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Relationships of *Asanthus* (Asteraceae, Eupatorieae)

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Abstract—Analysis of DNA sequence data was used to clarify the circumscription and relationships of the small Mexican genus *Asanthus*. Results from non-coding regions, including the nuclear ITS and the chloroplast *psbA-trnH* spacer showed that *Asanthus* is clearly monophyletic and distinct from *Steviopsis* and *Brickellia*, two genera with which it is sometimes lumped, as well as from the closely related *Brickelliastrum* and *Carminatia*. The DNA sequence data also agree with morphological observations in showing that *Asanthus* is composed of three species, *A. thrysiflorus* from the Sierra Madre Oriental and *A. squamulosus* and *A. solidaginifolius* from the Sierra Madre Occidental. Samples from the southern Sierra Madre Occidental that have been identified as *A. thrysiflorus* are suggested by the presence of an additive pattern of polymorphisms in the nuclear ITS region to be of interspecific hybrid origin between *A. thrysiflorus* and *A. solidaginifolius*, which is consistent with reports of their morphological intergradation in this area. The results suggest that geographic separation and divergence within *Asanthus* is now being followed by secondary contact, and point to a dynamic pattern of movement and change in the genus.

Keywords—*Brickellia*, *Brickelliastrum*, divergence times, Mexico, phylogenetics, *Steviopsis*.

Asanthus R. M. King & H. Rob. was proposed as part of a series of sweeping generic level reclassifications of Eupatorieae, summarized in the seminal volume by King and Robinson (1987). Their efforts were aimed primarily at the large and unwieldy *Eupatorium* L., but also extended to other genera such as *Brickellia* Elliott. *Brickellia* was traditionally recognized within Eupatorieae as distinct from *Eupatorium* s. l. based on its 8–10 ribbed cypselae (rather than five ribbed) and chromosome base number of $x = 9$ (vs. $x = 10$) combined with a pappus of bristles. There are, however, a number of species that proved difficult to place unequivocally because they have an overall habitat similar to *Brickellia* but possess either five ribbed cypselae or a chromosome base of $x = 10$. King and Robinson (1987) refined the circumscription of *Brickellia* and added several features that were considered to characterize it: a densely pubescent, enlarged node at the base of the style; style appendages that are usually clavate; and flattened outer surfaces of the pappus bristles. A number of species that mostly have cypselae with more than five ribs were segregated by King and Robinson (1987) into other genera, based in part on lack of the pubescent and enlarged style base, including *Asanthus* R. M. King & H. Rob., *Brickelliastrum* R. M. King & H. Rob., *Dyscritogyne* R. M. King & H. Rob., and *Steviopsis* R. M. King & H. Rob. (Table 1). Turner (1988, 1990, 1994, 1997), while accepting the segregation of these species from *Brickellia*, took a slightly different view and lumped them all together into an enlarged *Steviopsis*. This treatment produces what Turner (1997) admitted was “an heterogeneous group,” but has the apparent advantage of simplicity. Another genus, *Carminatia* Moc. ex DC, was briefly included in *Brickellia* (Keil and Pinkava 1976), but has generally been accepted as distinct although closely related. These species collectively occur in a biogeographically complex setting, primarily in Mexico, thus a sound taxonomy that reflects phylogenetic relationships is necessary to help interpret their evolutionary and biogeographic history. Robinson et al. (2009) provided an update that noted some problems in subtribal level classification

of Eupatorieae, but did not address the generic-level issues that are the focus of the current study. Notably, subtribes such as Gyptidinae and Alomiinae that include a mixture of Mexican/North American and South American genera are likely to be polyphyletic (Schilling 2008; Schilling and Panero unpublished data.)

As part of an ongoing study of the phylogeny of Eupatorieae, we undertook an analysis of the systematic status of *Asanthus* to assess whether it should be recognized as a distinct genus or lumped into either *Brickellia* or *Steviopsis*. Initial surveys based on plastid DNA sequence analysis (Panero et al. unpublished data) suggested that exemplars of the taxa included within *Steviopsis* as recognized by Turner (1997) were placed close to but not within *Brickellia*. In the current project, a survey was undertaken that utilized primarily herbarium material to conduct a comprehensive sampling of *Asanthus* and its close relatives. For molecular markers, we focused on two spacer regions that are short enough to amplify well from herbarium specimen-derived DNA preparations, the chloroplast *psbA-trnH* region and the complete nuclear ribosomal ITS region. Although problems in alignment limit the usefulness of these regions for phylogenetic comparisons across the entire tribe Eupatorieae, they proved to be sufficiently similar to be readily applied to *Asanthus* and its close relatives and to offer insight not only into its generic level classification but also into the circumscription, timing of divergence, and relationships of its species.

