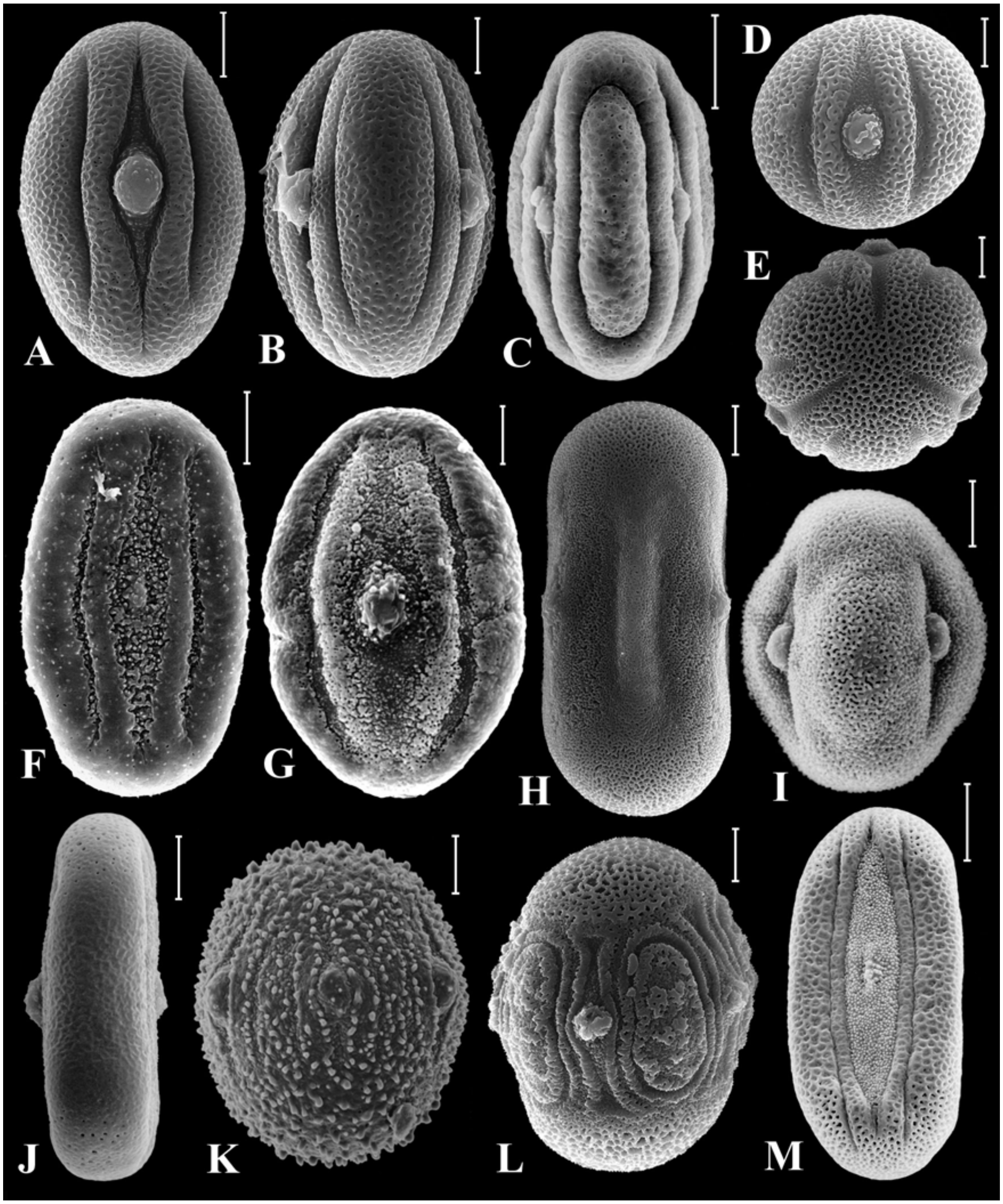
FIG. 2. Interapertural exine sculpture. A. Bireticulate exine of *Anisacanthus pumilis* (Dietr.) Nees (*Lott & Magallanes 313*). B. Bireticulate exine of *Fittonia albivenis* (*Daniel s.n.*). C. Reticulate exine of *Yeatesia viridiflora* (*Boufford & Wood 23871*). D. Foveolate-gemmate exine of *Mirandea huastecensis* (*Daniel & Baker 3616*). E. Subrugulate-gemmate exine of *Mirandea grisea* (*Daniel 852*). Scales = 3 µm.