MATERIALS AND METHODS

Taxonomic Sampling—A list of samples and vouchers is provided in Appendix 1. Assessment of the phylogenetic placement and species relationships of *Asanthus* utilized 11 samples representing each of the putative species, and at least one sample of all of the accepted species in the other members of *Steviopsis* sensu Turner (*Steviopsis*, *Brickelliastrum*, and *Dyscritogyne*). A set of representative species of *Brickellia*, including the type species *B. cordifolia*, and representing each of the major lineages based on ITS sequence analysis (Schilling et al. unpublished data) were also sampled, and samples were included of two other genera that are recognized to be close to but distinct from *Brickellia*, *Pleurocoronis*

TABLE 1. Morphological comparison of *Asanthus* and related genera. The two species originally proposed for *Dyscritogyne* are included in *Steviopsis*.

	<i>Asanthus</i>	<i>Brickelliastrum</i>	<i>Steviopsis</i>	<i>Dyscritogyne</i>	<i>Carminatia</i>	<i>Brickellia</i>
Number of species	3	2	5	(2)	4	ca. 90
Base Chromosome #	$x = 10$	$x = 10$	$x = 10$	$x = 10$	$x = 10$	$x = 9$
Cypselae ribs	8–10	5–7	5 or 8–10	4–5	5	8–10
Cypselae glands	Absent	Absent	Present	Present, dense	Absent	Absent
Corolla shape	Narrow	Broad	Narrow	Narrow	Narrow	Narrow
Corolla lobes	Erect	Erect	Spreading	Spreading	Erect	Erect
Style base	Glabrous	Glabrous	Glabrous	Glabrous	Glabrous	Pubescent
Style branches	Clavate	Clavate	Clavate	Clavate	Narrow	Clavate
Leaves	Sessile	Petiolate	Sessile / subsessile	Subsessile	Petiolate	Sessile or petiolate
Leaf base	Tapering	Truncate or cordate	Tapering	Varied	Truncate or cordate	Varied

R. M. King & H. Rob., and *Carminatia*. Most of the samples were made from herbarium material, although a few were collected as fresh material and either frozen in liquid nitrogen or preserved in silica gel. For outgroups, samples of *Ageratina* Spach were used, based on results from previous studies placing it as one of the basal-most diverging groups in Eupatorieae (Schmidt and Schilling 2000; Ito et al. 2000). Outgroups outside of Eupatorieae (Panero and Funk 2008) included two members of tribe Perityleae (*Galeana* La Llave and *Amauria* Benth.), as well as a member of tribe Madieae (*Monolopia* DC.), using sequences on deposit in GenBank.

Molecular Methods—Preparations of total DNA were performed with the Dneasy plant minikit (Qiagen, Valencia, California) and typically utilized a portion (ca. 0.1 g) of a single leaf. The crude DNA extracts of some samples required further purification using the Wizard kit protocol (Promega, Madison, Wisconsin). The PCR amplifications were performed in 20 μ l reactions using 10–20 ng of genomic DNA, 10 \times PCR buffer (Promega), 1.8–2.25 mM MgCl₂, 0.2 mM each dNTP, 1.25 units of *Taq* polymerase, and 0.2 μ M each primer. Amplification and sequencing of the chloroplast spacer *psbA-trnH* followed Panero and Crozier (2003). The PCR products were checked on 1% agarose gels before being cleaned with ExoSAP-IT (USB, Cleveland, Ohio). All DNA sequencing was performed with the ABI Prism BigDye terminator cycle sequencing ready reaction kit, v. 3.1 (Perkin-Elmer/Applied Biosystems, Foster City, California) and electrophoresed and detected on an ABI Prism 3100 automated sequencer (University of Tennessee Molecular Biology Resource Facility, Knoxville, Tennessee). The initial sequence data text files were edited following comparison with the same data displayed in four-color electropherograms before they were analyzed further. Sequence alignment was performed manually. GenBank accession numbers are provided in Appendix 1.