FIG. 3. Floral diversity and androecial characteristics in the *Tetramerium* lineage. A. *Gypsacanthus nelsonii* (from *Daniel 8357*), corolla opened to show androecium. B. Distal portion of stamens showing parallel to sagittate, bithecous, and unappendaged anthers (left: *Tetramerium carranzae* T.F. Daniel (from *Carranza & Cervantes 5470*), ventral view; middle: *Carlowrightia venturae* T.F. Daniel (from *Ventura & López 9336*), side view; right: *Tetramerium sagasteguianum* (from *Daniel & Butterwick 4319*), dorsal view. C. *Gypsacanthus nelsonii*, small, pleurotribic flower (redrawn from Flora del Valle de Tehuacán-Cuicatlán 23: 38. 1999). D. *Mirandea huastecensis*, nototribic flower (from *Daniel 762*). E. *Hoverdenia speciosa*, nototribic flower with subsaccate corolla throat (redrawn from Flora del Bajío y de Regiones Adyacentes 117: 63. 2003, and photograph of *Daniel & Baker 3739*). F. *Anisacanthus linearis* sternotribic flower with funnelform corolla tube (redrawn from Brittonia 34:173. 1982 and photograph of *Daniel 875*). G. *Mexacanthus mcvaughii*, pleurotribic flower with short corolla tube (from *Daniel & Bartholomew 4880*). H. *Yeatesia mabryi*, nototribic flower with elongate corolla tube (from *Daniel & Baker 3698*). I. *Angkalanthus oligophylla*, pendant flower with enlarged corolla throat (redrawn from Kew Bull. 49:472. 1994). J. *Carlowrightia arizonica*, sternotribic flower with central lobe of lower lip modified into a keel (redrawn from Flora of Chiapas 4:22. 1995). K. *Clinacanthus nutans* with corolla strongly bent so that positions of lips are reversed (from *Maxwell 90-244*). L. *Streblacanthus cordatus*, nototribic flower with narrowly cylindric and elongate corolla tube (from photos and specimens of *Almeda & McPherson 6040*). Scales for flowers = 2 mm. Scales for anthers = 0.5 mm. All drawings and redrawings by E. Hunter in 2006.

FIG. 4. Relationships among members of the *Tetramerium* lineage. A. Strict consensus of 15 MP trees (length = 2423, CI = 0.700, RI = 0.742; 632 parsimony informative characters (583 substitutions + 49 indels). This tree is congruent with the Bayesian MAP tree except (1) the *Megalochlamys* clade is sister to the New World (NW) *Tetramerium* lineage and (2) *Angkalanthus* is sister to the *Ecbolium* clade with strong and weak support from Bayes,

respectively (dashed lines). Weak support from parsimony bootstrap analysis for placement of *Chorisochora* with the *Ecbolium* clade + *Angkalanthus* is also indicated by a dashed line. Bayesian posterior probabilities are above clades; bootstrap values to the left, decay indices to the right below clades (*=100% Bayesian or BS support). Limited data (only nrITS) available for *Anisacanthus andersonii* and *A. quadrifidus* var. *wrightii* place these two taxa with the North American (NA) *Anisacanthus* clade but without further resolution of relationships. *Tetramerium nemorum, T. nervosum* (South American accession), *T. wasshausenii,* and *T. zeta* are placed by our data in a polytomy with *T. nervosum* (North American accession), *T. yaquianum, T. peruvianum* and *T. sagasteguianum*, but without further resolution of relationships. These taxa were excluded from bootstrap analyses because of time required to complete searches owing to large numbers of MP trees differing only in weakly supported alternative relationships among these eight accessions of *Tetramerium*. *Gypsacanthus nelsonii, Fittonia albivenis,* and *Mirandea sylvatica* are not placed with precision by our data and we treat them as members, incertae sedis, of the core *Tetramerium* lineage (*G. nelsonii*) and the NW *Tetramerium* lineage. B. Placement of *Justicia gonzalezii* in the South American (SA) *Anisacanthus* clade; parsimony analysis, bootstrap values above, decay indices below clades. C. Placement of *Yeatesia platystegia* and *Y. viridiflora*; parsimony analysis, bootstrap values above, decay indices below clades. See text for full explanation of B and C.