Phylogenetic Analyses—Phylogenetic relationships were analyzed using both maximum parsimony and Bayesian approaches. Parsimony analysis was implemented using PAUP 4.0b10* (Swofford 2003), using a heuristic search with 1,000 random addition replicates and with TBR branch swapping. Bootstrap analysis (Felsenstein 1985) was performed with 1,000 replicates. Bayesian analysis was implemented in MRBAYES 3.0B4 (Huelsenbeck and Ronquist 2001) run for ten million generations with four separate chains and trees saved every 100 generations. The number of trees to discard as “burn-in” was assessed by plotting likelihoods of trees sampled throughout the run and discarding all trees prior to the stable likelihood plateau (in this case the first 10% were discarded). An appropriate maximum likelihood model of sequence evolution (GTR + I + G; general time reversible model with a proportion of invariant sites and gamma distributed rates) for the Bayesian analysis was chosen separately for each locus using Modeltest (Posada and Crandall 1998). Results are submitted to TreeBASE (study number S13157). An ILD (incongruence length difference) test (Farris et al. 1994) was performed in PAUP* to determine whether the nuclear ITS and plastid *psbA-trnH* data were congruent.

We calculated divergence times using the program BEAST v. 1.6.1 (Drummond and Rambaut 2007). The analysis was performed using the CIPRES Science Gateway (<http://www.phylo.org/>, Miller et al. 2010). The input data matrix consisted of 31 Eupatorieae samples and two outgroup taxa, *Amauria*/*Galeana* (Perityleae) and *Monolopia*. Because fossils for Eupatorieae have yet to be discovered, we used a calibration from a rate calibrated Asteraceae analysis (Kim et al. 2005) to constrain the age of the Perityleae/Eupatorieae node. All members of Eupatorieae and *Amauria*/*Galeana* were enforced as a monophyletic group and a normal distribution prior with a median age of 14 Mya (million years ago) and a standard deviation of 1 was used to constrain this node. The analysis had the following parameters set: a relaxed, uncorrelated lognor-

mal clock and a GTR + G model with four categories, a Yule tree prior, and MCMC chains of 30 million generations sampled every 2,000 generations. We used Tracer 1.5 (Rambaut and Drummond 2009) to assess the effective sample sizes (ESS). We constructed a maximum clade credibility timetree using the program TreeAnnotator v. 1.4.8 (Drummond and Rambaut 2007) with no burnin and a posterior probability ≥ 0.95 .

RESULTS

Sequences from the chloroplast DNA *psbA-trnH* spacer region for samples of Eupatorieae varied in length from 245 bp in *Carminatia papagayana* to 400 bp in *Ageratina luciae-brauniae*. Size variation could be attributed to the presence of 16 insertions or deletions, 11 of which were five to eight bp in length and were most likely the result of duplication events. A large (139 bp) deletion characterized the three samples of *Carminatia*. Samples of *Brickelliastrum* and *Carminatia* shared a 24 bp inversion in the hairpin region that has been discussed by Bain and Jansen (2006) as having recurrent variation within Asteraceae; this region was recoded as a single character for phylogenetic analysis. Within *Asanthus*, sequence lengths were species-specific, and were 377 bp in *A. thyrsoiflorus*, 383 bp in *A. solidaginifolius*, and 384 bp in *A. squamulosus*. These varying lengths were inferred to result from three indels: a seven bp duplication in *A. squamulosus*, and an eight bp duplication and a two bp deletion in *A. solidaginifolius*. The amount of base pair variation among the *Asanthus* species was minimal: samples of *A. squamulosus* differed by three single bp differences from the other species, and there was a single base pair difference at a fourth position in *A. thyrsoiflorus* compared to the other two species. Gaps were coded as characters for the data analysis following the gap-coding method of Simmons and Ochoterena (2000).

The ITS region from samples of Eupatorieae varied in length from 636 bp in *Ageratina luciae-brauniae* to 653 bp in *Brickellia cordifolia*. The ITS sequence lengths within *Asanthus* were also species-specific, with 651 bp in *A. thyrsoiflorus*, 652 bp in *A. solidaginifolius*, and 647 bp in *A. squamulosus*. Relative to *A. thyrsoiflorus*, for ITS *A. solidaginifolius* exhibited a single base pair insertion (within a short poly-T region), and *A. squamulosus* exhibited a two bp deletion and two one bp deletions. There was only a single bp difference between the ITS sequences of *A. thyrsoiflorus* and *A. solidaginifolius*, and the two differed from *A. squamulosus* by 17 and 18 bp differences, respectively. Two samples (DNA# 2403, 2491) of *A. thyrsoiflorus* from the westernmost portion of its range in the Sierra Madre Occidental exhibited polymorphisms for the two differences (a bp and a length difference) in ITS sequence that separated the samples of *A. thyrsoiflorus* from those of *A. solidaginifolius*.

The results of phylogenetic analysis of the two individual spacer regions were congruent (PAUP* homogeneity partition test, $p = 0.05$), and only the results of the combined analysis of all regions are shown (Fig. 1). Parsimony and Bayesian analyses produced consensus trees of the same topology, in which most of the nodes received high statistical support (Fig. 1). Relative to the outgroups and *Ageratina*, there was a split that separated samples of *Brickellia* and *Pleurocoronis* from the remaining samples, with both branches of this split receiving modest statistical support. Within the first lineage, *Pleurocoronis* was placed as sister to a monophyletic *Brickellia*. Within the second lineage, there were clades that corresponded to a number of the previously described genera, including *Asanthus*, *Brickelliastrum*, and *Steviopsis* s. s. There was not, however, a clade that corresponded to *Steviopsis* sensu Turner: samples of *Brickelliastrum* were placed as sister to *Carminatia* in a clade that was further sister to *Steviopsis* s. s., and that clade was sister to *Asanthus*. There was also not a clade that corresponded to *Dyscritogyne*; the two species were placed as basally diverging branches within *Steviopsis* (Fig. 1).

Results of the dating analysis (Fig. 2) estimated the split between Perityleae and Eupatorieae at 12.85 Mya (95% CI 11.9–15.8), with the divergence between *Ageratina* and the chromosomally $x = 9/x = 10$ clades at 9.10 Mya (95% CI 5.8–12.4). Divergence between the $x = 9$ and $x = 10$ clades was 6.37 Mya (95% CI 3.9–8.3). Divergence between *Asanthus* and

closely related genera was dated at 6.0 Mya (95% CI 3.3–8.5), with divergence between *Steviopsis* and subsequently *Brickelliastrum* and *Carminatia* estimated at 4.93 Mya (95% CI 2.7–7.5) and 4.46 Mya (95% CI 1.9–6.0), respectively. Infrageneric divergence varied considerably among genera, with species level splits within *Asanthus*, *Brickelliastrum*, and *Steviopsis* dated from < 0.01 Mya to 1.91 Mya. In contrast, the divergence between the two species sampled from *Ageratina* was estimated at 3.98 Mya, and the oldest split within *Brickellia* at 6.1 Mya (Fig. 2). The effective sample sizes (ESS) for all parameters were above 1,000.

DISCUSSION

The molecular systematic results help us to resolve the issues regarding generic level classification of *Asanthus* by showing that it is a monophyletic group distinct from both *Brickellia* and *Steviopsis*. These results further help to clarify the circumscriptions of *Brickelliastrum* and *Steviopsis* by showing that they are also distinct from *Brickellia*, although they do not support the separation of *Steviopsis* and *Dyscritogyne*. Within *Asanthus*, there is support for three taxa, of which one (*A. squamulosus*) is distinct and the other two (*A. thyrsoflorus* and *A. solidaginifolius*) appear to be undergoing secondary intergradation.

The results of phylogenetic analysis of *Asanthus*, *Brickellia*, and related genera based on molecular data showed a clear