FIG. 5. Geographic distribution (key to abbreviations in box, upper left) and the evolution of floral traits associated with hummingbird pollination. Phylogeny is strict concensus of MP trees as in fig. 4; for simplicity, species names are omitted but taxa are positioned exactly as in fig. 4. All basal clades in the *Tetramerium* lineage are Old World, and New World members are monophyletic such that a single dispersal event to the New World is hypothesized (arrow). Within the New World note that numerous North America – South America exchanges are likely. Ellipses mark the evolution of long, funnelform, brightly colored flowers that are known or hypothesized to be pollinated by hummingbirds.


A
B
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D
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F
G
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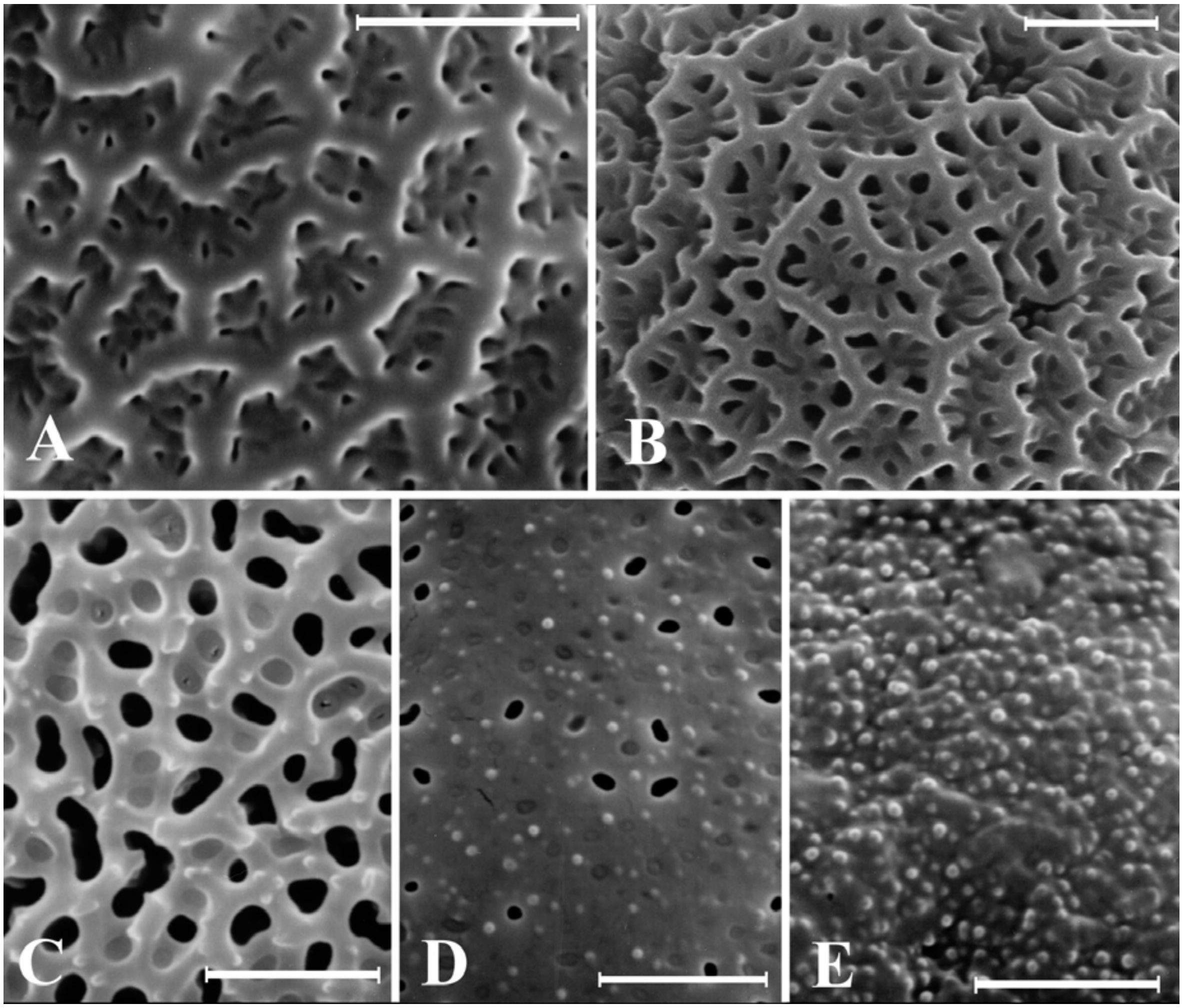
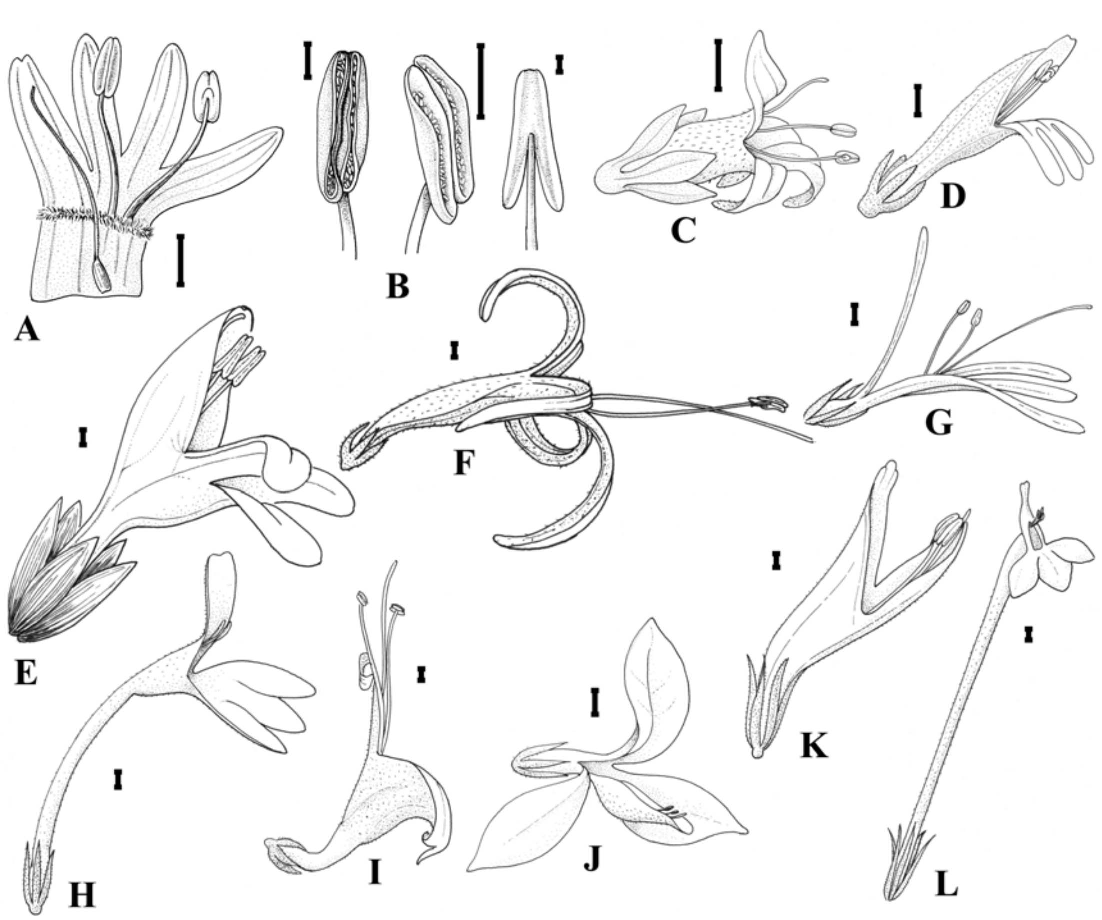
K
L
M

1





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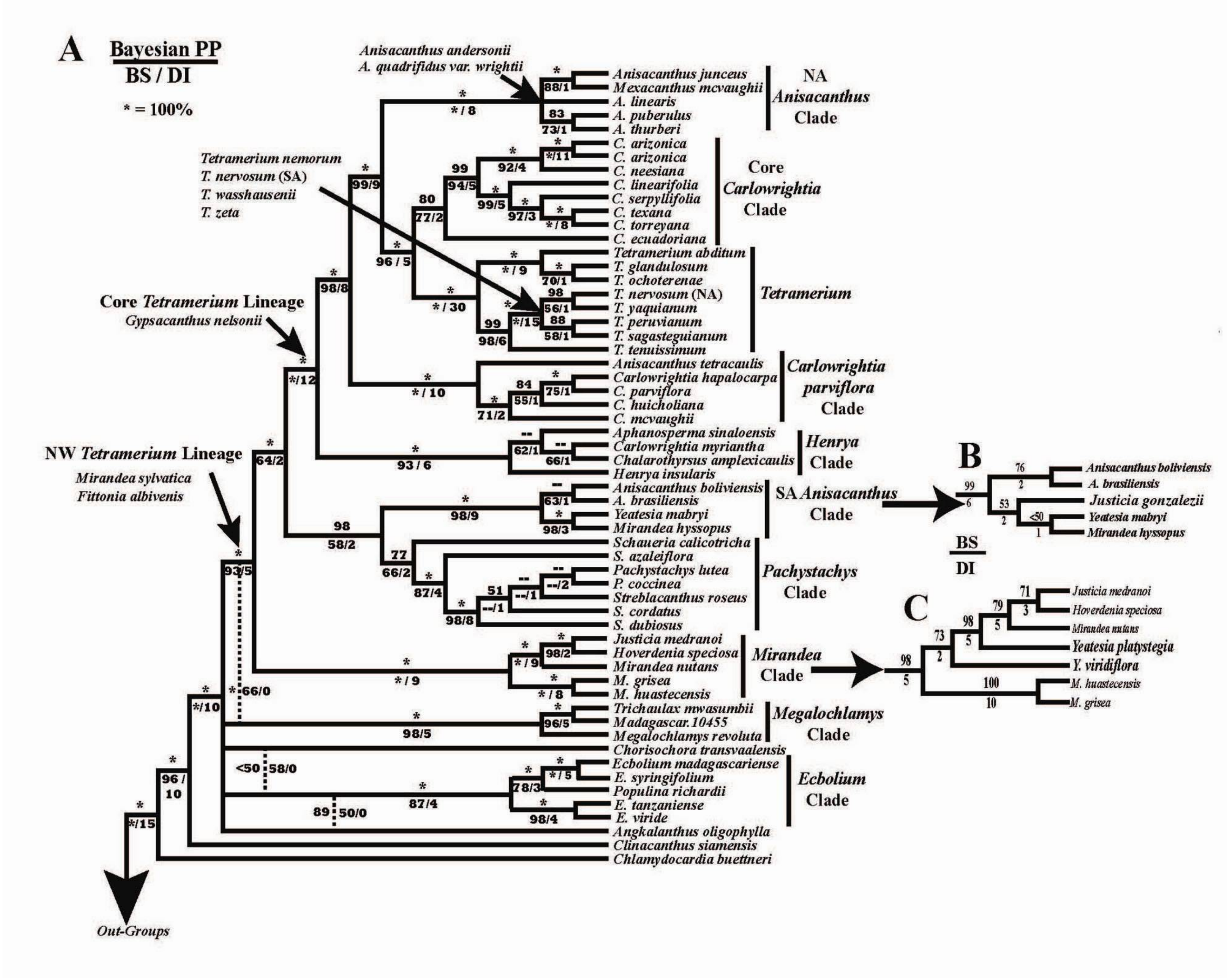
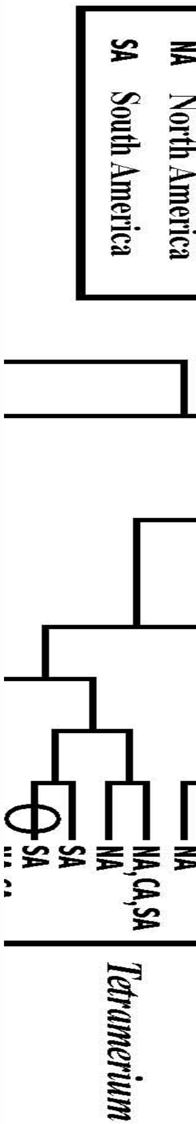
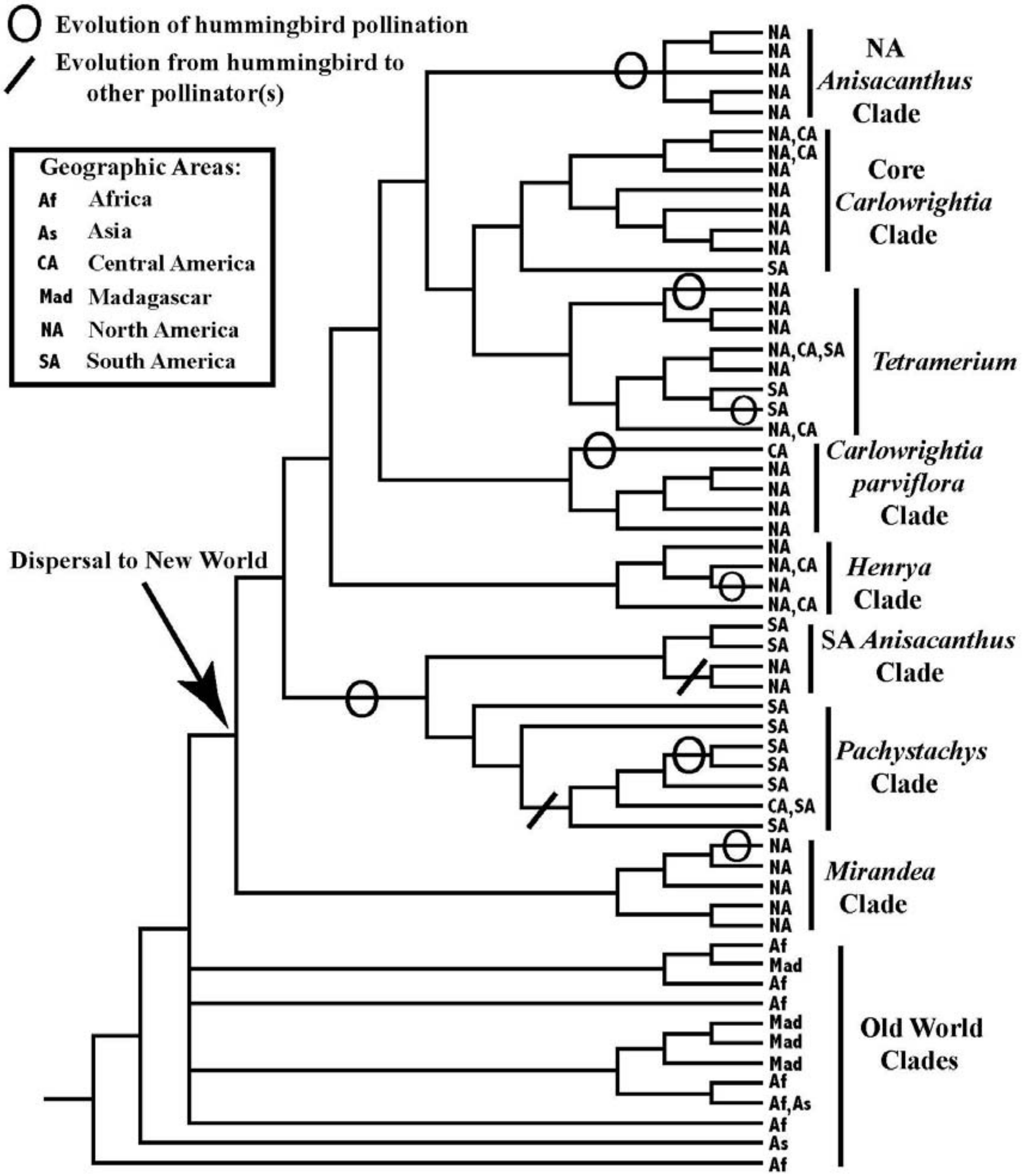

A
Bayesian PP
BS / DI
* = 100%
Anisacanthus andersonii
A. quadrifidus var. wrightii
Tetramerium nemorum
T. nervosum (SA)
T. wasshausenii
T. zeta
Core Tetramerium Lineage
Gypsacanthus nelsonii
NW Tetramerium Lineage
Mirandea sylvatica
Fittonia albivenis
Out-Groups
Anisacanthus junceus
Mexacanthus mcvaughii
A. linearis
A. puberulus
A. thurberi
NA Anisacanthus Clade
C. arizonica
C. arizonica
C. neesiana
C. linearifolia
C. serpyllifolia
C. texana
C. torreyana