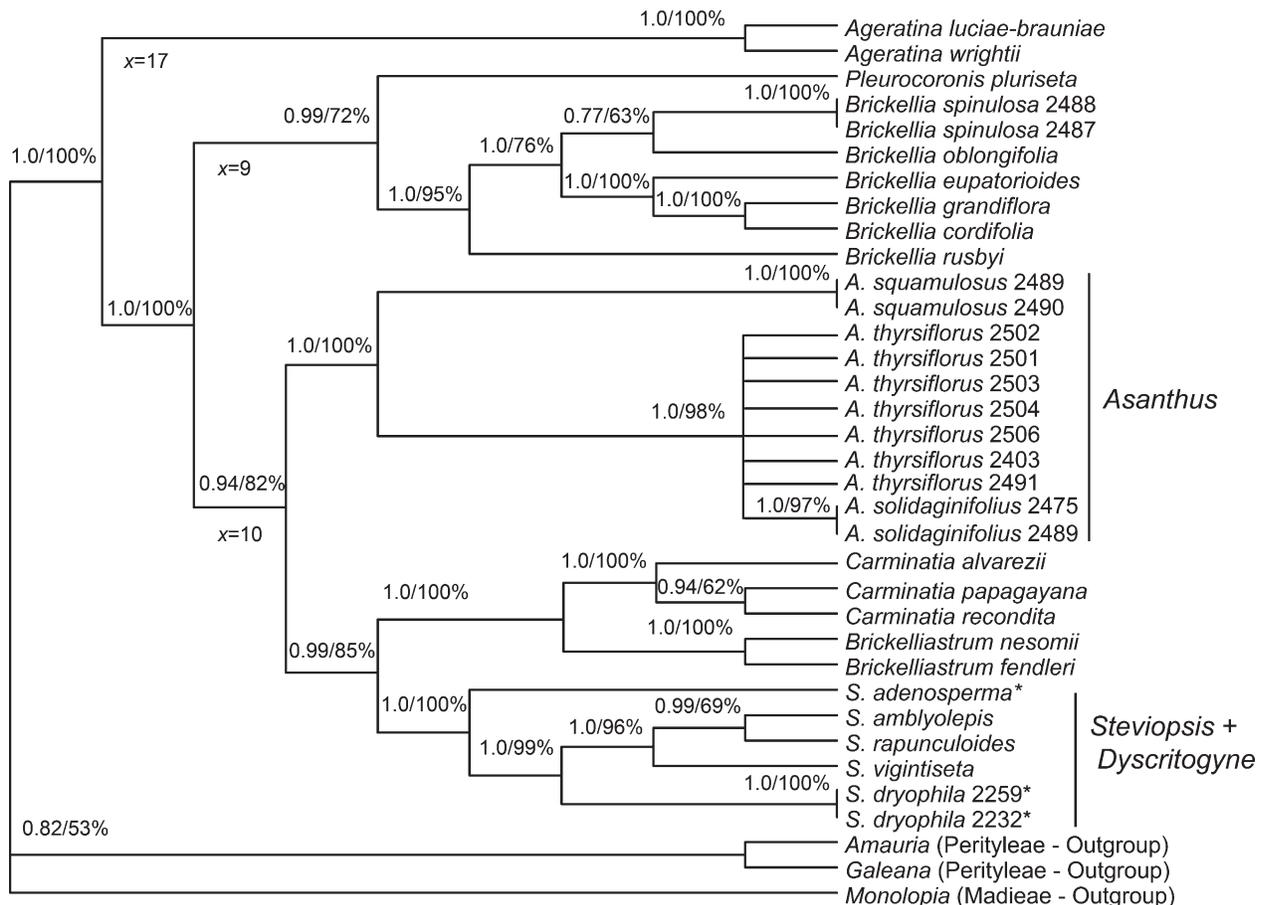


FIG. 1. Relationships among species of *Asanthus* and related genera based on analysis of combined nuclear ribosomal ITS and plastid *psbA-trnH* DNA sequence data. Bayesian posterior probabilities/parsimony bootstrap values shown above branches, $< 50\%$; chromosome base numbers shown below branches. Sample numbers shown for species represented by multiple samples; asterisk- species of *Dyscritogyne*.

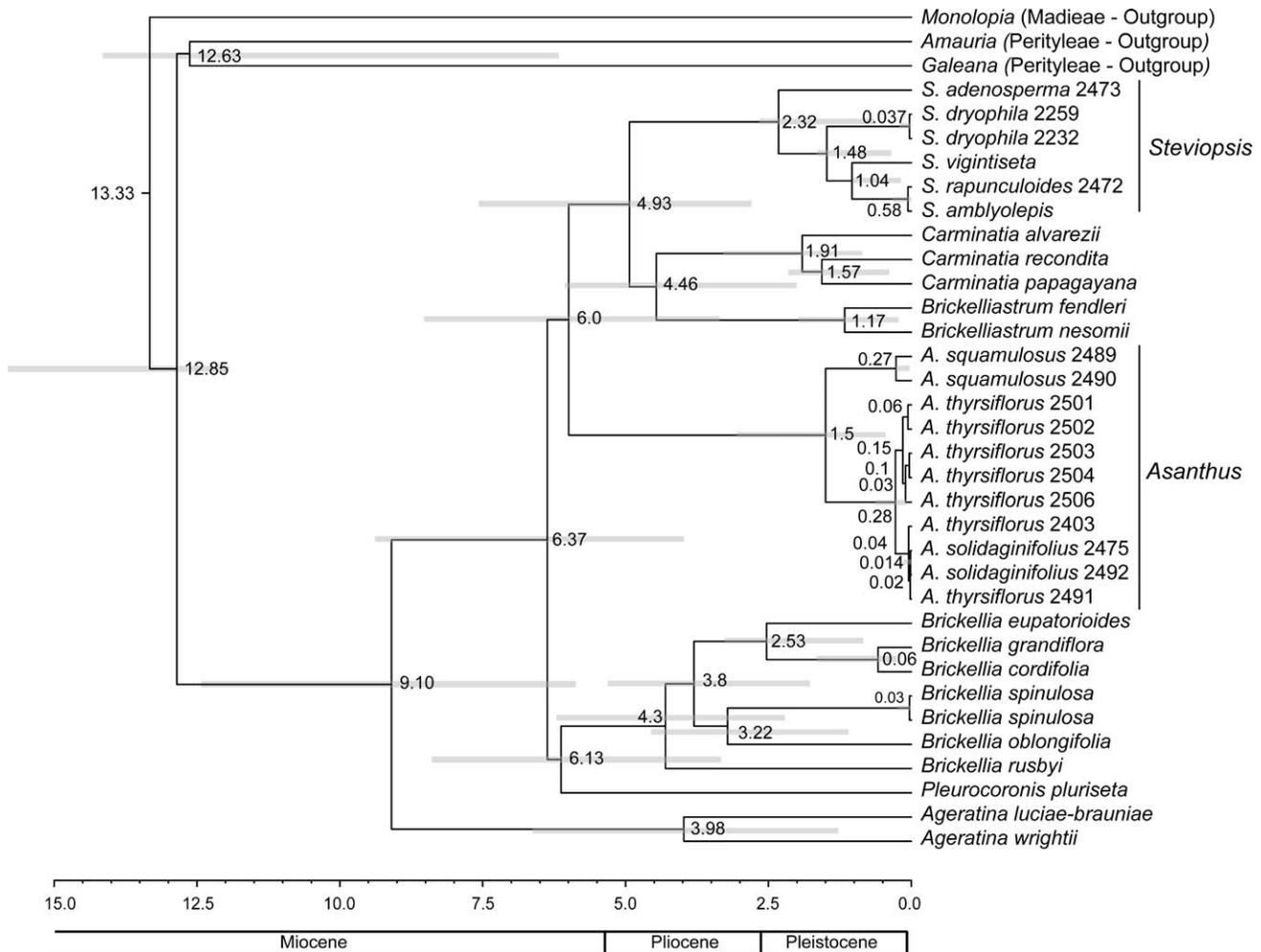


FIG. 2. Maximum clade credibility chronogram of *Asanthus* and related genera. Nodes represented by their mean ages (million years ago) under a relaxed lognormal uncorrelated molecular clock assumption using BEAST. The Perityleae/Eupatorieae node was used for calibration. Light gray bars represent 95% highest posterior density limits.

separation into two distinct clades that correlated with the base chromosome numbers of $x = 9$ and $x = 10$, respectively (Figs. 1, 2). This result is consistent with previously published information on Eupatorieae phylogeny (Schilling et al. 1999; Schmidt and Schilling 2000; Ito et al. 2000). If *Brickellia* were to be expanded to include *Asanthus* or *Brickelliastrum*, to remain monophyletic it would also have to include *Pleurocoronis* and *Carminatia*, both of which have been accepted as being unquestionably distinct morphologically from *Brickellia*. The molecular-based results also failed to support a broadly defined *Steviopsis* sensu Turner (1988), as any expansion of the genus to include *Asanthus* or *Brickelliastrum* would require inclusion also of *Carminatia* for it to remain monophyletic. The results (Fig. 1) showed that *Dyscritogyne* was paraphyletic to *Steviopsis* s. s., suggesting that the two be combined. The apparent close relationship between *Brickelliastrum* and *Carminatia* indicated by the molecular results has not been suggested previously, nor is there in retrospective analysis any striking morphological similarity. It can be noted, however, that there is strong support for the placement of *Brickelliastrum nesomii* with the other species of the genus, *B. fendleri* (Fig. 1), as was suggested by King and Robinson (1994, 1995), rather than in *Steviopsis* (Turner 1997).

The molecular phylogenetic results provided support for the significance of the morphological features summarized in King and Robinson (1987) that have been used to separate *Asanthus* and other genera from *Brickellia*, and are summarized in Table 1. A salient feature is the absence of the pubescent, enlarged node at the base of the style that is characteristic of all species of *Brickellia* but is absent in *Asanthus*, *Brickelliastrum*, and *Steviopsis*. *Asanthus* consistently has 8–10 ribbed eglandular cypselae in combination with narrow corollas with erect lobes and sessile leaves that have tapering bases. The cypselae in *Brickelliastrum* are five to seven ribbed, the corollas are broad, and the leaves are petiolate. The cypselae in *Steviopsis* may have five or 8–10 ribs but they are consistently glandular, and the corollas have spreading rather than erect lobes. *Dyscritogyne* was separated from *Steviopsis* based on the more densely glandular cypselae and somewhat broader style lobes, but it appears that these features do not define a monophyletic lineage.