C. ecuadoriana
Core Carlowrightia Clade
Tetramerium abditum
T. glandulosum
T. ochoterenae
T. nervosum (NA)
T. yaquianum
T. peruvianum
T. sagasteguianum
T. tenuissimum
Tetramerium
Anisacanthus tetracaulis
Carlowrightia hapalocarpa
C. parviflora
C. huicholiana
C. mcvaughii
Carlowrightia parviflora Clade
Aphanosperma sinaloensis
Carlowrightia myriantha
Chalarothyrsus amplexicaulis
Henrya insularis
Henrya Clade
Anisacanthus boliviensis
A. brasiliensis
Yeatesia mabryi
Mirandea hyssopus
SA Anisacanthus Clade
Schaueria calicotricha
S. azaleiflora
Pachystachys lutea
P. coccinea
Streblacanthus roseus
S. cordatus
S. dubiosus
Pachystachys Clade
Justicia medranoi
Hoverdenia speciosa
Mirandea nutans
M. grisea
M. huastecensis
Mirandea Clade
Trichaulax mwasumbii
Madagascar.10455
Megalochlamys revoluta
Megalochlamys Clade
Chorisochora transvaalensis
Ecbolium madagascariense
E. syringifolium
Populina richardii
E. tanzaniense
E. viride
Ecbolium Clade
Angkalanthus oligophylla
Clinacanthus siamensis
Chlamydocardia buettneri
88/1
83
73/1
*/8
*/11
92/4
99
94/5
99/5
97/3
*/8
80
77/2
99/9
96 / 5
*/9
70/1
98
56/1
88
58/1
*/15
99
98/6
*/30
98/8
84
75/1
55/1
71/2
*/10
*/12
62/1
66/1
93 / 6
64/2
63/1
98/9
98/3
98
58/2
77
66/2
87/4
51
--/2
--/1
--/1
98/8
98/2
*/9
*/8
*/9
93/5
66/0
96/5
98/5
*/10
<50
58/0
*/5
78/3
98/4
87/4
89
50/0
96 / 10
*/15
B
99
6
76
2
53
2
<50
1
Anisacanthus boliviensis
A. brasiliensis
Justicia gonzalezii
Yeatesia mabryi
Mirandea hyssopus
BS
DI
C
98
5
73
2
98
5
79
5
71
3
100
10
Justicia medranoi
Hoverdenia speciosa
Mirandea nutans
Yeatesia platystegia
Y. viridiflora
M. huastecensis
M. grisea


NA North America
SA South America
NA
NA,CA,SA
NA
SA
SA
Tetramerium


Evolution of hummingbird pollination
Evolution from hummingbird to other pollinator(s)
Geographic Areas:
Af Africa
As Asia
CA Central America
Mad Madagascar
NA North America
SA South America
Dispersal to New World
NA
NA
NA
NA
NA
NA Anisacanthus Clade
NA,CA
NA,CA
NA
NA
NA
NA
NA
SA
Core Carlowrightia Clade
NA
NA
NA
NA,CA,SA
NA
SA
SA
NA,CA
Tetramerium
CA
NA
NA
NA
NA
Carlowrightia parviflora Clade
NA
NA,CA
NA
NA,CA
Henrya Clade
SA
SA
NA
NA
SA Anisacanthus Clade
SA
SA
SA
SA
SA
CA,SA
SA
Pachystachys Clade
NA
NA
NA
NA
NA
Mirandea Clade
Af
Mad
Af
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Mad
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Af,As
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Old World Clades