Within *Asanthus*, the pattern of molecular variability fits well with earlier observations regarding species-level variability. The morphologically most distinctive species, *A. squamulosus*, was also the most distinctive at the molecular level. The habitual similarities between *A. squamulosus* and *Brickellia spinulosa*, which led Robinson (1917) to group them

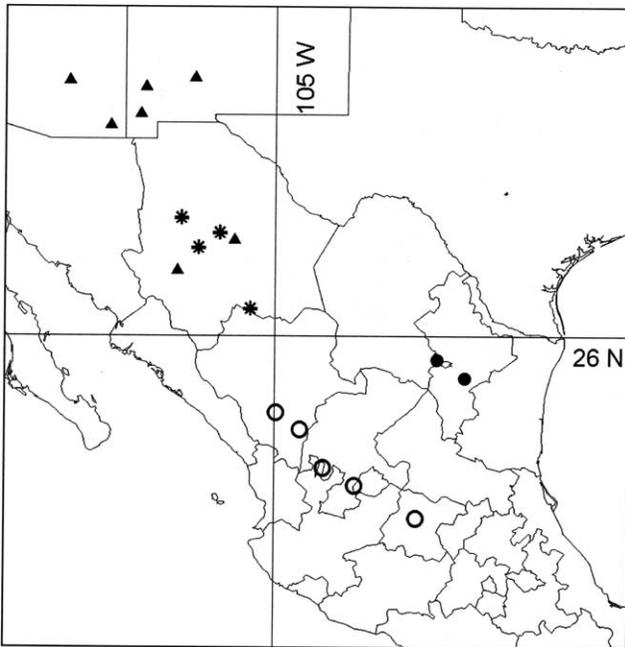


FIG. 3. Distribution of *Asanthus* in Mexico and the southwestern U. S. A. asterisk- *A. solidaginifolius*; triangle - *A. squamulosus*; solid circle- *A. thyrsoiflorus*; open circle- *A. thyrsoiflorus*, intermediates with *A. solidaginifolius*.

together in *Brickellia* sect. *Gemmipedium* B. L. Rob., were shown clearly by molecular data to be the result of convergence (Fig. 1). The other two species, *A. solidaginifolius* and *A. thyrsoiflorus*, are similar both morphologically and at the molecular level, and the decision to recognize them as distinct species or as varieties (or subspecies) within a species is still open. Flavonoid studies (Yu et al. 1986) as well as the molecular results suggest that *A. solidaginifolius* and *A. thyrsoiflorus* may be consistently distinct in the northwestern and eastern areas, respectively, of their combined range (Fig. 3). The details of the ITS sequences with the single nucleotide and indel polymorphisms suggested that the collections from the southwestern portion of the range, identified as *A. thyrsoiflorus*, which McVaugh (1984) and Turner (1997) characterized as morphologically intermediate between *A. thyrsoiflorus* and *A. solidaginifolius*, might actually be hybrids or hybrid derivatives between the two. Combined with the overall pattern of geographic distribution (Fig. 3), the molecular results suggested that *A. thyrsoiflorus* and *A. solidaginifolius* represent sister taxa that may have diverged from a common ancestor as they moved to occupy ranges in the Sierra Madre Oriental and Sierra Madre Occidental, respectively. The presence of morphological intermediates that are also intermediate at the molecular level suggests the possibility of hybridization after the two species re-established contact. The high degree of molecular similarity between *A. thyrsoiflorus* and *A. solidaginifolius* suggests that any changes in geographic ranges (Fig. 3) have occurred relatively recently, and thus both the initial dispersal to separate them and to subsequently bring them back into contact may have been rapid.

Results of divergence time estimates (Fig. 2) add to the growing evidence (Schmidt and Schilling 2000; Kim et al. 2005) that Eupatorieae is relatively recent in origin, and point to significant differences in times of origin among genera of the tribe. Note that the absolute time estimates must be

interpreted with caution, however, because they are based on a single nuclear region, ITS. The estimates within *Asanthus*, *Brickelliastrum*, *Carminatia*, and *Steviopsis* suggest that much of the species-level divergence has occurred in Pleistocene times, possibly related to climate changes driven by glacial/post-glacial variability (Rull 2011), and continuing until recently. Although sampling for this study was not dense, it is clear that crown divergence in the larger *Brickellia* occurred much earlier than the other smaller genera. Future studies to quantify levels of molecular divergence within a sound phylogenetic framework will allow elucidation of the evolutionary history of plant groups that occur in the geologically complex region of northern Mexico.

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- APPENDIX 1. Taxa and vouchers of plant material of *Asanthus* and related Eupatorieae from which DNA was extracted for sequence analysis, together with GenBank accession numbers [ITS; *psbA-trnH*]. Vouchers at TENN unless otherwise noted.
- Asanthus thyrsiflorus* (A. Gray) R. M. King & H. Rob.: Mexico, Panero 8815, DNA 2403 [JQ737006; JQ737036]; Villareal 5422 [TEX], DNA 2491 [JQ737007; JQ737037]; McVaugh & Koelz 112 [TEX], DNA 2501 [JQ737008; JQ737038]; Diaz Leon 13045 [TEX], DNA 2502 [JQ737009; JQ737039]; Feddema 2432 [TEX], DNA 2503 [JQ737010; JQ737040]; Villareal 4987 [TEX], DNA 2504 [JQ737011; JQ737041]; Warnock 2420 [TEX], DNA 2506 [JQ737012; JQ737042]. *Asanthus solidaginifolius* (A. Gray) R. M. King & H. Rob.: Mexico, Sundberg 2475 [TEX], DNA 2473 [JQ737013; JQ737043]; Sundberg & Lavin 2736 [TEX], DNA 2492 [JQ737014; JQ737044]. *Asanthus squamulosus* (A. Gray) R. M. King & H. Rob.: Mexico, Pringle 705 [TEX], DNA 2489 [JQ737015; JQ737045]; Bye 4077, DNA 2490 [JQ737016; JQ737046].
- Brickellia cordifolia* Elliott: U. S. A., MacDonald 7637, DNA 2285 [JQ737032; JQ737062]. *Brickellia eupatorioides* (L.) Shinn.: U. S. A., Schilling 04-48, DNA 1184 [JQ737028; JQ737058]. *Brickellia grandiflora* (Hook.) Nutt.: U. S. A., Schilling 2004, DNA 728 [JQ737031; JQ737061]. *Brickellia oblongifolia* Nutt.: U. S. A., Holmgren 3740, DNA 2289 [JQ737029; JQ737059]. *Brickellia rusbyi* A. Gray: U. S. A., Schilling 2003, DNA 732 [JQ737030; JQ737060]. *Brickellia spinulosa* A. Gray: Mexico, Hinton et al. 22722, DNA 2487 [JQ737033; JQ737063]; Powell et al. 2060, DNA 2488 [JQ737034; JQ737064].
- Brickelliastrum fendleri* (A. Gray) R. M. King & H. Rob.: U. S. A., Schilling 2012, DNA 724 [JQ737023; JQ737053]. *Brickelliastrum nesomii* (B. L. Turner) R. M. King & H. Rob.: Mexico, Hinton et al. 23958 [TEX], DNA 2474 [JQ737024; JQ737054].
- Carminatia alvarezii* Rzed. and Calderón: Mexico, Panero 6776, DNA 3290. *Carminatia papagayana* B. L. Turner: Mexico, Panero 6193, DNA 557 [JQ737025; JQ737055]. *Carminatia recondita* McVaugh.: Mexico, Panero 8833, DNA 2399 [JQ737026; JQ737056].
- Pleurocoronis pluriseta* (A. Gray) R. M. King & H. Rob.: U. S. A., Schilling 05-02, DNA 2078 [JQ737027; JQ737057].
- Steviopsis adenosperma* (Sch. Bip.) B. L. Turner: Mexico, Machaca 7591 [TEX], DNA 2473 [JQ737021; JQ737051]. *Steviopsis amblyolepis* (B. L. Rob.) R. M. King & H. Rob.: Mexico, Panero 6166, DNA 2471 [JQ737019; JQ737049]. *Steviopsis dryophila* (B. L. Rob.) B. L. Turner: Mexico, Panero 8819, DNA 2232 [JQ737017; JQ737047]; Panero 8855, DNA 2259 [JQ737018; JQ737048]. *Steviopsis rapunculoides* (DC.) R. M. King & H. Rob.: Mexico, Rzedowski 44954 [TEX], DNA 2472 [JQ737020; JQ737050]. *Steviopsis vigintiseta* (DC.) R. M. King & H. Rob.: Mexico, Panero 6754, DNA 559 [JQ737022; JQ737052].
- Ageratina luciae-brauniae* (Fern.) R. M. King & H. Rob.: U. S. A., Schilling 95-15, DNA 519 [AF177781/AF17782; HQ416183]. *Ageratina wrightii* (A. Gray) R. M. King & H. Rob.: Mexico, Panero 8813, DNA 2401 [JQ737035; JQ737065].